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Research Article

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Marginal ulcer after mini gastric by-pass and sleeve gastrectomy with transit bipartition

Muzaffer AL

Department of Surgery, Faculty of Medicine, Near East University, Nicosia, Cyprus

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Abstract

The aim of this study to present the incidence of marginal ulcer among patients who underwent laparoscopic mini gastric by-pass (MGBP) or laparoscopic sleeve gastrectomy with transit bipartition (SG-TB), and to determine demographic, clinical and surgical risk factors associated with marginal ulcer development. This case-control study was designed as a retrospective analysis of an SG-TB and MGBP surgical series. The marginal ulcer group consisted of 8 patients who developed marginal ulcers during their follow-up. The control group was formed with a randomly selected sample of 18 people matching according to age, sex and operation type among the remaining 626 patients. The median age of all patients included in the study was 51 (27-62) years. 75.0% of the marginal ulcer group and 66.7% of the control group were males (p = 1.000). Marginal ulcer developed in 1.26% of all patients who underwent bariatric-metabolic surgery, in 6.45% of patients who underwent MGBP, and in 0.99% of patients who underwent SG-TB. It was observed that the ulcers of all cases recovered completely. No perforation was observed in any patient, no surgical intervention was required, and no marginal ulcer-related mortality was observed. There was no significant difference between the marginal ulcer group and the control group in terms of surgical, clinical and demographic features. Considering our practices and results, it was thought that post-operative PPI treatment for at least 6 months had a preventive effect on marginal ulcer development, and PPI treatment used after marginal ulcer development had an important role in healing.

Keywords: obesity, marginal ulcer, mini gastric by-pass, sleeve gastrectomy with transit bipartition, bariatric-metabolic surgery

1. Introduction

Obesity is a growing public health problem which is comparable to a pandemic since it affects more than 1 billion people worldwide (1). Bariatric-metabolic surgery (BMS) has unique efficacy in the treatment of obesity and related complications, and its indications continue to expand (2). This increased frequency of administration inevitably increases the frequency of complications. In particular, late complications are not uncommon and can occur several years after surgery (2, 3). Sleeve gastrectomy (SG) has been specifically associated with incisura angularis stenosis and gastroesophageal reflux disease, gastric bypass (GBP) is specifically associated with marginal ulceration, gastrojejunal anastomotic stenosis, internal hernia, gastro-gastric fistula, while cholelithiasis, abdominal pain and undesirable weight gain may occur after either surgery (2, 4).

The incidence of marginal ulcer after BMS has been reported in a very wide range, from 0.35% to 25% (4, 5). Studies claim that various factors contribute to the pathophysiology of marginal ulcer, including high acidity due to large pouch size or gastro-gastric fistula, loss of protective effects provided by pancreatobiliary secretions in the jejunum (due to gastrojejunal anastomosis), helicobacter pylori, local ischemia, foreign bodies (e.g., suture material), suture technique, smoking, corticosteroid and non-steroidal anti-inflammatory drug (NSAID) use (2, 6, 7). However, the roles of these factors in the pathophysiology of marginal ulcers have not been clarified. Both laparoscopic SG and mini GBP (MGBP) are newer bariatric procedures compared to the classical Roux-en-Y GBP (RY-GBP). In particular, SG has become a mainstay procedure to treat morbid obesity. MGBP is also rapidly gaining global acceptance as an effective BMS procedure (8-11). First described by Santoro et al., the SG with transit bipartition (SG-TB) procedure is an adaptation that involves manipulation of the digestive tract to modulate neuroendocrine response of the distal small intestine in order to minimize malabsorption (12). Studies evaluating marginal ulcers have mainly focused on RY-GBP (7, 13) and the incidence or risk factors of marginal ulcers after MGBP (3, 14) or SG-TB (15, 16) have been researched relatively rarely.

In this study, we aimed to present the incidence of marginal ulceration in patients who underwent laparoscopic MGBP or laparoscopic SG-TB and to determine the risk factors for marginal ulcer development by comparing the clinicodemographic and surgical features of patients with and without marginal ulceration.

2. Material and Methods

This single-centered case-control study was designed as the retrospective analysis of a surgical series investigating laparoscopic SG-TB and laparoscopic MGBP for which data

*Correspondence: drmuzaffer61@hotmail.com

had been collected prospectively. The surgical procedures administered in our Bariatric Surgery Center of Exellence (COE), Department of General Surgery, Büyük Anadolu Hospital, Istanbul, Turkey was approved by the local Ethics Committe (No: YDU/2022/102-1515). The study was conducted in compliance with the 1964 Helsinki declaration and its later amendments.

2.1. Participants and data collection

The data of 634 patients with body mass index (BMI) ≥ 35 kg/m^2 who underwent BMS in our department between 2015 and 2021 were evaluated retrospectively. Laparoscopic SG-TB was performed to 603 obese patients with type 2 diabetes mellitus (T2DM) and a BMI of ≥35 kg/m^2, and laparoscopic MGBP was performed to 31 patients without T2DM and with a BMI of ≥40 kg/m^2. As a result of endoscopic controls, it was observed that marginal ulcer developed in 8 patients who comprised the marginal ulcer group. The "control group" was formed with a randomly selected sample of 18 subjects from the remaining 626 patients by matching according to age, sex and operation type. Patients younger than 18 years old and older than 65 years, patients who underwent revision surgery, patients who died, those whose data could not be reached, those with known peptic ulcer, Barrett's esophagus or liver cirrhosis, recipients of preoperative proton pump inhibitors (PPI), and those with major psychiatric disease were excluded from the study. Data of the cases, such as age, sex, BMI, type of operation, presence of diabetes, alcohol consumption and smoking, preoperative campylobacter-like organism (CLO) test results, drugs used, preoperative hemoglobin A1c (HbA1c) values, total cholesterol and triglyceride values were obtained from the digital records in the hospital database.

2.2. Surgery, follow-up and treatment

A routine surgical protocol was performed to all patients for BMS and routine follow-up was applied after surgery. Patients were called for controls 1 week, 1 month, 3 months, 6 months, 1 year after surgery and once a year thereafter. Oral PPI (pantoprazole 40 mg) was administered to all patients once a day for 6 months after the operation (14, 17). Upper gastrointestinal system endoscopy was performed before the operation and at 6-month intervals after the operation. In addition to the routine control examinations, upper gastrointestinal endoscopy was also performed in patients with relevant complaints. PPI treatment was resumed (if discontinued by patient) or continued in all patients with marginal ulceration, and nutritional regimens were adjusted according to the advice received from a dietitian. Patients with bleeding were hospitalized and necessary medical treatment and blood replacement procedures were applied, and the treatments of those using anticoagulants were altered if deemed necessary after hematology referral.

2.3. Statistical analysis

All analyses were performed in IBM SPSS v25.0 (IBM Corp., Armonk, NY, USA) with a significance threshold set at $p < 0.05$. The suitability of quantitative variables to normal distribution was evaluated with histogram and Q-Q plots. Quantitative variables were summarized as median (smallest value - largest value), while qualitative variables were summarized as frequency (percentage). Quantitative variable comparisons were performed with the Mann-Whitney *U* test. Qualitative variable distributions were compared with the Fisher's exact test.

3. Results

The median age of all patients included in the study was 51 (27-62) years. In terms of age, the marginal ulcer group [51 (32-61)] and the control group [51.5 (27-62)] were similar ($p = 0.802$). Males comprised 75.0% of the marginal ulcer group and 66.7% of the control group ($p = 1.000$).

Six of the patients who developed marginal ulcer also had T2DM. In patients with T2DM, marginal ulcer was observed on endoscopic examination performed at the first-year control examination. In patients without T2DM, marginal ulcer was observed 3 years after the operation in one patient and 4 years after the operation in the remaining patient. Two patients with T2DM applied with the complaint of bleeding and both of these patients were using anticoagulants. In the endoscopies of patients with bleeding, the bleeding ulcer was observed on the ileal side of the gastroileal anastomosis. The primary complaint of the remaining 4 patients with T2DM was epigastric pain. Both non-T2DM patients with marginal ulcers were active smokers and one had excessive alcohol consumption. The primary complaint of the patient with alcohol consumption was bleeding, while the other reported epigastric pain.

Overall, it was determined that marginal ulcer developed in 1.26% of all patients who underwent BMS. This percentage was 6.45% among MGBP recipients, and 0.99% among SG-TB recipients. The intergroup differences of all investigated variables are summarized in Table 1, and the demographic and clinical characteristics of all patients with marginal ulcers are summarized in Table 2. It was observed that all marginal ulcers were completely healed in the control upper gastrointestinal system endoscopies performed 3 months later. No perforation was observed, no surgical intervention was required, and no marginal ulcer-related mortality was observed in any of these patients.

4. Discussion

Marginal ulcer is a well-established complication that can occur after BMS. Although many factors have been associated with its pathophysiology, the contributions of these factors remain unclarified (2, 7). In the present study, the incidence of marginal ulcer was found to be 1.26% in all patients who underwent BMS. While this rate was 6.45% in MGBP patients, it was 0.99% in SG-TB patients. Asymptomatic marginal ulcer was not observed in any of our patients undergoing control endoscopy. There was no significant difference between the marginal ulcer group and the control group in terms of the parameters evaluated in the study.

Table 1. Patient characteristics and intergroup analysis results

	Marginal ulcer group (n=8)	Control group (n=18)	p
Age	51 (32 - 61)	51.5 (27 - 62)	0.802
Sex			
Male	6 (75.0%)	12 (66.7%)	1.000
Female	2 (25.0%)	6 (33.3%)	
Body mass index (kg/m^2)	35.95 (35.0 - 46.0)	35.15 (30.1 - 60.9)	0.278
Operation			
Mini gastric bypass	2 (25.0%)	4 (22.2%)	1.000
Sleeve gastrectomy with transit bipartition	6 (75.0%)	14 (77.8%)	
Diabetes mellitus	6 (75.0%)	14 (77.8%)	1.000
Alcohol consumption	2 (25.0%)	3 (16.7%)	0.628
Smoking	4 (50.0%)	6 (33.3%)	0.664
Preoperative CLO Test			
Negative	7 (87.5%)	15 (83.3%)	1.000
Pozitive	1 (12.5%)	3 (16.7%)	
Drug use			
Non-steroidal anti-inflammatory drug	5 (62.5%)	6 (33.3%)	0.218
Oral antidiabetic	6 (75.0%)	11 (61.1%)	0.667
Insulin	5 (62.5%)	7 (38.9%)	0.401
Anticoagulant	5 (62.5%)	9 (50.0%)	0.683
Antihypertensive	6 (75.0%)	6 (33.3%)	0.090
HbA1c (%)	9.4 (5.4 - 13.2)	10.05 (4.4 - 12.8)	0.846
Total cholesterol (mg/dL)	168 (57.8 - 281)	117.3 (47 - 281)	0.127
Triglyceride (mg/dL)	162.5 (118 - 666)	181.5 (45 - 606)	0.890

Quantitative variables are given as median (smallest value - largest value) and qualitative variables are given as frequency (percentage).
Abbreviations; CLO: Campylobacter-like organism, HbA1c: Hemoglobin A1c

Table 2. Characteristics of patients with marginal ulceration

No	Age	Sex	BMI	Operation	DM	Alcohol	Smoking	Preoperative CLO Test	NSAID	Oral antidiabetic	Insulin	Anticoagulant	Antihypertensive	HbA1c(%)	Total cholesterol(mg/dL)	Triglyceride(mg/dL)
1	38	Male	46.0	MGBP	-	+	+	-	-	-	-	-	-	5.4	144	124
2	32	Female	41.0	MGBP	-	-	+	-	-	-	-	-	-	5.6	78	132
3	51	Male	35.0	SG-TB	+	-	+	-	+	+	+	+	+	10.3	169	189
4	56	Male	35.4	SG-TB	+	-	-	-	+	+	+	+	+	8.1	57.8	366
5	52	Male	35.0	SG-TB	+	+	-	-	+	+	+	+	+	8.5	241	666
6	45	Male	36.0	SG-TB	+	-	+	+	-	+	-	-	+	13.2	281	222
7	61	Female	35.9	SG-TB	+	-	-	-	+	+	+	+	+	10.7	210	118
8	51	Male	37.4	SG-TB	+	-	-	-	+	+	+	+	+	13.0	167	136

Abbreviations: BMI: Body mass index, CLO: Campylobacter-like organism, DM: Diabetes mellitus, HbA1c: Hemoglobin A1c, MGBP: Mini gastric bypass, NSAID: Non-steroidal anti-inflammatory drugs, SG-TB: Sleeve gastrectomy with transit bipartition

Morbid obesity is associated with increased mortality and morbidity. Surgical treatment provides sustainable weight loss, reduction in comorbidity and improvement in quality of life (7). Despite these benefits of BMS, both surgeons and patients may face the risks of some important complications, such as the development of marginal ulcers in or just distal to the gastroenteral anastomosis (7). In previous studies, the incidence of marginal ulcer has been reported between 0.35% and 25% (4, 5). In this study, it was observed that marginal ulcer developed in 1.26% of all patients, 6.45% of patients who underwent MGBP, and 0.99% of patients who underwent SG-TB. In a retrospective study, marginal ulcer development rates in SG and MGBP patients followed for at least 5 years were found to be 0% and 1.4%, respectively (11). Ece et al. (n = 26) and Topart et al. (n = 71) did not observe any marginal ulcers in any of the patients who had SG-TB during their 1-year and 2-year follow-up periods, respectively. In these studies, it was also reported that marginal ulcers did not occur in patients who had undergone RY-GBP (n = 83, n = 71, respectively) (16, 18). In a systematic review of 12.807 patients who underwent

MGBP, the incidence of marginal ulcer was reported as 2.7% (10). Clapp et al. reported a marginal ulcer incidence of 0.35% in 44.379 GBP patients (4). In another study, the incidence of marginal ulcer reported by the surgeon after a total of 27,672 MGBP operations performed by 86 surgeons was investigated and this incidence was determined as 2.24% (14). It can be said that the frequency of marginal ulcer detected in our study is compatible with other studies. The reasons for the large differences in the incidence of marginal ulcer development after BMS may include different distributions of risk factors, patient characteristics, preoperative preparation, prophylaxis, surgical technique(s) and surgeon experience (14).

In this study, it was observed that the majority (6/8) of marginal ulcers developed within the first year. It was also noteworthy that all of them were identified in patients with T2DM who had undergone SG-TB. This result is consistent with the results of other studies reporting that marginal ulcers develop after 30 days of the intervention and within the first 2 years (4, 7, 19-21). If marginal ulcer is not diagnosed and treated early, it may cause serious complications such as massive bleeding and perforation and may require revision surgery. As a result of marginal ulcer after MGBP, perforation can be seen with a frequency of 8.2% and bleeding with a frequency of 9.5% (22, 23). A series of 7 cases with delayed anastomotic perforation caused by marginal ulcer after MGBP has been published (3). In a cohort study, 1-year outcomes of SG, MGBP and SG-TB operations were compared. While marginal ulcers were not observed in any patients in the SG group (0/104), marginal ulcer-induced perforation occurred in 1 patient (1/39) in the MGBP group and in 2 patients (2/34) in the SG-TB group (15). In the current study, none of the patients suffered from marginal ulcer perforation or had required revision surgery. Three of the 8 patients presented with the complaint of non-massive bleeding and completely recovered with conservative medical treatment.

Another important result of this study was that all patients with marginal ulcers were symptomatic. Baksi et al. argued that routine surveillance endoscopy performed 1 year after MGBP operation could detect asymptomatic marginal ulcers, and thus prevent ulcer-related complications (24). In this study, it was observed that none of the patients developed asymptomatic marginal ulcers. Therefore, it can be said that the necessity of routine control endoscopy in asymptomatic patients is not clear; however, since 6 of the 8 marginal ulcers were identified at the first year follow-up examination, the likelihood of asymptomatic marginal ulcer should not be overlooked in the earlier periods (23).

Risk factors that facilitate marginal ulcer occurrence after BMS have been the focus of attention for many researchers. Different risk factors for marginal ulcers have been identified in studies (6, 13, 21, 25). There was no significant difference between the patients with and without marginal ulcer in terms of the parameters we examined in this study, possibly due to the relatively low incidence of marginal ulcer in our patients. In a comprehensive study, increased BMI, need for percutaneous transluminal cardiac catheterization, history of deep vein thrombosis and pulmonary embolism were reported as significant risk factors for marginal ulcer after GBP (4). In the study of Mahawar et al., surgeons stated that the most important risk factors for the development of marginal ulcer after MGBP were smoking, NSAID use and alcohol consumption (14). In a population-based cohort study, risk factors for marginal ulcer development were investigated in 20,294 GBP patients, and it was stated that the presence of diabetes and a history of peptic ulcer were the most important risk factors, while hyperlipidemia, hypertension, chronic obstructive pulmonary disease, low-dose aspirin and NSAID use were not risk factors (26). In various studies, use of NSAIDs for more than 30 days, active smoking or smoking history, use of immunosuppressive drugs, preoperative gastroesophageal reflux disease, diabetes, dyslipidemia, coronary artery disease, chronic lung disease, time elapsed after surgery, inhaled steroid use, and gastric pouch location and its size were identified as risk factors for marginal ulcer development after RY-GBP (5, 6, 13, 21, 27, 28). The pathophysiological effects of these risk factors have not been clarified. Azagury et al. hypothesized that the increased incidence of marginal ulcers, particularly in patients with cardiovascular risk factors, may be related to impaired mucosal microcirculation around the gastro-jejunal anastomosis (21). Although it seems difficult to pinpoint definite risk factors, it can be said that the presence of diabetes, smoking and NSAID use in particular stand out as possible risks.

Acid exposure plays a role in the development of marginal ulcers and the effectiveness of PPI therapy in the treatment of marginal ulcers is clearly known (21). Coblijn et al. reported that 6 months of acid suppression therapy following surgery reduced marginal ulcer incidence from 7.3% to 1.2%, also Kang et al. showed that a 90-day PPI treatment was more effective than a 30-day treatment in preventing the occurrence of marginal ulcers (17, 29). In another study, it was argued that using PPI before the operation halved the probability of developing a marginal ulcer (13). In a survey study, 82.4% of 86 surgeons stated that they used PPIs prophylactically; however, it has been observed that there are differences in the drugs used, dosages and durations (14). Although there is no definite consensus regarding the duration of PPI use, many studies have suggested the use of PPIs in the first 6 months post-operatively (17, 24, 30). Some bariatric surgeons have argued that marginal ulcers after MGBP are more persistent, less responsive to PPI, and can lead to anastomotic perforation even years after surgery (31-33). We initiated PPI treatment in all patients undergoing BMS for at least 6 months postoperatively, and to patients who developed marginal ulcers until they were completely healed. We think that this approach has an important role in reducing marginal ulcer likelihood and the success of marginal ulcer management.

This study has some limitations. The fact that it was a single-centered study limited the generalizability of the results. Since the study was retrospective, data on some factors that may affect marginal ulcer development, such as gastric pouch width, sutures, staples or other foreign bodies, smoking and alcohol consumption could not be accessed, and therefore, their effects could not be investigated. Since marginal ulcer is a rare complication, the inevitably low number of cases may have affected the statistical results concerning the comparison of groups. The lack of follow-up of patients after inclusion in the study may have obscured the incidence of asymptomatic marginal ulcers that may occur later, and thus, the true incidence of marginal ulcers.

In conclusion, the overall incidence of marginal ulcer after BMS was found to be 1.26% (6.45% in patients who underwent MGBP, and 0.99% in patients who underwent SG-TB). There was no significant difference between the marginal ulcer group and the control group in terms of surgical, clinical and demographic characteristics. Considering our practices and results, it was thought that post-operative PPI treatment for at least 6 months had a protective effect in preventing marginal ulcer development, and PPI treatment in patients with marginal ulcer contributed to better management. It will be useful to carry out comprehensive and multicenter studies in order to determine the true incidence and risk factors of marginal ulcer and to establish a generally-accepted treatment and/or prevention algorithm.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

Concept: M.A., Design: M.A., Data Collection or Processing: M.A., Analysis or Interpretation: M.A., Literature Search: M.A., Writing: M.A.

References

1. Deffieux X, De Tayrac R, Huel C, Bottero J, Gervaise A, Bonnet K, et al. Vaginal mesh erosion after transvaginal repair of cystocele using Gynemesh or Gynemesh-Soft in 138 women: a comparative study. Int Urogynecol J Pelvic Floor Dysfunct. 2007; 18(1): 73-9.

2. Kumbhari V, Le Roux CW, Cohen RV. Endoscopic Evaluation and Management of Late Complications After Bariatric Surgery: a Narrative Review. Obes Surg. 2021; 31(10): 4624-33.

3. Aviran E, Rayman S, Yehuda AB, Goitein D. Marginal ulcer causing delayed anastomotic perforation following one anastomosis gastric bypass (OAGB). Surg Obes Relat Dis. 2021; 17(2): 379-83.

4. Clapp B, Hahn J, Dodoo C, Guerra A, De La Rosa E, Tyroch A. Evaluation of the rate of marginal ulcer formation after bariatric surgery using the MBSAQIP database. Surg Endosc. 2019; 33(6): 1890-7.

5. Coblijn UK, Goucham AB, Lagarde SM, Kuiken SD, Van Wagensveld BA. Development of ulcer disease after Roux-en-Y gastric bypass, incidence, risk factors, and patient presentation: a systematic review. Obes Surg. 2014; 24(2): 299-309.

6. Skogar ML, Sundbom M. Nonsteroid anti-inflammatory drugs and the risk of peptic ulcers after gastric bypass and sleeve gastrectomy. Surg Obes Relat Dis. 2022; 18(7): 888-93.

7. Bekhali Z, Sundbom M. Low Risk for Marginal Ulcers in Duodenal Switch and Gastric Bypass in a Well-Defined Cohort of 472 Patients. Obes Surg. 2020; 30(11): 4422-7.

8. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, et al. Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes. N Engl J Med. 2017; 376(7): 641-51.

9. Madsbad S, Holst JJ. Bariatric surgery-which procedure is the optimal choice? Lancet. 2019; 393(10178): 1263-4.

10. Parmar CD, Mahawar KK. One Anastomosis (Mini) Gastric Bypass Is Now an Established Bariatric Procedure: a Systematic Review of 12,807 Patients. Obes Surg. 2018; 28(9): 2956-67.

11. Kular KS, Manchanda N, Rutledge R. Analysis of the five-year outcomes of sleeve gastrectomy and mini gastric bypass: a report from the Indian sub-continent. Obes Surg. 2014; 24(10): 1724-8.

12. Santoro S. Adaptive and neuroendocrine procedures: a new pathway in bariatric and metabolic surgery. Obes Surg. 2008; 18(10): 1343-5.

13. Di Palma A, Liu B, Maeda A, Anvari M, Jackson T, Okrainec A. Marginal ulceration following Roux-en-Y gastric bypass: risk factors for ulcer development, recurrence and need for revisional surgery. Surg Endosc. 2021; 35(5): 2347-53.

14. Mahawar KK, Reed AN, Graham YNH. Marginal ulcers after one anastomosis (mini) gastric bypass: a survey of surgeons. Clin Obes. 2017; 7(3): 151-6.

15. Santoro S, Castro LC, Velhote MC, Malzoni CE, Klajner S, Castro LP, et al. Sleeve gastrectomy with transit bipartition: a potent intervention for metabolic syndrome and obesity. Ann Surg. 2012; 256(1): 104-10.

16. Ece I, Yilmaz H, Yormaz S, Çolak B, Calisir A, Sahin M. The Short-Term Effects of Transit Bipartition with Sleeve Gastrectomy and Distal-Roux-en-Y Gastric Bypass on Glycemic Control, Weight Loss, and Nutritional Status in Morbidly Obese and Type 2 Diabetes Mellitus Patients. Obes Surg. 2021; 31(5): 2062-71.

17. Coblijn UK, Lagarde SM, De Castro SM, Kuiken SD, Van Tets WF, Van Wagensveld BA. The influence of prophylactic proton pump inhibitor treatment on the development of symptomatic marginal ulceration in Roux-en-Y gastric bypass patients: a historic cohort study. Surg Obes Relat Dis. 2016; 12(2): 246-52.

18. Topart P, Becouarn G, Finel JB. Comparison of 2-Year Results of Roux-en-Y Gastric Bypass and Transit Bipartition with Sleeve Gastrectomy for Superobesity. Obes Surg. 2020; 30(9): 3402-7.

19. Palermo M, Acquafresca PA, Rogula T, Duza GE, Serra E. Late surgical complications after gastric by-pass: a literature review. Arq Bras Cir Dig. 2015; 28(2): 139-43.

20. Moon RC, Teixeira AF, Goldbach M, Jawad MA. Management and treatment outcomes of marginal ulcers after Roux-en-Y gastric bypass at a single high volume bariatric center. Surg Obes Relat Dis. 2014; 10(2): 229-34.

21. Azagury DE, Abu Dayyeh BK, Greenwalt IT, Thompson CC. Marginal ulceration after Roux-en-Y gastric bypass surgery: characteristics, risk factors, treatment, and outcomes. Endoscopy. 2011; 43(11): 950-4.

22. Baksi A, Kamtam DNH, Aggarwal S, Ahuja V, Kashyap L, Shende DR. Should Surveillance Endoscopy Be Routine After One Anastomosis Gastric Bypass to Detect Marginal Ulcers: Initial Outcomes in a Tertiary Referral Centre. Obes Surg. 2020; 30(12): 4974-80.

23. Coblijn UK, Lagarde SM, De Castro SM, Kuiken SD, Van Wagensveld BA. Symptomatic marginal ulcer disease after Roux-en-Y gastric bypass: incidence, risk factors and management. Obes Surg. 2015; 25(5): 805-11.

24. Noun R, Skaff J, Riachi E, Daher R, Antoun NA, Nasr M. One thousand consecutive mini-gastric bypass: short- and long-term outcome. Obes Surg. 2012; 22(5): 697-703.

25. Kalaiselvan R, Exarchos G, Hamza N, Ammori BJ. Incidence of perforated gastrojejunal anastomotic ulcers after laparoscopic gastric bypass for morbid obesity and role of laparoscopy in their management. Surg Obes Relat Dis. 2012; 8(4): 423-8.

26. Sverdén E, Mattsson F, Sondén A, Leinsköld T, Tao W, Lu Y, et al. Risk Factors for Marginal Ulcer After Gastric Bypass Surgery for Obesity: A Population-based Cohort Study. Ann Surg. 2016; 263(4): 733-7.

27. Dittrich L, Schwenninger MV, Dittrich K, Pratschke J, Aigner F, Raakow J. Marginal ulcers after laparoscopic Roux-en-Y gastric bypass: analysis of the amount of daily and lifetime smoking on postoperative risk. Surg Obes Relat Dis. 2020; 16(3): 389-96.

28. Yuce TK, Khorfan R, Soper NJ, Hungness ES, Nagle AP, Teitelbaum EN, et al. Post-Operative Complications and Readmissions Associated with Smoking Following Bariatric Surgery. J Gastrointest Surg. 2020; 24(3): 525-30.

29. Kang X, Zurita-Macias L, Hong D, Cadeddu M, Anvari M, Gmora S. A comparison of 30-day versus 90-day proton pump inhibitor therapy in prevention of marginal ulcers after laparoscopic Roux-en-Y gastric bypass. Surg Obes Relat Dis. 2016; 12(5): 1003-7.

30. Parmar CD, Mahawar KK, Boyle M, Carr WR, Jennings N, Schroeder N, et al. Mini Gastric Bypass: first report of 125 consecutive cases from United Kingdom. Clin Obes. 2016; 6(1): 61-7.

31. Scheffel O, Daskalakis M, Weiner RA. Two important criteria for reducing the risk of postoperative ulcers at the gastrojejunostomy site after gastric bypass: patient compliance and type of gastric bypass. Obes Facts. 2011; 4 Suppl 1(Suppl 1): 39-41.

32. Mahawar KK, Jennings N, Brown J, Gupta A, Balupuri S, Small PK. "Mini" gastric bypass: systematic review of a controversial procedure. Obes Surg. 2013; 23(11): 1890-8.

33. Mahawar KK, Borg CM, Kular KS, Courtney MJ, Sillah K, Carr WRJ, et al. Understanding Objections to One Anastomosis (Mini) Gastric Bypass: A Survey of 417 Surgeons Not Performing this Procedure. Obes Surg. 2017; 27(9): 2222-8.

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Prognostic factors affecting survival in breast cancer patients age 40 or younger

Can AKGÜN[1,*], Bekir KURU[2], Savaş YÜRÜKER[2], Ufuk KARABACAK[4] Necati ÖZEN[1]

[1]Department of General Surgery, Faculty of Medicine, İstinye University, İstanbul, Türkiye
[2]Department of General Surgery, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[3]Department of Surgical Oncology, Sivas Numune Hospital, Sivas, Türkiye
[4]Department of General Surgery, VM Medical Park Samsun Hospital, Samsun, Türkiye

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Abstract

The aims of this study were to identify the factors affecting survival and disease-free survival (DFS) in invasive breast cancer patients who underwent surgery for breast cancer age 40 or younger. Medical records of 216 women with breast carcinoma at age 40 or younger who underwent surgery at our Institution between October 2005 and May 2017 were retrospectively reviewed. One hundred and eighty eight invasive breast cancer patients that were eligible were categorized according to their clinical and pathological features. Univariate analyses of survival and DFS were performed by the Kaplan–Meier method and the log-rank test. Independent prognostic and predictive factors affecting survival and DFS were assessed by Cox regression proportional hazard method. 10-year survival and DFS were 85 and 74%, respectively. Axillary involvement, pathologic tumor size, HER 2 + subtype and Triple Negative subtype were found to be the prognostic factors that independently affected survival and DFS. The prognosis is worse in patients with axillary involvement, tumors larger than 2 cm, and HER 2+ and Triple Negative subtypes. These adverse prognostic factors should be considered during treatment and follow-up of patients age 40 or younger.

Keywords: breast cancer, prognosis, survival, disease-free survival, breast neoplasms

1. Introduction

Breast cancer is the most common cancer in women and is an important public health problem that causes approximately 2.088.000 new cases and 627.000 deaths worldwide each year (1). Although majority of patients (88.4%) are diagnosed over the age of 40, the 11.6% of patients are at age 40 or younger (1). In a study published from our country, 17.2% of patients diagnosed with breast cancer were found to be younger than 40 years old (2). Despite these high rates, there are few studies examining breast cancer and prognostic factors affecting survival or disease-free survival (DFS) in women at the age of 40 or younger (3-6).

Risk factors, clinical outcomes, and tumor biology could differ in women aged 40 or younger and may present more aggressive behaviour and worse prognosis (7, 8). It has been reported that the disease is at an advanced stage during the presentation in cases of breast cancer at the age of 40 or younger (9-11). These suggestions raise the question that prognostic factors for survival and disease-free survival in patients≤ 40 years old could be different than the older patients. Therefore, prognostic factors for survival and DFS have been investigated inpatients aged 40 or younger. Positive axillary lymph nodes, hormone receptors, HER2 status, molecular subtypes, tumor size, grade, age, and type of surgery have been defined as independent prognostic factors but controversies about prognostic factors still exist (3-6, 12, 13).

The aim of our study is to show the prognostic and predictive factors affecting survival and disease-free survival of invasive breast cancer patients age 40 years or younger.

2. Material and Methods

The procedures followed were in accordance with the ethical standards of the institutional or regional responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983. Informed consent was obtained from participants in the study and our Institution's ethics committee approved the study.

This study was conducted with the approval of our hospital's ethics committee (Approval number: 2019/663, approval date:17.10.2019). Medical records of 216 women with breast carcinoma age 40 or younger who underwent surgery at Ondokuz Mayis University School of Medicine, Department of General Surgery between October 2005 and May 2017 were retrospectively reviewed.

Two hundred and sixteen patients included in the present

*Correspondence: canakgun@outlook.com.tr

study were selected among 1587 patients who underwent surgery during this period (13.6%). Pathological and clinical data and follow-up information were retrieved from the medical records. Patients with pure ductal carcinoma in-situ (DCIS), with T4 tumor and patients who received neoadjuvant chemotherapy and patients whose postoperative examination, follow-up and treatment data were not available were not eligible and excluded from the study.

Prognostic and predictive factors for survival and DFS were analyzed for 188 invasive breast cancer patients. If 3 months elapsed after the last regular examination time during follow-up, recent status of patients was also updated by phone call. The follow-up times of the survivors continued to March 2019. All patients were followed up with 3-6 months' intervals for the first three years and every 6 months during years 4 and 5.

Histopathological subtypes were classified according to World Health Organization criteria as invasive ductal, invasive lobular carcinoma, or as pure special features such as medullary, tubular, mucinous, papillary, scirrhous, apocrine and adenoid cystic carcinoma. ER and PR status were defined by immunochemistry and staining of 1% of tumor cells was accepted ER or PR positive. Molecular subtypes of breast cancer were determined according to the classification on the St. Gallen International Expert Consensus in 2011 (14) .Adjuvant radiation therapy was applied to the breast of patients who underwent breast conserving surgery, and patients who underwent mastectomy and had pT3 tumor or had ≥ 4 axillary lymph node involvement received adjuvant radiotherapy to the chest wall. Patients with axillary involvement who did not undergo axillary lymph node dissection received axillary radiation therapy. Adjuvant radiotherapy was given also to the supraclavicular and internal mammary fields of the patients who had ≥ 4 positive axillary lymph nodes (15). Adjuvant chemotherapy was given to all patients except three patients whose tumors were classified as Luminal A subtype, had a tumor less than 1cm, and grade 1 tumor, and had not axillary lymph node involvement. All patients with ER and/or PR positive tumors were given adjuvant hormonotherapy. Patients with HER2+ status had trastuzumab treatment (16-22)

Potential prognostic and predictive factors included in this study were age (20-30, 31-40), type of surgery performed (BCS, mastectomy), axillary lymph node metastasis (pN0, pN+ and pN0, pN1, pN2, pN3), histopathological type (invasive ductal carcinoma, invasive lobular carcinoma, other), pathological tumor size (pT1, pT2, pT3), pathological grade (1, 2, 3), estrogen, progesterone, and HER2 status (Negative, Positive), molecular subtypes (Luminal A, Luminal B, HER2 +, Triple Negative) and lymphovascular invasion (LVI) (Yes, No)(Table 1).

Table 1. 10-year survival and disease-free survival by characteristics of patients

	No (%)	10-year OS %	p (log rank)	10-year DFS %	p (log-rank)
Age					
<31	32 (17)	87		63	NS
31-41	156 (83)	84	N	76	
Type of surgery					
BCS	98 (52)	89		80	
Mastectomy	90 (48)	81	0.062	70	NS
Axillary involvement					
pN0	89 (47)	91		80	
pN+	99 (53)	80	0.028	69	NS
Axillary involvement					
pN0	89 (47)	91	0.	80	0.05
pN1 (1-3 positive nodes) LN)LN)	62 (33)	79		68	
pN2 (4-9 positive nodes) LLNLLLLN)LN) nodes	28 (15)	82		73	
pN3 (≥10 positive nodes)	9 (5)	78		56	
Histopathological Type					
Invasive ductal	173 (92)	85		76	
Invasive lobular	7 (4)	86	NS	86	NS
Other	8 (4)	88	NS	49	NS
Pathological Tumor Size					
pT1	66 (35)	96		93	
pT2	95 (51)	80	0.029	66	0.00
pT3	27 (14)	60	0.027	49	0.00
Grade					
1	17 (9)	91		91	
2	95 (51)	89	NS	77	NS

3	76 (41)	66	0.054	64	NS
Estrogen Receptor					
Positive	152 (81)	88		78	
Negative	38 (19)	73	0.061	61	0.02
Progesterone Receptor					
Positive	129 (69)	88		77	
Negative	59 (31)	77	NS	67	NS
HER2					
Positive	75 (40)	87		79	
Negative	113 (60)	80	NS	67	NS
Molecular Subtype					
Luminal A	73 (39)	90		88	
Luminal B	79 (42)	92	NS	71	0.025
HER2+	12 (6)	68	0.004	54	0.001
Triple Negative	24 (13)	54	0.021	53	0.009
Lymphovascular Invasion					
Yes	75 (40)	82	NS	71	NS
No	113 (60)	91		81	

2.1. Statistical Analysis

SPSS (Statistical Package for Social Sciences) for Windows 15.0 program was used for statistical analysis. The follow-up interval calculated in months and defined as the time between the date of surgery and the date of event (death, local, regional or distant recurrence, or contralateral breast cancer) or last follow-up. The first documented recurrence of disease or occurrence of contralateral breast cancer, whichever occurred earlier was defined as event of endpoint for DFS, and death was defined as event of endpoint for survival. Descriptive statistical methods (Median) were used while evaluating the data regarding age, follow-up and recurrence time. The ca0tegorical data were expressed as numbers and percentages, and the continuous data and the follow-up time were expressed as median (range). Comparisons of categorical data were made with the Chi-square test. Kaplan Meier method and Log Rank test were used in survival analysis. Stepwise Cox Regression Proportional Hazards model was used to assess the independent prognostic and predictive factors affecting survival and DFS. Variables that were found to have a significant effect on OS or DFS at univariate analysis were included in the multivariate analysis. The results were evaluated in the 95% confidence interval and P values of less than 0.05 were considered significant.

3. Results

Median age was 36 years (range, 22-40) and the median follow-up was 75 months (range, 8 - 166). Ninety-eight patients underwent sentinel lymph node biopsy (SLNB) and 90 underwent axillary lymph node dissection (ALND) with or without SLNB. There were 18 deaths due to breast carcinoma. Total number of events was 34. Median recurrence time was 32 months (range, 4-114). Ten-year survival was 85% and 10-year disease-free survival was 74%. Characteristics of patients and the factors that are potentially affecting survival or DFS were shown in Table 1.

Local, regional recurrence or distant metastasis were found in 2 (1%), 2 (1%) and 19 (10%) patients, respectively. Single organ metastasis and multiple distant metastases were detected in 9 and 10 of those 19 patients, respectively. Out of 33 organ metastasis bone, lung, brain and liver metastases were found in 11, 11, 6 and 5 patients, respectively. Two patients had contralateral breast cancer.

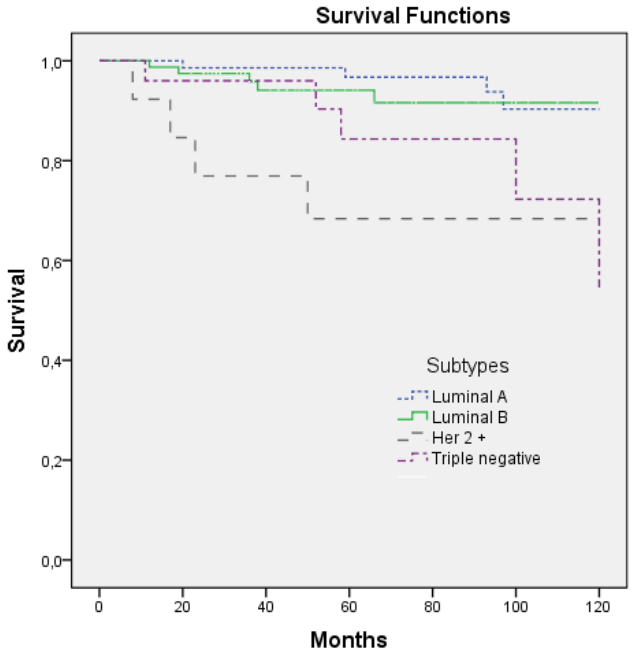

Fig.1. Overall survival by molecular subtypes (p=0.011)

Among the prognostic and predictive variables entered into the univariate analysis axillary involvement (pN0, pN+), pathological tumor size and molecular subtype (Fig.1) correlated with survival. Pathological tumor size (pT), ER status and molecular subtype (Fig. 2) were found to be associated with DFS (Table 1).

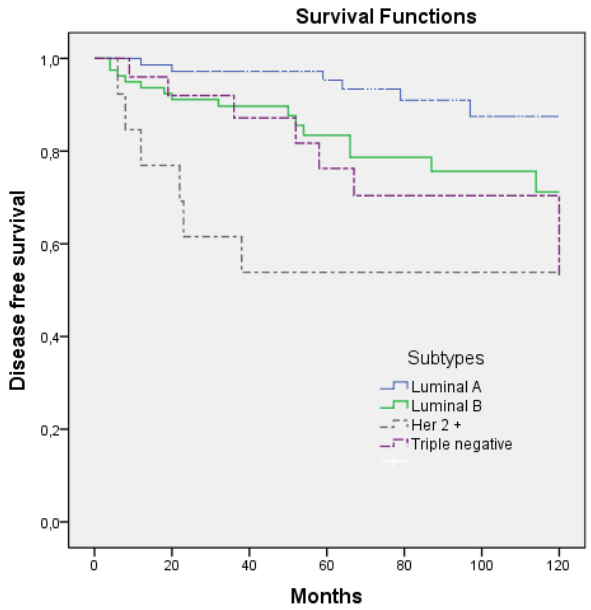

Fig. 2. Disease free survival by molecular subtypes (p=0.004)

Multivariate analysis revealed that axillary lymph node involvement (pN0, pN+), (HR (95% CI): 4.05 (1.30-12.64), p = 0.016), HER2 + subtype (HR (95% CI): 5.83 (1.44- 23.60), p = 0.013) and Triple Negative subtype (HR (95% CI): 5.63 (1.48-21.41), p = 0.011) were independent prognostic factors affecting 10-year survival (Table 2). pT2 (HR (95% CI): 3.51 (1.20-10.28), p = 0.022) and pT3 (HR (95% CI): 4.85 (1.44-16.23), p = 0.010), HER2 + molecular subtype (HR (95% CI): 5.33 (1.70-16.64), p = 0.004) and Triple Negative subtype (HR (95% CI): 3.04 (1.02-9.12), p = 0.047) were found as independent prognostic factors for 10-year DFS (Table 3).

Table 2. Independent prognostic factors affecting overall survival in multivariate analysis

	Hazard ratio (confidence interval)	p
Axillary involvement	4.05 (1.30-12.64)	0.016
HER2 + Subtype	5.83 (1.44-23.60)	0.013
Triple Negative	5.64 (1.48-21.41)	0.011

Table 3. Independent prognostic factors affecting disease-free survival in multivariate analyzes

	Hazard ratio (confidence interval)	p
Pathologic Tumor Size (pT2)	3.51 (1.20-10.28)	0.022
Pathologic Tumor Size (pT3)	4.85 (1.44-16.23)	0.010
HER2+ Subtype	5.33 (1.70-16.64)	0.004
Triple Negative Subtype	3.04 (1.02-9.12)	0.047

4. Discussion

Breast cancer is the most common and most fatal form of cancer in women, leaving lung cancer behind. According to GLOBOCAN 2018 data, 25% of cancers in women and 15% of cancer-related women deaths are related to breast cancer. Approximately 11.6% of breast cancer patients newly diagnosed in 2018 are at the age of 40 or younger. This rate was determined as 6.2% in Europe, 5.6% in the USA and 16.6% in our country (1, 2). It was 13.6% in our center.

In the POSH study by Copson et al. which consists of 2956 patients at age ≤40, the median age was 36 years. 10.7% of patients were at 30 or younger, and 89.3% were at age 31-40 (3). The age groups in POSH study are similar to our findings (Table 1). Ten-year survival and disease-free survival were 85%, and 74%, respectively in our study. In the POSH study, 5-year survival, and disease-free survival were 81.9%, 76.6%, respectively (3). In the study of Thomas et al. based on the Surveillance, Epidemiology, and End Results (SEER) registries database, 10-year survival was 76% in 38.411 women with stage I-III breast cancer younger than 40 years (23). In a large series from SEER database, 10-year survival was 84% for stage I-II breast cancer (6). In our study, 10-year survival was found to be higher as 85%. We agree with Mahmood et al. (6) who reported that there was no significant difference in survival in 14.764 patients aged 20-39 years from SEER database who underwent BCS and mastectomy (83.5% vs 83.6% for BCS or mastectomy) and we agree also with the other two studies which reported that type of surgery was not an independent factor for survival (13, 17).

In the present study ten-year survival was significantly shorter in patients with pathological axillary involvement. Our findings are also compatible with the previous studies in literature which reported that survival and/or DFS decreases significantly in patients with axillary involvement (4, 6, 13, 24). However, Keegan et al. reported that it was not a significant factor for survival. (6) Our findings overlap with the studies which reported that survival and/or DFS decreases as tumor size increases in patients ≤ 40 years old, (4, 6, 24) but contrast with study by Yoshida et al (13).

Among the studies which analysed the molecular classification in patients younger than 40years old, Keegan et al. based on data for 5.331 breast cancers aged 15-39 years obtained from the California Cancer Registry reported that survival in HER2 + and Triple Negative subtypes was significantly shorter than in HR+/HER2- breast cancersubtype (5). Yoshida et al.also reported that survival was significantly shorter in patients with Triple Negative subtype (13). The findings of the present study are in agree with those studies.In our study, 10-year survivaland 10-year DFS were significantly shorter in the Triple Negative and HER2 + subtypes than in Luminal A, B subtypes. Fredholm et al. stated that Luminal B subtype has the worst breast cancer specific survival in compared with the other subtypes in patients under the age of 40. However, in that analysis it was stated that Luminal-HER2 (ER+ and Her2+, any PR or Ki67), and HER2-positive were combined (12).

The retrospective nature, the limited number of patients analysed in the present study and lack of details of the adjuvant treatments are some limitations of the present study.

Independent prognostic factors affecting 10-year survival and DFS in breast cancer patients ≤ 40 years old were determined as pT, axillary involvement, HER2 + and Triple Negative subtypes. Considering these adverse factors could play an important role in the course of the disease during the diagnosis, treatment and follow-up processes in patients aged 40 or younger.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: C.A., S.S.Y., Design: U.K., N.Ö., Data Collection or Processing: C.A., S.S.Y., Analysis or Interpretation: C.A., B.K., Literature Search: U.K., N.Ö., Writing: C.A., B.K.

References

1. World Health Organization I. Estimated number of new cases in 2018 GLOBOCAN web page: World Health Organization, IARC; 2018 [cited 2018]. Available from: https://gco.iarc.fr/today/online-analysis-table?v=2018&mode=cancer&mode_population=continents&population=900&populations=900&key=asr&sex=0&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=5&group_cancer=1&include_nmsc=1&include_nmsc_other=1.

2. Ozmen V, Ozmen T, Dogru V. Breast Cancer in Turkey; An Analysis of 20.000 Patients with Breast Cancer. Eur J Breast Health. 2019;15(3):141-6. Epub 2019/07/18. doi: 10.5152/ejbh.2019.4890. PubMed PMID: 31312788; PubMed Central PMCID: PMCPMC6619786.

3. Copson E, Eccles B, Maishman T, Gerty S, Stanton L, Cutress RI, et al. Prospective observational study of breast cancer treatment outcomes for UK women aged 18-40 years at diagnosis: the POSH study. J Natl Cancer Inst. 2013;105(13):978-88. Epub 2013/06/01. doi: 10.1093/jnci/djt134. PubMed PMID: 23723422.

4. Karihtala P, Winqvist R, Bloigu R, Jukkola-Vuorinen A. Long-term observational follow-up study of breast cancer diagnosed in women ≤40 years old. Breast (Edinburgh, Scotland). 2010;19(6):456-61. Epub 2010/06/24. doi: 10.1016/j.breast.2010.05.001. PubMed PMID: 20570150.

5. Keegan TH, Press DJ, Tao L, DeRouen MC, Kurian AW, Clarke CA, et al. Impact of breast cancer subtypes on 3-year survival among adolescent and young adult women. Breast Cancer Res. 2013;15(5):R95. Epub 2013/10/18. doi: 10.1186/bcr3556. PubMed PMID: 24131591; PubMed Central PMCID: PMCPMC3978627.

6. Mahmood U, Morris C, Neuner G, Koshy M, Kesmodel S, Buras R, et al. Similar survival with breast conservation therapy or mastectomy in the management of young women with early-stage breast cancer. Int J Radiat Oncol Biol Phys. 2012;83(5):1387-93. Epub 2012/02/04. doi: 10.1016/j.ijrobp.2011.10.075. PubMed PMID: 22300561.

7. Hartley MC, McKinley BP, Rogers EA, Kalbaugh CA, Messich HS, Blackhurst DW, et al. Differential expression of prognostic factors and effect on survival in young (< or =40) breast cancer patients: a case-control study. The American surgeon. 2006;72(12):1189-94; discussion 94-5. Epub 2007/01/16. PubMed PMID: 17216817.

8. Love RR, Duc NB, Dinh NV, Quy TT, Xin Y, Havighurst TC. Young age as an adverse prognostic factor in premenopausal women with operable breast cancer. Clinical breast cancer. 2002;2(4):294-8. Epub 2002/03/20. doi: 10.3816/cbc.2002.n.005. PubMed PMID: 11899361.

9. Partridge AH, Hughes ME, Ottesen RA, Wong YN, Edge SB, Theriault RL, et al. The effect of age on delay in diagnosis and stage of breast cancer. Oncologist. 2012;17(6):775-82. Epub 2012/05/05. doi: 10.1634/theoncologist.2011-0469. PubMed PMID: 22554997; PubMed Central PMCID: PMCPMC3380876.

10. Vogel JE, Chu C, McCullough M, Anderson E, Losken A, Carlson GW. Breast cancer in women under age 40 years: treatment by total mastectomy and reconstruction. Annals of plastic surgery. 2011;66(5):557-60. Epub 2011/04/01. doi: 10.1097/SAP.0b013e318216b648. PubMed PMID: 21451370.

11. Zabicki K, Colbert JA, Dominguez FJ, Gadd MA, Hughes KS, Jones JL, et al. Breast cancer diagnosis in women < or = 40 versus 50 to 60 years: increasing size and stage disparity compared with older women over time. Ann Surg Oncol. 2006;13(8):1072-7. Epub 2006/07/26. doi: 10.1245/aso.2006.03.055. PubMed PMID: 16865599.

12. Fredholm H, Magnusson K, Lindstrom LS, Garmo H, Falt SE, Lindman H, et al. Long-term outcome in young women with breast cancer: a population-based study. Breast Cancer Res Treat. 2016;160(1):131-43. Epub 2016/09/15. doi: 10.1007/s10549-016-3983-9. PubMed PMID: 27624330; PubMed Central PMCID: PMCPMC5050247.

13. Yoshida M, Shimizu C, Fukutomi T, Tsuda H, Kinoshita T, Akashi-Tanaka S, et al. Prognostic factors in young Japanese women with breast cancer: prognostic value of age at diagnosis. Japanese journal of clinical oncology. 2011;41(2):180-9. Epub 2010/10/16. doi: 10.1093/jjco/hyq191. PubMed PMID: 20947623.

14. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Annals of oncology : official journal of the European Society for Medical Oncology. 2011;22(8):1736-47. Epub 2011/06/29. doi: 10.1093/annonc/mdr304. PubMed PMID: 21709140; PubMed Central PMCID: PMCPMC3144634.

15. Recht A, Edge SB, Solin LJ, Robinson DS, Estabrook A, Fine RE, et al. Postmastectomy radiotherapy: clinical practice guidelines of the American Society of Clinical Oncology. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(5):1539-69. Epub 2001/03/07. doi: 10.1200/jco.2001.19.5.1539. PubMed PMID: 11230499.

16. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast cancer before age 40 years. Seminars in oncology. 2009;36(3):237-49. Epub 2009/05/23. doi: 10.1053/j.seminoncol.2009.03.001. PubMed PMID: 19460581; PubMed Central PMCID: PMCPMC2894028.

17. Baselga J. Clinical trials of Herceptin(trastuzumab). European journal of cancer (Oxford, England : 1990). 2001;37 Suppl 1:S18-24. Epub 2001/02/13. PubMed PMID: 11167087.

18. Goldhirsch A, Coates AS, Gelber RD, Glick JH, Thürlimann B, Senn HJ. First--select the target: better choice of adjuvant treatments for breast cancer patients. Annals of oncology : official journal of the European Society for Medical Oncology.

2006;17(12):1772-6. Epub 2006/10/31. doi: 10.1093/annonc/mdl398. PubMed PMID: 17071934.

19. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thürlimann B, Senn HJ. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. Annals of oncology : official journal of the European Society for Medical Oncology. 2007;18(7):1133-44. Epub 2007/08/07. doi: 10.1093/annonc/mdm271. PubMed PMID: 17675394.

20. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. The New England journal of medicine. 2005;353(16):1659-72. Epub 2005/10/21. doi: 10.1056/NEJMoa052306. PubMed PMID: 16236737.

21. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE, Jr., Davidson NE, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. The New England journal of medicine. 2005;353(16):1673-84. Epub 2005/10/21. doi: 10.1056/NEJMoa052122. PubMed PMID: 16236738.

22. Smith I, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, et al. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. Lancet (London, England). 2007;369(9555):29-36. Epub 2007/01/09. doi: 10.1016/s0140-6736(07)60028-2. PubMed PMID: 17208639.

23. Thomas A, Rhoads A, Pinkerton E, Schroeder MC, Conway KM, Hundley WG, et al. Incidence and Survival Among Young Women With Stage I-III Breast Cancer: SEER 2000-2015. JNCI Cancer Spectr. 2019;3(3):pkz040. Epub 2019/08/09. doi: 10.1093/jncics/pkz040. PubMed PMID: 31392297; PubMed Central PMCID: PMCPMC6668585.

24. Kim IK, Park S, Hwang H, Lee JS, Ko SM, Kim SI, et al. Clinical Significance of Age at the Time of Diagnosis among Young Breast Cancer Patients. Journal of breast cancer. 2011;14(4):314-21. Epub 2012/02/11. doi: 10.4048/jbc.2011.14.4.314. PubMed PMID: 22323919; PubMed Central PMCID: PMCPMC3268929.

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Change in GFR in relation to pulse rate, dipping blood pressure, anti-hypertensives, NLR and PLR in patients with chronic kidney disease

Hüseyin DURU[*]

Department of Internal Medicine, Rize Satate Hospital, Rize, Türkiye

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Abstract

This study aimed to evaluate the change in glomerular filtration rate (GFR) in chronic kidney disease (CKD) patients in relation to certain 24-h ambulatory blood pressure monitoring (ABPM) parameters and anti-hypertensives and inflammatory markers. This retrospective study included 206 adult CKD patients (mean±SD age: 51.3±17.1 years, 54.9% females). We recorded the data on patient demographics, comorbidity and medications, 24-h ABPM parameters (pulse rate and dipping systolic and diastolic BP), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and GFR. We evaluated the change in e-GFR values from baseline concerning study variables. There was a mean -1.5mL/min/1.73m^2(range -80.2 to 18.1) decline from baseline GFR during the study period. The decrease in GFR from baseline was significantly lower in patients with vs without diuretic therapy (median 1.2 vs 0 mL/min/1.73m^2, p=0.017). The GFR change from baseline was positively correlated with the patient age (r=0.145, p=0.040) as well as with the total (r=0.198, p=0.005), day-time (r=0.184, p=0.009) and night-time (r=0.219, p=0.003) pulse rate. We noted no significant difference in the GFR change from baseline concerning gender, anti-hypertensive medications other than diuretics, dipping systolic or diastolic BP values or inflammatory markers. Our findings revealed a significant correlation between age, pulse rate and diuretic usage but not dipping systolic or diastolic BP or inflammatory markers with the GFR change from baseline.

Keywords: chronic kidney disease, hypertension, renal progression, dipping blood pressure, anti-hypertensive medications, inflammatory markers

1. Introduction

Chronic kidney disease (CKD) is an important public health concern in association with an increased risk of adverse outcomes such as the development of end-stage renal disease (ESRD), cardiovascular events, psychiatric problems and mortality in the advanced stage (1-4). Given the continued risk of progression to ESRD and the mortality despite several measures devoted to managing CKD, identifying the risk factors of kidney function decline is considered crucial for patients with CKD (4, 5).

Hypertension leads to an increased risk of CKD development and progression to ESRD, while CKD is also a common cause and a sequel of uncontrolled hypertension (4-6).

Although the exact mechanisms of circadian pattern alterations in CKD patients remain unknown, the diurnal variability of blood pressure (BP) and pulse rate (reduced or absent decrease in nighttime BP levels in particular) is considered likely to be associated with end-organ damage and cardiovascular events in hypertensive CKD patients (7-13).

The 24-h ambulatory blood pressure monitoring (ABPM) is therefore considered the gold standard for assessing hypertension in CKD patients to assess the progression of renal dysfunction and prevent cardiovascular complications (5,14).

In addition, neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have also been proposed recently as markers of inflammation and predictors of renal decline in patients with CKD (15-19).

This study aimed to evaluate the change in GFR values in CKD patients in relation to certain 24-h ABPM parameters (pulse rate, dipping systolic and diastolic BP), anti-hypertensive medications and inflammatory markers (NLR, PLR).

2. Materials and Methods

2.1. Study population

We included 206 adult CKD patients (mean±SD age: 51.3±17.1 years, 54.9% females) in this retrospective descriptive study conducted between January 2013 and December 2017.

We obtained written informed consent from each subject, and the institutional ethics committee approved the study (Approval number: 2021/208, approval date: 9.12.2021).

*Correspondence: huseyindurudr@gmail.com

2.2. Assessments

We recorded each patient's data on demographics (age, gender), body mass index (BMI, kg/m^2), active smoking, comorbid diseases, anti-hypertensive medications, 24-h ABPM parameters, including pulse (bpm, total daytime, nighttime) and dipping systolic and diastolic BP (mmHg) via inflammatory markers (NLR, PLR) and glomerular filtration rate (GFR; baseline, last visit and the change from baseline values).

A decrease of ≥10% in BP value measured at night compared to daytime is considered dipping. We calculated the patients' e-GFR values (mL/min/1.73m^2) using the CKD-EPI formula. We evaluated the change in e-GFR values from baseline concerning demographics, anti-hypertensive medications, pulse, dipping BP and inflammatory markers.

2.3. Statistical analysis

We conducted statistical analyses using MedCalc® Statistical Software version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). We used Mann-Whitney U test to analyze the parametric variables and analyzed the correlation of GFR difference from baseline with study parameters via Spearman correlation analysis. We expressed the data as mean ± standard deviation (SD), median (min-max) and percent (%) where appropriate and considered $p < 0.05$ statistically significant.

3. Results

3.1. Demographic and clinical characteristics

Mean±SD patient age was 51.3±17.1 years, and 54.9% of patients were females. Concomitant hypertension was evident in 90.3% of CKD patients, while diabetes in 25.7%. The most commonly used anti-hypertensive medications were calcium channel blockers (CCB, 40.8%), beta-blockers (28.9%) and diuretics (23.3%) (Table 1).

Table 1. Demographic and clinical characteristics

Age (year)	mean±SD	51.3±17.1
	Median (min-max)	52.5(17-86)
Gender, n(%)		
Male		93(45.1)
Female		113(54.9)
BMI (kg/m^2), mean±SD		31.1±6.4
Active smoking, n(%)		46(22.3)
Comorbid diseases, n(%)		
Diabetes mellitus		53(25.7)
Hypertension		186(90.3)
Antihypertensive medications, n(%)		
CCB		84(40.8)
Beta blocker		59(28.9)
Diuretic		48(23.3)
ACEi		44(21.5)
ARB		39(19.0)
Alpha-blocker		34(16.5)

BMI: Body mass index; CCB: Calcium channel blocker; ACEi: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin receptor blocker

3.2. Cardiac parameters, inflammatory markers and GFR change from baseline

Mean±SD total daytime and nighttime pulse rate values were 74.1±10, 76.3±10.7 and 66.5±8.9, respectively. Median dipping systolic and diastolic BP values were 6.8mmHg (range, -17.5 to 100 mmHg) and 9.7mmHg (range, -14.5 to 100mmHg), respectively (Table 2).

Mean±SD NLR values were 2.3±2.2, while PLR values were 130.2±57.0 (Table 2).

There was a mean -1.5 mL/min/1.73m^2 (range -80.2 to 18.1mL/min/1.73m^2) decline from baseline GFR during study period (Table 2).

Table 2. 24-h ABPM parameters, inflammatory markers and GFR change from baseline

24-h ABPM parameters		
Pulse rate (bpm), mean±SD	Total	74.1±10
	Day-time	76.3±10.7
	Nigh-time	66.5±8.9
Dipping blood pressure (mmHg), median (min-max)	Systolic	6.8 (-17.5-100)
	Diastolic	9.7 (-14.5-100)
Inflammatory markers, mean±SD		
NLR		2.3±2.2
PLR		130.2±57.0
GFR (mL/min/1.73m^2)		
Baseline	mean±SD	74.8±34.6
	Median (min-max)	78.9(0.4-166.1)
Last visit	mean±SD	73.4±33.4
	Median(min-max)	74.5(6.1-147.1)
Change from baseline	mean±SD	-1.5±15.4

NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; GFR: Glomerular filtration rate

3.3. GFR change from baseline with respect to study parameters

We noted no significant difference in GFR change from baseline with respect to gender or anti-hypertensive medications other than diuretics. The decrease in GFR from baseline was significantly lower in patients with vs without diuretic therapy (median 1.2 vs 0 mL/min/1.73m^2, p=0.017) (Table 3).

3.4. Correlation of GFR change with continuous variables

GFR change from baseline was positively correlated with patient age (r=0.145, p=0.040) as well as with the total (r=0.198, p=0.005), day-time (r=0.184, p=0.009) and night-time (r=0.219, p=0.003) pulse rate values. We noted no significant change from baseline GFR with the dipping systolic or diastolic BP values or inflammatory markers.

Table 3. GFR change from baseline with respect to gender and antihypertensive medications

		GFR change from baseline (mL/min/1.73m^2) median (min-max)	p value
Gender			
Male		-0.1±13.6	0.444
Female		0(-29.5-18.1)	
Antihypertensive medications			
ACEi	No	0(-80.2-18.1)	0.983
	Yes	0(-49-62.2)	
ARB	No	0(-49.7-18.1)	0.757
	Yes	0(-80.2-17.5)	
Beta blocker	No	0(-80.2-62.2)	0.527
	Yes	0(-22.9-18.1)	
CCB	No	0(-49-62.2)	0.192
	Yes	0(-80.2-18.1)	
Alpha blocker	No	0(-80.2-18.1)	0.295
	Yes	0(-19.8-27.9)	
Diuretic	No	0(-80.2-62.2)	**0.017**
	Yes	1.2(-19.8-18.1)	

ACEi: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin receptor blocker; CCB: Calcium channel blocker; GFR: Glomerular filtration rate
Mann Whitney U test

4. Discussion

Our findings revealed that eGFR declined by 1.5 units on average during follow up in CKD patients, along with a significant correlation of age, pulse rate and diuretic usage with the change in GFR over time. The gender, BMI, other types of anti-diabetic medications, dipping systolic or diastolic BP or inflammatory markers (NLR, PLR) had no significant impact on GFR change over time in CKD patients.

Notably, a past study with 190 hypertensive patients reported patients with reduced eGFR (60 mL/minute/1.73 m^2) to have an earlier time of systolic BP to reach a significantly higher level above the midnight systolic BP, to be older and to include more non-dippers (57.8% vs 39.3%) than those with a normal eGFR, while the time course of pulse rate was similar in reduced vs normal GFR groups (9). The 5-year data of APrODiTe-2 study revealed faster eGFR progression and adverse renal outcomes in patients with poor BP control at baseline or over one year and higher systolic BP and pulse pressure (5).

In this regard, the association of total daytime and nighttime pulse rates with the amount of decline in GFR in CKD patients in the current study seems to be in accordance with the consideration of pulse rate as a good surrogate marker of adverse renal outcomes and eGFR progression, as well as the association of lower BP burden over at least one year with increased likelihood of better renal outcomes over five years (5).

Although the non-dipper HT pattern is considered to be more prevalent in CKD patients as a known indicator of rapid end-organ damage, the direct interaction between non-dipper HT and renal progression remains unclear (20,21). Past studies based on 24-h ABPM recording revealed the likelihood of increased DBP variability associated with better renal outcomes (13) and no significant impact of 24-hour systolic and diastolic BP variability on the progression of CKD (21-23). Our findings revealed no association between dipping systolic or diastolic BP and GFR decline from baseline. Likewise, we have previously reported in 186 adult patients with CKD and HT that the dipper HT pattern was prevalent (45.8%) in them, possibly concerning the presence of severe proteinuria and no significant association of BP variability or non-dipper HT pattern with renal progression (21).

However, other studies indicated the increased systolic BP variability to be a significant determinant of increased risk of CKD and ESRD (11,12). Also, a past study with 436 CKD patients reported non-dipper HT as a significant risk factor for CKD progression through an analysis adjusted for 24-hour ABPM, cardiovascular history, proteinuria and other risk factors (10). Similarly, the authors of a past study with 46 CKD patients reported a significant decrease in dipping diastolic BP during the night, whereas there was no change in nighttime systolic dipping, mean BP values or pulse wave velocity after a one-year observation period (7). The authors noted the likelihood of peripheral and central BP not participating in the CKD progression and no change in their levels over a 1-year follow up despite the significant decline of eGFR (7). The authors also suggested that the reduced magnitude of the diastolic dipping had a key role in the pathogenesis of deterioration of kidney function (7). Also, patients with IgA-nephropathy and non-dipping BP patterns were reported to have lower eGFR and more extensive renal tissue damage than those with preserved dipping BP patterns (24). Moreover, 1-year data from the APrODiTe-2 study with 400 CKD patients revealed the association of good BP control and the dipper BP pattern with subtler eGFR and proteinuria changes (25).

Indeed, given the association of non-dipping BP profile and nocturnal hypertension with hypertension-mediated organ damage in CKD patients, ABPM is suggested to be more extensively used for applying individual risk assessment and personalized anti-hypertensive treatment in CKD patients (26), and the daily BP variability on 24-hour ABPM rather than visit-to-visit BP variability is considered more valuable in reflecting the renal survival (5,21). Notably, a past study with 10271 hypertensive patients (3227 with CKD) from the

Hygia Project reported patients with vs without CKD to have older age, higher nocturnal systolic BP, higher ambulatory pulse pressure, and lower daytime ambulatory diastolic BP along with the higher prevalence of non-dipping and the riser BP (elevated asleep systolic BP) pattern (27). The authors emphasized the increased prevalence of a blunted nocturnal BP decline and the riser BP pattern, and the elevated pulse pressure as a marker of increased arterial stiffness. They enhanced CVD risk in hypertensive patients with CKD to indicate a need for ABPM to be considered necessary for proper diagnosis and CVD risk assessment in CKD patients (27).

In fact, our findings on the significant age-dependency of GFR decline seem notable given the reported association of age with SBP or DBP variability in the past studies (21,22,28). In addition, the association of diuretic treatment with GFR change from baseline in our study also seems to support the likelihood of the different impacts of different anti-hypertensive medications on BP variability (i.e., a decline in variability with calcium channel blockers and non-loop diuretics and an increase in variability with ACE inhibitors) (29).

Although previous studies indicated a likelihood of NLR to serve as an independent risk factor for hypertension and renal progression in patients with IgA nephropathy (18,19) and both NLR and PLR to be potential markers for predicting renal outcomes in patients with rapidly progressive glomerulonephritis (RPGN) (15), our findings revealed no correlation of NLR or PLR values with GFR decline during follow up.

In conclusion, our findings revealed a significant correlation between age, pulse rate, and diuretic usage but not dipping systolic or diastolic BP or inflammatory markers with the change of GFR in CKD patients. Future longer-term large-scale studies addressing BP patterns via 24-hour ABPM in CKD patients are needed to understand the exact role of BP variability and dipping status in controlling hypertension and predicting renal progression in CKD patients.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: H.D., Design:H.D., Data Collection or Processing: H.D., Analysis or Interpretation: H.D., Literature Search: H.D., Writing: H.D.

References

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. Lancet. 2013; 382(9888): 260-272.
2. Grams ME, Yang W, Rebholz CM, Wang X, Porter AC, Inker LA, et al. Risks of adverse events in advanced CKD: the chronic renal insufficiency cohort (CRIC) study. Am J Kidney Dis. 2017; 70(3): 337-346.
3. Duru H. The prevalence and severity of mental health problems and sexual dysfunction in hemodialysis patients before and during the COVID-19 pandemic. Ther Apher Dial. 2022 Jan 27. Epub ahead of print. doi: 10.1111/1744-9987.13805.
4. Jhee JH, Nam BY, Lee CJ, Park JT, Han SH, Kang SW, et al. Soluble Urokinase-Type Plasminogen Activator Receptor, Changes of 24-Hour Blood Pressure, and Progression of Chronic Kidney Disease. J Am Heart Assoc. 2021; 10(1): e017225.
5. Cha RH, Lee H, Lee JP, Kim YS, Kim SG. The influence of blood pressure patterns on renal outcomes in patients with chronic kidney disease: The long-term follow up result of the APrODiTe-2 study. Medicine (Baltimore). 2020; 99(8): e19209.
6. Hamrahian SM. Management of Hypertension in Patients with Chronic Kidney Disease. Curr Hypertens Rep. 2017; 19(5): 43.
7. Kuczera P, Kwiecień K, Adamczak M, Bączkowska T, Gozdowska J, Madziarska K, et al. Different Relevance of Peripheral, Central or Nighttime Blood Pressure Measurements in the Prediction of Chronic Kidney Disease Progression in Patients with Mild or No-Proteinuria. Kidney Blood Press Res. 2018; 43(3): 735-743.
8. Salles GF, Reboldi G, Fagard RH, Cardoso CR, Pierdomenico SD, Verdecchia P, et al. Prognostic Effect of the Nocturnal Blood Pressure Fall in Hypertensive Patients: The Ambulatory Blood Pressure Collaboration in Patients With Hypertension (ABC-H) Meta-Analysis. Hypertension. 2016; 67(4): 693-700.
9. Kuramoto M, Aizawa M, KuramotoY, Okabe M, Sakata Y, Aizawa Y. Blood Pressure Increases Before Pulse Rate During the Nocturnal Period in Hypertensive Patients. Int Heart J. 2020; 61(3): 579-584.
10. Minutolo R, Agarwal R, Borrelli S, Chiodini P, Bellizzi V, Nappi F, et al. Prognostic role of ambulatory blood pressure measurement in patients with nondialysis chronic kidney disease. Arch Intern Med. 2011; 171(12): 1090-1098.
11. Whittle J, Lynch AI, Tanner RM, Simpson LM, Davis BR, Rahman M, et al. Visit-to-Visit Variability of BP and CKD Outcomes: Results from the ALLHAT. Clin J Am Soc Nephrol. 2016; 11(3): 471-480.
12. McMullan CJ, Lambers Heerspink HJ, Parving H-H, Dwyer JP, Forman JP, de Zeeuw D. Visit-to-visit variability in blood pressure and kidney and cardiovascular outcomes in patients with type 2 diabetes and nephropathy: a post hoc analysis from the RENAAL study and the Irbesartan Diabetic Nephropathy Trial. Am J Kidney Dis. 2014; 64(5): 714-722.
13. Sahutoglu T, Sakaci T. Diastolic blood pressure variability in 24 hour-ABPM and outcomes of chronic kidney disease. Clin Nephrol. 2018; 90(1): 46-52.
14. Pickering TG, Shimbo D, Haas D. Ambulatory blood-pressure monitoring. N Engl J Med. 2006; 354(22): 2368-2374.
15. Mae Y, Takata T, Ida A, Ogawa M, Taniguchi S, Yamamoto M, et al. Prognostic Value of Neutrophil-To-Lymphocyte Ratio and Platelet-To-Lymphocyte Ratio for Renal Outcomes in Patients with Rapidly Progressive Glomerulonephritis. J Clin Med. 2020; 9(4): 1128.
16. Turkmen K, Guney I, Yerlikaya FH, Tonbul HZ. The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. Ren Fail. 2012; 34(2): 155-159.

17. Turkmen K, Erdur FM, Ozcicek F, Ozcicek A, Akbas EM, Ozbicer A, et al. Platelet-to-lymphocyte ratio better predicts inflammation than neutrophil-to-lymphocyte ratio in end-stage renal disease patients. Hemodial Int. 2013; 17(3): 391-396.

18. Wang S, Dong L, Pei G, Jiang Z, Qin A, Tan J, et al. High Neutrophil-To-Lymphocyte Ratio Is an Independent Risk Factor for End Stage Renal Diseases in IgA Nephropathy. Front Immunol. 2021; 12: 700224.

19. Li Q, Chen P, Shi S, Liu L, Lv J, Zhu L, et al. Neutrophil-to-lymphocyte ratio as an independent inflammatory indicator of poor prognosis in IgA nephropathy. Int Immunopharmacol. 2020; 87: 106811.

20. Davidson MB, Hix JK, Vidt DG, Brotman DJ. Association of impaired diurnal blood pressure variation with a subsequent decline in glomerular filtration rate. Arch Intern Med. 2006; 166(8): 846-852.

21. Duru H, Kara E. The relationship between blood pressure variability and renal progression in hypertensive patients with chronic kidney disease. Gevher Nesibe J Med Health Sci. 2021; 6(14): 80-88.

22. Di Iorio B, Pota A, Sirico ML, Torraca S, Di Micco L, Rubino R, et al. Blood pressure variability and outcomes in chronic kidney disease. Nephrol Dial Transplant. 2012; 27(12): 4404-4410.

23. Manios E, Tsagalis G, Tsivgoulis G, Barlas G, Koroboki E, Michas F, et al. Time rate of blood pressure variation is associated with impaired renal function in hypertensive patients. J Hypertens. 2009; 27(11): 2244-2248.

24. Briet M, Collin C, Karras A, Laurent S, Bozec E, Jacquot C, et al. Arterial remodeling associates with CKD progression. J Am Soc Nephrol. 2011; 22(5): 967-974.

25. Cha RH, Lee H, Lee JP, Kang E, Song YR, Kim YS, et al. Changes of blood pressure patterns and target organ damage in patients with chronic kidney disease: results of the APrODiTe-2 study. J Hypertens. 2017; 35(3): 593-601.

26. Stróżecki P, Pluta A, Donderski R, Włodarczyk Z, Manitius J. Abnormal diurnal blood pressure profile and hypertension-mediated organ damage in nondiabetic chronic kidney disease G1-G3b patients. Blood Press Monit. 2021; 26(1): 22-29.

27. Mojón A, Ayala DE, Piñeiro L, Otero A, Crespo JJ, Moyá A, et al. Comparison of ambulatory blood pressure parameters of hypertensive patients with and without chronic kidney disease. Chronobiol Int. 2013; 30(1-2): 145-158.

28. Benetos A, Salvi P, Lacolley P. Blood pressure regulation during the aging process: the end of the 'hypertension era'?. J Hypertens. 2011; 29(4): 646-652.

29. Webb AJ, Fischer U, Mehta Z, Rothwell PM. Effects of antihypertensive-drug class on interindividual variation in blood pressure and risk of stroke: a systematic review and meta-analysis. Lancet. 2010; 375(9718): 906-915.

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Establishment of biocidal activity evaluation study protocol in healthcare facility for routine monitoring of antibacterial power of disinfectants

Mostafa Essam EISSA[1,*], Engy Refaat Rashed[2], Dalia Essam EISSA[3]

[1]Independent Researcher Project Section, Pharmaceutical Research Facility, Cairo, Egypt,
[2]National Centre for Radiation Research and Technology, Cairo, Egypt
[3]Royal Oldham Hospital, Oldham, United Kingdom

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Abstract

Mitigation of the nosocomial infection risk is of prime importance in any healthcare facility to protect patients as well as the working staff's health. To ensure accomplishing this target, one of the crucial good practices is the establishment of rigorous sanitization and disinfection programs that should be executed as part of GxP activity in hospitals. The cornerstone of this task is the implementation of a biocidal validation protocol to evaluate the commercially available antimicrobial formulae in the market against the microorganisms that could be found in the environment. In the present study, a biochemically identified – at least to the genus level -Gram-negative microorganism was found on three different occasions belonging to the *Burkholderia cepacia* complex (BCC). The disinfectant validation program was executed using four chemically different antimicrobial formulae that embraced alcohols, amphoteric detergent and peroxygen products. A preliminary neutralization study design evaluation was established before the biocidal assessment to ensure effective stoppage of the antimicrobial activity after a specific contact time with the microorganism using a bacterial count range between 40 and 100 Colony Forming Unit (CFU) which was determined by serial dilution. An acceptable neutralization (in terms of toxicity and efficacy) was achieved through a combination of chemical and dilution methods. Contact surface method for testing the biocides was applied using three different coupons that were made for the material of construction of the covers or lining of the walls, floors and metallic surfaces. Microbial reduction level exceeded 15,800 times the original count of $\geq 1 \times 10^6$ CFU/coupon with all groups and replicates at zero- and two-weeks storage time of the prepared and diluted disinfecting solution. The disinfectants that have been challenged in this study showed acceptable activity against the vegetative organism of concern in the healthcare industry with 2-hydroxypropane being slightly the least active among the group.

Keywords: disinfection, neutralizer, efficacy, toxicity, peroxygen, alcohol

1. Introduction

Nosocomial infections are a major problem of concern in healthcare buildings, especially in hospital facilities (1). They can be caused by various microorganisms, notably bacteria. Cross-contamination might also occur due to the transfer of the infectious agents between different sections of the plant and between departments through various means, including personnel, tools, instruments, furniture and equipment (2). Individuals with variable health defects might be at great risk from not only pathogenic microorganisms but also opportunistic and even commensal microbes (3). Depending on the state of illness, the exposure to these microorganisms may encounter health complications and even death depending on different factors such as route of entry, inoculum size, exposure length presence or absence of natural body barriers (4).

One of the major strategies in limiting the transmission and spreading of microorganisms through surfaces is the implementation of an effective sanitization program in the hospitals and other healthcare organizations using a validated disinfection protocol as a part of the GxP program for minimizing contamination and infection risk in healthcare facilities (5, 6). Several experimental designs have been adopted to test the antimicrobial power of biocidal products (7). It is important for the test protocol to be able to demonstrate an acceptable kinetic of the microbial death by the biocidal formula using a high microbial count to ensure the ability to control the bioburden level in a short time when high workload and traffic are expected (8 – 10).

The present study aimed to study the biocidal effect of four selected commercial disinfectant formulae from market retail in a healthcare setting. These disinfectants were used to test the antimicrobial effect on simulated surfaces that are common and could be found in the building. The study would be projected as a routine activity that should be implemented using simple tools and instruments in the laboratory as an integral part of GxP in hospitals and other healthcare facilities.

2. Materials and Methods

Biocidal validation protocol was established as a part of GxP activity that was aimed to be used in healthcare organizations,

*Correspondence: mostafaessameissa@yahoo.com

especially hospitals which will be implemented through standard procedures after an acceptable evaluation and verification program (11, 12). The test group in this work will be referred to as treatment synonymously as well as column in electronic dataset generation.

2.1. Selection of the biocidal agents

Disinfectant products were purchased from a market retail involving four types comprising three main classes of the biocidal agents: two alcohols (ethyl hydroxide and dimethyl carbinol) amphoteric detergent (Ampholyte with pH ≈ 8)), and Peroxygen compounds (acetyl hydroperoxide/perhydroxic acid in acidic formula) (13 - 15). A detailed description of very close products could be found in other sanitization study series for preparation and dilution.

2.2. Identification of the study subject

Establishment of an environmental microorganism library database was established electronically through dynamic inclusion of the biochemically identified microorganisms after appropriate Gram-stain implementation for microscopical examination. The identification was conducted using suitable miniaturized identification kit system such as API® and VITEK® 2 BioMerieux (16, 17). Upon the validation study execution, randomly selected microbial samples were used based on the health risk encountered with the critically ill patients, specifically for those in the Intensive Care Units (ICUs).

2.3. Preliminary neutralization study design

Three distinct neutralization groupings should be established as comparison treatments to evaluate the neutralization process. General procedure and steps were performed as outlined by researchers in other studies (18 – 20).

2.3.1. Viability group

This treatment involved only the microorganism without a chemical neutralizer alone or biocidal compounds. This group was prepared and serially diluted in sterile saline or buffer to deliver microbial plate count between 30 and 100 Colony Forming Unit (CFU) per nutrient agar plate. This treatment was used as a reference control to assess the possible toxicity on the microorganism that could be stemmed from the neutralizer components.

2.3.2. Neutralizer toxicity group

This treatment was composed of the microorganism plus the chemical neutralizer as a diluent. This group served as toxicity test against the viability group but represented the control for the evaluation of the efficacy of the neutralization procedure in the presence of the biocidal test materials. There should not be any adverse effect from the neutralizer components on the microbial viability which would be indicated by the relative recovery from that of the concurrent control treatment. The selected neutralizer was prepared and sterilized as described in a previous research study.

2.3.3. Neutralizer efficacy group

This treatment included the working concentration of the disinfectant that was diluted and mixed well with the neutralizer and adding the microorganisms. This group is the test treatment that was assessed against the toxicity group as a control. Thus, four lines of analysis were streamed based on the number of disinfectants to be evaluated. Judgment on a successful neutralization procedure would be based on the relative recovery as in the toxicity analysis.

2.4. Disinfectant validation experiment (13, 15)

Antimicrobial efficacy of the freshly prepared disinfectants was evaluated after dilution as recommended by the manufacturer to 70% (v/v) for alcohols, 2% (v/v) for peroxygens and 1% (v/v) for the ampholyte with purified water. The biocidal agents were added over representative coupon surface materials that were inoculated with the test microorganisms at ≥ 1,000,000 CFU/coupon which was determined using a serial dilution of the microorganisms in saline or buffer in tubes. The surfaces were made from representative materials for the construction of walls, floors and metallic surfaces (tools, instruments and furniture such as tables, and chairs). Antimicrobial Activity of the stored disinfectant over the study period (biocidal stability) after 14 days was evaluated using the same experimental procedures and conditions with the same microorganisms on the same surfaces.

2.5. Statistical evaluation of the study (21)

2.5.1. Neutralization design evaluation

This computation analysis should be performed to assess the validity and the quality of the neutralization design to avoid any unintentional bias in the results due to exaggerated effect from the residual amounts of antimicrobial component(s) in the medium (22).

2.5.1.1. Neutralizer toxicity and efficacy acceptance criterion

The accepted microbial recovery percent of the test from the reference control should not be less than 75%. Any result that could not meet the acceptance criterion must be assessed statistically to elucidate the significance before judging the outcome as true failure of the neutralization (22). The statistical significance of the difference between the reference values of the control values and the experiment groups was tested at significance level of 0.05. Statistical evaluation between the recovery ratio of the treatment groups against reference target values was also assessed using nonparametric analogue of t-test.

2.5.1.2. Recovery ratio and groups comparison

Recovery ratios of the experimental groups were compared using Analysis of Variance (ANOVA) at $\alpha = 0.05$. When the result is significant, a pairwise multiple comparison test was executed to elucidate the source of variation (22). When no evidence of significance could be found, the second line of comparison was performed on the separate treatments (as CFU count per plate) to determine the test group that shows a significant difference from that of control at $P = 0.05$. Cases

must be evaluated individually to decide to either proceed with the next step or halt the study of the concerned biocidal agent till further investigation.

2.5.2 Biocidal activity evaluation

The microbial count decline over the preselected contact time (i.e., five minutes in the current work) should not be less than 1000 times from the initial bioburden (23). A conservative threshold was set to 10,000 times decline in the number of the CFUs. The observed results would be recorded in Excel sheet database and any non-conforming input cells would be highlighted to spot the aberrant values. Unless there is no microorganism could be detected, the variation in log count of the recovered bioburden from exposure to disinfectant at zero- and two-weeks storage period should be within 0.3 – 0.5 (23).

3. Results

It would be plausible to start the analysis of the output results by visualizing data by describing the set of the results followed by a more comprehensive study of the neutralization design followed by the disinfectant evaluation.

3.1. Descriptive statistical interpretation of results of the treatments

The mean microbial recovery of Amphoteric Detergent, Ethyl Hydroxide, Dimethyl Carbinol and Acetyl hydroperoxide/Perhydroxic acid (abbreviated as TX, AD, EH, DC and AP, respectively) were 0.8820, 0.8243, 0.8637, 0.8257 and 0.8813, respectively whereas the standard deviations 0.03830, 0.02055, 0.007572, 0.02237 and 0.01002 in the same order. On the other hand, the calculated standard error of the mean (SEM) was 0.02211, 0.01186, 0.004372, 0.01291 and 0.005783. The lower 95% Confidence Interval (CI) of the (mean), (median) and (geometric mean) was (0.7869, 0.7733, 0.8449, 0.7701, 0.8565), (0.8490, 0.8030, 0.8550, 0.8000, 0.8700) and (0.7919, 0.7746, 0.8450, 0.7713, 0.8567). The computed upper 95% CI of (mean), (median) and (Geometric Mean (GM)) – in the same order – was (0.9771, 0.8754, 0.8825, 0.8812, 0.9062), (0.9240, 0.8440, 0.8690, 0.8410, 0.8890) and (0.9812, 0.8769, 0.8827, 0.8834, 0.9066). On the same line, the calculated (actual median), (discrepancy) and (GM) was (0.8730, 0.8260, 0.8670, 0.8360, 0.8850), (-0.1230, -0.07600, -0.1170, -0.08600, -0.1350) and (0.8815, 0.8242, 0.8636, 0.8255, 0.8813), respectively. Fig. 1 and 2 showed relative recoveries expressed as GM with CI and medians with Interquartile Ranges (IQR), respectively.

3.1. Recovery of tests groups vs. control count and the reference criterion

The plate count results of the test groups were not significantly different from that of the control of either neutralizer toxicity or biocidal neutralization efficiency which was indicated by the exact P-value of 0.25. For paired neutralizer validation experimental design, a two-sided (two-tailed) Wilcoxon matched-pairs signed-rank test was implemented at a statistical significance of $P < 0.05$ for raw data. The sum of positive and negative ranks was 0 and -6, respectively. The sum of the signed ranks (W) was -6. The median of the differences between control and test for toxicity and efficacy groups of AD, EH, DC and AP were -8, -12, -8, -10 and -7, respectively. The pairing was perfect with a significant correlation at $P > 0.05$ and the value of P (one-tailed) was 0.1667.

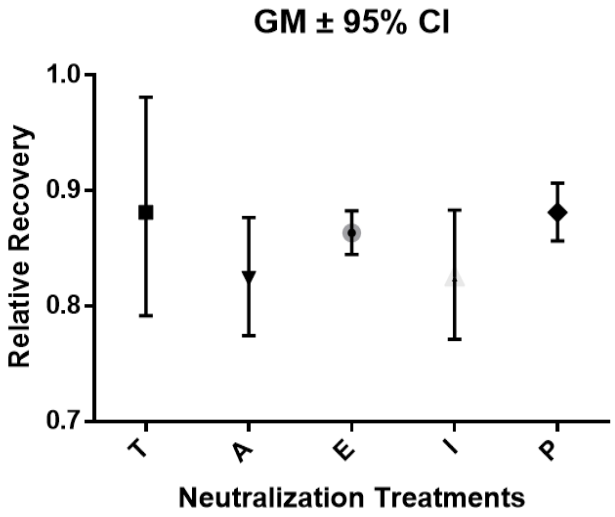

Fig. 1. Geometric Mean (GM) with 95% Confidence Interval (CI) for the relative recoveries of the neutralizer toxicity (T) and efficacy treatments of Amphoteric detergent, Ethyl hydroxide, Dimethyl carbinol and Acetyl hydroperoxide/perhydroxic acid (abbreviated as T, A, E, D and A, respectively)

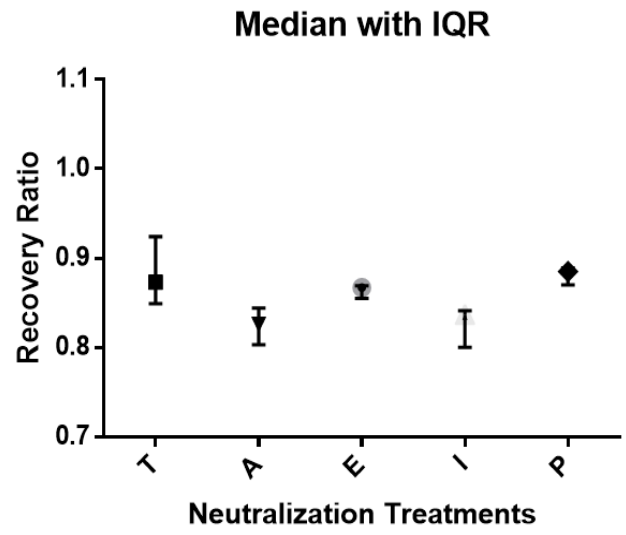

Fig. 2. Medians with Interquartile Ranges (IQR) for the relative recoveries of the neutralizer toxicity (T) and efficacy treatments of Amphoteric detergent, Ethyl hydroxide, Dimethyl carbinol and Acetyl hydroperoxide/perhydroxic acid (abbreviated as T, A, E, D and A, respectively)

The result of all treatment groups had met the acceptance criterion of 75% at $\alpha = 0.05$ with 95% confidence of the true mean to be within 0.75 and 1.00 recovery ratio. The microbiological count recovery for each group is shown in Fig. 3 on a logarithmic scale (at the y-axis) with the Error percent of the mean plate count expressed as CFU.

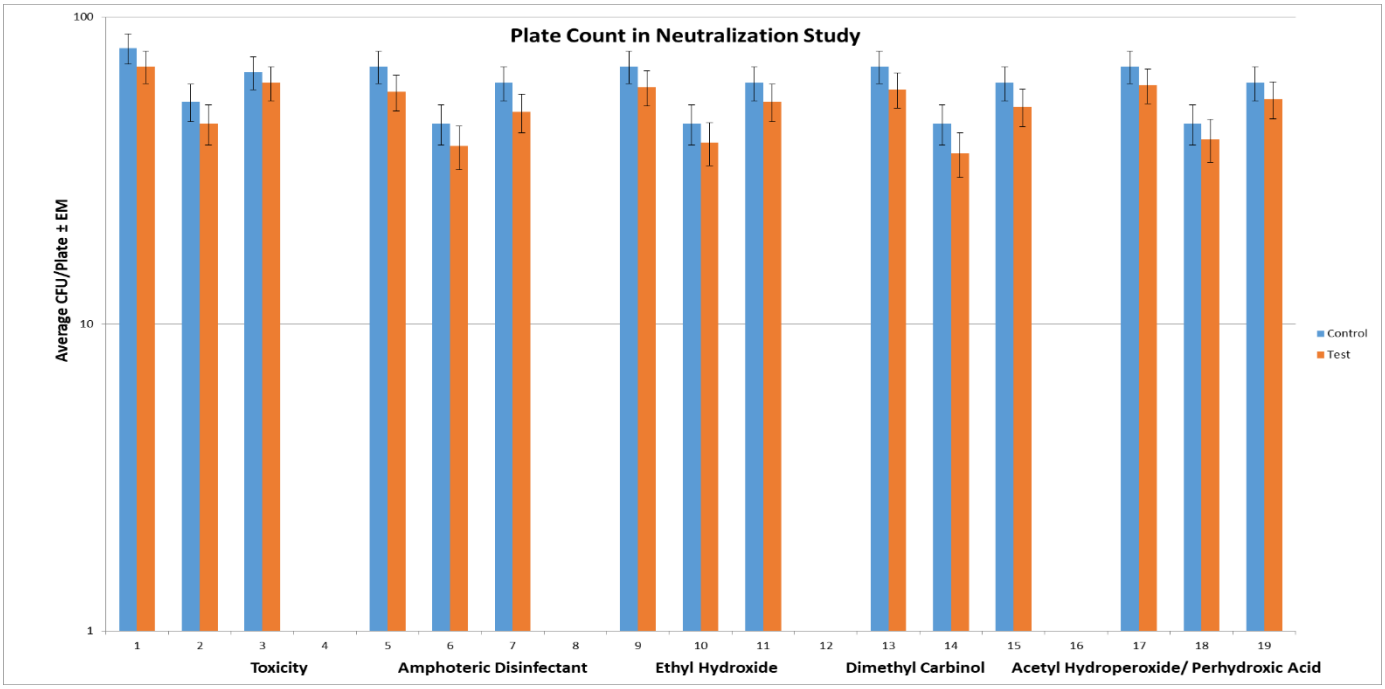

Fig. 3. Neutralization validation study showing the recovery of the test groups against controls. (EM: Error percent of the mean plate count expressed as CFU)

3.2. Analysis of the relative recovery between neutralization treatments

One-Way ANOVA test ($P < 0.05$) conducted on the relative bacterial recovery for three subjects over five treatments, showed significant difference between groups with exact P value of 0.0151 and the statistic value of 9.867. Table 1 demonstrated the recovery of microbial plate counts as transformed data of CFU with Standard Deviation (SD) and acceptable Relative Standard Deviation (RSD < 2%) were shown. The relative microbial recovery ± SD of all test groups was demonstrated in Fig. 4.

Table 1. Preliminary neutralization study design evaluation for selected biocidal agents to be used in a healthcare facility

Experiment Ω	Viability Control	Toxicity Group	Recovery ¥	Mean Recovery
I	1.90	1.84	0.969	0.970
II	1.72	1.65	0.959	SD = 0.011
III	1.82	1.79	0.981	RSD =1.157 %
Experiment Ω	Efficacy Control	Amphoteric Disinfectant Group £	Recovery ¥	Mean Recovery
I	1.84	1.76	0.955	0.952
II	1.65	1.58	0.956	SD = 0.005
III	1.79	1.69	0.947	RSD = 0.518 %
Experiment Ω	Efficacy Control	Ethyl Hydroxide Group	Recovery ¥	Mean Recovery
I	1.84	1.77	0.963	0.964
II	1.65	1.59	0.962	SD = 0.002
III	1.79	1.72	0.966	RSD = 0.188 %
Experiment Ω	Efficacy Control	Dimethyl Carbinol Group	Recovery ¥	Mean Recovery
I	1.84	1.76	0.959	0.952
II	1.65	1.56	0.941	SD = 0.010
III	1.79	1.71	0.956	RSD = 0.999 %
Experiment Ω	Efficacy Control	Acetyl Hydroperoxide/ Perhydroxic Acid Group €	Recovery ¥	Mean Recovery
I	1.84	1.78	0.967	0.969
II	1.65	1.60	0.969	SD = 0.002
III	1.79	1.73	0.970	RSD = 0.175 %

¥ An acceptance criterion was set to 0.938 which is equivalent to the recovery of three fourth of the control group count.SD: Standard Deviation.
Ω Individual experiments were performed in duplicates and the results were expressed as a logarithm of the average CFU/plate.RSD: Relative Standard Deviation.
£ The commercial product is basically composed of amines, n-C10-16-alkyltrimethylenedi-, as reaction products with chloroacetic acid.
€ The commercial product includes also acetic acid at 7.5 ± 2.5 % concentration.

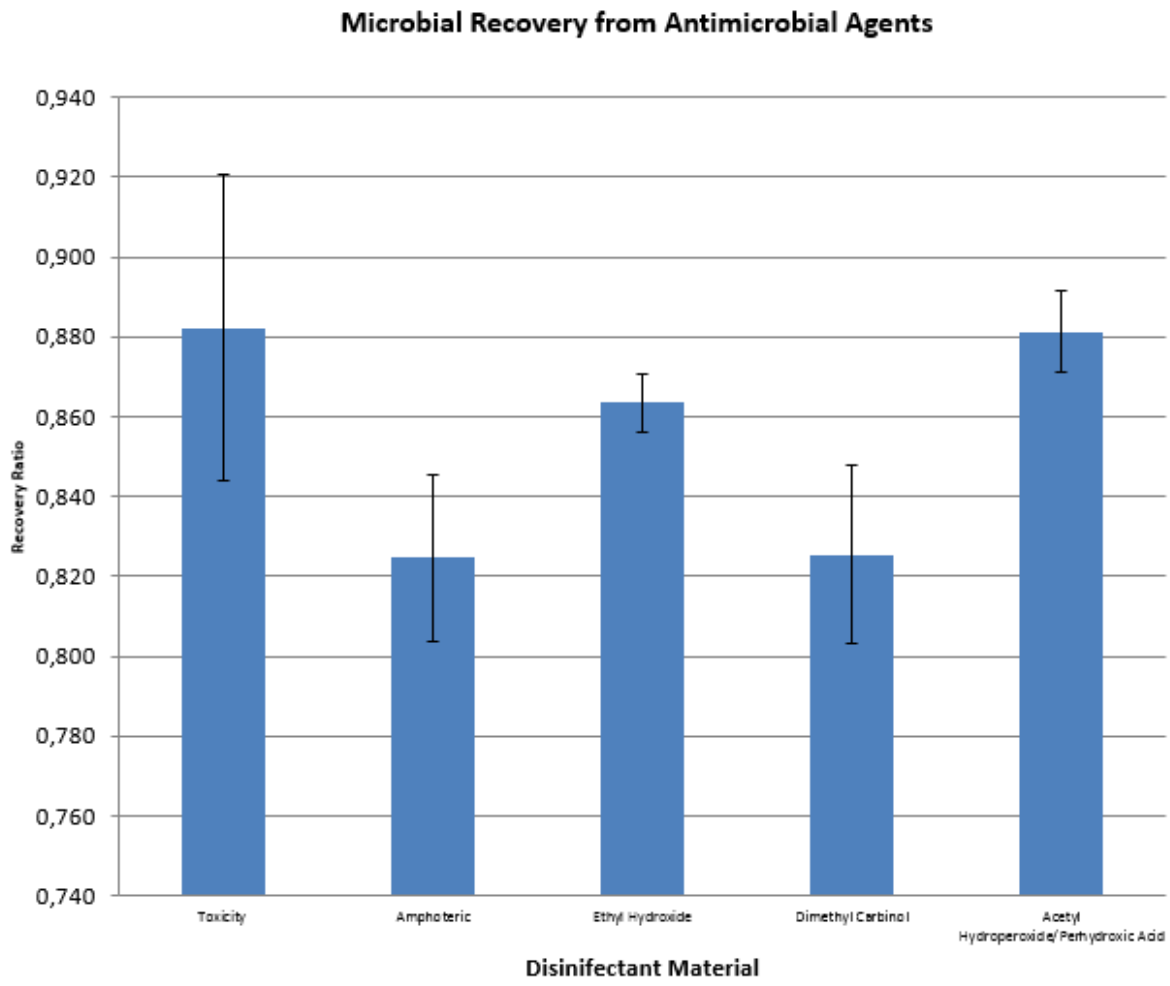

Fig. 4. *Burkholderia cepacia* complex (BCC) in the pooled result of the Neutralizer Toxicity (NT) and Neutralizer Efficacy (NE) tests along with standard deviation bars.

Multiple comparisons test between the values of the relative recovery of the original raw data did not reveal significant difference between groups at $\alpha = 0.05$ for five treatments (columns) and three subjects (rows). The Δ rank sum of rank sum (I – II) with adjusted P values for toxicity and efficacy groups of TX, AD, EH, DC and AP were AD vs. TX -9 (4 – 13) 0.2018, EH vs. TX -3 (10 -13) > 0.9999, DC vs. TX -8 (5 – 13) 0.3892, AP vs. TX 0 (13 – 13) > 0.9999, EH vs. AD 6 (10 – 4) > 0.9999, DC vs. AD 1 (5 – 4) > 0.9999, AP vs. AD 9 (13 – 4) 0.2018, DC vs. EH -5 (5 – 10) > 0.9999, AP vs. EH 3 (13 – 10) > 0.9999 and AP vs. DC 8 (13 – 5) 0.3892.

3.3. Pairwise analysis for the source of significance in microbial recovery between groups

Variance analysis ($P < 0.05$) of the plate count data showed exact P value < 0.0001 with the statistical value of 14.62. Multiple comparison was conducted for pairwise investigation of the treatments for the source of this variation at $\alpha = 0.05$ and the comparison included Test (T) against Control (C). The examination was conducted by calculating rank sum I from the first group minus Rank sum II from the second comparison to yield rank sum difference with P value was computed. The only significant difference was found between the amphoteric detergent test and the toxicity control group with adjected P value 0.0339 and the mean rank difference of (4 - 18) = -14.

3.4. Antibacterial activity of disinfectants over the study period

Biocidal activity examination over five minutes period showed more than 10,000 times reduction of the microbial population using surface contact test (Table 2). The average reduction factor for AD, EH and AP for wall, floor and metallic surfaces – pooled for the two test time periods of fresh and stored biocidal agents - exceeded 1.42×10^5, 1.50×10^5 and 1.68×10^5, respectively. Thus, the mean logarithmic reductions were > 5.14, > 5.17 and > 5.21 with (maximum – minimum) range of (5.36 – 5.00) and estimated approximate standard errors of 0.048, 0.045 and 0.055, respectively. The average reduction factor for DC for wall, floor and metallic surfaces – pooled for the two test time periods of fresh and stored biocidal agents - was 2.14×10^4, 2.03×10^4 and 6.32×10^4, respectively. Thus, the logarithmic reductions were 4.32, 4.30 and 4.79 with estimated standard errors of 0.043, 0.035 and 0.034, respectively. The mean ± standard deviation of the difference in the logarithmic microbial count reduction of the three experiments for wall, floor and metallic surfaces for DC was 0.029 ± 0.016, 0.045 ± 0.037 and 0.106 ± 0.22, respectively. The variation in the log reduction of bioburden

between wall and floor materials was insignificant being in the range of 0.007 to 0.032. While the microbial reduction from metallic (Stainless Steel) surfaces was considered significant compared to wall and floor structures. The reduction factor ± SD of the metallic surface versus both wall and floor materials in the triplicate was (0.447 ± 0.053, 0.474 ± 0.10, 0.486 ± 0.026) and (0.479 ± 0.048, 0.492 ± 0.009, 0.512 ± 0.028), respectively.

Table 2. Disinfectant validation using surface contact test on representative materials from the common surfaces found in the healthcare facility

Biocidal Agent£ / ¥CFU Recovery	Control Count			Freshly Prepared Disinfectant			Prepared Stored Disinfectant for 313 to 359 hours€		
	Coupon Material Mean Recovery (RSD%)			Coupon Material Mean Recovery (SE)			Coupon Material Mean Recovery (SE)		
I	PC	FL	MT	PC	FL	MT	PC	FL	MT
AD	6.18 (1.62)	6.20 (0.62)	6.27 (0.26)	<1	<1	<1	<1	<1	<1
EH				<1	<1	<1	<1	<1	<1
DC				1.81 (0.90)	1.85 (0.92)	1.36 (0.68)	1.83 (0.91)	1.88 (0.94)	1.46 (0.73)
AP				<1	<1	<1	<1	<1	<1
II	PC	FL	MT	PC	FL	MT	PC	FL	MT
AD	6.04 (0.93)	6.07 (1.70)	6.09 (2.05)	<1	<1	<1	<1	<1	<1
EH				<1	<1	<1	<1	<1	<1
DC				1.78 (0.89)	1.80 (0.90)	1.30 (0.65)	1.76 (0.88)	1.85 (0.92)	1.34 (0.67)
AP				<1	<1	<1	<1	<1	<1
III	PC	FL	MT	PC	FL	MT	PC	FL	MT
AD	6.20 (1.88)	6.22 (1.76)	6.28 (1.77)	<1	<1	<1	<1	<1	<1
EH				<1	<1	<1	<1	<1	<1
DC				1.83 (0.91)	1.89 (0.94)	1.43 (0.71)	1.85 (0.92)	1.90 (0.95)	1.52 (0.76)
AP				<1	<1	<1	<1	<1	<1

PC: Partition Construct, FL: Floor Lining, MT: Metal Tool, SE: Standard Error, RSD: Relative Standard Deviation
¥ Results of the plate count were expressed as logarithmically transformed results to the base ten
£Amphoteric Detergent, Ethyl Hydroxide, Dimethyl Carbinol and Acetyl hydroperoxide/Perhydroxic acid (abbreviated as AD, EH, DC and AP, respectively)
€ Storage was done at room temperature under ordinary working conditions

4. Discussion

The newly emerging Gram-negative bacterium is of significant health concern in healthcare facilities, notably hospitals (24). While BCC imposes little concern on healthy individuals, it demonstrated infection risk in hospitalized patients with defective health issues such as weak immunity and lung diseases (24, 25). Hence, the biochemically identified microorganism was included in the dynamic library database of the critically important environmental isolates. As a part of GxP in healthcare plants, regular examination of the efficacy of the sanitizers against the microbial isolates in the organization would be a critical task as an integral part of controlling nosocomial infections and minimization of cross-contamination risk, in addition to the Surgical Site Infections (SSI) issues (26). The disinfection protocol involved a spectrum of the biocidal agents in an application rotation program that minimizes the problem with prolonged use of each one (27). Materials, tools and equipment's corrosion and exposure toxicity are among the main mitigated challenges, in addition to the possible minimization of the antimicrobial resistance risk using the biocidal rotation concept.

To assess the antimicrobial activity of the disinfectants correctly, a complete stoppage of the biocidal action after a predefined contact period must be ensured (28). This was ensured using a combination of both dilution 1:10 (v/v) and chemical neutralization (28). To validate the neutralization technique, two comparisons analyses must be made (29). The first requirement is the proof of non-toxicity of the neutralizer on the test microbe. The second criterion is the evidence of the effectiveness of the neutralization process through the absence of evidence of suppression of microbial growth after mixing the neutralizer with the disinfectant agents at the working concentration (29). Each study involved its own control group as viability (microorganism) treatment for toxicity study and the toxicity (microorganism plus neutralizer) group for neutralizer efficacy. It should be noted that the microbial toxicity might originate from the neutralizer ingredients, disinfectant components (from its trace amounts, if complete neutralization was not ensured) and/or the byproduct of the chemical neutralization process due to neutralizer-biocide interaction (28,30-32). The selected microbial count range for the serial dilution of > 40 to 100 CFU/plate was selected as an optimal bioburden range to minimize count error.

Statistical inference was performed at different stages. The first one was broadly based on the initial examination of the recovery ratio that should meet the acceptance criterion (22). The second one gets it deeper into investigating the significance of the difference between the found results from the reference lower acceptance value and the upper microbial count result of the control (33). By virtue of the non-Gaussian nature of the microbiological distribution, the Wilcoxon signed-ranks test was used as a non-parametric that is equivalent to the paired t-test (34). It is most widely used to investigate for a difference in the median or mean of recorded

data - whether measurements were based on pairing examination or before and after readings on the same test subject (35). Also, it could be applied as a one-sample test to determine whether a particular sample was raised from a population with a predefined median value such as 0.75 recovery ratios of the raw data for CFU count/plate or logarithmically transformed figure for this microbial count of 0.938 as an acceptance criterion. In the present study, the microbial recovery ratios had met the acceptance criterion without significant difference from the control values.

The variations of the bacterial recoveries between different groups were examined using multiple comparison analyses. Interestingly, the detailed one-by-one comparison did not mark any significant difference at $\alpha = 0.05$. Nevertheless, the ANOVA test pinpointed a significant difference ($P = 0.05$). A situation that has been observed statistically in other previous works (36). Treatments were segregated as columns of CFU/plate recovery for individual group examination. A remarkable difference was detected to be possibly stemmed from the variation between the control plate count and the AD biocidal group. This is even though fact that this disinfectant did not fail apparently in the other preliminary statistical examination. Accordingly, a decision has been made to proceed in the testing procedure with disinfectant.

A disinfectant evaluation was executed by mimicking the application in the actual use – including storage time – on the most common surface materials that could be found in the building (37). These surfaces were made in the form of coupon shapes from the lining of the floor, wall material and metallic objects (mostly steel) (38). The surface contact was used instead of the suspension test because it is more realistic and challenging. This is because the microbial particles might be protected from the microscopic irregularities of the hard objects that hinder the full exposure of the cells to the biocidal environment in contrast to the suspended microbial cells where the surface area of exposure could be maximized accelerating the effect of the antimicrobial compounds (30). All investigated disinfectant formulae were effective directly after the preparation and after two weeks of storage at the ordinary facility-controlled environment in a well closed container. Interestingly, freshly prepared DC showed very low microbial recovery which was not significantly different from that after about 336 hours. The more significant microbial reduction observed with the metallic surfaces compared with wall and floor materials, notably with DC disinfectant, could suggest the unfavorable survivability conditions for the microorganism on the metal with possible antimicrobial effect on the viable cells.

Finally, it could be concluded that the commercially used disinfectants in the healthcare facility were effective in killing a large population ($\geq 1 \times 10^6$ CFU/coupon) of the microorganism under study (BCC) using a challenging over-kill strategy to account for a high load of bioburden that could be inoculated on the surfaces during the traffic of a heavy workload (39 - 41). This could be ensured only after an effective neutralization plan to avoid any overestimation of the biocidal agent activity due to the crippling residual amount of the disinfectant after the proposed contact period (42). This trace amount of the antimicrobial components might halt the microbial growth in the agar media leading to an exaggerated impression of the true potency of the biocidal agents. After successful implementation of this study, other microorganisms could be included in the validation program after identification to build a growing database for better control of microbial dissemination and contamination. The result of the ampholyte antimicrobial product might require further extended investigation in another study in the future including a greater number of replicates to confirm or exclude the observed significant difference. The presence of a toxic trace level of antimicrobial constituents – that is not enough to block the full growth of the microorganism totally - cannot be ruled out. However, this would be needed to be addressed in a new study that would require deeper analysis. It is also advisable to select the materials of construction for both equipment, tools, furniture, wall and floor surfaces carefully. Implementation of smooth surface materials that possess antimicrobial properties in healthcare buildings will be an advantage in hygiene control policy in the organization.

Conflict of interest

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Authors' contributions

Concept: D.E.E., E.R.R., Design: E.R.R., D.E.E., Data Collection or Processing: D.E.E., M.E.E., Analysis or Interpretation: M.E.E., Literature Search: E.R.R., Writing: M.E.E.

References

1. Scherbaum M, Kösters K, Mürbeth R, Ngoa U, Kremsner P, Lell B, Alabi A. Incidence, pathogens and resistance patterns of nosocomial infections at a rural hospital in Gabon. BMC Infectious Diseases. 2014;14(1): 14-124.

2. Weinstein R, Hota B. Contamination, Disinfection, and Cross-Colonization: Are Hospital Surfaces Reservoirs for Nosocomial Infection?. Clinical Infectious Diseases. 2004;39(8):1182-1189.

3. Casadevall A, Pirofski L. Host-Pathogen Interactions: Basic Concepts of Microbial Commensalism, Colonization, Infection, and Disease. Infection and Immunity. 2000;68(12):6511-6518.

4. Burrell C, Howard C, Murphy F. Epidemiology of Viral Infections. Fenner and White's Medical Virology. 2017;185-203.

5. Rutala WA, Weber DJ, Healthcare Infection Control Practices Advisory Committee (HICPAC). Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008 | AHE [Internet]. AHE.

2022 [cited 2022 May 16]. Available from: https://www.ahe.org/guideline-disinfection-and-sterilization-healthcare-facilities-2008

6. Rutala W, Weber D. Disinfection, Sterilization, and Control of Hospital Waste. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2015;3294-3309.e4.

7. Habboush Y, Benham M, Louie T, Noor A, Sprague R. New York State Infection Control [Internet]. Ncbi.nlm.nih.gov. 2022 [cited 2022 May 16]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK565864/

8. Johnston M, Simons E, Lambert R. One explanation for the variability of the bacterial suspension test. Journal of Applied Microbiology. 2000;88(2):237-242.

9. EISSA M. Assessment for Sporicidal Activity of Two Types of Peroxygen/Silver-Based Disinfectants: A Comparative Study. Journal of Pharmaceutical Technology, Research and Management. 2016;4(2):161-169.

10. Essam Eissa M. A novel approach in assessing the antimicrobial efficacy of eye drop products, European Pharmaceutical Review. 2016; 21(2): 40-43.

11. Eissa M. Validation of Microbial Recovery of Pharmaceutically Important Gram-negative Bacteria from Peroxygen/Silver based Disinfectants and Evaluation of their Degree of Corrosiveness, Journal of Advanced Microbiology. 2017; 1(2): 41-58.

12. Essam Eissa M. Limitations of the current chemical neutralization tests for assessment of microbial recovery from antimicrobials: A new prospective approach, International Journal of Microbiology and Allied Sciences (IJOMAS). 2016; 2(4):1-5.

13. Eissa M, Abd El Naby M, Beshir M. Bacterial vs. fungal spore resistance to peroxygen biocide on inanimate surfaces. Bulletin of Faculty of Pharmacy, Cairo University. 2014;52(2):219-224.

14. Eissa M, Kamel M, Beshir M. Study of Antimicrobial Power of Amphoteric Disinfectants of Tego Series Used in Pharmaceutical Industry. Egyptian Academic Journal of Biological Sciences, G Microbiology. 2014;6(1):17-27.

15. Eissa M, Abd El Naby M, Saber Nouby A. Assessment of antımıcrobıal actıvıty of novel dısınfectant based on peroxygen/bıguanıde/alcohol combınatıon. Indonesıan Journal Of Pharmacy. 2014;25(3):153.

16. Pincus DH. Encyclopedia of Rapid Microbiological Methods. Hazelwood, MO: bioMérieux, Inc.; 2006.

17. API® [Internet]. bioMérieux. 2022 [cited 2022 May 16]. Available from: https://www.biomerieux-usa.com/clinical/api

18. Eissa M, Mahmoud a. Establıshment of method for bıoburden recovery: Non-Antibiotic Oral Tablets (STUDY II). 2015;4(7): 234-243.

19. Kratzer C, Tobudic S, Assadian O, Buxbaum A, Graninger W, Georgopoulos A. Validation of AKACID Plus as a Room Disinfectant in the Hospital Setting. Applied and Environmental Microbiology. 2006;72(6):3826-3831.

20. S. Sheraba N. Efficacy and toxicity of neutralizers against disinfectants and antiseptics used in vaccine production facility. African Journal of Microbiology Research. 2012;6(36).

21. Motulsky H. GraphPad Prism. San Diego, Calif.: GraphPad Software; 2003.

22. Sutton SV, Proud DW, Rachui S, Brannan DK. Validation of microbial recovery from disinfectants. PDA Journal of Pharmaceutical Science and Technology. 2002;56(5):255-66.

23. Clontz L. Microbial limit and bioburden tests. Boca Raton: Taylor & Francis; 2009.

24. Tavares M, Kozak M, Balola A, Sá-Correia I. *Burkholderia cepacia* Complex Bacteria: a Feared Contamination Risk in Water-Based Pharmaceutical Products. Clinical Microbiology Reviews. 2020;33(3).

25. Sousa S, Ramos C, Leitão J. *Burkholderia cepacia* Complex: Emerging Multihost Pathogens Equipped with a Wide Range of Virulence Factors and Determinants. International Journal of Microbiology. 2011;2011:1-9.

26. Jain V, Karibasappa G, Dodamani A, Prashanth V, Mali G. Comparative assessment of antimicrobial efficacy of different hand sanitizers: An in vitro study. Dental Research Journal. 2016;13(5):424.

27. Claverley Group company. Lifecycle approach to cleaning and disinfection rotation [Internet]. Cleanroomtechnology.com. 2022 [cited 2022 May 16]. Available from: https://www.cleanroomtechnology.com/news/article_page/Lifecycle_approach_to_cleaning_and_disinfection_rotation/140448

28. Eissa ME, Nouby AS. Validation of spore-forming organisms recovery from peroxygen-based disinfectants. J Pharm Res Clin Pract. 2014;4(2):23-32.

29. Klein D. Disinfectant Efficacy Testing [Internet]. Basic medical Key. 2021 [cited 2022 May 16]. Available from: https://basicmedicalkey.com/disinfectant-efficacy-testing/

30. Eissa M. Studies of microbial resistance against some disinfectants: microbial distribution & biocidal resistance in pharmaceutical manufacturing facility. Chisinau: LAP Lambert Academic Publishing; 2014.

31. Sozzi E, Baloch M, Strasser J, Fisher M, Leifels M, Camacho J, et al. A bioassay-based protocol for chemical neutralization of human faecal wastes treated by physico-chemical disinfection processes: A case study on benzalkonium chloride. International Journal of Hygiene and Environmental Health. 2019;222(2):155-167.

32. Spengler J, Amy G. Disinfectants and disinfectant by-products. Geneva: World Health Organization; 2000.

33. GraphPad Software LLC. GraphPad Prism 9 Statistics Guide - Nonparametric tests [Internet]. Graphpad.com. 2022 [cited 2022 May 16]. Available from: https://www.graphpad.com/guides/prism/latest/statistics/stat_nonparametric_tests.htm

34. Eissa M. Evaluation of microbiological purified water trend using two types of control chart. European Pharmaceutical Review. 2018;23(5):36-8.

35. Illowsky B, Dean S. Matched or Paired Samples [Internet]. Opentextbc.ca. 2022 [cited 2022 May 16]. Available from: https://opentextbc.ca/introstatopenstax/chapter/matched-or-paired-samples/

36. Eissa M, Rashed E, Eissa D. Dendrogram Analysis and Statistical Examination for Total Microbiological Mesophilic Aerobic Count of Municipal Water Distribution Network System. HighTech and Innovation Journal. 2022;3(1):28-36.

37. McLaren I, Wales A, Breslin M, Davies R. Evaluation of commonly used farm disinfectants in wet and dry models of *Salmonella* farm contamination. Avian Pathology. 2011;40(1):33-42.

38. Eissa ME, Ashour MS, Mansy MS. Impact of synthetic detergent on sporicidal activity in pharmaceutical facility. Egyptian Academic Journal of Biological Sciences, G. Microbiology. 2013;5(1):43-50.

39. Dancer S. Controlling Hospital-Acquired Infection: Focus on the Role of the Environment and New Technologies for Decontamination. Clinical Microbiology Reviews. 2014;27(4):665-690.

40. Gouse M, Jayasankar V, Patole S, Veeraraghavan B, Nithyananth M. Clinical outcomes in musculoskeletal involvement of *Burkholderia Pseudomallei* infection. Clinics in Orthopedic Surgery. 2017;9(3):386-91.

41. Torbeck L, Raccasi D, Guilfoyle D, Friedman R, Hussong D. *Burkholderia cepacia*: This Decision Is Overdue. PDA Journal of Pharmaceutical Science and Technology. 2011;65(5):535-543.

42. Rutala WA, Weber DJ. Guideline for disinfection and sterilization in healthcare facilities. CDC, Atlanta: U.S. Department of Health & Human Services; 2008

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Cupping therapy combined with conventional physical therapy improves pain and health related quality of life among female patients with low back pain

Nida KİANİ*, Furqan AHMED SIDDIQI, WardahAjaz QAZİ, Ruqia BEGUM Arva NAEEM, Kanwal ZAFAR, Shoaib KAYANİ, Sanna PERVAİZ

Foundation University College of Physical Therapy, Islamabad, Pakistan

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Abstract

Cupping therapy is a traditional therapy treatment used since ancient era. It is a therapy of alternate medicine, gaining popularity in physical medicine because of minimal adverse effects, reduction in pain and muscle tenderness. Very little literature is available on the combine effect of cupping therapy and conventional physical therapy in order to treat low back pain in female patients. To evaluate effects of cupping therapy combined with conventional Physical therapy in order to improve the pain and health related quality of life among female patients with low back pain. Randomized control trial done on diagnosed cases of 40 low back pain female patients in Fauji Foundation Hospital, Rawalpindi. Experimental Group (A) received cupping therapy once every two weeks for 4 weeks combined with conventional physical therapy including hot pack, interferential therapy and strengthening exercises for back done 3 times per week for 4 weeks. Control group (B) did not received cupping therapy, only conventional Physical therapy was given and their outcomes were observed at the baseline and at the end of 4 weeks sessions. Results showed significant improvement in the measured variables of pain and low back disability as well as in physical health, psychological health, and social relations whereas no such significant improvement was seen in environmental health. Combine effect of cupping therapy along with conventional physical therapy helps in reducing not only low back pain symptoms but also improve the health related quality of life among female patients.

Keywords: cupping therapy, health related quality of life, low back pain, musculoskeletal disorders, physical therapy

1. Introduction

Pain is one of the most common causes of seeking medical care (1). While cardiac infarction, stroke and many other infectious diseases including cancer and diabetes have high mortality rates, chronic pain is the leading cause of human suffering and disability. Pain, along with many diseases resulting from chronic pain, is not life-threatening. People can also continue to live with the pain. Both the developed and underdeveloped countries are facing the problem (3, 4).

Pain in the low back region is usually referred to as pain, muscle tension and stiffness that is localized below the region of the costal margin and also above the region of the inferior gluteal folds, which may or may not involve leg pain. Sciatica involving low back pain occurs in about 60 – 80% of the people at some point in their lives, and there are some cases where it begins in childhood (5, 6).

Chronic back pain may cause emotional, physical, and also socioeconomic changes (7, 8) which also includes high dose and frequent usage of medicines and other health resources (9). Sometimes the search for de-medicalization results in an increase in the usage of integrative and complementary practices, such as the traditional Chinese medicine (TCM) in order to complement the pain related to allopathic care (10). One of the recommended therapies of TCM for reducing chronic pain is cupping therapy (11). Cupping therapy involves application of cups of different materials (12) placed at acupoint or any area causing pain with the help of heat or any vacuum apparatus (13).

Manual therapy is one of the most commonly used and applicable among the various physical therapy procedures. It aims at relieving pain through the use of the vertebral manipulations along with relaxation of the muscles and hence improving the biomechanical functions present in the tissues (11). Besides the manual therapy, the physical therapy can also depend upon various types of resources and different techniques which are mainly used for analgesic purposes, which include kinesiotherapy, electrotherapy. Pilates is also commonly performed on patients who are complaining of low back pain (14).

However, in addition to the techniques and resources mentioned for the treatment of the pain in the low back region, the cupping therapy is most commonly used today as a complementary therapy for the reduction of the symptoms

*Correspondence: nida.mushtaq@fui.edu.pk

caused by chronic pain, helping in lowering the problems faced by the patient suffering from the pain in the low back region. The procedure of cupping therapy is a very common practice in traditional Chinese medicine. There is a history of applying it in order to minimize the symptoms experienced due to the chronic pain. Currently, an increasing number of patients are displaying their interest in the use of cupping therapy in treatment of the reducing pain in the low back region, as it is believed to be very effective and a lot safer (11). Many techniques utilizing the cupping therapy procedure which may include both dry and wet suction cups, holding the cups, stirring fast cups, setting in motion and balancing the cups. The safest form of application is the dry suction cup, which is mostly utilized in clinical practice. This technique involves sucking the skin through the suction cups without drawing blood. Negative pressure is generated through this type of suction, which in turn is used for increasing the removal of toxin materials at the location of application and thus in return activates the anti-inflammatory enzymes which causes relief from pain and increases the relaxation of muscles (11) .In 2017 Wang et al. (11), in 2018 Teut et al.(15), in 2011 Kim et al.(16)and the other authors analyzed the efficacy and safety of using cupping therapy on different pain levels in treating patients for back pain. Significant results were obtained, revealing that this was potentially very safe and also economical, with an average reduction in pain ranging from 50 % to 70%. In this study, the effectiveness of using different types of cupping therapy for lowering symptoms of pain in the low back area revealed that no such significant difference was present among the different types of the suction cups. Whereas in other studies it has been said that the cupping therapy may lower the pain among patients with nonspecific pain in the low back area, however its effect on the variables of neuromuscular region which are responsible for causing low back pain is still not very well-known. Among different causes, the back pain is said to be associated with various imbalances in neuromuscular region, resulting in less stabilization of the trunk and causing an overload on the structures of the joint while performing the task of daily activities(17). The difficulty in spine stabilization is connected to recruitment inefficiency among stabilizing deep muscles, increase in the superficial muscle contraction in the trunk region and the reduction in the fatigue resistance in the capacity of extensor muscles of the trunk (14) .

The cupping therapy is a traditional technique used in Chinese medicine. This technique has been practiced for nearly around thousands of years and is considered very beneficial for treating various conditions which include pain, stroke and hypertension (18), (19), (12). However, the clinical efficacy is still uncertain. The process of the action is not clearly explained and also the methodology and research are not of good quality which represents many research biases (18,20).

Although cupping has been used successfully for thousands of years in order to treat the pain and a variety of other complains, the cupping therapy usage has greatly diffused during last few decades as the preliminary systematic clinical trials indicating that it is very useful in managing the very painful conditions (20, 21). Moreover, the cupping procedure has a very broad indication in the therapeutic properties, simplicity in applying the cups, low cost, with very few adverse effects, and immediate results after the treatment of various kinds of diseases (12, 22). Cupping therapy is now regularly observed in clinical practice, in order to bring relief from pain and also to improve the general feeling of well-being in a patient (23).

The purpose of this present study is to analyze the patients with low back pain and to perform the comparison between the control and experimental group.

The main goal of doing this research is to find out if there are any additional impacts and benefits of cupping therapy combined with the conventional physical therapy. The study objective was to determine the cupping therapy effects combined with conventional physical therapy in a female patient population with pain in the low back region and also to find out the health related quality of life.

2. Materials and Methods

The study design used was Randomized Control trial. This study was performed in the physical therapy department of Fauji Foundation University Hospital Rawalpindi, Pakistan. It was a 6- month study from July 1st, 2019 to December 30th, 2019.

This study was conducted with the approval of Foundation University Review Committee on 18 August 2020. (Approval number FF/FUMC/215 Phy/20)

2.1. Participants

Forty-three female patients were initially assessed for eligibility criteria. Diagnosed cases of low back pain referred from the rehabilitation department of Fauji Foundation hospital were included in our study. The sample collected was selected by purposive sampling technique and then participants were randomly assigned by using toss and trial method to group A and B. Patients were randomly assigned to two different groups. Group A (experimental group) received cupping therapy along with conventional physical therapy which included ultrasound modality and McKenzie exercises for back pain. The questionnaire used to check the disability related to back pain was Oswestry low back pain disability questionnaire and WHOQOL-BREF questionnaire was utilized to assess the quality of life at base line and at the end of the treatment. Sample selection was done on the basis of the inclusion and exclusion criteria. The criteria of inclusion for this study included the (I) female population; (ii) they were between the ages of 30 and 50 years; and (iii) diagnosed cases of the mechanical low back pain. Whereas the patients excluded consists of (I) patients having spinal tumor; (ii)

cases of herniated discs problems; (iii) patients suffering from spondylolisthesis or spondylosis and any case of systemic illness.

2.2. Data collection procedure

Data was collected on Demographics and general information. Chronic pain was assessed with the help of VAS (Visual Analog scale). The health related to the quality of life was evaluated by a set questionnaire (WHOQOL- BREF). Experimental Group (A): This group received cupping therapy every month combined with conventional physical therapy which included: Moist Heat Pack for 10 min as well as McKenzie Extension Protocol which involves prone lying position, the prone lying position in extension, the sustained extension and the correction of the posture in static condition having 3 sets of 10 repetitions each. In dynamic condition the McKenzie extension protocol was extension in lying, extension in lying with overpressure as well as extension mobilization with the same 3 sets of the 10 repetitions, following for 3 days in a week, for a period of 4 weeks. The treatment duration extended from 40 to 50 minutes. Control group (B): This group received conventional Physical therapy same as experimental group. Outcomes will be observed at the baseline and at the end of the session. All post treatments were measured at the end of 12 sessions. The data was analyzed through SPSS 21.0.

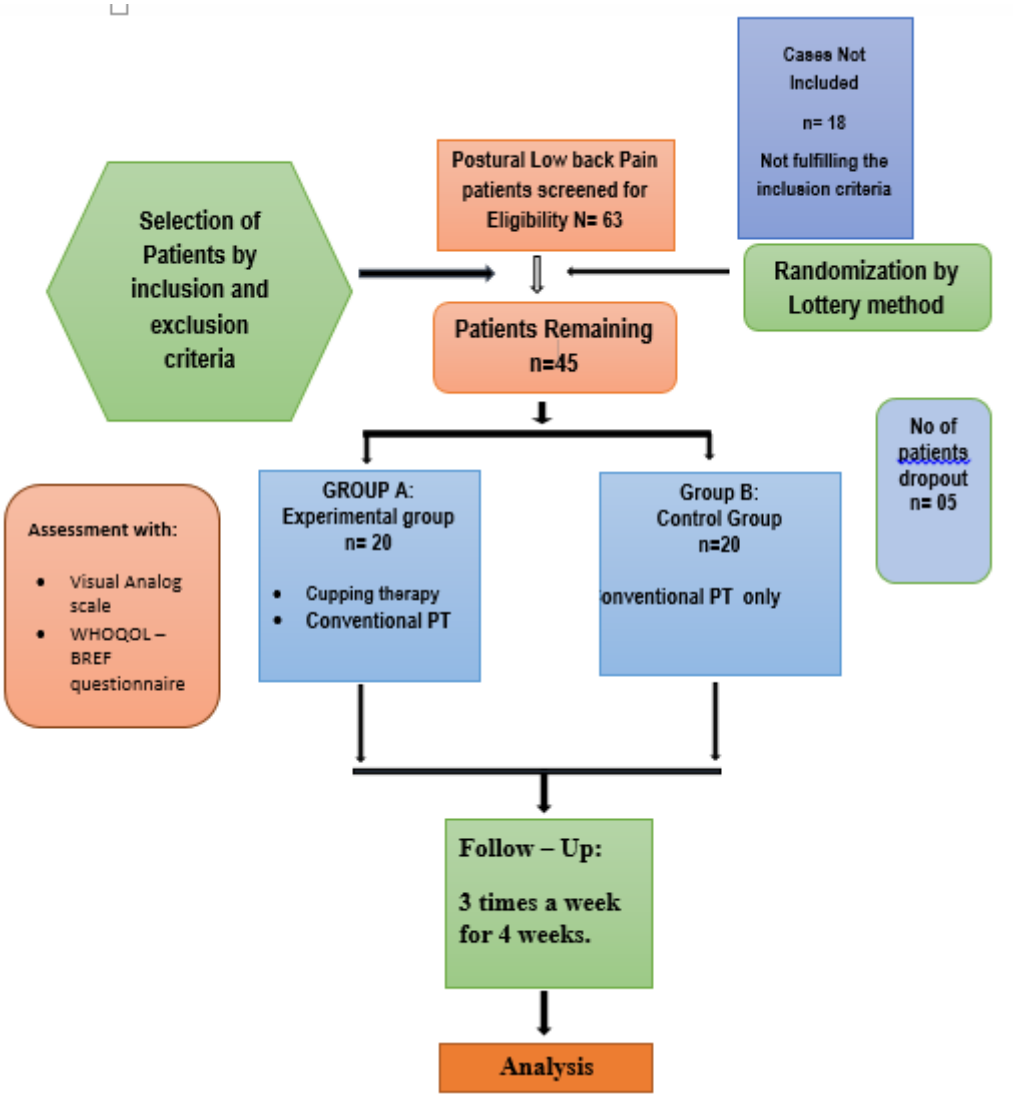

Fig 1. Study flow diagram

3. Results

Among 63 approached cases, 45 of the females met the inclusion criteria and were included in this study. After taking proper consent from the patients, only 40 patients completed the treatment protocol 20 in each group. The treatment protocol involved cupping therapy along with conventional physical therapy exercises in the experimental group(group A) and for the patients included in the control group(Group B) they were treated using conventional physical therapy exercises only . Base line values for evaluation of patient demographics and duration of back pain were taken. The patients were assessed at the time of the first visit and also at the end of the last visit after completion of 12 sessions on a 4-week treatment protocol. A self-reported structured questionnaire was filled and the oswestry low back pain disability questionnaire, visual analog scale and WHOQOL-

BREF questionnaire were calculated to compare the outcome measures in patients.

Table 1. Demographic distribution

Variables		Group A (Experimental)	Group B (Control)
Age in years		Mean ± SD	Mean ± SD
		42.85±6.98	39.35±7.06
		f (%)	f(%)
Occupation	Working women	08(40%)	11(55%)
	House wife	12(60%)	09(45%)
Marital status	Single	06(30%)	04(20%)
	Married	14(70%)	16(80%)
Duration of illness	<1 year	07(35%)	09(45%)
	>1 year	13(65%)	11(55%)

The normality of the data was checked by applying Q-Q plots with a normality test to identify the normal distribution of data. Shapiro Wilk test showed the data for Oswestry low back pain disability questionnaire was not normally distributed at baseline so the non- parametric test Mann Whitney U test was applied.

Table 2. Group analysis between OLBPDQ and VAS by Mann Whitney U test

Variable		Median	IQ	P Value
OLBPDQ	Pre	51.0	9.75	.53
	Post	40.0	8.75	.01
VAS	Pre	7.0	1.0	.60
	Post	5.5	2.0	.00

OLBPDQ, Oswestry Low Back Pain disability Questionnaire; VAS, Visual Analog Scale; IQ, interquartile

Table 3. Comparison of the WHOQOL-BREF Mean Scores in four Domains

Variable		Experimental Group(A)	Control Group (B)	P value
PHD	Pre	40.51±5.92	45.69±6.99	0.07
	Post	40.55±5.96	66.86±8.56	0.00
PSHD	Pre	38.32±4.30	43.90±5.22	0.09
	Post	38.33±4.30	49.89±6.34	0.00
SHD	Pre	30.20±3.5	35.23±4.38	0.07
	Post	30.22±3.5	38.45±4.99	0.05
EHD	Pre	27.50±2.8	34.54±3.33	0.88
	Post	27.50±2.8	35.11±3.52	0.78

PH, Physical Health; PSH, Psychological Health; SR, Social Relation; ED, Environmental Domain

Significant difference was seen between the experimental group (A) and control group (B) after following the intervention in the following domains of WHOQOL-BREF, Physical Health Domain (P <0.05), Psychological health domain (P = 0.00) and Social Relations Domain (P= 0.05). There was found to be no significant difference observed between groups for Environmental Domain (P= 0.78).

4. Discussion

The Low back pain is one of the most prevalent health condition experienced by the elderly population especially females(24, 25). Nowadays there is an increase trend among people to start using alternative and complementary medicine to treat many different types of pain including the pain in the low back region (26). Cupping therapy is one of the most common among them. (26)

In the present study, it was revealed that the mean age used for the young adult female population was between 18 to 35 years. This was in accordance with a study conducted in Brazil in 2020, which stated that the female population mainly suffers from low back pain with an age ranging from 20 to 55 years (27). It is believed that women are at a higher risk in comparison to the male population due to their anatomical structure. Their bodies usually consist of small stature, bone density, less muscle mass, less adaption to physical effort and a greater amount of joint fragility. In addition to all this, the sum of the burden imposed on the body due to the performance of the household or domestic tasks can also result in an increase in the risk of developing low back pain (28).

Individuals receiving the cupping therapy, which was used for the treatment of their pain in the low back region, showed marked improvement on the visual analog scale. Another similar study conducted showed a significant reduction in pain level after cupping therapy application (29).

Low Back pain is accompanied often by either musculoskeletal, neurological or psychiatric disorders resulting in a negative impact on health related quality of life of a patient(30).The health related quality of life was assessed by WHOQOL – BREF questionnaire (31). This questionnaire was modified according to the requirement in order to include the 17 questions which involve the domain of Physical Health (PH), Psychological Health (PSH), Social health (SH) and environmental health domain (EHD). According to the results obtained wet cupping therapy proved to be effective in improving the quality of life in many domains especially the physical, social and Psychological domain. The two different studies were done which proved that by using various sessions of cupping therapy treatment or even one session of cupping therapy can notably improve the pain and enhance the physical function improving quality of life of the patients complaining of chronic pain (32, 33). Another Quasi experimental study was done in 2017, in Kind Abdul-Aziz University Saudi Arabia which reported that

cupping helps in improving the quality of life especially in domain of physical health, involving different pain types and other conditions related to medical issues(34).

One limitation in this study was that there was only a female population involved. The sample size used was also small which may limit the conclusiveness of this study finding. Therefore, it cannot be generalized for the whole population which can also contribute to selection bias. Other limitations include short periods of follow ups and the limited number of cupping therapy treatment sessions having a high rate of dropout in the female population. Further studies are needed to be done on cupping therapy to increase awareness and knowledge about cupping therapy sessions and its uses along with conventional physical therapy.

One of the few limitations observed in this study was that the participants included were only female. Most of the patients did not complete the follow up. The sample size used was also small. Further researches are recommended to include both female and male participants and also increase the time duration required in the study and also the sample size**.**

The study concluded that there are a lot of promising effects that not only help use the cupping therapy treatment to reduce the symptoms of pain in the low back region but also improve the quality of life of the female patients.

Conflict of interest

There was no conflict of interest declared among the authors.

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Authors' contributions

Concept: N.M.K., W.A.Q., Design: F.A.S., R.B., Data Collection or Processing: S.K., A.N., Analysis or Interpretation: K.Z., S.P., Literature Search: F.A.S., W.A.Q., Writing: N.M.K., R.B.

References

1. Mäntyselkä P, Kumpusalo E, Ahonen R, Kumpusalo A, Kauhanen J, Viinamäki H, et al. Pain as a reason to visit the doctor: a study in Finnish primary health care. Pain. 2001;89(2-3):175-80.
2. Goldberg DS, McGee SJ. Pain as a global public health priority. BMC public health. 2011;11(1):1- 5.
3. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. European journal of pain. 2006;10(4):287-333.
4. Dureja GP, Jain PN, Shetty N, Mandal SP, Prabhoo R, Joshi M, et al. Prevalence of chronic pain, impact on daily life, and treatment practices in India. Pain Practice. 2014;14(2):E51-E62.
5. Manek NJ, MacGregor A. Epidemiology of back disorders: prevalence, risk factors, and prognosis. Current opinion in rheumatology. 2005;17(2):134-40.
6. Calvo-Muñoz I, Gómez-Conesa A, Sánchez-Meca J. Prevalence of low back pain in children and adolescents: a meta-analysis. BMC pediatrics. 2013;13(1):14.
7. Sielski R, Rief W, Glombiewski JA. Efficacy of biofeedback in chronic back pain: a meta-analysis. International journal of behavioral medicine. 2017;24(1):25-41.
8. Bergström G, Hagberg J, Busch H, Jensen I, Björklund C. Prediction of sickness absenteeism, disability pension and sickness presenteeism among employees with back pain. Journal of occupational rehabilitation. 2014;24(2):278-86.
9. Gore M, Sadosky A, Stacey BR, Tai K-S, Leslie D. The burden of chronic low back pain: clinical comorbidities, treatment patterns, and health care costs in usual care settings. Spine. 2012;37(11):E668-E77.
10. Sita Ananth M. Complementary Alternative Medicine Survey of Hospitals-Summary of Results. Alexandria, VA: Samueli Institute. 2011.
11. Wang Y-T, Qi Y, Tang F-Y, Li F-M, Li Q-H, Xu C-P, et al. The effect of cupping therapy for low back pain: A meta-analysis based on existing randomized controlled trials. Journal of back and musculoskeletal rehabilitation. 2017;30(6):1187-95.
12. Aboushanab TS, AlSanad S. Cupping therapy: an overview from a modern medicine perspective. Journal of acupuncture and meridian studies. 2018;11(3):83-7.
13. Cao H, Li X, Yan X, Wang NS, Bensoussan A, Liu J. Cupping therapy for acute and chronic pain management: a systematic review of randomized clinical trials. Journal of Traditional Chinese Medical Sciences. 2014;1(1):49-61.
14. Silveira APdB, Nagel LZ, Pereira DD, Morita ÂK, Spinoso DH, Navega MT, et al. Efeito imediato de uma sessão de treinamento do método Pilates sobre o padrão de cocontração dos músculos estabilizadores do tronco em indivíduos com e sem dor lombar crônica inespecífica. Fisioterapia e Pesquisa. 2018;25(2):173-81.
15. Teut M, Ullmann A, Ortiz M, Rotter G, Binting S, Cree M, et al. Pulsatile dry cupping in chronic low back pain–a randomized three-armed controlled clinical trial. BMC complementary and alternative medicine. 2018;18(1):115.
16. Kim J-I, Kim T-H, Lee MS, Kang JW, Kim KH, Choi J-Y, et al. Evaluation of wet-cupping therapy for persistent non-specific low back pain: a randomised, waiting-list controlled, open-label, parallel-group pilot trial. Trials. 2011;12(1):146.
17. de Morais Kobill AF, de Almeida Silveira AL, de Lima AI, Paidosz A, Siqueira AF, Penteado D, et al. Influência da estabilização segmentar core na dor e funcionalidade da coluna lombar. Fisioterapia Brasil. 2017;18(2):148-53.
18. Cao H, Li X, Liu J. An updated review of the efficacy of cupping therapy. PloS one. 2012;7(2):e31793.
19. Qureshi NA, Ali GI, Abushanab TS, El-Olemy AT, Alqaed MS, El-Subai IS, et al. History of cupping (Hijama): a narrative review of literature. Journal of integrative medicine. 2017;15(3):172-81.
20. Kim J-I, Lee MS, Lee D-H, Boddy K, Ernst E. Cupping for treating pain: a systematic review. Evidence-Based Complementary and Alternative Medicine. 2011;2011.
21. Farhadi K, Schwebel DC, Saeb M, Choubsaz M, Mohammadi R, Ahmadi A. The effectiveness of wet-cupping for nonspecific low

back pain in Iran: a randomized controlled trial. Complementary therapies in medicine. 2009;17(1):9-15.

22. Rubinstein SM, van Middelkoop M, Kuijpers T, Ostelo R, Verhagen AP, de Boer MR, et al. A systematic review on the effectiveness of complementary and alternative medicine for chronic non-specific low-back pain. European Spine Journal. 2010;19(8):1213-28.

23. Kanodia AK, Legedza AT, Davis RB, Eisenberg DM, Phillips RS. Perceived benefit of complementary and alternative medicine (CAM) for back pain: a national survey. The Journal of the American Board of Family Medicine. 2010;23(3):354-62.

24. Prince MJ, Wu F, Guo Y, Robledo LMG, O'Donnell M, Sullivan R, et al. The burden of disease in older people and implications for health policy and practice. The Lancet. 2015;385(9967):549-62.

25. Leopoldino AAO, Diz JBM, Martins VT, Henschke N, Pereira LSM, Dias RC, et al. Prevalence of low back pain in older Brazilians: a systematic review with meta-analysis. Revista brasileira de reumatologia. 2016;56:258-69.

26. Arslan M, Gökgöz N, Dane Ş. The effect of traditional wet cupping on shoulder pain and neck pain: A pilot study. Complementary therapies in clinical practice. 2016;23:30-3.

27. Spinoso DH, Pereira LC. Effect of cupping therapy on the level of pain and neuromuscular variables in women with low back pain. Manual Therapy, Posturology & Rehabilitation Journal. 2020;18:1-8.

28. Guedes KN, dos Santos RR, de Sá dpc. Eficácıa da osteopatıa na lombalgıa ınespecífica comparada a fısıoterapıa convencıonal. Hígıa-revısta de cıências da saúde e socıaıs aplıcadas do oeste baıano. 2021;6(1).

29. Al-Bedah AM, Elsubai IS, Qureshi NA, Aboushanab TS, Ali GI, El-Olemy AT, et al. The medical perspective of cupping therapy: Effects and mechanisms of action. Journal of traditional and complementary medicine. 2019;9(2):90-7.

30. Buse DC, Rupnow MF, Lipton RB, editors. Assessing and managing all aspects of migraine: migraine attacks, migraine-related functional impairment, common comorbidities, and quality of life. Mayo Clinic Proceedings; 2009: Elsevier.

31. Barcaccia B, Esposito G, Matarese M, Bertolaso M, Elvira M, De Marinis MG. Defining quality of life: a wild-goose chase? 2013.

32. Lauche R, Cramer H, Langhorst J, Dobos G. Cupping for chronic nonspecific neck pain: a 2-year follow-up. Complementary Medicine Research. 2013;20(5):328-33.

33. Lauche R, Cramer H, Hohmann C, Choi K-E, Rampp T, Saha FJ, et al. The effect of traditional cupping on pain and mechanical thresholds in patients with chronic nonspecific neck pain: a randomised controlled pilot study. Evidence-Based Complementary and Alternative Medicine. 2012;2012.

34. Al Jaouni SK, El-Fiky EA, Mourad SA, Ibrahim NK, Kaki AM, Rohaiem SM, et al. The effect of wet cupping on quality of life of adult patients with chronic medical conditions in King Abdulaziz University Hospital. Saudi medical journal. 2017;38(1):53.

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The comparison of the therapeutic effects of piroxicam gel and oral vitamin E in cyclic mastalgia

Razieh BEHZADMEHR[1], **Tahmineh Ezazi BOJNORDİ**[2*]

[1]Department of Radiology, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran
[2]Department of Obstetrics and Gynecology, Mostafa Khomeini Teaching Hospital, Tabas, Iran

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Abstract

Cyclic mastalgia is a common condition among women. There are different methods in reducing pain in such patients, but there is yet no consensus in this regard. This study aims to compare the therapeutic effects of topical piroxicam gel and oral vitamin E in patients with cyclic mastalgia. Seventy females with mild to moderate cyclic mastalgia were recruited from an outpatient clinic in this randomized, double-blind, placebo-controlled clinical trial. The patients received either topical 0.5% Piroxicam gel four times daily or vitamin E 400IU capsules once daily for two consecutive months. The visual analogue scale (VAS) was used to report pain one and two months later. Thirty-five patients completed the study in each group. Although both medications were effective and safe in reducing pain severity, topical piroxicam led to a significantly better pain control outcome. Almost half the patients required analgesics five months after the study stopped. In conclusion, comparing topical piroxicam gel and oral vitamin E in patients with cyclic mastalgia showed that the former is more efficient in pain control in mid-term. Both medications were safe and well-tolerated.

Keywords: mastalgia; NSAIDs, vitamin E, Visual Analogue Scale

1. Introduction

Mastalgia or breast pain is a very common annoying experience. Some estimates indicate that almost 65% of women experience varying degrees of mastalgia during their reproductive age (1).

It could be cyclic, noncyclic, or extramammary (2). The cyclic form is the most frequent subtype, comprising about two-thirds of the cases who seek medical comfort. (3, 4) Although the main ethology of cyclic mastalgia is yet to be defined, its relation to the menstrual cycle, pregnancy, lactation, and menopause suggests underlying hormonal causes or aggravators (5, 6).

Cyclic pain resolves spontaneously in 20-30% of patients. However, in 60% of patients, recurring episodes might be seen (7). It has been reported that in up to 40% of patients with cyclic mastalgia the pain interferes with the normal life. Sexual and physical activities, work, and social interactions could all be negatively impacted in patients with cyclic mastalgia (8).

Reassurance alone or following nonpharmacological methods such as stress-management, relaxation techniques and wearing well-fitted and supportive brassieres have been found effective in relieving pain in many patients with cyclic mastalgia (9). In some cases, however, the pain is still bothersome and resistant to conservative managements. Pharmacologic therapy with Danazol, Bromocriptine, and oral contraceptives is considered the standard method of treatment. Side-effects and complications, however, are sometimes associated with using these medications and limit their liberal use (9). Employment of alternative treatments such as using oral and topical nonsteroidal anti-inflammatory drugs (NSAIDs) and vitamin E has been advocated by some physicians but the clinical trials in support or against their use in cyclic mastalgia are still insufficient (10, 11).

The objective of the present double-blind clinical trial is to examine and compare the efficacy of topical piroxicam gel and oral vitamin E in reducing pain in women with mild-to-moderate cyclic mastalgia.

2. Materials and Methods

2.1. Study design and population

A total of 70 women with mild to moderate cyclic mastalgia, who came to our clinic during a 14 -month period of time (starting February 2021) was enrolled in this prospective, double-blind, randomized clinical trial.

This clinical trial study is registered by the Australian New Zealand Clinical Trial Registry (ANZCTR) under this registration number: ACTRN12622000103763.

This study was conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at

*Correspondence: lily_rasouli2005@yahoo.com

http://www.wma.net/e/policy/17-c_e.html). Written informed consent was obtained from the participants. This study was approved by the ethics committee of Zabol University, date March 2020, no KH\432-12.

Patients were premenopausal females aged 18-45 years at the time of enrolment with a pain score of ≥3 on the visual analogue scale (VAS), measured in their first visit. They had no history of treatment for their pain during the last three months before the study. Pregnant women, those with concomitant or previous other medical condition (s) including a positive history of cancer, women with irregular menses, patients with abnormal findings in breast physical examination or imaging studies suggesting a possible underlying cause of breast pain rather than cyclic mastalgia were not included. Participants were tested nonallergic to the used medications before recruitment.

2.2. Study protocol

The patients were alternatively assigned to two groups. A colleague pharmacologist who was not involved in conducting the study prepared and provided medications as follows: The first group received topical Piroxicam gel (0.5% gel, RAZAK pharmaceutical Co., Tehran, Iran) four times daily plus placebo capsules (identical to vitamin E capsules in appearance, filled with distilled water) once daily both for two consecutive months (group PG). The second group received vitamin E capsules (E-Zavit, 400IU, Zahravi, Tabriz, Iran) once daily plus topical placebo gel (identical to Piroxicam gel tubes in appearance, filled with paraffin) four times daily both for two consecutive months (group VitE).

The study started from the beginning of a new menstrual cycle in each patient after she received adequate information regarding proper medication use, accurate documentation and reporting of breast pain severity, wearing well-fitted and comfortable brassieres, and avoiding stressful events and other pharmacological or non-pharmacological pain-reduction methods during the study period.

The VAS, a continuous scale ranging from 0 (no pain) to 10 (worst pain ever), was used to report the pain severity.

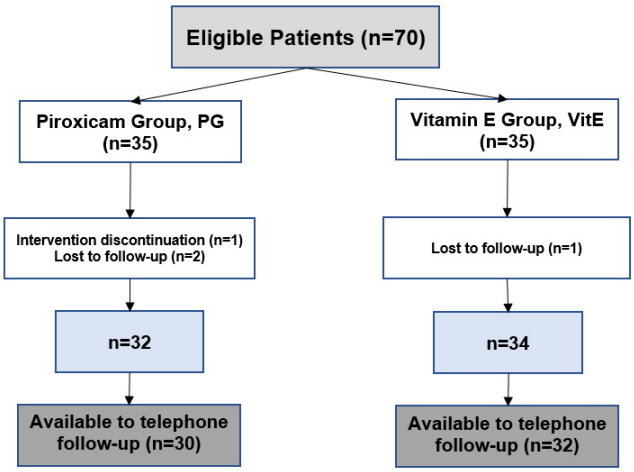

Fig. 1 Flowchart of the study groups

In-person follow-up visits were performed at the end of the first and second months after the interventions initiated. A telephone follow-up was performed five months after the interventions stopped (Fig. 1).

For blinding purposes, neither the patients nor the main investigators were aware of the grouping until after data analysis.

2.3. Statistics

The SPSS software version 25.0 (IBM Corporation, New York, USA) was used for statistical analysis. The normal distribution of numeric data was confirmed using the Kolmogorov-Smirnov analysis. The Contingency tables (Chi-square), independent samples t-test, and repeated measures analysis were used. A $P \leq 0.05$ was considered statistically significant.

3. Results

Patients' demographics and general information are summarized and compared between the two study groups in Table 1.

Table 1. Demographics and general information of the study groups

Variable	Piroxicam Group	Vitamin E group	P
Age (y)	31.7 (±8.4)	29.6 (±6.8)	0.28
Menarche (y)	12.8 (±1.8)	13.6 (±1.6)	0.08
Marital Status (Single)	12 (37.5%)	11 (32.4%)	0.66
Occupation (Housewife)	10 (31.3%)	11 (32.4%)	0.92
Regular Exercise	8 (25%)	9 (26.5%)	0.89

The PG and VitE groups were comparable in terms of age, age of menarche, marital status, occupation, and the history of regular exercise.

The mean duration of mastalgia was 4.6±2.0 years in the PD group and 5.1±1.5 years in the VitE group (P=0.23).

The mean pain severity at baseline did not differ significantly between the two groups (5.0±0.8 in PD group and 4.7±0.7 in VitE group; P=0.7). The pain severity decreased significantly in both groups at the end of the first and second months of intervention (P<0.001) (Fig. 2).

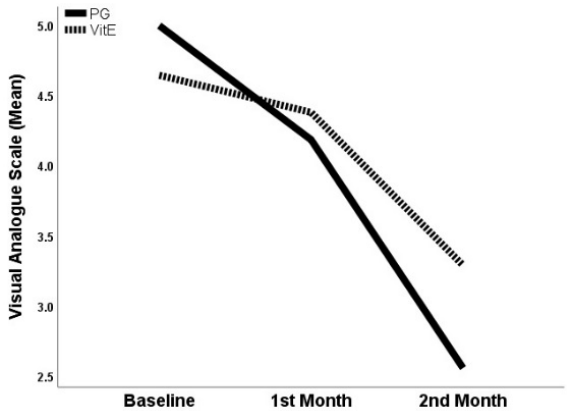

Fig. 2. Mean pain severity at baseline and one month and two months after starting interventions in two study groups

Percent decrease in pain severity was marginally more in the PG group than in the VitE group after one month, post-intervention (P=0.05). However, a difference was significant after two months, post-intervention (p<0.01) (Fig. 3).

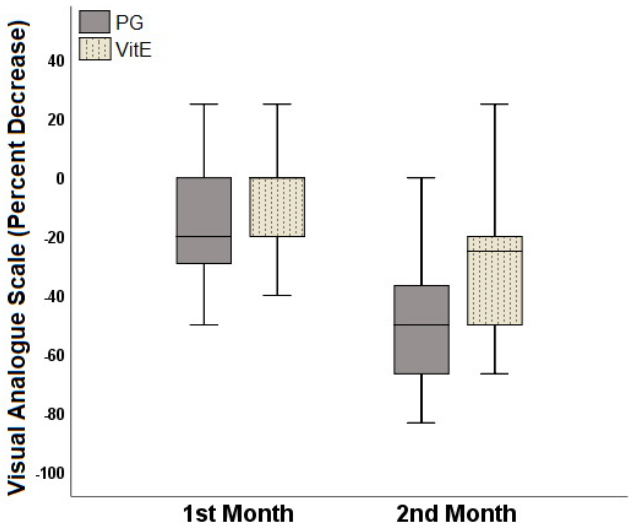

Fig. 3. Mean pain percent decrease one month and two months after starting interventions in two study groups

No significant complications or side-effects were reported by the patients in either group during or at the end of the study period.

Five months after the end of study 16 out of 30 reachable patients in the PG group (53.3%) had recurred mastalgia requiring treatment. This rate was 37.5% (12 out of 32 reachable patients). The difference, however, did not reach a statistically significant level (P=0.21).

4. Discussion

In this study, we showed that topical piroxicam gel was significantly superior to oral vitamin E in reducing cyclic breast pain after two months. The patients did not report any significant side-effects in either group.

There are a limited number of studies in the literature that have investigated the effect of topical non-steroidal anti-inflammatory drugs (NSAIDs) in treating mastalgia. In one of the first studies, the authors were successful in reducing pain in 11 out of 13 patients with cyclic breast pain (12) by using topical NSAIDs. Like in our study, the authors found the intervention safe and rapidly effective.

Colak et al. (13) administered topical NSAIDs or placebo three times daily for at least 6 months in 60 patients with cyclic mastalgia. They also found topical NSAIDs effective and safe in treating their patients.

In another study, Qureshi et al. (14) compared the oil of evening primrose and topical NSAIDs in the treatment of mastalgia in an open, non-randomized study. Fifty female patients with moderate to severe pain were recruited. A clinically significant response to topical NSAIDs was found in 92% of patients, with no significant side effects or complications.

In a study by Ahmadinejad et al (11), the authors compared the analgesic efficacy of piroxicam and diclofenac topical gels in patients with breast pain. Both topical medications were found successful in reducing pain after two months of application. However, the piroxicam gel was significantly along with better results. Only one patient was found to have side effects in the diclofenac group.

Regarding the use of vitamin E in mastalgia, however, the results of studies are more conflicting compared to the results of using NSAIDs. In some older studies, for example, the authors did not report clinically significant findings in this regard (15-17).

In contrast and in conformity with our findings, Pruthi et al (18) found that daily doses of 1200 IU vitamin E could effectively decrease the severity of cyclical mastalgia. Likewise, Shobeiri et al (19) showed that vitamin E supplementation, compared to the placebo, yielded significantly better results in pain control in patients with cyclic mastalgia.

The beneficial effect of vitamin E in the management of breast pain has been attributed to its antioxidant property. (9) To the best of the authors' knowledge, this is the first double-blind, randomized clinical trial that compares the pain-reduction efficacy of a topical NSAID and oral vitamin E in patients with mild-to-moderate mastalgia.

In a recent metanalysis, Groen et al (10) concluded that topical NSAIDs can reduce pain by almost 60% in patients with cyclic mastalgia. This is similar to the amount of pain reduction we saw by using topical piroxicam gel after two months of application (Fig. 3).

The rate of spontaneous remission up to 3 years has been found high in patients with cyclic mastalgia (20). However, about 60% of patients with cyclic mastalgia show a relapsing and remitting pattern of pain episodes, and in some patients, the pain recurs 2 years after therapy (7). In the present study, the rate of subjective remission, which needed pain killers was 53% in patients who received topical piroxicam and 38% in patients who were treated by oral vitamin E after 5 months of treatment discontinuation. Therefore, it seems that although both topical piroxicam and oral vitamin E are effective in ameliorating pain in cyclic mastalgia, they are required to be used regularly until the physician makes sure that the pain would not recur after the treatment stopped.

Further studies with larger sample size and comparing results with other convention al methods of treatment are suggested for future studies.

In conclusion, both topical piroxicam gel and oral vitamin E are safe and effective treatments in reducing pain in patients with mild-to-moderate cyclic mastalgia. The topical piroxicam is along with better results than the oral vitamin E, but discontinuation of the treatment may lead to pain recurrence severe enough to require analgesics in almost half

of the patients.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: R.B., T.E.B., Design: R.B., T.E.B., Data Collection or Processing: R.B., T.E.B., Analysis or Interpretation: R.B., T.E.B., Literature Search R.B., T.E.B., Writing: R.B., T.E.B.

References

1. Niazi A, Rahimi VB, Hatami H, Shirazinia R, Esmailzadeh-Dizaji R, Askari N, et al. Effective Medicinal Plants in the Treatment of the Cyclic Mastalgia (Breast Pain): A Review. J Pharmacopuncture. 2019;22(3):131-9. Epub 2019/11/02. doi: 10.3831/KPI.2019.22.017. PubMed PMID: 31673442; PubMed Central PMCID: PMCPMC6820470.

2. Lochner J, Larson M, Torell E, Schrager S. How best to address breast pain in nonbreastfeeding women. J Fam Pract. 2019;68(7):379;82;84;88. Epub 2019/09/19. PubMed PMID: 31532814.

3. Hafiz SP, Barnes NLP, Kirwan CC. Clinical management of idiopathic mastalgia: a systematic review. J Prim Health Care. 2018;10(4):312-23. Epub 2019/05/02. doi: 10.1071/HC18026. PubMed PMID: 31039960.

4. Stachs A, Stubert J, Reimer T, Hartmann S. Benign Breast Disease in Women. Dtsch Arztebl Int. 2019;116(33-34):565-74. Epub 2019/09/27. doi: 10.3238/arztebl.2019.0565. PubMed PMID: 31554551; PubMed Central PMCID: PMCPMC6794703.

5. Ooi SL, Watts S, McClean R, Pak SC. Vitex Agnus-Castus for the Treatment of Cyclic Mastalgia: A Systematic Review and Meta-Analysis. J Womens Health (Larchmt). 2020;29(2):262-78. Epub 2019/08/30. doi: 10.1089/jwh.2019.7770. PubMed PMID: 31464546.

6. Jaafarnejad F, Adibmoghaddam E, Emami SA, Saki A. Compare the effect of flaxseed, evening primrose oil and Vitamin E on duration of periodic breast pain. J Educ Health Promot. 2017;6:85. Epub 2017/11/09. doi: 10.4103/jehp.jehp_83_16. PubMed PMID: 29114553; PubMed Central PMCID: PMCPMC5651668.

7. Goyal A. Breast pain. BMJ Clin Evid. 2011;2011. Epub 2011/04/12. PubMed PMID: 21477394; PubMed Central PMCID: PMCPMC3275318.

8. Scurr J, Hedger W, Morris P, Brown N. The prevalence, severity, and impact of breast pain in the general population. Breast J. 2014;20(5):508-13. Epub 2014/07/22. doi: 10.1111/tbj.12305. PubMed PMID: 25041468.

9. Cornell LF, Sandhu NP, Pruthi S, Mussallem DM. Current Management and Treatment Options for Breast Pain. Mayo Clin Proc. 2020;95(3):574-80. Epub 2020/03/07. doi: 10.1016/j.mayocp.2019.12.014. PubMed PMID: 32138883.

10. Groen JW, Grosfeld S, Wilschut JA, Bramer WM, Ernst MF, Mullender MM. Cyclic and non-cyclic breast-pain: A systematic review on pain reduction, side effects, and quality of life for various treatments. Eur J Obstet Gynecol Reprod Biol. 2017;219:74-93. Epub 2017/10/24. doi: 10.1016/j.ejogrb.2017.10.018. PubMed PMID: 29059585.

11. Ahmadinejad M, Delfan B, Haghdani S, Hashemi M, Khan IA, Tafti MT. Comparing the effect of diclofenac gel and piroxicam gel on mastalgia. Breast J. 2010;16(2):213-4. Epub 2009/12/25. doi: 10.1111/j.1524-4741.2009.00870.x. PubMed PMID: 20030650.

12. Irving AD, Morrison SL. Effectiveness of topical non-steroidal anti-inflammatory drugs in the management of breast pain. J R Coll Surg Edinb. 1998;43(3):158-9. Epub 1998/07/09. PubMed PMID: 9654874.

13. Colak T, Ipek T, Kanik A, Ogetman Z, Aydin S. Efficacy of topical nonsteroidal antiinflammatory drugs in mastalgia treatment. J Am Coll Surg. 2003;196(4):525-30. Epub 2003/04/15. doi: 10.1016/S1072-7515(02)01893-8. PubMed PMID: 12691925.

14. Qureshi S, Sultan N. Topical nonsteroidal anti-inflammatory drugs versus oil of evening primrose in the treatment of mastalgia. Surgeon. 2005;3(1):7-10. Epub 2005/03/26. doi: 10.1016/s1479-666x(05)80003-4. PubMed PMID: 15789786.

15. Ernster VL, Goodson WH, 3rd, Hunt TK, Petrakis NL, Sickles EA, Miike R. Vitamin E and benign breast "disease": a double-blind, randomized clinical trial. Surgery. 1985;97(4):490-4. Epub 1985/04/01. PubMed PMID: 3885456.

16. London RS, Sundaram GS, Murphy L, Manimekalai S, Reynolds M, Goldstein PJ. The effect of vitamin E on mammary dysplasia: a double-blind study. Obstet Gynecol. 1985;65(1):104-6. Epub 1985/01/01. PubMed PMID: 3880876.

17. Meyer EC, Sommers DK, Reitz CJ, Mentis H. Vitamin E and benign breast disease. Surgery. 1990;107(5):549-51. Epub 1990/05/01. PubMed PMID: 2185569.

18. Pruthi S, Wahner-Roedler DL, Torkelson CJ, Cha SS, Thicke LS, Hazelton JH, et al. Vitamin E and evening primrose oil for management of cyclical mastalgia: a randomized pilot study. Altern Med Rev. 2010;15(1):59-67. Epub 2010/04/03. PubMed PMID: 20359269.

19. Shobeiri F, Oshvandi K, Nazari M. Clinical effectiveness of vitamin E and vitamin B6 for improving pain severity in cyclic mastalgia. Iran J Nurs Midwifery Res. 2015;20(6):723-7. Epub 2016/01/23. doi: 10.4103/1735-9066.170003. PubMed PMID: 26793260; PubMed Central PMCID: PMCPMC4700694.

20. Talimi-Schnabel J, Fink D. Praxis (Bern 1994). 2017;106(20):1101-6. Epub 2017/10/05. doi: 10.1024/1661-8157/a002795. PubMed PMID: 28976254.

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Understanding physicians' moral distress in the Covid 19 pandemic

Selin KESKİN KIZILTEPE [1,*], **Aslı KURTGÖZ** [2]

[1]Department of Nursing, Faculty of Health Science, Düzce University, Düzce, Türkiye
[2]Department of Therapy and Rehabilitation, Sabuncuoglu Serefeddin Health Services Vocational School, Amasya University, Amasya, Türkiye

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Abstract

Moral distress is a significant problem for health care professionals, resulting in reduced job satisfaction, burnout and job retirement. This study aims to explore the experiences of emergency physicians related to moral distress during the Covid-19 pandemic. We conducted in-depth semi-structured interviews of 25 physicians working in the emergency department to describe their experiences related to moral distress. Data were analyzed using thematic analysis through the six-step process. Three major themes were identified: (I) The impact of the Covid-19 pandemic on moral distress; (II) The effect of moral distress on physicians; (III) Suggestions to prevent/reduce moral distress. In light of the results of our study, the moral distress experienced by physicians has been exacerbated due to the increase in the factors causing moral distress during the pandemic period.

Keywords: Covid-19, moral distress, pandemic, physicians

1. Introduction

The novel infectious disease Covid-19 was first seen in China in December 2019. The virus spread rapidly around the world and affected many people. In this process, the large numbers of infected patients loaded into the emergency services increased, and healthcare professionals, especially those working in the emergency services, have struggled on the front lines. The Covid-19 disease has led to new challenges for physicians, nurses, respiratory therapists, social workers, patients, and their families (1-3). They faced many unprecedented levels of death, changes to care delivery, and provided community-centred care rather than patient-centred care. Besides these situations, they are obliged to provide infection prevention practices and social distancing precautions (2). Therefore, they limited face-to-face interaction with the patient and their families; they had to use protective equipment, limiting them from interacting with the patient (1, 2, 4). With this, they experienced excessive workload, difficulties in accessing high-quality personal protective equipment (5), death anxiety (death of family, colleagues, patients) (1), witnessed patients dying in isolation away from loved ones (6), worried about not being able to do enough for Covid-19 patients (7). Many health care professionals fear being contaminated with the coronavirus in the ED, infecting and losing their patients, families, and colleagues (9), and the anxiety of something terrible happening (8). Most of the time, they could not offer quality care to their patients and felt a conflict between fear and conscience (1). These situations brought ethical problems to clinical settings. This process inevitably contributed to increased moral distress in the emergency department. Moral distress includes a range of moral events and is related to various ethics-related distress experiences of healthcare professionals (10,11), particularly among physicians who had to force choices that conflicted with their values and ethical values (12-15). Some physicians had to make difficult triage decisions, life and death decisions and prioritize which patients got life-saving equipment on account of limited resources; they may have to decide to stop the support of some to save others, such as ventilators, dialysis, and hospital beds, etc. (16-18). In addition, they were forced to do unethical behaviour they did not want to do due to the pressure from the patients' families or social pressure. More easily withhold or withdraw from their job and/or coworkers, inappropriate treatments, and rapidly changing guidelines-protocols regarding Covid-19 are added to the table and cause exacerbated moral distress among physicians (4, 16, 19). During the pandemic, physicians reported high anxiety levels (20) and moral distress (15). They were highly prone to exposure to potentially moral injury events related to physical and emotional impact (21). In a study on healthcare professionals during this period, moral distress is related to family, fear of infecting others, and work-related concerns (7). Moral distress is a multidimensional phenomenon that can characterize a variety of negative physical and

*Correspondence: selinkeskin@duzce.edu.tr

psychological effects on healthcare professionals (4,9). According to studies carried out during the pandemic, there are numerous negative emotions associated with moral events such as burnout, compassion fatigue, frustration, guilt, weakness, despair, regret, anger, grief, anxiety, helplessness, shame, embarrassment, loss, sadness, fear, sleep difficulties, post-traumatic stress disorder symptoms and anguish (7, 22, 23). Moral distress also causes job-patient dissatisfaction, decreasing care quality, and even wanting to leave their profession and position (22, 24). Starting with this point, we aimed to explore the experiences of emergency physicians related to moral distress during the Covid-19 pandemic.

2. Material and Methods

2.1. Aim

This study aims to explore the experiences of emergency physicians related to moral distress during the Covid-19 pandemic.

2.2. Study Design

This exploratory qualitative study explicitly employed the moral distress that emergency physicians experienced during the Covid-19 pandemic.

2.3. Data collection and sample

This study was carried out in a research and training hospital's emergency department from August to December 2021. In this study, emergency physicians were selected based on the purposive sampling method. The data was collected through 25 semi-structured in-depth interviews by second author. The inclusion criteria were as follows: (1) Being an emergency physician; (2) Having worked for at least one year in the emergency department in the Covid-19 pandemic process; (3) Volunteering to participate in this study. The socio-demographic information of the 25 physicians is shown in Table 1.

Table 1. Socio-demographic characteristics of the participants

Participants	Age	Gender	Workıng times as a physıcıan	Working times in the emergency department
1. Participant	28	Male	23 month	23 month
2. Participant	27	Male	1 year	1 year
3. Participant	27	Female	20 month	20 month
4. Participant	35	Male	8 year	3 year
5. Participant	25	Female	1 year	1 year
6. Participant	36	Male	10 year	10 year
7. Participant	27	Male	2 year	2 year
8. Participant	37	Male	13 year	12 year
9. Participant	29	Male	16 month	16 month
10. Participant	32	Male	10 year	10 year
11. Participant	28	Female	3 year	3 year
12. Participant	29	Male	1,5 year	1,5 year
13. Participant	25	Female	1 year	1 year
14. Participant	25	Male	1 year	1 year
15. Participant	29	Female	3,5 year	1 year
16. Participant	36	Male	8 year	7 year
17. Participant	27	Female	3,5 year	3,5 year
18. Participant	28	Male	23 month	15 month
19. Participant	30	Female	7 year	4 year
20. Participant	28	Male	4 year	4 year
21. Participant	25	Male	1 year	1 year
22. Participant	29	Female	4 year	4 year
23. Participant	36	Female	10 year	6 year
24. Participant	38	Male	9 year	6 year
25. Participant	32	Male	4 year	3 year

2.4. Ethical approval

Before starting the study, written permission was obtained from the hospital, Ministry of Health and Ethics Committee (E-15386878-044-24640). All participants were informed about the purpose of the study, and audio recordings would be taken during the interviews. They were also informed that their names would not be used in the voice recordings of the interviews and that the data collected would be used only for this study.

2.5. Data collection

Semi-structured in-depth interviews took approximately 20–35 minutes. The interviews were conducted by the second author in a private room outside the emergency services. At

the beginning of each interview, the purpose of the study was described, and a code name was assigned by the interviewer to each participant. The interviews were conducted following open-ended questions. The interview questions were:" Share with me your experiences related to moral distress during the Covid-19 pandemic", "How did you feel when you experienced moral distress", and "What do you think were the factors that caused you to have this experience? "and "What are your suggestions to reduce/prevent moral distress?". The interview continued according to the answers provided by the physicians, more in-depth questions such as "what do you mean?", and "please detail and/or explain more about this issue' ' were asked.

2.6. Data analysis

Data were analyzed using thematic analysis according to Braun and Clark's six-step process (25). All interviews were recorded and transcribed, and the transcripts were checked according to the original sound to familiarise with the data. Then we carefully read the transcripts several times to build and examine them line by line in detail and assign paragraphs or segments of the text. Descriptive codes were found for meaningful statements. The same meanings unit were grouped and named. Then, major themes and sub-themes were formed, reviewed, and discussed by all researchers. Both of them were revised by comparing with the participants' statements. Then, final changes were conducted on the themes and subthemes.

2.7. Rigour

Rigour was established by following the four criteria defined by Lincoln and Guba (1985); credibility, dependability, transferability, and confirmability (26). All coding was done by the second author to ensure consistency. Interview transcriptions were analyzed independently, and all authors reviewed identified themes several times to provide credibility. Confirmability and transferability were ensured by the original interview recordings and reflection notes taken by the second author during the data collection process.

3. Results

Of the 25 participants, nine were women, and sixteen were men. Their ages ranged from 25 to 38 (mean 29.92, SD 4.13). The length of experience in physicians ranged from one to thirteen years (mean 12.6, SD 5.9), and the length of experience in the emergency department ranged from 1–12 years (mean 5.3, SD 3.3) (Table 1). While 80% (n=20) of the participants thought about quitting their job, 20% (n=5) did not. Thematic analysis revealed the following three major themes; (I) The impact of the Covid-19 pandemic on moral distress; (II) The effect of moral distress on physicians; (III) Suggestions to prevent/reduce moral distress.

3.1. Theme 1: The impact of the Covid-19 pandemic on moral distress

In the direction of the participant statements, the effects of the Covid-19 pandemic on moral distress were grouped as "inappropriate working conditions", "inappropriate management of pandemic process", and "situations/problems stemming from society and management".

Sub-Theme 1: Inappropriate working conditions

The participants stated that an inappropriate working environment prepared the ground for moral distress during the pandemic period due to long/variable working hours, restrictions on personal rights (inability to use leave, resignation, etc.), insufficient number of health personnel, and inadequate physical conditions (emergency service capacity, lack of medical equipment). Regarding this theme, participant statements are as follows:

"We are undergoing a burnout due to unwilling people to obey the rules taken to protect public health and inappropriate working conditions. In addition, there were restrictions on many personnel rights of healthcare workers and an increase in working tempo density due to the pandemic. These situations prepare a ground for developing morally distressing events (Participant 6).

"I was desperate because of the restrictions such as not being able to take leave or resign" (Participant 18).

"Sometimes I feel I cannot do my job properly due to the high patient density, inappropriate physical conditions, and the prolonged pandemic process that makes us fatigued (Participant 23).

Sub-Theme 2: Failure to properly manage the pandemic process

The participants stated that some deteriorations occurred in the functioning of healthcare services due to a lack of proper management of the pandemic process. They especially stated that transferring the patients to the emergency for Covid-19 diagnostic tests increased the work burden in emergencies. In addition, they stated that situations such as lack of planning, admission of patients to emergency services without emergency indications, and constantly changing and uncertain protocols regarding the Covid-19 process (diagnosis, treatment, prevention, vaccine, etc.) affected the management of the pandemic process. The participant statements regarding this theme are as follows:

There is an additional provision in the universal declaration of physician's rights saying that "the time a physician allocates for one patient is a minimum of 15 minutes which administrative measures cannot shorten". It is impossible not to experience moral problems unless we meet this standard. Specifically for the Covid-19 pandemic, I believe that the increase in patient numbers and the already insufficient number of staff together with lack of planning negatively affect the functioning of healthcare services inevitably" (Participant 17).

"During the first months, there was only one physician in a very busy patient circulation, without a defined rest time in

24 hours, dealing with anamnesis, filling out forms, giving information to more than 100 patients, sampling for PCR, requesting tests, observing, evaluating the results and consultations in which condition the brain and body are under a great burden. As such, we do not even have time to inform patients. Although things work this way all over the world, it is not in line with professionalism in health." (Participant 12).

"From a Covid-19 aspect, I agree that those with serious complaints such as shortness of breath apply to the emergency services; however, I sadly saw that the entire burden was again on the emergencies as always and this responsibility was not shared with our colleagues in both family medicine and other institutions and organizations. Covid diagnostic tests for people with symptoms started to be performed only in emergencies, and patients from many other outpatient clinics were directed to the emergencies for a test without even being examined. We had to examine all patients referred to the emergency with personal protective equipment to determine the emergency. This increased the workload of emergency physicians (Participant 22).

Sub-Theme 3: Situations originating from society and management

The participants expressed the social and management-related situations that cause moral distress during the pandemic process as follows; the pressure of the management, inappropriate demands of the management, unfair treatment, not being respected and valued, not being appreciated, lack of support from the management to the healthcare professionals, the pressure and violence on healthcare professionals by the society, lack of timely and correct information by the management to the society, laying at all of the burdens to the healthcare professionals, and unconscious behaviours of the society.

"Situations such as the burden on our shoulders in business life, excessive responsibility, not being valued, appreciated, and ignorance of material and moral rights cause moral distress-related problems. In addition, these situations were not only present during the pandemic period, but they existed earlier but before, but they got denser during this process" (Participant 6)

Even people who do not need urgent intervention are intervened due to the demands of the managers. For example, we send an ambulance, or when an intensive care bed is evacuated, another patient is admitted to the intensive care unit, not the one who needs it most." (Participant 25)

"Even if we accept that the pandemic process is a state of emergency where healthcare professionals are given the actual responsibility, we could not see the necessary respect and support. It was a great responsibility for us where all the workload of the pandemic was tried to be compensated with our self-dedication. This was made a necessity and shown to the public as a normal situation. This greatly increased the times we felt stuck between our self-dedication while doing our job and our burnout." (Participant 5)

"The pressure and violence coming from the society on healthcare professionals negatively affects our performance." (Participant 2)

"I think that the managers and the media did not share some data (such as vaccines, number of cases) transparently with the society and healthcare professionals during the pandemic. I think that all information about the pandemic period should be shared on the website of the Ministry of Health, news sources, and public spots through a transparent system. Or else, information pollution occurs in social media about this process in the society, and people do not want to be vaccinated, they are worried..." (Participant 10)

3.2. Theme 2: The effect of moral distress on physicians

In line with the statements of the participants, it was determined that the moral problems experienced during the pandemic period affected the physicians in terms of exhausting (physical, emotional, spiritual, psychological), fatigue, not enjoying life, feeling worthless, desire to leave the profession (resigning), feelings of regret and guilt, sadness, burnout, decrease in job satisfaction, distrust against management, feeling of dilemmas, ignorance, questioning the profession and one's self.

It affected us in every way. It wore off and took away our joy of life" (Participant 8).

"Theoretical and practical knowledge and experience we have about the emergency created a routine expectation from us. Due to the new process during the pandemic period, the unreliable and slippery ground caused us to be stuck between a constantly changing work schedule, treatment methods, and new procedures instead of this routine. In addition to the intense working conditions and the loss of our rights, the pandemic period caused physical and psychological fatigue for many of us." (Participant 5).

"I cannot enjoy life. There is a serious state of fatigue and exhaustion" (Participant 7).

"Despite not being physically affected, it made me feel worthless for myself and my profession emotionally and spiritually" (Participant 19).

"In all aspects, it was an exhausting process that I cannot count how many times I thought about resigning. Even when our physician friends were infected, we tried not to disrupt the hospital by working overtime due to our insufficient number of employees. To be able to take a 1-week annual leave, we had to work at the hospital every day or every other

day before or after they leave. I remember the times when I was working in the emergency as the only doctor and at the same time went to collect samples from the accumulating patients for the covid test every hour. While I was taking samples for the test, some patients needed urgent intervention in the emergency. Why was a test that had nothing to do with the emergency response, such as the Covid test, given to the emergency doctors? After a while, this process wears people out so much that they start to give up on everything" (Participant 22).

"The situation of not being treated equally due to the conditions causes guilt and sadness in me." (Participant 14).

3.3. Theme 3: Suggestions to prevent/reduce moral distress

The recommendations to eliminate/reduce moral distress in line with the statements of the participants are grouped as "regulation of working conditions at emergencies", "appropriate behaviour of managers", and "public awareness".

Sub-Theme 1: Regulation of emergency service working conditions

Many participants expressed their ideas about eliminating/reducing moral distress, which was associated with working conditions in emergency services. The participants made some suggestions such as preventing the misuse of emergency services, imposing penal sanctions, reducing patient density and working hours, increasing the number of personnel, having a stable working schedule, standardizing practices, and protecting the personal rights of healthcare professionals.

"Working conditions of emergencies must be improved, and the number of patients must be reduced" (Participant 1).

"It must be possible to carry out urgent applications without going beyond the standards, nor considering special requests" (Participant 4).

"Compliance with generally accepted preventive and treatment methods will reduce our dilemma" (Participant 19).

"First of all, the working conditions of the employees must be improved." (Participant 5).

"There must be sanctions against the unnecessary use of emergency services by individuals in the community" (Participant 17).

"The number of healthcare professionals must be increased to alleviate the intense working conditions" (Participant 16).

"Hospitals and emergencies must be used in line with the intended purpose. Citizens who do not have an emergency, in other words, a "life-threatening" complaint, can be examined by their family doctors, and a covid test can be done there. This process (meaning the pandemic) will not end; the burden on the emergencies must be reduced; otherwise, resignations will be unpreventable and experienced doctors will not be available in the emergency services. I say all these sincerely as a physician who loves this profession and is on the brink of resigning within 4 years" (Participant 22).

"The number of personnel must be increased, working hours and conditions must be reduced to a humane level. There must be an environment where we can do our job without any pressure" (Participant 24).

"There must be stability in our work schedule. Our resting periods such as holidays and vacations must be respected. Thus, our burnout will be reduced" (Participant 6).

Sub-Theme 2: Managers exhibit appropriate behaviour

Some offers of the participants to eliminate/reduce moral distress were discussed under the sub-theme of "an exhibition of appropriate behaviours by the managers". About this issue, the participants stated that there must be managers who treat everyone fairly, focus on solving the problems, defend the rights of employees, and do not have a punitive understanding.

"Managers must do their job properly and be fair" (Participant 4).

"I use all my means to solve these problems, but this is not enough. This situation will be solved through the improvement of conditions by the authorities, not by individual means" (Participant 8).

"The management approach must be far from populism, human-oriented, and protect and watch over the employee instead of seeking for faults" (Participant 25).

Sub-Theme 3: Raising awareness of the society

The participants stated that moral distress could be eliminated/reduced by raising awareness in the society about the use of emergency services, appropriate attitudes and behaviours towards healthcare professionals (verbal and physical violence, rude orders), observing the necessities of the pandemic period, and increasing the education level. The statements of the participants on this subject are as follows:

"In my opinion, raising awareness about the necessities of the pandemic and bringing more appropriate sanctions to integrate these necessities into daily life will reduce our conflict with the public" (Participant 6).

"The education level of the society must be increased" (Participant 7).

4. Discussion

The emergency department is a crowded, uncontrolled-unpredictable, stressful environment in which physicians make critical decisions under pressure in a short time. They are faced with unethical challenges and are exposed to moral distress, which can have negative outcomes. The present study was conducted on physicians' experiences with moral distress during the pandemic. In our study, the factors that may cause the formation of moral distress were associated with inappropriate working conditions, failure to manage the pandemic process properly, and the situations originating from society and management. It has been stated that many factors such as directing all cases to the emergency service due to lack of planning during the Covid-19 pandemic, demolition of all applications related to the process to emergency services, the uncertainties experienced in this process, and changing protocols cause problems in the functioning of the health service. In addition, the inappropriate demands of the managers, the unequal distribution of resources to the patients, the pressure exerted on the health workers by the society, the fact that the society was not informed correctly in the process, and the unconscious behaviour of the society also caused the moral distress to be exacerbated. Compared to other studies conducted on pandemic period; working long hours and excessive workload, lack of personal protection equipment (PPE), difficulties access to clean (PPE) (4), lack of treatment guidelines, lack of control, unavailability of Covid-19 testing capabilities, uncertain most of the patient deaths, clinical status (27), worrying spreading to their patient or family, lack of training to allocate scarce resources (5), social or family pressure, poor communication with colleagues and patients (16), etc. were found to be associated with increased stress. These constraints make physicians' clinical or triage decisions difficult, likewise who will die/ who will live (16,17). All this originated the stage for developing events that cause moral distress (22).

In this study, physicians experienced anxiety, distress, fatigue, regret, guilt, sadness, burnout, decreased desire to work, and request to resign due to moral distress in this period. According to the quantitative results, most physicians considered quitting due to moral distress. In addition, it has also been revealed that they experienced distrust towards managers, feeling in between, neglect, and questioning their profession and themselves. These effects have been associated with moral distress in many studies (11, 19, 24). At the peak of the first wave, cognitive, emotional, and physical stress symptoms almost doubled among healthcare professionals (7,9,20). Several recent studies have shown that during the pandemic, healthcare professionals had a higher level of stress and post-traumatic stress disorder, fear of being infected and infecting their families, emotional-mental exhaustion, burnout, depressive symptoms, sleep disturbances, psychological impact on professionals working in emergency departments (28-30). These symptoms may be related to the timing of studies. For example, one study found that physicians have high levels of injurious moral events and are prone to it during the pandemic (21). After the pandemic, moral distress levels were generally low among healthcare professionals (15). Our study was conducted from August to December 2020, during which time the epidemic in Turkey had been controlled. Therefore, our findings -effects of moral distress on emergency physicians- are less severe than other studies. In interviews, physicians reported that they devised individual solutions for situations that caused moral distress without the support of managers. Considering the factors that will reduce or prevent the development of moral distress in our study, it is expected that the working conditions of the emergency service will be improved and that the managers should be fair, solution-oriented, and supportive of health professionals. It has come to the fore that it is very important to raise society's awareness in this direction (not using the emergency services unnecessarily, their behaviour towards healthcare workers, etc.), especially during such crisis periods. Some studies have suggested that to resolve moral distress, healthcare professionals mostly tried to cope by taking support from their colleagues, friends, and families (31). To diminish moral distress, managers should identify the factors that create stress in the workplace, implement support programs to alleviate the stress experienced and allow healthcare professionals to express themselves more comfortably (31, 32). However, in this process, they had limited contact with their family and friends due to isolation, and most of them reported engaging in stress reduction activities, such as physical activity/exercise, talk therapy, virtual support groups, and religious/ spiritual practices (3, 27). Looking at the studies related to coping with moral distress; all levels of government, hospital management, and the community promote readiness to protect healthcare workers in the Covid-19 pandemic, adopting more guidelines for defining appropriate care, end-of-life applications, appropriate end-of-life treatments, end of life decision making, work to decrease the role of hierarchy (16, 27).

The study has some limitations. The study was conducted in one hospital's emergency department with small groups. And the participants had different cultural values and work experience. Therefore, the findings of this study cannot be generalized the other research results.

According to findings, working during the Covid-19 pandemic, participants reported negative impacts on them. The moral distress experienced by physicians has been exacerbated due to the increase in the factors also leading to cause moral distress during the pandemic period. Suggestions to reduce/prevent moral distress included regulating a supportive work environment, the need for qualified managers, and raising public awareness about this issue.

Conflict of interest
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Authors' contributions
Concept: S.K.K., A.K., Design: S.K.K., A.K., Data Collection or Processing: S.K.K., A.K., Analysis or Interpretation: S.K.K., A.K., Literature Search: S.K.K., A.K., Writing: S.K.K., A.K.

References

1. Galehdar N, Kamran A, Toulabi T, Heydari H. (2020). Exploring nurses' experiences of psychological distress during care of patients with COVID-19: A qualitative study. BMC psychiatry 2020; 20(1):1 9.

2. Morley G, Sese D, Rajendram P, Horsburgh CC. Addressing caregiver moral distress during the COVID-19 pandemic. *Cleveland Clinic journal of medicine* 2020;2(6):1-5.

3. Patterson JE, Edwards TM, Griffith JL, Wright S. Moral distress of medical family therapists and their physician colleagues during the transition to COVID-19. *Journal of Marital and Family Therapy* 2021; *47*(2): 289-303.

4. Ness MM, Saylor J, DiFusco L A, Evans K. Leadership, professional quality of life and moral distress during COVID-19: A mixed-methods approach. *Journal of Nursing Management* 2021;*29*(8): 2412-2422.

5. O'Neal L, Heisler M, Mishori R, Haar RJ. Protecting providers and patients: results of an Internet survey of health care workers' risk perceptions and ethical concerns during the COVID-19 pandemic. *International journal of emergency medicine* 2021;*14*(1):1-11.

6. Williams RD, Brundage JA, Williams EB. Moral injury in times of COVID-19. *Journal of health service psychology* 2020; *46*(2): 65-69.

7. Norman SB, Feingold JH, Kaye-Kauderer H, Kaplan CA, Hurtado A, Kachadourian L, ... Pietrzak RH. Moral distress in frontline healthcare workers in the initial epicenter of the COVID-19 pandemic in the United States: Relationship to PTSD symptoms, burnout, and psychosocial functioning. *Depression and anxiety* 2021;*38*(10):1007-1017.

8. Hancock J, Witter T, Comber S, Daley P, Thompson K, Candow S, ... Kits O. Understanding burnout and moral distress to build resilience: A qualitative study of an interprofessional intensive care unit team. *Canadian Journal of Anesthesia/Journal canadien d'anesthésie* 2020; *67*(11):1541-1548.

9. Hesselink G, Straten L, Gallée L, Brants A, Holkenborg J, Barten DG, Schoon Y. Holding the frontline: a cross-sectional survey of emergency department staff well-being and psychological distress in the course of the COVID-19 outbreak. *BMC health services research* 2021; *21*(1): 1-10

10. Hines SE, Chin KH, Glick DR, Wickwire EM. Trends in moral injury, distress, and resilience factors among healthcare workers at the beginning of the COVID-19 pandemic. *International Journal of Environmental Research and Public Health* 2021; *18*(2):488.

11. Morley G, Bradbury-Jones, C, Ives J. What is 'moral distress' in nursing? A feminist empirical bioethics study. *Nursing ethics* 2020; *27*(5): 1297-1314.

12. Borges LM, Barnes SM, Farnsworth JK, Bahraini NH, Brenner LA. A commentary on moral injury among health care providers during the COVID-19 pandemic. *Psychological Trauma: Theory, Research, Practice, and Policy* 2020; *12*(S1):S138.

13. Haller M, Norman SB, Davis BC, Capone C, Browne K, Allard CB. A model for treating COVID-19–related guilt, shame, and moral injury. *Psychological Trauma: Theory, Research, Practice, and Policy* 2020;*12*(S1):S174.

14. Maguen S, Price MA. Moral injury in the wake of coronavirus: Attending to the psychological impact of the pandemic.*Psychological Trauma: Theory, Research, Practice, and Policy* 2020; *12*(S1), S131.

15. Miljeteig I, Forthun I, Hufthammer KO, Engelund IE, Schanche E, Schaufel M, Onarheim KH. Priority-setting dilemmas, moral distress and support experienced by nurses and physicians in the early phase of the COVID-19 pandemic in Norway. *Nursing ethics* 2021; *28*(1):66-81.

16. Rosenwohl-Mack S, Dohan D, Matthews T, Batten JN, Dzeng E. Understanding experiences of moral distress in end-of-life care among US and UK physician trainees: A comparative qualitative study. *Journal of general internal medicine* 2021;*36*(7): 1890-1897.

17. Sprung CL, Joynt GM, Christian MD, Truog RD, Rello J, Nates JL. Adult ICU triage during the coronavirus disease 2019 pandemic: who will live and who will die? Recommendations to improve survival. *Critical care medicine* 2020; *48*(8): 1196.

18. Truog RD, Mitchell C, Daley GQ. The toughest triage—allocating ventilators in a pandemic. *New England Journal of Medicine* 2020; *382*(21):1973-1975.

19. Kherbache A, Mertens E, Denier Y. Moral distress in medicine: An ethical analysis. *Journal of Health Psychology* 2021; 13591053211014586.

20. Mosheva M, Hertz-Palmor N, Dorman Ilan S, Matalon N, Pessach IM et al. nxiety, pandemic-related stress and resilience among physicians during the COVID-19 pandemic. *Depression and anxiety* 2020; *37*(10): 965-971.

21. Maftei A, Holman AC. The prevalence of exposure to potentially morally injurious events among physicians during the COVID-19 pandemic. *European Journal of Psychotraumatology* 2021;*12*(1):1898791.

22. Morley G, Bradbury-Jones C, Ives J. The moral distress model: An empirically informed guide for moral distress interventions.*Journal of Clinical Nursing* 2021; 00:1–18.

23. Wang Z, Harold KG, Tong Y, Wen J, Sui M, Liu H, ... Liu G. Moral injury in Chinese health professionals during the COVID-19 pandemic. *Psychological Trauma: Theory, Research, Practice, and Policy* 2021.

24. Laurs L, Blaževičienė A, Capezuti E, Milonas D. Moral distress and intention to leave the profession: Lithuanian nurses in municipal hospitals. *Journal of Nursing Scholarship* 2020; *52*(2):201-209.

25. Braun V, Clarke V. Using thematic analysis in psychology. *Qual Res Psychol* 2006; 3(2): 77–101.

26. Lincoln Y, Guba EG. Naturalistic Inquiry. California, CA: Sage Publication; 1985.

27. Shechter A, Diaz F, Moise N, Anstey DE, Ye S, Agarwal S, ... & Abdalla M. Psychological distress, coping behaviors, and preferences for support among New York healthcare workers during the COVID-19 pandemic. *General hospital psychiatry* 2020; *66*: 1-8.

28. An Y, Yang Y, Wang A, Li Y, Zhang Q, Cheung T, ... Xiang YT. Prevalence of depression and its impact on quality of life among frontline nurses in emergency departments during the COVID-19 outbreak. *Journal of affective disorders* 2020; *276*, :312-315.

29. Song X, Fu W, Liu X, Luo Z, Wang R et al.Mental health status of medical staff in emergency departments during the Coronavirus disease 2019 epidemic in China. *Brain, behavior, and immunity* 2020; *88*, 60-65.

30. Rodriguez RM, Montoy JCC, Hoth KF, Talan DA, Harland KK et al. Symptoms of anxiety, burnout, and PTSD and the mitigation effect of serologic testing in emergency department personnel during the COVID-19 pandemic. *Annals of emergency medicine* 2021;78(1):35-43.

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Examining the prevalence of allergic diseases in hairdressers in the Black Sea region

Nida KIYICI[1,*], Şefika İlknur KÖKÇÜ KARADAĞ[2], Gonca HANCIOĞLU[2], Recep SANCAK[2]

[1]Department of Nursing Faculty of Healty Science, Ondokuz Mayıs University, Samsun, Türkiye

[2]Department of Pediatrics, Division of Pediatric Allergy and Immunology, Ondokuz Mayıs Univesity, Samsun, Türkiye

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Abstract

In the present study, the prevalence of allergic diseases among occupational diseases in hairdressers was examined in Samsun Province, which is located in the Black Sea Region. The purpose was to determine whether hairdresser employees use adequate protective equipment, and to increase the training levels of hairdressers in this respect. The Study Group consisted of hairdressers working in the Black Sea Region. A total of 700 hairdressers were included in the study. The data were prepared to determine working conditions, occupational and environmental allergic diseases. The ISAAC Questionnaire Form was modified according to our Study Group. The data obtained were analyzed in computer medium by using the IBM SPSS V23 Package Program. The Mann Whitney U and McNemar Tests were used in the evaluation of the data. It was determined that 310 of the hairdressers who participated in the study worked in female hairdressers, and 390 worked in male hairdressers. It was determined that 97.4% of the female hairdressers who worked in female hairdressers and who participated in the study used protective equipment, 33.5% of them had respiratory complaints before professional life, 9.4% had itching on the skin; 54.5% of them had respiratory complaints after their professional life, and 28.4% had skin itching. A total of 44.2% had an allergic reaction to hair dye, and 42.9% to decolorizer. A total of 25.5% of had obstruction, and 24.2% had cough as allergic reactions. It was determined that 31.6% of those who did not have respiratory complaints before professional life had respiratory complaints after their professional lives. In male hairdressers who participated in the study, it was determined that 63.8% of them used protective equipment, 11.3% had respiratory complaints before professional life, and 31.0% had respiratory complaints after professional life. It was also determined that 30.0% had allergic reactions to hair spray, and 26.2% had allergic reaction to perfume. It was determined that 23.3% had allergic reactions as sneezing and 20.5% obstruction. In the present study, the relation between the allergic diseases of hairdressers and their professions was examined. It was determined that the hairdressing profession increases allergic diseases.

Keywords: asthma, allergic diseases, hairdressers, occupational disease

1. Introduction

Those who work in hairdresser salons face the negative effects of various chemicals and mechanical practices at work periodically. The products used is these salons (hair dye, hair spray, permanent oils, decolorization agents, shampoo, etc.), and workplace conditions (dust, smoke, steam, cigarette smoke, etc.) cause occupational diseases especially for inexperienced employees with both allergen and irritable effects (1, 2). It was reported in previous studies that the most common disease faced because of occupational hazards employees are exposed to in hairdressing profession is occupational asthma. The UK Health and Safety Agency reported in 2005 that hairdressers had the highest frequency of occupational skin disease (3). It was stated by professionals in this field that very few of the hairdresser employees had asthma before starting the profession; however, 40% were receiving asthma and eczema treatment at the time of the study (4). Esin et al. (5) reported that young people working in hairdressers between the ages of 15-21 in our country had more skin and respiratory system complaints than other occupational employees of similar age.

In planning healthcare for employees, it is necessary that the existing problems of the employees are determined first. It is possible to argue that that studies on health problems and related factors are inadequate in terms of occupational diseases especially in risky professions. For this reason, this study was planned to determine the risks and factors that affect occupational asthma in people in hairdressing profession.

2. Material and Methods

This study aimed at examining the prevalence of allergic diseases of male and female hairdressers in the Black Sea Region. The study was conducted between 20.02.2016 and 20.05.2016 with the participation of the hairdressers who were willing and voluntary to participate in the study. The ISAAC Questionnaire Form was modified according to our Study Group. The Questionnaire Form was pre-applied in a 10-person hairdresser salon group, determining and correcting the questions that were incomprehensible or missing. After the pilot study, the final form was applied to the Study Group. The data were collected with face-to-face interview with the hairdressers by the researcher. The hairdressers who

*Correspondence: drilknurkokcu@gmail.com

participated in the study were informed about the study, their informed consents were obtained, and questions were asked about the introductory information form and professional diseases.

According to previous questionnaire studies, power analysis was made, and the sample size calculated for 14.6% allergy difference, 80% test power, 95% Confidence Interval was determined to be 680 people. The Kolmogorov-Smirnov, Shapiro Wilk, Mann Whitney U, Chi-Square, and McNemar Test were used in the analyses of the data. The reliability of the data was examined with the Cronbach Alpha Coefficient. The significance level was taken as $p<0.05$. The study was started after the approval of the Ethics Committee of the Faculty of Medicine at the University of Ondokuz Mayıs (19.11.2015/issue; B.30.2.ODM.020.08/2137). Informed consents of the people who were included in the study were also obtained.

The questions that were modified from the ISAAC Test given in Table 1 were asked to the patients.

Table 1. The ISAAC Questionnaire Form was modified according to our Study Group.

1. How many years have you been a hairdresser?
a) 1-5 b) 6-10 c) 11-15 d) 16-20 e) more than 20 years

2. Do you find the ventilation in your workplace sufficient?
a) Yes b) No

3. Do you use protective equipment while working?
a) Yes b) No

4. Which equipment do you use while working? (you may choose more than one)
a) Gloves b) Mask c) Glasses d) Apron e) Other:

5. Did you have any complaints about breathing before your professional life?
a) Yes b)No

6. Which respiratory complaints did you have?
a) Cough b)Shortness of breath
c) Wheezing d) Chest pain

7. Did you have any complaints about breathing after your professional life?
a) Yes b)No

8. Which complaints did you have after you started hairdresser profession?
a) Cough b)Shortness of breath
c) Wheezing d) Chest pain

9. Were you diagnosed with **allergic bronchitis or asthma** by your doctor?
a) Yes b) N o

10. Did you have nasal flow, nasal obstruction, sneezing, itchiness in the nose, postnasal drip complaints before you started your professional life?
a) Yes b) No

11. Did you have nasal flow, nasal obstruction, sneezing, itchiness in the nose, postnasal drip complaints after you started your professional life?
a) Yes b) No

12. Were you diagnosed with **allergic rhinitis or allergic seasonal rhinitis** by your doctor?
a) Yes b) No

13. Did you have redness, watering, burning sense in your eyes before you started job as a hairdresser?
a) Yes b) No

14. Did you have redness, watering, burning sense in your eyes after you started job as a hairdresser?
a) Yes b) No

15. Were you diagnosed with **allergic** conjunctivitis **or eye flu** by your doctor?
a) Yes b) No

16. Did you have **itching, rash, redness** in your skin before you started your professional life?
a) Yes b) No

17. Did you have **itching, rash, redness** in your skin after you started your professional life?
a) Yes b) No

18. Were you diagnosed with **eczema (itchy rashes)** by your doctor?
a) Yes b) No

19. Where did you have **eczema (itchy rashes)** in your body?
The place of the eczema:

20. Do you have an allergic medication you use constantly?
a) Yes b) No

21. Does your parents have asthma, allergic rhinitis, eczema, allergic conjunctivitis? Please specify?

3. Results

The questionnaires were applied to 700 hairdressers in the scope of the study. A total of 233 of the hairdressers who participated in the study were women, and 467 were men. There were a total of 390 male hairdressers, and had a mean age of 34.2±8.7. The number of the female hairdressers was 310, and 75.2% of them were female (n:233), and 24.8% (n:77) were male. The mean age of the groups was 30.8±8.4.

A total of 82.9% of the female hairdressers found the ventilation of their workplace as sufficient, and 97.4% used protective equipment. A total of 92.3% of the hairdressers who used protective equipment used gloves, 38.7% used masks, 5.2% used glasses, and 71.9% worked with aprons. A total of 84.1% of the male hairdressers found workplace ventilation sufficient, and 63.8% used protective equipment. A total of 57.9% of the hairdressers who used protective equipment used aprons, 25.6% used gloves, 1% used masks, and none of them used protective glasses. It was determined that those who used protective equipment had lower asthma risks.

According to Table 2, 33.5% of the participants had complaints about breathing, 20.3% had cough, 17.4% had shortness of breath, 3.5% wheezing, and none had chest pain. When the condition of female hairdressers after their professional life was evaluated, it was determined that 54.5% complained about breathing, 37.4% had cough, 35.5% had shortness of breath, 13.9% had wheezing, and 4.5% had chest pain. The diagnosis of allergic asthma and bronchitis was 31.3%.

Before their professional life, although 25.2% of the female hairdressers had complaints about nasal flow and sneezing, after professional life, the rate of having nasal flow and sneezing complaint was 45.5%. The allergic rhinitis diagnosed by doctor was 22.9%. Before their professional life, although female hairdressers had itching and redness complaints in the

eyes was 20.0%, after the professional life, the itching and redness complaints in the eyes was 49.4%. Doctor-diagnosed allergic conjunctivitis rate was 20.0%.

Table 2. Evaluation of allergic diseases of the employees working in women hairdressers before and after their professional lives

Working in Women Hairdressers (n:310)	Before professional life	After professional life	p
Complaints about breathing	33.5%	54.5%	p<0.01
Cough	20.3%	37.4%	p<0.01
Shortness of breath	17.4%	35.5%	p<0.01
Wheezing	3.5%	13.9%	p<0.01
Chest pain	0%	4.5%	p<0.01
Nasal flow, sneezing	25.2%	45.5%	p<0.01
Itchiness in the eyes, rash	20%	49.4%	p<0.01
Itchiness in the skin, rash	9.4%	28.4%	p<0.01

McNemar Test

The rate of female hairdressers having itching and redness complaint in the skin before their professional life was 9.4%. After professional life, the complaint of itching and redness in the skin was 28.4%. The number of people diagnosed with eczema by doctor was 14.8%. Eczema was seen at 11.9% in the hands, 3.5% in the arms, 3.5% in the feet, 2.3% in the scalp, and 0.6% in the face.

According to Table 3, 11.3% of the male hairdressers had respiratory complaints, 9.7% had cough, 4.9% had shortness of breath, 2.1% had wheezing, and none had chest pain. When their conditions after professional life were evaluated, it was determined that 31.0% had breathing complaints, 26.7% had cough, 15.1% had shortness of breath, 5.9% had wheezing, and 2.1% had chest pain. Doctor-diagnosed allergic asthma and bronchitis diagnosis rate was 12.3%.

The incidence of nasal flow and sneezing complaints before professional life in those who worked in male hairdressers was 9.7%, and the incidence of nasal flow and sneezing complaints after professional life was 37.9% in male hairdressers. Doctor-diagnosed allergic rhinitis rate was 13.6%. Before professional life, the rate of itching and redness in the eyes was 1.3%, and after professional life, the rate of itching and redness in the eyes was 18.5%. Doctor-diagnosed allergic conjunctivitis rate was 2.6%. Before professional life, the complaint of itching and redness on the skin was 1.0%. After professional life, the complaint of itching and redness on the skin was 10.3%. The number of people who were diagnosed with eczema by doctor was 3.1%. When examined in terms of the place where eczema was detected, it was detected 2.8% in the feet, 2.6% in the hands, 1.8% in the arms, 1.8% in the face, and 0.5% in the scalp. A statistically significant difference was detected in terms of allergic diseases before and after the professional life.

Table 3. Evaluation of the allergic diseases of the employees working in men's hairdressers before and after the profession

Working in Women Hairdressers (n: 390)	Before professional life	After professional life	p
Complaints about breathing	%11,3	%31	p<0,01
Cough	%9,7	%26,7	p<0,01
Shortness of breath	%4,9	%15,1	p<0,01
Wheezing	%2,1	%5,9	p<0,01
Chest pain	%0,5	%2,1	p<0,01
Nasal flow, sneezing	%9,7	%37,9	p<0,01
Itchiness in the eyes, rash	%1,3	%18,5	p<0,01
Itchiness in the skin, rash	%1	%10,3	p<0,01

McNemar Test

When the allergic reactions of the hairdressers were examined for the last 12 months, it was determined that 51.0% of female hairdressers had watering in the eyes, 41.6% of them had itching in the nose, nasal flow, obstruction, 33.9% had complaints of waking up with cough attacks, 27.1% had redness in the skin and itching, 26.8% of them had complaints of waking up with shortness of breath, 25.2% had wheezing. A total of 36.9% of the male hairdressers had itching, nasal flow and obstruction in the nose, 18.2% had watering in the eyes, 10.5% had wheezing, 8.7% had itching and redness on the skin, and 8.5% had complaints of waking up with shortness of breath. When smoking rates were examined, it was determined that 65.8% of the female hairdresser smoked, and 40.5% of the male hairdressers smokes.

Although the female hairdressers used 23.9% allergy medication, male hairdressers used allergy medication at a rate of 11.5%. It was determined that the most frequently used medication was inhaler steroid at a rate of 27.7% in female hairdressers, and at a rate of 11.5% in male hairdressers.

4. Discussion

The present study was conducted to evaluate the risk of occupational asthma in hairdressers in the Black Sea Region in northern Turkey. Although the daily usage of cosmetics containing chemicals is quite high today, it was also determined that employees did not use precautions to reduce their exposure to these chemicals. The risk of occupational asthma increases because of lack of good ventilation conditions in workplaces, and the lack of adequate use of protective equipment. In the study that was conducted by Soy (6) to evaluate the ergonomic suitability of female hairdresser salons by employees, it was determined that 60.6% of the female employees at female hairdressers and 75.0% of the male

employees at female hairdresser salons found that the ventilation in the workplace was sufficient, which supports the research findings. When personal protective equipment usage rates at work were examined, it was determined that 76.7% of the female employees, and 83.3% of the male employees used protective equipment. In our study, on the other hand, it was determined that female hairdressers used protective equipment at a rate of 97.4%, and male hairdressers used protective equipment at a rate of 63.8%. It is considered that the use of protective equipment will reduce the rate of exposure to chemicals and the risk of occupational asthma allergies may decrease by improving ventilation systems.

It was determined that hairdressers did not pay adequate attention to the use of protective clothing and gloves, which are extremely important in terms of protection of the skin and exposure to chemicals. The rate of apron use was 64%, wearing gloves was 55%, wearing masks was 17%, and wearing glasses was 2%. Mandıracıoğlu et al. (7) determined that 41.2% of the participants wore gloves in each task at work, and 15.2% used protective wear. In the study of Gül et al. (8), the rate of using gloves was determined to be 72.5%. Chemicals that have allergic and irritant effects often cause health problems like respiratory tract reactions, asthma, dermatitis, rhinitis, and ocular diseases in barbers. The use of protective equipment for hairdressers and barbers, where atopic dermatitis, allergic rhinitis, and conjunctivitis are seen commonly, are very important in this respect.

Cosmetic products like shampoos, creams, hair dyes, sprays and conditioners that have hundreds of chemicals are used in barber shops and hair salons. The substances female hairdressers reacted at the highest rate was hair dye with 44.2%, decolorization agent, and the nail polish odor had the lowest rate as 2.6%. In male hairdressers, hair spray had the highest reaction at a rate of 30.0%, perfume at a rate of 26.2%, hair dye at a rate of 23.3%, and perm medication had the lowest reaction at a rate of 3.1%. Higher eczema rates in female hair dressers were associated with being exposed to more chemicals.

Compared to those who have asthma, it is difficult for hairdressers who have eczema to continue their professions regularly. It causes early leave of work because of constant hand washing during the day and exposure to various chemicals. It was reported that the risk of leaving work because of an allergic disease was more than 20% in hairdressers (11). Studies show that the frequency of eczema decreases, while asthma increases with furthering age (1). Hairdressers who suffer from respiratory symptoms continue their work despite findings like nasal obstruction, sneezing, wheezing and shortness of breath. It was determined that as the number of years spent at work increased, so did the prevalence of allergic reactions. More respiratory symptoms, prolonged encounter with chemicals and inadequate ventilation were associated with the increasing age and years spent at work. In the study that was conducted by Akpınar et al., it was determined that the frequency of occupational asthma was 3.6 times higher in those who had more contact with chemicals because of the intensity of work.

However, it was observed that there are more occupational asthma and allergic diseases in people who had atopy history in their families.

Holland et al. (1) conducted a study with hairdressers, and reported that the prevalence of respiratory symptoms suggesting asthma was 45%. Mandıracıoğlu et al. (7) conducted a study in Izmir province to determine the occupational health risks of barbers and hairdressers, and reported that 35% of hairdressers had allergic complaints. When the complaints of the female hairdressers participating in this study related to allergic diseases before and after their professional life were evaluated, it was determined that 33.5% complained about respiratory functions before their professional life, and 54.5% complained about respiratory functions after their professional life. The rate of the complaints of the male hairdressers was 11.5% before their professional lives, and after their professional lives, this rate was 31%.

In the study conducted by Hollund et al. it was determined that 71% had nasal flow, 41% shortness of breath, 39% watering in the eyes, 37% wheezing, 34% cough complaints lasting more than two weeks, and 34% had eczema. In our study, on the other hand, 51.0 of the female hairdressers had watering the eyes, 41.6% had itching, nasal flow, and obstruction in the nose, 33.9% had the complaint of waking up with a cough attack, 27.1% had redness and itching in the skin, 26.8% had the complaint of waking up with shortness of breath, 25.2% had wheezing. In 36.9% of the male hairdressers, the complaints were itching, nasal flow, and obstruction; watering in the eyes was at a rate of 18.2%, wheezing at a rate of 10.5%, itching and redness on the skin at a rate of 8.7%, shortness of breath at a rate of 8.5%.

In our study, 20% of the patients had doctor-diagnosed asthma, 17% had rhinitis, 10% had conjunctivitis, and 8% had eczema. In a previous study conducted by Akpınar et al. in our country, asthma rate was 14.6%. Today, it is considered that the use of more chemicals increased these rates.

In the study conducted by Gül et al. (8) to Determine the Health Problems of Employees in Hair Salons, 21.1% of the hairdressers were diagnosed with allergy, 61% were diagnosed with asthma, and 14.0% were diagnosed with skin disease by the doctor. It was determined that 18.7% of the participants had skin rash in their bodies. In the study that was conducted by Çelenk (9) on the basic qualities and problems faced in workplaces of hairdressers department students in Ordu Vocational Training Center, it was determined that 11.3% had skin diseases, and 11.9% had respiratory diseases. Içbay (10) conducted a study to determine the physical conditions of

female hairdressers in the city center of Gaziantep and to evaluate the health-related complaints and practices of the employees due to infectious diseases, it was determined that 25.9% of employees had respiratory tract problems, 52.4% had allergic complaints, and 32.6% complained about their skins.

In the light of the findings obtained in the study, it is recommended that initiatives are planned to pay more attention to the use of protective equipment for hairdressers, it is ensured that hairdressers are aware of occupational diseases, and trainings are organized on the measures that can be taken, working conditions are regulated and improved. It is necessary that the workplace environment is safe, ventilation is improved, chemicals used are inspected, protective equipment are used appropriately, and legal sanctions are imposed in this respect.

Conflict of interest

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Authors' contributions

Concept: N.K, R.S, Ş.İ.K.K., Design: N.K, R.S, Data Collection or Processing: N.K, R.S, Ş.İ.K.K., G.H, Analysis or Interpretation: N.K, R.S, Ş.İ.K.K, Literature Search: N.K, Ş.İ.K.K, G.H , Writing: N.K, Ş.İ.K.K

References

1. Hollund BE, Moen BE, Lygre SH, Florvaag E, Omenaas E. Prevalence of airway symptoms among hairdressers in Bergen, Norway. Occup Environ Med 2001;58:780-5.

2. Hollund BE, Moen BE. Chemical exposure in hairdresser salons: effect of local exhaust ventilation. Ann Occup Hyg 1998;42:277-81.

3. O'Connell RL, White IR, Mc Fadden JP, White JML. Hairdressers with dermatitis should always be patch tested regardless of atopy status. Contact Dermatitis, 2010; 62: 177-81.

4. Amodeo M, Boudot H, Desfray E, Ducrot-Henry L, Gomis C, Seneque B, Demmuid JP, Regin S, Guinot P. "La Coiffure: Une Enquete De Terrain En Cote-d'Or, Pour Les Medecins Du Travail, No: 99, 367- 381, trimestre 3, 2004.

5. Esin MN, Bulduk S, Ince H. Work related risksand health problems of working children in urban Istanbul, Turkey. J Occup Health, 2005; 47 (5): 431-6.

6. Soy, T, Ağaç, S. Kadın Kuaför Salonlarının Ergonomik Açıdan Uygunluğunun Çalışanlar Tarafından Değerlendirilmesi. Yüksek Lisans Tezi. Gazi Üniversitesi. Eğitim Bilimleri Enstitüsü. Ankara. 2013;9-91.

7. Mandıracıoğlu A, Köse Ş, GözaydınA, Türken M, Kuzucu L. Berber ve Kuaförlerin Mesleki Sağlık Riskleri. 12. Ulusal Halk Sağlığı Kongresi, 2125 Ekim 2008, Ankara, 489.

8. Gül, H., İşveren, H., Babaoğlu, Ü., Hapçıoğlu, B., Özyıldırım, B. Kuaför Salonlarında Çalışanların Sağlık Sorunları. 3. Çevre Hekimliği Kongresi, İstanbul Üniversitesi, İstanbul Tıp Fakültesi, Halk Sağlığı Anabilim Dalı. 2009.

9. Çelenk, B. Ordu İli Mesleki Eğitim Merkezi'ndeki Kuaförlük Bölümü Öğrencilerinin Temel Nitelikleri ve İşyerinde Karşılaştıkları Sorunlar. Yayımlanmamış Yüksek Lisans Tezi, Gazi Üniversitesi Eğitim Bilimleri Enstitüsü, Ankara. 2007.

10. İçbay, E. Gaziantep Merkezinde Kadın Kuaförlerin Fiziksel Koşullarının Çalışanlarının Sağlıkla İlgili Yakınmalarının ve Bulaşıcı Hastalıklarla İlgili Bilgi ve Uygulamalarının Değerlendirilmesi. Uzmanlık Tezi, Gaziantep Üniversitesi Tıp Fakültesi, Gaziantep.2011.

11. Leino T, Tuomi K, Paakkulainen H, Klockars M. Health reasons for leaving the profession as determined among Finnish hairdressers in 1980-1995. Int Arch Occup Environ Health 1999;72:56-9.

12. Holm JO, Veierød MB. An epidemiological study of hand eczema. Acta Derm Venereol (Stockh) 1994;187:8-27.

13. Akpinar M, Elci O. Importance of work intensity on respiratory problems in hairdressers. Occup Environ Med 2002;59:649-50.

14. Helaskoski E. Suojalehto H., Virtanen H., Airaksinen L., Kuuliala O.,Occupational asthma, rhinitis, and contact urticaria caused by oxidative hair dyes in hairdressers. Ann Allergy Asthma Immunol 112 (2014) 46-52

15. Lysdal S., Mosbech H., Johansen J. Sosted H., Asthma and Respiratory Symptoms Among Hairdressers in Denmark: Results from a Register Based Questionnaire Study American Journal of Industrial Medicine 57:1368–1376 (2014)

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Evaluation of patients undergoing colpocleisis: A single-center experience

Selim GÜLÜCÜ*, Neşet GÜMÜŞBURUN
Department of Obstetrics and Gynecology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Türkiye

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Abstract

The aim was to evaluate patients who underwent colpoclesis due to pelvic organ prolapse (POP). This cross-sectional study included patients who had undergone colpoclesis surgery. Age, gravity, parity, systemic diseases, examination findings, anesthetic methods used, surgical complications, additional operations, and length of hospital stay were recorded. At evaluation, patients were divided into two groups: partial colpoclesis and total colpoclesis. The mean age of patients was 73.25±5.45 (60-80), gravidity was 4.58±2.38 (2-11), and parity was 4.08±1.45 (2-7). While uterine prolapse was observed in 47 (97.9%) patients, one patient was found to have vaginal cuff prolapse. Partial colpoclesia was performed in 28 (58.3%) of the patients. While 16 of the patients (33.3%) had no additional systemic disease, the remaining patients had at least one systemic disease, with hypertension being the most common at 58.3%. Twenty-eight of the patients (58.3%) underwent surgery under general anesthesia and the rest under spinal anesthesia. While no postoperative complication occurred in 46 (95.8%) of the patients, blood transfusion (erythrocyte suspension) was observed in one patient and delirium was observed in another patient after surgery. The postoperative discharge time of patients was 3.66±2.10 (2-9) days. Statistically significant differences were found between the partial and total colpoclesis groups in terms of age, gravity and parity (p=0.002, p=0.022, and p=0.030, respectively). There were no significant differences between groups in discharge time (p = 0.143) and type of anesthesia (p=0.104). Colpoclesis surgery can be safely performed in elderly patients diagnosed with pelvic organ prolapse who are not sexually active. This method should be recommended as an option, especially in patients with complicated systemic diseases. Although short-term serious complications are not uncommon in patients, routine follow-up of patients with partial colpoclesis for long-term complications should be continued.

Keywords: colpoclesis, pelvic organ prolapse, hysterectomy, pelvic floor disorder, LeFort

1. Introduction

Protrusion of pelvic organs (uterus, rectum, bladder) toward or out of the vagina is called pelvic organ prolapse (POP). The main purpose of treatment POP is to improve symptoms, and reconstructive or obliterative surgical approaches are available (1, 2). The treatment approach depends on the patient's expectations and preferences. Colpocleisis is an obliterative method in which the vaginal canal is closed and the pelvic organs are returned to the pelvis. It can be performed by two methods: partial (LeFort) and complete (1). It can be recommended for older women who no longer want to use the vagina for sexual intercourse, for women who want to avoid hysterectomy, and for patients who prefer surgery with the lowest risk of complications and a short duration (2,3). The advantages of this surgery are its short duration, minimal blood loss, rapid discharge from the hospital, and the fact that only local anesthesia is required. However, it also has disadvantages, such as the lack of sexual activity after the procedure, the development of de novo incontinence or urinary incontinence, and the impossibility of taking an endometrial biopsy because the cervical canal cannot be reached (4). Cervical lesions should be evaluated and treated as appropriate before prolapse repair (2). The procedure has a high success rate and long-term patient satisfaction (3).

The aim of the present study was to evaluate the demographic characteristics and surgical methods of patients who underwent colpocleisis due to POP in our clinic.

2. Materials and Methods

Patients undergoing colpocleisis due to POP in the Department of Obstetrics and Gynecology at the Gaziosmanpasa University Faculty of Medicine between 2015 and 2021 were included in the study. Medical history, age, gravidity, parity, systemic diseases, examination results, anesthesia methods used, surgical complications, additional surgeries, and length of hospital stay were recorded in the patients' records.

All patients who had undergone colpocleisis and whose data were accessible were included in the study. All patients who were diagnosed with pelvic organ prolapse (POP -Q) were classified as grade 3-4 prolapse, and the surgical method used was partial and total colpocleisis. Patients were divided into two groups, partial colpocleisis and total colpocleisis.

In partial colpocleisis, a rectangular area on the anterior and posterior vaginal walls was marked and excised with a scalpel, and bleeding was controlled with a cautery. A 2-cm-deep bilateral vaginal mucosal bridge was created to create a lateral tunnel. The tunnel walls were sutured with late absorbable sutures. The posterior and anterior layers of the vaginal muscles were sutured together. After the uterus and

*Correspondence: selim.gulucu@gop.edu.tr

vagina were inverted, the upper and lower edges of the rectangle were sutured, and the vagina was obliterated.

Total colpocleisis was performed in patients who had already had a hysterectomy or who were scheduled for a hysterectomy in addition to surgery. In the surgical procedure of total colpocleisis, all vaginal walls were incised circularly with a scalpel to the edge of the vaginal cuff, taking into account the borders of the bladder above and the rectum below, and then cautery was used to ensure hemostasis. The muscularis layers were sutured together. The vaginal epithelium was closed transversely. Patients who underwent surgery at an external center and were referred to our center and whose information could not be obtained were excluded from the study.

The study was approved by the local ethical committee of Tokat Gaziosmanpaşa University (2022/04 22-KAEK-023).

Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used in the analysis of the study data to obtain information on the general characteristics of the groups. Differences between groups were analyzed for quantitative values using the independent samples t-test and for qualitative values using Fisher's exact chi-square test. It was considered statistically significant when p values below 0.05 were calculated. Prepackaged statistical software IBM SPSS Statistics 19 was used for statistical analysis. Ethical approval was obtained before the study.

3. Results

The study found that 48 female patients underwent colpocleisis due to POP. The mean age of these patients was 73.25±5.45 (60-80), gravidity was 4.58±2.38 (2-11), and parity was 4.08±1.45 (2-7). Twenty-eight of the patients (58.3%) underwent partial colpocleisis (LeFort). All patients had undergone a normal vaginal delivery. While 47 patients (97.9%) had uterine prolapse, one patient had vaginal cuff prolapse. Vaginal hysterectomy was performed in 19 patients who underwent total colpocleisis, and TOT (transobturator tape) was applied as an additional surgery in one patient who underwent partial colpocleisis. While 16 patients (33.3%) had no additional systemic diseases, the remaining patients had at least one systemic disease, with hypertension being the most common systemic disease at 58.3%. Twenty-eight patients (58.3%) underwent surgery under general anesthesia, and the remaining patients underwent surgery under spinal anesthesia. While no postoperative complications occurred in 46 patients (95.8%), blood transfusion (red blood cell suspension) was observed in one patient and delirium was observed in one patient after surgery. The postoperative discharge time of the patients was 3.66±2.10 (2-9) days. The demographic characteristics of the patients are shown in table 1. When the patients who had undergone partial and total colpocleisis were evaluated by dividing them into two groups, there were statistically significant differences between the groups in age, gravidity, and parity (p = 0.002, p = 0.022, and p = 0.030, respectively). No significant differences were found between groups in terms of discharge time (p = 0.143) and type of anesthesia (p = 0.104).

Table 1. The patients' demographic characteristics

	Total Kolpoklezis (n=20)	Parsiyel Kolpoklezis (n=28)	p
Age (year)	69.40±6.09 (60-78)	76.00±2.94 (72-80)	0.002*
Gravity	3.40±0.52 (3-4)	5.43±2.87 (2-11)	0.022*
Parity	3.41±0.52 (3-4)	4.57±1.74 (2-7)	0.030*
Discharge time (day)	3.00±1.15 (2-5)	4.14±2.45 (2-9)	0.143

Values are expressed as mean ± standard deviation (minimum-maxium).
p: Independent Sample T-Test. * p value is significant at the 0.05 level ($p<0.05$)

4. Discussion

Nowadays, the incidence of POP is predicted to increase with the increasing life expectancy of women. Mortality and morbidity rates are higher in elderly patients undergoing urogynecologic surgery for POP than in young patients (5). In elderly patients with symptomatic POP and high morbidity, surgical intervention must be carefully selected and performed (6). If there is an alternative treatment (such as a pessary) to surgery in this group of patients, it may be considered in the first instance. However, this alternative treatment does not definitively eliminate POP, and long-term use of this treatment is uncomfortable for the patient (7). Colpocleisis is a valuable surgical procedure for patients with prolapse who have undergone unsuccessful reconstructive surgery and no longer desire sexual intercourse (8,9).

The colpocleisis method ensures that the POP operation is both simple and short. It also has a significantly lower recurrence rate than reconstructive methods. Although there is no large-scale study on this topic, the success of colpocleisis varies from 91% to 100% (10,11). In our study, patients were examined a total of 3 times, at the first postoperative week, first month, and first year, and no recurrence was observed in any patient. The ability to perform the procedure under local anesthesia and the short hospital stay are also major advantages of colpocleisis (12). In our study, it was found that patients were operated under general and spinal anesthesia. This was attributed to the fact that the surgery was performed in the general operating room of our hospital and patients did not desire local anesthesia under these conditions.

Colpocleisis is associated with general complications in terms of mortality and morbidity. The most common complication is the need for blood transfusion after surgery. Venous thromboembolism, pulmonary embolism, hypovolemic shock, heart failure, sepsis, and psychiatric disorders can be mentioned as other important complications (13,14). In our study, one patient developed a postoperative

hematoma. Because the hematoma was self-limiting, no additional procedure was performed on the patient, and blood substitution was subsequently performed. In a retrospective study of 1104 women who had undergone urogynecologic surgery by Solomon et al, the incidence of venous thromboembolism was reported to be 0.3% (15). All patients in the study wore antithromboembolic stockings for prophylaxis and received Low-molecular-weight heparin (LMWH) appropriate doses postoperatively. None of the patients experienced a thromboembolic event. One patient had postoperative delirium. The higher average age of patients in the partial colpocleisis group compared with the complete colpocleisis group was attributed to the fact that this method was used to shorten the duration of the procedure and keep the risk of complications to a minimum.

Urinary incontinence may develop after colpocleisis (16). The occurrence of new postoperative incontinence or worsening of preexisting incontinence was noted by Hoffman et al (17) in three of 27 patients and by Hanson et al (18) in twenty-two of 288 patients. One patient in the study had stage 3 uterine prolapse according to the POP -Q classification and stress incontinence. This patient underwent complete colpocleisis and TOT. At the patient's postoperative follow-up, her urinary incontinence improved and no recurrence was observed. No effect of colpocleisis on bowel function was reported, and von Pechmann (19) noted that rectal prolapse, although rare, may occur after surgery. Consistent with the literature, no problems with bowel function were noted in our patients.

Sexual desire has been shown to persist in advanced age (20), and Huang et al. reported that 30% of women older than 65 years continue to have moderate sexual desire (21). Women who are sexually active or considering being sexually active are more likely to prefer reconstructive procedures because the vaginal structure is preserved. However, patient satisfaction is higher with obliterative procedures, where both complications and recurrence of prolapse are less common (9, 10). We think that the advanced average age of the patients, the fact that 20 patients (83.3%) did not have a spouse and the rest did not want sexual activity facilitated the determination of the treatment method as colpocleisis. The limitations of our study are that it is retrospective and includes a small number of cases. After ruling out the risk of malignancy in POP patients, colpocleisis is an appropriate treatment option after comprehensive counseling considering sexual activity status.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: S.G., N.G., Design: S.G., N.G., Data Collection or Processing: S.G., N.G., Analysis or Interpretation: S.G., Literature Search: S.G., N.G., Writing: S.G., N.G.

References

1. Raju R., Occhino JA, Linde BJ. LeFort partial colpocleisis: tips and technique. Int Urogynecol J. 2020;31:1697-99.

2. Grzybowska ME, Futyma K, Kusiak A, Wydra DG. Colpocleisis as an obliterative surgery for pelvic organ prolapse: is it still a viable option in the twenty-first century? Narrative review. Int Urogynecol J. 2022;33(1):31-46.

3. Farghali MM, Abdelzaher A, Abdelazim IA. Surgical and quality of life outcomes after pelvic organ prolapse surgery in older postmenopausal women. Prz Menopauzalny. 2021;20(1):21-28.

4. Buchsbaum GM, Lee TG. Vaginal Obliterative Procedures for Pelvic Organ Prolapse: A Systematic Review. Obstet Gynecol Surv. 2017;72(3):175-83.

5. Hong MK, Ding DC. Current Treatments for Female Pelvic Floor Dysfunctions. Gynecol Minim Invasive Ther. 2019;8(4):143-48.

6. Joukhadar R, Radosa J, Paulus V, Hamza A, Solomayer EF, Herr D, Wöckel A, Baum S. Influence of Patient's Age on the Outcome of Vaginal and Laparoscopic Procedures in Urogynaecology. Geburtshilfe Frauenheilkd. 2019;79(9):949-58.

7. Oh S, Namkung HR, Yoon HY, Lee SY, Jeon MJ. Factors associated with unsuccessful pessary fitting and reasons for discontinuation in Korean women with pelvic organ prolapse. Obstet Gynecol Sci. 2022;65(1):94-99.

8. Braga A, Serati M, Salvatore S, Torella M, Pasqualetti R, Papadia A, Caccia G. Update in native tissue vaginal vault prolapse repair. Int Urogynecol J. 2020 Oct;31(10):2003-2010.

9. Pizarro-Berdichevsky J, Clifton MM, Goldman HB. Evaluation and Management of Pelvic Organ Prolapse in Elderly Women. Clin Geriatr Med. 2015;31(4):507-21.

10. Park JY, Han SJ, Kim JH, et al. Le Fort partial colpocleisis as an effective treatment option for advanced apical prolapse in elderly women. Taiwan J Obstet Gynecol 2019;58:206-211.

11. Petcharopas A, Wongtra-Ngan S, Chinthakanan O. Quality of life following vaginal reconstructive versus obliterative surgery for treating advanced pelvic organ prolapse. Int Urogynecol J. 2018;29(8):1141-46.

12. Manonai J, Wattanayingcharoenchai R. Surgical treatment for pelvic organ prolapse in elderly women. Journal of Obstetrics and Gynaecology. 2015;35(1):82-4.

13. Alshankiti H, Houlihan S, Robert M; Calgary Women's Pelvic Health Research Group. Incidence and contributing factors of perioperative complications in surgical procedures for pelvic organ prolapse. Int Urogynecol J. 2019;30(11):1945-53.

14. Haya N, Feiner B, Baessler K, Christmann-Schmid C, Maher C. Perioperative interventions in pelvic organ prolapse surgery. Cochrane Database Syst Rev. 2018;8(8):CD013105.

15. Solomon ER, Frick AC, Paraiso MFR, et al. Risk of deep venous thrombosis and pulmonary embolism in urogynecologic surgical patients. American journal of obstetrics and gynecology. 2010;203(5):510.e1-e4.

16. Pratt JH, Baker RK. Urinary incontinence following the Le Fort operation: report of a case. Obstetrics & Gynecology. 1960;16(6):722-3.

17. Hoffman MS, Cardosi RJ, Lockhart, et al. Vaginectomy with pelvic herniorrhaphy for prolapse. American journal of obstetrics

and gynecology. 2003;189(2):364-70.

18. Hanson GE, Keettel WC. The Neugebauer-Le Fort operation: a review of 288 colpocleises. Obstetrics & Gynecology. 1969;34(3):352-7.

19. von Pechmann WS, Mutone M, Fyffe J, et al. Total colpocleisis with high levator plication for the treatment of advanced pelvic organ prolapse. American journal of obstetrics and gynecology. 2003;189(1):121-6.

20. Stentagg M, Skär L, Berglund JS, Lindberg T. Cross-Sectional Study of Sexual Activity and Satisfaction Among Older Adult's ≥60 Years of Age. Sex Med. 2021;9(2):100316.

21. Huang AJ, Subak LL, Thom DH, et al. Sexual function and aging in racially and ethnically diverse women. Journal of the American Geriatrics Society. 2009;57(8):1362-8.

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Research Article

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Analysis of microscopic characteristics of cartilage, synovial membrane, and subchondral bone in collagenase induction model of knee osteoarthritis *Rattus Norvegicus*

Caesar Haryo BIMOSENO[1,*], Dwikora Novembri UTOMO[1], Lukas WIDHIYANTO[1]

[1]Department of Orthopedic and Traumatology, Faculty of Medicine, Universitas Airlangga/ Soetomo General Hospital, Surabaya, Indonesia

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Abstract

Osteoarthritis (OA) is a degenerative arthritis disease of the synovial joints and is one of the most common causes of disability in adults. A study requires experimental animal models to be well understood. Collagenase was found to be able to induce OA in experimental animals. This study was conducted with the aim of analyzing the microscopic characteristics of cartilage tissue, synovial membrane, and subchondral bone in the knee of *Rattus norvegicus* rats after collagenase injection. In-vivo experiment using experimental animals *Rattus norvegicus* which was injected with collagenase was conducted. The sample will be divided into 3 groups: evaluation time at 7 days (group 1), 14 days (group 2), and 21 days (group 3). Synovial and cartilage will be evaluated based on OARSI while subchondral bone will be evaluated based on Subchondral Bone Score (SBS). It was found that cartilage erosion and synovial membrane damage were the most severe in group 3. The level of damage based on OARSI and SBS scoring was also found to be progressive and worsened over time. Subchondral bone values increased in both control and treatment with the control peak at 0.87 and the treatment peak at 3.2. It was found that there was a clear and microscopically progressive difference 3 weeks after collagenase injection. Induction of OA using collagenase injection in experimental animals *Rattus norvegicus* can be a good model for making secondary OA.

Keywords: osteoarthritis, knee, collagenase, cartilage, synovial membrane, subchondral bone

1. Introduction

Osteoarthritis (OA) is a very complex degenerative arthritis disease of the synovial joints and is one of the most common causes of disability in older adults. According to the United Nations, by 2050 people over the age of 60 will make up more than 20% of the world's population, which means it is estimated that by 2050, 130 million people will suffer from OA worldwide, of which 40 million will be severe disability due to the disease (1). Patients with OA in the United States alone have reached 27 million people in 2008 (2).

Synovial membrane inflammation has emerged as another major feature of the pathophysiology of OA. Synovial histological changes include synovial hypertrophy and hyperplasia with an increased number of lining cells often accompanied by sublining tissue infiltration with scattered lymphocyte foci. Activated synovium can produce proteases and cytokines that accelerate damage to the area around the cartilage (3).

In addition to cartilage tissue and synovial membrane, various characteristic changes in subchondral bone are also found which are also considered to be very related in the pathogenesis of OA, thus implying a strong relationship between OA and subchondral bone. Other researchers have published findings that an increase in subchondral bone mass that is rigid (stiff) is an important factor in the pathogenesis of OA, because it contributes to cartilage degeneration, in addition to inflammation of the synovial membrane (4,5).

Research on the pathogenesis of OA is a challenge in itself considering the very complex pathogenesis of OA. The main obstacle faced by researchers in the study of OA is that the pathogenesis of OA is slow and difficult to predict, and clinical symptoms that appear only in advanced OA so that they cannot describe the various structural changes in the joint cavity (6). Therefore, a research model is needed that can be carried out in a shorter time, in this case using an experimental animal model (7).

The small animal trial model is the model of choice as an initial study of the pathogenesis of OA considering the practicality and financing aspects, as well as a foothold before being implemented in a larger model. In addition to the types of experimental animals, the model for making OA is also divided into primary and secondary OA models. Secondary models, both surgical and chemical induction models, have become an option in consideration of making models that are shorter and more practical. Chemical induction has recently

*Correspondence: caesarbimoseno@gmail.com

become the choice of many researchers given its practicality. Chemical induction can use monosodium iodoacetate (MIA) and collagenase, but because MIA is much faster than collagenase, collagenase is considered more capable of representing OA itself (8).

Given the importance of a good model to support future studies for OA, this study was conducted with the aim of analyzing the microscopic characteristics of cartilage, synovial membrane, and subchondral bone in a collagenase-induced model of knee osteoarthritis of *Rattus norvegicus*. This study is expected to be the basis for modeling OA in mice.

2. Material and Methods

This type of research is an in-vivo experiment using *Rattus norvegicus* experimental animals with an analytical research design in the form of a post-test only design. This study has been approved by Animal Care and Use Committee of Universitas Airlangga, Surabaya, Indonesia with the number of 2.KEH.029.03.2022.

Experimental animals used in research must meet the following criteria: (1) experimental animals are *Rattus norvegicus*; (2) rats aged 2 months; (3) rats weighed 200-300 grams; (4) male rats; and (5) in an healthy condition with signs of active movement and solid stools. In the event that *Rattus norvegicus* fell ill and/or rats died during the acclimatization period or during the study, the sample was not included in the data for this study. All institutional and national guidelines for the care and use of laboratory animals were followed.

The sample will be divided into 3 groups. Grouping is based on the time of evaluation: 7 days (group 1), 14 days (group 2), and 21 days (group 3). Each group will be subdivided into 2 subgroups: control group and treatment group. Treatment group will receive injection of collagenase type 7 as much as 1 ml and control group will receive injection of only 1 ml of NaCl. At each time, the sample will be terminated and the knee tissue sample taken for microscopic evaluation: cartilage, synovial membrane, and subchondral bone. Synovial and cartilage will be evaluated based on OARSI (9) while subchondral bone based on Subchondral Bone Score (10).

In brief, OARSI score is calculated based on 4 parameters: hematoxylin eosin staining, cartilage structure, chondrocyte density, and cluster formation with a total score ranging from 0-24. On the other hand, subchondral bone score is based on 3 parameters: subchondral plate condition, bone volume (based on formula), and observable osteophytes. Maximum score of 12 can be achieved with higher score indicating worse subchondral bone.

Based on the calculation of the sample formula, it was found that the total number of experimental *Rattus norvegicus* rats for this study should be 5 for each group. Therefore minimum required sample would be 30 samples (3 treatment groups and 3 control groups).

All research was carried out at the Laboratory of the Faculty of Veterinary Medicine (FKH) Universitas Airlangga, Surabaya, Indonesia from March 2022 to April 2022.

All rats will go through the acclimatization stage for one week. Experimental animals were placed in groups placed in cages, within 12 hours on a light-dark cycle at 24°C and had access to water and food in a veterinary laboratory facility.

OA was induced unilaterally in one knee of each *Rattus norvegicus* by 2 intra-articular injections of 3 collagenase type VII units (Sigma-Aldrich) on the first day of observation (day 0). All intra-articular injections were applied with an injection volume of 6 L using a 50 L glass syringe (Hamilton Company, Ghiroda, Romania) and a 30 G needle. The contralateral knee control was injected intraarticularly using 1 ml NaCl.

All specimens were fixed with 10% buffered formalin for 24 hours, decalcified with 5% formic acid for 5 days and then put into paraffin blocks. A series of 3 sagittal sections (4 m thick) were created for each of the 4 compartments, through the large diameter of the cartilage lesion. Hematoxylin-eosin staining was used to evaluate synovial, cartilage, and subchondral bone scores. Evaluation was done by two anatomic pathology specialist and mean score from the two will be used as the final score on this patient. All pathology anatomist were blinded by the sample's group.

The data collected will be analyzed descriptively by totaling each scoring parameter and averaging the results of the sample used. Calculations were carried out using the Microsoft Excel 2016 program. The data will be presented in tabular form.

3. Results

From the results of the study it was found that cartilage erosion was the most severe in group 3 and the lightest in group 1 (Fig. 1 and fig. 3). The signs of inflammation were most severe in group 1 and mild in group 3. From the results of immunohistochemical analysis, it was also found that cartilage and synovial tissue damage occurred mainly in the area of collagenase injection (Fig. 2). This suggests that collagenase may need to be administered in larger volumes or injected more than once to create a more evenly-spread breakdown.

The average results of the assessment of the synovial membrane for all samples are described in table 1. Overall, the cumulative total OARSI value of the synovial membrane in the control group on average is the same, ranging from 0.6-0.9. Compared with the group treated with collagenase injection, there was a large increase of up to 7.53 on day 21.

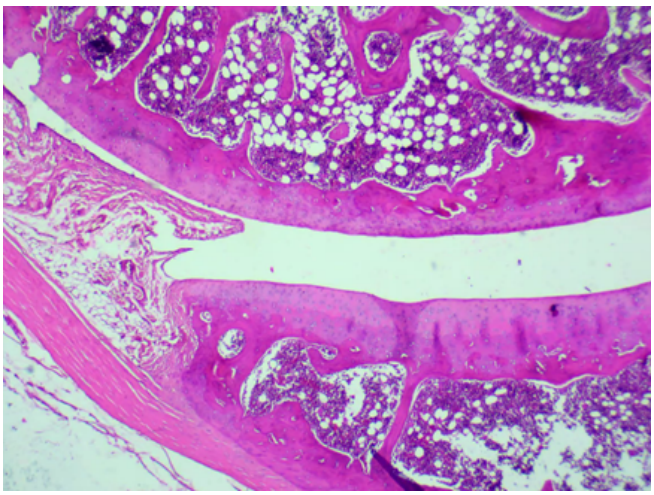

Fig. 1. Microscopic image of the control group 3. A normal picture was found. No erosions or leukocytes were seen in the joint space. There was also no inflammatory cell infiltration in the synovial membrane. This image is used as a reference for detecting pathology in other sections

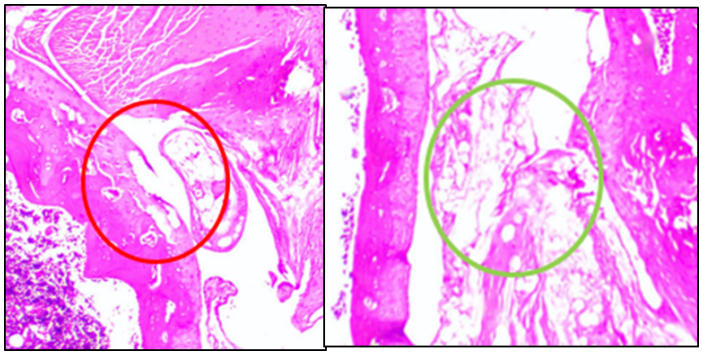

Fig. 2. Microscopic image of treatment group 1 sections. The left image shows an irregularity of the cartilage surface. In the image on the right, there are inflammatory cells infiltrating the synovial membrane. This picture is an early symptom of mild OA

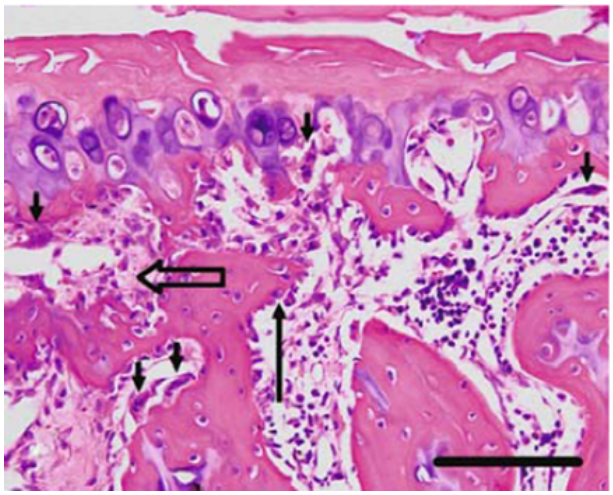

Fig. 3. Subchondral bone changes in treatment group 3. Focal fibrous tissue proliferation (blank fill arrow), increased osteoclasts along the junction between damaged cartilage and subchondral bone (short arrow), and several adjacent trabeculae lined with osteoblasts (long arrow) are seen.

In terms of cartilage microscopy, it was found that the various parameters of cartilage tissue had increased quite a lot from day to day and the difference was large with the control, especially on day 21 (control vs. treatment: 5.13 and 0.6).

Subchondral bone values increased in both control and treatment with the control peak at 0.87 and the treatment peak at 3.2. Although both experienced improvement, there was a significant difference between the two from day 7 to day 21. This indicates the progression of subchondral bone damage, especially in the treatment group.

Table 1. Results of OARSI assessment on synovial membrane, cartilage tissue, and subchondral bone of *Rattus norvegicus* rat after treatment

		Day of Observation		
Group	**Scoring Criteria**	Group 1 (7 Days) Mean Score	Group 2(14 Days) Mean Score	Group 3 (21 Days) Mean Score
Control	Synoviocyte proliferation	0	0.07	0
	Hypertrophy	0	0.13	0
	Inflammatory infiltrate	0.2	0.13	0.07
	Fibrinous exudate	0.07	0	0.2
	Lymphoplasmacytic infiltrate	0	0	0.07
	Lymphoplasmacytic aggregates	0	0	0.13
	Synovial stroma villous hyperplasia	0.07	0	0.07
	Proliferation of fibroblasts/fibrocytes	0.07	0.13	0.07
	Proliferation of blood vessels	0.13	0.07	0.2
	Cartilage/bone detritus	0	0	0.07
	Hemosiderosis	0.07	0.13	0
	Cumulative Microscopic OARSI Synovial Membrane Score	0.6	0.67	0.87
	Hematoxylin-eosin Staining	0.2	0.2	0.13
	Structure	0.47	0.4	0.27
	Chondrocyte Dencity	0.13	0.13	0.13
	Cluster formation	0.07	0.07	0.07
	Cumulative Microscopic OARSI Cartilage Score	0.87	0.8	0.6
	Subchondral Bone Plate	0.13	0.13	0.47
	Bone Volume	0.13	0	0.33
	Osteophyte	0.07	0.13	0.07
	Cumulative Microscopic Cartilage Score	0.33	0.27	0.87

Perlakuan: Injeksi Kolagenase 1x	Synoviocyte proliferation	0.33	0.47	0.67
	Hypertrophy	0.53	0.6	0.47
	Inflammatory infiltrate	0.47	0.73	0.4
	Fibrinous exudate	0.53	0.87	0.87
	Lymphoplasmacytic infiltrate	0.47	0.67	0.67
	Lymphoplasmacytic aggregates	0.47	0.6	0.53
	Synovial stroma villous hyperplasia	0.47	0.67	0.8
	Proliferation of fibroblasts/fibrocytes	0.6	0.53	0.87
	Proliferation of blood vessels	0.53	0.6	0.67
	Cartilage/bone detritus	0.47	0.67	0.93
	Hemosiderosis	0.53	0.87	0.67
	Cumulative Microscopic OARSI Synovial Membrane Score	5.4	7.27	7.53
	Hematoxylin-eosin Staining	0.47	0.87	1.27
	Structure	0.53	1.27	2.27
	Chondrocyte Dencity	0.4	0.6	0.8
	Cluster formation	0.47	0.47	0.8
	Cumulative Microscopic OARSI Cartilage Score	1.87	3.2	5.13
	Subchondral Bone Plate	0.6	0.8	1.47
	Bone Volume	0.47	0.87	0.93
	Osteophyte	0.53	0.73	0.8
	Cumulative Microscopic Cartilage Score	1.6	2.4	3.2

4. Discussion

Collagenase is an enzyme that will damage most joint structures such as tendons, ligaments, and meniscus. Intra-articular injection of collagenase to induce osteoarthritis has been known since 1990. In the previous literature it was found that one injection of collagenase was able to cause significant and progressive osteoarthritis for 2 weeks. Maximum effect occurs at 10 weeks, after which repair will begin to return to normal when collagenase is applied to young *Rattus norvegicus*. The success rate in inducing OA is 100% with cartilage fibrillation occurring within 7 days, cartilage erosion within 21 days and osteophyte formation within 42 days.(11,12)

Apart from the knee joint, collagenase administration was also found to cause OA of the facet joints in the lumbar spine. At all doses (1U, 10U, and 50U), collagenase resulted in cartilage fibrillation and cartilage calcification within 1 week. In addition, hypertrophy and inflammation of the subsynovial tissue and changes in the subchondral bone occur within 1 week as well. Osteoclasts were also found to be elevated, indicating the role of collagenases not only in joint structure but also in bone(13).

When assessed based on OARSI, collagenase administration had a progressive difference over time. This indicates that the progression of OA caused by collagenase is significant every week (13). The effect of giving collagenase in this study was similar to the description of previous studies. Where there are changes in the synovial structure, cartilage, and subchondral bone in *Rattus norvegicus* compared to those not given collagenase.(14)

Another thing that is interesting in this study is that the administration of collagenase, which is small compared to previous studies, has proven to be successful in inducing the occurrence of osteoarthritis. Previous studies generally used 10 collagenase units compared to the 3 units used in this study. This shows that the effectiveness of collagenase is indeed quite large(11,14,15).

It is interesting to note that the collagenase injection technique must be of the right volume, because as previously stated. It was found that the distribution of joint destruction was uneven. This suggests that perhaps collagenase needs to be administered in larger volumes or injected more than once to create a more even breakdown.

It was found that there was a clear and microscopically progressive difference 3 weeks after collagenase injection. Induction of OA using collagenase injection in experimental animals *Rattus norvegicus* can be a good model for making secondary OA because various microscopic changes in cartilage, synovial membrane, and subchondral bone can be clearly observed.

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship and/or publication of this article.

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Authors' contributions

Concept: C.H.B, D.N.U, L.W Design : C.H.B, L.W, A.R.H Data Collection : C.H.B, A.R.H Literature search : C.H.B, A.R.H Writing : C.H.B, D.N.U

References

1. World Health Organization. Essential medicines and health products - Priority diseases and reasons for inclusion - Osteoarthritis. World Heal Organ. 2013;12:6–8.
2. Dillon CF, Rasch EK, Gu Q, Hirsch R. Prevalence of knee osteoarthritis in the United States: Arthritis data from the Third National Health and Nutrition Examination Survey 1991-94. J Rheumatol. 2006;33(11):2271–9.
3. Firestein GS. Textbook of Rheumatology , 8th ed . Strategies. 2009. 1–5 p.
4. Widhiyanto L, Desnantyo AT, Djuari L, Kharismansha M. Correlation Between Knee Osteoarthritis (OA) Grade and Body Mass Index (BMI) in Outpatients of Orthopaedic and Traumatology Department RSUD Dr. Soetomo. J Orthop Traumatol Surabaya [Internet]. 2019 Dec 9;6(2):71. Available from: https://e-journal.unair.ac.id/JOINTS/article/view/16557
5. Fachrizal A, Irianto KA. Clinical Evaluation of Post Total Hip/ Knee Replacement in Hip/ Knee Spine Syndrome. J Orthop Traumatol Surabaya [Internet]. 2017 Apr 30;6(1):40. Available from: https://e-journal.unair.ac.id/JOINTS/article/view/18033
6. Haviv B, Bronak S, Thein R. The complexity of pain around the knee in patients with osteoarthritis. Isr Med Assoc J [Internet]. 2013 Apr;15(4):178–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23781753
7. Utomo DN, Mahyudin F, Wijaya AM, Widhiyanto L. Proximal fibula osteotomy as an alternative to TKA and HTO in late-stage varus type of knee osteoarthritis. J Orthop [Internet]. 2018 Sep;15(3):858–61. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0972978X18302563
8. Kuyinu EL, Narayanan G, Nair LS, Laurencin CT. Animal models of osteoarthritis: classification, update, and measurement of outcomes. J Orthop Surg Res [Internet]. 2016 Dec 2;11(1):19. Available from: https://josr-online.biomedcentral.com/articles/10.1186/s13018-016-0346-5
9. Waldstein W, Perino G, Gilbert SL, Maher SA, Windhager R, Boettner F. OARSI osteoarthritis cartilage histopathology assessment system: A biomechanical evaluation in the human knee. J Orthop Res. 2016;34(1):135–40.
10. Nagira K, Ikuta Y, Shinohara M, Sanada Y, Omoto T, Kanaya H, et al. Histological scoring system for subchondral bone changes in murine models of joint aging and osteoarthritis. Sci Rep [Internet]. 2020 Dec 22;10(1):10077. Available from: http://www.nature.com/articles/s41598-020-66979-7
11. Van Der Kraan PM, Vitters EL, Van Beuningen HM, Van De Putte LBA, Van Den Berg WB. Degenerative knee joint lesions in mice after a single intra-articular collagenase injection. A new model of osteoarthritis. J Exp Pathol. 1990;71(1):19–31.
12. Widhiyanto L, Utomo DN, Perbowo AP, Hernugrahanto KD. Macroscopic and histologic evaluation of cartilage regeneration treated using xenogenic biodegradable porous sponge cartilage scaffold composite supplemented with allogenic adipose derived mesenchymal stem cells (ASCs) and secretome: An in vivo experiment. J Biomater Appl [Internet]. 2020 Sep 19;35(3):422–9. Available from: http://journals.sagepub.com/doi/10.1177/0885328220934938
13. Yeh T Te, Wen ZH, Lee HS, Lee CH, Yang Z, Jean YH, et al. Intra-articular injection of collagenase induced experimental osteoarthritis of the lumbar facet joint in rats. Eur Spine J [Internet]. 2008 May 26;17(5):734–42. Available from: http://link.springer.com/10.1007/s00586-008-0594-0
14. Botter SM, van Osch GJVM, Waarsing JH, van der Linden JC, Verhaar JAN, Pols HAP, et al. Cartilage damage pattern in relation to subchondral plate thickness in a collagenase-induced model of osteoarthritis. Osteoarthr Cartil [Internet]. 2008 Apr;16(4):506–14. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1063458407002750
15. Kikuchi T, Sakuta T, Yamaguchi T. Intra-articular injection of collagenase induces experimental osteoarthritis in mature rabbits. Osteoarthr Cartil [Internet]. 1998 May;6(3):177–86. Available from:https://linkinghub.elsevier.com/retrieve/pii/S1063458498901108

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Research Article

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Does the frequency of ureaplasma increase in human papillomavirus positive women?

Asena AYAR MADENLİ *

Department of Obstetrics and Gynecology, Liv Hospital Vadistanbul, Istanbul,Turkey

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Abstract

This study aimed to investigate the positive prevalence rate of Ureaplasma in Human papillomavirus (HPV) positive women. The role of sexually transmitted diseases (STD) in developing cervical cancer is evident. Clinical consequences can be prevented by screening and identifying risk factors. This retrospective cohort study was approved by the Ethics Committee of Sisli Memorial Hospital. Ureaplasma and HPV and PAP smear tests were performed for 526 sexually active women aged between 21 and 45 years (mean 31.66±4.91 years) visiting a Sisli Memorial Hospital for routine cervical screening. The association of HPV and Ureaplasma detection with cytological results were examined. The prevalence of Ureaplasma in asymptomatic women was 31%, high-risk HPV (HR-HPV) and Ureaplasma coincidence as 56.6% and the prevalence of Ureaplasma in HR-HPV was 91.5%. Most positive HPV was observed in the age range of 26-30 (50%). The most positive Ureaplasma was observed in the age range of 26-30 (51%). The highest simultaneous occurrence of two infections was observed in the age range of 26-30 (51%). Cervical screening abnormality was observed more in Ureaplasma-positive women. There was a statistically significant association between pap smear result and Ureaplasma (p-value < 0.001). There was a statistically significant association between HPV-positive and Ureaplasma (p-value < 0.001). There was a statistically significant association between HR-HPV and Ureaplasma (p-value < 0.001). Regarding the high prevalence of Ureaplasma in HR-HPV women, screening in women, especially in the age range of 26-30 years, is a breakthrough and can prevent adverse clinical consequences.

Keywords: human papillomavirus, ureaplasma, cervical cancer, sexually transmitted disease

1. Introduction

Based on epidemiological and laboratory studies all over the world, Human Papillomavirus (HPV) is the main factor of cervical cancer (1). The prevalence of persistent genital high risk-HPV (HR-HPV) in the cause of cervical cancer is approximately 99.7% (2). It is one of the most common causes of sexually transmitted diseases (STD) in women below 35 all over the world (3). Cervical cancer is the fourth most common cancer among women, and is almost found in poor and middle-income countries (4,5).

HPV infection is self-limiting, without symptoms, and associated with benign or malignant squamous mucosa proliferation. There are more than 190 types of this virus, which are classified into low-risk and high-risk groups. This infection also affects the quality of women's sexual activities (6-8).

Factors such as smoking, high parity, long-term use of oral contraceptives, other sexually transmitted infections, and co-infection affect the growth of cervical cancer in women with HPV. There is much evidence of a significant association between STD and the development of cervical cancer in HPV-positive women (9).

Ureaplasma is a common STD. Vaginal colonization with Ureaplasma spp. occurs in 40–80% of asymptomatic sexually active women (10). Ureaplasma is a frequent reason for vaginitis, cervicitis, spontaneous abortion, urethritis, and infertility (11). This infection is mainly transmitted through unprotected vaginal sex (12). Small and fastidious bacteria called Ureaplasma species often colonize the lower genitalia of asymptomatic hosts. Studies have shown that these bacteria cause neonatal infection, chorioamnionitis, urethritis, and surgical site infection in transplant recipients (13,14).

This study investigated the positive prevalence rate of Ureaplasma in HPV-positive women. In addition, the prevalence of these two infections was investigated separately and concurrently in different age groups. HR-HPV in women with Ureaplasma were also evaluated.

2. Materials and Methods

The Ethics Committee of Sisli Memorial Hospital approved this retrospective study (Decision no:6, Date:26.02.2021). Five hundred twenty-six asymptomatic healthy women who had Ureaplasma and pap smear tests simultaneously participated in this study from January 2016 - January 2021. The frequency of Ureaplasma infection accompanying abnormal and normal cytology is also planned to be

*Correspondence: asenaayar@yahoo.com.tr

controlled by dividing them into dysplasia subgroups. In addition, it is designed to investigate whether there is an increased incidence of infection in high-grade lesions.

HPV was tested using the digene1 HC2 HR-HPV DNA Test1 (QIAGEN, Gaithersburg, MD) with the Rapid Capture System, which is based on signal amplification using RNA probes to target the entire HR-HPV genome. All steps were performed according to the manufacturer's protocols. Simply, cervical brush samples collected in preserve cytological solution underwent a process that included denaturation, hybridization, capture, and amplification of chemiluminescent signal detection.

Cervical secretion specimens were tested for UU using the CT/NG/UU nucleic acid test kit (HybriBio Ltd, Chaozhou, China). A PCR-fluorescent probe method (48 copies/ box) was used to detect UU.

The inclusion criteria were: (1) the premenopausal women between the ages of 18 and 50. The exclusion criteria were: (1) pregnant women and women in the breastfeeding period; (2) absence of chronic disease, immune suppression and ; (3) women with cervical operation and history of malignities and treatments.

In this study, two groups were considered for comparison: the low-risk group and the high-risk group for HPV infection. Types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 are considered HR-HPV types.

The Kolmogorov-Smirnov test was performed to check the normality, and the nonparametric tests were performed given the non-normality of the groups before the statistical analyses. Mean and standard deviations (SD) were measured to check each continuous variable, such as age. SPSS v20 was used for statistical analyses. A value of $p < 0.05$ was accepted as statistically significant.

When the sample size was calculated with the G Power 3.1 (http://www.gpower.hhu.de/) program, the difference between two independent proportions based on a chi-square tests measure with the effect size of 80%, power of 75% and 0.05 type 1 error, was found to be at least 532 patients (15).

3. Results

The study included five hundred twenty six women (mean age ± SD: 33.66 ± 4.91). The mean age in HPV(+) and Ureaplasma(+) was 31.11±4.81 and 31.80±4.48, respectively. Table 1 shows age information in groups.

As stated in Table 2, a chi-square test found a statistically significant association between HR-HPV and Ureaplasma (p-value < 0.001). There was a statistically significant association between cervical smear result and Ureaplasma (p-value < 0.001). Data in tables 2, 3 and 4 are presented as numbers (percentages).

Table 1. Age information in groups

Variable	N	Minimum	Maximum	Mean	SD
Age(yr)	526	21.00	45.00	31.66	4.91
Age(yr) in HPV (+)	526	22.00	45.00	31.11	4.81
Age(yr) Ureaplasma (+)	526	22.00	45.00	31.80	4.48

Table 2. The relationship between ureaplasma and tests results

Variable		Ureaplasma (-) (n=125) n(%)	Ureaplasma (+) (n=163) n(%)	P
HPV positive	Low-Risk	39(31.2)	14(8.5)	<0.001*
	High-Risk	86(68.8)	149(91.5)	
Cervical smear result	Normal	24(19.2)	8(4.9)	<0.001*
	AGUS	20(16)	28(17.2)	
	ASCUS	53(42.4)	68(41.7)	
	LGSIL	21(16.8)	39(23.9)	
	HGSIL	7(5.6)	12(7.4)	
	Servisit	0(0)	8(4.9)	

HPV: Human papilloma virus, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, CIN: Cervical intraepithelial neoplasia. (+), positive; (-), negative
*A Chi-square test

As stated in Table 3, a chi-square test found a statistically significant association between HPV and Ureaplasma (p-value < 0.001). Data in tables 2, 3 and 4 are presented as numbers (percentages).

Table 3. The relationship between HPV results and Ureaplasma

Variable		HPV (-) (n=238) n(%)	HPV (+) (n=288) n(%)	P
Ureaplasma	No	238(100)	125(43.4)	<0.001*
	Yes	0(0)	163(56.6)	

*A Chi-square test. (+), positive; (-), negative.

As stated in Table 4, the highest frequency of Pap smear results were ASCUS (38.6%), Normal (27.9%), LGSIL (11.8%), AGUS (11%), Servisit (7%) and HGSIL (3.6%). These results were similar in all three age groups.

Table 4. Distribution of age by PAP smear results

Cervical smear result	Total N= 526	18-25 N= 84 (16%)	26-30 N= 100 (19%)	31-45 N= 342 (65%)
Normal	147(27.9)	32(38.1)	21(21.0)	94(27.5)
AGUS	58(11.0)	10(11.9)	15(15.0)	33(9.6)
ASCUS	203(38.6)	29(34.5)	40(40.0)	134(39.2)
LGSIL	62(11.8)	9(10.7)	18(18.0)	35(10.2)
HGSIL	19(3.6)	1(1.2)	4(4.0)	14(4.1)
Servisit	37(7.0)	3(3.6)	2(2.0)	32(9.4)

Table 5 shows distribution of age by infection of HPV and Ureaplasma. Most HPV and Ureaplasma positives were observed in the age group between 26-30. In the age group of 18-25, HPV and Ureaplasma positives had the lowest frequency. The age group over 30 years was similar to the total frequency in cases HPV and Ureaplasma positives.

Table 5. Distribution of age by infection of HPV and Ureaplasma

Infection	Total N= 526	18-25 N= 84 (16%)	26-30 N= 100 (19%)	31-45 N= 342 (65%)
HPV (+)	288(54.7)	50(59.5)	67(67)	171(50)
HPV (-)	238(45.3)	34(40.5)	33(33)	171(50)
Ureaplasma (+)	163(31)	12(14.3)	51(51)	100(29.3)
Ureaplasma (-)	363(69)	72(85.7)	49(49)	242(70.7)
HPV (+) Ureaplasma (+)	163(31)	12(14.3)	51(51)	100(29.3)
HPV (+) Ureaplasma (-)	125(23.7)	38(45.2)	16(16)	71(20.7)
HPV (-) Ureaplasma (+)	122(23.3)	3(3.5)	16(16)	103(30.1)
HPV (-) Ureaplasma (-)	116(22)	31(37)	17(17)	68(19.9)

(+), positive; (-), negative.

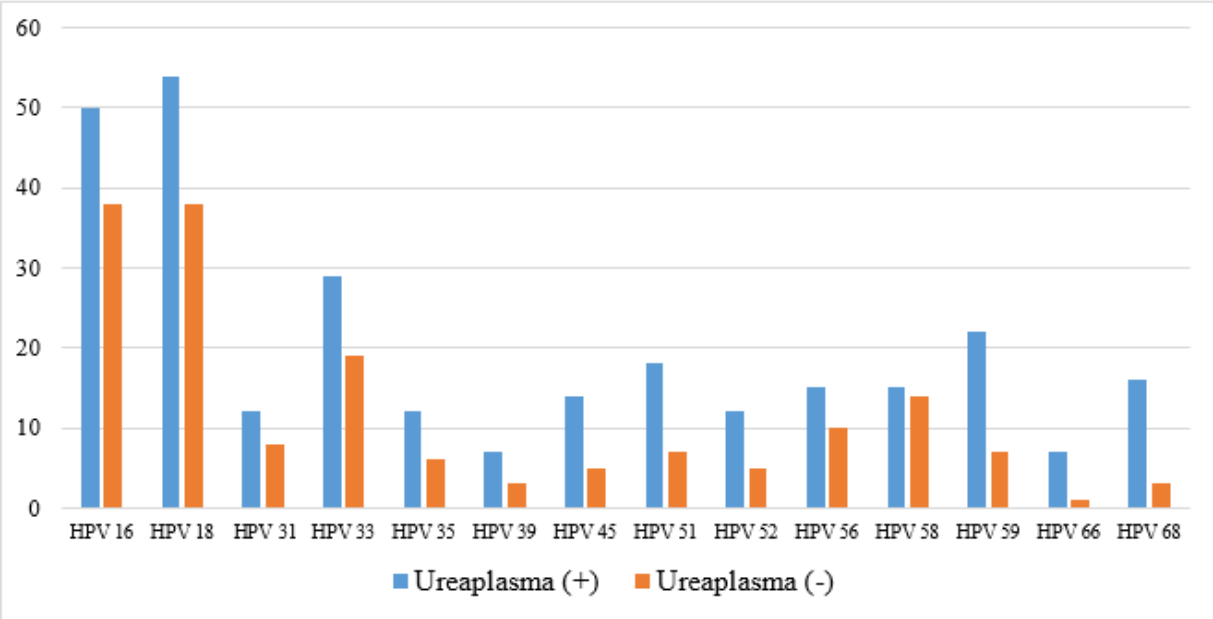

Fig. 1. Comparison of HR- HPV prevalence between ureaplasma positive and ureaplasma negative

There are about 14 HR-HPV types including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. In this study, a comparison was made between the Ureaplasma positive and negative in terms of the prevalence high-risk HPV types (Fig. 1). All high-risk types of HPV were more in the Ureaplasma positive

4. Discussion

The relationship between HR-HPV and cervical cancer is clear. There is evidence that facilitating factors such as sexually transmitted diseases increase the risk of cervical cancer. This study investigated the frequency of Ureaplasma as a sexually transmitted disease in women with HPV infection. In the current study, the Ureaplasma positivity rate was 56.6% in HPV-positive women. The prevalence of Ureaplasma in asymptomatic women was 31%, and the prevalence of Ureaplasma in HR-HPV was 91.5%. This study examined the frequency of Ureaplasma infection in both low and high-risk HPV groups; it was observed that the frequency of Ureaplasma infection is higher in women with HR-HPV infection. By studying different age ranges, women between 26-30 years old were more to have HPV and Ureaplasma. After this group, women between 30-45 years old were more to have Ureaplasma and HPV infection than women under 25. A significant difference was found when the HPV type and cervical smear results were compared in terms of the Ureaplasma result. This study indicated that HR-HPV infection was significantly associated with Ureaplasma, meaning that Ureaplasma may serve as a cofactor in developing Cervical cancer.

Previous studies have shown that Ureaplasma infection increases the risk of HPV infection. In their study on 1200 married Chinese women, Zhang et al. (11)showed that the prevalence of Uroplasma was 35.5%, and the coexistence of HPV and Ureaplasma was 8.6%. Xiaolei et al. (16) reported the prevalence of HPV and Ureaplasma coincidence as 53.2% in 233 Chinese women. Kim et al. (17) reported the prevalence of HPV as 33.1%, Ureaplasma (U>104 CFU/ml) as 36%, and HPV and Ureaplasma coincidence as 19.5% in 264 Chinese asymptomatic women. Lv et al. (18) reported the prevalence of HR-HPV as 30.7%, Ureaplasma as 11.9%, and HPV and Ureaplasma coincidence as 15.4% in 826 asymptomatic Chinese women. Parthenis et al. (19) noted the prevalence of Ureaplasma as 18.2%, and HR-HPV and Ureaplasma coincidence as 25.4% in 345 asymptomatic

Greek women. Lopez et al. (20) reported the prevalence of HR-HPV as 17.1%, different types of Ureaplasma between 0.08% to 32.9%, and HR-HPV and Ureaplasma coincidence as 16.7% in 258 asymptomatic Mexican women. Tantengco et al. (21) noted the prevalence of different types of HPV as 75%, and HR-HPV and Ureaplasma coincidence as 22.7% in 44 asymptomatic Filipino women.

Zhang et al. (11) reported a significant relationship between Ureaplasma infection and HR-HPV infection. Camporiondo et al. (22), Kim et al. (17), and Xiaolei C et al. (16) reported a significant relationship between Ureaplasma and HPV. In contrast, Tantengco et al. (21) reported no a significant relationship between these two infections by examining 44 Filipino women. Also, by studying 37 Turkish women, Biyik et al. (23) concluded that there is no significant relationship between Ureaplasma and HPV. The sample size is very small in these two studies, where no significant relationship has been reported.

In conclusion, a high prevalence of Ureaplasma was observed in HR-HPV women, which indicates that Ureaplasma was a risk factor in HR-HPV. These results showed the importance of simultaneous diagnosis of sexually transmitted infections in the genital tract as an essential strategy to prevent adverse clinical outcomes of uterine cancer. Screening for the simultaneous diagnosis of Ureaplasma and HPV infection in asymptomatic women is recommended, especially between 26-30 years of age. It is hoped that this study will attract more attention to the common sexually transmitted diseases, that these diseases will be included in the routine screening, and will inform the patients thus helping reduce cervical cancer a little.

Conflict of interest

The author declared no conflict of interest.

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Authors' contributions

Concept: A.A.M., Design: A.A.M., Data Collection or Processing: A.A.M., Analysis or Interpretation: A.A.M., Literature Search: A.A.M., Writing: A.A.M.

References

1. Erbıyık HI, Palalıoğlu RM. HPV Infection, HPV Vaccines and Cervical Cancer Awareness: A Multi-Centric Survey Study in Istanbul, Turkey. Women Health. 2021; 61(8): 771-82.
2. Okunade KS. Human papillomavirus and cervical cancer. J Obstet Gynaecol. 2020; 40(5): 602-8.
3. Jin H, Kim DH, Lee KE. Human Papillomavirus Prevalence in Urine Samples of Asymptomatic Male Sexual Partners of Women with Sexually Transmitted Diseases. Int J Environ Res Public Health. 2021; 18(21): 11706.
4. WHO. Cervical cancer. 2022 [cited 2022 08.07.2022]; Available from: https://www.who.int/health-topics/cervical-cancer#tab=tab_1.
5. Hull R, Mbele M, Makhafola T, Hicks C, Wang SM, Reis RM, et al. Cervical cancer in low and middle-income countries. Oncol Lett. 2020; 20(3): 2058-74.
6. Gürbüz T, Dokuzeylül Güngör N, Tanrıdan Okçu N, Yurci A. Effects of contraception methods on female sexual function and quality of life. J Surg Med. 2020; 4(12): 1231-35.
7. Dokuzeylül Güngör N. , Gürbüz T. , Yurci A. Hysteroscopic Evaluation of Chronic Endometritis Incidence in Unexplained Infertile Women with Recurrent Implantation Failure: Six Years Experience. Ahi Evran Med J. 2022; 6(1): 64-70.
8. Dokuzeylül Güngör N, Gürbüz T, Bozkurt S, Dokuzeylül B, Or E, Cengiz F. Evaluation of Toxoplasmosis Risk on Anxiety And Perception of Cat Owner Pregnant Women. J. Exp. Clin. Med.. 2021; 38(2): 154-58.
9. Kashyap N, Krishnan N, Kaur S, Ghai S. Risk Factors of Cervical Cancer: A Case-Control Study. Asia Pac J Oncol Nurs. 2019; 6(3): 308-14.
10. Rittenschober-Böhm J, Habermüller T, Waldhoer T, Fuiko R, Schulz SM, Pimpel B, et al. Maternal Vaginal *Ureaplasma* spp. Colonization in Early Pregnancy Is Associated with Adverse Short- and Long-Term Outcome of Very Preterm Infants. Children (Basel). 2021; 8(4): 276.
11. Zhang D, Li T, Chen L, Zhang X, Zhao G, Liu Z. Epidemiological investigation of the relationship between common lower genital tract infections and high-risk human papillomavirus infections among women in Beijing, China. PLoS One. 2017; 12(5): e0178033.
12. Kallapur SG, Kramer BW, Jobe AH. Ureaplasma and BPD. Semin Perinatol. 2013; 37(2): 94-101.
13. Lanao AE, Chakraborty RK, Pearson-Shaver AL. Mycoplasma Infections. [Updated 2022 Apr 22]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.Available from: https://www.ncbi.nlm.nih.gov/books/NBK536927/
14. Jhaveri VV, Lasalvia MT. Invasive *Ureaplasma* Infection in Patients Receiving Rituximab and Other Humoral Immunodeficiencies-A Case Report and Review of the Literature. Open Forum Infect Dis. 2019; 6(10): ofz399.
15. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009; 41(4): 1149-60.
16. Xiaolei C, Taot H, Zongli S, Hongying Y. The role of ureaplasma urealyticum infection in cervical intraepithelial neoplasia and cervical cancer. Eur J Gynaecol Oncol. 2014; 35(5): 571-5.
17. Kim SI, Yoon JH, Park DC, Lee DS, Lee SJ, Choe HS, et al. Co-infection Of *Ureaplasma urealyticum* And Human Papilloma Virus In Asymptomatic Sexually Active Individuals. Int J Med Sci. 2018; 15(9): 915-20.
18. Lv P, Zhao F, Xu X, Xu J, Wang Q, Zhao Z. Correlation between Common Lower Genital Tract Microbes and High-Risk Human Papillomavirus Infection. Can J Infect Dis Med Microbiol. 2019; 2019: 9678104.
19. Parthenis C, Panagopoulos P, Margari N, Kottaridi C, Spathis A, Pouliakis A, Konstantoudakis S, et al. The association between sexually transmitted infections, human papillomavirus, and cervical cytology abnormalities among women in Greece. Int J Infect Dis. 2018; 73: 72-77.
20. Lopez-Arias M, Vazquez-Jimenez S, Martinez-Abundis E, Gomez-Crisostomo NP, Chavez-Blanco A, Contreras-Paredes A,

et al. Genital association of human papillomavirus with *Mycoplasma* and *Ureaplasma* spp. in Mexican women with precancerous lesions. Int J STD AIDS. 2019; 30(10): 969-77.

21. Tantengco OAG, Nakura Y, Yoshimura M, Nishiumi F, Llamas-Clark EF, Yanagihara I. Co-infection of human papillomavirus and other sexually transmitted bacteria in cervical cancer patients in the Philippines. Gynecol Oncol Rep. 2022; 40: 100943.

22. Camporiondo MP, Farchi F, Ciccozzi M, Denaro A, Gallone D, Maracchioni F,et al. Detection of HPV and co-infecting pathogens in healthy Italian women by multiplex real-time PCR. Infez Med. 2016; 24(1): 12-7.

23. Bıyık İ,Keskin N,Gülcan A,İnce O Erten Ö, Şişman Hİ.Coinfection of Ureaplasma urealyticum/Mycoplasma hominis in HPV Positive Women: Frequency, Risk Factors and Multidrug Resistance J Clin Obstet Gynecol.2020; 30(3): 92-9.

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The effects of sperm parameters and sperm DNA damage on pregnancy outcomes of women undergoing intrauterine insemination

Murat ÖNAL[1], Müstecep KAVRUT[2], Nur DOKUZEYLÜL GÜNGÖR[3], Tuğba GÜRBÜZ[4*]

[1]Gynolife IVF Clinic, Lefkosa, Cyprus
[2]Private Clinic,İstanbul, Türkiye
[3]Department of Obstetrics and Gynecology IVF Clinic Bahçeşehir University Göztepe Medikal Park Hospital İstanbul, Türkiye
[4]Department of Obstetrics and Gynocology, Medistate Hospital, İstanbul, Türkiye

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Abstract

In this study, we compared the effects of sperm parameters and sperm DNA damage on pregnancy outcomes of women undergoing intrauterine insemination (IUI) because of an ovulatory dysfunction (OD) and unexplained infertility (UEI). This study was retrospective, and semen samples were collected from records of 88 infertile couples referred to private clinic for infertility treatment from December 2019 to March 2020. The study has two groups: Groups 1: couples with UEI, and Group 2: fertile males with their partners having OD. The participants' age and body mass index (BMI) were 28.20 ± 3.08 and 25.45±2.25, respectively. In the control group, the pregnancy rate was 9/41 (%21.9), and one out of nine patients had a miscarriage. The pregnancy rate in the study group (UEI) was 8/47 (17%), and half of the pregnancies ended as miscarriage. Our results showed that sperm DNA damage increases the abortion rate but has not influenced the pregnancy rate in IUI.

Keywords: sperm DNA damage, pregnancy, unexplained infertility, intrauterine insemination

1. Introduction

According to the report of the World Health Organization, pregnancy failure has affected more than 80 million people in the world (1,2). The origin of infertility can be due to male or female factors or both. Based on this, 40% of male factors, 40% of female factors, and 20% of both male and female factors are involved in infertility (2). Many factors like age, autoimmune diseases and body mass index can affect the sperm parameters (3, 4).

Spermatozoa of infertile men often have different functional and structural defects (5). Standard analysis of semen, which includes concentration, motility, and sperm morphology, is considered a sensitive biological marker. However, these markers cannot give us information about the health of the genetic material of male gametes and cannot be used as predictors of fertility ability (5). Several tests have been introduced based on the physiological and molecular function of sperm in the fertilization process, including the ability of sperm to attach to the transparent layer around the egg in the first stage of fertilization, the chemical penetration power of sperm, and examining the state of DNA damage (5-8).

Sperm makes up half of the genetic material of the embryo resulting from fertilization, so half of the percentage of fertility success depends on the flawless transfer of sperm DNA during its journey from the testicle to the fallopian tubes in women (7). Sperm DNA damage can be caused by DNA fragmentation, improper chromatin packaging, and epigenetic defects (8, 9). Clinical evidence shows that sperm DNA damage has harmful and destructive effects on fertility results, and the amount of these damages is much higher in infertile men than in fertile men (5,6). In addition, various studies indicate that DNA fragmentation can have a negative effect on sperm parameters and the rate of pregnancy (6-9).

Defects in sperm chromatin structure are typically associated with abnormal content of nuclear proteins or DNA strand breaks (10), which are detected using different techniques such as Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), Sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD) test, aniline blue,acridine orange (AO) and Chromomycin A3 (CMA3) (7, 8, 10, 11).

The first step for infertility treatment in many couples is the treatment of intrauterine sperm insemination (IUI). This method is less invasive and less expensive than other assisted reproductive treatment methods.

In this study, the sperm parameters and DNA damage of patients in two groups are retrospectively compared to investigate their effects on pregnancy outcomes.

*Correspondence: drtgurbuz@hotmail.com

2. Materials and Methods

This study was retrospective, and semen samples were collected from records of 88 infertile couples referred to private clinic for infertility treatment from December 2019 to March 2020. The Ethics Committee of Beykoz University approved this retrospective study (Decision no: 1 Date: 26.10.2020). The sperm parameters and DNA damage of subjects are compared to investigate their effects on pregnancy outcomes. The study has two groups: Groups 1: Couples with unexplained infertility (UEI), and Group 2: fertile males with their partners having ovulatory dysfunction (OD). The inclusion criteria were: (1) Men between the ages of 25 and 40. The exclusion criteria were: (1) Absence of diabetes, thyroid dysfunction, and systemic diseases. (2) Men with systemic disease and known history of varicocele are excluded. A total of 47 people in the first and 41 in the second groups were included. All parameters related to participants' sperm were extracted from electronic records and analyzed. After 3-5 days of sexual abstinence semen analyses were performed. Aniline blue staining was used to determine sperm DNA damage as described in the literature (8).

The Kolmogorov-Smirnov test was performed to check the normality, and the nonparametric tests were performed given the groups' non-normality before the statistical analyses. Mean and standard deviations (SD) were measured to check each continuous variable, including age, body mass index (BMI), follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, prolactin (ng/ml), estradiol, spermiogram parameters: volume, numbers (Millions), mobility (%). The Mann-Whitney test was performed to study the difference between the two groups. SPSS v22 was used for statistical analyses. A value of $p<0.05$ was accepted as statistically significant.

Data analysis was performed on SPSS 21 (SPSS Inc., IBM, Armonk, NY, USA). Normality of distribution was evaluated with the Shapiro-Wilk test. Normally distributed variables were analyzed with the independent samples t-test. Non-normally distributed variables were analyzed with the Mann-Whitney U test. Spearman correlation coefficients were calculated for the assessment of relationships between continuous variables. The distributions of categorical variables were evaluated using Pearson Chi-square tests or Fisher's exact tests. Logistic regression analysis (backward conditional method) was performed to determine risk factors affecting fertility status. Data were given as mean ± standard deviation for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables. Differences were considered statistically significant if the p-value <0.05. To calculate the sample size with the G-Power 3.1 (http://www.gpower.hhu.de/) program, two groups' total mean was measured based on the Mann-Whitney test with the power of 95%, effect size of 50%, and 0.05 type 1 error for at least 92 patients (12).

3. Results

This study was conducted on 88 males in two groups: Case-Couples with UEI who underwent IUI, and control- fertile males with their wife having OD. The average age of males and females and the BMI of both groups were not significantly different. The demographic and laboratory characteristics of both groups are shown in Table 1.

Table 1. Demographic and laboratory characteristics of both groups

Study parameters	Case-UEI (n=47) Mean±SD	Control-OD (n=41) Mean±SD	*p-value*
Male age	33.17±3.33	33.1±3.92	0.977*
Female age	30.02±3.48	30.05±3.09	0.969**
Male BMI	24.61±1.78	24.68±1.52	0.651*
Duration of infertility ±year	3.26±1.47	3.22±1.06	0.976*
FSH	5.95±2.48	4.76±1.42	0.027*
LH	4.46±1.64	4.52±1.37	0.587*
Total testosterone ±ng/ml	5.8±2.69	4.68±1.28	0.062*
E2 ±pg/ml	17.61±5.43	14.88±2.06	0.060*
Prolactin ±ng/ml	13.27±3.56	14.47±2.09	0.074*
Varicocele	0±0%	0±0%	0.862***
Cigarette	10±24.4%	24±51%	**0.036*** **
Alcohol	41±100%	45±95.7%	**0.025*** **

* Mann-Whitney test **Independent t-test ***Pearson Chi-square test

No significant difference was observed in the variables duration of infertility, FSH, LH, total testosterone, E2, prolactin and varicocele between the two groups.

An interesting result was the significantly higher consumption of cigarettes and alcohol in the control group, whose patients partners had infertility complications, and the participants themselves had no fertility complications (p=0.036 and p= 0.025). Participants in the case group (UEI) who had infertility problems themselves had significantly less alcohol and cigarette consumption than the control group. Table 2 shows the comparison of sperm characteristics of the two groups.

Table 2. Comparison of sperm characteristics of two groups

Sperm quality parameters	Case-UEI (n=47) Mean±SD	Control (n=41) Mean±SD	*p*
Sperm count ±/mL	33.92±24.49	35.73±19.85	0.351*
Total motility	59.3±15.89	61.73±9.53	0.870*
Sperm morphology	1.74±0.71	2.2±0.68	**0.006***
DNA damage ±%	38.19±15.55	25.15±5.69	**<0.001***

* Mann-Whitney test

There was no significant difference in sperm count and total motility between the two groups. Also, the sperm morphology and DNA damage were significantly different between the two groups (p-value= 0.006 and p-value= 0.001). Fig. 1 shows the information related to pregnancy and abortion in two groups. In the control group, the pregnancy rate was 9/41 (21.9%), and one out of nine patients miscarried. The pregnancy rate in the study group was 8/47 (17%), and half of the participants miscarried.

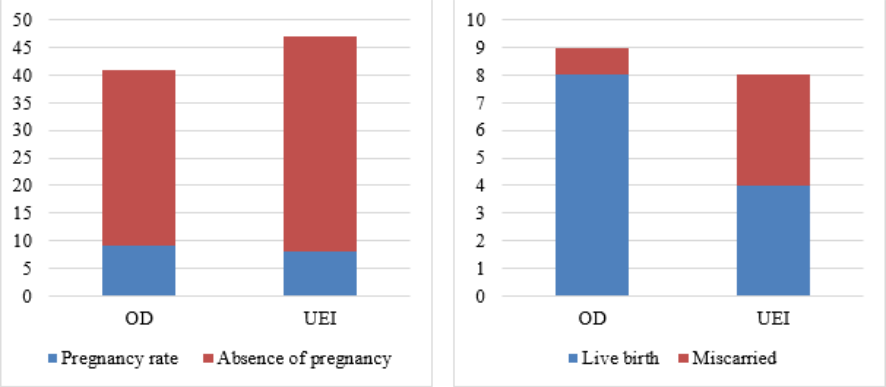

Fig. 1. Information related to pregnancy and abortion in two groups

4. Discussion

15-20% of couples are infertile despite trying to have children, which is the cause of half of the infertility cases due to the male factor. The majority of male infertility is associated with abnormal sperm parameters (11). Therefore, these people are candidates for assisted reproductive methods and may have experienced these treatments many times. One of the essential factors in the success of gamete fertilization is the health of sperm DNA and chromatin. Various studies show that the lower the quality of these sperm parameters, the more problems the sperm DNA health faces (10,12-16). Therefore, before choosing the appropriate treatment method, evaluating the amount of sperm DNA damage is recommended (16). In many of these cases, if there is damage to sperm DNA, it is possible to improve sperm quality by performing appropriate therapeutic interventions. Ensuring the health of sperm DNA is one of the most critical things in the egg fertilization process, the continuation of embryo development, and the success of assisted reproductive methods (17).

Therefore, sperm DNA health is one of the most critical characteristics of sperm, and its evaluation can provide valuable information regarding fertility ability (18). Therefore, in the present research, we investigated the effect of DNA damage on fertility and abortion rates. In this study, patients used IUI as a treatment method. Our results showed a significant difference in sperm DNA damage between the two study groups and the control group, which is comparable to the results of previous studies in this field (15-19).

This study showed a significant relationship between DNA damage and abnormal sperm morphology. These results agree with the previous research that there is a significant relationship between semen parameters and DNA damage (20-22). Therefore, it can be said that the sperm of infertile people with abnormal morphology probably have more DNA damage compared to fertile people with normal seminal fluid parameters. Also, considering that this relationship is statistically significant but has a low correlation coefficient, it cannot be concluded that every sperm that is normal in terms of morphology is also healthy in terms of genetic material or aneuploidy. Therefore, some people have normal sperm parameters but different degrees of DNA damage, which can cause much UEI (20). In addition, sperm DNA damage is probably more affected by improper chromatin packaging than sperm morphology abnormalities during spermatogenesis (21).

This study showed a significant relationship between DNA damage and vitamin deficiency. These findings indicate that vitamin deficient sperm are more susceptible to DNA damage. The results obtained from our study are comparable to those of previous studies in this field (15, 22, 23).

The effect of DNA damage on the pregnancy rate is conflicting among different studies (14,16, 21,24). There are many reasons for these contradictions, which can be related to factors such as the sperm preparation process, the fertilization process (CSI, IUI, IVF), the DNA damage evaluation method (TUNEL, SCSA, SCD, AO, COMET), and the way the test is performed (manual, automatic). In the present study, no correlation was observed between DNA damage and the IUI pregnancy rate of the two groups, which is consistent with the results of other findings in this field (22-24). However, a significant relationship between DNA damage and increased miscarriage was observed, so sperm containing damaged DNA have more miscarriage risk. The results of this study are compatible with previous studies in this field (23-26). Despite the conflicting results presented in this field in different studies, our findings indicate that sperm DNA damage has no effect on the pregnancy rate in IUI patients of the two groups, but this factor can probably affect the miscarriage rate. The main limitation of this study was the small sample size. It is suggested to investigate the impact of DNA damage on pregnancy outcomes in a higher sample size.

Seminal fluid samples are generally heterogeneous and probably contain sperm with different defects. These defects can be related and likely affect pregnancy and the fetus's early development. Also, the method of sperm selection, based on its functional capacity, can play an essential role in advancing the treatment result. From this study and other studies, it can be concluded that the defects of the sperm DNA damage affect the abortion rate, but it has not influenced the pregnancy rate. However, the effect of these defects on babies

resulting from assisted reproductive techniques needs further study.

Conflict of interest
The authors declared no conflict of interest.

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Authors' contributions
Concept: M.Ö., N.D.G., T.G., Design: M.K., T.G., Data Collection or Processing: N.D.G., M.Ö., M.K., Analysis or Interpretation: M.K., T.G., Literature Search: M.K., N.D.G., Writing:M.Ö., T.G.

References
1. Agarwal A, Panner Selvam MK, Baskaran S, Cho C-L. Sperm DNA damage and its impact on male reproductive health: a critical review for clinicians, reproductive professionals and researchers. Expert review of molecular diagnostics. 2019; 19(6): 443-57.
2. Antonouli S, Papatheodorou A, Panagiotidis Y, Petousis S, Prapas N, Nottola SA, et al. The impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes. Archives of gynecology and obstetrics. 2019; 300(1): 207-15.
3. Beaud H, Tremblay AR, Chan PT, Delbes G. Sperm DNA damage in cancer patients. Genetic Damage in Human Spermatozoa: Springer; 2019; 189-203.
4. Casanovas A, Ribas-Maynou J, Lara-Cerrillo S, Jimenez-Macedo AR, Hortal O, Benet J, et al. Double-stranded sperm DNA damage is a cause of delay in embryo development and can impair implantation rates. Fertility and sterility. 2019; 111(4): 699-707.
5. Chen Q, Zhao J-Y, Xue X, Zhu G-X. The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta analysis. Reproductive Toxicology. 2019;86:50-5.
6. Colacurci N, De Leo V, Ruvolo G, Piomboni P, Caprio F, Pivonello R, et al. Recombinant FSH improves sperm DNA damage in male infertility: a phase II clinical trial. Frontiers in endocrinology. 2018; 9: 383.
7. Deng C, Li T, Xie Y, Guo Y, Yang Qy, Liang X, et al. Sperm DNA fragmentation index influences assisted reproductive technology outcome: A systematic review and meta-analysis combined with a retrospective cohort study. Andrologia. 2019; 51(6): e13263.
8. Güngör K, Dokuzeylül Güngör N,Basar MM, Cengiz F, Ersahin SS, Cil K. Relationship between serum vitamin D level ,semen parameters and sperm DNA damage in men with unexplained infertility Eur Rew Med Pharmacol Sci 2022; 26(2): 499-505.
9. Esteves SC. Interventions to prevent sperm DNA damage effects on reproduction. Genetic damage in human spermatozoa: Springer; 2019; 119-48.
10. Gill K, Rosiak-Gill A, Jakubik J, Patorski L, Lukaszuk M, Piasecka M. The higher risk for sperm DNA damage in infertile men. Ginekologia polska. 2019; 90(12): 684-91.
11. Esteves SC, Santi D, Simoni M. An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men. Andrology. 2020; 8(1): 53-81.
12. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods. 2007; 39(2): 175-91.
13. Haddock L, Gordon S, Lewis SE, Larsen P, Shehata A, Shehata H. Sperm DNA fragmentation is a novel biomarker for early pregnancy loss. Reproductive BioMedicine Online. 2021; 42(1): 175-84.
14. Kabukçu C, Çil N, Çabuş Ü, Alataş E. Effect of ejaculatory abstinence period on sperm DNA fragmentation and pregnancy outcome of intrauterine insemination cycles: A prospective randomized study. Archives of Gynecology and Obstetrics. 2021; 303(1): 269-78.
15. Kim GY. What should be done for men with sperm DNA fragmentation? Clinical and experimental reproductive medicine. 2018; 45(3): 101.
16. McQueen DB, Zhang J, Robins JC. Sperm DNA fragmentation and recurrent pregnancy loss: a systematic review and meta-analysis. Fertility and sterility. 2019; 112(1): 54-60.
17. Nicopoullos J, Vicens-Morton A, Lewis SE, Lee K, Larsen P, Ramsay J, et al. Novel use of COMET parameters of sperm DNA damage may increase its utility to diagnose male infertility and predict live births following both IVF and ICSI. Human Reproduction. 2019; 34(10): 1915-23.
18. Paoli D, Pallotti F, Lenzi A, Lombardo F. Fatherhood and sperm DNA damage in testicular cancer patients. Frontiers in endocrinology. 2018; 9: 506.
19. Ribas-Maynou J, Benet J. Single and double strand sperm DNA damage: different reproductive effects on male fertility. Genes. 2019; 10(2):105.
20. Ribas-Maynou J, Yeste M, Becerra-Tomás N, Aston KI, James ER, Salas-Huetos A. Clinical implications of sperm DNA damage in IVF and ICSI: updated systematic review and meta-analysis. Biological Reviews. 2021; 96(4): 1284-300.
21. Selvam MKP, Agarwal A. A systematic review on sperm DNA fragmentation in male factor infertility: laboratory assessment. Arab Journal of Urology. 2018; 16(1): 65-76.
22. Sugihara A, Van Avermaete F, Roelant E, Punjabi U, De Neubourg D. The role of sperm DNA fragmentation testing in predicting intra-uterine insemination outcome: A systematic review and meta-analysis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2020; 244: 8-15.
23. Tvrdá E, Arroyo F, Gosálvez J. Dynamic assessment of human sperm DNA damage I: The effect of seminal plasma-sperm co-incubation after ejaculation. International Urology and Nephrology. 2018; 50(8): 1381-8.
24. Yang H, Li G, Jin H, Guo Y, Sun Y. The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle. Translational andrology and urology. 2019; 8(4): 356.
25. Zhang J, Xue H, Qiu F, Zhong J, Su J. Testicular spermatozoon is superior to ejaculated spermatozoon for intracytoplasmic sperm injection to achieve pregnancy in infertile males with high sperm DNA damage. Andrologia. 2019; 51(2): e13175.
26. Zheng W-W, Song G, Wang Q-L, Liu S-W, Zhu X-L, Deng S-M, et al. Sperm DNA damage has a negative effect on early embryonic development following in vitro fertilization. Asian journal of andrology. 2018; 20(1): 75.

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Evaluation of mammographic features in women with adenomyosis

Nezaket KADIOĞLU[1,*], Ayçağ YORGANCI[2], Harika GUMGUMCU[2], M. Kuntay KOKANALI[2], Şebnem ÖZYER[2], Yaprak ENGIN USTUN[3]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Yüksek Ihtisas University, Ankara, Türkiye
[2]Department of Obstetrics and Gynecology, University of Health Sciences, Ankara City Hospital, Ankara, Türkiye
[3]Department of Obstetrics and Gynecology, University of Health Sciences, Etlik Zübeyde Hanım Women's Health Education and Research Hospital, Ankara, Türkiye

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Abstract

This study aimed to investigate the mammographic features in women with adenomyosis to determine the relationship between adenomyosis and breast disease. In this study, the mammographic features of women with adenomyosis were recorded. For the control group, women who had mammography without any gynaecologic complaints and with normal pelvic ultrasound were selected. The adenomyosis group had higher breast density, more micro and macro calcifications, and higher BIRADS-mammography classification than the control group. When patients with low mammographic density (Density A - B, n = 80) and high mammographic density (Density C - D, n = 60) were compared, there were no statistically significant differences between the groups except the presence of adenomyosis. When patients were compared according to the BIRADS 1 - 2 (n=114) and BIRADS 3 - 4 (n=26) category, age ≥ 49.5, gravidity ≥ 3, parity ≥ 2, and the presence of adenomyosis were significantly higher in the BIRADS 3 - 4 category. In the logistic regression analysis, the presence of adenomyosis was found to be the sole factor for BIRADS 3-4 category. The results of our study suggested that patients with adenomyosis have an increased risk of higher mammographic breast density and BIRADS 3 classification.

Keywords: BIRADS, breast density, gynecology, mammography, uterine adenomyosis

1. Introduction

Adenomyosis is characterized by the presence of ectopic endometrial glands and stroma within the myometrium. Abnormal uterine bleeding, pelvic pain, and sub infertility are the typical symptoms of adenomyosis (1, 2). It is difficult to report the true prevalence of adenomyosis since histopathological confirmation is required although clinical symptoms and imaging modalities suggest adenomyosis. (3). Besides, leiomyoma and endometriosis that cause similar symptoms are often associated with adenomyosis (1, 4). The traditional belief of adenomyosis as a disease of multiparous women of perimenopausal age has begun to change with the awareness of the disease and the advances in diagnostic technologies (3). Nevertheless, most women who present with the full symptoms of adenomyosis require hysterectomy during the perimenopausal period.

The etiopathogenesis of adenomyosis has not been fully elucidated. Several factors have been suggested facilitating the invasion of the myometrium with the endometrial cells or transformation of Müllerian remnants to adenomyosis (5-7). Local sex steroid hormonal imbalance and inflammatory status are the main ones among these factors (5-7). Given that these factors are also involved in benign and malignant breast diseases (8, 9), we aimed to investigate the relationship between adenomyosis and breast disease. For this purpose, we evaluated the mammographic features in a cohort of women with histopathologically proven adenomyosis.

2. Materials and Methods

In this retrospective study, the histopathological records of women who had undergone hysterectomy in our Hospital between 2013-2017 were reviewed. Approval was obtained from the review board of the institution (10-11/2018). Patients who had clinically adenomyosis symptoms and histopathologically proven diagnosis of diffuse adenomyosis or adenomyoma were included in the study. Histopathological reports showing focal adenomyosis, uterine leiomyoma > 1cm in hysterectomy specimen, and other premalignant and malignant uterine, cervical or ovarian pathologies were excluded. Other exclusion criteria were patients with a history of local or systemic hormonal treatments due to adenomyosis symptoms, prior use of oral contraceptive pills, benign or

*Correspondence: nzkt81@gmail.com

malignant breast disease, breast biopsy, treatment or operation due to endometriosis. Demographic and clinical characteristics of patients who had mammograms with/without breast ultrasound (USG) six months before or after the hysterectomy were included in the analysis. Uterine volume was calculated according to the pelvic ultrasound measurements performed in the preoperative evaluation period. Endometrial biopsy results performed in the preoperative evaluation period were also recorded. The control group consisted of women who were admitted for routine gynaecologic follow-up. Inclusion criteria for the control group were women who had mammography and/or breast USG without any gynaecologic complaints and with normal pelvic ultrasound. Similarly, women with a history of benign or malignant breast disease, breast biopsy, treatment or operation due to endometriosis, and other malignant diseases were also excluded from the control group.

The Breast Imaging Reporting and Data System (BIRADS) is being used since 1993 for the standard reporting of breast pathology seen on mammograms and ultrasound (10). For mammography, the BIRADS lexicon includes the following principal headlines for reporting: 1) Breast density is the comparison of the fat tissue and fibroglandular tissue in the breast and classified as (A) if the breasts are almost entirely fatty; (B) if there are scattered areas of fibroglandular density; (C) if the breasts are heterogeneously dense, which may obscure small masses; and (D) if the breasts are extremely dense, which lowers the sensitivity of mammography. 2) Mass shape classified as oval, round, and irregular. 3) Calcifications are reported as benign, intermediate, and suspicious. 4) Architectural distortion 5) Asymmetries classified as asymmetry, global asymmetry, developing asymmetry, and focal asymmetry. 6) Intramammary lymph nodes 7) Skin lesions 8) Solitary dilated duct 9) Associated findings. 10) Location of the lesion. The features on mammography are categorized as BIRADS 0 to 6 according to benign and malign characteristics. The reports of mammography were reviewed for both study groups and the reported features were noted.

Data analysis was performed by the IBM SPSS Statistics version 22 program (SPSS, Chicago, IL, USA). The suitability of continuous variables to normal distribution was examined with the Shapiro-Wilk test. Since not all the variables were normally distributed, the values were given as median (min. – max.). The descriptive statistics of continuous variables were done by the Mann-Whitney test. The descriptors of the questionnaires were shown in numbers and percentages. Crosstables were created for the categorical variables and a Chi-square test was applied to investigate the intergroup differences. Data were analyzed at a 95% confidence level and $p<0.05$ was considered to be significant. The effects of the confounding variables to BIRADS 3-4 classifications were sought by logistic regression analysis. BIRADS 3-4 was the dependent variable. The area under the receiver-operating characteristics (ROC) curve was used to determine the cut-off values. The following predictors were examined: age < 49.5, and ≥ 49.5, gravidity < 3 and ≥ 3; parity < 2 and ≥ 2, and the presence of adenomyosis (yes or no). Odds ratios (OR) for the predictors including 95 % CI's were calculated.

3. Results

There were 70 patients in the adenomyosis group who met the inclusion and exclusion criteria. The flowchart of the adenomyosis group is shown in Fig. 1.

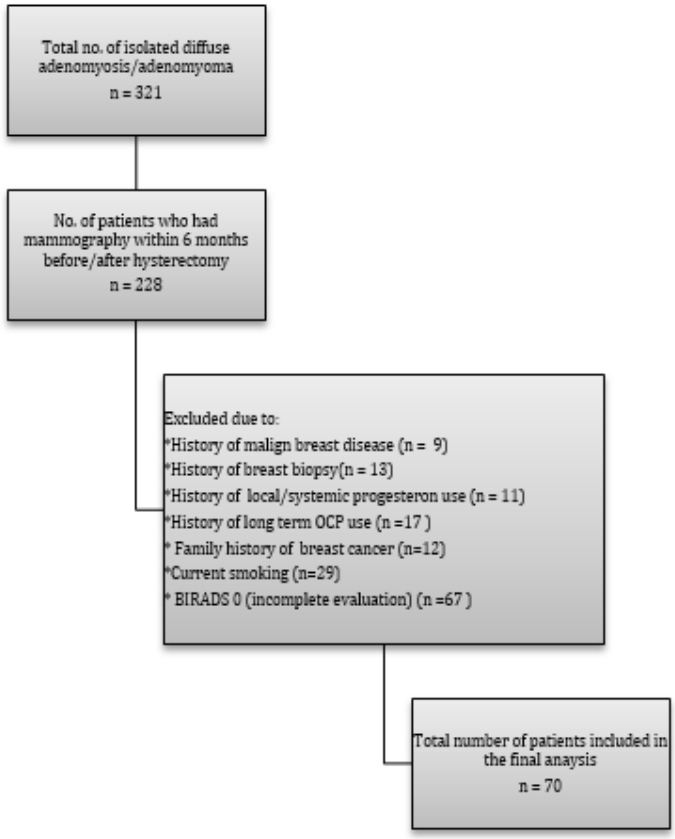

Fig. 1. Flowchart of the adenomyosis group

For the control group, 70 patients who met the criteria were selected from the routine follow-up patients. The demographic and clinical characteristics of the adenomyosis and the control groups are shown in Table 1. The median age of the adenomyosis group was significantly higher than the control group [49 (40-55) vs. 47 (41-55), $p = 0.012$; respectively]. While the number of gravidity, parity, and voluntary pregnancy terminations were significantly higher in the adenomyosis group than the controls, the BMI, age of menarche, and first birth were similar between groups. The median uterine volume was 177 (40-582) cm^3 in the adenomyosis group. Pathological reports of preoperative endometrial biopsies of the adenomyosis group revealed benign findings (30%), endometrial polyp (32.8%), endometrial hyperplasia without atypia (28.6%), and endometrial hyperplasia with atypia (8.6%).

Table 1. The demographic and clinical characteristics of the adenomyosis and the control group

	Adenomyosis group (n=70)	Control group (n=70)	P value
Age	49 (40-55)	47 (41-55)	**0.012**
BMI	30 (21-46)	30 (21-47)	0.9
Gravida	4 (0-8)	2 (0-10)	**< 0.001**
Parity	3 (0-6)	2 (0-7)	**< 0.001**
Abortus	0 (0-4)	0 (0-3)	0.13
D&C	0 (0-3)	0 (0-3)	**0.05**
Age of Menarche	13 (11-16)	13 (11-16)	0.53
Age at first birth	27 (17-35)	26 (17-35)	0.56
Uterine volume (cc)	177 (40-582)	N/A	N/A
Endometrial biopsy			
Benign	21 (30%)		
Endometrial polyp	23 (32.8%))		
Endometrial hyperplasia w/o atypia	20 (28.6%)	N/A	N/A
Endometrial hyperplasia w atypia	6 (8.6%)		

BMI: Body mass index; D&C: Dilation and curettage
P value <0.05, statistically significant

The mammographic findings of the adenomyosis and the control group are shown in Table 2. The adenomyosis group had higher breast density, more micro and macro calcifications, and higher BIRADS-mammography classification than the control group. All calcifications were reported as benign calcifications.

Table 2. Mammographic findings of the adenomyosis and the control group

	Adenomyosis group (n=70)	Control group (n=70)	P value
Breast density			
A	7 (10%)	11 (15.7 %)	
B	24 (34.3%)	38 (54.3 %)	**0.024**
C	27 (38.6%)	15 (21.4 %)	
D	12 (17.1%)	6 (8.6 %)	
Calcifications			
None	25 (35.7%)	45 (64.3%)	
Micro	24 (34.3%)	12 (17.1%)	**0.001**
Macro	8 (11.4%)	10 (14.3%)	
Micro + Macro	13 (18.6%)	3 (4.3%)	
Extra			
None	38 (54.3)	48 (68.6%)	
Nodular density	14 (20.0%)	11 (15.7%)	0.201
Focal asymmetry	18 (25.7%)	11 (15.7%)	
Intramammary lymph nodes			0.13
No	58 (82.9%)	64 (91.4%)	
Yes	12 (17.1%)	6 (8.6%)	
BIRADS-mammography			
1	13 (18.6 %)	35 (50.0%)	**<0.001**
2	35 (50.0%)	31 (44.3%)	
3	22 (31.4%)	4 (5.7%)	
4	-	-	

P value <0.05, statistically significant

Since high breast density is an independent risk factor for breast cancer, we compared patients with low mammographic density (Density A - B, n = 80) and high mammographic density (Density C - D, n = 60). There were no statistically significant differences between the groups except the presence of adenomyosis. While there were 31 (38.8%) patients with adenomyosis in the Density A-B group, there were 39 (65%) patients in the Density C-D group (p = 0.002).

Lastly, we grouped patients according to the BIRADS 1 - 2 and BIRADS 3 - 4 categories. There were 114 patients in the BIRADS 1 - 2 group and 26 in the BIRADS 3 - 4 group. Age ≥ 49.5, gravidity ≥ 3, parity ≥ 2, and the presence of adenomyosis were significantly higher in the BIRADS 3 - 4 category. When the effects of the confounding variables to BIRADS 3 - 4 classifications were sought by logistic regression analysis, the presence of adenomyosis was found to be the sole factor for BIRADS 3 - 4 category [OR 0.19 (95% CI: 0.055-0.636), p = 0.007] (Table 3).

Table 3. Logistic regression analysis of the BIRADS 3-4 group with regard to confounding variables

	Wald	S. E.	P value	Odds Ratio (95% CI)
Age ≥ 49.5	3.158	0.479	0.076	0.43 (0.167- 1.092)
Gravida ≥ 3	0.019	0.563	0.89	1.08 (0.359-3.255)
Parite ≥ 2	2.310	0.624	0.129	0.41 (0.132- 1.292)
Adenomyosis	7.216	0.395	**0.007**	**0.19 (0.055-0.636)**

P value <0.05, statistically significant

4. Discussion

The results of our study suggested that patients with adenomyosis have an increased risk of higher mammographic breast density and BIRADS 3 classification. However, we could not conclude that these mammographic findings will lead to an increased risk of breast cancer in women with adenomyosis.

Breast density is a mammographic finding that is strongly associated with breast cancer risk (11, 12). The fibroglandular tissue appears as white on mammograms as it attenuates X-rays more than fatty tissue (12). Since the majority of breast cancers arise from the glandular and stromal cells, the risk of breast cancer increases with the increase in mammographic breast density. Moreover, underlying cancer may not be visible due to radio-opaque dense tissue. When the extremely dense breasts (Category D) were compared with the almost entirely fatty breasts (Category A), there is a 4.64-fold increase in the risk of breast cancer for the extremely dense breasts (11).

The Breast Imaging Reporting and Data System lexicon has been developed to report mammographic features among radiologists in a standardized manner and for clinicians to standardize their follow-up and management according to the final classification. Women with BIRADS 1 (negative) and 2 (benign findings) categories have the lowest risk of breast cancer (13). On the other hand, BIRADS 3 (probably benign) category is still questionable as it means that the risk of malignancy is lower than 2%; however, it also implies that these probably benign findings should be reassessed within six months (14). According to the results of a recent study with the largest series of BIRADS 3 cases, Berg et al.

reported 1.86% breast cancer over two years, and 57.8% of detected cancers were diagnosed in the first 6 months or earlier, confirming the role of short-range follow-up (15).

The next problem is to explain why the mammographic breast findings of adenomyosis patients are in the higher risk group for breast cancer compared to the control group. Classically both adenomyosis and breast neoplasia are defined as oestrogen-dependent diseases. Indeed, the coexistence of adenomyosis and breast tumour has been shown in many ancient animal studies (16-18). In mice with different breast tumor potentials, it has been shown that all mammary tumour-bearing animals develop uterine adenomyosis (16, 18). High levels of prolactin and growth hormone by pituitary grafting also resulted in both uterine adenomyosis and mammary tumours (17). In addition, tamoxifen use due to chemoprevention of breast cancer is highly associated with uterine adenomyosis in postmenopausal women (19, 20). Eutopic endometrium in adenomyosis shows altered metabolism of steroid sulphatase, aromatase, and 17ß-hydroxysteroid dehydrogenase enzymes leading to local hyperoestrogenism (21-23). In terms of adenomyosis pathophysiology, local hyperoestrogenism plays an active role both in epithelial-mesenchymal transition (24) and hyperperistalsis of the sub endometrial myometrium activating “tissue injury and repair” mechanisms (25). On the other hand, the place of these three main steroidogenic enzymes in breast cancer is indisputable (9, 26, 27). Thus, we can speculate that similar epigenetic, inflammatory, and hormonal pathways might be involved in the pathophysiology of the two lesions. However, the relationship between adenomyosis and cancers has been sought in a couple of studies (28, 29). Although no relationship was found between adenomyosis and breast cancer in both studies, Kok et al. found an increased risk of ovarian, endometrial, and colorectal cancers (28), while Yeh et al. found higher risks of endometrial and thyroid cancers in women with adenomyosis (29).

This study has several limitations to consider. First of all, we could not exclude selection bias due to the retrospective design of the study. Secondly, the radiologic features were noted from the reports of the mammograms, thus the interobserver bias could not be evitable. Lastly, there were a high number of patients excluded due to BIRADS 0 (incomplete evaluation), which might be the reason for not finding a higher classification other than BIRADS 3. On the other hand, we have included patients who have not used any hormonal medications due to adenomyosis or contraception. In addition, since we excluded those with a history of breast biopsy and a personal and family history of malignant breast disease, we only tried to examine the effects of adenomyosis on the breast. Furthermore, all our patients with adenomyosis were histopathologically proven cases.

In conclusion, the results of our study point out the importance of breast screening of women with adenomyosis. We hope that our study will lead to prospective studies that will investigate both the molecular history and clinical consequences of breast diseases in women with adenomyosis.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors’ contributions

Concept: N.K., K.K., S.O., Design: N.K., K.K., S.O., Data Collection or Processing: N.K., Y.E.U., Analysis or Interpretation: N.K., H.G., A.Y., Literature Search: N.K., K.K., S.O., Writing: N.K.

References

1. Li X, Liu X, Guo SW.Clinical profiles of 710 premenopausal women with adenomyosis who underwent hysterectomy.J Obstet Gynaecol Res.2014;40(2):485-94.

2. Szubert M, Kozirog E, et al.Adenomyosis and Infertility-Review of Medical and Surgical Approaches.Int J Environ Res Public Health.2021;18(3).

3. Protopapas A, Grimbizis G, et al.Adenomyosis: Disease, uterine aging process leading to symptoms, or both?Facts Views Vis Obgyn.2020;12(2):91-104.

4. Leyendecker G, Bilgicyildirim A, Inacker M, Stalf T, Huppert P, Mall G, Böttcher B, Wildt L. Adenomyosis and endometriosis. Re-visiting their association and further insights into the mechanisms of auto-traumatisation. An MRI study. Arch Gynecol Obstet. 2015 Apr;291(4):917-32. doi: 10.1007/s00404-014-3437-8. Epub 2014 Sep 21. PMID: 25241270; PMCID: PMC4355446.

5. Donnez J, Donnez O, Dolmans MM.Uterine adenomyosis, another enigmatic disease of our time.Fertil Steril.2018;109(3):369-70.

6. Bourdon M, Santulli P, et al.Immunological changes associated with adenomyosis: a systematic review.Hum Reprod Update.2021;27(1):108-29.

7. Stratopoulou CA, Donnez J, Dolmans MM.Origin and Pathogenic Mechanisms of Uterine Adenomyosis: What Is Known So Far.Reprod Sci.2020.

8. Grebic D, Gulic T, et al.The Role of Innate Immunity in the Pathogenesis of Breast Cancer.Breast Care (Basel).2021;16(1):1-5.

9. Hilborn E, Stal O, Jansson A.Estrogen and androgen-converting enzymes 17beta-hydroxysteroid dehydrogenase and their involvement in cancer: with a special focus on 17beta-hydroxysteroid dehydrogenase type 1, 2, and breast cancer.Oncotarget.2017;8(18):30552-62.

10. Spak DA, Plaxco JS, Santiago L, Dryden MJ, Dogan BE. BI-RADS((R)) fifth edition: A summary of changes. Diagn Interv Imaging.2017;98(3):179-90.

11. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev.2006;15(6):1159-69.

12. Boyd NF, Martin LJ, Bronskill M, Yaffe MJ, Duric N, Minkin S. Breast tissue composition and susceptibility to breast cancer. J Natl Cancer Inst. 2010 Aug 18;102(16):1224-37. doi: 10.1093/jnci/djq239. Epub 2010 Jul 8. PMID: 20616353; PMCID: PMC2923218.

13. Kerlikowske K, Cook AJ, Buist DS, Cummings SR, Vachon C, Vacek P, et al. Breast cancer risk by breast density, menopause, and postmenopausal hormone therapy use.J Clin Oncol.2010;28(24):3830-7.

14. Boyer B, Canale S, et al. Variability and errors when applying the BIRADS mammography classification.Eur J Radiol.2013;82(3):388-97.

15. Berg WA, Berg JM, et al. Cancer Yield and Patterns of Follow-up for BI-RADS Category 3 after Screening Mammography Recall in the National Mammography Database.Radiology.2020;296(1):32-41.

16. Nagasawa H KR, Naito T, Ohmiya S, Mori T. Relationship between mammary tumourigenesis and uterine adenomyosis in four strains of mice.In Vivo.1987;1(4):237-40.

17. Huseby RA SM, Talamantes F. Ectopic Pituitary Grafts in Mice: Hormone Levels, Effects on Fertility, and the Development of Adenomyosis Uteri, Prolactinomas, and Mammary Carcinomas.Endocrinology.1985;116(4):1440-8.

18. Nagasawa H, Naito T. Enhanced potentials for mammary tumourigenesis and uterine adenomyosis in (SLN x C3H/He)F1 virgin mice.Lab Anim.1992;26(1):23-4.

19. Cohen I, Beyth Y, et al. Adenomyosis in postmenopausal breast cancer patients treated with tamoxifen: a new entity?Gynecol Oncol.1995;58(1):86-91.

20. Cohen I, Beyth Y, et al. High frequency of adenomyosis in postmenopausal breast cancer patients treated with tamoxifen.Gynecol Obstet Invest.1997;44(3):200-5.

21. Kitawaki J, Noguchi T, et al. Expression of aromatase cytochrome P450 protein and messenger ribonucleic acid in human endometriotic and adenomyotic tissues but not in normal endometrium.Biol Reprod.1997;57(3):514-9.

22. Rizner TL. The Important Roles of Steroid Sulfatase and Sulfotransferases in Gynecological Diseases.Front Pharmacol.2016;7:30.

23. Kitawaki J, Koshiba H, et al. Progesterone induction of 17beta-hydroxysteroid dehydrogenase type 2 during the secretory phase occurs in the endometrium of estrogen-dependent benign diseases but not in normal endometrium.J Clin Endocrinol Metab.2000;85(9):3292-6.

24. Chen YJ, Li HY, et al. Oestrogen-induced epithelial-mesenchymal transition of endometrial epithelial cells contributes to the development of adenomyosis.J Pathol.2010;222(3):261-70.

25. Leyendecker G, Wildt L. A new concept of endometriosis and adenomyosis: tissue injury and repair (TIAR).Horm Mol Biol Clin Investig.2011;5(2):125-42.

26. Sang X, Han H, Poirier D, Lin SX. Steroid sulfatase inhibition success and limitation in breast cancer clinical assays: An underlying mechanism.J Steroid Biochem Mol Biol.2018;183:80-93.

27. Peleg Hasson S et al. Adjuvant endocrine therapy in HER2-positive breast cancer patients: systematic review and meta-analysis. ESMO Open. 2021;6(2):100088.

28. Kok VC, Tsai HJ, Su CF, Lee CK. The Risks for Ovarian, Endometrial, Breast, Colorectal, and Other Cancers in Women With Newly Diagnosed Endometriosis or Adenomyosis: A Population-Based Study. Int J Gynecol Cancer. 2015;25(6):968-76.

29. Yeh CC, Su FH, Tzeng CR, Muo CH, Wang WC. Women with adenomyosis are at higher risks of endometrial and thyroid cancers: A population-based historical cohort study. PLoS One. 2018;13(3):e0194011..

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The relationship between human papilloma virus and anxiety, depression and sexual dysfunction in women

Naziye GÜRKAN[1,*], **Tuğba GÜRBÜZ**[2]

[1]Department of Obstetrics and Gynecology, Medical Park Samsun Hospital, Samsun Türkiye
[2]Department of Obstetrics and Gynecology, Medistate Hospital, İstanbul, Türkiye

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Abstract

We aimed to investigate the impact of Human Papillomavirus **(**HPV) positivity on women's mental health and sexual dysfunction (SD). Anxiety, hopelessness, and depression were investigated in this study as common psychological problems. This prospective cross-sectional case-control study was done on 213 participants who attended gynecology clinic from February 2021 to May 2022. The Turkish version of Female Sexual Function Index (FSFI)-6 was used to measure SD in women. The Turkish version of Beck Depression Inventory (BDI), Beck Anxiety Inventory *(BAI), and* Beck Hopelessness Scale *(BHS)* was used to measure depression, anxiety and hopelessness in women. According to the HPV test results, the subjects were divided into two groups: 1st group as HPV positive and 2nd group as HPV negative. 97 women in the 1st group and 116 patients in the 2nd group were included in the study. The participants' age and body mass index (BMI) were 33.91±3.74 and 24.88±3.01, respectively. There was a significant difference between HPV-positive and HPV-negative women in FSFI total scores and all subdomains (p-value<0.05). There was a significant difference in BDI, BHS, and BAI (p-value<0.05). There was a significant correlation between the FSFI and BAI in HPV-positive group. There was a significant correlation between the FSFI and BAI in HPV-negative group. There was a significant correlation between the FSFI and BDI in HPV-negative group. The health system should support women with HPV in terms of mental health. These women are more exposed to depression, anxiety, hopelessness, and SD.

Keywords: human papillomavirus, female sexual function ındex, sexual dysfunction, quality of life

1. Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted diseases globally, and is closely related to cervical cancer as the fourth leading cause of cancer-related death in women. More than 90% of the women with this cancer are HPV-positive (1, 2). The virus generally affects the epithelial tissue of the genital area, especially in women. Its effects are usually removed by the person's immune system within two years (3). Viral infection or damage to the mucous and skin tissues of the target area begins, leading to a wound or wart at the site, indicating the ability of the HPV virus to differentiate the infected tissue of the host cellularly (4). In general, HPV virus is present in 32.1% of women worldwide. A comprehensive and multicenter study in Turkey with 2234 women reported a prevalence of 38.05%, and the 16th type had the highest prevalence (5). The prevalence of HPV is strongly correlated with the country's vaccination rate.

Infertility in women has attracted the attention of researchers for decades (6). In the conducted research, the factors affecting female infertility are studied. The effect of sexual dysfunction (SD) and psychological problems such as stress, hopelessness, depression, and anxiety on infertility has received increasing attention in recent years. Besides women's sexual health, their mental health also affects their fertility (7). Identifying each factor besides its effect on the infertility treatment process is essential. In most societies, hopelessness, depression, and anxiety are more common in women than men. Environmental factors and some diseases can cause frustration, stress, depression, and anxiety in women. HPV has a negative effect on women's emotional and sexual health (8). HPV can cause shame in women, disrupt their relationship with their partner by reducing their femininity, and increase fear, anxiety, and stress (9). Identifying the roots of depression and anxiety in women and curing them can increase their chances of pregnancy (7).

The prevalence of SD varies among populations and is influenced by psychological, medical, economic, social, cultural, and ethnic factors (10). SD and infertility are significantly associated with each other. The effect of HPV virus on SD was assessed in some studies (6). However, further studies are needed to determine changes in sexual function and psychological problems in women after the

*Correspondence: drtgurbuz@hotmail.com

diagnosis of HPV.

This study assessed the SD, hopelessness, depression, and anxiety rate in HPV-positive women and the control group. The main aim of this study was to evaluate the effect of this virus on the sexual and mental function of HPV-positive women. The contribution of this study is to evaluate the effect of a positive HPV test result on the mental health of Turkish women.

2. Materials and Methods

The Ethics Committee of Beykoz University approved this prospective cross-sectional case-control study. 213 women participated in this study between February 2021 and May 2022 at Medistate Hospital Gynecology and Obstetrics Clinic. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee. Before enrolling in the study, all participants gave their informed consent.

116 women were included in the control group, and 97 women infected with HPV in the last year were included in the study group.

The inclusion criteria were: (1) being between the ages of 20 and 45. The exclusion criteria were: (1) being pregnant or in the breastfeeding period; (2) absence of diabetes, thyroid dysfunction, and systemic diseases.

The Turkish version of Female Sexual Function Index (FSFI-6) was used to measure SD in women (11). The total FSFI scores and each subdomain score of HPV-positive women and the controls were compared. We included six domains, including desire (the desire to have sexual experience), arousal (having interest in sexual relation before stimulations), lubrication, orgasm (reaching orgasm following arousal), satisfaction, and pain calculated based on the patients' self-report in the FSFI score. The six domains of the scale items are desire (2 questions), arousal (4 questions), orgasm (3 questions), lubrication (4 questions), satisfaction (3 questions), and pain (3 questions). The total FSFI score was the sum of all scores obtained in all six domains. A higher score indicates improved sexuality.

The Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) are a 21-item inventory assessing the symptoms of depression and anxiety, respectively. The total score may range from 0 to 63. Beck Hopelessness Scale (BHS) is a 20-item inventory assessing the symptoms of hopelessness. Hisli, Durak, and Ulusoy assessed the reliability and validity of the Turkish version of these surveys (12-14).

The Kolmogorov-Smirnov test was performed to check the normality. All six sexual function subdomains and total sexual functioning mean scores were measured to compare the groups. The relationship between the case and control groups was studied using the chi-square test to check the variables of infertility time, smoking, dyspareunia, menstruation, and hirsutism. The Spearman test was used to examine examined a significant relationship between the questionnaire variables. The Mann-Whitney test was performed to study the difference between the two groups in all FSFI subdomains. SPSS v20 was used for statistical analyses. A value of $p < 0.05$ was accepted as statistically significant.

To calculate the sample size with the G-Power 3.1 (http://www.gpower.hhu.de/) program, two groups' total mean was measured based on the Mann-Whitney test with the power of 95%, effect size of 50%, and 0.05 type 1 error for at least 92 patients (15).

3. Results

The study included two 213 (mean age ± SD: 33.91 ± 3.74). The participants' mean body mass index (BMI) was 24.88±3.01. Table 1 shows descriptive information on the study parameters. Descriptive characteristics of other variables were omitted omit for brevity.

Table 1. Descriptive information on the study parameters

Study parameters		N	Minimum	Maximum	Mean	SD
Age(yr)		213	21.00	45.00	33.91	3.74
Body mass index(BMI) kg/m2		213	18.00	30.00	24.88	3.01
	Total	Study group (HPV-positive) (n=97)		Control group (HPV- negative) (n=116)		*p*-value
Working Status						
Working	108(50.7)	52(53.6)		53(45.7)		0.250
Unemployed	105(49.3)	45(46.4)		63(54.3)		
Cigarette						
Yes	97(45.5)	32(33)		65(56)		<0.001
No	116(54.5)	65(67)		51(44)		
Education						
Middle School	5(2.3)	5(5.2)		0(0)		<0.001
High School	105(49.3)	44(45)		61(52.5)		
Bachelor	84(39.4)	46(47)		38(32.7)		
Master	19(8.9)	2(2.8)		17(14.8)		
Parity						
Nulliparous	142(66.7)	72(74.3)		68(58.6)		0.031
Multiparous	71(33.3)	25(25.7)		48(41.4)		

As stated in Table 1, a chi-square test did not find a statistically significant association between study and control groups in terms of working status (p-value > 0.05). There was a statistically significant association between study and control groups in terms of cigarettes, education, and parity (p-value < 0.05).

Table 2 shows a significant difference between HPV-positive and HPV-negative women in FSFI total scores and all FSFI subdomains (p-value<0.05). FSFI subdomains scores were higher in control group women. It means that HPV-positive affects sexual function negatively.

Table 2. Comparing HPV- and HPV+ patients in terms of mean FSFI total scores and subdomain sexual function scores

Category	Study group (HPV-positive) (mean ± SD)	Control group (HPV- negative) (mean ± SD)	p-value
Desire	3.43 ± 1.22	4.60 ± 1.08	<0.001
Arousal	4.04 ± 1.10	4.36 ± 1.12	0.034
Lubrication	3.27 ± 0.94	4.18 ± 1.15	<0.001
Orgasm	3.14 ± 1.00	3.88 ± 1.12	<0.001
Satisfaction	3.03 ± 1.13	3.69 ± 1.26	<0.001
Pain	2.58 ± 1.34	3.66 ± 1.77	<0.001
Total FSFI score	19.49 ± 2.92	24.16 ± 3.63	<0.001

FSFI: Female Sexual Function index, HPV: Human papillomavirus, SD: Standard deviation

Table 3 shows a significant difference between HPV-positive and HPV-negative women in BDI, BHS, and BAI (p-value<0.05). It means that HPV-positive affects mental health negatively.

Table 3. Age, BMI, BDI, BHS, and BAI mean values according to the human papillomavirus result

Category	Study group (HPV-positive) (mean ± SD)	Control group (HPV- negative) (mean ± SD)	p-value
Age(year)	33.81 ± 2.48	33.99 ± 4.55	0.939
BMI(kg/m2)	23.39 ± 2.89	26.13 ± 2.49	<0.001
BDI	24.05 ± 7.44	10.51 ± 7.67	<0.001
BHS	12.72 ± 4.47	1.46 ± 1.14	<0.001
BAI	19.54 ± 6.08	3.46 ± 2.56	<0.001

BDI: Beck Depression Inventory, BAI: Beck Anxiety Inventory, FSFI: Female Sexual Function Index, BHS: Beck Hopelessness Scale, SD: Standard deviation, HPV: Human papillomavirus

Fig. 1 shows the effect of HPV on mental health issues and sexual dysfunction.

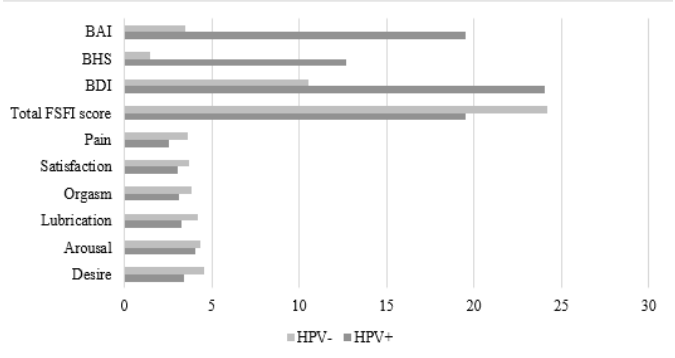

Fig. 1. The effect of HPV on mental health issues and sexual dysfunction

Table 4 shows the correlation between FSFI and mental health scales according to HPV result. Spearman's rho correlation coefficient was used to assess the relationship between FSFI total score and three scales (BDI, BHS and BAI). No significant correlation was found between the FSFI and BDI in study group (r= 0.022, p-value > 0.05). There was no significant correlation between the FSFI and BHS in study group (r= -0.122, p-value > 0.05). There was significant correlation between the FSFI and BAI in study group (r= 0.250, p-value < 0.05). FSFI was significantly correlated with the BDI in control group (r= -0.270, p-value < 0.05). No significant correlation was found between the FSFI and BHS in control group (r= -0.052, p-value > 0.05). There was significant correlation between the FSFI and BAI in control group (r= -0.189, p-value < 0.05).

Table 4. The correlation between FSFI and mental health scales according to the human papillomavirus result

		BDI	BHS	BAI
HPV-positive	FSFI			
	Correlation Coefficient	0.022	-0.122	0.250
	p-value	0.830	0.236	**0.014**
HPV-negative	FSFI			
	Correlation Coefficient	-0.270	-0.052	-0.189
	p-value	**0.003**	0.582	**0.043**

4. Discussion

This study dealt with the effect of HPV-positivity on sexual dysfunction, BDI, BAI, and BHS in women. Our findings showed that HPV-positivity harmed women's mental health. Hopelessness, anxiety, and depression were significantly more common in HPV-positive women. According to the results, sexual dysfunctions were substantially more common in HPV-positive women. Both case and control groups were found to have similar correlations between SD and anxiety.

The current study observed a statistically significant relationship between mental health problems (hopelessness, anxiety and depression) and positive HPV. Studies highlighted the negative effects of HPV- positivity on mental health in women. Several studies showed that anxiety was more common in HPV-positive women. Depression and hopelessness were reported in fewer studies than anxiety in HPV-positive women. Lin et al. (16) reported negative emotions in HPV-positive women, including fear, anxiety and suspicion, by interviewing 20 Taiwanese women. McCaffery et al. (17) showed HPV-positivity had negative effects on mental health problems such as anxiety and stress in a study of 271 white, black, and Asian women. Lin et al. (18) showed increased anxiety and stress as a positive HPV outcome (19). Felden et al. reported HPV-positive causes reluctance to engage in sexual activity, stress, and anxiety in women. Mercan et al. (20) studied anxiety and depression in the case and control groups of 133 HPV-positivity Turkish women. In this study, six weeks after the positive HPV result, an evaluation of anxiety and depression was performed, and a significant relationship was found between HPV-positivity and depression, but not anxiety. Heinonen et al. (21)

measured the effect of HPV-positivity on 238 Finnish women over 12 months. The negative effects of HPV-positivity on quality of life, sleep, sexual activity, anxiety, and distress were shown. Maissi et al. (22) showed that anxiety, distress, and concern were higher in HPV-positive women. The negative impact on mental health problems increased when women were less aware of the meaning of their test results. Waller et al. (23) reported a significant relationship between HPV-positive and negative emotions. With increasing awareness of the prevalence of this virus, the negative psychological consequences and anxiety were significantly reduced. Uzun et al. (24) reported HPV-positivity as the cause of increased anxiety and depression, which has decreased over time. Rask et al. (25) found that HPV-positivity was associated with reduced quality of life and anxiety in a study of Swedish women. Results of this study reported that a significant relationship was found between anxiety and HPV-positivity but not depression. Alay et al. (6) reported a negative effect of different types of HPV on anxiety in 80 Turkish women. Highest level of anxiety reported in women with 16/18 HPV.

In line with similar studies in the literature, according to the results, HPV-positive women face sexual dysfunction problems. All subdomains of the FSFI index were lower in the HPV-positive women. These values indicate that HPV-positive had a significant effect on female sexual function. Ferenidou et al. (26) showed a decrease in sexual desire in HPV-positive women. Uzun et al. (24) did not observe a significant relationship between sexual dysfunction and HPV-positivity with continuous visits of women in the first, third, sixth, and twelfth months. In this study, women's employment, financial status, and age were considered influential in sexual satisfaction. Caruso et al. (27) showed a decline in all aspects of women's sexual quality of life following a positive HPV outcome. Ekmez and his brother (28) reported increased anxiety and sexual dysfunction after the diagnosis of HPV-positivity. According to the research findings, the partner's sex life is also negatively affected by the positive HPV result. Fornage et al. (29) reported no significant association between sexual health and HPV-positivity while showed a positive HPV result as an increase in anxiety. Ilgen et al. (30) showed no significant relationship between female sexual function and HPV. Physicians should be aware of the adverse effects on the sexual function of HPV-positive women. However, more comprehensive and detailed research is required to prove the association between sexual dysfunction and HPV, especially over the long term.

The most important limitations of this study are the lack of sufficient information about sexual dysfunction, hopelessness, anxiety, and depression in HPV-positive women before the test. Continuous evaluation of these patients during 3-month and 6-month periods could better demonstrate the durability of adverse effects of HPV-positivity Further research is suggested to show a significant relationship between the negative effects and women's awareness of this virus as part of the research.

HPV-positivity causes hopelessness, anxiety, depression, and sexual dysfunction in women. The health system should support women with HPV in terms of mental health. Increasing women's awareness of this virus, its different types, and its high prevalence can significantly affect the reduction of negative emotions. Free vaccination and giving necessary information about its positive impact among health centers can increase women's sexual and mental health.

Conflict of interest

The authors declared that there was no conflict of interest in this study.

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This study was approved by Beykoz University Clinical Studies Ethics Committee. (Date: 21.01.2021, Decision No: 1)

Authors' contributions

Concept: N.G.,T.G., Design: N.G.,T.G., Data Collection or Processing: N.G.,T.G., Analysis or Interpretation: N.G.,T.G., Literature Search: N.G.,T.G., Writing: N.G.,T.G.

References

1. Preston SM, Darrow WW. Improving human papillomavirus-related knowledge and attitudes among ethnically diverse young adults. Health Equity 2019; 3: 254-63.

2. Graham SV. The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. Clin Sci (Lond). 2017; 131: 2201-21.

3. Shanmugasundaram S, You J. Targeting persistent human papillomavirus infection. Viruses 2017; 9: 229.

4. Kombe Kombe AJ, Li B, Zahid A, Mengist HM, Bounda GA, Zhou Y, et al. Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation. Front Public Health. 2021; 8: 552028.

5. Hancer VS, Buyukdogan M, Bylykbashi I, Oksuz B, Acar M. Prevalence of human papilloma virus types in Turkish and Albanian women. J Cytol 2018; 35: 252-4.

6. Alay I, Kaya C, Karaca I, Yildiz S, Baghaki S, Cengiz H, et al. The effect of being diagnosed with human papillomavirus infection on women's sexual lives. J Med Virol. 2020; 92(8): 1290-7.

7. Markovic-Denic L, Djuric O, Maksimovic N, Popovac S, Kesic V. Effects of human papillomavirus awareness and knowledge on psychological state of women referred to cervical cancer screening. J Low Genit Tract Dis 2018; 22: 178-83.

8. Ngu SF, Wei N, Kwan TTC, Chu MMY, Tse KY, Chan KKL, et al. Impact of different educational interventions on psychosocial well-being of women with a positive high-risk human papillomavirus and normal cervical cytology: a randomised trial. J Psychosom Obstet Gynaecol. 2018; 39(2): 146-55.

9. de Kok IMCM, Korfage IJ, van den Hout WB, Helmerhorst TJM, Habbema JDF, Essink-Bot ML,et al. Quality of life assumptions

determine which cervical cancer screening strategies are cost-effective. Int J Cancer. 2018; 142(11): 2383-93.

10. Omani-Samani R, Amini P, Navid B, Sepidarkish M, Maroufizadeh S, Almasi-Hashiani A. Prevalence of sexual dysfunction among infertile women in iran: a systematic review and meta-analysis. Int J Fertil Steril 2019; 12: 278-83.

11. Aygin D, ETİ FA. Kadın cinsel işlev ölçeği'nin Türkçeye uyarlaması. Türkiye Klinikleri Tıp Bilimleri Dergisi 2005; 25: 393-9.

12. Hisli N. Beck Depresyon Envanterinin gecerliligi uzerine bit calisma (A study on the validity of Beck Depression Inventory.). Psikoloji Dergisi 1988; 6: 118-22.

13. Ulusoy M, Sahin NH, and Erkmen H. Turkish version Of The Beck Anxiety Inventory: Psychometric Properties. J Cognitive Psychother 1998; 12: 163.

14. Durak A, Palabiyikoğlu R. Beck umutsuzluk ölçeği geçerlilik çalişmasi. Kriz Dergisi 1994; 2: 311-9.

15. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007; 39: 175-91.

16. Lin H, Jeng CJ, Wang LR. Psychological responses of women infected with cervical human papillomavirus: a qualitative study in Taiwan. Taiwan J Obstet Gynecol 2011; 50: 154-8.

17. McCaffery K, Waller J, Forrest S, Cadman L, Szarewski A, Wardle J. Testing positive for human papillomavirus in routine cervical screening: examination of psychosocial impact. BJOG 2004; 111: 1437-43.

18. Li CC. The Impact of HPV Diagnosis on Taiwanese Women's Quality of Life and Sexual Health. Sigma's 30th International Nursing Research Congress, 2019.

19. Dominiak-Felden G, Cohet C, Atrux-Tallau S, Gilet H, Tristram A, Fiander A. Impact of human papillomavirus-related genital diseases on quality of life and psychosocial wellbeing: results of an observational, health-related quality of life study in the UK. BMC Public Health 2013; 13: 1065.

20. Mercan R, Mercan S, Durmaz B, Sur H, Kilciksiz CM, Kacar AS, et al. Sexual dysfunction in women with human papilloma virus infection in the Turkish population. J Obstet Gynaecol. 2019; 39(5): 659-63.

21. Heinonen A, Tapper AM, Leminen A, Sintonen H, Roine RP. Health-related quality of life and perception of anxiety in women with abnormal cervical cytology referred for colposcopy: an observational study. Eur J Obstet Gynecol Reprod Biol 2013; 169: 387-91.

22. Maissi E, Marteau TM, Hankins M, Moss S, Legood R, Gray A. Psychological impact of human papillomavirus testing in women with borderline or mildly dyskaryotic cervical smear test results: cross sectional questionnaire study. BMJ 2004; 328: 1293.

23. Waller J, Marlow LA, Wardle J. The association between knowledge of HPV and feelings of stigma, shame and anxiety. Sex Transm Infect 2007; 83: 155-9.

24. Uzun SB, Sakin Ö, Çetin H, Şimşek EE. The Effects of HPV Test on Anxiety, Emotion and Depression in Women. J Acad Res Med 2020; 10: 149-54.

25. Rask M, Swahnberg K, Oscarsson M. Swedish women's awareness of human papillomavirus, and health-related quality of life, anxiety, and depression after a notification of an abnormal Pap smear result: a cross-sectional study. Eur J Cancer Prev 2019; 28: 96-101.

26. Ferenidou F, Salakos N, Vaidakis N, Paltoglou G, Bakalianou K, Papadimitriou G,et al. The impact of HPV diagnosis on women's sexual and mental health: preliminary findings. Clin Exp Obstet Gynecol 2012; 39: 79-82.

27. Caruso S, Bruno mt, cianci s, di pasqua s, minona p, cianci a. sexual Behavior of Women With Diagnosed HPV. J Sex Marital Ther 2019; 45: 569-73.

28. Ekmez M, Ekmez F. Effects of HPV Positivity In Women On Couples Sexual Behavior. J Sex Marital Ther 2022: 1-8.

29. Fornage S, Schweizer A, Lepigeon K, Bianchi Demicheli F, Faouzi M, Jacot-Guillarmod M. Is women's sexual function impaired by an abnormal pap smear? Eur J Contracept Reprod Health Care 2022; 27: 184-8.

30. Ilgen O, Kurt S, Kula H, Celiloglu M. Sexual dysfunction in women who are human papillomavirus positive. J Reprod Med Gynecol Obstet 2020; 5: 61.

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The role of inflammatory markers in the diagnosis of extraperitoneal endometriosis

Selim GÜLÜCÜ*, Neşet GÜMÜŞBURUN

Department of Obstetrics and Gynecology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Türkiye

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Abstract

This study was concerned with the examination of patients who underwent surgery for subcutaneous endometriosis in our clinic and the relationship between subcutaneous endometriosis and inflammatory markers.Patient demographics and information on history and duration of previous surgery, lesion size, number of lesions, location, recurrence, symptoms, type and number of deliveries, recurrence status, and imaging method were recorded. Laboratory analysis recorded TSH, blood count (Hb), WBC, mean platelet volume (MPV), neutrophil/lymphocyte ratio (NLR), monocyte/platelet ratio (MPR), lymphocyte/monocyte ratio (LMR), platelet/lymphocyte ratio (PLR) and CA -125 values of patients.The study included 28 patients and it was found that the mean age of the patients was 32.67±5.56 years. Five (17.9%) and 18 (64.3%) of the patients complained of a palpable mass and cyclic pain, respectively. Five patients (17.9%) were asymptomatic. Endometriosis associated with the scar line was localized in 18 (64.3%) of the patients. In three (10.7%) of the patients, the endometriosis was localized in the perineal line and in 7 (25%) of the patients in the rectus abdominis. No significant difference was found in the patients' routine laboratory results and inflammatory markers. In the present study, there was no significant association between the levels of inflammatory markers in patients who underwent surgery for subcutaneous endometriosis at different sites and with different symptoms.

Keywords: endometriosis, scar line, episiotomy line, abdominal wall, extraperitoneal, inflammatory

1. Introduction

Endometriosis is recognized by the presence of endometrial glands and stroma outside the uterine cavity (1). The prevalence of endometriosis is up to 40% of women at reproductive age. Although endometriosis most commonly occurs in the pelvic region, it has also been found in areas outside the pelvis (1). Less common locations include the abdominal wall, cesarean section scar line, and perineal endometriosis associated with episiotomy scarring. It usually occurs after obstetric or gynecologic surgery (2). The incidence of this pathology ranges from 0.04% to 1% for abdominal wall endometriosis (3) and from 0.03% to 0.4% for surgical scar endometriosis (4).Their incidence has increased worldwide due to high cesarean section rates. This particular form of endometriosis has been only partially recognized, and the diagnosis is usually made late. The incidence of the disease increases under the influence of postpartum estrogen exposure and concomitant endometrial inoculation during surgery, variable immunity, chronic inflammation, and local growth factors (5). Nowadays, the first treatment option is surgical excision of subcutaneous endometriosis. Neutrophil/lymphocyte ratio (NLR), lymphocyte/monocyte ratio (LMR), monocyte/platelet ratio (MPR), and platelet/lymphocyte ratio (PLR) in peripheral blood are easy to calculate, inexpensive, and can reveal systemic inflammation. It is believed that these markers can be a prognostic factor for many diseases.

This study focused on the examination of patients who had undergone subcutaneous endometriosis surgery in our clinic and the relationship between subcutaneous endometriosis and inflammatory markers.

2. Materials and Methods

The study included patients who underwent surgery for extraperitoneal subcutaneous endometriosis at the Gynecology and Obstetrics Department of Tokat Gaziosmanpaşa University Research and Application Hospital between 2015 and 2021. Prior to the study, approval was obtained from the Clinical Research Ethics Committee of Gaziosmanpaşa University (project number: 21-KAEK-173/ 05.08.2021). The study was planned as a retrospective study in which 28 patients with extraperitoneal subcutaneous endometriosis were enrolled. Patient data were obtained from hospital records. As for the inclusion criteria, patients who were operated for extraperitoneal endometriosis and whose pathology was endometriosis externa were included in the study. Regarding the exclusion criteria, patients who underwent surgery at an external center and whose data could not be collected were excluded from the study. All patients underwent a physical examination, a detailed medical history, and routine hematologic and biochemical analysis. Patient demographics and information on age, gravid, parity, history and duration of previous surgery, lesion size, number of lesions, location, recurrence, complaints, history of systemic

*Correspondence: selim.gulucu@gop.edu.tr

diseases, type and number of deliveries, recurrence status, and follow-up and imaging were recorded. Laboratory analyzes included TSH, free T4, blood count (hemogram) (Hb), neutrophils, monocytes, lymphocytes, white blood cells (WBC), platelets, MPV, PLR, NLR, LMR, and CA -125 values of patients. A regularly maintained device (Mindray BC-6800, China) was used for complete blood count. A regularly maintained device (Roche Cobas e601, Roche Diagnostics GmbH; Germany) was also used for other tests. In the evaluation, patients were grouped according to their complaint status (asymptomatic, pain, palpable mass) and extra-abdominal and local location (subcutaneous, perineal, rectus-related). Descriptive statistics were performed to obtain data on the general characteristics of the study groups. Continuous variable information is presented as mean±standard deviation and min-max. Differences between groups were examined with Kruskal-Wallis analysis of variance considering parametric conditions. The Bonferroni-corrected Mann-Whitney U test was used for multiple comparisons. Differences were analyzed with the nonparametric test considering the number of subjects in the groups. P values of less than 0.05 were considered statistically significant. Ready-made statistical software was used for calculations (IBM SPSS Statistics 19, SPSS inc, an IBM Co, Somers, NY).

3. Results

It was found that the mean age of the patients was 32.67±5.56 years and the parity was 2.32±0.94. Twenty-four (85.7%) of these patients had a cesarean section, 3 (10.7%) had a normal vaginal delivery, and 1 (3.6%) had a previous subcutaneous endometriosis operation with cesarean section. The mean duration after current delivery in these patients was 4.17±1.92 years. The mean lesion diameter was 3.02±1.52 cm2. Five (17.9%) and 18 (64.3%) of the patients complained of a palpable mass and cyclic pain, respectively, and 5 patients (17.9%) were asymptomatic. While 18 (64.3%) of the patients had the localization of endometriosis associated with the scar line, 3 (10.7%) of them had the localization of endometriosis with the episiotomy line, and 7 (25%) of them had the localization of endometriosis with the rectus abdominis. Twenty-seven of the patients (96.4%) were operated for the first time for endometriosis, and 1 patient (3.6%) was operated for recurrence. It was found that 13 (46.4%) of the patients had a history of cesarean section, 7 (25%) had two cesarean sections, 5 (17.9%) had three cesarean sections, and 3 patients (10.7%) had a normal vaginal delivery. Diagnosis was made by ultrasound in 21 (75%) patients, magnetic resonance imaging (MRI) in 2 (7.1%), and computed tomography (CT) and clinical examination in 2 (7.1%) and 3 (10.7%), respectively.

TSH, T4, Hb, Ca-125, neutrophils, lymphocytes, platelets, monocytes, MPR, PLR, NLR, and LMR were calculated in the patients. The laboratory results of the patients are shown in Table 1. Comparison of symptoms (palpable mass, cyclic pain, asymptomatic) and location (under the scar, episiotomy line, rectus-related) and laboratory results were summarized in Table 2 and Table 3. No statistically significant differences were found between inflammatory markers and other laboratory results in the comparisons. It was noted that the patients with excision had no recurrence.

Table 1. Laboratory results of the patients

	Laboratory results (n=28)
TSH (mIU/L)	1.87 ±1.39
T4(ng/dL)	1.24 ± 0.24
Neutrophil (10^2/µL)	5209.00 ± 3109.95
Lymphocyte (10^2/µL)	2263.57 ± 1254.82
Platelet (lakhs/mm^3)	248232.14 ± 55039.30
WBC (10^5/mL)	7.95 ± 3.14
Hb (gr/dl)	12.19 ±1.32
Monocytes (10^2/µL)	540.61 ± 143.84
MPV (fL)	9.73 ± 1.34
NLR	2.51 ±1.56
PLR	124.68 ± 49.82
MPR	0.0022 ± 0.0005
LMR	4.25 ± 1.60
Ca-125 (U/mL)	27.96 ± 17.01 (10.13-92.51)

Values are expressed as mean ± standard deviation. TSH: thyroid-stimulating hormone; T4: thyroxine,Hb: blood count, WBC:white-blood cell, MPV: mean platelet volume, MPR: Monosit/platalet, LMR: Lenfosit/monosit, NLR: neutrophil / lymphocyte ratio, PLR: platelet / lymphocyte ratio

Table 2. Comparison of laboratory values according to symptoms

	Symptom				
	Asymptomatic (n=5)	Cyclic pain (n=18)	Palpable mass (n=5)	KW	p
TSH (mIU/L)	1.1±0,7	2.1±1.47	1.82±1.57	2.882	0.237
T4(ng/dL)	1.39±0.31	1.23±0.24	1.13±0.14	2.263	0.323
Neutrophil (10^2/µL)	5320±1536.91	5310.67±3706.11	4732±2049.72	1.536	0.464
Lymphocyte (10^2/µL)	2020±761.97	2357.78±1516.29	2168±388.48	0.782	0.676
Platelet (lakhs/mm^3)	210460±13925.09	258455.56±62942.38	249200±34083.72	5.163	0.076
WBC (10^5/mL)	8.07±1.83	7.99±3.68	7.7±2.36	0.798	0.671
Hb (gr/dl)	12.39±0.67	12.2±1.42	11,94±1.64	0.204	0.903
Monocytes (10^2/µL)	520±109.77	532.61±154.04	590±151.33	0.791	0.673
MPV (fL)	10.14±1.13	9,53±1.51	10,04±0.75	1.814	0.404
NLR	2.91±1.23	2.48±1.8	2.2±0.93	1.715	0.424
PLR	116.24±42.36	128.92±57.67	117.85±24.37	0.044	0.978
MPR	0.0025±0.0005	0.0005±0.0025	0.0024±0.0004	1.93	0.381
LMR	4.12±1.89	4.41±1.7	3.82±1.01	0.334	0.846
Ca-125 (U/mL)	30.75±13.29	28.91±19.53	21.76±9.7	1.534	0.464

Values are expressed as mean ± standard deviation, KW: Kruskal Wallis Varyans Analysis

Table 3. Distribution of laboratory values according to localization

	Localization				
	Scar line (n=18)	Episiotomy line (n=3)	Associated with rectus abdominis (n=7)	KW	p
Tsh (mIU/L)	1.62±0.84	3.84±2.92	1.67±1.3	1.572	0.456
T4(ng/dL)	1.29±0.24	1.34±0.3	1.07±0.16	5.837	0.054
Neutrophil (10^2/μL)	5335±2970.49	3286.67±337.24	5708.86±4037.02	3.754	0.153
Lymphocyte (10^2/μL)	2087.78±517.82	2050±160.93	2807.14±2420.12	0.059	0.971
Platelet (lakhs/mm^3)	256322.22±51702.4	208500±43511.49	244457.14±66518.34	1.395	0.498
Wbc (10^5/mL)	8.14±3.03	6.03±0.56	8.31±4.04	3.049	0.218
Hb (gr/dl)	12.37±1.31	12.32±1.8	11.67±1.22	2.325	0.313
Monocytes (10^2/μL)	527.22±126.81	496.67±112.4	593.86±196.28	0.601	0.740
MPV (fL)	9.68±0.97	10.19±2.01	9.65±1.97	0.121	0.941
NLR	2.74±1.83	1.6±0.09	2.3±0.96	3.924	0.141
PLR	131.88±51.23	101.53±19.09	116.09±55.68	1.523	0.467
MPR	0.0021±0.0005	0.0005±0.0021	0.0025±0.0006	2.386	0.303
LMR	4.14±1.36	4.25±0.84	4.54±2.43	0.134	0.935
Ca-125 (U/mL)	24.55±10.97	41.22±44.51	31.07±12.9	1.426	0.490

Values are expressed as mean ± standard deviation, KW: Kruskal Wallis Varyans Analysis

4. Discussion

Abdominal wall endometriosis is a form of endometriosis characterized by a painful or painless lump in the previous incision scar. It is most commonly seen after cesarean section, laparotomy, and hysterectomy. It can also be observed after gynecologic laparoscopic surgery (2, 4). In the literature, case series of scar endometriosis after episiotomy have been reported rarely (6). In our study, 64.3% of patients were found to have endometriosis associated with the scar line, 10.7% had endometriosis associated with the episiotomy line, and 25% had endometriosis associated with the rectus abdominis. The time between surgery and diagnosis of endometriosis varied from 3 months to 20 years, and the mean age at diagnosis was 35 years (7). In the present study, the mean age at diagnosis was found to be 32.6 years, and the time to diagnosis and surgery after surgery in our study was 4.17 years. Concurrent pelvic lesion was not observed in the patients. Tatli et al. in their series of 14 cases also reported that there was no concurrent pelvic lesion (7). Sumathy et al. in their series of 16 cases found that 18.9% of patients had concurrent endometriosis (8). Most patients had symptoms of cyclic pain associated with menstruation at the surgical incision site (4). Occasionally, a palpable mass may be found without pain. These symptoms must be a warning for scar endometriosis. In our study, 17.9% of patients had a palpable mass, 64.3% of them had cyclic pain complaints, and 17.9% of them were asymptomatic. A noninvasive examination is beneficial to differentiate the mass from surrounding tissues in terms of location, size, density, and homogeneity. Ultrasonography is a practical, accessible, reliable, and inexpensive method (9). On ultrasound, the appearance of a vascularized, hypoechoic, heterogeneous solid lesion is a supportive finding for scar endometriosis. If there is doubt about the diagnosis, CT and MRI can be used. In the present study, 75% of patients were diagnosed by ultrasound, 7.1% by MRI, and 7.1% and 10.7% by CT and clinical examination, respectively. Fine needle aspiration cytology (FNAC) is a simple, convenient, and cost-effective method that can be used in cases with uncertain diagnosis (10). However, the use of this method is controversial. It is argued that this technique increases the risk of developing new endometriotic implants at the administration site and the risk of injury to internal organs (incisional hernia is the differential diagnosis of endometrioma) (11). The final diagnosis is made by histopathologic evaluation after complete excision. In our study, the final histopathologic diagnosis was compatible with the provisional diagnosis in all patients. The recognized most appropriate treatment model is wide surgical excision, which provides the final diagnosis and treatment simultaneously. In wide surgical excision, it is important to operate at least 1 cm from the margin of the lesion (12). The recurrence rate is very low for excisions with appropriate surgical margins. Yela et al. reported a high recurrence rate of 36.1% in patients with positive surgical margins (13). Because of the likelihood of recurrence, it is necessary to monitor patients after surgery. If recurrence is detected, surgical resection must be repeated. None of the patients in our series had a recurrence of the disease. Only 1 patient had previously been operated on at another center and was operated on again at our center. Our patients had no recurrence. It was assumed that the reason was resection of at least 1 cm of endometriosis. It was found that the mean value of Ca-125 was within normal limits. Its use in patients was not found to be beneficial. To the best of our knowledge, this is the first study to investigate the systemic inflammatory markers MPR, NLR, LMR, and PLR in subcutaneous endometriosis. MPR, NLR, LMR, and PLR are systemic inflammatory markers associated with various diseases such as coronary artery disease, peripheral vascular disease, rheumatologic disease, gastrointestinal tract disease, and malignancy (14,15). In the literature, some authors suggest that NLR may be a prognostic factor for many diseases. Endometriosis is a chronic inflammatory disease associated with findings such as dysmenorrhea, dyspareunia, and chronic pain. In their study of 467 patients with endometrioma, Tokmak et al. reported that NLR and Ca-125 levels were correlated and higher than other patients with benign ovarian cysts (16). In another clinical study, Cho et al. found that

NLR levels were higher in 231 patients with endometriosis compared with the other group with benign ovarian cysts and the control group (17). While Kim et al. performed laparoscopic endometrioma surgery in 419 patients and found no association between NLR and endometrioma (18), Yavuzcan et al. reported no association between PLR and NLR and endometriosis in 33 patients who underwent endometriosis surgery (19). While the retrospective and sectional nature of the study and the limited number of patients are the weaknesses of the study, the facts that inflammatory markers were studied for the first time in the subcutaneous endometriosis series and that it includes results from the only tertiary hospital in the region are the strengths of the work.

When evaluating masses near the incision site in patients with a history of surgery, cyclic pain that increases with menstruation should be especially investigated, and endometriosis should always be considered. It is necessary to reduce the possibility of recurrence by performing a comprehensive surgical excision in these cases. Our study showed that there was no significant association between MPR, NLR, LMR and PLR values in patients operated for subcutaneous endometriosis at different sites and with different symptoms. There is a need for studies with larger patient populations to determine the role of these markers in subcutaneous endometriosis.

Conflict of interest

The authors have no conflict of interest.

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None to declare.

Authors' contributions

Concept: S.G., Design: S.G., N.G., Data Collection or Processing: S.G., Analysis or Interpretation: S.G., Literature Search: S.G., N.G.,, Writing: S.G., N.G.

References

1. de Almeida GJ, Migliore R, Kistenmacker FJN, Oliveira MM, Garcia RB, Bin FC, et al. Malignant transformation of abdominal wall endometriosis to clear cell carcinoma: case report. Sao Paulo Med J 2018; 136: 586-90.

2. Koninckx PR, Ussia A, Wattiez A, Zupi E, Gomel V. Risk Factors, Clinical Presentation, and Outcomes for Abdominal Wall Endometriosis. J Minim Invasive Gynecol 2018; 25: 342-3.

3. Nominato NS, Prates LF, Lauar I, Morais J, Maia L, Geber S. Caesarean section greatly increases risk of scar endometriosis. Eur J Obstet Gynecol Reprod Biol 2010; 152: 83-5.

4. Mistrangelo M, Gilbo N, Cassoni P, Micalef S, Faletti R, Miglietta C, et al. Surgical scar endometriosis. Surg Today 2014; 44(4): 767-72.

5. Grigore M, Socolov D, Pavaleanu I, Scripcariu I, Grigore AM, Micu R. Abdominal wall endometriosis: an update in clinical, imagistic features, and management options. Med Ultrason 2017; 19: 430-7.

6. Maillard C, Cherif Alami Z, Squifflet JL, Luyckx M, Jadoul P, Thomas V, et al. Diagnosis and Treatment of Vulvo-Perineal Endometriosis: A Systematic Review. Front Surg. 2021;8:637180.

7. Tatli F, Gozeneli O, Uyanikoglu H, et al. The clinical characteristics and surgical approach of scar endometriosis: A case series of 14 women. Bosn J Basic Med Sci 2018; 18: 275-8.

8. Sumathy S, Mangalakanthi J, Purushothaman K, et al. Symptomatology and Surgical Perspective of Scar Endometriosis: A Case Series of 16 Women. J Obstet Gynaecol India 2017; 67: 218-23.

9. Youssef AT. The ultrasound of subcutaneous extrapelvic endometriosis. J Ultrason. 2020; 20(82): 176-80

10. Medeiros F, Cavalcante DI, Medeiros MA, Eleuterio J, Jr. Fine-needle aspiration cytology of scar endometriosis: study of seven cases and literatüre review. Diagn Cytopathol 2011; 39(1): 18-21

11. Corrêa G, Pina L, Korkes H, Guazzelli T, Kenj G, Toledo A. Scar endometrioma following obstetric surgical icisions: retrospective study on 33 cases and review of literature. Sao Paulo Med J 2009; 127(5): 270–7.

12. Andres MP, Arcoverde FVL, Souza CCC, Fernandes LFC, Abrão MS, Kho RM. Extrapelvic Endometriosis: A Systematic Review. J Minim Invasive Gynecol 2020; 27(2): 373-89.

13. Yela DA, Trigo L, Benetti-Pinto CL. Evaluation of cases of abdominal wall endometriosis at Universidade Estadual de Campinas in a period of 10 years. Rev Bras Ginecol Obstet 2017; 39: 403-7.

14. Fest J, Ruiter R, Ikram MA, Voortman T, van Eijck CHJ, Stricker BH. Reference values for white blood-cell-based inflammatory markers in the Rotterdam Study: a population-based prospective cohort study. Sci Rep. 2018; 8(1): 10566

15. Fan Y, Li X, Zhou XF, Zhang DZ, Shi XF. [Value of neutrophil lymphocyte ratio in predicting hepatitis B-related liver failure]. Zhonghua Gan Zang Bing Za Zhi 2017; 25: 726-31.

16. Tokmak A, Yildirim G, Öztaş E, Akar S, Erkenekli K, Gülşen P, et al. Use of neutrophil-to-lymphocyte ratio combined with ca-125 to distinguish endometriomas from other benign ovarian cysts. Reprod Sci 2016; 23: 795-802.

17. Cho S, Cho H, Nam A, Kim HY, Choi YS, Park KH, et al. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. Fertil Steril 2008; 90: 2073-9.

18. Kim SK, Park JY, Jee BC, Suh CS, Kim SH. Association of the neutrophil-to-lymphocyte ratio and CA 125 with the endometriosis score. Clin Exp Reprod Med 2014; 41: 151-7.

19. Yavuzcan A, Cağlar M, Ustün Y, Dilbaz S, Ozdemir I, Yıldız E, et al. Evaluation of mean platelet volume, neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in advanced stage endometriosis with endometrioma. J Turk Ger Gynecol Assoc 2013; 14: 210-5.

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The effects of maternal anticoagulant therapy on cord blood levels of VEGF-A and soluble Flt-1 in women with recurrent miscarriage

Şahin TAKCI[1,*], Ayşe KORKMAZ TOYGAR[2], Şule YİĞİT[3], Murat YURDAKÖK[3]

[1]Department of Neonatology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[2]Department of Neonatology, Faculty of Medicine, Acibadem University, İstanbul, Türkiye
[3]Department of Neonatology, Faculty of Medicine, İhsan Doğramacı Children's Hospital, Hacettepe University, Ankara, Türkiye

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Abstract

Angiogenic imbalance of the placenta is one of the prominent pathophysiologic mechanisms leading to pregnancy complications like recurrent miscarriages. Low molecular weight heparin and low dose aspirin are frequently used to manage recurrent miscarriages. Vascular endothelial growth factor (VEGF) and its soluble receptor-sFlt-1-plays a major role in angiogenesis. This study investigates cord blood VEGF-A and sFlt-1 levels of women with recurrent miscarriages who receive anticoagulant treatment. We included term newborns whose mothers were treated with LMWH and low-dose aspirin due to recurrent miscarriages. The control group consisted of healthy gestational age-matched infants born in the same period without an adverse perinatal outcome. We assayed the concentrations of VEGF-A and sFlt 1 in umbilical cord blood by ELISA and compared the study and control groups. We included in the study forty-four infants with a maternal LMWH and low dose aspirin treatment during pregnancy and 42 healthy infants as the control group. There were no significant differences between the groups' demographics and cord blood VEGF-A and sFlt-1 levels. There was also no correlation between the cumulative LMWH dosage and serum levels of these angiogenic factors. Cord blood VEGF-A and sFlt-1 levels were comparable in women with recurrent miscarriage under anticoagulant treatment and healthy subjects. Further studies are needed to compare women with recurrent miscarriages with or without heparin treatment to understand better the effects of anticoagulant treatment on the circulatory profile of cord blood angiogenic factors.

Keywords: cord blood, recurrent miscarriage, heparin, VEGF-A, sFlt-1

1. Introduction

Recurrent miscarriage is a primary health problem for women of reproductive age, with at least two consecutive miscarriages before 20 weeks of gestation. It affects approximately 1% to 5% of couples trying to conceive (1). Several conditions are involved in recurrent miscarriages, including antiphospholipid syndrome, acquired thrombophilia, and heritable thrombophilia, such as factor V Leiden and the prothrombin G20210A mutation, but half of the pregnancy losses remain unexplained (2).

It is likely that unexplained recurrent pregnancy loss may reflect a prothrombotic situation and that miscarriage in affected women may be caused by thrombosis in decidual vessels. However, beyond the "thrombosis hypothesis", angiogenic imbalance is an important cause of insufficient utero-placental circulation in the pathophysiology of recurrent miscarriage (3). Despite various discrepancies about the pathophysiology in the literature, many studies show that the treatment with heparin, aspirin or both is associated with a significantly higher rate of live births in women with a history of recurrent miscarriage (4, 5). Heparin has been reported to organize angiogenesis and support endovascular communication between the trophoblast and endothelial cells besides the anticoagulant effects (6). Regulation of angiogenesis is mainly controlled by the pro-angiogenic and anti-angiogenic factors released from the placental unit, such as placenta growth factor (PGF), vascular endothelial growth factor (VEGF), soluble FMS-like tyrosine kinase-1 (sFlt-1) and soluble endoglin. Heparin could demonstrate its effectiveness through complex interactions with pro-and anti-angiogenic factors (7). The literature includes several in-vivo and in-vitro studies evaluating the effects of anticoagulation therapy on angiogenesis; however, little is known about the fetal side (7-9). It is well known that the abnormality of angiogenesis plays a critical role in certain prematurity complications such as retinopathy of prematurity and bronchopulmonary dysplasia (10).

*Correspondence: stakci@gmail.com

In the current case-controlled prospective study, we aimed to investigate cord blood VEGF-A and sFlt-1 levels of women with recurrent miscarriages receiving anticoagulant treatment.

2. Materials and Methods

We conducted this study prospectively over 11 months (April 2012–March 2013) at Hacettepe University School of Medicine. Our institution's ethics committee (12-HEK-033) approved the study, and we obtained informed consent from all families before including the infants in the study.

2.1. Study population

We included term infants (≥ 37 weeks of gestation) in the study. We prospectively included newborns whose mothers were treated with LMWH and low dose aspirin due to recurrent miscarriages. The control group consisted of healthy gestational age-matched infants born in the same period without an adverse perinatal outcome. We excluded infants with a history of antenatal cigarette or alcohol exposure, maternal infection, hypertension, pre-eclampsia, chronic renal or cardiac disease, thyroid disease, epilepsy active asthma. We also excluded infants with congenital heart disease and other congenital and chromosomal abnormalities if determined after birth.

Two or more failed pregnancies before 20 weeks of gestation are considered recurrent miscarriages. We did not include biochemical, ectopic, and molar pregnancies. We excluded women who had uterine anatomic abnormalities, endocrine or chromosomal disorders, history of venous or arterial thromboembolism, and an exact indication for anticoagulant treatment during pregnancy rather than a recurrent miscarriage. We tested all participating women with recurrent miscarriages for acquired thrombophilia, antiphospholipid syndrome and heritable thrombophilia such as factor V Leiden, prothrombin G20210A, plasminogen activator inhibitor mutations and plasma activity levels of Protein C, Protein S and antithrombin.

2.2. Treatment protocol

We treated pregnant women diagnosed with recurrent miscarriages with enoxaparin (Clexane; Rhone-Poulet Rorer, France) subcutaneously in combination with low dose aspirin administered orally. We started both LMWH and aspirin treatments before six weeks of gestation in all cases. We maintained heparin treatment throughout delivery while stopping aspirin treatment approximately one week before delivery and noted the daily and cumulative doses of LMWH within the initiation time of the treatment. We calculated cumulative LMWH dosage between the multiplication of daily drug dose and total days of treatment.

2.3. Analytic procedure

We obtained mixed arterial-venous cord blood samples and temporarily stored them on ice upon delivery. We centrifuged all the samples at 3000 rpm within 15 min of collection. A technician, unaware of the patient's condition until assayed, aliquoted and froze serum at -80°C. We assayed the concentrations of VEGF-A and sFlt 1 in cord blood by ELISA (eBioscience, Bender MedSystems, Vienna, Austria), according to the protocol recommended by the manufacturer. The minimum detectable concentrations in the assays for VEGF-A and sFlt1 were 7.9 pg/ml and 30 pg/ml, respectively. The calculated overall inter-assay and intra-assay coefficients of variation were 4.3% and 6.2% for VEGF-A and 5.1% and 5.5% for sFlt-1, respectively.

2.4. Statistical analysis

We carried out statistical analysis using Statistical Package for Social Sciences (SPSS) version 19 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp; 2010) and calculated statistical differences between the studied groups using the Student t-test for the data normally distributed. We used the Mann-Whitney U-test for the data not normally distributed and the chi-square or Fisher's exact tests to compare categorical variables. We performed correlation between studied parameters using Spearman's coefficient of correlation. We considered differences to be statistically significant at $P<0.05$.

3. Results

We included Forty-four infants with a maternal LMWH and low dose aspirin usage during pregnancy in the study group and 42 healthy infants in the control group. There were no significant differences between the two groups' mean gestational ages, birth weight, gender and maternal ages (Table I). Median gravity was 3.5 (3-7) in the study group and 2 (1-5) in the control group (p=0.000). Median parities were 1 (1-3) and 1 (1-4) in the study and control groups, respectively (p=0.75). Mean first and fifth minute Apgar scores were significantly lower in the study group than in the control group (p=0.000).

Table 1. Comparison of the characteristics of the study and control group

Gestational age (weeks)*	37.2±0.6	37.4±0.7	0.18
Birth weight (g)*	3013±358	3106±344	0.22
Maternal age (years)*	30.4±5	30.7±4.7	0.79
Gender M/F (%)	30/14 (68.2/31.8)	25/17 (59.5/40.5)	0.54
Type of birth (NVD/CS)	6/38 (13.6/86.4)	9/33 (21.4/78.6)	0.40
Apgar 1.minute*	8.3±0.9	8.9±0.4	0.00
Apgar 5.minute*	9.2±1	9.9±0.3	0.00
Gravida (min-max)**	3.5 (3-7)	2 (1-5)	0.00
Parity (min-max)**	1 (1-3)	1 (1-4)	0.70
VEGF (pg/ml)*	301.4±220.9	360.5±174.9	0.16
sFlt-1 (pg/ml)*	530±610	380±520	0.52
Cumulative LMWH dosage (mg)*	6009±2067	-	

*Mean±standard deviation, **Median, M: male, F: female, NVD: Normal Vaginal Delivery, CS: Cesarean Section, VEGF: Vascular Endothelial Growth Factor, sFlt-1: soluble FMS-like tyrosine kinase-1, LMWH: Low Molecular Weight Heparin

We found the detectable thrombophilia etiologies of recurrent miscarriages as the following: Factor V Leiden heterozygous mutation in 8, plasminogen activator inhibitor heterozygous mutation in 2, Prothrombin G20210 A heterozygous mutation in 3 women, Protein C deficiency in 1, and Protein S deficiency in 1 woman (some women had two diagnoses)

We measured the serum VEGF-A levels in all samples. The mean cord blood VEGF-A level was 301.4±220.9 pg/ml in the study group and 360.5±174.9 pg/ml in the control group (p=0.16). Twenty-nine of 86 samples were not within the detectable limit for sFlt-1 (18 in the study group and 11 in the control group). Cord blood sFlt-1 levels were 530 ± 610 pg/ml and 380 ± 520 pg/ml in study and control groups, respectively (p=0.52).

Mothers received LMWH with a range of 20 to 60 mg per day. The cumulative mean maternal heparin dosage was 6009±2067 mg. Women received 100 mg of aspirin per day and stopped approximately before one week of delivery. There was no correlation between the cumulative heparin dosage and serum VEGF-A level (r=-0.082 and p=0.59). Although it did not reach statistical significance, there was a trend of negative correlation between the cumulative heparin dose and serum sFlt-1 (r=-0.41 and p=0.051).

4. Discussion

This study shows that the circulating VEGF-A and sFlt-1 levels do not alter in women with recurrent miscarriages receiving anticoagulant treatment. While there is no correlation between the cumulative dose of enoxoparine and cord blood VEGF-A, there is a negative correlation between total enoxoparine dose and cord blood sFlt-1.

Adequate uteroplacental blood flow during healthy pregnancies is necessary to maintain optimal fetal growth. Angiogenic growth factors play an essential role in cytotrophoblast differentiation, the remodeling of the spiral arteries, and the vascular development of the fetus (11). Angiogenic imbalance of the placenta is one of the prominent pathophysiologic mechanisms underlying pregnancy complications like recurrent miscarriage, pre-eclampsia, intrauterine growth restriction, and death (2, 12).

VEGF and sFlt-1 appear to be promising biomarkers for understanding the pathophysiology of angiogenesis in such conditions (7). VEGF is a pro-angiogenic key mediator of other angiogenic pathways with mitogenic, anti-antiapoptotic, and vascular permeability-enhancing activities primarily for vascular endothelium. VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D and PGF. VEGF-A is the most important member of this family (13, 14). The effects of VEGF are mediated by binding to tyrosine kinase receptors, the two main ones being FMS-like tyrosine kinase 1 (Flt-1) and the fetal liver kinase-1 (Flk-1). Flt-1 is a weak transmembrane tyrosine kinase-type VEGF receptor expressed in vascular endothelial cells, placental trophoblast cells, macrophages and monocytes. The soluble form of the Flt-1 receptor (a variant with a ligand-binding domain) is generated through alternative splicing of the same pre mRNA that encodes Ft-1 and proteolytic cleavage of Flt's ectodomain sFlt-1. It captures free VEGF with high affinity and functions as a circulating VEGF antagonist. Thus, sFlt can inhibit the pro-angiogenic role of VEGF competitively (15, 16).

It is known that VEGF and its receptor, sFlt-1, play a key role in cytotrophoblast differentiation and survival during placentation (17). In normal pregnancies, sFlt-1 increases excessively during gestation and decreases sharply soon after delivery. This condition suggests that the placenta is the primary origin of this circulating factor (18). Growing evidence shows that an altered balance between the levels of VEGF and sFlt-1 has been proposed as an indicator of placental hypoxia-ischemia and endothelial dysfunction leading to recurrent miscarriage (19). There are conflicting results on the levels and mRNA expressions of VEGF and sFlt-1 in women with recurrent miscarriage. In some studies, decreased sFlt-1 levels in maternal blood are proposed as a potential marker for predicting the risk of recurrent miscarriage (20-22). On the other hand, other reports found placental expressions of sFLt-1 and VEGF mRNA significantly higher in women with recurrent miscarriage than in the control group (19, 23). Wide variation of the study design and relatively low sample size might be the reasons for inconsistent results.

A recent metanalysis showed that LMWH could decrease recurrent miscarriage with a history of women with three or more miscarriages (24). Although more solid and assured results are needed to clarify LMWH treatment's benefit, it is increasingly used to prevent recurrent miscarriage (25, 26).

Favorable effects of heparin on trophoblast implantation and apoptosis inhibition suggest this treatment is a significant potential therapeutic agent in managing recurrent miscarriage (27). Most angiogenic proteins bind heparin and depend on heparan sulfate for their biological activities. Systemic heparin treatment may affect these proteins and vascular endothelial cells (28). Searle et al. suggested that heparin mobilized the sFlt-1 bound to heparan sulfate proteoglycans of the extracellular matrix into the circulation (2011). It has been shown that LMWH (enoxoparine) increases circulating levels of sFlt-1 and enhances urinary elimination in preeclamptic mothers (29). Also, a couple of other studies demonstrated that heparin elevates serum levels of sFlt-1 in vivo and in vitro (8, 9, 30, 31). Again, in the study by Searle et al. (8), heparin strongly induced sFlt-1 release in patients who underwent coronary angiography and sFlt-1 returned to baseline values 6 to 10 hours after heparin administration. In the study of Rosenberg et al. (9), sFlt-1 levels increased, and VEGF levels did not alter in women receiving heparin treatment in the third trimester compared to healthy controls.

The same study evinced that upregulation in the levels of

sFlt-1 is generated from heparin-induced shedding of Flt-1 receptor ectodomain both in vivo and in vitro (9). Recently, Moore et al. (32) clearly demonstrated that the placenta is a source of a large amount of sFlt-1, and chronic unfractionated heparin can display matrix-bound sFlt to the maternal circulation. In our study, cord blood sFlt-1 and VEGF-A levels did not alter within the maternal LMWH and low dose aspirin usage. Although numerous studies investigated the serum or plasma levels of sFlt-1 and VEGF in heparin-treated pregnant women, the circulatory profile of these factors has not been studied in the fetal site yet. As LMWH does not cross the placenta, non-altered cord blood levels may be a common condition. However, complex molecular interactions between the fetal and maternal side of the placenta under maternal anticoagulant therapy could not be ignored. The recognized or unrecognized causes of recurrent miscarriage and heparin usage may have different effects on circulating cord blood VEGF-A and sFlt-1 levels. Thus, further studies comparing women who have recurrent miscarriages with or without heparin treatment may be helpful to understand better the effects of anticoagulant treatment on the circulatory profile of cord blood angiogenic factors. It is common knowledge that heparin exerts its effects on VEGF and VEGF receptors in a dose and time manner (9, 33). Therefore, we calculated the cumulative LMWH dose for each woman during pregnancy and did not find any correlation between the heparin dose and the levels of both factors. Although the result did not reach statistical significance, the results indicate that there may be an association between the cumulative heparin dose and sFlt-1 level ($p=0.051$ and $r=-0.46$).

In conclusion, cord blood VEGF-A and sFlt-1 levels are not affected in women with recurrent miscarriage by maternal LMWH and low-dose aspirin treatment. These findings will improve our understanding of the complex interactions between maternal anticoagulant therapy and angiogenic factors on the fetal side. On the other hand, it is difficult to make a strong comment on the cord blood sFlt-1 level due to the low sample size.

Conflict of interest

The authors have no conflicts of interest relevant to this article to disclose.

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Authors' contributions

Concept: Ş.T., M.T., Design: A.K.T, Ş.Y., Data Collection or Processing: Ş.T., A.K.T, Ş.Y., M.T., Analysis or Interpretation: Ş.T., Literature Search: Ş.T., Writing: Ş.T.

References

1. Allison JL, Schust DJ. Recurrent first trimester pregnancy loss: revised definitions and novel causes. Curr Opin Endocrinol Diabetes Obes 2009; 16: 446-50. http://doi.org/10.1097/MED.0b013e3283327fc5
2. Matthiesen L, Kalkunte S, Sharma S. Multiple pregnancy failures: an immunological paradigm. Am J Reprod Immunol 2012; 67 :334-340. http://doi.org/ 10.1111/j.1600-0897.2012.01121.x
3. Rana S, Burke SD, Karumanchi SA. Imbalances in circulating angiogenic factors in the pathophysiology of pre-eclampsia and related disorders. Am J Obstet Gynecol 2020; S0002-9378 :31196-0. http://doi.org/10.1016/j.ajog.2020.10.022
4. Elmahashi MO, Elbareg AM, Essadi FM, Ashur BM, Adam I. Low dose aspirin and low-molecular-weight heparin in the treatment of pregnant Libyan women with recurrent miscarriage. BMC Res Notes 2014; 7: 23. http://doi.org/10.1186/1756-0500-7-23.
5. Shaaban OM, Abbas AM, Zahran KM, Fathalla MM, Anan MA, Salman SA. Low-Molecular-Weight Heparin for the Treatment of Unexplained Recurrent Miscarriage With Negative Antiphospholipid Antibodies: A Randomized Controlled Trial. Clin Appl Thromb Hemost 2017; 23: 567-572. http://doi.org/10.1177/1076029616665167
6. Kwak-Kim J, Agcaoili MS, Aleta L, Liao A, Ota K, Dambaeva S, et al. Management of women with recurrent pregnancy losses and antiphospholipid antibody syndrome. Am J Reprod Immunol. 2013 Jun;69(6):596-607. doi: 10.1111/aji.12114.
7. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of pre-eclampsia. N Engl J Med 2004; 350: 672– 683. http://doi.org/10.1056/NEJMoa031884.
8. Searle J, Mockel M, Gwosc S, Datwyler SA, Qadri F, Albert GI, et al. Heparin strongly induces soluble fms-like tyrosine kinase 1 release in vivo and in vitro--brief report. Arterioscler Thromb Vasc Biol 2011; 31: 2972-2974. http://doi.org/10.1161/ATVBAHA.111.237784.
9. Rosenberg VA, Buhimschi IA, Lockwood CJ, Paidas MJ, Dulay AT, Ramma W, et al. Heparin elevates circulating soluble fms-like tyrosine kinase-1 immunoreactivity in pregnant women receiving anticoagulation therapy. Circulation 2011; 124: 2543-2553. http://doi.org/10.1161/CIRCULATIONAHA.111.046821.
10. Levesque BM, Kalish LA, Winston AB, Parad RB, Hernandez-Diaz S, Phillips M, et al. Low urine vascular endothelial growth factor levels are associated with mechanical ventilation, bronchopulmonary dysplasia and retinopathy of prematurity. Neonatology 2013; 104: 56-64. http://doi.org/ 10.1159/000351040.
11. Catarino C, Rebelo I, Belo L, Rocha S, Castro EB, Patrício B, et al. Fetal and maternal angiogenic/anti-angiogenic factors in normal and preeclamptic pregnancy. Growth Factors. 2009 ;27: 345-351. http://doi.org/10.3109/08977190903184670
12. Osol G, Moore LG. Maternal uterine vascular remodeling during pregnancy. Microcirculation 2014; 21: 38-47. http://doi.org/10.1111/micc.12080
13. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. Genes Cancer 2011; 2: 1097-1105. http://doi.org/10.1177/1947601911423031
14. Andraweera PH, Dekker GA, Roberts CT. The vascular endothelial growth factor family in adverse pregnancy outcomes. Hum Reprod Update. 2012; 18: 436-457. http://doi.org/10.1093/humupd/dms011
15. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A 1993; 90: 10705-10709. http://doi.org/10.1073/pnas.90.22.10705.

16. Kappas NC, Zeng G, Chappell JC, Kearney JB, Hazarika S, Kallianos KG, et al. The VEGF receptor Flt-1 spatially modulates Flk-1 signaling and blood vessel branching. J Cell Biol 2008; 181: 847-858. http://doi.org/10.1083/jcb.200709114

17. Brockelsby JC, Anthony FW, Johnson IR, Baker PN. The effects of vascular endothelial growth factor on endothelial cells: a potential role in pre-eclampsia. Am J Obstet Gynecol 2000; 182: 176-183. http://doi.org/10.1016/s0002-9378(00)70510-2

18. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, et al. A vascular endothelial growth factor antagonist is produced bythe human placenta and released into the maternal circulation. Biol Reprod 1998; 59: 1540-1548. http://doi.org/10.1095/biolreprod59.6.1540

19. Pang L, Wei Z, Li O, Huang R, Qin J, Chen H, et al. An increase inb vascular endothelial growth factor (VEGF) and VEGF soluble receptor-1 (sFlt-1)are associated with early recurrent spontaneous abortion. PLoS One 2013; 8: e75759. http://doi.org/10.1371/journal.pone.0075759

20. Daponte A, Pournaras S, Polyzos NP, Tsezou A, Skentou H, Anastasiadou F, et al. Soluble FMS-like tyrosine kinase-1 (sFlt-1) and serum placental growth factor (PlGF) as biomarkers for ectopic pregnancy and missed abortion. J Clin Endocrinol Metab 2011; 96: E1444-51. , http://doi.org/10.1210/jc.2011-0037

21. Kaitu'u-Lino TJ, Whitehead CL, Ngian GL, Permezel M, Tong S. Serum concentrations of soluble Flt-1 are decreased among women with a viable fetus and no symptoms of miscarriage destined for pregnancy loss. PLoS One 2012; 7: e32509. http://doi.org/10.1371/journal.pone.0032509

22. Muttukrishna S, Swer M, Suri S, Jamil A, Calleja-Agius J, Gangooly S, et al. Soluble Flt-1 and PlGF: new markers of early pregnancy loss? PLoS One 2011; 6: e18041. http://doi.org/ 10.1371/journal.pone.0018041

23. Pang LH, Li MJ, Li Yang DM, Shi L. Vascular endothelial growth factor (VEGF) and the VEGF soluble receptor-1 (sFlt-1) in chorionic villus tissue from Chinese women with early recurrent spontaneous abortion. J Int Med Res 2011; 39: 830-837. http://doi.org/10.1177/147323001103900316

24. Wang G, Zhang R, Li C, Chen A. Evaluation of the effect of low molecular weight heparin in unexplained recurrent pregnancy loss: a meta-analysis of randomized controlled trials. J Matern Fetal Neonatal Med. 2021: 1-8. http://doi.org/10.1080/14767058.2021.1957819

25. Kaandorp SP, Goddijn M, van der Post JA, Hutten BA, Verhoeve HR, Hamulyák K, et al. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med 2010; 362: 1586-1596. http://doi.org/ 10.1056/NEJMoa1000641

26. Clark P, Walker ID, Langhorne P, Crichton L, Thomson A, Greaves M, et al. SPIN (Scottish Pregnancy Intervention) study: a multicenter, randomized controlled trial of low-molecular-weight heparin and low-dose aspirin in women with recurrent miscarriage. Blood 2010; 115: 4162-4167. http://doi.org/10.1182/blood-2010-01-267252

27. Nelson SM, Greer IA. The potential role of heparin in assisted conception. Hum Reprod Update 2008; 14: 623-645. http:doi.org/10.1093/humupd/dmn031

28. Norrby K, Nordenhem A. Dalteparin, a low-molecular-weight heparin, promotes angiogenesis mediated by heparin-binding VEGF-A in vivo. APMIS 2010; 118: 949-957. http://doi.org/10.1111/j.1600-0463.2010.02635.x

29. Hagmann H, Bossung V, Belaidi AA, Fridman A, Karumanchi SA, Thadhani R, et al. Low-molecular weight heparin increases circulating sFlt-1 levels and enhances urinary elimination. PLoS One 2014; 9: e85258. http://doi.org/ 10.1371/journal.pone.0085258

30. Sela S, Natanson-Yaron S, Zcharia E, Vlodavsky I, Yagel S, Keshet E. Local retention versus systemic release of soluble VEGF receptor-1 are mediated by heparin-binding and regulated by heparanase. Circ Res 2011; 108: 1063-1070. http://doi.org/10.1161/CIRCRESAHA.110.239665.

31. Denizot Y, Leguyader A, Cornu E, Laskar M, Orsel I, Vincent C, et al. Release of soluble vascular endothelial growth factor receptor-1 (sFlt-1) during coronary artery bypass surgery. J Cardiothorac Surg 2007; 2: 38. http://doi.org/10.1186/1749-8090-2-38

32. Moore KH, Chapman H, George EM. Unfractionated heparin displaces sFlt-1 from the placental extracellular matrix. Biol Sex Differ 2020; 11: 34. http://doi.org/10.1186/s13293-020-00311-w

33. Park M, Lee ST. The fourth immunoglobulin-like loop in the extracellular domain of FLT-1, a VEGF receptor, includes a major heparin-binding site. Biochem Biophys Res Commun 1999; 264: 730-734. http://doi.org/10.1006/bbrc.1999.1580

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Impact of COVID-19 pandemic on the diagnosis of gastrointestinal cancer

Tuğba ŞENEL*, Talat AYYILDIZ

Department of Gastroenterology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

Coronavirus disease 2019 (COVID-19) pandemic has had a major impact every aspect of life all over the world with severe consequences. This study aimed to evaluate the impact of COVID-19 pandemic on the number of gastrointestinal endoscopy procedures and resulting cancer detection rate at our center. This was a retrospective and single-center study. The 6-month period from March 11 to September 11, 2020 (lockdown period) was compared with the same months of 2018 and 2019 (pre-pandemic period) in terms of the number of endoscopic procedures performed at our gastroenterology unit, malignancy detection rate and clinicopathological characteristics of patients. The data were analyzed using the SPSS Statistics 22.0 software package. A 33% reduction was observed in the number of endoscopic procedures during the pandemic compared to pre-pandemic years and the difference was significant ($p<0.001$). Despite the decrease in endoscopic activity, cancer detection increased during the pandemic ($p=0.057$). Male sex and age 65 years or older were non-significantly more common among patients diagnosed with cancer on endoscopic biopsy during the pandemic compared to the pre-pandemic era but the difference was non-significant ($p=0.983$, $p=0.241$). Patients diagnosed with cancer during the pandemic were more likely to present at an advanced stage. The most common cancers were those originating from the colon and rectum and adenocarcinoma was the most prevalent pathological diagnosis. The distributions of tumor location and pathological diagnosis of the patients were not significantly different among the years ($p=0,494$, $p=0,849$). In conclusion, a reduction was found in the overall number of endoscopic procedures during the lockdown. However, despite the decrease in the number of procedures, cancer detection rate and the rate of admission at advanced stages were increased at a non-significant level.

Keywords: COVID-19, gastrointestinal endoscopy, gastrointestinal cancer, tumor stages, tumor locations

1. Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spread rapidly from China to almost all parts of the world and was declared a pandemic by the WHO (World Health Organization) on March 2020. This infection typically presents with fever, cough, muscle pain and fatigue. Patients having gastrointestinal disturbances such as diarrhea and nausea as the initial symptoms have also been reported (1). While transmission of SARS CoV-2 occurs mainly through respiratory droplets, SARS-CoV-2 RNA has also been demonstrated in the stools of infected patients (2). Gastrointestinal endoscopy is an invasive procedure that carries a risk for the spread of SARS-CoV-2 via respiratory droplets, aerosol inhalation, conjunctival contact and potentially fecal-oral route (3).

COVID-19 pandemic has affected healthcare systems globally irrespective of differences in political structure and economic status among countries. Both emergency room and intensive care unit beds have been heavily used in the fight against COVID-19, leading to inevitable disruptions to routine and non-emergency health services. Thus, as with other countries, endoscopic procedures have been performed for the diagnosis and treatment of prioritized patients and those requiring emergency care only during the pandemic. Consistently, in our hospital, endoscopic procedures were limited to carefully selected patients who were deemed clinically to be at greatest need. The current study aimed to determine and discuss the impact of COVID-19 on the number of endoscopic procedures performed in our hospital, the rate of cancer detection on endoscopic biopsy and clinicopathological characteristics of the patients.

2. Materials and Methods

In this study, the number of patients (18 years of age or older) who underwent esophagogastroduodenoscopy, colonoscopy and rectosigmoidoscopy in the 6-month period between March 11, 2020 (the date of first COVID-19 case identified) and September 11, 2020, cancer detection rate, pathologic subtypes of tumors in patients diagnosed with a malignancy, tumor locations and stages, and age (≥ 65 and <65 years) and sex distribution of these patients were compared to the patients

*Correspondence: tugbasenel-89@hotmail.com

who had the same procedures during the same time intervals in 2018 and 2019. Patient information was retrieved from the Nucleus v9.35.1 medical information system (MONAD, Turkey) database. This was a single-center study with a retrospective design. The study was approved with the decision of the Ethics Committee of our hospital, dated 13.10.2021 and numbered 2021/479.

2.1. Statistical analysis

The study data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) Version 22 (IBM Corp., Armonk, NY). Descriptive statistics were presented as numbers and percentages for categorical variables. Pearson's chi-square test was applied to compare categorical variables using contingency tables, and likelihood ratio test and Bonferroni correction were used for significant results. In addition, frequency distributions were presented and interpreted using clustered bar charts by categorical variables. Statistical significance levels were set at $p<0.05$, $p<0.01$ and $p<0.001$.

3. Results

It was identified that a total of 6828 esophagogastroduodenoscopy, 1116 flexible rectosigmoidoscopies and 3266 colonoscopies were performed in our center at the specified time intervals in 2018, 2019 and 2020. Fig. 1 shows the distribution of these procedures by time intervals. The total number of endoscopic procedures was 3616 in 2018, 4773 in 2019 and 2821 in 2020. A significant 33% reduction was observed in the total number of endoscopic procedures performed in 2020 compared to 2018 and 2019 ($p<0.001$).

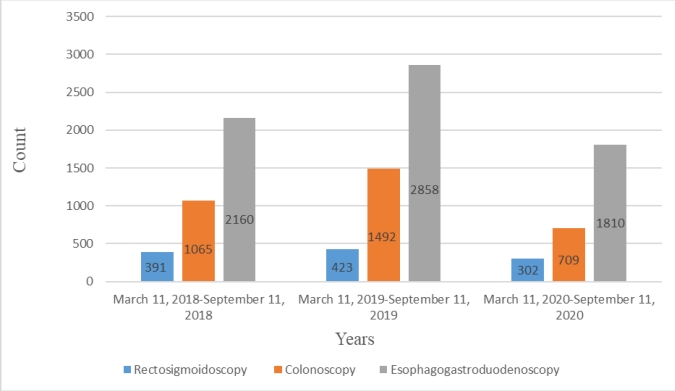

Fig. 1. Number of endoscopic procedures by years

A total of 309 patients were identified as having received a diagnosis of gastrointestinal cancer following endoscopic procedures during this time period. The monthly distribution of the cancer patients is shown in Fig. 2. Among these patients, 27.2% (n=84) were diagnosed in 2018, 37.2% (n=115) in 2019 and 35.6% (n=110) in 2020. The rate of cancer detection per procedure was 0.023% in 2018, 0.024 in 2019 and 0.038 in 2020. It was observed that despite the reduction in the total number of endoscopic procedures in 2020, the increase in the cancer detection rate was non-significant at 95% confidence interval (CI) but significant at 90% CI (p=0.057).

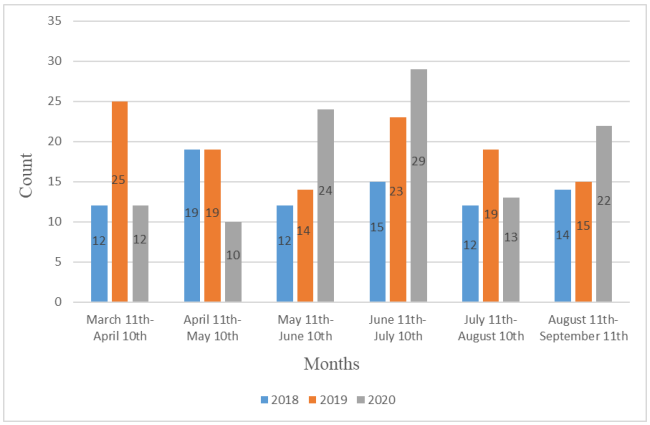

Fig. 2. Frequency of monthly gastrointestinal cancer detection by years

Table I shows the distribution of demographic variables by years. Although the annual cancer detection rates were higher in male patients than in female patients, there was no significant difference between sexes over the study years (p=0.983). When the age groups were examined by years, there was a greater number of patients aged 65 years or older in 2020 than patients under 65 years of age but the difference was not significant (p=0.241). The age cut-off of 65 years was specifically chosen for the study because a curfew was imposed for persons aged 65 years or older during the pandemic in Turkey. No significant differences were found in terms of sex and age group among years in patients diagnosed with cancer (p2018=0.095, p2019=0.946, p2020=0.791).

Table 1. Distributions of demographic variables by years

		Years			p value
		2018	2019	2020	
		n (%)	n (%)	n (%)	
Sex	Female	29 (34.5)	40 (34.8)	37 (33.6)	0.983
	Male	55 (65.5)	75 (65.2)	73 (66.4)	
Age (years)	<65	49 (58.3)	57 (49.6)	51 (46.4)	0.241
	≥65	35 (41.7)	58 (50.4)	59 (53.6)	

A review of the pathology results of 309 patients diagnosed with cancer showed that 81.9% (n=253) of the patients had adenocarcinoma, 8.7% (n=27) had a neuroendocrine tumor, 4.9% (n=15) had squamous cell carcinoma and 4.5% (n=14) had other tumors (lymphoma, gastrointestinal stromal tumor, malignant melanoma). In patients with cancer, the distribution of tumors was as follows: colorectal cancer (46.6%, n=144), gastric cancer (37.9%, n=117), small intestine cancer (8.4%, n=26) and esophageal cancer (7.1%, n=22).

The distribution of tumor locations by years is presented in Table II. Colorectal cancer was the most common malignancy in all years. There was no significant difference in the tumor location distribution among the years (p=0.494).

Table 2. Distribution of tumor locations by years

		Years			p value
		2018	2019	2020	
		n (%)	n (%)	n (%)	
Location	Esophagus	7 (8.3)	8 (7.0)	7 (6.4)	0.494
	Stomach	35 (41.7)	41 (35.7)	41 (37.3)	
	Small intestine	5 (6.0)	7 (6.1)	14 (12.7)	
	Colon and rectum	37 (44.0)	59 (51.3)	48 (43.6)	

The distribution of pathologies by years is summarized in Table III. No significant difference was found among tumor locations (p=0.849).

Table 3. Distribution of pathologies by years

		Years			p value
		2018	2019	2020	
		N (%)	N (%)	N (%)	
Pathology	Adenocarcinoma	67 (79.8)	99 (86.1)	87 (79.1)	0.849
	Squamous cell carcinoma	4 (4.8)	4 (3.5)	7 (6.4)	
	Neuroendocrine tumor	9 (10.7)	8 (7.0)	10 (9.1)	
	Others	4 (4.8)	4 (3.5)	6 (5.5)	

Among 309 patients included in the study, the tumor stage was 0, 1 or 2 in 45.3% (n=140) and 3 or 4 in 54.7% (n=169). Table IV shows the distribution of tumor stages by years. It was seen that stage 3 tumors were more common in all years and also, the number of patients with stage 3 or 4 tumors increased in 2019 and 2020 compared to 2018. However, there was no significant difference in tumor stage among the years (p=0.374).

Table 4. Distribution of tumor stages by years

		Years			p value
		2018	2019	2020	
		n (%)	n (%)	n (%)	
Stages	0-2	40 (47.6)	56 (48.7)	44 (40.0)	0.374
	3, 4	44 (52.4)	59 (51.3)	66 (60.0)	

4. Discussion

Endoscopy plays an important role in the diagnosis and treatment of gastrointestinal diseases. Endoscopic screening enables early detection and treatment of gastrointestinal tumors. However, considering the invasive nature of endoscopy and associated high risk for transmission of viral infection as well as prioritization of fight against COVID-19 in healthcare services, elective endoscopic procedures were postponed in most clinics across Turkey after the reporting of first case on March 11, 2020. Naturally, this has led to concerns that cancerous lesions would be diagnosed at a late and advanced stage. The current study was conducted on the consideration that from a scientific standpoint, it is of vital importance to present quantitative data on cancer outcomes from the pandemic period and compare them with literature data.

In a study involving 252 endoscopy units in 55 countries around the world, over 80% reduction was reported in endoscopic procedures during the COVID-19 pandemic (4). Similarly, in a study by D'Ovidio et al., it was shown that while the number of colonoscopies performed was substantially reduced, the detection rate of colorectal cancers and high-risk adenomas was significantly high during the COVID-19 lockdown (5). Turkington et al. reported that the number of patients diagnosed with esophagogastric cancer and Barrett's esophagus decreased significantly during the pandemic compared to previous years (6). In a single-center study from Australia, a significant reduction was observed in the overall number of endoscopic procedures during the pandemic versus pre-pandemic period, there was no significant difference in the number of cancerous and precancerous lesions (7). A study from the United Kingdom reported an 88% decrease in the number of endoscopic procedures during the pandemic. Additionally, weekly cancer detection rate dropped by 58%. However, cancer detection rate per procedure was significantly increased from 1.91% in the pre-pandemic period to 6.61% during the COVID-19 period (8). Likewise, in two separate studies from China, although a considerable reduction in the endoscopic activity was found during the pandemic, there was a significantly greater percentage of patients diagnosed with malignancy (9, 10). In a study comparing the 6-month period from the beginning of the pandemic in the UK (April to October 2020) to the same period of the previous year, it was shown that colonoscopies performed across the country dropped by 46% and colorectal cancer diagnosis was missed in over 3500 patients. While the number of colonoscopies was reduced by 92% in April 2020, the figures increased to an expected level in October 2020 (11). In the current study, a 6-month interval during the pandemic period was compared to the same period of 2018 and 2019 in terms of the number of endoscopies performed and a significant reduction was found in all three endoscopic procedures (flexible rectosigmoidoscopy, esophagogastroduodenoscopy, colonoscopy) ($p<0.001$) during the lockdown. The cancer detection rate per procedure was 0.023% in 2018, 0.024% in 2019 and 0.038% in 2020. In line with literature data, increased cancer detection rate was observed despite a reduction in the overall number of endoscopic procedures during the pandemic (p=0.057). Contrastingly, the cancer detection rate that we found both before and during the pandemic was lower compared to those previously reported in the literature. We think that this may be related to the fact that endoscopic procedures are easily accessible and relatively inexpensive in our country and they are also performed in secondary care centers. Therefore, it can be assumed that fewer cases of cancer were diagnosed in tertiary referral hospitals like our hospital.

Looking at the demographic characteristics of the patients, the mean age of the patients was and the percentage of male

patients undergoing endoscopic screening were found to be slightly but not significantly lower during the lockdown compared to the pre-pandemic period in D'Ovidio et al.'s study (5). In another study, it was reported that advanced age and male sex were significantly more common in patients undergoing an endoscopic procedure during the pandemic versus pre-pandemic era (8). In our study, male sex and age 65 years or older were non-significantly more common among patients diagnosed with cancer on endoscopic biopsy during the pandemic compared to the pre-pandemic period (p=0.983, p=0.241).

In a study from Japan, a group of patients with colorectal cancer undergoing surgery during and before the pandemic were analyzed retrospectively. While the number of patients undergoing colonoscopy was reduced significantly during the pandemic, no significant difference was found in the number of colorectal cancer surgeries performed. However, there was a significantly higher rate of obstructive colorectal cancers requiring emergency surgery in that patient group compared to pre-pandemic period. The authors reported that numerically, more patients presented with stage 3 or 4 CRC and fewer patients presented with stage 0, 1 or 2 CRC during the pandemic period (12). Similarly, it was found that more patients admitted with stage 3 or 4 cancer and fewer patients presented with stage 0, 1 or 2 cancers during the pandemic versus the pre-pandemic period but the difference was non-significant (p=0.374).

Regarding tumor location, the most prevalent gastrointestinal cancer was colorectal cancer, followed by gastric, esophageal and small intestine cancers (13). The most common pathological diagnosis was adenocarcinoma. Other pathological diagnoses included squamous cell carcinoma, neuroendocrine tumor, lymphoma, gastrointestinal stromal tumor and malignant melanoma. In line with previous reports, the largest patient population comprised of colorectal cancer patients, followed by patients with gastric cancer. Adenocarcinoma was the most common pathological diagnosis, followed by neuroendocrine tumor, squamous cell carcinoma and others (gastrointestinal stromal tumor, malignant melanoma). In the present study, no significant changes were found in the distributions of tumor location and pathological diagnosis of study patients during the pandemic compared to pre-pandemic years (p=0.494, p=0.849).

A number of limitations should be noted for our study. Since this was a retrospective study, data on the indications for endoscopic procedures were not available. In our center, routine COVID-19 testing has not been done before and after endoscopic procedures, especially in the beginning of the pandemic. Therefore, information about the safety of our endoscopic procedures in relation to infection control could not be obtained.

Our study stands out from previous studies because we examined the distributions of tumor locations and pathological diagnoses from the pandemic period and the study adds valuable relevant data to the literature.

A remarkable finding of our study was the increase in cancer detection rate, albeit non-significantly, despite the decline in the number of endoscopic procedures during the lockdown.

Strictly ensuring safety in the endoscopy units in terms of infection prevention, implementation of infection control measures to protect patients and healthcare personnel and sharing these safety strategies on social media will help individuals requiring an endoscopic procedure make informed decisions.

In conclusion; the experience from the pandemic has clearly shown the need for the development of different, easily applicable and non-invasive techniques to detect malignancies. Survey studies with an in-depth analysis of the opinions of individuals choosing or refusing to undergo endoscopic procedures during the pandemic may provide valuable insights. Interruptions in access to gastrointestinal endoscopy may lead to delays in the treatment of patients with gastrointestinal cancer. We believe that studies on larger patient series using different methodologies and assessments will improve our understanding of the impact of COVID-19 on healthcare utilization and patient outcomes, and help guide future planning.

Conflict of interest

All authors declare no conflict of interest regarding this manuscript.

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Authors' contributions

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References

1. Zhang H, Kang Z, Gong H, Xu D, Wang J, Li Z, et al. Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. Gut, 2020. 69(6): p. 1010-1018. doi: 10.1136/gutjnl-2020-320953

2. Cheung KS, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, et al. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. Gastroenterology, 2020. 159(1): p. 81-95. doi: 10.1053/j.gastro.2020.03.065

3. Soetikno R, Teoh AYB, Kaltenbach T, Lau JYW, Asokkumar R, Prodigalidad PC, et al. Considerations in performing endoscopy during the COVID-19 pandemic. Gastrointest Endosc, 2020. 92(1): p. 176-183. doi: 10.1016/j.gie.2020.03.3758

4. Parasa S, Reddy N, Faigel DO, Repici A, Emura F, Sharma P. Global İmpact of the COVID-19 Pandemic on Endoscopy: An

International Survey of 252 Centers From 55 Countries. Gastroenterology, 2020. 159(4): p. 1579-1581. e5. doi: 10.1053/j.gastro.2020.06.009

5. D'Ovidio V, Lucidi C, Bruno G, Miglioresi L, Lisi D, Emilio M, et al. Impact of COVID-19 Pandemic on Colorectal Cancer Screening Program. Clin Colorectal Cancer, 2021. 20(1): p. e5-e11. doi: 10.1016/j.clcc.2020.07.006

6. Turkington RC, Lavery A, Donnelly D, Cairnduff V, McManus DT, Coleman HG. The Impact of the COVID-19 Pandemic on Barrett's Esophagus and Esophagogastric Cancer. Gastroenterology, 2021. 160(6): p. 2169-2171. e1. doi: 10.1053/j.gastro.2021.01.208

7. He T, Maclsaac MB, Hume SJ, Thompson AJ, Schulberg JD. COVID-19 and its impact on endoscopy services: what is the threshold for missed malignant diagnosis? Gut, 2020. doi: 10.1136/gutjnl-2020-322769

8. Rutter MD, Brookes M, Lee TJ, Rogers P, Sharp L. Impact of the COVID-19 pandemic on UK endoscopic activity and cancer detection: a National Endoscopy Database Analysis. Gut, 2021. 70(3): p. 537-543. doi: 10.1136/gutjnl-2020-322179

9. Zhu L, Cai MY, Shi Q, Wang P, Li QL, Zhong YS, et al. Analysis of selective endoscopy results during the epidemic of coronavirus disease 2019 (COVID-19). Zhonghua wei chang wai ke za zhi, 2020. 23(4): p. 327-331. doi: 10.3760/cma.j.issn.1671-0274.2020-0316-00147

10. Huang K, Zhao X, Chen X, Gao Y, Yu J, Wu L. Analysis of Digestive Endoscopic Results During COVID-19. J Transl Int Med, 2021. 9(1): p. 38. doi: 10.2478/jtim-2021-0006

11. Morris EJ, Goldacre R, Spata E, Mafham M, Finan PJ, Shelton J, et al. Impact of the COVID-19 pandemic on the detection and management of colorectal cancer in England: a population-based study. Lancet Gastroenterol Hepatol, 2021. doi: 10.1016/S2468-1253(21)00005-4

12. Mizuno R, Ganeko R, Takeuchi G, Mimura K, Nakahara H, Hashimoto K, et al. The number of obstructive colorectal cancers in Japan has increased during the COVID-19 pandemic: A retrospective single-center cohort study. Ann Med Surg (Lond), 2020. 60: p. 675-679. doi: 10.1016/j.amsu.2020.11.087

13. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer, 2019. 144(8): p. 1941-1953. doi: 10.1002/ijc.319

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Knowledge and perceptions of the use of complementary and alternative medicine in dental clinic students in Makassar city, Indonesia

Ayub Irmadani ANWAR*

Department of Dental Public Health, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia

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Abstract

To find out the knowledge and perspectives of professional students of the Faculty of Dentistry of Hasanuddin University regarding complementary and alternative medicine in the field of dentistry. The clinical students of class 2014 and 2015 are the respondents in this study. Each received an online questionnaire in the form of Google Form, which consists of questions about knowledge and perspectives on complementary and alternative medicine, along with additional questions about the characteristics of the respondents (gender, class). The data are then analyzed with statistical analysis. The mean correct answers to five items of the clinical question from 148 participating students were 2.22 ± 1.14. Of the 740 correct answers expected (5 correct answers from each student) for 5 clinical questions, only 329 correct answers (44.45%) were obtained. The percentage of students who have little to no knowledge about CAM is 85.8% (127 students). Only 29 students (19.6%) disagreed that CAM was necessary for health and dental services. Twenty-one out of one hundred forty-eight students stated that they knew CAM. More than half of the students are interested in learning more about CAM and agree that CAM education should be integrated into the curriculum.

Keywords: complementary and alternative medicine, knowledge, perspective, physical and mental health

1. Introduction

The history of palliative care is currently attracting public attention through traditional treatments that have developed and are used for various medical and dental conditions because they have had a positive impact on society's history. Health efforts, in addition to conventional medicine, are also mostly done with complementary and alternative medicine (1).

Complementary and Alternative Medicine is a nonconventional treatment aimed at improving the health status of the community, including promotive, curative, preventive, and rehabilitative efforts obtained through structured education with high quality, safety, and effectiveness based on biomedical science, which has not been accepted in conventional medicine (2, 3).

Indonesia has cultural diversity and the habit of using herbs, which is one of the treatment methods of CAM that has been used for a long time. Based on the results of Riskesdas 2018, traditional health services are seen from the use of family medicinal plants, the proportion is 24.6%. The proportion of traditional health service utilization increased slightly, from 30.4% (Riskesdas 2013) to 31.4% (4).

The popularity of CAM is rapid in the field of medical science and is now considered an important branch of the health care system (5). Popular CAM interventions include vitamins and nutraceuticals, herbal and homeopathic products, which can also be applied in dentistry. Common dental indications, including symptom relief, occur in acute oral conditions. About 10% of dental patients use topical herbal products for pain relief. However, it is important for patients and healthcare professionals, including dentists, to adhere to evidence-based practice when using CAM (3).

Traditional practice is based on a holistic approach to people in the wider fields of health, religion, and culture (4, 5). CAM can be classified into the categories of biologically based therapies such as herbal and dietary supplements, alternative medical systems such as acupuncture or Ayurveda, energy therapies such as Reiki, manipulatives, and body-based systems such as chiropractic or massage, and mind-body interventions such as tai chi or yoga (6-8).

About 80% of sick individuals, mainly in developing countries, are more dependent on complementary therapies than conventional health care, while the percentage using CAM therapies has decreased by half among the population in industrialized countries. It has always been a treatment for millions of patients around the world (9, 10).

Although some understanding comes from research abroad, no studies have yet assessed whether dentists in Indonesia

*Correspondence: ayubanwar.dds@gmail.com

graduated with adequate knowledge to treat patients using CAM, nor have demonstrated behavior toward CAM, in college students. Indonesian Dentist Competency Standards do not have guidelines on the role of CAM in dentistry. Currently, there are no CAM courses available for clinical dental students at the Faculty of Dentistry, Hasanuddin University.

2. Materials and Methods

This research is a cross-sectional study conducted online through social networks in August 2020. A total of 148 clinical students of the Faculty of Dentistry, Hasanuddin University are actively registered and willing to be respondents.

The instrument used in this study was a questionnaire consisting of a knowledge question questionnaire (clinical knowledge and self-reported), perspective questions, and additional questionnaires regarding the characteristics of the respondents (gender and class).

The clinical knowledge indicator consists of 5 clinically relevant case sketch questions, where there is only one correct answer among the four possible answer choices provided. The self-reported knowledge and interest indicator consisted of 2 questions, which were assessed using a Likert scale (score 4 'yes' to score 1 'no at all). The perspective indicator consists of five perspective-based questions measuring behavior towards CAM. Self-reported behavior about CAM to treat dental disease, safety, effectiveness, necessity, and integration into the dental curriculum was assessed using a Likert scale (score 4 'strongly agree' to score 1 'strongly disagree').

This study was approved by the Ethics Committee of the Faculty of Dentistry, Hasanuddin University based on Attachment Number: 0086/PL.09/KEPK FKG-RSGM UNHAS/ 2020.

Data were processed using SPSS 25 and analyzed using Fisher's test, Chi-square test, Mann Whitney test with statistical significance $p<0.05$.

3. Results

Most of the respondents were women as many as 117 respondents (79.1%), while male respondents were 31 (20.9%). Based on the class, the results showed that the respondents in the 2014 batch were 42 respondents (28.4%) and 106 respondents (71.6%) in the 2015 batch (Table 1).

Table 1. Distribution of study subjects by gender and class (N=148)

Respondents' Characteristics		n	%
Gender	Male	31	20.9
	Female	117	79.1
Class	2014	42	28.4
	2015	106	71.6

A total of 81 (54.7%) respondents got the correct answer regarding CAM. Of the 5 question topics, only on the topic of CAM interaction, there was a significant difference between class years ($p<0.05$) (Table 2).

Table 2. Knowledge of dental clinic student about CAM (N=148)

Topic	Correct answers (%)	Mean ± SD	Gender[a] (p)	Class[b] (p)
CAM Interactions	45 (30.4)	0.30 ± 0.46	0.277	0.014*
CAM-nutraceutical Interactions	61 (41.2)	0.41 ± 0.49	0.414	0.799
CAM used in dentistry	81 (54.7)	0.55 ± 0.49	0.155	0.270
Acupuncture	80 (54.1)	0.54 ± 0.50	1.00	0.401
CAM side effect reaction	62 (41.9)	0.42 ± 0.49	0.838	0.604

[a] Fisher's exact test; [b] Chi-square test; * Statistical Significance ($p<0.05$)

Table 3 shows that 21 respondents (14.2%) know CAM. Based on the question about the interest in learning more about CAM, it was found that 115 respondents (77.7%) were interested in learning more about CAM. Statistically, there was no significant difference between gender and year of class on the question of knowledge and interest in learning more about CAM ($p>0.05$).

Table 3. Knowledge and interest in CAM subjects (N=148)

Question	Number of responses (%)		Gender[a] (p)	Class[b] (p)
	Yes	Not		
Knowledge about CAM	21 (14.2)	127 (85.8)	0.567	0.983
Interest in learning more about CAM	115 (77.7)	33 (22.3)	0.569	0.300

[a] Fisher's exact test; [b] Chi-square test; * Statistical Significance ($p<0.05$)

Table 4 shows 5 questions related to the perspective on the role of CAM in oral health, statistically, no significant differences were found between sex and year of class ($p>0.05$).

Table 4. Perspective of dental clinic student about the role of CAM (N=148)

Question	Number of responses (%)		Mean ± SD	Gender[a] (p)	Class[b] (p)
	Agree	Disagree			
CAM can help direct common dental diseases	114 (77)	34 (23)	2.83 ± 0.56	0.349	0.779
CAM can affect the safety of dental procedures	110 (74.3)	38 (25.7)	2.80 ± 0.57	0.648	0.612
CAM is ineffective and has no effect	43 (29.1)	105 (70.9)	2.25 ± 0.57	0.661	0.935
CAM is considered necessary for health and dental services	119 (80.4)	29 (19.6)	2.89 ± 0.51	0.135	0.572
CAM education is required in the dentistry curriculum	129 (87.2)	19 (12.8)	3.08 ± 0.61	0.765	0.448

[a] Fisher's exact test; [b] Chi-square test

Table 5 shows the gender variable, the results showed that there was no significant difference between the genders in knowledge and perspective on CAM (p>0.05). In the variable class, it was found that there was a significant difference between the class in the knowledge of CAM (p<0.05), but there was no significant difference between the class in the perspective of CAM (p>0.05).

Table 5. Differences in knowledge and perspectives on complementary and alternative medicine by gender and class

Variable	n	Knowledge		Perspective	
		Mean±SD	p	Mean±SD	p
Gender					
Male	31	2.22 ± 1.17	0.841	14.12 ± 1.74	0.105
Female	117	2.22 ± 1.13		13.76 ± 1.53	
Class					
2014	42	2.54 ± 1.06	0.025*	13.57 ± 1.41	0.230
2015	106	2.09 ± 1.14		13.95 ± 1.63	

* Statistical Significance (p<0.05), Mann-Whitney test

4. Discussion

This study is the first study to analyze the knowledge and perspectives of clinical students of the Faculty of Dentistry of Hasanuddin University towards complementary and alternative medicine, which has been investigated by many authors in the international literature (1, 11).

A dentist must always act not to provide treatment beyond his expertise and competence, including the decision to perform CAM, whether it is an adjunct or a substitute for conventional therapy. In discussing with patients, a dentist must always provide an accurate and objective professional opinion, which must be supported by sound clinical judgment and informed by scientific studies (12).

In this study, it was found that quite a several clinical students of the dental faculty of Hasanuddin University were able to correctly answer clinical questions about CAM, especially questions on the topic of CAM used in dentistry and acupuncture used in the treatment of the temporomandibular joint disorder. However, on questions about CAM interactions, CAM-nutraceutical interactions, and side effects related to CAM, more students did not answer correctly. These findings indicate that clinical knowledge of CAM among students is still lacking, which is consistent with a study conducted by Park (2020) that found a clear knowledge gap among clinical dental students in Australia (6).

In this study, the majority of students (74.3%) agreed that CAM can affect the safety of dental procedures, which is by Sekhri's (2013) research on the need to provide knowledge about CAM among dental students about and also considerations of its safety about the effects of CAM side (12). Health professionals, including dentists, must know and understand the safe use of CAM, as any other treatment, to ensure that patients are treated appropriately (13).

One in six clinical students (14.2%) stated that they knew about CAM, as also shown in a study by Kameyama A (2017) at two dental schools in Japan (14). As with studies in other countries, most respondents wanted the introduction of CAM into in the curriculum in the form of lectures during the preclinical stage of the dentistry program (15, 16).

In this study, more than two-thirds of clinical dental students (77.7%) expressed their interest in learning more about CAM and there was no significant difference between interest in learning more about CAM and gender, this is contrary to the class B study (2017) in Germany, which found that German female dentists were more supportive of CAM to patients (17).

In addition, more than two-thirds of clinical dental students (77%) consider CAM to be beneficial for common dental diseases. This confirms the findings in Japan.11 Only one in three clinical students from the Faculty of Dentistry of Hasanuddin University (29.1%) stated that CAM was ineffective and had no effect, which is in agreement with a Malaysian cross-sectional survey (16, 18).

Meanwhile, 80.4% of students think that CAM is considered necessary for oral health, this figure is significantly higher than the results of a survey of clinical dental students in Japan. According to the results of a survey by Newadkar UR (2013) in India, where only 12% of students are unaware of its implications in oral health care services (19).

Most of the students (87.2%) agreed that CAM education was necessary for the dental curriculum given its clinical relevance, which doubled the proportion identified in a cross-sectional study conducted in the United States, where 40% of the students surveyed requested that CAM be included in the study of their dental curriculum (20). Results obtained in the United States are similar to those of Australia, Pakistan, Malaysia, and India (17, 21).

Twenty-one out of one hundred forty-eight students stated that they knew CAM. More than half of the students are interested in learning more about CAM and agree that CAM education should be integrated into the curriculum.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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References

1. Suganya M, Vikneshan M, Swathy U. Usage of complementary and alternative medicine: A survey among Indian dental professionals. Complement Ther Clin Pract. 2017 Feb;26:26-29. doi: 10.1016/j.ctcp.2016.11.005.
2. Satria D. Complementary and alternative medicine (CAM): fakta

atau janji? Idea Nursing Journal. 2013; 4(3): 82-90. ISSN: 2087-2879

3. Erry, Susyanty AL, Raharni, Sasanti RH. Kajian implementasi kebijakan pengobatan komplementer alternatif dan dampaknya terhadap perijinan tenaga kesehatan praktek pengobatan komplementer alternatif akupuntur. Buletin Penelitian Sistem Kesehatan. 2014; 17(3): 275–284. ISSN: 14102935

4. World Health Organization. Diabetes-Fact Sheet No. 312. Geneva, Switzerland: World Health Organization; 2013.

5. AlRawi SN, Khidir A, Elnasha MS, Abdelrahim HA, Killawi AK, Hammoud MA, et al. Traditional Arabic and Islamic medicine: Validation and empirical assessment of a conceptual model in Qatar. BMC Complement Altern Med 2017;17:157.

6. Andira D, & Huda MK. Kewajiban Pemerintah atas Hak Imbalan Jasa Pelayanan Kesehatan Dokter Internsip. YUSTISIA MERDEKA: Jurnal Ilmiah Hukum, 2021, 7.2: 126-134.

7. Peraturan Konsil Kedokteran Indonesia Nomor 30 Tahun 2014 Tentang Standar Pendidikan Profesi Dokter Gigi Indonesia.

8. Fakultas Kedokteran Gigi Universitas Hasanuddin. Available from http://dent.unhas.ac.id/struktur-kurikulum/. Accessed on August, 16th 2020.

9. G. Bodeker, "Lessons on integration from the developing world's experience," British Medical Journal, vol. 322, no. 7279, pp. 164–167, 2001.

10. Alzahrani SH, Bashawri J, Salawati EM, Bakarman MA. Knowledge and Attitudes towards Complementary and Alternative Medicine among Senior Medical Students in King Abdulaziz University, Saudi Arabia. Evid Based Complement Alternat Med. 2016;2016:9370721. doi:10.1155/2016/9370721.

11. Ashraf M, Saeed H, Saleem Z, Rathore HA, Rasool F, Tahir E., et al. A cross-sectional assessment of knowledge, attitudes and self-perceived effectiveness of complementary and alternative medicine among pharmacy and non-pharmacy university students. BMC Complement Altern Med. 2019; 19(95): 1-12. DOI: 10.1186/s12906-019-2503-y

12. Use of Complementary and Alternative Therapies in Dental Practice: Practice Advisory. Toronto: Royal College of Dental Surgeons of Ontario; 2014. H. 1-4.

13. K. Sekhri, Bhanwra S, Nandha R. Herbal products: a survey of students' perception and knowledge about their medicinal use. Int. J. Basic Clin Pharmacol. 2013; 2(25): 71-6. DOI: 10.5455/2319-2003.ijbcp20130114.

14. Kameyama A, Toda K. Survey of dental students' attitude regarding oriental medicine/complementary and alternative medicine: Comparison between two Japanese dental schools. Afr J Tradit Complement Altern Med. 2017; 14(3): 287–295. DOI: 10.21010/ajtcam.v14i3.30.

15. Baatsch B, Zimmer S, Rodrigues Recchia D, Bussing A. Complementary and alternative therapies in dentistry and characteristics of dentists who recommend them. Complement Ther Med. 2017; 35: 64–69. DOI: http://dx.doi.org/10.1016/j.ctim.2017.08.008.

16. Babar MG, Syed SH, Naing CM, Hamzah NHB. Perceptions and self-use of Complementary and alternative medicine (CAM) among Malaysian dental students. Eur J Integr Med. 2012; 4(1): e63–e69. DOI: 10.1016/j.eujim.2011.11.001.

17. Loh KP, Ghorab H, Clarke E, Conroy R, Barlow J. Medical students' knowledge, perceptions, and interest in complementary and alternative medicine. J Altern Complement Med 2013;19:360-6.

18. De Sylvia D, Stuber M, Fung CC, Bazargan-Hejazi S, Cooper E. The knowledge, attitude and usage of complementary and alternative medicine of medical students. Evid Based Complement Altern Med 2011;2011:728902.

19. Spector ML, Kummet CM, Holmes DC. Complementary and alternative medicine in predoctoral dental curricula: an exploratory survey of U.S. dental schools. J Dent Educ. 2013; 77(12): 1610–1615. DOI: 10.1002/j.0022-0337.2013.77.12.tb05639.x.

20. Newadkar UR, Chaudhari L, Khalekar YK. Knowledge, awareness, and practices of complementary and alternative medicine for oral health-care management among dental students. Int J Yoga. 2017; 10(1)44-6. DOI: 10.4103/0973-6131.14562.

21. Sagar, Sandra; Ganapathy, Dhanraj M.; Thangavelu, Lakshmi. Knowledge and perception of the usage of alternative medicine in general dental practice among dental practitioners. Drug Invention Today, 2020, 14.7.

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Research Article

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Investigation of pediatric innocent murmur with echocardiography

Naile TUFAN PEKKÜÇÜKSEN[1], Tuğba TAŞ[2,*], Sadi TÜRKAY[3]

[1]Division Pediatric Nephrology, Department of Pediatrics, University of Florida, Gainesville, USA
[2]Department of Pediatrics, Hisar Intercontinental Hospital, Istanbul, Türkiye
[3]Private Practice, Ankara, Türkiye

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Abstract

Innocent murmurs are common in childhood. Usually, they are heard without any structural and physiological abnormalities. The murmur is usually weak and short. But even if it is thought to be innocent, echocardiography (ECHO) is occasionally requested by primary doctor or families. The aim of this study is evaluation of childhood innocent murmur with ECHO and possible minor findings with normal clinical status. This is a descriptive - retrospective chart review. We reviewed the charts of children and adolescents who were referred with asymptomatic murmur to Pediatric cardiology clinic at Fatih University Hospital between 01/ 2010 – 12/2011. Any type of congenital/acquired cardiac diseases are excluded. We only included first to third degrees of murmurs. All participants are analyzed based on demographic, clinical, and ECHO finding data. It was reviewed 610 charts, 82% (495) of the chart were included to study. 209 (42.2%) of cases were female. Minimal Tricuspid valve regurgitation (TVR) was seen in 200 children (40.4%) and minimal TVR+ physiological Mitral valve regurgitation (MVR) was seen in 65 children (11.6%). Patent foramen ovale (PFO) was seen in 306 children (54,8%). Left ventricular (LV) muscular band (false tendon) was seen in 171 children (30,6%). Minimal TVR + physiological MVR+ LV muscular band (false tendon) was seen in 26 children (4,7%), trivial arteria valve regurgitation (AVR) was seen in 7 children (1,4%), and minimal TVR + minimal pulmonary regurgitation (PR) was seen in 11 cases (2%). ECHO evaluation is common to use to rule out pathological murmur. Included cases exhibited some cardiac findings but none of those cause any shown cardiac dysfunction in near future. We advise innocent murmur group to be followed by primary physician unless they have any cardiac symptoms

Keywords: innocent murmur, murmurs, echocardiography, pediatric cardiology

1. Introduction

Innocent heart murmurs (IHMs) are accepted as functional and harmless with the absence of any anatomical or physiological cardiac pathology (1). 50-90% of murmurs that are observed between 3 to 7 years of age are innocent. There is no reported difference between genders and races (2-4). Definition of innocent murmur is that the murmur is heard during the systole or continuous and intensity rises to a maximum of 3 degrees without trills. Both innocent and pathologic murmurs become more intense with increased cardiac output, such as anemia, fever, exercise, acute anxiety, and acute illness (1).

ECHO is accepted as the gold standard to evaluate murmurs in children and more sensitive than physical exam. With the increased accessibility to ECHO, all children who have murmur should be evaluated by a cardiologist with ECHO (5).

For that reason, we retrospectively evaluated medical records of children who were referred for IHM and underwent ECHO imaging.

2. Material and Methods

This is a single centered retrospective chart review. All demographic, clinical, laboratory data were extracted from patient medical records of patients between 1-17 years old, who were referred to Pediatric Cardiology clinic due to first time evaluation of murmur. The study was approved by Institutional Research Board (IRB). The ethical approval for this study was obtained from Fatih University Faculty of Medicine Ethics Committee (approval number: 2008-15).

Patients who were on medication or who had history of prematurity, NICU admission, cardiac or other chronic diseases or positive family history of cardiac diseases were excluded. All anthropometric measurements with vital sign records are investigated. Patients who have less than 3rd percentile or more than 97th percentile or abnormal vital signs were excluded from study.

Patients who have been clinically diagnosed with pathological murmur after detailed physical examination were

*Correspondence: tubahalici@gmail.com

also excluded.

Philips Envisor® was used for Echocardiographic (ECHO) investigations. All patients were evaluated in supine and left lateral positions with 2-6 MHz of ultrasound transducers. All measurements were gathered in M-mode and assessed with respect to the recommended normal values by the American Society of Echocardiography (ASE) (6-9). Left ventricular systolic function was calculated by the machine with fractional shortening and ejection fraction. Atrioventricular (mitral and tricuspid) and ventriculoarterial (pulmonic and aortic) valves mean gradient measurements were gathered by Doppler ECHO. Patients who have a significant gradient impedance that was more than 1 cm by the valve coaptation point on any valves were excluded.

All statistical analysis was done in SPSS for Windows 16th edition (SPSS Inc, Chicago, Illinois, USA). T-test was used to compare the mean of continuous variables, and Chi-square is used to compare categorical data. P-value <0.05 is accepted as significant.

3. Results

Electronic medical record system screened with "ECHO" and "innocent murmur" words. Based on screening, 610 charts were reviewed, and 495 of them were included in the study. 115 patients excluded due to having one of these exclusion criteria: history of prematurity, NICU admission, cardiac or other chronic disease or being on medication or positive family history of cardiac diseases, less than 3rd percentile or more than 97th percentile or abnormal vital signs, physical examination concluded with pathological murmur. Table 1 shows the characteristics of the study population.

Table 1. Characteristics of study population

Characteristics of patients	
Age, months, mean (SD)	52.2 (±41.9)
Gender, male, number (%)	286 (57.8)
Degree of murmur, number (%)	
- First degree	78 (15.8)
- Second degree	408 (82.4)
- Third degree	9 (1.8)
Type of murmur:	
- Midsystolic	487 (98.4)
- Pansystolic	8 (1.6)
Extra heard sounds, number (%)	3 (0.6) *

*A patient was with S3 and 2 patients were with midsystolic click

Based on physical examination notes that were completed by a cardiologist, 98.4% of innocent murmur was defined as midsystolic and 1.6% of them was pansystolic. Only 0.6% of patients had extra heart sounds like S3 and midsystolic click. 98.2% of murmur was first- and second-degree strength.

Only 6.8% (34/495) of cases were evaluated with electrocardiography (EKG) due to being clinically normal and having normal rhythm. There was no significant rhythm abnormality in EKGs and all EKGs were reported as within normal limit for age. All cases were evaluated by CBC. Based on the records, none of the participants had anemia or cytopenia. ECHO findings are listed in table 2.

Table 2. ECHO findings

ECHO findings	(n)	(%)
Minimal tricuspid regurgitation (TR)	200	40,4
Physiologic Mitral regurgitation (MR)	2	0,4
Left ventricular aberrant band (LVAB)	9	1,6
Pulmonary artery turbulent flow (PATF)	3	0,5
PATF+ Minimal TR	8	1,4
Minimal TR+ Physiologic MR	65	11,6
LVAB+ PATF	1	0,2
LVAB+ Minimal TR	128	22,9
Minimal TR+ minimal ductus flow	13	2,2
Minimal TR+ physiologic MR+ LVAB	26	4,7
Minimal TR+ Minimal pulmonary regurgitation (PR)	11	2
Physiologic MR+LVAB	2	0,4
Minimal TY+ Physiologic MR+ Trivial aorta regurgitation (AR)	11	2
Minimal TR+ Trival AI	3	0,5
Minimal TR+ Minimal PR+ LVAB	3	0,5
Trivial AR+ Physiologic MR+ LVAB	2	0,4
Transient narrowing in neonates.	1	0,2
Minimal TR+ Physiologic MR+ trivial AR	2	0,4

In doppler analysis, 85.8% of cases were found with minimal (clinically insignificant) TVR. 45.4% of those had additional pathological findings like PVR (1.6%), MVR (15.7%), and AVR (3.4%).

Left ventricular muscular bands (false tendons) (LVMB) is seen in 30.6% of cases. All LVMBs were longitudinal. Additional clinically insignificant findings like TVR was contributing to 70.8% of LVMB cases.

Patent foramen ovale (PFO) was seen in 54% of cases by itself or with other insignificant findings. It was observed more commonly in males than females (F:0.69 x M).

When we looked at the overall insignificant minimal positive findings, all minimal pathologies were seen in patients under five years old ($p<0.01$) and there was no significant difference by gender ($p>0.05$).

4. Discussion

Murmurs constitute approximately 70% of pediatric cardiology consultations. Many opinions have been put forward regarding the formation mechanisms of murmurs. Various methods are used to understand whether murmurs are pathological or innocent. Innocent murmurs are murmurs that are common in childhood for which normal values are observed as a result of examinations for the murmur etiology.

The frequency of innocent murmur is reported to be 50-90% in the literature (3). In the study conducted by Fogel in the 1960s, the frequency of innocent murmur was found to be 63% in the childhood population (10). Castellotti et al. reported innocent murmur rate 41% in 256 children who were admitted due to murmur (11). Similar studies were conducted in various regions of Turkey. Üner et al. showed that the murmur frequency is 4% in school age children and 86% of them (3.7% of all study population) were innocent (12). Kozan et al., reported this rate as 10%, and it was emphasized that the low

result compared to the literature was that the study was conducted in a tertiary hospital and the applications were generally referred for pathological reasons (13). Congenital heart disease (CHD) in children was found between 0.8-1% in the literature (2). In studies conducted with all school-age children in Turkey, the rate of CHD was 0.21% for Şanlıurfa province (Koç et al.), and 1% for Elazig province (Aygün et al.), 0.14% for Adana province (Altıntaş et al.), and 0.44% and 0.2% for Diyarbakır province (Elevli et al. and Yıldırım et al.), respectively (14-18). Children with CHD or other rheumatic heart diseases on ECHO were excluded from our study.

The character of innocent murmurs depends on postural changes and Valsalva maneuver (3, 19). However, innocent murmurs as defined by Bronzetti et al. are systolic, short, soft, inaudible in all areas, not accompanied by click or additional sounds, are not noisy, and their characteristics do not change with respiration and/or postural changes (20). In our study, it was observed that the character of the murmur changed with postural changes and Valsalva maneuver in children with innocent murmur.

In the study of Üner et al., the severity of the murmur in children with an innocent murmur was found to be 99.5% first and second degree (12). In our study, all children had a murmur of varying severity. 98.2% of these murmurs were first and second degree soft midsystolic short and non-invasive murmurs. 98.9% of the murmurs were murmurs that were not accompanied by any additional sounds.

Many studies have been conducted on the necessity of ECG and telecardiography in the evaluation of murmurs. In the study of Kozan et al., Cardiothoracic rates (CTO) were found within normal limits in children with innocent murmur in telecardiography and no difference was found with the control group (13). In the study of Mackie et al., it was shown that ECG in newborns does not contribute to the differential diagnosis of murmur (21). Since the American Heart Academy (AHA) 2008 did not recommend telecardiography in children who were thought to have innocent murmur with physical examination findings, none of the children participating in our study had telecardiography (22).

In the study of Smythe et al., for 161 patients whose physical examination and ECHO and ECG findings were examined, the sensitivity of physical examination was found to be 96%, specificity 95%, positive predictive value 88%, and negative predictive value 98% (23). In a series of 200 cases by Mellies et al., the sensitivity and positive predictive value of physical examination were 92% and 99%, while the specificity was found to be 50-60%. In the study of Mackie et al., the sensitivity of physical examination in newborns was found to be 80.5%, specificity 90.9%, positive predictive value 91.9%, and negative predictive value 78.4% (24). The American Heart Academy (AHA) does not recommend performing ECG as a routine examination in children who are considered to have an innocent murmur with physical examination findings in the 2008 guideline (22). ECGs were performed on 34 children who participated in our study for different reasons. No rhythm changes requiring treatment and follow-up were detected. There was LVMB and TVR together in the LV in 12 cases and only minimal TVR in 16 cases. All ECG results were evaluated as normal.

In the literature, when two groups before and after school are compared, the frequency of innocent murmur was found to be 1 to 2 times higher in the school age group compared to the preschool group (18). In the study of Kozan et al., 36.7% of the cases diagnosed with innocent murmur were from 0 to 6 age group and the rest were the school age group (13). When the age distribution of the children included in our study was examined, it was seen that 62.4% were in the preschool age group (<60 months) and 37.6% were in the school age group (>60 months). The frequency of innocent murmurs was found 1.6 times more common in preschool age than in school period.

In the literature, false negativity was found with a rate of 0.7-4% in children with suspected innocent murmur with auscultation (25). The most common pathologies that could not be detected by auscultation were reported as small ASD, VSD, MVP, and mild PS (26). In our study, PDA showing a fine flow pattern without hemodynamic significance in minimal TVR coexistence was observed in 13 children with innocent murmur and trivial AR was observed in 18 cases.

The causes of innocent murmurs are unclear. According to Van Oort et al, vibratory innocent murmurs are caused by turbulence caused by physiological narrowing of the LV outflow tract due to the high myocardial contractility in children (19). Since both myocardial contractility and systolic velocity are higher in children compared to adults, it supports this view. The murmur relationship with the bands in the LV has also been the subject of many studies. Brenner et al. reported the incidence of innocent murmur in healthy children as 29.3%. Its relation with innocent murmurs has been found with a rate of 63.6-77% in different studies (27). In the study of Özme et al., the relationship between the structure of the bands in the LV and the severity and shape of the murmur was examined, and it was found that longitudinal bands were associated with pulmonary ejection murmurs (28). ECHO findings were examined in our study. While minimal TVR was detected in 429 cases (85.8%) in color doppler echocardiographic examination, an LVMB was observed in 171 cases (30.6%). All of the LVMB's detected in our study were longitudinal bands. Also, there was no difference between genders. There are a few studies in the literature on valve regurgitation in normal subjects. In a study conducted by Kostucki et al. 92% minimal PVR, 40% minimal MVR, 44% minimal TVR, and 33% minimal AS were found in normal cases in adults (29). In the study conducted by Kozan et al. in children from Turkey, minimal regurgitation was found at the same rates in the tricuspid and pulmonary valve (13). In the study of Üner et al., TR was found in 25% of the patients with

an innocent murmur, and PY was found in 4% (12). In the study of Van Dijk et al. in 173 school-age children with murmurs, minimal TR was seen in 75% of children (30). In our study, the most common minimal valve regurgitation was seen in 429 cases (85.8%) in the Tricuspid valve, this was the mitral valve in 85 cases (15.7%), the aortic valve in 18 cases (3.4%), and the pulmonary valve was observed to follow with 9 cases (1.8%).

In this study, minor pathological findings were investigated by evaluating the children's ECHO findings. Considering the known causes of innocent murmur, minimal deficiency in AV valves was detected in 86% of all children diagnosed with innocent murmur. There was no rheumatic heart disease or systemic disease that could cause this together with additional pathological findings accompanying these deficiencies. Therefore, if the cases that may cause AV valve regurgitation are ruled out, the failure does not progress, and there is no impairment in cardiac functions, then minimal valve regurgitation should be considered as innocent murmur and unnecessary examinations and monitoring should be avoided. However, this study was planned cross-sectionally and conducted with a selected group. Further double-blinded controlled studies are needed to investigate the relationship between minimal valve regurgitation and innocent murmur.

Murmurs were heard during routine examination of healthy children cause serious anxiety in families. Even if the pediatrician thinks that the murmur is innocent, it is often inadequate to relieve families' anxiety. However, it has been shown that anxiety decreases in families of children diagnosed with innocent murmur by a pediatric cardiologist (31, 32). Although the diagnosis of CHD is desired early, great care should be taken in making the diagnosis. Unnecessary long-term cardiac monitoring and unnecessary drug use should be avoided in a large group with innocent murmur. It should be explained to the family that innocent murmurs can be seen quite frequently in the childhood age group, that there will be no problems in the future due to murmur, no restriction of physical activities, and long-term cardiac monitoring is not required. Therefore, we think that it is important to evaluate all children with murmurs at least once by a pediatric cardiologist and demonstrate the accuracy of the diagnosis with ECHO in terms of relieving the anxiety of both the family and the child.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: N.T.P., S.T., Design: N.T.P., Data Collection or Processing: N.T.P., T.T., Analysis or Interpretation: N.T.P., Literature Search: N.T.P., Writing: N.T.P.

References

1. Frank JE, Jacobe KM. Evaluation and management of heart murmurs in children. Am Fam Physician. 2011;84(7):793-800.
2. Pelech AN. The cardiac murmur. When to refer? Pediatr Clin North Am. 1998;45(1):107-22.
3. Martins P, Dinis A, Canha J, Ramalheiro G, Castela E. Innocent heart murmurs. Rev Port Cardiol. 2008;27(6):815-31.
4. Castle RF. Clinical recognition of innocent cardiac murmurs in children. JAMA. 1961;177:1-5.
5. Sachdeva R, Douglas PS, Kelleman MS, McCracken CE, Lopez L, Stern KW, et al. Effect of Release of the First Pediatric Appropriate Use Criteria on Transthoracic Echocardiogram Ordering Practice. Am J Cardiol. 2016;118(10):1545-51.
6. Oran B, Bodur AS, Arslan D, Cimen D, Guvenc O. Normal M mode values in healthy Turkish children. Turk J Med Sci. 2014;44(5):756-63.
7. Pettersen MD, Du W, Skeens ME, Humes RA. Regression equations for calculation of z scores of cardiac structures in a large cohort of healthy infants, children, and adolescents: an echocardiographic study. J Am Soc Echocardiogr. 2008;21(8):922-34.
8. Kim HJ, Yoon JH, Lee EJ, Oh JH, Lee JY, Lee SJ, et al. Normal left ventricular torsion mechanics in healthy children: age related changes of torsion parameters are closely related to changes in heart rate. Korean Circ J. 2015;45(2):131-40.
9. Dallaire F, Slorach C, Hui W, Sarkola T, Friedberg MK, Bradley TJ, et al. Reference values for pulse wave Doppler and tissue Doppler imaging in pediatric echocardiography. Circ Cardiovasc Imaging. 2015;8(2):e002167.
10. Fogel DH. The innocent systolic murmur in children: a clinical study of its incidence and characteristics. Am Heart J. 1960;59:844-55.
11. Castellotti DS, Makssoudian A, Mendes MC, Fisberg M. Heart murmur in pediatrics: innocent or pathologic? Rev Paul Med. 1992;110(1):29-33.
12. Uner A, Dogan M, Bay A, Cakin C, Kaya A, Sal E. The ratio of congenital heart disease and innocent murmur in children in Van city, the Eastern Turkey. Anadolu Kardiyol Derg. 2009;9(1):29-34.
13. Kozan MT, Saylam G, Dundar B, Hudaoglu S, Akcoral A Cocukluk cagindaki Masum Ufurumlu Olgularin Laboratuar ve Ekokardiyografik Olarak Degerlendirilmesi. . Dokuz Eylul Tip Fakultesi Dergisi. 1998;12:249-59.
14. Altıntaş G, Acartürk E, Tokcan A, Dikmengil M. Adana ili ilkokul çocuklarında kalp üfürümleri taraması. Ç Ü Tıp Fakültesi Dergisi. 1988;3:211-4.
15. Denizmen Aygün A, Kocaman S, Akarsu S, Yaşar F, Türkbay D. İlkokul çocuklarında kalp üfürümlerinin sıklığı ve önemi. Türkiye Klinikleri Pediatri Dergisi 1998.
16. Elevli M, Yakut Y, Devecioğlu C, Günbey S, Taş MA. Diyarbakır il merkezinde iki ilkokuldayapılan anemi ve üfürümleri taraması. Dicle Tıp Bülteni. 1991:145-53.
17. Koç A, Kösecik M, Ataş A, Kılınç M. İlköğretim çağı çocuklarında kalp üfürümleriprevelans çalışması. Türk Pediatri Arşivi 1997;32:28-33.
18. Yıldırım MS, Müftüoğlu E, Kepekçi Y, Yazıcıoğlu N. Diyarbakr ili belediye hudutları dahilinde 7-18 yaşları arasındaki ilk ve orta dereceli okul öğrencilerinde doğumsal kalp hastalığı oranı. Türk Kardiyoloji Derneği Arşivi. 1986;14:21.

19. Advani N, Menahem S, Wilkinson JL. The diagnosis of innocent murmurs in childhood. Cardiol Young. 2000;10(4):340-2.

20. Bronzetti G, Corzani A. The Seven "S" Murmurs: an alliteration about innocent murmurs in cardiac auscultation. Clin Pediatr (Phila). 2010;49(7):713.

21. Mackie AS, Jutras LC, Dancea AB, Rohlicek CV, Platt R, Beland MJ. Can cardiologists distinguish innocent from pathologic murmurs in neonates? J Pediatr. 2009;154(1):50-4 e1.

22. Bonow RO, Carabello BA, Chatterjee K, de Leon AC, Jr., Faxon DP, Freed MD, et al. 2008 focused update incorporated into the ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines Endorsed by the Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. J Am Coll Cardiol. 2008;52(13):e1-142.

23. Smythe JF, Teixeira OH, Vlad P, Demers PP, Feldman W. Initial evaluation of heart murmurs: are laboratory tests necessary? Pediatrics. 1990;86(4):497-500.

24. Mellies U, Hentrich F, Hofelich B, Neudorf U, Schmaltz AA. [The incidental heart sound. Study of clinical diagnostic certainty and possible causes]. Klin Padiatr. 1992;204(5):323-7.

25. Shub C. Echocardiography or auscultation? How to evaluate systolic murmurs. Can Fam Physician. 2003;49:163-7.

26. Van Oort A, Hopman J, De Boo T, Van Der Werf T, Rohmer J, Daniels O. The vibratory innocent heart murmur in schoolchildren: a case-control Doppler echocardiographic study. Pediatr Cardiol. 1994;15(6):275-81.

27. Brenner JI, Baker K, Ringel RE, Berman MA. Echocardiographic evidence of left ventricular bands in infants and children. J Am Coll Cardiol. 1984;3(6):1515-20.

28. Ozme S, Ozkutlu S, Muftuoglu S, Baysal K, Saraclar M, Tuncali T. Left ventricular aberrant bands in children with innocent murmurs. Turk J Pediatr. 1987;29(2):73-9.

29. Kostucki W, Vandenbossche JL, Friart A, Englert M. Pulsed Doppler regurgitant flow patterns of normal valves. Am J Cardiol. 1986;58(3):309-13.

30. Van Dijk AP, Van Oort AM, Daniels O. Right-sided valvular regurgitation in normal children determined by combined colour-coded and continuous-wave Doppler echocardiography. Acta Paediatr. 1994;83(2):200-3.

31. Haney I, Ipp M, Feldman W, McCrindle BW. Accuracy of clinical assessment of heart murmurs by office based (general practice) paediatricians. Arch Dis Child. 1999;81(5):409-12.

32. Hansen LK, Birkebaek NH, Oxhoj H. Initial evaluation of children with heart murmurs by the non-specialized paediatrician. Eur J Pediatr. 1995;154(1):15-7.

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Study of bacteria isolated from COVID-19 and non-COVID-19 intensive care units and determination of their antibiotic susceptibility profiles

Çetin KILINÇ[1], Nilay ÇÖPLÜ[2], Melike YAŞAR DUMAN[1], Büşra ÇALIŞIR[1] Enis Fuat TÜFEKCİ[3,*], Muhammet GÜLHAN[4], Ayşe YILMAZ[5], Veysel Garani SOYLU[6]

[1]Department of Microbiology, Kastamonu Training and Research Hospital, Kastamonu, Türkiye
[2]Department of Microbiology, Ankara City Hospital, Ankara, Türkiye
[3]Department of Medical Microbiology, Faculty of Medicine, Kastamonu University, Kastamonu, Türkiye
[4] Department of Infectious Disease and Clinical Microbiology, Kastamonu Training and Research Hospital, Kastamonu, Türkiye
[5]Department of Anesthesiology and Reanimation, Kastamonu Training and Research Hospital, Kastamonu, Türkiye
[6] Department of Intensive Care Science, Kastamonu Training and Research Hospital, Kastamonu, Türkiye

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Abstract

Nosocomial infections occur 48-72 hours after hospitalization, especially caused by bacteria, and pose a high risk for patients in intensive care units (ICUs), including COVID-ICUs. This study aimed to reveal bacteria distribution and antibiotic susceptibility profiles isolated from various clinical samples of non-COVID-ICU and COVID-ICU patients. We included in this study bacterial strains isolated from ICUs patients in Kastamonu Training and Research Hospital between March 2020 and October 2020. We identified the strains using the Vitek 2 compact automated system (BioMerieux, France) and standard microbiological methods. Using the Vitek 2 automated system, we analyzed antibiotic susceptibility tests and interpreted the results based on the European Committee for Antimicrobial Susceptibility Tests (EUCAST) guideline. There were 302 patients in the non-COVID-ICUs and 440 patients in the COVID-ICUs. We isolated a total of 470 strains, 370 from non-COVID-ICUs and 100 from COVID-ICUs. *Acinetobacter* spp. was the most frequently isolated strains for both ICUs. *Acinetobacter* spp. isolated from non-COVID-ICUs had higher resistance rates to meropenem ($p= 0.043$), ceftazidime ($p= 0.014$), and levofloxacin ($p<0.001$) antibiotics than strains from COVID-ICUs. Antibiotic susceptibility profiles of other strains were similar for both ICUs. As a result, the incidence of nosocomial infections in COVID-ICU patients was lower than in non-COVID-ICU patients. Health personnel working in COVID-ICUs may have played an important role in this, as they were more careful about using personal protective equipment and complying with hygiene rules. However, antibiotic resistance continues to be a serious problem in ICUs, including COVID-ICUs.

Keywords: *Acinetobacter*, antibiotic resistance, COVID-intensive care unit, nosocomial infections

1. Introduction

COVID-19 was described in December 2019 and, has become a pandemic in March 2020. The disease is transmitted by aerosols and shows a course ranging from asymptomatic to severe respiratory failure (1). About 20% of the patients are treated in hospitals due to severe lung involvement, and 5-10% of them are hospitalized in intensive care units (ICUs) and need respiratory support. The COVID-19 pandemic has become a severe burden on health systems, especially ICUs (2).

The widespread use of ventilators, catheters, and antibiotics and the prolonged hospital stay period predispose the development of nosocomial infections in ICUs patients. Nosocomial infections are infectious diseases that usually occur 48 hours after hospitalization and are frequently bacterial in origin. Nosocomial infections are troublesome for all ICUs patients, including the COVID-ICUs, as they adversely affect the prognosis and increase the mortality rate (3). On the other hand, viral infections may predispose the host to secondary bacterial infections due to their effects on the immune system (4). In addition, high-dose steroid therapy can be used to alleviate the symptoms of COVID-19 patients with severe symptoms. In this case, COVID-ICU patients may become prone to nosocomial infections or secondary bacterial infections of flora (endogenous) origin (5).

Periodic monitoring of bacteria distribution and antibiotic susceptibility profiles isolated from COVID-ICU and other ICUs patients is essential for infection control. This study

*Correspondence: etufekci@kastamonu.edu.tr

aimed to reveal bacteria distribution and antibiotic susceptibility profiles isolated from various clinical samples in non-COVID-19 intensive care units (non-COVID-ICUs) and COVID-19 intensive care units (COVID-ICUs) patients.

2. Materials and Methods

This study was approved by the Kastamonu University (Turkey), Faculty of Medicine Medical Research Ethical Committee (Date: 14.12.2020 and Decision number: 2020-KAEK-143-04).

We conducted this study in the Microbiology Laboratory of Kastamonu Training and Research Hospital (TRH) and the COVID-19 diagnosis using RT-PCR (Bio-Speedy COVID-19 RT-qPCR Detection kit; Bioeksen, Istanbul, Turkey) and CORONEX (MOTAKK, Ankara, Turkey) from respiratory tract samples. According to the manufacturer's instructions, we performed qPCR using the C1000 Touch CFX96 system (Bio-Rad, USA).

We included in this study bacterial strains isolated from various clinical samples (respiratory secretions, blood, urine, wound, pleural and peritoneal fluids) of COVID-ICU and non-COVID-ICU patients in Kastamonu TRH between March and October 2020 and the first isolates of the patients. We identified the strains using the Vitek 2 compact automated system (BioMerieux, France) besides standard microbiological methods (culture examination, Gram reaction, catalase, and oxidase tests). We analyzed antibiotic susceptibility tests using the Vitek 2 automated system and interpreted the results based on the European Committee for Antimicrobial Susceptibility Tests (EUCAST) guideline (6).

2.1. Statistical analysis

We used the chi-square test for the statistical analysis of the data on the SPSS 23.0 for Windows (IBM Inc., Armonk, NY, USA) and took the significance of the p-value as <0.05.

3. Results

There were 302 patients in the non-COVID-ICUs and 440 patients in the COVID-ICUs. We isolated a total of 470 strains, 370 from non-COVID-ICUs and 100 from COVID-ICUs, as infectious agents. Table 1 shows the distribution of bacterial strains between ICUs. *Acinetobacter* spp. was the most frequently isolated strains (33.6%) for both ICUs.

Table 1. Comparison of the distribution of inpatients and isolated bacteria in non-COVID-ICU and COVID-ICU

Bacteria	non-COVID-ICU	COVID-ICU	Total (n, %)
Gram-negative bacteria	*Acinetobacter* spp. (n=123, 26.2%)	*Acinetobacter* spp. (n=35, 7.4%)	158 (33.6%)
	Klebsiella spp. (n=89, 18.9%)	*Klebsiella* spp. (n=22, 4.7%)	111 (23.6%)
	E. coli (n=57, 12.1%)	*E. coli* (n=18, 3.8%)	75 (15.9%)
	Pseudomonas spp. (n=52, 11.1%)	*Pseudomonas* spp. (n=5, 1.1%)	57 (12.2%)
	Others (n=5, 1.1%)	Others (n=3, 0.6%)	8 (1.7%)
Total (n)	**326 (69.4%)**	**83 (17.6%)**	**409 (87.0%)**
Gram-positive bacteria	*S. aureus* (n=29, 6.2%)	*S. aureus* (n=13, 2.8%)	42 (9.0%)
	Enterococcus spp. (n=11, 2.3%)	*Enterococcus* spp. (n=2, 0.4%)	13 (2.7%)
	Others (n=4, 0.9%)	Others (n=2, 0.4%)	6 (1.3%)
Total (n)	**44 (9.4%)**	**17 (3.6%)**	**61 (13.0%)**
Overall (n)	**370 (78.8%)**	**100 (21.2%)**	**470 (100%)**

Among the clinical samples of ICU patients, we isolated most bacteria from respiratory secretions (n= 251), followed by blood (n= 112), urine (n= 89), wound (n= 13), pleural fluid (n= 4), and peritoneal fluid (n= 1). Of respiratory secretion samples, 214 were sent from non-COVID-ICUs and 37 from COVID-ICUs. The most common strains isolated from respiratory secretions for both ICUs were *Acinetobacter* spp. The most frequently isolated bacteria from blood and urine cultures for both ICUs were *Staphylococcus aureus* and *Escherichia coli*, respectively (Table 2).

Table 3 and Table 4 show the resistance rates of Gram-negative and Gram-positive bacteria to commonly used antibiotics, respectively. We did not present colistin susceptibility results in this study due to EUCAST criteria. *Acinetobacter* spp. isolated from non-COVID-ICUs had higher resistance rates to meropenem (p= 0.043), ceftazidime (p= 0.014), and levofloxacin (p<0.001) than isolates from COVID-ICUs. Moreover, *Pseudomonas* spp. isolated from non-COVID-ICUs had a higher resistance rate to levofloxacin (p= 0.047) than isolates from COVID-ICUs. Antibiotic susceptibility profiles of other bacteria were similar for both ICUs.

Table 2. Bacterial distribution among clinical specimens from non-COVID-ICU and COVID-ICU

Clinical specimen	Bacteria	non-COVID-ICU (n, %)	COVID-ICU (n, %)	Total (n, %)
Respiratory secretions	*Acinetobacter* spp.	98 (20.8%)	23 (4.9%)	121 (25.7%)
	Klebsiella spp.	55 (11.7%)	11 (2.3%)	66 (14.0%)
	Pseudomonas spp.	45 (9.6%)	0 (0.0%)	45 (9.6%)
	E. coli	7 (1.5%)	1 (0.2%)	8 (1.7%)
	Other	9 (1.9%)	2 (0.4%)	11 (2.3%)
	Total (n, %)	**214 (45.5%)**	**37 (7.8%)**	**251 (53.3%)**
Blood	*S. aureus*	21 (4.5%)	11 (2.3%)	32 (6.8%)
	Acinetobacter spp.	21 (4.5%)	10 (2.1%)	31 (6.6%)
	Klebsiella spp.	13 (2.8%)	3 (0.6%)	16 (3.4%)
	E. coli	12 (2.6%)	4 (0.9%)	16 (3.4%)
	Enterecoccus spp.	9 (1.9%)	1 (0.2%)	10 (2.1%)
	Pseudomonas spp.	2 (0.4%)	1 (0.2%)	3 (0.6%)
	Other	1 (0.2%)	3 (0.6%)	4 (0.9%)
	Total (n, %)	**79 (16.9%)**	**33 (6.9%)**	**112 (23.8%)**
Urine	*E. coli*	35 (7.4%)	11 (2.3%)	46 (9.8%)
	Klebsiella spp.	16 (3.4%)	7 (1.5%)	23 (4.9%)
	Pseudomonas spp.	3 (0.6%)	4 (0.9%)	7 (1.5%)
	Enterococcus spp.	2 (0.4%)	2 (0.4%)	4 (0.9%)
	Other	7 (1.5%)	2 (0.4%)	9 (1.9%)
	Total (n, %)	**63 (13.3%)**	**26 (5.5%)**	**89 (18.8%)**
Wound	*Klebsiella* spp.	4 (0.9%)	1 (0.2%)	5 (1.0%)
	E. coli	3 (0.6%)	2 (0.4%)	5 (1.0%)
	Acinetobacter spp.	1 (0.2%)	1 (0.2%)	2 (0.4%)
	Pseudomonas spp.	1 (0.2%)	0 (0.0%)	1 (0.2%)
	Total (n, %)	**9 (1.9%)**	**4 (0.8%)**	**13 (2.7%)**
Pleural fluid	*S. aureus*	3 (0.6%)	0 (0.0%)	3 (0.6%)
	Stenotrophomonas maltophilia	1 (0.2%)	0 (0.0%)	1 (0.2%)
	Total (n, %)	**4 (0.8%)**	**0 (0.0%)**	**4 (0.8%)**
Peritoneal fluid	*Pseudomonas* spp.	**1 (0.2%)**	**0 (0.0%)**	**1 (0.2%)**
	Overall (n, %)	**370 (78.4%)**	**100 (21.2%)**	**470 (100%)**

Table 3. The resistance rates of Gram-negative bacteria to commonly used antibiotics

Bacteria	ICUs	Amoxicillin clavulanic acid	Piperacillin tazobactam	Meropenem	Amikacin	Ceftazidime	Cefepime	Levofloxacin
E. coli	non-COVID-ICU (n=57)	19 (33.3%)	6 (10.5%)	3 (5.3%)	2 (3.5%)	14 (24.6%)	13 (22.8%)	-
	COVID-ICU (n=18)	7 (38.9%)	4 (22.2%)	0 (0.0%)	0 (0%)	4 (22.2%)	4 (22.2%)	-
Klebsiella spp.	non-COVID-ICU (n=89)	60 (68.2%)	66 (75.0%)	60 (67.4%)	47 (53.4%)	69 (78.4%)	66 (75.0%)	-
	COVID-ICU (n=22)	14 (63.6%)	14 (63.6%)	10 (45.4%)	9 (40.9%)	13 (59.1%)	13 (59.1%)	-
Acinetobacter spp.	non-COVID-ICU (n=123)	-	122 (99.2%)	**120 (97.6%)[1]**	97 (78.9%)	**120 (97.6%)[2]**	-	**122 (99.2%)[3]**
	COVID-ICU (n=35)	-	33 (94.3%)	**31 (88.6%)**	26 (74.3%)	**30 (85.7%)**	-	**27 (77.1%)**
Pseudomonas spp.	non-COVID-ICU (n=52)	-	33 (63.5%)	36 (69.2%)	4 (7.7%)	24 (46.2%)	24 (46.2%)	**36 (69.2%)[4]**
	COVID-ICU (n=5)	-	2 (40.0%)	2 (40.0%)	1 (20.0%)	2 (40.0%)	2 (40.0%)	**1 (20.0%)**

[1]p= 0.043, [2]p= 0.014, [3]p<0.001, [4]p= 0.047

Table 4. The resistance rates of Gram-positive bacteria to commonly used antibiotics

Bacteria	ICUs	Methicillin	Vancomycin	Ampicillin	Ciprofloxacin	Clindamycin	Erythromycin	Tetracycline	Tigecycline
S. aureus	non-COVID-ICU (n=29)	14 (48.3%)	0 (0.0%)	-	10 (34.5%)	10 (34.5%)	12 (41.4%)	9 (31.0%)	2 (6.9%)
	COVID-ICU (n=13)	8 (61.5%)	0 (0.0%)	-	4 (30.8%)	6 (46.2%)	7 (53.8%)	4 (30.8%)	1 (7.7%)
Enterococcus spp.	non-COVID-ICU (n=11)	-	2 (18.2%)	9 (81.8%)	9 (81.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (18.2%)
	COVID-ICU (n=2)	-	0 (0.0%)	2 (100.0%)	2 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

4. Discussion

This study determined bacteria distribution and antibiotic susceptibility profiles isolated from COVID-ICUs and other ICUs patients. The results showed that non-COVID-ICU patients (78.8%) had a higher incidence of nosocomial infections than COVID-ICUs (21.2%). This striking difference in the frequency of nosocomial infections might stem from healthcare personnel working in COVID-ICUs being more careful about using personal protective equipment and complying with hygiene rules in pandemic conditions.

Considering the distribution of bacteria, we found that gram-negative bacteria were dominant in both ICUs in this study. Many researchers stated that gram-negative bacteria were dominant among bacteria isolated from ICUs patients (7-9). This can be attributed to gram-negative bacteria being more resistant than gram-positive bacteria by their structure. So, resistant gram-negative strains become dominant in the hospital environment due to the selective pressure of antibiotics (10).

The distribution of the clinical samples from which the strains were isolated evinced that involvement in the respiratory system was the most common, followed by blood, urine, and wounds in both ICUs. However, although there was risk factor such as the use of ventilators for the development of nosocomial infections in the COVID-ICUs (11), the frequency of bacteria isolated from respiratory secretions was 32.7% in COVID-ICUs and 56.7% in non-COVID-ICUs. This may indicate that the antibiotics recommended in the COVID-19 treatment protocol play an active role in protecting against respiratory system infections.

In the presented study, *Acinetobacter* spp. were the most frequently isolated bacteria from both ICUs. *Acinetobacter* spp. can survive for a long time in the hospital environment and on dry surfaces with their simple nutritional requirements, ability to grow in a broad pH and temperature range, resistance to disinfectants and antiseptics, and the ability to form biofilms on living and non-living surfaces (12-14). Therefore, they are frequently isolated from inpatients in hospitals (15). In addition, many studies reported that *Acinetobacter* spp. was the most frequently isolated bacteria from COVID-ICU and other ICUs patients (16-19).

The antibiogram results of the strains revealed that *Acinetobacter* spp. had the highest resistance rate for both ICUs. We actually expected this result, as these bacteria, especially *Acinetobacter baumannii* strains, have intrinsic resistance to many antibiotics (20). In addition, they can easily acquire resistance to antibiotics with acquired resistance mechanisms. In particular, carbapenem-resistant *Acinetobacter* strains emerge as urgent threats (21). We found the resistance rates of *Acinetobacter* strains isolated from COVID-ICU and other ICUs patients against meropenem, a carbapenem class antibiotic, as 88.6% and 97.6%, respectively. Meropenem resistance was statistically significant in *Acinetobacter* strains isolated from other ICUs compared to those isolated from COVID-ICUs ($p= 0.043$). However, this may be due to the number of *Acinetobacter* isolated from COVID-ICUs being lower than those isolated from other ICUs.

As a result, the COVID-ICU patients had a lower incidence of bacterial infection than other ICU patients in Kastamonu TRH. While there are many risk factors for COVID-ICU patients to get bacterial infections, the lower incidence of infection than in other ICUs shows that successful infection control is implemented in COVID-ICUs of Kastamonu TRH. However, antibiotic resistance continues to be a serious problem in ICUs, including COVID-ICUs.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors' contributions

Concept: Ç.K., N.C., Design: N.C., M.Y.D., B.Ç., Data Collection or Processing: M.G., A.Y., V.G.S., Analysis or Interpretation: Ç.K., E.F.T., B.Ç., Literature Search: E.F.T.,

M.G., A.Y., V.G.S., Writing: E.F.T., M.Y.D.

References

1. He F, Deng Y, Li W. Coronavirus disease 2019: What we know? J Med Virol. 2020; 92(7): 719–725.
2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020; 323(13): 1239–1242.
3. Monegro AF, Muppidi V, Regunath H. Hospital acquired infections. [Internet]. 2021 [Updated 2021 Aug 30; cited 01 Apr 2022]. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441857/
4. Manna S, Baindara P, Mandal SM. Molecular pathogenesis of secondary bacterial infection associated to viral infections including SARS-CoV-2. J Infect Public Health. 2020; 13(10): 1397–1404.
5. Obata R, Maeda T, Rizk D, Kuno T. Increased secondary infection in COVID-19 patients treated with steroids in New York City. Jpn J Infect Dis. 2021; 74(4): 307–315.
6. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 10.0, 2020. Available from: http://www.eucast.org/clinical_breakpoints/
7. Bulut ME, Öncül A. Nosocomial infection agents of Şişli Hamidiye Etfal training and research hospital: Comparison of 1995 and 2017 data. Med Bull Sisli Etfal Hosp 2020; 54(1): 78–82.
8. Genç Y, Gürkan Y, Mumcuoğlu İ, Kanyılmaz D, Aksoy A, Aksu N. Yoğun bakım hastalarında hastane kaynaklı pnömoni olgularının değerlendirilmesi ve sık görülen bakteriyel etkenlerin antimikrobiyallere dirençlerinin araştırılması. Turk Hij Den Biyol Derg. 2016; 73(4): 355–364.
9. Barış A, Bulut ME, Öncül A, Bayraktar B. Yoğun bakım ünitelerinde yatan hastalara ait klinik izolatların tür dağılımı ve antibiyotik duyarlılıkları. J Turk Soc Intens Care. 2017; 15: 21–27.
10. Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules. 2020; 25(6): 1340.
11. Pasero D, Cossu AP, Terragni P. Multi-drug resistance bacterial infections in critically Ill patients admitted with COVID-19. Microorganisms. 2021; 9(8): 1773.
12. Almasaudi SB. *Acinetobacter* spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi J Biol Sci. 2018; (3): 586-596.
13. Dekic S, Hrenovic J, Ivankovic T, van Wilpe E. Survival of ESKAPE pathogen *Acinetobacter baumannii* in water of different temperatures and pH. Water Sci Technol. 2018; (5-6): 1370-1376.
14. Nowak P, Paluchowska P. *Acinetobacter baumannii*: biology and drug resistance - role of carbapenemases. Folia Histochem Cytobiol. 2016; 54(2): 61-74.
15. Rebic V, Masic N, Teskeredzic S, Aljicevic M, Abduzaimovic A, Rebic D. The importance of *Acinetobacter* species in the hospital environment. Med Arch. 2018; 72(5): 325-329.
16. Ceparano M, Baccolini V, Migliara G, Isonne C, Renzi E, Tufi D, et al. *Acinetobacter baumannii* isolates from COVID-19 patients in a hospital intensive care unit: Molecular typing and risk factors. Microorganisms. 2022; 10: 722.
17. Sharifipour E, Shams S, Esmkhani M, Khodadadi J, Fotouhi-Ardakani R, Koohpaei A, et al. Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. BMC Infect Dis. 2020; 20(1): 646.
18. Syed RF, Yelamanchili S, Thati S. A comparative study of *Acinetobacter* infections in COVID and non-COVID patients. J Infect Dis Epidemiol. 2022; 8: 250.
19. Yu Y, Xu D, Fu S, Zhang J, Yang X, Xu L, et al. Patients with COVID-19 in 19 ICUs in Wuhan, China: A cross-sectional study. Crit Care. 2020; 24(1): 219.
20. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. *Acinetobacter baumannii* antibiotic resistance mechanisms. Pathogens. 2021; 10(3): 373.
21. CDC, Centre for Disease Control. Antibiotic resistance threats in the United States, 2019. [Internet]. 2019 [Updated 2019 Dec; cited 04 Apr 2022]. U.S. Department of Health and Human Services, Atlanta, GA. Available from: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

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The impact of family history of preeclampsia and alteration of MMP-13/TIMP-1 balance in the occurrence of preeclampsia

Asparuh NIKOLOV[1,*], Nikola POPOVSKI[2], Irena HRISTOVA[3]

[1]Cardiovascular Research Working Group, Division of Medicine, Institute for Scientific Research, Medical University-Pleven, Pleven, Bulgaria
[2]Department of Obstetrics and Gynaecology, Medical University-Pleven, Pleven, Bulgaria
[3]Department of Midwifery, Faculty of Health Care, Medical University-Pleven, Pleven, Bulgaria

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Abstract

Tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) suppresses the activity of matrix metalloproteinase-13 (MMP-13). The MMP-13/TIMP-1 complex has been proposed as one of the collagen types I and III turnover regulators in a healthy pregnancy. This study intends to investigate the distribution of serum levels of MMP-13, TIMP-1 and the ratio of MMP-13/TIMP-1 in women with preeclampsia (PE) with a family history of preeclampsia. We examined 37 patients with PE, with a mean age of 24.9±6 years, while the mean age of the control group of 32 healthy subjects was 24.7±5.4 years. We divided patients into two subgroups: subjects with a family history of PE (n=18); (PE+FHPE) and subjects without a family history of PE (n=19); (PE-FHPE). We measured MMP-13 and TIMP-1 levels via enzyme-linked immunosorbent assay (ELISA), and calculated the ratio. Either MMP-13 or TIMP-1 alone did not exhibit any obvious differences between normal and PE pregnancies. MMP-13/TIMP-1 ratio was statistically significantly higher in PE than healthy pregnant women: 0.2 (0.1÷0.5) vs. 0.065 (0.05÷0.2) ($p<0.05$). Patients with PE+FHPE showed statistically significantly lower MMP-13/TIMP-1 ratio compared with PE-FHPE 0.085 (0.05÷0.25) vs. 0.22 (0.12÷0.35) (KW=5.71; p=0.02). These results indicate an altered balance between MMP-13 and TIMP-1 in PE patients with a family history of preeclampsia. Further studies with more precise analysis and genetic methods are needed to elucidate how the imbalance between MMP-13 and TIMP-1 contributes to PE susceptibility and pathogenic mechanisms determining preeclampsia development.

Keywords: matrix metalloproteinase-13, tissue inhibitor of matrix metalloproteinase-1, family history of preeclampsia, collagen types I and III

1. Introduction

Preeclampsia (PE) is a hypertensive disorder of pregnancy, defined by the occurrence of new-onset hypertension (140/90 mmHg) and either proteinuria (0.3g in a 24h urine sample) or end-organ dysfunction developing after 20 weeks of gestation (1). It is a major cause of maternal and perinatal morbidity and mortality (2, 3). However, it has not been thoroughly explored.

The two major proteins of the human uterine wall are collagen type I and type III. In a healthy pregnancy, collagen metabolism increases as the uterus develops. Preeclampsia has been associated with altered collagen turnover leading to impaired modification of the uterine wall structure. Type I and III collagen play a central role in this abnormal process (4, 5). Therefore, it is proposed that these fibrillar proteins' turnover (the primary function of which in normal pregnancy is to maintain the uterine consistency and support the scaffold's stability) could be pathologically affected.

Matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) are essential regulators of extracellular matrix (ECM) remodeling. It is well known that dysregulation of the MMP/TIMP complex expression/activity leads to structural collagen damage (6, 7). During pregnancy, abnormally expressed MMPs have been reported to cause hypertensive disorders. In preeclampsia, these mechanisms have also been assumed to play a key role in altered uterine and vascular ECM turnover characterized by abnormal vasodilation, placentation, and the development of generalized vascular damage (8, 9). MMP-13 breaks down ECM proteins such as collagens and fibronectins. It degrades "triple helical collagens, including type I, II, and III collagens, but has the highest soluble type II collagen activity. It can also degrade

*Correspondence: a_nicoloff@yahoo.com

type IV, type XIV, and type X collagens and play a role in wound healing and tissue remodeling, in cell migration and tumor cell invasion" (10). MMP-13 plays a vital role in tissue regeneration and the pathogenic mechanisms of certain diseases such as atherosclerosis, aneurysms, and cancer. In reference to TIMP-1, it is a glycoprotein. This biomolecule is a crucial part of the TIMPs family (11).

Several tissues have been described to express TIMP-1 (12). The primary function of this protein is to inhibit the activity of matrix metalloproteinases, thus suppressing ECM degradation (13). With the accumulation of additional knowledge about the structure and function of TIMP-1, it was revealed that it potently inhibits the activity of most MMPs except MMP-2 and MT1-MMP (14). Hence, it can be theorized that TIMP-1 plays a role as an inhibitor of MMP-13.

It can be concluded that failure of the regulation of MMPs/TIMPs complex occurs in preeclampsia. This leads to pathological collagen I and III turnover and abnormal uterine wall remodeling, which results in impaired modification of uterine wall collagen structure. It has been postulated that control of expression and regulation of MMP-13 and TIMP-1 might be crucial in complicated pregnancy. However, there are no data in the literature on a parallel examination of MMP-13 and TIMP-1 serum concentrations and their ratio in preeclamptic women with a family history of preeclampsia.

The present study aimed to investigate the distribution of serum levels of MMP-13, TIMP-1 and the ratio of MMP-13/TIMP-1 in women with preeclampsia with a family history of preeclampsia.

2. Material and methods

2.1. Study population

The current research was a cross-sectional study and part of a university scientific project (N1/2020). The Ethics Committee of the Medical University- Pleven approved the project with Protocol N51/2020. All participants signed informed consent. Study procedures followed all guidelines for ethical standards of the responsible committee on human experimentation and the Helsinki Declaration of 1975, as revised in 2000.

All participants were in-patients of the Clinic of Obstetrics and Gynecology, University Hospital "G. Stranski" Pleven. The sera of subjects were taken from October 2019 to March 2021. The study group consisted of 37 women with preeclampsia, with a mean age of 24.9±6 years, while the mean age of the control group of 32 women with normal pregnancies was 24.7±5.4 years. We divided the patients into two subgroups: Subjects with a family history of PE (n=18); (PE+FHPE) and subjects without a family history of PE (n=19); (PE-FHPE).

Criteria for inclusion in the study were as follows: Pregnant women with clinical symptoms and laboratory criteria for preeclampsia (We used the 2018 European Society of Cardiology Guideline for the management of cardiovascular diseases during pregnancy for the diagnostic criteria of preeclampsia); gestational hypertension with significant proteinuria (>300mg/24h urine collection or the extrapolated amount from a timed collection) (15); maintaining a current diet and exercise during the study; signed informed consent to participate in the study; dysfunction of mother's organ such as HELLP syndrome, renal failure, neurological involvement, hepatic involvement or fetal growth retardation. Criteria for exclusion from the study were as follows: diabetes mellitus, kidney and heart disease, signs of chorioamnionitis, and the presence of a fetus with a chromosomal abnormality.

2.2. ELISA

We used ELISA to determine MMP-13 and TIMP-1 and measured MMP-13 and TIMP-1 levels in serum samples using ELISA kits (Human MMP-13 ELISA kit Reagent Genie; Human TIMP-1 Quantikine ELISA kit) according to the manufacturer's instructions. We calculated the MMP-13/TIMP-1 ratio.

2.3. Statistical analysis

We used the following computer programs to analyze the research data: Excel (Microsoft Corporation, Redmond, WA), SPSS and Statgraphics Plus (Manugistics, Rockville, MD) for Windows. We used tables, graphs, and numerical values to describe all results. We determined the level of significance as $p<0.05$. We used standard skewness and kurtosis to check the normality of distribution and equality of variances. We used student's t-test and ANOVA with mean±SD in cases with normal distribution to find significant differences between groups (LSD, Tukey HSD, Scheffe, Bonferroni, Newman-Keuls, Duncan). We used □2 and K-W H-tests with a median (M) value in cases without normal distribution, together with first and third quartile Q1 and Q3; (twenty-fifth and seventy-fifth percentile P25 and 75P).

3. Results

Either MMP-13 or TIMP-1 alone did not exhibit any obvious differences between normal and PE pregnancies (Table 1) (Fig. 1 and 2). However, MMP-13/TIMP-1 ratio was statistically significantly higher in PE than healthy pregnant women: 0.2 (0.1÷0.5) vs. 0.065 (0.05÷0.2) ($p<0.05$) (Fig. 3). Moreover, patients with PE+FHPE showed statistically significantly lower MMP-13/TIMP-1 ratio compared to PE-FHPE 0.085 (0.05÷0.25) vs. 0.22 (0.12÷0.35) (KW=5.71; p=0.02) (Fig. 4).

Table 1. Serum levels of MMP-13 and TIMP-1 in healthy pregnant women and preeclampsia

	Healthy pregnant women	Preeclampsia	P
MMP-13 (ng/ml)	0.17 (0.15÷0.2)	0.18 (0.16÷0.2)	p>0.05
TIMP-1 (ng/ml)	3.02 (1.28÷3.58)	2.41 (1.01÷4)	p>0.05

MMP-13: matrix metalloproteinase 13, TIMP-1: tissue inhibitor of matrix metalloproteinase 1; Data are expressed as median (interquartile range)

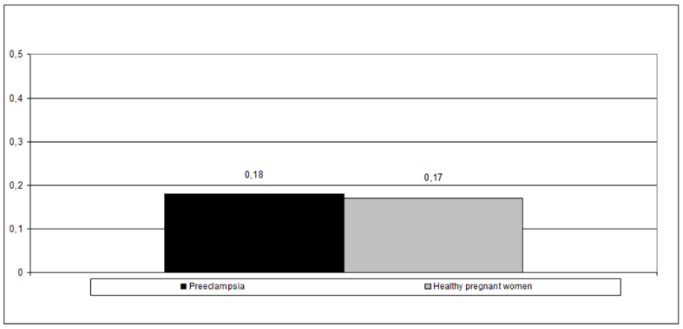

Fig. 1. Serum levels of MMP-13 (ng/ml) in women with preeclampsia vs healthy pregnant women; MMP-13: matrix metalloproteinase 13; Data are expressed as median

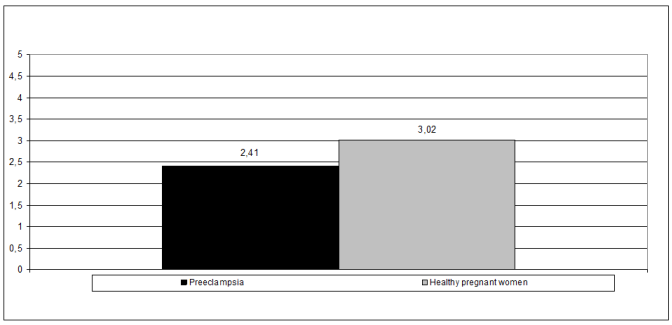

Fig. 2. Serum levels of TIMP-1 (ng/ml) in women with preeclampsia vs healthy pregnant women; TIMP-1: tissue inhibitor of matrix metalloproteinase 1; Data are expressed as median

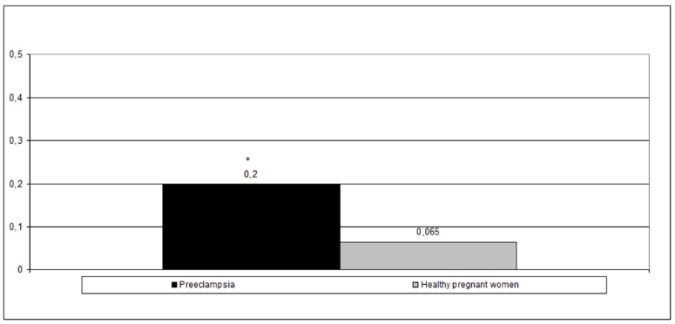

Fig. 3. MMP-13/TIMP-1 serum ratio in women with preeclampsia vs healthy pregnancy; MMP-13/TIMP-1 ratio was significantly higher in preeclampsia than in healthy pregnant women: 0.2 vs 0.065 (*p<0.05); Data are expressed as median

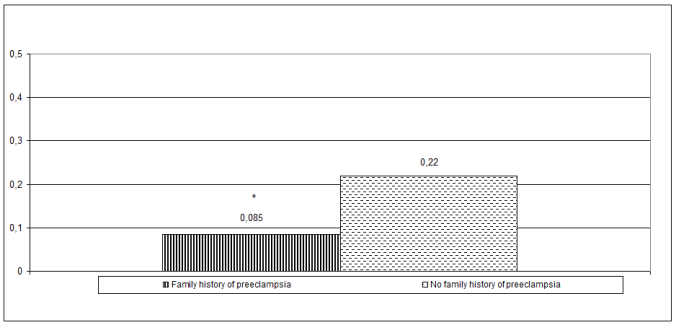

Fig. 4. MMP-13/TIMP-1 serum ratio in women with preeclampsia according to the presence or absence of a family history of preeclampsia; Patients with a family history of preeclampsia showed a significantly lower MMP-13/TIMP-1 ratio than subjects without a family history: 0.085 vs 0.22 (p=0.02); Data are expressed as median

4. Discussion

Preeclampsia is a vital pregnancy complication and one of the most common pregnancy disorders. Preeclampsia is characterized by a mother's high blood pressure, often with proteinuria. Fetal growth restriction is also generally observed. Early preeclampsia detection is paramount for risk stratification and preventing further complications. There is evidence that uterine ECM metabolism is altered during preeclampsia. However, data in the medical literature about the modifications of collagen in the human uterus is limited (16, 17). The courses of collagen types I and III synthesis and degradation in the uterine ECM are "dynamic and reflect healthy and complicated pregnancy" (18). Accumulating data proves that collagen type I and III turnover is impaired in preeclampsia. This might be a consequence of disrupted uterine ECM homeostasis and MMP/TIMP system imbalance (19).

Considering the conception of MMPs and TIMPs' crucial role in the pathogenic pathways in preeclampsia, Karthikeyan et al. (20) studied these proteins and reported that plasma and genetic alterations in the MMP/TIMP system are associated with hypertensive disorders of pregnancy. Authors have found that if the regulation of the MMP/TIMP system fails in the control of the ECM remodeling, this may lead to diseases such as gestational hypertension and preeclampsia. Furthermore, vascular "remodeling disorders of the uterine and placenta and placenta hypoperfusion have been generally recognized" (21). The following literature data represents current studies exploring levels of MMP-13 and TIMP-1 in samples of patients with hypertensive disorders of pregnancy:

In 2017, Laskowska tested the hypothesis of whether maternal serum MMP- 2, 3, 9, and 13 levels have different values in early- and late-onset preeclampsia and uncomplicated pregnancies. “The levels of MMP-13 were higher in both preeclamptic groups of pregnant women than in the healthy controls, but these differences were statistically insignificant. A critical finding of the present study was that MMP-3 appears to be involved solely in early-onset preeclampsia but not in late-onset preeclampsia. Higher levels of MMP-2 and MMP-13 and lower levels of MMP-9 seem to be related to both early- and late-onset severe preeclampsia" (22). Using ELISA, Tayebjee et al. (2005) investigated TIMP-1 and -2 levels in women with gestational hypertension, normotensive women with normal pregnancies and healthy non-pregnant control subjects. Levels of TIMP-1 and TIMP-2 were significantly different among the three groups. Authors concluded that "altered MMP/TIMP ratios in maternal blood during gestational hypertension. These observations suggest pregnancy-related changes in ECM breakdown and turnover. Given the importance of changes in ECM composition to vascular and cardiac structure in hypertension, investigators suggest that these observations may be related to the pathophysiology of human gestational hypertension" (23).

Luizon et al. (2014) examined TIMP-1 polymorphism, plasma TIMP-1 levels, and antihypertensive therapy responsiveness in hypertensive disorders of pregnancy. They measured plasma MMP-9 and TIMP-1 levels using ELISA. Gestational hypertension patients with the GG genotype for the TIMP-1 polymorphism had lower MMP-9 levels and MMP-9/TIMP-1 ratios than those with the TT genotype. Preeclampsia patients with the TG genotype had higher TIMP-1 levels (24).

Gupta M et al., 2016, explored the serum concentrations of MMP-1, TIMP-1 and their ratio in the second and third trimesters of normal and preeclamptic pregnancy. The investigators examined these biomolecules via the ELISA method. Serum levels of MMP-1, TIMP-1, and their ratio during the progression of preeclampsia did not show statistical significance compared to normal pregnancy (25). Myers JE et al.'s (2005) investigation involved women who subsequently developed preeclampsia. Authors studied MMP-2 and-9 levels, TIMP-1 and TIMP-2 in the plasma of this contingent of patients. Plasma samples were taken from women whose pregnancies were subsequently complicated by preeclampsia and from normal pregnant women at 22 and 26 weeks and delivery or diagnosis. "Following equal protein loading, MMP-2 and 9 and TIMP-1 and 2 were quantified using zymography and Western blot analysis, respectively. TIMP-1 levels were significantly reduced in the preeclampsia group at 26 weeks ($p = 0.0002$), but TIMP-2 levels were not quantifiable. Authors conclude that at all three gestational time points an imbalance in the MMP-2/TIMP-1 ratio was found in patients who subsequently developed preeclampsia" (26). In their research, Palei et al., 2008, focused on patients with preeclampsia and gestational hypertension and compared their data with those of normotensive pregnancies and healthy non-pregnant women. The investigators examined the concentrations of TIMP-1 and TIMP-2 in the subjects mentioned above. TIMP concentrations were measured in plasma samples by gelatin zymography and ELISA. The results of Palei et al. showed higher TIMP-1 levels in PE than in GH and normotensive pregnant women (27). In another study, Ab Hamid et al. (2012) determined the total levels of TIMP-1and 2 by ELISA. The contingent taking part in the study involved women with gestational hypertension and normotensive pregnant women. The expression levels of TIMP-1 and TIMP-2 in the gestational hypertension group were low (28).

In 2014, forty-seven biomarkers involved in the pathogenesis of preeclampsia were determined in 5623 pregnant women, part of a prospective investigation named "Early Pregnancy Prediction of Preeclampsia in Nulliparous Women, Combining ClinicalRisk and Biomarkers the Screening for Pregnancy Endpoints (SCOPE) International Cohort Study ". These biomolecules' levels were surveyed in the plasma of subjects sampled at 14 to 16 weeks gestation. Preeclampsia developed in 278 women, approximately 5% of all subjects participating in the study. TIMP-1 showed higher levels in these patients than in the no preeclampsia group (29). In order to estimate the role of MMP-2 and -9, along with their inhibitors TIMP-1 and-2, Montagnana et al. (2009) evaluated these indicators using ELISA in preeclamptic, normotensive pregnant and non-pregnant women. The serum levels of TIMP-1 were significantly higher in preeclamptic compared to both non-pregnant and normotensive pregnant women (30).

In our study, serum MMP-13 levels in preeclampsia were higher than in women with normal pregnancies, but not significantly. Current findings are consistent with the study of Laskowska mentioned above (22). As for TIMP-1, patients' levels were insignificantly lower than healthy pregnant women. Our results are in line with those of Gupta et al. (25) but in a contradiction with the reports of the researchers Luizon et al. (24), Montegrana et al. (30), as well as Paley and co-authors (27). A possible explanation for the lack of a TIMP-1 significant difference in our investigation was the likely predominance of different TIMP-1 polymorphisms in which TIMP-1 levels did not increase significantly (24). In addition, there are also options for some differences in the sample types used between the different studies [plasma (23, 24, 26, 27, 29) vs. serum (25, 28, 30)], methods [ELISA (23-25, 27, 28, 30) vs. gelatin zymography (26, 27)], difference in the examined contingent of patients [gestational hypertension (23, 24, 27, 28) or preeclampsia (24-27, 29, 30)].

In the light of our observations, we continued our investigation and further analyzed the ratio of MMP-13/TIMP-1 between the study groups. In this context, we reported two compelling pieces of evidence: (1) significantly higher serum MMP-13/TIMP-1 ratio in preeclampsia than in normal pregnancy subjects and (2) significantly lower MMP-13/TIMP-1 ratio in PE+FHPE compared to PE-FHPE. Given the present results, a question of great interest arises. So, what could be the reason favoring patients with a history of preeclampsia to indicate a lower MMP-13/TIMP-1 ratio than subjects without a family history? The following theory might possibly explain our findings. We hypothesize that in preeclampsia, the precise balance between the degradation activity of collagenase MMP-13 and tissue inhibitor TIMP-1 is disturbed. As a result of this dysregulation, collagen type I and III turnover are impaired. This may favor abnormal vascular and uterine ECM changes at the maternal-fetal interface. These processes lead to the over deposition of collagen, which may affect uterine remodeling. It should be considered that we just assessed the levels of circulating MMP-13, TIMP-1 and their ratios, so the role of these components' expression/activity cannot be commented upon. Of note, we closely monitored the subgroup of patients with a history of preeclampsia during the whole investigation, also following them post-study. We marked their pregnancy outcomes, possible later hypertension development, and other results. We are still analyzing all these data to be used for future publications.

The major finding of the present study was the disrupted

balance between MMP-13 and TIMP-1 in preeclamptic women with a family history of preeclampsia. Hereby, we suggest that altered steadiness between collagen type I and III synthesis and degradation might play an essential role in PE susceptibility and the development of preeclampsia. To the best of our knowledge, our study has provided for the first time the equilibrium between serum MMP-13 and TIMP-1 in preeclamptic women with a family history of preeclampsia.

Our study revealed an altered balance between the MMP-13 and TIMP-1 in PE patients with a family history of preeclampsia. The net effect of this imbalance might contribute to the development and progression of PE, but the exact mechanisms are yet unclear. The small sample size and the cross-sectional design were limitations of the current study. More extensive and longitudinal studies, including more sensitive methods like Western blot, gelatin zymography and analysis of the genes encoding MMP-13 and the protein segment of TIMP-1 are needed to explore in detail the clinical significance of MMP-13/TIMP-1 complex in preeclampsia.

Our findings provided evidence for diminished serum MMP-13/TIMP-1 ratio in preeclamptic women with a family history of preeclampsia. The imbalance between MMP-13 and TIMP-1 could be a factor contributing to the pathogenic mechanisms determining susceptibility and development of preeclampsia.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.N., N.P., Design: A.N., N.P., Data Collection or Processing: N.P., Analysis or Interpretation: A.N., N.P., Literature Search: N.P., I.H., Writing: A.N., N.P.

References

1. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. Obstetrics and gynecology. 2013 Nov; 122(5): 1122-1130.
2. Eiland E, Nzerue C, Faulkner M. Preeclampsia 2012. J Pregnancy. Jul 11 2012, 586578.
3. Prakash J, Ganiger VC. Acute Kidney Injury in Pregnancy-specific Disorders. Indian J Nephrol. 2017; 27(4):258-70.
4. Poon LC.,Nicolaides KH. Early prediction of preeclampsia. Obstet Gynecol Int. 2014; 1–11.
5. Pulkkinen M, Lehto M, Jalkanen M, Näntö-Salonen K. Collagen types and fibronectin in the uterine muscle of normal and hypertensive pregnant patients. Am J Obstet Gynecol. 1984; 149: 711–717.
6. Mansell JP, Bailey AJ. Collagen Metabolism. In Encyclopedia of Endocrine Diseases; Martini L, Ed.; Elsevier: Amsterdam, The Netherlands 2004, pp. 520–529.
7. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med. 2003; 348: 2543–2556.
8. Tryggvason L, Patrakka J.Alport's Disease and Thin Basement Membrane Nephropathy. In Genetic Diseases of the Kidney; Lifton RP, Somlo S, Giebisch GH, Eds.; Elsevier: Amsterdam, The Netherlands 2009, Chapter 4: pp. 77–96.
9. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure function and biochemistry. Circ Res. 2003; 92: 827–839.
10. Kim YS, Kim SH, Kang JG, Ko JH. Expression level and glycan dynamics determine the net effects of TIMP-1 on cancer progression. BMB Rep. 2012; 45: 623–628.
11. Creemers EE, Cleutjens JP, Smits JF, Daemen MJ. Matrix metalloproteinase inhibition after myocardial infarction, a new approach to prevent heart failure? Circ Res. 2001; 389: 201–210.
12. Morgunova E, Tuuttila A, Bergmann U, Tryggvason K. Structural insight into the complex formation of latent matrix metalloproteinase 2 with tissue inhibitor of metalloproteinase. Proc Natl Acad Sci USA. 2002; 99: 7414–7419.
13. Gross J, Lapiere CM. Collagenolytic activity in amphibian tissues: A tissue culture assay. Proc Natl Acad Sci USA. 1962; 48: 1014–1022.
14. Bourboulia D, Stetler-Stevenson WG. Matrix metalloproteinases (MMPs) and tissue inhibitors ofmetalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. Semin Cancer Biol. 2010; 20: 161–168.
15. European Society of Cardiology (ESC) Guideline for the management of cardiovascular diseases during pregnancy. European Heart Journal 2018; 39(34): 3165–3241.
16. Eiland E, Nzerue C, Faulkner M. Preeclampsia 2012. J. Pregnancy 2012, 1–7.
17. Pulkkinen MO, Kivikoski AI, Nevalainen TJ. Group I and group II phospholipase A2 in serum during normal and pathological pregnancy. Gynecol Obstet Invest. 1993; 36:96–101.
18. Henriksen K, Karsdal MA. Type I Collagen. In Biochemistry of Collagens, Laminins and Elastin Structure, Function and Biomarkers, 2nd ed.; Karsdal MA., Ed.; Elsevier: Amsterdam, The Netherlands 2019; Chapter 1: pp. 1–12.
19. Nielsen MJ, Karsdal MA. Type III Collagen. In Biochemistry of Collagens, Laminins and Elastin Structure, Function and Biomarkers, 2nd ed.; Academic Press: Cambridge, MA, USA, 2019; Chapter 3: pp. 21–30.
20. Karthikeyan VJA, Lane D, Beevers DG, Lip GYH, Blann AD. Matrix metalloproteinases and their tissue inhibitors in hypertension-related pregnancy complications. J Hum Hypertens. 2012; 27: 72–78.
21. Wang Z, Lu S, Liu C, Zhao B, Pei K, Tian L, et al. Expressional and epigenetic alterations of placental matrix metalloproteinase 9 in preeclampsia. Gynecol Endocrinol. 2010; 26: 96–102.
22. Laskowska M. Altered maternal serum matrix metalloproteinases MMP-2, MMP-3, MMP-9, and MMP-13 in severe early-and late-onset preeclampsia. BioMed Res Int. 2017; 1–9.
23. Tayebjee M, Karalis I, Nadar S, Beevers DJ, MacFadyen R, Lip GYH. Circulating matrix metalloproteinase–9 and tissue inhibitors of metalloproteinases–1 and –2 levels in gestational hypertension. AJH 2005; 18: 325–329.

24. Luizon MR, Palei ACT, Sandrim VC, Amaral LM, Machado JSR, Lacchini R. Tissue inhibitor of metalloproteinase (TIMP)-1 is a major endogenous inhibitor of matrix metalloproteinase (MMP)-9, which may affect the responsiveness to therapy in hypertensive disorders of pregnancy. Pharmacogenom J. 2014; 14: 535–541.

25. Gupta M, Chari S. Assessment of matrix metalloproteinase-1 and its tissue inhibitor of metalloproteinase-1 in pre-eclampsia. Int J Sci Study. 2016; 3: 70–73.

26. Myers JE, Merchant SJ, MacLeod M, Mires GJ, Baker PN, Davidge ST. MMP-2 levels are elevated in the plasma of women who subsequently develop preeclampsia. Hypertens Pregnancy. 2005; 24: 103–115.

27. Palei AC, Sandrim VC, Cavalli RDC, Tanus-Santos JE. Comparative assessment of matrixmetalloproteinase (MMP)-2 and MMP-9, and their inhibitors, tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2 in preeclampsia and gestational hypertension. Clin Biochem. 2008; 41: 875–880.

28. Ab Hamid J, Mohtarrudin N, Osman M, Asri AA, Hassan WHW, Aziz R. Matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases 1 and 2 as potential biomarkers for gestational hypertension. Singap Med J. 2012; 53: 681–683.

29. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers the screening for pregnancy endpoints (SCOPE) international cohort study. Hypertension 2014; 64: 644–652.

30. Montagnana M, Lippi G, Albiero A, Scevarolli S, Salvagno GL, Franchi M, et al. Evaluation of metalloproteinases 2 and 9 and their inhibitors in physiologic and pre-eclamptic pregnancy. J Clin Lab Anal. 2009; 23: 88–92.

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The relationship between demographic indicators and mortality rate of COVID-19 disease comparatively and retrospectively in different waves of COVID-19 disease in Iran

Kamran AGHAKHANI[1], Siamak SOLTANI[1], Azadeh MEMARIAN[2,*], Shahrokh MEHRPISHEH[3]

[1]Department of Forensic Medicine, Iran University of Medical Science, Tehran, Iran
[2]Department of Forensic Medicine, Mazandaran University of Medical Science, Sari, Iran
[3]Department of Neonatology, Mazandaran University of Medical Science, Sari, Iran

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Abstract

Coronavirus disease-19 (COVID-19) is a novel emerging infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In this study, we aimed to examine the relationship between demographic indicators and mortality rates in Covid-19 disease in different Covid-19 waves in Iran. In this retrospective cross-sectional study, the study population consisted of 9874 patients of Covid-19 admitted to Hazrat Rasoul Akram Hospital of Tehran, from the beginning of the disease to the end of the fifth wave. Demographic variables such as age and sex as well as clinical variables such as hospitalization date and mortality rate were collected and evaluated. The analysis was performed using SPSS software version 26. The mean age of 9874 participants was 58.9 ± 17.0 years. In this study 5510 (55.8%) of patients were male. 1762 (17.8%) patients died. The fifth wave had the highest number of patients (31.1%) and the trend in the number of patients was increasing from wave second to fifth. However, the percentage of death was lower in waves fourth (14.5%) and fifth (15.3%). The mean age of deceased patients was significantly greater than alive patients (69.25 ± 14.60 vs. 56.76 ± 16.75, P=0.0001). The frequency of male deaths was significantly higher than female deaths (P=0.0001). The results of the present study indicate that the frequency of mortality in recent waves, despite a significant increase in hospitalization, has been decreased. It can also be said that mortality increases with age as well as male gender, and males are more prone to death due to covid-19 disease with age.

Keywords: COVID-19, demographic indicators, mortality rate, Iran

1. Introduction

In late December 2019, a novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spread in Wuhan, China. The illness caused by SARS-CoV-2 termed coronavirus disease-19 (COVID-19) (1). The COVID-19 was declared a pandemic by World Health Organization (WHO) on 11 March 2020 (2). COVID-19 virus is mostly spread from person to person via respiratory droplet transmission, which happens while a person is in close contact with an infected person. This happens via exposure of the host's mucosal surfaces, including nose, mouth, and eyes to the incoming infective droplets (3, 4). The covid-19 median incubation duration is expected to be 5.1 days, and most of the patients will progress symptoms in 11.5 days of infection (5). 17.9% of covid-19 patients is estimated to remain asymptomatic (6). However, the symptoms of symptomatic patients commonly include fever, cough, as well as shortness of breath. Sore throat, anosmia, nausea, dysgeusia, anorexia, malaise, diarrhea, and myalgias are reported as less common symptoms (7, 8). RT-PCR was introduced as the standard test for the diagnosis of the virus, however, the importance of chest CT scans in those whose RT-PCR test is false-negative was reported with a sensitivity of 98% (7). Presently, different therapeutic choices are existing for covid-19 including antiviral drugs such as molnupiravir, remdesivir, and paxlovid; anti-SARS-CoV-2 monoclonal antibodies such as bamlanivimab/etesevimab and casirivimab/imdevimab; anti-inflammatory drugs like dexamethasone; and immunomodulators agents such as baricitinib and tocilizumab (9). According to the evidence, older age, suppressed immune systems, presence of underlying cardiovascular, metabolic, and respiratory diseases are risk factors for adverse outcomes (10).

Accurate estimation of epidemiological information, in particular infection and mortality rates, demographic information, and comorbidities, is required to decide on

*Correspondence: memarian_azadeh@yahoo.com

epidemic control interventions and regulations (11). In this study, we aim to use the information recorded in the Statistics Center of Rasoul Akram Hospital to examine the relationship between demographic indicators and mortality rate in covid-19 disease in a comparative and retrospective study between different waves of covid-19. The information of this study can be useful in coronavirus management policies and evaluation of the results of vaccination in Iran. So far, no comparative study has been conducted between different waves of Covid-19 in Iran, and with the results of this study, we can examine the difference between the mortality rate of the disease in different waves and the factors affecting the mortality rate of Covid-19 disease in Iran.

2. Materials and Methods

In this retrospective cross-sectional study, a total of 9874 patients were admitted with a definitive diagnosis of Covid-19 disease in Hazrat Rasool Akram Teaching, Research and Treatment Hospital in Tehran, from the beginning of the pandemic to the end of the fifth were included in the study. The sampling method was census.

In order to collect information, after ethical approval, the researcher obtained the list of Covid-19 patients, from the medical records (hospital statistics center unit) of the hospital. The demographic variables such as age and sex as well as clinical variables such as hospitalization date and patient status at discharge (alive and deceased) were collected and evaluated.

In order to determine the disease waves, with the cooperation of the Vice Chancellor for Health of Iran University of Medical Sciences and Health Services, the following dates were determined and announced. The first wave was determined from March 2019 to spring 2020; The second wave was in summer 2020; The third wave was from autumn 2020 and the first half of winter 2021; The fourth wave was from the second half of winter 2021 to spring 2021, and the fifth wave was in summer 2021.

2.1. Statistical analysis

SPSS software version 26 was used for the statistical analysis of data. The results were expressed as mean and standard deviation (mean ± SD) for quantitative variables and as a percentage for qualitative variables. It should be noted that in order to investigate the relationship between qualitative variables, Chi-square and Fisher exact tests were used, and also the relationship of quantitative variables, Spearman or Pearson tests were used. In order to measure the relationship between quantitative variables and qualitative variables in parametric conditions, the Student T-test and in non-parametric condition Mann-Whitney U test were used. Paired t-test and its non-parametric equivalent (Wilcoxon) were also used to evaluate quantitative variables before and after the parametric test. A P-value less than 0.05 was considered significant.

2.2. Ethical consideration

The study was performed according to the principles of the Declaration of Helsinki and the ethics committee of Iran University of Medical Sciences (Code of ethics: IR.IUMS.REC.1400.619).

3. Result

The mean age of patients at the time of diagnosis was 58.9 ± 17.0 years. In this study, out of 9874 patients, 5510 (55.8%) were male. Also, 8112 (82.2%) of patients were alive while discharging from the hospital and 1762 (17.8%) died.

The frequency of covid-19 patients in different waves is demonstrated in Table 1. Out of 9874 patients, 3075 (31.1%) were in the fifth wave.

Table 1. The frequency of patients in different waives

Waves	No.	%
First	1453	14.7
Second	1157	11.7
Third	1807	18.3
Fourth	2382	24.1
Fifth	3075	31.1

The mean age of patients and the frequency of different sex and the mortality are shown in Table 2. From the second wave to the fifth wave the mean age of patients was decreased. In all waves, the number of males was higher than females, although from wave third to fifth the number of females increased. Moreover, the percentage of mortality was decreased in waves fourth and fifth.

Table 2. The status of Age, sex, and mortality in patients by disease waves

Waves	Age, mean ± SD [min, max]	Sex, No. (%)		Mortality, No. (%)	
		Male	Female	Alive	Deceased
First	59.92 ± 18.27 [10, 98]	877 (60.4)	576 (39.6)	1186 (81.6)	267 (18.4)
Second	61.03 ± 16.76 [8, 101]	675 (58.3)	482 (41.7)	892 (77.1)	265 (22.9)
Third	60.88 ± 17.07 [< 1, 100]	1073 (59.4)	734 (40.6)	1391 (77)	416 (23)
Fourth	58.95 ± 16.88 [3, 98]	1267 (53.2)	1115 (46.8)	2037 (85.5)	345 (14.5)
Fifth	56.69 ± 16.47 [< 1, 100]	1618 (52.6)	1457 (47.4)	2606 (84.7)	469 (15.3)

The mean age of deceased patients was significantly greater than alive patients. The rate of death was higher in male patients (Table 3).

Table 3. Relationship of age and gender with mortality of patients

	Alive	Deceased	P-value
Age*, mean ± SD	56.76 ± 16.75	69.25 ± 14.60	0.0001
Sex, No. (%)**			0.0001
Male	4445 (45%)	1065 (10.8%)	
Female	3667 (37.1%)	697 (7.1%)	

* t-test, ** Chi-Squared

Table 4 shows that in all waves the mean age was significantly higher in deceased patients.

Table 4. Age status according to mortality in patients in different waves

Waves	Age		P-value
	Alive	Deceased	
First	57.18 ± 17.96	72.11 ± 14.22	0.0001
Second	58.92 ± 16.87	68.11 ± 14.28	0.0001
Third	57.97 ±16.80	70.62 ± 14.13	0.0001
Fourth	57.05 ± 16.49	70.13 ± 14.72	0.0001
Fifth	54.95 ± 16.13	66.41 ± 14.84	0.0001

t-test

According to Table 5, the mean age of patients was significantly different between males and females in the first, second, and fourth waves and it was higher in females.

Table 5. Age status according to sex in patients in different waves

Waves	Age, Mean ± SD		P-value
	Male	Female	
First	58.92 ± 18.20	61.46 ± 18.29	0.010
Second	59.98 ± 16.85	62.50 ± 16.54	0.011
Third	60.49 ± 17.53	61.47 ± 16.38	0.231
Fourth	58.22 ± 17.15	59.77 ± 16.54	0.025
Fifth	56.48 ± 16.72	56.93 ± 16.18	0.443

t-test

4. Discussion

In this study, the Covid-19 hospitalized cases in Rasoul Akram Hospital in Tehran from the beginning of hospitalization of patients due to Covid-19, March 26, 2019, to the end of the fifth wave of the disease, September 22, 2021, in terms of age, gender, mortality and the relationship between these variables were evaluated.

In a total of 9874 patients, the male population was about 11.6% more than the female population. It should also be noted that the frequency of male deaths was significantly higher than female deaths. The mean age of all patients was 58.9 ± 17 years. Also, the average age of the deceased patients was significantly higher than the alive patients (about 13 years), which indicates that mortality in the covid-19 disease is more likely with older age and the probability of death in older people are more.

Concerning the frequency of patients in each wave, it can be seen that from the second to the fifth wave, the frequency has increased significantly. Regarding the average age of patients in different waves of the disease, it can be seen that from the first to the second wave, the average age has increased by about a year, and after that, from the second wave to the fifth, the mean age has decreased so that the mean difference of the age of the second to fifth waves is about 4.5 years, which may indicate that newer strains of the virus (such as Delta) cause involvement at younger ages especially in the fourth and fifth waves of the disease. About the gender frequency of people in different waves of the disease, it can be seen that in the first to third waves, the frequency of sexes was close to each other, but in the fourth and fifth waves, compared to the previous three waves, the incidence decreased in males and increased in females.

With regards to the frequency of mortality in different waves, it can be said that the death rate from the first to the third wave is upward and in the fourth and fifth waves, the mortality rate is significantly lower than the previous three waves. Perhaps this obvious and significant decline, despite the fact that the frequency of patients in the fourth and fifth waves was much higher than the previous three waves, can be attributed to the widespread vaccination of the community against this disease.

In a systematic review and meta-analysis by Dessie et al. on 42 studies and 423,117 patients, the results presented that the pooled occurrence of mortality in Covid-19 hospitalized patients was 17.62% (95% CI = 14.26-21.57%). In the current study, 17.8% of patients died as a result of the disease. Similar to the present study, advanced age was increased the mortality risk and the pooled odds ratio and hazard ratio were 2.61(95%CI = 1.75-3.47) and 1.31(95%CI = 1.11-1.51). Also, they found a significant association between Covid-19 mortality and male sex, which is found in this study, with pooled odds ratio and hazard ratio equal to 1.45 (95% CI= 1.41-1.51) and 1.24 (95% CI= 1.07-1.41) (12). In a meta-analysis in Iran by Parohan et al. on publications up to 1 May 2020 (14 studies and 29,909 patients), a significant relation was reported between age older than 65 (pooled OR = 4.59, [95%CI = 2.61-8.04]) and male gender (pooled OR = 1.50, [95%CI = 1.06-2.12]) and the risk of mortality from Covid-19, which is consistent with the result of the present study (13). Alimohammadi et al. study reported that the pooled case fatality rate (CFR) of Covid-19 in hospitalized patients was 13.0% (95% CI= 9.0-17.0) and in patients with age more than 50 years old it was 19.0% (95% CI= 13.0-24.0) (14). In a study in Iran by Nikpouraghdam et al. on 12870 patients from February 2020 to April 2020, most of the cases were in the age range of 50-60 years old. The male to female ratio was 1.93 to 1. The overall CFR was 8.06% among hospitalized Covid-19 patients (15). In another study in southwest of Iran by

Azarbakhsh et al. on 7313 patients with Covid-19 from February to June 2020, 53.5% were male which is close to the result of this study. The CFR was 4.84%. The highest mortality rate was related to patients with different cancers as well as those with age over 80 years (16). Emami et al. assessed the features of patients after the first peak in Fars province in Iran. From the 3702 confirmed covid-19 cases, 87 patients died so the fatality rate was determined 2.35. They also showed that male sex, older age, and comorbidity of diseases particularly diabetes were the main features of deceased patients (17). In the first wave of this study, 18.4% of patients have died. Similar to Emami et al.'s study male sex and older age were associated with mortality. Rahmanian et al. evaluated Covid-19 deceased cases in Jahrom, in the south of Iran, from March to November 2020. 57.54% were men and 42.36% were women. The mean age of the deceased men was 68.7±18.33 and women were 68.82±14.24 years. The result of their study represented that the mortality rate was higher in men than women (18). In Khosravi Shadmani et al.'s study, the epidemiological characteristics of 103,179 Covid-19 patients were evaluated. The average age of men was 52.40 years and females were 52.41 years. 55.2% of the patients were men and 44.8% were women. 60.9% of deaths occurred in the male gender and 39.1% in the female gender and their results were consistent with the result of the current study (19). In Barek et al.'s meta-analysis study on 55 studies and 10014 Covid-19 patients, male patients (OR = 2.41, P < 0.00001) and patients with an age of more than 50 years (RR = 3.36, P=0.0002) were affected by SARS-CoV-2 severely (20). According to Zali et al.'s study on 16000 Covid-19 cases from 19 hospitals of Shahid Beheshti University of Medical Sciences, in Tehran, Iran, 1612 patients died. The uppermost rate of death was determined among the age group of more than 65 years as well as intensive care unit (ICU) and critical care unit (CCU) patients. Total CFR was 10.05% and the highest CFR was reported in patients with age higher than 65 years, those with underlying comorbidities as well as ICU/CCU patients (11).

The results of the present study indicate that the percentage of mortality in recent waves of Covid-19 disease, despite a significant increase in hospitalization, has a decreasing trend and the factors affecting this issue can be widespread vaccination in the community, obtaining more experience in treating this disease, presence of more equipment and facilities in identifying and treating the disease, more safety in re-infection of this disease, and starting treatment earlier due to public awareness. The result also shows that mortality increases with age as well as male sex, and males are more prone to death due to this disease with increasing age.

Conflict of interest

There are no conflicts of interest for the present study.

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Authors' contributions

Concept: K.A., A.M., Design: K.A., A.M., Data Collection or Processing: S.S., S.M. Analysis or Interpretation: S.S., S.M. Literature Search: S.S., S.M. Writing: K.A., A.M., S.S.

References

1. WHO. Novel Coronavirus (2019-nCoV), situation [Internet]. 2020 [updated 2020 Jan 20; cited 2020 Jan 21]. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019-ncov.pdf?sfvrsn=20a99c10_4
2. WHO. Director-General's Opening Remarks at the media Briefing on COVID-19 [Internet]. 2020 [updated 2020 March 11; cited 2020 Jan 21]. Available from: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020
3. Liu J, Liao X, Qian S, Yuan J, Wang F, Liu Y, et al. Community Transmission of Severe Acute Respiratory Syndrome Coronavirus 2, Shenzhen, China, 2020. Emerg Infect Dis. 2020;26(6):1320-3.
4. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020;382(13):1199-207.
5. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med. 2020;172(9):577-82.
6. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Euro Surveill. 2020;25(10).
7. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, Evaluation, and Treatment of Coronavirus (COVID-19). StatPearls. Treasure Island (FL)2022.
8. Stokes EK, Zambrano LD, Anderson KN, Marder EP, Raz KM, El Burai Felix S, et al. Coronavirus Disease 2019 Case Surveillance - United States, January 22-May 30, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(24):759-65.
9. Coopersmith CM, Antonelli M, Bauer SR, Deutschman CS, Evans LE, Ferrer R, et al. The Surviving Sepsis Campaign: Research Priorities for Coronavirus Disease 2019 in Critical Illness. Crit Care Med. 2021;49(4):598-622.
10. CDC COVID-19 Response Team. Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) - United States, February 12-March 16, 2020. MMWR Morb Mortal Wkly Rep. 2020 Mar 27;69(12):343-346. doi: 10.15585/mmwr.mm6912e2.
11. Zali A, Gholamzadeh S, Mohammadi G, Azizmohammad Looha M, Akrami F, Zarean E, et al. Baseline Characteristics and Associated Factors of Mortality in COVID-19 Patients; an Analysis of 16000 Cases in Tehran, Iran. Arch Acad Emerg Med. 2020;8(1):e70.
12. Dessie ZG, Zewotir T. Mortality-related risk factors of COVID-19: a systematic review and meta-analysis of 42 studies and 423,117 patients. BMC Infect Dis. 2021;21(1):855.
13. Parohan M, Yaghoubi S, Seraji A, Javanbakht MH, Sarraf P, Djalali M. Risk factors for mortality in patients with Coronavirus disease 2019 (COVID-19) infection: a systematic review and meta-analysis of observational studies. Aging Male. 2020;23(5):1416-24.

14. Alimohamadi Y, Tola HH, Abbasi-Ghahramanloo A, Janani M, Sepandi M. Case fatality rate of COVID-19: a systematic review and meta-analysis. J Prev Med Hyg. 2021;62(2):E311-E20.

15. Nikpouraghdam M, Jalali Farahani A, Alishiri G, Heydari S, Ebrahimnia M, Samadinia H, et al. Epidemiological characteristics of coronavirus disease 2019 (COVID-19) patients in IRAN: A single center study. J Clin Virol. 2020;127:104378.

16. Azarbakhsh H, Jokari K, Moftakhar L, Ghelichi Ghojogh M, Karimyan A, Salmanzadeh S, et al. Epidemiological characteristics of patients with COVID-19 in Southwest of Iran from February 19 to June 20, 2020. Med J Islam Repub Iran. 2021;35:116.

17. Emami A, Javanmardi F, Akbari A, Moghadami M, Bakhtiari H, Falahati F, et al. Characteristics of deceased patients with CoVID-19 after the first peak of the epidemic in Fars province, Iran. Infect Ecol Epidemiol. 2020;10(1):1781330.

18. Rahmanian F, Hatami N, Haghbeen M, Raoufi R, Abbasi AR, Shakeri H, et al. Gender Differences in COVID-19 Deceased Cases in Jahrom City, South of Iran. Bull Emerg Trauma. 2021;9(2):80-5.

19. Khosravi Shadmani F, Amanollahi A, Zali A, Najafi F, Karami M, Moradi G, et al. Description of Epidemiological Features, Symptoms and Mortality of the Patients with COVID-19 in Some Provinces of Iran. Arch Iran Med. 2021;24(10):733-40.

20. Barek MA, Aziz MA, Islam MS. Impact of age, sex, comorbidities and clinical symptoms on the severity of COVID-19 cases: A meta-analysis with 55 studies and 10014 cases. Heliyon. 2020;6(12):e05684.

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Investigation, design and synthesis of new anticancer agents with anticancer effect potential on MCF-7 breast cancer cells by machine learning method

Suat Utku KESKİN[1], Volkan BÜLBÜL[2], Mervenur KALENDER[3], Sümeyye ÖZYAMAN[3] Arif MERMER[4,*]

[1]Robert College, İstanbul, Türkiye
[2]Department of Histology and Embryology, Faculty of Medicine, Nişantaşı University, İstanbul, Türkiye
[3]Department of Histology and Embryology, Faculty of Medicine, İstanbul Medipol University, İstanbul, Türkiye
[4]Experimental Medicine Application and Research Center, University of Health Sciences, İstanbul, Türkiye

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Abstract

Cancer is one of the diseases with a high mortality rate, which occurs when cells multiply uncontrollably, acquire an invasive character and metastasize. Breast cancer is one of the cancer types with an increasing incidence worldwide. Chemotherapy is a method used in the treatment of cancer diseases, and the chemotherapeutic drugs used inhibit the growth and proliferation of cancer cells due to their cytotoxic properties. Today, machine learning techniques offer significant advantages by helping several steps of the drug discovery process, reducing the time spent in the laboratory, the use of consumables and chemical materials, and the maximum time predicted for the discovery of a drug with traditional methods. In our study, it was aimed to determine the 3 Schiff base derivatives with the most active cytotoxic effect on breast cancer cells from the large data set using machine learning. In our study, 7 Schiff base derivatives were determined from a large data set containing 98 compounds, and the 3 most active compounds with cytotoxic properties on breast cancer cells and their IC50 values were determined by machine learning method. In the future, it is thought that compound 1 can be used as an alternative to pharmacological applications to be used in preclinical studies as a therapeutic agent, supported by *in vitro* and *in vivo* applications, in order to be used in cancer treatments.

Keywords: machine learning, drug discovery, breast cancer, MCF-7

1. Introduction

Breast cancer is the second most frequently diagnosed cancer worldwide, with a frequency of up to 11.9%. It constitutes 25.2% of all newly diagnosed cancers and is the leading type of cancer encountered in women. It has been observed that only 5-10% of these cases are caused by genetic disorders, while the remaining 90-95% are due to environmental factors and lifestyle (1, 2). Hormonal factors, overexposure to estrogen, and subsequent differentiation of breast cells can affect a woman's risk of developing breast cancer (3). While obesity and weight gain in the postmenopausal period are associated with a higher risk of breast cancer, this association is not present for premenopausal women. The number of first-degree female relatives with a history of breast cancer significantly influences an individual's risk of breast cancer (4-6).

The use of drugs in cancer treatment has been extensively studied since the 1940s (7). Chemotherapy is an important part of treatment for many types of cancer. For this reason, the effort to develop new anti-cancer drugs constitutes one of the largest areas in the pharmaceutical industry (8). It is known that a wide variety of chemotherapeutic agents are used clinically in sending cancer cells to death by apoptosis. Some of the most commonly used of these are Cisplatin, Taxol and Doxorubucin (9). While an increase has been observed in the number of anti-cancer agents developed in the last 10 years, the number of agents that can successfully progress clinically from these products is less than 10%. The two most important reasons why anti-cancer agents cannot be accepted for treatment are their lack of clinical efficacy and high toxicity values. The product intended to be used as an anti-cancer agent must pass the phase-III stage during clinical tests. Drug development studies have various difficulties due to the high cost of approximately 1.8 billion dollars and the 9 to 12 years to be approved (10).

Traditional methods of cancer treatment today, including radiotherapy and chemotherapy, are expensive and often have harmful side effects on healthy cells. In addition, cancer cells have the ability to develop resistance to existing chemotherapeutic drugs (11). Therefore, there is a constant need for the development of new anticancer drugs to reduce the proliferation of cancer cells. While traditional *in vitro* prediction strategies developed in this context face time and

*Correspondence: arif.mermer@sbu.edu.tr

cost constraints, potential interaction candidates can be predicted more efficiently by computational or *in silico* methods, which have recently become widespread (12). For all these reasons, the interest in the use of machine learning (ML) methods in the pharmaceutical industry is increasing in order to reduce the time spent and increased costs.

Machine learning algorithms and software have been developed and used at all stages of drug discovery and development, including clinical trials. ML provides various facilities to identify new targets, provide stronger evidence for target-disease relationships, improve small molecule compound design and optimization, increase understanding of disease mechanisms, increase understanding of disease and non-disease phenotypes, and develop new biomarkers for prognosis (13). ML, a branch of artificial intelligence, is based on the idea of learning data from systems, identifying patterns and making decisions with minimal human intervention. There are several different ML methods implemented within the framework of artificial intelligence. For example, drug candidates can be optimized in drug discovery through a combination of models that predict favorable physicochemical properties (eg, solubility and permeability), pharmacokinetic properties, safety, and likely efficacy (14). ML techniques are very powerful tools in helping several steps of the drug discovery process, reducing the time spent in the laboratory, the use of consumables and chemical materials, and the maximum time anticipated for the discovery of a drug by conventional methods (15).

Although there are a wide variety of chemotherapeutics in current use, scientific studies continue rapidly to produce new agents. In these studies, it is aimed to develop new agents that are more effective and have fewer side effects than the agents currently in use. However, successful results cannot be obtained from the vast majority of new agents whose efficacy is estimated and *in vitro* cytotoxicity tests are performed after synthesis. In this direction, in our study, it was aimed to select new anticancer compounds that can be effective on breast cancer by using large data sets with machine learning method. In this way, it is planned to avoid cost, time loss and ethical concerns arising from unsuccessful experiments by conducting trials with compounds with cytotoxic potential in future *in vitro* and *in vivo* studies.

2. Materials and Methods

In this study, Health Sciences University, Experimental Medicine Application and Research Center Organic Synthesis laboratory facilities were used.

2.1. Data set

Compounds showing anti-cancer activity were obtained for the analyses, with the support of the literature. The collected data was uploaded to the OCHEM database, an easy-to-use and web-based platform designed to store experimental results and biological activity results of compounds that form the basis of *in silico* modeling. This dataset (98 compounds) prepared to develop the models consisted of carbazones, coumarins, quinolones, azoles, barbiturates and different chemical series. Concentrations of compounds with various IC50 values were applied to the data set in micromolar (Fig. S1). The dataset was used to develop both classification and regression models. To develop the regression models, the concentration of selected compounds against anti-cancer inhibition was determined in μM. After uploading the dataset to the website (www.ochem.eu), an independent test set was created by randomly selecting approximately 20%-25% of the compounds to obtain validation. Molecular formulas and anti-cancer activity results of all compounds generated in the training and validation test sets are available and accessible online.

2.2. Machine learning

It is the products that have the basic features that have the basic features that are basic in a data set consisting of those who have this collection prepared for a data set consisting of those in the literature. EU). ML method available in OCHEM is used to create in-silico models based on different sets (ASNNN, XGBOOST, WEKA-RF).

2.2.1. Validation of Models

Fivefold cross-validation and external reading set are used to validate models.

2.2.2. Statistical Parameters

Classification models (SN) and specificity (SP) will be calculated as follows;

SN=TP/(TP+FN) and SP=TN/(TN+FP)

In this case, it can be accurately calculated, verified as accurately as possible, accurately calculated, which is absolutely not true.

2.2.3. Molecular Identification

The following molecular identifiers from the OCHEM database are used.

-E-state indices: Electro-topological state indices are 2D descriptors that combine both electronic and topological properties of the compounds analyzed.

-ALogPS: The program calculates the 1-octanol/water distribution coefficient and solubility in water.

-ChemAxon descriptors: ChemAxon supports the calculation of six descriptive groups from 0D to 3D: fundamental analysis, charge, geometry, partitioning, protonation states.

-ADRIANA.Code: The software uses a set of methods for the creation of 3D-structures, calculation of physicochemical descriptors and molecular properties based on experimental models.

2.3. Classification methods

The data set (98 compounds) was randomly divided into two groups: the training (80) and the test set (18). The models were

developed after unidentified filtering of molecular descriptors. In addition, unsupervised forward selection (UFS) was used in models 1, 2 and 4 (Table 1) for better filtering of molecular descriptors. The RF algorithm used random subsets of descriptors as it was less subject to the problem of correlation between molecular descriptors. Therefore, the best WEKA-RF model was obtained without the use of UFS.

Initially, all descriptor sets available on the OCHEM website were scanned and the latest models with the highest predictive accuracy were calculated using four different descriptor sets. Improved models were summarized in Table 1 and Fig. S2. All models showed approximate results in terms of sensitivity, specificity and mean accuracy (BA), calculated by averaging all other methods with the training set consensus model.

Table 1. Statistical coefficients calculated for classification models obtained from the data set

		Sensitivity (%)		Specificity (%)		Average Accuracy (%)	
N	Methods	Practice[a]	Test[a]	Practice[a]	Test[a]	Practice	Test
1	ASNN	86.1	76.4	62.5	100.0	81.0 ± 6.0	90.0 ± 10.0
2	*k*NN	91.6	78.6	50.0	50.0	59.0 ± 7.0	70.0 ± 10.0
3	XGBOOST	86.5	76.4	53.8	100.0	75.0 ± 7.0	90.0 ± 10.0
4	WEKA-RF	89.7	81.1	66.7	100.0	70.0 ± 7.0	80.0 ± 10.0
5	Consensus	89.8	86.6	72.7	100.0	75.0 ± 3.0	89.0 ± 5.00

2.3.1. Regression models

The data set used in classification is also used in this model. Three methods for regression model analysis, ASNN, *k*NN and XGBOOST, have been developed to give the highest performance. 3 different molecular descriptors Adriana, ALOGPS and E-state were used on OCHEM to ensure better performance of the above methods used in the calculation of regression models. The results obtained (R^2, q^2) are summarized in Table 2.

Table 2. Statistical coefficients of regression models

		Training Set		Test Set	
N	Methods	R^2	q^2	R^2	q^2
1	ASNN	0.09 ± 0.07	0.05 ± 0.06	0.6 ± 0.2	0.3 ± 0.1
2	*k*NN	0.02 ± 0.03	0.00 ± 0.00	0.3 ± 0.2	0.06 ± 0.04
3	XGBOOST	0.09 ± 0.01	0.04 ± 0.1	0.5 ± 0.3	0.4 ± 0.2
4	Consensus	0.08 ± 0.09	0.06 ± 0.09	0.5 ± 0.2	0.3 ± 0.1

2.3.2. Activity estimation of new compounds

A virtual database of 7 Schiff base derivatives with different substituents at the R1, R2 and R3 positions was created (Fig. 1). Compounds identified using the consensus classification model were screened for being active or inactive against the MCF-7 cancer cell line, and all of these compounds were found to be active (Table 3)

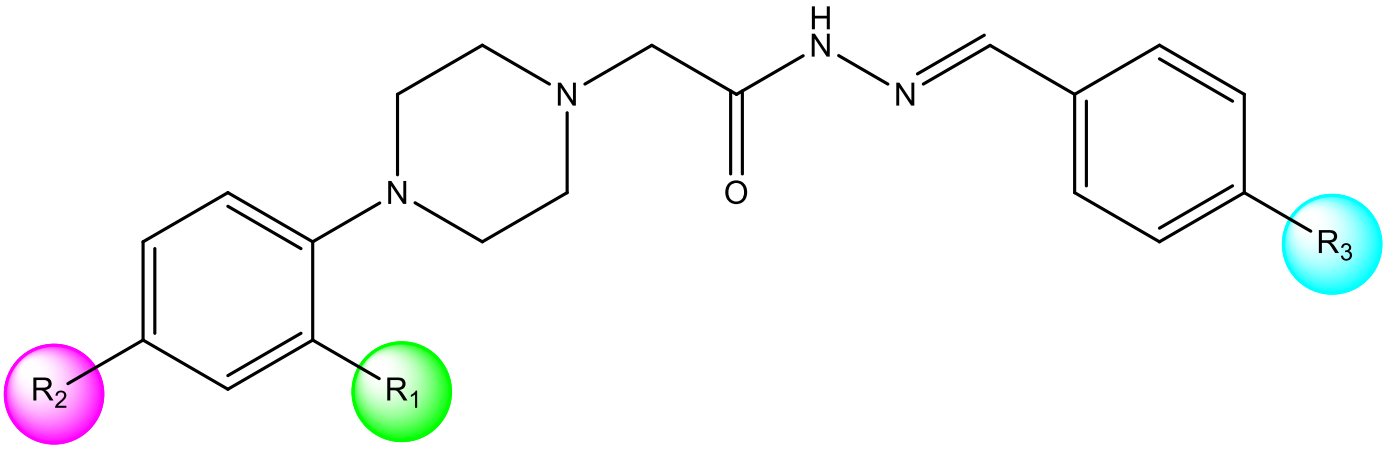
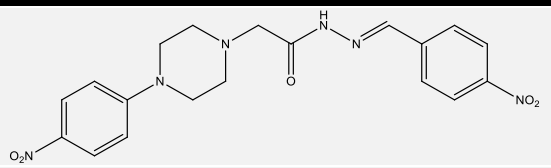
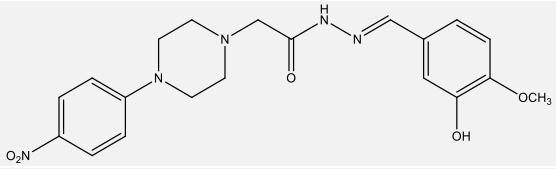
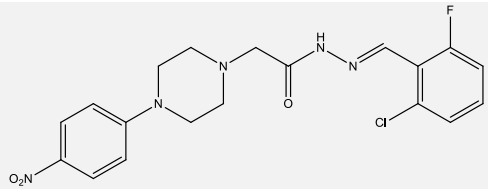
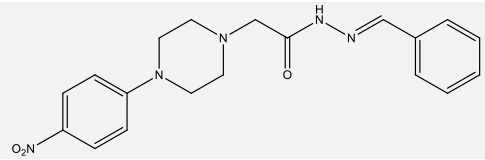
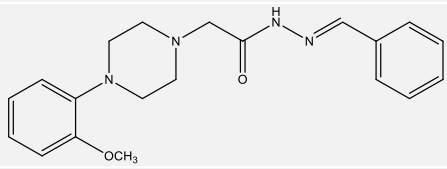
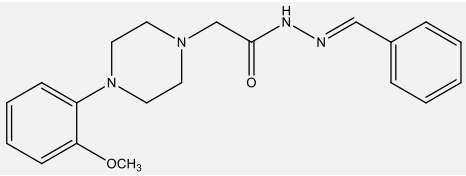
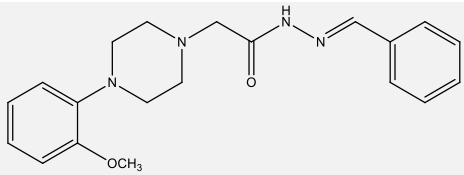


Fig. 1. 7 Virtual library of Schiff base derivative; R1, R2 and R3 were chosen based on the availability of starting materials for the synthesis

Table 3. Compounds tested against the MCF-7 cancer cell line and their estimated activity accuracies, the most active (marked in red), and their IC_{50} values (µM)

Compound	Active/Inactive	Predicted accuracy (%)	Predicted activity (IC_{50}, µM)
	active	88	29.50
	active	88	29.50
	active	96	38.00
	active	92	41.00
	active	88	39.00
	active	91	43.00
	active	87	28.00

2.4. Synthesis procedure

Compound 1 was selected among those with the highest activity results (compounds 1, 3 and 7), which were found to be active in our study, and its synthesis was carried out step by step as follows (Fig. 2).

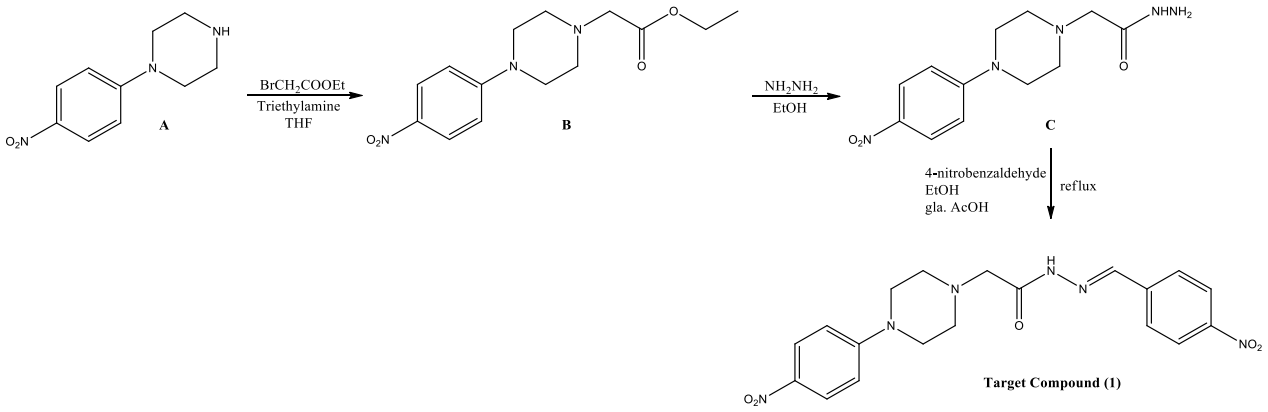


Fig. 2. Synthetic pathway for the synthesis of target compound (1)

Synthesis of Compound B (Ethyl 2-(4-(4-nitrophenyl)piperazin-1-yl)acetate) [16]

Ethyl bromoacetate (10 mmol) was added dropwise to the solution of 1-(4-nitrophenyl) piperazine (10 mmol) in tetrahydrofuran in a round-bottom flask, and the resulting mixture was stirred at room temperature in the presence of triethylamine (10 mmol) for 24 hours. After the salt formed was removed by filtration, the solvent was evaporated under reduced pressure and the solid obtained was crystallized from ethanol and purified.

Melting point: 115-116 ºC, Reaction yield: 99%. Yellow solid. FT-IR (υ_{max}, cm^{-1}): 3080 (ar-CH), 1742 (C=O). ^{1}H NMR (DMSO-d_6, δppm): 1.24 (t, 3H, *J*=8.0 Hz, CH_3), 2.69 (s, 4H, $2CH_2$), 3.34 (s, 2H, CH_2), 3.50 (s, 4H, $2CH_2$), 4.14 (q, 2H, *J*=8.0 Hz, CH_2), 7.06 (d, 2H, *J*=8.0 Hz, arH), 8.09 (d, 2H, *J*=8.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): 14.70, 46.90, 52.00, 58.64, 60.48, 113.20, 113.24, 126.28, 126.30, 137.44, 155.29, 170.41. LC-MS m/z: 316.12 ($[M+Na]^+$).

Synthesis of Compound C (2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide) [16]

Hydrazine hydrate (30 mmol) was added to the solution of compound no. 2 (10 mmol) in ethanol in a round-bottom flask, and the mixture was boiled under reflux for 5 hours. After completion of the reaction, the solvent was evaporated under reduced pressure to give a solid. The resulting solid was crystallized from ethanol and purified.

Melting point: 163-164 ºC, Reaction yield: 97%. Yellow solid. FT-IR (υ_{max}, cm^{-1}): 3328 and 3248 (NH_2 + NH), 3006 (ar-CH), 1631 (C=O). ^{1}H NMR (DMSO-d_6, δppm): 2.58-2.63 (m, 4H, $2CH_2$), 3.06 (s, 2H, CH_2), 3.55 (s, 4H, $2CH_2$), 4.32 (s, 2H, NH_2), 7.09 (s, 2H, arH), 8.11 (s, 2H, arH), 9.06 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 46.88, 52.89, 60.16, 113.22, 126.36, 137.39, 155.31, 168.65. LC-MS m/z: 302.11 ($[M+Na]^+$).

Synthesis of Compound 1 ((E)-N'-(4-nitrobenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl) acetohydrazide)

4-nitrobenzaldehyde (10 mmol) and 3-4 drops of acetic acid were added to the solution of compound 3 (10 mmol) in ethanol in a round-bottom flask, and the mixture was refluxed for 6 hours. After completion of the reaction, the solid formed was filtered and dried. Purification was carried out by crystallization from ethyl acetate.

Melting point: 236-237 ºC, Reaction yield: 91%. Yellow solid. FT-IR (υ_{max}, cm^{-1}): 3380 (NH), 1684 (C=O), 1584 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 2.59 (t, 2H, *J*=4.0 Hz, CH_2), 2.74 (t, 2H, *J*=4.0 Hz, CH_2), 3.18 (s, 2H, CH_2), 3.46 (t, 2H, *J*=4.0 Hz, CH_2), 3.60 (t, 2H, *J*=4.0 Hz, CH_2), 6.85 (d, 2H, *J*=4.0 Hz, arH), 7.80 (d, 2H, *J*=8.0 Hz, arH), 7.87 (d, 2H, *J*=4.0 Hz, arH), 8.22 (d, 2H, *J*=4.0 Hz, arH), 8.50 (s, 1H, CH), 10.74 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δppm): 50.36, 51.54, 57.62, 112.41, 124.48, 126.40, 127.95, 138.88, 139.94, 148.02, 148.64, 158.23, 165.42. LC-MS m/z: 413.23 ($[M+1]^+$).

3. Results

3.1. Calculation of IC_{50} Value with Machine Learning Method

Cross-validated average accuracy results for all models (5-fold cross validation) for all models using the ML method range from 59% to 81%. With the same models, the mean accuracy was also calculated for the test sets, and significant values were found in the range of 70%-89%. Consensus model, which is calculated by averaging all models, has been chosen for the highest results efficiency.

Statistical calculations of the regression models were made by applying ASNN, *k*NN, XGBOOST and consensus models as the average of the three methods. R2 and q2 values in the training and test sets are shown in Table 2. Anti-cancer activity predictions of new compounds were made with the help of classification and regression models created by ML method. All 7 determined compounds were found to be active with the models created, and 3 compounds were determined to be synthesized with an estimated accuracy result (≥87) and estimated activity value (≤30 μM) by making an evaluation from these results (Table 3).

4. Discussion

Using the OCHEM web-based platform, in silico models based on different ML techniques and various molecular descriptors were created. The models created, cross validation methods and estimation of external test sets were made to ensure the validation of the model. With these methods, the activities of the new Schiff base derivatives designed based on the literature data against the MCF-7 cancer cell line were estimated and it was determined that the models created showed high stability, robustness and predictive power. All compounds engineered as a result of the ML models were found to be active against the MCF-7 cancer cell line. This result confirms that ML approaches facilitate a rational search for active molecules within budget and time constraints, which is especially important in academic settings. In addition, the developed models are available to researchers working in this field and can be used to predict the anti-cancer activity of new compounds. Compound 1 was synthesized by ML method among the 3 most active compounds on MCF-7 cells. Traditional treatment methods are now being replaced by personalized cancer treatment. The structure of the cells taken from the tumour tissue of the patient diagnosed with cancer can be examined under laboratory conditions, and the treatment of the patient can be directed. With this method, it is aimed to find the right treatment method without time, energy and financial loss. Personalized cancer treatment is a more effective method than traditional therapies (17). In parallel, in our study, the anticancer effect of compound 1, whose cytotoxic effect was determined by machine learning method, was also demonstrated in vitro. Therefore, it has been demonstrated that personalized treatments can also be performed using machine learning method. One of the main problems with the

development of cancer therapy is the low reproducibility of results observed in animal models and patients. It has been reported that the correlation of data obtained from animal models with human tissue is less than 10% (18). More physiological human models are needed to reduce this attrition rate, improve preclinical screening, and reduce animal use. Monolayer and three-dimensional cell culture methods, such as cancer spheroids, are emerging as an important tool for high-throughput screening (19, 20). There is a large gap between in vitro two-dimensional cell culture and in vivo. 3D cell culture models provide more realistic spatial, biochemical and cellular parameters compared to 2D models and can bridge this gap. It has been shown that 3D cell culture models are pioneers in elucidating molecular and cellular mechanisms, facilitating the development and screening of new drugs because they reflect intercellular interactions more realistically (21-23). It is planned to switch to in vivo studies after the compounds synthesized by showing the potential for cytotoxic effects on MCF-7 will be used in 2D and 3D in vitro studies in the future.

Conflict of interest

The authors have no conflict of interest.

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None to declare.

Authors' contributions

Concept: S.K., A.M., M.B., Design: S.K., A.M., M.B Data Collection or Processing: S.K., A.M., M.B., M.K., Analysis or Interpretation: S.K., A.M., M.B., M.K., S.O., Literature Search: S.K., A.M., M.B., M.K., S.O., Writing: S.K., A.M., M.B.

References

1. Kolak A, Kamińska M, Sygit K, Budny A, Surdyka D, Kukiełka-Budny B, et al. Primary and secondary prevention of breast cancer. Ann Agric Environ Med. 2017;24(4):549-53.
2. Anastasiadi Z, Lianos GD, Ignatiadou E, Harissis HV, Mitsis M. Breast cancer in young women: an overview. Updates Surg. 2017 Sep;69(3):313-317.
3. Enger SM, Ross RK, Paganini-Hill A, Bernstein L. Breastfeeding experience and breast cancer risk among postmenopausal women. Cancer Epidemiol Biomarkers Prev. 1998 May;7(5):365-9.
4. Colditz GA, Willett WC, Hunter DJ, Stampfer MJ, Manson JE, Hennekens CH, et al. Family history, age, and risk of breast cancer: prospective data from the Nurses' Health Study. Jama. 1993;270(3):338-43.
5. Collaborative Group on Hormonal Factors in Breast Cancer. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet. 2001 Oct 27;358(9291):1389-99.
6. Pharoah PD, Day NE, Duffy S, Easton DF, Ponder BA. Family history and the risk of breast cancer: a systematic review and meta-analysis. Int J Cancer. 1997 May 29;71(5):800-9.
7. Lu Z-R, Steinmetz NF, Zhu H. New Directions for Drug Delivery in Cancer Therapy. ACS Publications; 2018.
8. Chabner BA, Roberts TG. Chemotherapy and the war on cancer. Nature Reviews Cancer. 2005;5(1):65-72.
9. Fuertes MA, Alonso C, Pérez JM. Biochemical modulation of cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance. Chemical reviews. 2003;103(3):645-62.
10. Breslin S, O'Driscoll L. Three-dimensional cell culture: the missing link in drug discovery. Drug discovery today. 2013;18(5-6):240-9.
11. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. 2018;68(6):394-424.
12. Chen R, Liu X, Jin S, Lin J, Liu J. Machine learning for drug-target interaction prediction. Molecules. 2018;23(9):2208.
13. Vamathevan J, Clark D, Czodrowski P, Dunham I, Ferran E, Lee G, et al. Applications of machine learning in drug discovery and development. Nature Reviews Drug Discovery. 2019;18(6):463-77.
14. Talevi A, Morales JF, Hather G, Podichetty JT, Kim S, Bloomingdale PC, et al. Machine Learning in Drug Discovery and Development Part 1: A Primer. CPT: pharmacometrics & systems pharmacology. 2020;9(3):129-42.
15. Lima AN, Philot EA, Trossini GHG, Scott LPB, Maltarollo VG, Honorio KM. Use of machine learning approaches for novel drug discovery. Expert opinion on drug discovery. 2016;11(3):225-39.
16. Mentese M, Demirbas N, Mermer A, Demirci S, Demirbas A, Ayaz FA. Novel Azole-Functionalited Flouroquinolone Hybrids: Design, Conventional and Microwave Irradiated Synthesis, Evaluation as Antibacterial and Antioxidant Agents. Letters in Drug Design and Discovery. 2018;15:46-64.
17. Jackson SE, Chester JD. Personalised cancer medicine. Int J Cancer. 2015 Jul 15;137(2):262-6.
18. Mak IW, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res. 2014 Jan 15;6(2):114-8.
19. Anastasov N, Höfig I, Radulović V, Ströbel S, Salomon M, Lichtenberg J, et al. A 3D-microtissue-based phenotypic screening of radiation resistant tumor cells with synchronized chemotherapeutic treatment. BMC Cancer. 2015 Jun 10;15:466.
20. Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. Nat Rev Cancer. 2006 Oct;6(10):813-23.
21. Kim MJ, Chi BH, Yoo JJ, Ju YM, Whang YM, Chang IH. Structure establishment of three-dimensional (3D) cell culture printing model for bladder cancer. PLoS One. 2019 Oct 22;14(10):e0223689.
22. Knight E, Przyborski S. Advances in 3D cell culture technologies enabling tissue-like structures to be created in vitro. J Anat. 2015 Dec;227(6):746-56.
23. Sokolova V, Rojas-Sánchez L, Białas N, Schulze N, Epple M. Calcium phosphate nanoparticle-mediated transfection in 2D and 3D mono- and co-culture cell models. Acta Biomater. 2019 Jan 15;84:391-401.

Supplementary materials

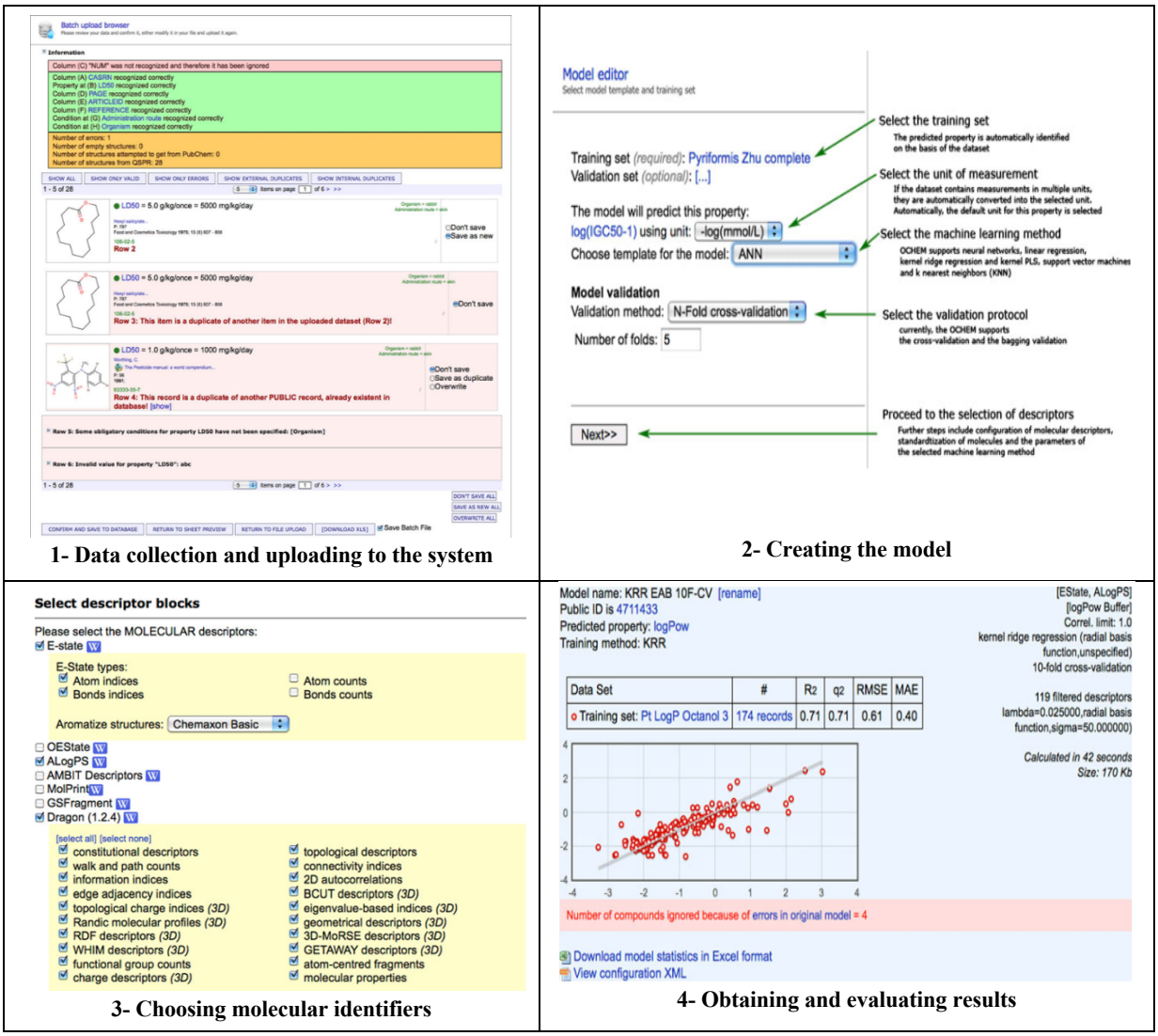
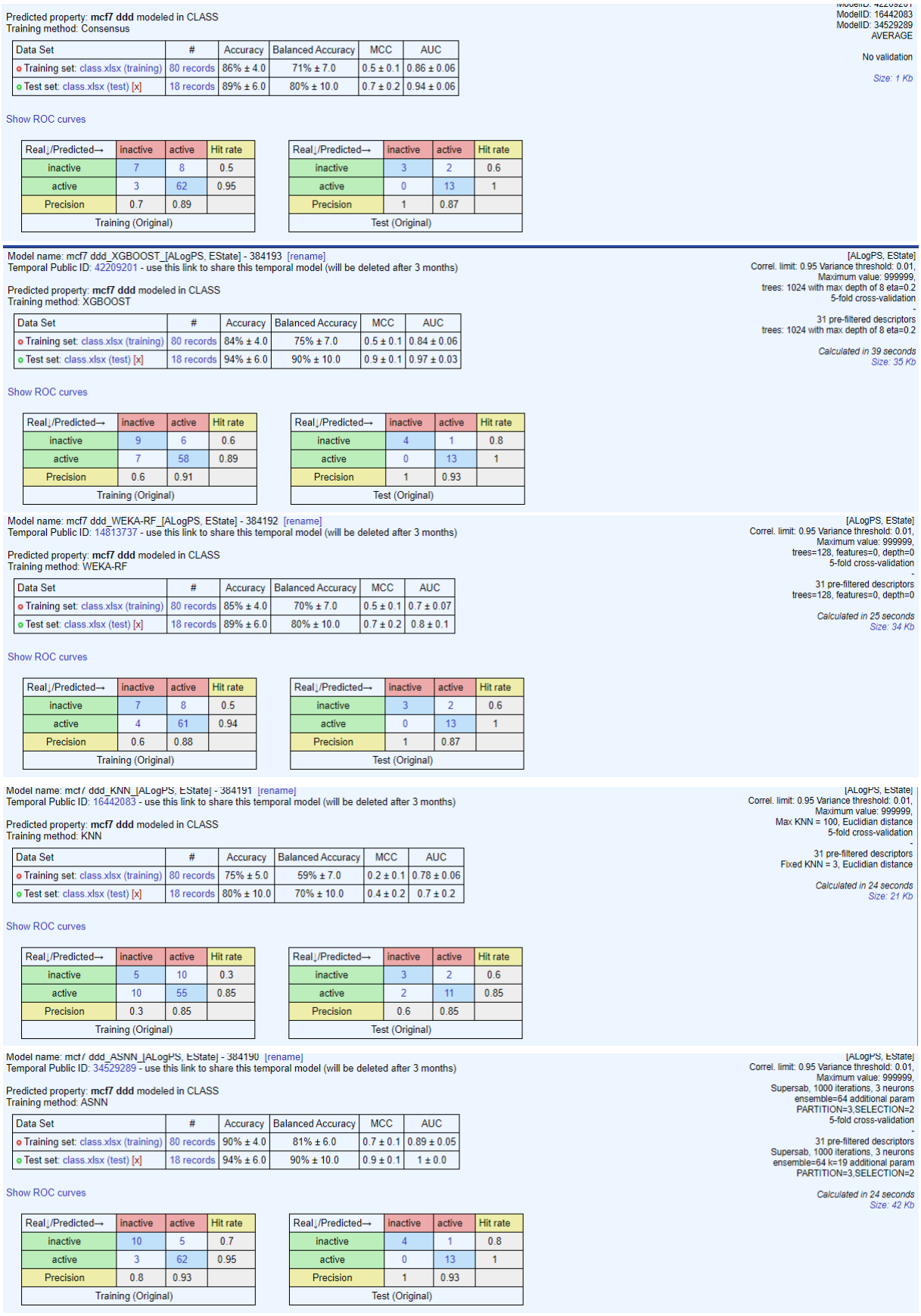


Fig. S1. Application of machine learning method



Data Set	#	Accuracy	Balanced Accuracy	MCC	AUC
Training set: class.xlsx (training)	80 records	86% ± 4.0	71% ± 7.0	0.5 ± 0.1	0.86 ± 0.06
Test set: class.xlsx (test) [x]	18 records	89% ± 6.0	80% ± 10.0	0.7 ± 0.2	0.94 ± 0.06

Real↓/Predicted→	inactive	active	Hit rate
inactive	7	8	0.5
active	3	62	0.95
Precision	0.7	0.89	

Training (Original)

Real↓/Predicted→	inactive	active	Hit rate
inactive	3	2	0.6
active	0	13	1
Precision	1	0.87	

Test (Original)

Data Set	#	Accuracy	Balanced Accuracy	MCC	AUC
Training set: class.xlsx (training)	80 records	84% ± 4.0	75% ± 7.0	0.5 ± 0.1	0.84 ± 0.06
Test set: class.xlsx (test) [x]	18 records	94% ± 6.0	90% ± 10.0	0.9 ± 0.1	0.97 ± 0.03

Real↓/Predicted→	inactive	active	Hit rate
inactive	9	6	0.6
active	7	58	0.89
Precision	0.6	0.91	

Training (Original)

Real↓/Predicted→	inactive	active	Hit rate
inactive	4	1	0.8
active	0	13	1
Precision	1	0.93	

Test (Original)

Data Set	#	Accuracy	Balanced Accuracy	MCC	AUC
Training set: class.xlsx (training)	80 records	85% ± 4.0	70% ± 7.0	0.5 ± 0.1	0.7 ± 0.07
Test set: class.xlsx (test) [x]	18 records	89% ± 6.0	80% ± 10.0	0.7 ± 0.2	0.8 ± 0.1

Real↓/Predicted→	inactive	active	Hit rate
inactive	7	8	0.5
active	4	61	0.94
Precision	0.6	0.88	

Training (Original)

Real↓/Predicted→	inactive	active	Hit rate
inactive	3	2	0.6
active	0	13	1
Precision	1	0.87	

Test (Original)

Data Set	#	Accuracy	Balanced Accuracy	MCC	AUC
Training set: class.xlsx (training)	80 records	75% ± 5.0	59% ± 7.0	0.2 ± 0.1	0.78 ± 0.06
Test set: class.xlsx (test) [x]	18 records	80% ± 10.0	70% ± 10.0	0.4 ± 0.2	0.7 ± 0.2

Real↓/Predicted→	inactive	active	Hit rate
inactive	5	10	0.3
active	10	55	0.85
Precision	0.3	0.85	

Training (Original)

Real↓/Predicted→	inactive	active	Hit rate
inactive	3	2	0.6
active	2	11	0.85
Precision	0.6	0.85	

Test (Original)

Data Set	#	Accuracy	Balanced Accuracy	MCC	AUC
Training set: class.xlsx (training)	80 records	90% ± 4.0	81% ± 6.0	0.7 ± 0.1	0.89 ± 0.05
Test set: class.xlsx (test) [x]	18 records	94% ± 6.0	90% ± 10.0	0.9 ± 0.1	1 ± 0.0

Real↓/Predicted→	inactive	active	Hit rate
inactive	10	5	0.7
active	3	62	0.95
Precision	0.8	0.93	

Training (Original)

Real↓/Predicted→	inactive	active	Hit rate
inactive	4	1	0.8
active	0	13	1
Precision	1	0.93	

Test (Original)

Fig. S2. Classification method results

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Research Article

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Development and pilot testing of remote active learning tool as antimicrobial stewardship co-interventions

I Wayan SURANADİ[1,*], I Gusti Agung Gede Utara HARTAWAN[1], Antonius Budi SANTOSO[1], Ni Nengah Dwi FATMAWATİ[2]

[1]Department of Anesthesiology and Intensive Care, Faculty of Medicine, Udayana University/Sanglah General Hospital, Denpasar, Bali, Indonesia
[2]Department of Clinical Microbiology, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia

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Abstract

An online education tool is known for its cost-effectivity as an antimicrobial stewardship intervention, yet only a limited number of studies have been done in developing country settings. This pilot was therefore conducted to assess the feasibility, acceptability, and effect of the prescriber-directed distance learning model and analyze feedback for future intervention. The research procedure was outlined in the preparation, conduct, and evaluation phases under antimicrobial resistance control committee supervision. The preparatory phase included learning tool planning and finalization to be disseminated in the conduct phase. A pre- and post-test was used to assess the attendees' knowledge, attitude, and practice regarding AMS and to collect feedback on intervention components before and after the educational intervention was delivered. Test scores were compared using paired t-test, and feedback was analyzed as aggregates as barriers or facilitators of AMS intervention. A total of 203 subjects were included in the analysis, with a 99% recruitment rate and a 96.7% adherence rate. Proportions of subjects with adequate knowledge and attitudinal scores improved from initially 36.9% and 21.2% before intervention into 83.3% and 51.7%, respectively. The mean knowledge score in the pretest (6.1±1.2) increased significantly in the posttest (7.6±1.1) with a p-value less than 0.001. Knowledge of antimicrobial classification and prescription workflow were the main topics with the least number of correct answers. Facilitators identified in the feedback were access to workflow, guideline, resistance pattern, and course, while antimicrobial availability was regarded as a barrier to optimal AMS implementation. The findings suggest the feasibility and acceptability of the evaluated protocol. Protocol modification and expansion of study recruitment hold the potential to improve AMS intervention efficacy.

Keywords: antibiotic resistance, antimicrobial stewardship, distance education, multidisciplinary communication, pilot study, prescriber-directed

1. Introduction

Misuse and inappropriate antimicrobial prescription couples are the main drivers of antimicrobial resistance worldwide. The trend of this erroneous practice continues to increase at an alarming rate, yet the ideal practice standard establishment remains an arduous journey (1). Accumulating body of evidence revealed that higher mortality risk and healthcare costs, particularly in developing countries, were directly imposed by antimicrobial resistance (2). It has been demonstrated that the limited discovery of novel antimicrobial agents lags far behind resistance rate growth, leaving antimicrobial efficacy preservation as the most strategically viable option (3).

Antimicrobial stewardship (AMS) endorsed by the centers for disease control and prevention presents a multidimensional approach in combating antimicrobial resistance which factors in realistic hospital workflow and key stakeholders' involvement (4). Nation-wide adoption of the AMS was formulated through recently published national guideline by the Indonesian Ministry of Health (5). The actual implementation of AMS fundamentally relies on the small-scale units at the organizational level; hence, uniquely tailored AMS measures are imperative to achieve their effectiveness. Integral multidisciplinary participation is also a prerequisite to constituting an impactful AMS (6).

The effectiveness of AMS in reverting the consequences of antimicrobial resistance was well established, and the online platform was recognized for its cost-effectiveness. However, studies performed in developing countries are lacking and render the proposed successful models of AMS biased toward developed countries. Furthermore, behavior change was rarely assessed in the previous studies (7–9). Despite the recommendation against education as a sole AMS intervention, (10) pilot testing may reveal actual feasibility, and the effect of the isolated intervention, hence, provides constructive

*Correspondence: wayan.suranadi@unud.ac.id

feedback for future development as AMS co-intervention is in larger research. This study sought to develop and pilot test an online learning tool intended for future use as co-intervention paired with compatible AMS efforts. The pilot testing process was performed as a part of a continuing quality improvement project to promulgate the world antimicrobial awareness week campaigned by the World Health Organization (WHO).

2. Material and methods

2.1. General study design

A prospective quasi-experimental study with one-group pretest-posttest design was performed. The design was opted/chosen in this pilot study instead of a randomized controlled study to test for field testing purpose and optimize uptake and feasibility. Primary outcomes of interest include feasibility and acceptability. Secondary outcomes include parameters constituting effect size and barriers and facilitators for actual AMS intervention. The tested tool would undergo further improvement under local antimicrobial resistance control committee oversight to develop an openly accessible tool to help strengthen AMS practice in supervised regional primary and secondary healthcare facilities. Research protocol complied with CONSORT 2010 statement extension for pilot and feasibility trials (11) and was approved by the research committee of Universitas Udayana/Sanglah General Hospital (approval number 63/UN14.2.2.VII.14/LT/2021), and individual consent was obtained from all study participants.

2.2. Study setting

The intervention occurred in a tertiary hospital in Indonesia with the capacity of 710 beds which translates to 259,150 bed-days, targeting active antimicrobial prescribers throughout the study period of December 2020 to January 2021. The antimicrobial resistance control committee consists of experts in infectious disease and related subspecialties from various departments (intensivist, clinical microbiology, internal medicine, pediatrics, and surgery).

2.3. Study population and sample

Study participants included attending physicians responsible for antimicrobial prescription who fulfilled the following sets of eligibility criteria. Inclusion criteria include on-duty healthcare worker and direct participation in antimicrobial prescription during the study period. Decline to participate and failure to finish the posttest were assigned as exclusion and drop-out criteria, respectively. The minimum sample size required was gauged from the paired t-test sample size formula with a minimal clinically important difference set as 30%. The sampling frame was extracted from the hospital employee list as a sampling frame while taking different departments and a drop-out rate of 10% into consideration to produce a total of 151 samples.

2.4. Tool development and intervention

Learning tool

Education materials were chosen by the antimicrobial resistance control committee members based on their respective expertise to cover the leading issues regarding antimicrobial prescription in the light of international, national, and local guideline. The framework of which followed a realistic workflow in prescribing practice encompassing prophylactic, therapeutic, and prudent implementation of antimicrobial use. Overall outline and duration for each material components were as follows: prophylactic antimicrobial administration principles (15 minutes), fundamentals of therapeutic antimicrobial use (15 min), prudent antimicrobial use implementation (15 min), practical insights from a microbiological perspective (15 min), and problem-based discussion (1 h). Methods of delivery include didactic teaching, clinical case discussion, and guideline promotion. The combined materials of approximately 2 h in total duration were then disseminated in a pre-recorded webinar format for time-limited asynchronous online learning.

Survey

A questionnaire with suitable content was constructed to assess knowledge, attitude, and practice and was divided into dedicated sections thereof. The knowledge section incorporated ten multiple-choice questions with one correct answer and four plausible distractors provided for each. One point was assigned for each correctly answered question and zero point otherwise, yielding a maximum score of ten. The attitude and practice sections featured 5-point Likert-type items (1 = strongly disagree, 2 = disagree, 3 = undecided, 4 = agree, 5 = strongly agree) in response to 5 statements, yielding a maximum score of 25 for each section. Higher scores reflected good practice in all sections of the survey. Informed consent and participant characteristics obtention were mandatory before access to the pretest questionnaire was granted. Additional questions on supporting and inhibiting factors for AMS implementation with free-text responses were added to the posttest questionnaire.

Procedure

The research protocol was made up of three phases, namely preparation, conduct, and evaluation. In the initial preparatory phase, the original education tool and questionnaire draft were assembled and piloted to 37 subjects with equivalent authority and qualification recruited from external institution. The objective of the preliminary pilot was to ensure adequate internal consistency of the questionnaire through content revision, and none of the results were included in the current analysis. The finalized questionnaire was created using an online form readily accessible in multiple platforms for user convenience. In the following phase, participants were invited to take a pretest and given a seven-day period to use the tool before the posttest form was accessible. Unique credentials for each participant were generated from individual initials to access the webinar and posttest online forms. The pilot data was then presented to antimicrobial resistance control committee members for evaluation and feedback.

2.5. Study outcomes and statistical analysis

Primary outcomes

Feasibility was assessed by the enrollment percentage of eligible subjects, while acceptability was represented by the percentage of subjects who finished the posttest.

Secondary outcomes

Univariate analysis was used to tabulate the frequency of baseline subject characteristics and survey results. Scores were deemed adequate with the lowest thresholds of 7 for knowledge section and 20 for attitude and practice sections. The effect size of the intervention was analyzed quantitatively using a paired t-test for pre- and posttest knowledge, attitude, and practice scores. Barriers and facilitators to the intervention were analyzed qualitatively as aggregates.

3. Results

A total of 212 of 251 subjects (84.5%) retrieved in the sampling frame fulfilled the inclusion criteria. Two subjects were excluded and another 7 subjects dropped out, resulting in 210/212 (99%) recruitment rate and 203/210 (96.7%) adherence. The subjects had a mean age of 48.1±9.3 years and were mostly (69%) males (Table 1). Almost one-fourth of whom represented the department of surgery with over ten years of working experience in nearly two-third of the subjects. Less than half of the subjects participated in previous learning course and completed it in 1–5 years prior.

The proportion of subjects with adequate pretest (36.9%) and posttest (83.3%) knowledge and adequate pretest (21.2%) and posttest (51.7%) attitude increased after intervention. The reverse was true for adequate pretest (88.7%) and posttest (82.3%) practice score. Mean knowledge, attitude, and practice scores in pretest were 6.1±1.2 (range 3–9), 17.8±2.7 (range 13–25), and 21.9±2.5 (range 6–25), respectively. Posttest scores for aforementioned section in consecutive order were 7.6±1.1 (range 5–10), 19.2±2.7 (range 12–25), and 22.1±2.7 (range 13–25). Score improvements for knowledge, attitude, and practice section were 1.6, 1.3, and 0.2 in decreasing order. The improvement was statistically significant in knowledge section ($p < 0.001$) and insignificant in attitude ($p = 0.898$) and practice ($p = 0.194$) sections.

Table 1. Baseline characteristics

	N	%
Gender		
Male	140	69.0
Female	63	31.0
Department		
Surgery	47	23.1
Pediatrics	31	15.3
Internal medicine	25	12.3
Obstetrics and gynecology	20	9.9
Neurology	16	7.9
Ophthalmology	14	6.9
Cardiology and vascular medicine	13	6.4
Otorhinolaryngology head and neck surgery	13	6.4
Dermatology and venereology	10	4.9
Anesthesiology and intensive care	8	3.9
Pulmonology	6	3.0
Working experience		
<5 years	28	13.8
5–10 years	55	27.1
>10 years	120	59.1
Previous antimicrobial stewardship learning course		
Yes	99	48.8
No	104	51.2
Time elapsed since course completion		
<1 year	9	9.1
1–5 years	46	46.5
>5 years	44	44.4

The majority of subjects answered correctly to Q1 in pretest and Q1 and Q10 in posttest. Q6 had the lowest proportion of correct response after intervention. The highest knowledge score improvement was observed in Q8, leveraging its correct responses proportion which was initially the lowest in pretest (Table 2). Q11, on the other hand, had the highest proportion of maximum attitudinal score at both the pre- and post-test. The lowest proportion of maximum attitudinal score was observed for Q13 in pretest and Q12 in posttest. The only increment in the proportion of the maximum attitudinal score was at Q13 (Table 3). The proportion of the maximum practical score for Q17 and Q18 remained the lowest and highest after intervention occurred. Improvement in the proportion of maximum practical score was evident solely for Q16 (Table 4).

Table 2. Responses on AMS knowledge survey

Topics	N (%) correct		Difference
	Pretest	Posttest	
1. Antimicrobial mechanism of action	92.6	94.6	2.0
2. Appropriate prophylactic antimicrobial administration practice	42.9	62.6	19.7
3. Prophylactic antimicrobial prescribing principles	69.9	91.6	21.7
4. Principles of prudent antimicrobial use	85.7	87.2	1.5
5. AWaRe antimicrobial classification	62.1	77.8	15.7
6. Antimicrobials under reserve classification of AWaRe	20.2	41.4	21.2
7. Fundamental terms relating to antimicrobial resistance	89.2	92.6	3.4
8. Antimicrobial prescribing practice workflow	4.4	69.5	65.1
9. Clinical decision following culture and sensitivity test results	49.7	50.7	1.0
10. The role of attending physicians in AMS implementation	90.1	94.6	4.5

Table 3. Responses on AMS attitude survey

Statements	Test	SDA (%)	DA (%)	U (%)	A (%)	SA (%)
11. The importance of pharmacology and microbiology comprehension in rational antimicrobial prescribing	Pre	0.5	0	0	4.9	94.6
	Post	0	0	0	6.4	93.6
12. Resistance pattern is not considered when choosing antimicrobial agent to treat severe infection	Pre	51.2	22.2	6.9	7.4	12.3
	Post	52.2	18.7	7.4	10.8	10.8
13. Antimicrobial resistance control committee involvement in prescribing antimicrobial agents within "access" category is optional	Pre	42.4	22.7	12.8	10.3	11.8
	Post	12.8	18.7	14.3	23.6	30.5
14. The rate of new antimicrobial agents' discovery is disproportionate to the development of resistance	Pre	3.0	4.4	5.9	25.1	61.6
	Post	3.0	2.0	6.4	20.2	68.5
15. Prevalent third-generation cephalosporins use leads to increased risk of *C. difficile* colitis	Pre	1.5	3.0	12.8	39.4	43.3
	Post	0.5	3.9	11.8	36.9	46.8

Table 4. Responses on AMS practice survey

Statements	Test	SDA (%)	DA (%)	U (%)	A (%)	SA (%)
16. Single-dose antimicrobial prophylaxis in 100 ml normal saline is administered intravenously for 15–30 min starting 30–60 min before surgical incision	Pre	3.9	2.0	8.4	27.6	58.1
	Post	1.0	0.5	7.4	30.5	60.6
17. Ceftriaxone has dose-dependent characteristic and is administered intravenously at 24 h interval	Pre	10.3	10.8	9.9	25.6	43.3
	Post	10.8	10.8	8.9	26.1	43.3
18. Culture specimen should be obtained before antimicrobial administration in sepsis management	Pre	0.5	0.5	3.0	9.8	86.2
	Post	0	1.5	4.9	12.8	80.8
19. Coordination with the antimicrobial resistance control committee is warranted in the absence of improvement despite sensitivity test guided antimicrobial treatment	Pre	1.5	0	2.5	16.3	79.8
	Post	1.0	1.0	2.0	23.6	72.4
20. Local guideline serves as a reference in hospital antimicrobial administration practice	Pre	7.4	3.0	11.3	19.2	59.1
	Post	2.5	3.0	7.9	27.6	59.1

Barriers and facilitators for AMS implementation were recapitulated in five main themes (Table 5). Access to workflow, guideline, resistance pattern, and course were regarded as facilitators, while antimicrobial availability was regarded as a barrier to optimal AMS implementation. Only 5.4% of subjects did not provide any remark on antimicrobial availability, as opposed to nearly half of all subjects that of workflow access.

Table 5. Barriers and facilitators to AMS implementation

Themes	Adequate (%)	Inadequate (%)	Abstain (%)
Availability of antimicrobials listed in local guideline	36.0	58.6	5.4
Access to antimicrobial administration workflow	37.4	13.3	49.3
Access to local guideline	66.0	22.2	11.8
Access to local resistance pattern	50.7	38.4	10.8
Learning course	51.7	13.3	35.0

4. Discussion

The current feasibility study attempted to guide AMS efforts reinvention, especially focusing on educational intervention for its excellent versatility. Development and dissemination of the learning tool encompassed education, persuasion, and enablement functions of behavior change interventions (7). The innovative distance learning model was matched with field testing methods to ensure real assessment of resources and management in addition to procedural and scientific evaluation. Single intervention delivery followed by feedback analysis allowed to generate future directions to modify educational cointervention and plan its complementary intervention. To the best of our knowledge, this study was the first to investigate prescriber-directed distance learning intervention in lower-middle-income countries such as Indonesia. High recruitment and adherence rates with minor refusal and drop-out rates confirmed the feasibility and acceptability of the study protocol, while a postintervention survey validated the effectiveness of learning tools in improving knowledge.

Based on the knowledge survey results, more emphasis should be given to some aspects of the knowledge section particularly on the WHO Access, Watch, and Reserve (AWaRe) classification and antimicrobial prescribing practice workflow. The two topics are interrelated in the sense that decision-making in antimicrobial dispensing is closely regulated within the workflow according to the AWaRe classification system. These are the cornerstone of daily clinical practice for prescribers as primary decision-maker in healthcare facilities, thus suggesting the propensity for "gatekeeper" intervention to be a direct problem solver. Similarly, the transfiguration of attitude and practice would benefit from intervention with a restriction and/or enablement function. In the current hospital-wide pilot study, for instance, AMS efforts would benefit from online preauthorization prescribing practice and adequate AWaRe classification-compliant pharmacy supply.

AMS practice postulates good governance, monitoring and feedback, support, and research as educational accompaniment (12). Clearer descriptions and stakeholder roles in AMS regulation and practice policy should be instilled by the governance counterpart in AMS implementation. Ongoing monitoring and support by multidisciplinary healthcare

personnel partaking in antimicrobial dispensing encourage AMS implementation sustenance. An ideal future large-scale intervention and education cointervention should set out population-wide thorough knowledge survey and monitoring and feedback analysis ahead of definitive intervention formulation on the basis of best available evidence. The current initiative calls for forthcoming AMS advancement and implementation outreach.

There were some limitations to this study that may or may not directly affect the study conclusion. The lack of randomization was the primary concern when opting for field testing strategy. Nonetheless, the high uptake and retention rate demonstrated its importance in the referring conclusion. Nature characteristics of this intervention rendered the Hawthorne effect as inevitable. Other limitations may include regression to the mean and unmeasured covariates such as assessment duration.

The pilot study of this quality improvement model was critical in ensuring a feasible protocol and outlining its potential pitfalls for actual implementation. Current protocol and tool were appropriate for further module modification and paired with restricting and/or enabling intervention. Future work with more extensive involvement is required in reshaping the future of AMS intervention models in developing areas.

Conflict of interest

The author reports no conflicts of interest in this work.

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None to declare.

Authors' contributions

Concept: I.W.S., I.G.A.G.U.H., A.B.S., N.N.D., Design: I.W.S., A.B.S., Data Collection or Processing: I.W.S., I.G.A.G.U.H., A.B.S., N.N.D., Analysis or Interpretation: I.W.S., I.G.A.G.U.H., A.B.S., N.N.D., Literature Search: A.B.S., Writing: I.W.S., A.B.S.

References

1. Dadgostar P. Antimicrobial Resistance: Implications and Costs. Infect Drug Resist. 2019;12:3903.
2. Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PLoS One. 2017;12(12).
3. Romo AL, Quirós R. Appropriate use of antibiotics: an unmet need. Ther Adv Urol. 2019;11:175628721983217.
4. Centers for Disease Control and Prevention. The core elements of hospital antibiotic stewardship programs: Antibiotic stewardship program assessment tool [Internet]. 2019 [updated 2021 Apr 28; cited 2022 Jul 10]. Available from: https://www.cdc.gov/antibiotic-use/healthcare/pdfs/assessment-tool-P.pdf
5. Ministry of Health of the Republic of Indonesia. In-hospital antimicrobial stewardship guidance. Jakarta; 2020.
6. Pereira NR, Castro-Sanchez E, Nathwani D. How can Multi-Professional Education Support Better Stewardship? Infect Dis Rep. 2017;9(1):1–7.
7. Davey P, Marwick CA, Scott CL, Charani E, McNeil K, Brown E, et al. Interventions to improve antibiotic prescribing practices for hospital inpatients. Cochrane Database Syst Rev. 2017;2017(2).
8. Nathwani D, Varghese D, Stephens J, Ansari W, Martin S, Charbonneau C. Value of hospital antimicrobial stewardship programs [ASPs]: a systematic review. Antimicrob Resist Infect Control. 2019;8(1).
9. Kyaw BM, Car LT, Galen LS van, Agtmael MA van, Costelloe CE, Ajuebor O, et al. Health Professions Digital Education on Antibiotic Management: Systematic Review and Meta-Analysis by the Digital Health Education Collaboration. J Med Internet Res. 2019;21(9).
10. Satterfield J, Miesner AR, Percival KM. The role of education in antimicrobial stewardship. J Hosp Infect. 2020;105(2):130–41.
11. Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016;355.
12. Hawes L, Buising K, Mazza D. Antimicrobial Stewardship in General Practice: A Scoping Review of the Component Parts. Antibiotics. 2020;9(8):1–13.

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Is Hashimoto Thyroiditis a risk factor in male infertility?

Murat ÖNAL[1], Mehmet AĞAR[2,*], Aynur ADEVİYE ERŞAHİN[3], Kağan GÜNGÖR[4]

[1]Gynolife IVF Clinic, Lefkoşa, Northern Cyprus
[2]Private Office, Şanlıurfa, Türkiye
[3]Department of Obstetrics and Gynecology, Bahçeşehir University, Göztepe Medikal Park Hospital, İstanbul, Türkiye
[4]Department of Endocrinology, Prof. Dr. Süleyman Yalçın Şehir Hastanesi, İstanbul, Türkiye

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Abstract

Hashimoto's thyroiditis is known as thyroid dysfunction and is one of the most common endocrine diseases worldwide. This study seeks to investigate thyroid dysfunction in the male reproductive system. Normal thyroid function is crucial for male reproduction. Semen quality parameters are among the most critical factors affecting male fertility. The main aim of this research was to investigate the effect of thyroid dysfunction on semen quality parameters such as sperm motility, number, and morphology. This cross-sectional retrospective case-control study was conducted between December 2021 and May 2022 in Bahcesehir University Göztepe Medicalpark Hospital Invitro Fertilization center. Patients with Hashimoto's thyroiditis (n=52) were included in the study as the case group. The control group was selected from age and body mass index (BMI)-matched patients who underwent semen analysis (n=57). The participants' age and BMI were 30.90±4.45 and 24.49±1.46, respectively. When we evaluated semen analysis results, we found the number of sperms ($p<0.001$), motility ($p<0.01$), and morphology ($p<0.001$) were significantly better in the controls than in the patients with Hashimoto's thyroiditis. Oligozoospermia, asthenozoospermia, and oligo-asthenozoospermia as sperm abnormalities were observed in patients with Hashimoto's thyroiditis. Hashimoto's thyroiditis has a negative effect on semen quality parameters such as sperm motility, number, and morphology. Evaluation of infertile men is recommended in terms of thyroid dysfunction.

Keywords: male infertility, Hashimoto's thyroiditis, semen quality, sperm abnormality

1. Introduction

Male infertility affects at least 7% of men worldwide as a common condition (1). Male infertility responds poorly to the initial treatments and is mostly cured with secondary treatments (2). About 30 to 50% of male infertility cases are known as idiopathic due to low-quality of spermatozoa (3). The main causes of male infertility in the world are defective spermatozoa due to reduced sperm counts, abnormal structure/morphology, and poor motility which have been reported in several publications (4, 5).There has been a global decline in the semen quality in the past few decades (6).

The thyroid gland is the most important gland affecting the body's metabolism and actions. Although thyroid dysfunction is more common in women than men, these disorders also occur in men, affecting their physical and sexual health (7). The most significant adverse effects include a decrease in the number and motility of sperm, which reduces male fertility. Male sexual function is affected by thyroid function, and men with thyroid disorders experience varying degrees of sexual disorders, including delayed ejaculation, erectile dysfunction, premature ejaculation, and reduced sexual desire (8, 9).

Hashimoto's thyroiditis as thyroid dysfunction is one of the prevalent diseases worldwide (8). Hashimoto thyroiditis is characterized by tertiary lymphoid follicles development, chronic inflammation, and higher concentrations of circulating autoantibodies against thyroglobulin (anti-TG) and thyroid peroxidase (anti-TPO) as an organ-specific autoimmune disease (9). Measuring serum anti-TG and anti-TPO levels enables the diagnosis of chronic autoimmune thyroiditis. Since anti-TG will be found to be high in almost all patients with autoimmune thyroid disease and anti-TPO positivity, this antibody has no significant effect on the diagnosis. The frequency of autoimmune thyroid disease with low anti-TPO and high anti-TG levels is around 5%. Anti-TPO and anti-TG are positive at a rate of 95-100% in Hashimoto's thyroiditis (10).

Although male infertility has been increasing and difficult

*Correspondence: drmehmetagar@yahoo.com

to treat, limited studies on risk factors and the main causes of male infertility (11). Considering the sustainable effects of thyroid dysfunction on male fertility and sexual function, timely screening and treatment of thyroid diseases in men with sperm abnormalities and erectile dysfunction should be considered to keep and improve men's health. The present study was conducted to study the impact of Hashimoto's thyroiditis on men's sexual function and fertility.

2. Materials and Methods

The Ethics Committee of Medeniyet University approved this cross-sectional case-control study (Decision no: 2021/0009 Date: 27.01.2021). One hundred nineteen men aged between 25 and 40 were included in this study during December 2021 - May 2022. Out of this group, 57 men were included in the control group, and 52 men with Hashimoto's thyroiditis were included in the case group. A man with fewer than 15 million sperm per ml of semen was accepted as oligozoospermia. Asthenospermia is defined as a less than 40% sperm motility or less than 32% progressive motility (12).

ELISA (BioVendor, Heidelberg, Germany) was used to measure serum Anti-TG, Anti-TPO, fT4, and TSH concentrations. DIAPLUS kit (Toronto, Canada) protocol was followed to perform this hormone assay. The reference ranges of T4 and TSH were 4.4-10.8 µg/dl and 0.39-5.95 µg/dl, respectively. The anti-TG measurement range is 10–4000 IU/mL, and the anti-TPO measurement range is 5–600 IU/mL. Anti-TPO< 35 IU/mL, and anti-Tg< 115 IU/mL were accepted as negative.

Semen samples were collected through masturbation after a 3-4-day sexual abstinence based on the WHO guidelines of 2010 (12). Complete semen analyses were done after liquefaction for 15 to 30 min at room temperature. Once liquefaction was performed, motility of each sample was evaluated at room temperature and its heated microscope stage was standardized for our laboratory.

2.1. Statistical analysis

The normality was checked based on the Kolmogorov-Smirnov test, and the nonparametric tests were conducted considering the non-normality of the groups before the statistical analyses. Mean and standard deviations (SD) measured to check each continuous variable, including age, BMI, FSH, LH, FT4, TSH, Testosterone, Prolactin (ng/ml), volume, number (Millions), motility (%), and morphology. The Mann-Whitney U test performed to study the difference between the two groups. SPSS v22 used for statistical analyses. $p < 0.05$ was regarded as statistically significant.

To calculate the sample size with the GPower 3.1 program, two groups' total mean was measured based on the Mann-Whitney test with the power of 95%, effect size of 50%, and 0.05 type 1 error for at least 92 patients (13).

3. Results

This study included one hundred nine age-matched (30.90 ± 4.45) and BMI-matched (24.49±1.46) men. Table 1 shows descriptive statistics of study parameters.

Table 1. Descriptive statistics of study parameters in the all group (n=119)

Study parameters	median (range)	mean ± SD
Age	32 (25-39)	30.90 ± 4.45
BMI	24.6 (21-29.21)	24.49 ± 1.46
FSH	6 (3.44-9)	6.09 ± 1.11
LH	5 (3.28-8)	5.34 ± 1.08
FT4	1 (0.9-1.41)	1.08 ± 0.11
TSH	2 (1.5-3.2)	2.24 ± 0.39
Testosterone	4.45 (2.44-7.28)	4.49 ± 0.73
Prolactin (ng/ml)	14.8(4.23-19.67)	14.96 ± 2.82
Anti-TPO	11 (3-65)	29.22 ± 24.55
Anti-TG	2.1 (1.5-5)	21.47 ± 25.21
Volume	3 (1.5-5)	3.44 ± 0.74
Number (10^6/ml)	32 (12-95)	35.63 ± 17.31
Motility (%)	56 (19-81)	54.11 ± 13.21
Morphology	5 (2-10)	4.43 ± 1.73

SD, standard deviation

Table 2 shows comparison of case and control groups on the study parameters.

Table 2. Comparison of case and control groups

Study parameters	Case (n=52) M±SD	Control (n=57) M±SD	*p*
Age	30.84±4.27	30.96±4.65	0.907
BMI	24.51±1.49	24.47±1.45	0.442
FSH	6.60±1.13	5.99±0.88	0.058
LH	5.43±1.30	5.27±0.83	0.822
FT4	1.06±0.10	1.09±0.12	0.303
TSH	2.22±0.34	2.26±0.43	0.981
Testosterone	4.48±0.67	4.50±0.78	0.400
Prolactin (ng/ml)	14.98±2.42	14.95±3.16	0.496
Anti-TPO	54.65±3.21	6.03±2.32	**<0.001**
Anti-TG	43.46±1.41	20.10±0.31	**<0.001**
Volume	3.46±0.65	3.42±0.82	0.987
Number (Millions)	28.38±15.71	42.24±16.13	**<0.001**
Motility (%)	46.46±13.26	61.10±8.51	**<0.001**
Morphology	3.34±1.54	5.42±1.23	**<0.001**

M: Mean; N: Number of subjects; BMI: Body mass index; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; FT4: Free T4; TSH: Thyroid-stimulating hormone; T4: Thyroxine; *All variables tested by a Mann-Whitney U test

As stated in Table 2, a Mann-Whitney test did not find a statistically significant association between case and control in regard to age and BMI (*p*-value>0.05).

There was not a statistically significant difference between case group and controls in regard to FSH(*p*-value=0.058), LH (*p*-value=0.822), FT4 (*p*-value=0.303), TSH (*p*-value=0.981), Total Testosterone (P-value=0.400), Prolactin (*p*-value=0.496) and Volume (*p*-value=0.987). Case group and controls showed a statistically significant difference in terms of number of sperm (*p*-value<0.001). The case group had significantly lower count than the controls (M=28.38; SD=15.71 vs. M=42.24; SD=16.13).

Case group and controls showed a statistically significant difference between in regard to Anti-TPO and Anti-TG (*p*-value<0.001).

The group with thyroid dysfunction (M=46.46; SD=13.26) and the healthy group (M=61.10; SD=8.51) showed a significant difference in terms of Motility (p-value <0.001). There was a statistically significant difference between case group and controls in terms of morphology (p-value<0.001). The case group had significantly lower morphology score than the controls (M=3.34; SD=1.54 vs. M=5.42; SD=1.23).

Table 3. Comparison of various sperm abnormalities in case and control groups

Sperm Abnormality	Normal (n=87)	Oligozoospermia (n=8)	Asthenozoospermia (n=14)	Oligo-asthenozoospermia (n=4)
Case	30 (34.5%)	8 (100%)	14 (100%)	4 (100%)
Control	57 (65.5%)	0 (0%)	0 (0%)	0 (0%)

Oligozoospermia was observed in 7.3% (n=8) of our patients (all in thyroid dysfunction group). Also, asthenozoospermia was seen in 12.8% (n=14) of our participants (all in thyroid dysfunction group). Four patients (3.6%) in this study suffered from two (Oligozoospermia and asthenozoospermia). Fig. 1 clearly shows that sperm abnormalities were more common in patients with thyroid dysfunction.

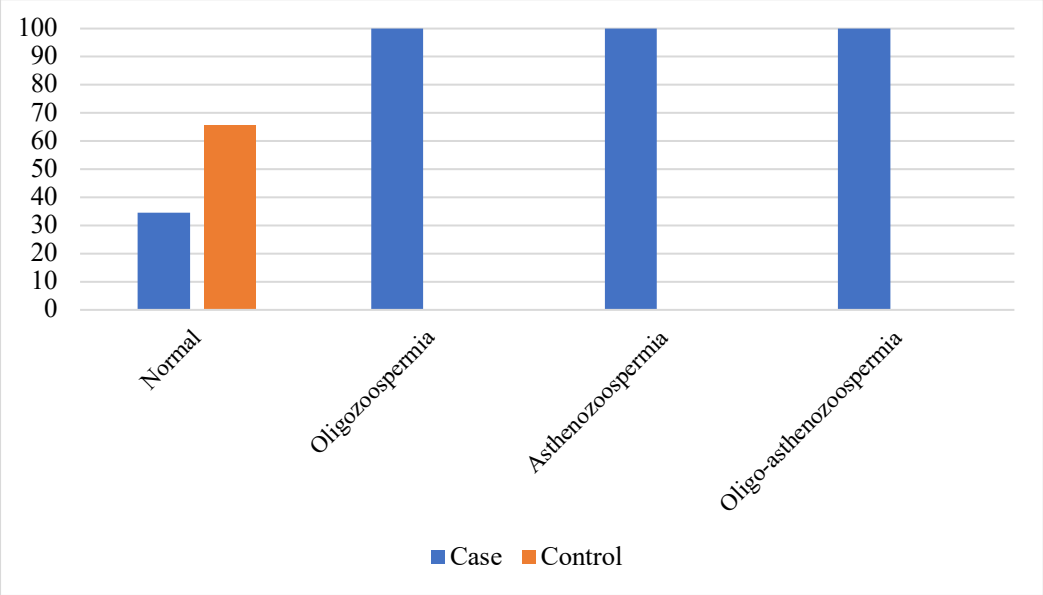

Fig. 1. Frequency of sperm abnormalities in case and control groups

4. Discussion

In this study, the effect of Hashimoto's thyroiditis on semen quality was analyzed. Based on the obtained results, Hashimoto's thyroiditis have significant effects on sperm motility, sperm count, and sperm morphology. These parameters in Hashimoto patients had much lower values than ordinary individuals in the control group. Oligozoospermia, asthenozoospermia, and oligo-asthenozoospermia were observed in the case group. In this regard, Hashimoto's thyroiditis significantly affect the men's reproductive system, and this effect includes reduced sperm motility, reduced sperm count, and a negative impact on morphology.

According to WHO, the quality of semen in men is decreasing (6, 14). Several studies have been conducted in recent years on the factors affecting the quality of semen (15-17). These studies are important because they can provide effective treatment by identifying the factors that reduce the quality. Semen quality is affected by nutritional, socioeconomic and environmental factors, such as rurality (18), phthalate levels (19), air pollution, harmful chemicals, and excessive heat (20). In addition to these factors, autoimmune diseases also affect the quality of semen (21). In the current study, thyroid dysfunction was also identified as a factor in reducing the quality of semen. Abalovich et al.(22) found a significant relationship between thyroid dysfunction and semen quality. Among the semen quality parameters, Hyperthyroidism had the most negative effect on the motility parameter. Krassas et al. (23) reported adverse effects of thyroid dysfunction on sperm morphology and sperm motility. Li et al. (24) reported the negative effect of Triiodothyronine levels on semen measures. Krassas et al. (25) recognized thyroid dysfunction as the cause of reduced sperm motility. These results were in line with studies by, Sengupta (26) and Clyde (27). Kidd et al.(28) reported reduced sperm counts in hyperthyroid men. Lisovskaya et al. (29) observed more thyroid dysfunction in men with sperm abnormalities. Niroomand et al. (30) studied 28 patients with Hyperthyroidism and Hypothyroidism regarding sperm abnormalities. Normozoospermia was seen in 68.75%, pathozoospermia was observed in 32.14%, and asthenospermia in 17.85%.

This study has several limitations. One of the important

limitations is that this is a retrospective design. Another limitation is that the sample size, which is relatively small due to financial constraints. Semen quality is affected by abstinence time for semen analysis, smoking habits, season, and time of sample collection. It is suggested that the samples of semen be collected considering the above-mentioned cases. Therefore, more samples should be studied to show the effect of thyroid disorders on the men's reproductive system.

As a result, Hashimoto's thyroiditis negatively affects the semen quality. Sperm motility and sperm count are lower in men with thyroid dysfunction. Sperm morphology is also negatively affected by Hashimoto's thyroiditis. Evaluation of thyroid antibodies to rule out Hashimoto's thyroiditis, is recommended in all infertile men.

Conflict of interest

The authors have no conflict of interest.

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None to declare.

Authors' contributions

Concept: M.Ö., M.A., Design: M.A., A.A.E., K.G., Data Collection or Processing: A.A.E., K.G., Analysis or Interpretation: M.Ö., M.A., Literature Search: M.Ö., M.A., A.A.E., KG., Writing: M.A., M.Ö.

References

1. Houston BJ, Riera-Escamilla A, Wyrwoll MJ, Salas-Huetos A, Xavier MJ, Nagirnaja L, et al. A systematic review of the validated monogenic causes of human male infertility: 2020 update and a discussion of emerging gene–disease relationships. Hum Reprod Update. 2022; 28(1): 15-29.
2. Yurci A, Gungor N. Impact of Male Aging on Semen Parameters. Gynecol Reprod Health.2021; 5 (2): 1-5.
3. Kups M, Gill K, Rosiak-Gill A, Harasny P, Machalowski T, Grabowska M, et al. Evaluation of selected semen parameters and biomarkers of male infertility–preliminary study. F1000Research. 2022; 11(591): 591.
4. Güngör K, Güngör N, Başar M, Cengiz F, Erşahin S, Çil K. Relationship between serum vitamin D levels semen parameters and sperm DNA damage in men with unexplained infertility. Eur Rev Med Pharmacol Sci. 2022; 26(2): 499-505.
5. Yurci A, Güngör ND. Use of frozen-thawed sperm for ICSI improves fertility outcome in men with azoospermia. JHSM. 2021; 4(3): 272-76.
6. Tocci A, Lucchini C. WHO reference values for human semen. Human Reprod Update. 2010; 16(5): 559.
7. Alahmar A, Dutta S, Sengupta P. Thyroid hormones in male reproduction and infertility. Asian Pac J Reprod. 2019; 8: 203-10.
8. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev. 2014; 13(4-5): 391-7.
9. Feghali K, Atallah J, Norman C. Manifestations of thyroid disease post COVID-19 illness: Report of Hashimoto thyroiditis, Graves' disease, and subacute thyroiditis. J Clin Transl Endocrinol Case Rep. 2021; 22:100094.
10. Gungor K, Dokuzeylul Gungor N. Antithyroid antibodies may predict serum beta HCG levels and biochemical pregnancy losses in euthyroid women with IVF single embryo transfer. Gynecol Endocrinol. 2021; 37(8): 702-5.
11. Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, et al. Male infertility. Lancet. 2021; 397(10271): 319-33.
12. WHO laboratory manual for the examination and processing of human semen, sixth edition. Geneva: World Health Organization; 2021.
13. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009; 41(4): 1149-60.
14. Sengupta P, Dutta S, Tusimin MB, Irez T, Krajewska-Kulak E. Sperm counts in Asian men: Reviewing the trend of past 50 years. Asian Pac J Reprod. 2018; 7: 87-92.
15. Candemir C. Estimating the semen quality from life style using fuzzy radial basis functions. Int J Mach Learn Comput. 2018; 8(1): 44-8.
16. Blay RM, Pinamang AD, Sagoe AE, Owusu EDA, Koney NK, Arko-Boham B. Influence of Lifestyle and Environmental Factors on Semen Quality in Ghanaian Men. Int J Reprod Med. 2020; 2020: 6908458.
17. Reddy NS, Vembu R. Thyroid Dysfunction and Infertility. J J Glob Obstet Gynecol. 2021; 1(1).
18. Rabinowitz M, Bowring M, Kohn T, Levy J, Herati A. Impact of Environmental and Socioeconomic Factors on Semen Quality in the United States. JSM. 2022; 19(4): S111-S2.
19. Jurewicz J, Hanke W, Radwan M, Bonde JP. Environmental factors and semen quality. Int J Occup Med Environ Health. 2009;22(4):305-29.
20. Kumar N, Singh AK. Impact of environmental factors on human semen quality and male fertility: a narrative review. Environ Sci Eur. 2022; 34(1): 1-13.
21. Kavrut M, Güngör ND, Yurci A, Güngör K, Çil K. Semen analysis results are negatively affected in patients with vitiligo: A cross-sectional study from Turkey. Zeynep Kamil Med J. 2022; 53(2): 68-74.
22. Abalovich M, Levalle O, Hermes R, Scaglia H, Aranda C, Zylbersztein C, et al. Hypothalamic-pituitary-testicular axis and seminal parameters in hyperthyroid males. Thyroid. 1999 Sep;9(9):857-63.
23. Krassas GE, Pontikides N, Deligianni V, Miras K. A prospective controlled study of the impact of hyperthyroidism on reproductive function in males. J Clin Endocrinol Metab. 2002 Aug;87(8):3667-71.
24. Li X, Gao H, Li P, Chen W, Tang S, Liu L, et al. Impaired sperm quantity and motility in adult rats following gestational and lactational exposure to environmentally relevant levels of PBDE-47: A potential role of thyroid hormones disruption. Environ Pollut. 2021 Jan 1;268(Pt A):115773.
25. Krassas GE, Pontikides N. Male reproductive function in relation with thyroid alterations. Best Pract Res Clin Endocrinol Metab. 2004 Jun;18(2):183-95.
26. Sengupta P, Dutta S. Thyroid Disorders and Semen Quality. Biomed Pharmacol J 2018;11(1).
27. Clyde HR, Walsh PC, English RW. Elevated plasma testosterone and gonadotropin levels in infertile males with hyperthyroidism. Fertil Steril. 1976 Jun;27(6):662-6.
28. Kidd GS, Glass AR, Vigersky RA. The hypothalamic-pituitary-

testicular axis in thyrotoxicosis. J Clin Endocrinol Metab. 1979 May;48(5):798-802.

29. Lisovskaya TV, Dubrovina OS, Treshchilov IM, Senturina LB, Sevostyanova OY, Mayasina EN, et al. Thyroid disorders and pathospermia in the ART clinic patients. Gynecol Endocrinol. 2021;37(sup1):4-7.

30. Niroomand H, Binaafar S, Nasiri M, Mansournia N, Bagheri Behzad A. Sperm abnormalities: Adverse effects of thyroid dysfunction. JBRMS. 2018; 5 (1) :47-50.

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Effect of irisin on the epilepsy induced by penicillin G: An electrophysiological study

Yasemin ŞAHİN YILDIZ[1,*], Şerif DEMİR[2], Ersin BEYAZÇİÇEK[2], Ali GÖK[3], Özge BEYAZÇİÇEK[2]

[1]Department of Home Patient Care, Vocational College of Health Services, Bartın University, Bartın, Türkiye
[2]Department of Physiology, Faculty of Medicine, Düzce University, Düzce, Türkiye
[3]Experimental Animals Application and Research Center, Düzce University, Düzce, Türkiye

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Abstract

Epilepsy is a neurological disease characterized by sudden and synchronized seizures caused by abnormal and excessive electrical discharges in brain neurons. The purpose of this study was to electrophysiologically examine the effects of acute administration of irisin, which is thought to be neuroprotective and increase cell proliferation, at different doses (10 and 100 nM) on the penicillin-induced experimental epilepsy in rats. Forty-nine adult male Wistar rats were used in the study. The rats were divided into 7 groups: sham, control group (penicillin), irisin group, the pre- and during-seizure groups of 10 nM and 100 nM irisin. All the substances except penicillin were administered intraperitoneally. The rats were anesthetized using urethane. The bone tissue on the left cerebral cortex was removed and the electrodes were placed in the somatomotor cortex. Thirty minutes before penicillin administration, irisin was administered to the pre-seizure penicillin group at doses of 10 nM and 100 nM. Then, penicillin (500 IU/2 µl) was injected intracortically, and ECoG recording was continued for 120 minutes. On the other hand, 10 nM and 100 nM of irisin were administered to the during-seizure penicillin group after penicillin was injected intracortically and the seizure occurred, and ECoG recording was continued for 120 minutes. The ECoG recordings were analyzed using the PowerLab Chart v.8 software. In conclusion, it was found that irisin prolonged the latency of initial epileptic activity and decreased the number and amplitude of spike-waves in the penicillin-induced experimental epilepsy model. These results suggest that irisin might have an antiepileptic potential.

Keywords: electrocorticography, epilepsy, epileptiform activity, irisin, rat

1. Introduction

Epilepsy is a serious neurological disease characterized by the disruption of the balance between cerebral inhibition and excitation, which affects millions of people around the world (1). Epileptic seizures occur as a result of a decrease in inhibitors such as γ-aminobutyric acid (GABA) or an increase in excitatory neurotransmitters such as glutamate (2). Epileptic seizures induced by abnormal neuronal discharges lead to significant neurobiological, cognitive, psychological and social consequences (3). The incidence of epilepsy is known to be higher in developing countries compared to developed countries (4, 5). Common head trauma, perinatal injury and CNS infections are associated with the risk of epilepsy (4). It very important to find out the underlying biological mechanisms in epilepsy for developing new and more effective drugs in the treatment of patients (6). The use of antiepileptic drugs is the mainstay of treatment in epilepsy (7). Antiepileptic drugs act by increasing the activity of GABA in the brain or by blocking the glutamate receptors (8). Antiepileptic drug therapy is very effective in providing seizure control, however, approximately 30% of patients continue to have seizures (9). Resistance to therapy leads to an increase in mortality and morbidity (10). Despite the increased diversity of antiepileptic drugs, seizures cannot be completely controlled. For these reasons, it is very important to develop new antiepileptic drugs that have less toxicity and are more effective and tolerable. To this end, the antiepileptic properties of different chemicals were investigated in many experimental epilepsy models (11).

Irisin, which was identified by Böstrom et al. in 2012, is a product of fibronectin type III domain 5 (FNDC5), a transmembrane protein. This myokine, which is considered to mediate positive effects on exercise-induced muscle metabolism, was first identified for its role in adipocyte browning and thermogenesis in mice and humans (12). In addition to muscle and adipose tissue, irisin is found in many organs and tissues such as cerebrospinal fluid, cerebellum, thyroid, pineal gland, pancreas, liver, testis, spleen (13). It is considered that irisin may be a molecular mediator of positive effects on different tissues and organs, including brain health (14). It has been reported that the administration of irisin, which has protective effects on central nervous system neurons

*Correspondence: yasemin-omu@hotmail.com

(15–20), at pharmacological doses can prevent neuronal damage in the brain and be effective in neurodegenerative diseases (21). Irisin revealed significant features in memory improvement and synaptic remodeling in the experimental models of neurodegenerative disorders (16, 17 ,22). Furthermore, it was also demonstrated that irisin could mitigate the brain damage and protect the blood-brain barrier (BBB) from disruption after focal cerebral ischemia/reperfusion (23). In parallel, it was concluded that irisin protected against ischemia-induced neuronal injury and reduced the levels of oxidative stress parameters (20). In the study conducted by Elhady et al., serum irisin levels were found to be significantly higher in children with epilepsy, especially in those with uncontrolled seizures, compared to the control groups (24). Inflammation in the brain is known to increase neuronal hyperexcitability and seizures (25). With many recent studies, it has been determined that irisin has an anti-inflammatory effect (26–30). It has been demonstrated that the treatment of irisin reduces inflammation (30) and modulates macrophage activity by reducing the overproduction of reactive oxygen species (ROS) (29).

The increase in irisin levels may occur as a protective mechanism against seizure-induced neuroinflammation by increasing the anti-inflammatory and antioxidant effect (24). The antiepileptic effect of irisin has not been studied much. Erkeç et al. conducted one of the studies with pentylenetetrazole (PTZ). They demonstrated that in seizures induced by PTZ, serum and brain FNDC5/irisin levels were significantly higher compared to the control group (31).

In this study, we investigated the antiepileptic effects of irisin in an experimental model of epilepsy induced by penicillin G. It was aimed to evaluate the effects of acute administration of different doses (10 and 100 nM) of irisin electrophysiologically. Furthermore, in the literature reviews, we observed that the antiepileptic effects of irisin in penicillin model of experimental epilepsy were not investigated. In this respect, our study is the first to demonstrate the antiepileptic effects of irisin in experimental epilepsy.

2. Materials and Methods

2.1. Animals

The study was conducted with the approval of Düzce University Animal Experiments Local Ethics Committee (DÜHADYEK) (2020.11.04). Forty-nine wistar male rats aged 2-3 months and weighing 240±30 g obtained from Düzce University Experimental Animals Application and Research Center were used in this study. The experimental protocols were carried out in accordance with the European Union Directive (2010/63/EU) on the use and care of experimental animals and the ethical rules of Düzce University Animal Experiments Ethics Committee. The rats given free access to food and water were kept under standard laboratory conditions in a 12-hour light–dark cycle, at a humidity of 60± 5% and a room temperature of 23 ºC.

2.2. Experimental Groups, Chemicals and Their Doses

The rats used in the experiments were randomly selected and divided into 7 different groups, each consisting of 7 rats, as sham, control group (penicillin), irisin group, pre-seizure and during-seizure 10 nM and 100 nM irisin groups.

Urethane (Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA) at a dose of 1.25 g/kg was administered as anesthetic, and 500 IU of penicillin G potassium salt (İ.E. Ulagay İlaç Sanayii Türk A.Ş., İstanbul, Istanbul, Turkey) in a volume of 2 µl was administered intracortically to induce epilepsy. Irisin (Phoenix Pharmaceuticals Inc., Burlingame, USA) was prepared at two different concentrations (low dose, 10 nM and high dose, 100 nM) by dissolving in saline. All drugs were prepared daily.

2.3. Surgical procedure and the establishment of epileptiform activity

Each rat in the groups was anesthetized with urethane just before the experiment. The rats that were found to be anesthetized were fixed in a stereotaxic frame after lying down (Harvard Instruments, South Natick, MA, USA). The scalp was incised along the midline from front to back with a scalpel, and the bone structure was reached by opening it sideways. The soft tissue on the bone structure was stripped and it was ensured that the Bregma line was clearly visible. Then, the bone tissue on the left cerebral cortex was removed by thinning with circular movements with a touring motor (FST Rechargeable Microdrill, KF Technology, Rome, Italy). Epileptiform activity was established by administering 500 IU/2 µl of penicillin G potassium intracortically (i.c.) with a Hamilton microinjector to 1.5-2 mm lateral, 1 mm anterior and 1.2 mm depth of the bregma line.

2.4. Electrophysiological recordings

Two Ag-AgCl ball electrodes were placed on the left hemisphere in the somatomotor cortex area opened lateral to the Bregma line. The reference electrode was fixed to the right ear of the rats. After the electrodes were placed, ECoG recordings were taken with the PowerLab/8SP data collection recording system (PowerLab/8SP, ADInstruments Pty Ltd. Castle Hill, NSW, Australia). A five-minute baseline activity recording was taken before the groups were injected with substances. After the baseline activity recording, irisin and saline were administered intraperitoneally the pre-seizure penicillin group and the control (penicillin) group, respectively, and ECoG recording was taken for 30 minutes. After this 30-minute ECoG recording, penicillin was injected intracortically and recording was taken for 120 minutes. The penicillin was injected intracortically after five-minute baseline activity recording to the during-seizure penicillin group, irisin was administered intraperitoneally after the occurrence of seizure, and ECoG recording was taken for 120 minutes. Only in the irisin group, after five-minute baseline activity recording, 100 nM irisin was injected and ECoG recording was taken for 120 minutes. The obtained recordings were analyzed using the PowerLab Chart v.8 software

program. Epileptiform activity occurring in bipolar spike and spike wave complexes was examined. No epileptiform activity was observed in the sham group and the irisin-alone group.

2.5. Statistical Analysis

The analyses of the data were calculated automatically via the software program (Lab Chart 8, ADInstruments Pty Ltd, Castle Hill, NSW, Australia). Epileptiform activity recordings were divided into five-minute periods and analyzed. The differences between the groups in terms of latency and spike-wave frequency and spike-wave amplitude measurements in each period were examined by Kruskal-Wallis test, and different groups were determined by post-hoc Dunn's test. SPSS program was used for the analysis of the data and $p<0.05$ was considered statistically significant.

3. Results

3.1. Latency of the epileptiform activity

There was a statistically significant difference between the groups in terms of mean latency values of epileptiform activity (P=0.005). It was observed that the onset latency of epileptic activity was quite delayed in the pre-seizure 10 nM (722.8 sec) and 100 nM (509.2 sec) irisin groups compared to the control group (282.5 sec). This delay differed according to dose rates, and it was determined that the group that the pre-seizure 10 nM irisin group mostly delayed the onset latency of epileptic activity. There was a statistically significant difference between the pre-seizure 10 nM irisin group and the control group (P=0.013) (Fig. 1).

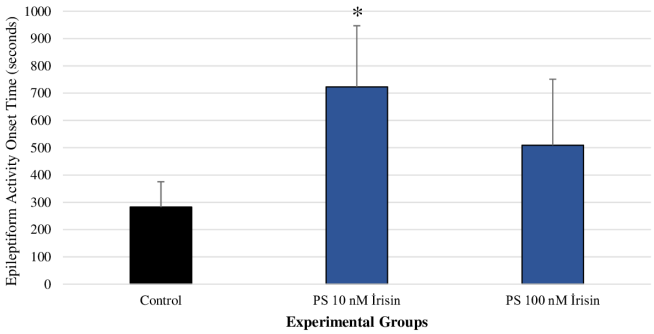

Fig. 1. Onset Latency of epileptiform activity in the control and irisin groups; (*Significant compared to the control group, P<0.05); PS: Pre-Seizure

3.2. Effects of irisin on spike wave frequency

There was a statistically significant difference in terms of mean spike-wave frequency of epileptiform activity between all groups at the 0th-120th minutes (except at the 6th-10th and 81st-85th minutes) after penicillin administration (P<0.05) (Fig. 2).

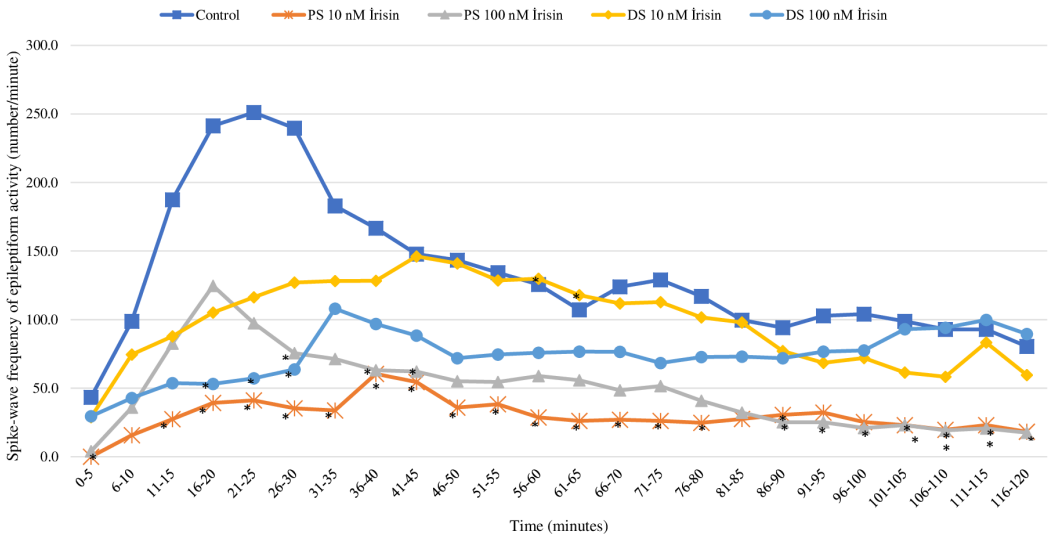

Fig. 2. Mean values of spike-wave frequency (number/minute) observed in the ECoG recording after penicillin G (intracortical) injection; (*Significant compared to the control group, P<0.05); DS: During-Seizure

3.3. Effects of irisin on total spike-wave frequency of epileptiform activity

The total spike wave frequency was significantly reduced in irisin groups, and there was a statistically significant difference between the groups in terms of total mean values of spike-wave frequency (P=0.001) (Fig. 3).

3.4. Effects of irisin on spike-wave amplitude

There was a statistically significant difference in terms of mean spike-wave amplitude of epileptiform activity between all groups at the 0th-120th minutes (except at the 0th-5th minutes) after penicillin administration (P<0.05) (Fig. 4).

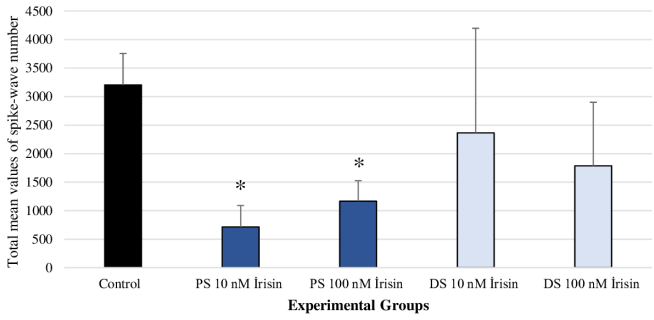

Fig. 3. Total mean values of spike-wave number of all groups; (*Significant compared to the control group, P<0.05)

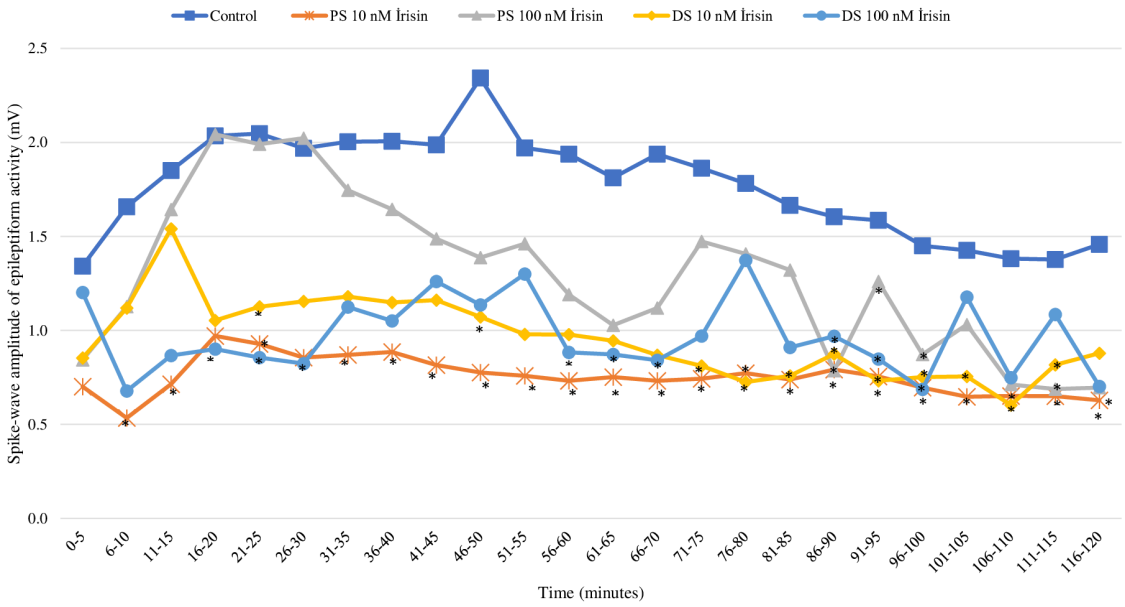

Fig. 4. Mean values of spike-wave amplitude (mV) observed in the ECoG recording after penicillin G (intracortical) injection; (*Significant compared to the control group, P<0.05)

4. Discussion

Irisin was initially identified as a skeletal muscle-derived myokine that increases with exercise to improve energy and glucose homeostasis by supporting the browning of white adipose tissue. In the following studies, it was shown to be secreted by various tissues and organs, including the brain. There are new studies investigating the effects of irisin on brain functions. In addition to its protective role against ischemia-induced neuronal damage, it also plays a role in the regulation of neuronal differentiation, metabolism and energy consumption (24). The gene expression of PGC1α (PPARγ coactivator-1α), a transcriptional coactivator that mediates many biological programs related to energy metabolism, stimulates the increase in FNDC5 gene expression. The formation of irisin, a product of FNDC5, is mainly induced by PGC1α (12).

Irisin, which is considered as a mediator of physical activity, has been extensively studied in recent years. In many studies, it was concluded that irisin levels increased with exercise (32–36). Physical activity has beneficial effects on brain health and cognitive functions. Furthermore, it is known that physical activity has protective effects against neurodegenerative diseases and improves the symptoms (37). Physical exercise is recommended as an alternative treatment to reduce epileptiform discharges and seizure frequency, to increase cardiorespiratory fitness and muscle strength, and to provide better health conditions (38). When these benefits of exercise are taken into account, it can be considered that the increase in the levels of irisin, which is defined as an intermediate in exercise, may contribute to the improvement of epileptic activity.

Mitochondrial dysfunction is considered as one of the potential causes of epileptic seizures (39). The preservation of mitochondrial function is one of the most important therapeutic approaches to prevent the development and progression of neurodegenerative diseases (40). It has been demonstrated that irisin, an exercise-related hormone, can improve the mitochondrial functions and protect against many diseases by reducing the production of reactive oxygen species (ROS) (41). With various studies conducted in parallel with this, it has been confirmed that exogenous irisin treatment improved the mitochondrial dysfunction and significantly reduces the ROS levels (42, 43).

The neuroprotective roles of irisin have been demonstrated with many studies (15–20, 37, 44). Brain-derived neurotrophic factor (BDNF) is one of the most important neurotrophic factors in the regulation of neuronal survival and neurogenesis (45, 46). Physical exercise increases BDNF levels through PGC-1α activation and modulation of FNDC-5 expression (46). With the studies conducted in recent years, it has been confirmed that irisin exerts its neuroprotective effects by increasing the expression of BDNF (43,46). Considering the neuroprotective roles of irisin in accordance with previous studies, it is considered that both exogenous and endogenous irisin may provide protection against epilepsy-induced neuronal damage (43). Neuroinflammation is known to cause neuronal hyperexcitability and an increase in seizures. Inflammation control may provide new strategies to treat seizures and epilepsy (47). In addition to its anti-inflammatory properties (26–30), irisin has protective effects against hypoxia, oxidative stress and apoptosis (48, 49). When it is also considered that inflammation promotes the occurrence of epilepsy (50), irisin level may be increased as a protective mechanism against seizure-induced neuroinflammation (24).

In seizures induced by pentylenetetrazole (PTZ), Erkeç et al. demonstrated that serum and brain FNDC5 / irisin levels of rats were significantly higher compared to the control group (31). In the study conducted by Elhady et al., epileptic children

without seizure control had higher serum irisin levels compared to control groups (24), however, in a study in which Erkeç et al. investigated possible changes in irisin levels between adult epileptic patients and healthy subjects, no significant difference was found in serum irisin levels between the groups (51). These controversial results may be associated with various factors such as patients' age, duration of antiepileptic drug treatment or antiepileptic drug type.

Irisin expression is rich in GABAergic cells that secrete GABA, a major inhibitory neurotransmitter that suppresses neuropathic pain (52-54). Exercise increases circulating GABA (55) and inhibits the reduction in glutamic acid decarboxylase, which catalyzes the decarboxylation of glutamate to GABA in the nervous system (56). Although some studies have reported that exercise supports GABA signaling (57–59), how irisin regulates the GABA pathway remains uncertain and further studies are needed in this regard (60).

Many experimental epilepsy models have been developed to evaluate the pathophysiology of epileptic seizures and to investigated new molecules with antiepileptic effect (61). The penicillin model of epilepsy was used in this study. The reason why we preferred this model was that the effects of irisin on the experimental model of epilepsy induced by penicillin have not been demonstrated previously, and our study is original in this respect. Penicillin leads to rhythmic epileptiform discharges by increasing glutamate release by inhibition of the GABAA receptor (62).

In this study, the effects of irisin administered acutely at different doses (10 nM and 100 nM) in the penicillin model of experimental epilepsy were investigated. The possible mechanism is that PGC1-α increases the circulating GABA concentration and the same substance exerts an anti-glutaminergic effect by decreasing the NMDA (N-Methyl-D-aspartate) receptor activity and increasing glutamate reuptake in astrocytes (55, 63). In the literature, no study in which the effects of irisin on epileptic seizures were investigated electrophysiologically was found, and our study is the first research in this regard.

In our study, it was determined that irisin prolonged the onset latency of epileptic activity and decreased the spike wave number and amplitude in the experimental model of epilepsy induced by penicillin. These results suggest that irisin may have antiepileptic potential. The results presented in our study show that irisin may have an antiepileptic effect. The dose values between 10 nM and 100 nM should be supported by studies in order to determine the efficacy of irisin in epilepsy. There is a need for further studies in which biochemical analyses are performed to show the change at the molecular level.

Conflict of interest

The authors declare there are no conflicts of interest.

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Authors' contributions

Concept: Y.Ş.Y, Ş.D., Design: Y.Ş.Y, Ş.D., E.B, Data Collection or Processing: Y.Ş.Y, Ş.D., E.B, A.G. Analysis or Interpretation: Y.Ş.Y, Ş.D., E.B, Ö.B, Literature Search: Y.Ş.Y, Ş.D., E.B, Writing: Y.Ş.Y, Ş.D.

References

1. Aygun H. Exendin-4 increases absence-like seizures and anxiety–depression-like behaviors in WAG/Rij rats. Epilepsy Behav [Internet]. 2021 Oct [cited 2022 Feb 11];123:108246. Available from: https://doi.org/10.1016/j.yebeh.2021.108246
2. Sarlo GL, Holton KF. Brain concentrations of glutamate and GABA in human epilepsy: A review. Seizure. 2021 Oct 1;91:213–27.
3. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE Official Report: A practical clinical definition of epilepsy. Epilepsia. 2014;55(4):475–82.
4. Newton CR, Garcia HH. Epilepsy in poor regions of the world. Lancet [Internet]. 2012 [cited 2022 Feb 16];380:1193–201. Available from: www.thelancet.com
5. Espinosa-Jovel C, Toledano R, Aledo-Serrano Á, García-Morales I, Gil-Nagel A. Epidemiological profile of epilepsy in low income populations. Seizure Eur J Epilepsy [Internet]. 2018 [cited 2020 Jul 16];56:67–72. Available from: https://doi.org/10.1016/j.seizure.2018.02.002
6. do Canto AM, Donatti A, Geraldis JC, Godoi AB, da Rosa DC, Lopes-Cendes I. Neuroproteomics in Epilepsy: What Do We Know so Far? Front Mol Neurosci [Internet]. 2021 Jan 7 [cited 2022 Feb 21];13:254. Available from: https://www.frontiersin.org/articles/10.3389/fnmol.2020.604158/full
7. Lee-Lane E, Torabi F, Lacey A, Fonferko-Shadrach B, Harris D, Akbari A, et al. Epilepsy, antiepileptic drugs, and the risk of major cardiovascular events. Epilepsia [Internet]. 2021 Jul 1 [cited 2022 Feb 22];62(7):1604–16. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/epi.16930
8. Kobayashi K, Endoh F, Ohmori I, Akiyama T. Action of antiepileptic drugs on neurons. Brain Dev [Internet]. 2020;42(1):2–5. Available from: https://doi.org/10.1016/j.braindev.2019.07.006
9. Govil N, Chahal S, Gupta N, Kaloti AS, Nadda A, Singh P. Factors Associated with Poor Antiepileptic Drugs Adherence in below Poverty Line Persons with Epilepsy: A Cross-Sectional Study. J Neurosci Rural Pract [Internet]. 2021 Jan 1 [cited 2022 Feb 22];12(1):95–101. Available from: http://www.thieme-connect.de/products/ejournals/html/10.1055/s-0040-1721200
10. Taşkiran E, Bebek N. Drug Resistance and Resistance Mechanisms in Epilepsy. J Turkish Epilepsi Soc. 2015;21(2):43–53.
11. Erdoğan MA, Erbaş O. Anticonvulsant Effect of Oxolamine

Citrate in Pentylenetetrazole Induced Experimental Epilepsy Model in Rats. Suleyman Demirel Univ J Heal Sci. 2020;11(1):92–9.

12. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1a dependent myokine that derives browning of white fat and thermogenesis. Nature. 2012;481(7382):463–8.

13. Leustean L, Preda C, Teodoriu L, Mihalache L, Arhire L, Ungureanu MC. Role of Irisin in Endocrine and Metabolic Disorders—Possible New Therapeutic Agent? Appl Sci 2021, Vol 11, Page 5579 [Internet]. 2021 Jun 16 [cited 2022 Mar 2];11(12):5579. Available from: https://www.mdpi.com/2076-3417/11/12/5579/htm

14. Pignataro P, Dicarlo M, Zerlotin R, Zecca C, Dell'abate MT, Buccoliero C, et al. Fndc5/irisin system in neuroinflammation and neurodegenerative diseases: Update and novel perspective [Internet]. Vol. 22, International Journal of Molecular Sciences. MDPI AG; 2021 [cited 2021 May 5]. p. 1–14. Available from: /pmc/articles/PMC7915567/

15. Wu H, Guo P, Jin Z, Li X, Yang X, Tang C, et al. Serum levels of irisin predict short-term outcomes in ischemic stroke. Cytokine [Internet]. 2019 Oct 1 [cited 2021 Apr 29];122:2–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043466618300577

16. Tu T, Yin S, Pang J, Zhang X, Zhang L, Zhang Y, et al. Irisin Contributes to Neuroprotection by Promoting Mitochondrial Biogenesis After Experimental Subarachnoid Hemorrhage. Front Aging Neurosci [Internet]. 2021 Feb 3 [cited 2021 Apr 29];13:1–15. Available from: https://www.frontiersin.org/articles/10.3389/fnagi.2021.640215/full

17. Lourenco MV, Frozza RL, de Freitas GB, Zhang H, Kincheski GC, Ribeiro FC, et al. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. Nat Med [Internet]. 2019 Jan 1 [cited 2021 Apr 29];25(1):165–75. Available from: https://pubmed.ncbi.nlm.nih.gov/30617325/

18. Asadi Y, Gorjipour F, Behrouzifar S, Vakili A. Irisin Peptide Protects Brain Against Ischemic Injury Through Reducing Apoptosis and Enhancing BDNF in a Rodent Model of Stroke. Neurochem Res [Internet]. 2018 Aug 1 [cited 2021 Apr 29];43(8):1549–60. Available from: https://doi.org/10.1007/s11064-018-2569-9

19. Tu WJ, Qiu HC, Cao JL, Liu Q, Zeng XW, Zhao JZ. Decreased Concentration of Irisin Is Associated with Poor Functional Outcome in Ischemic Stroke. Neurotherapeutics [Internet]. 2018 Oct 14 [cited 2021 Apr 29];15(4):1158–67. Available from: https://pubmed.ncbi.nlm.nih.gov/30030698/

20. Li DJ, Li YH, Yuan H Bin, Qu LF, Wang P. The novel exercise-induced hormone irisin protects against neuronal injury via activation of the Akt and ERK1/2 signaling pathways and contributes to the neuroprotection of physical exercise in cerebral ischemia. Metabolism [Internet]. 2017 Mar 1 [cited 2021 Apr 29];68:31–42. Available from: https://pubmed.ncbi.nlm.nih.gov/28183451/

21. Zarbakhsh S, Safari M, Aldaghi MR, Sameni HR, Lagmouj YK, Jaberi KR, et al. Irisin protects the substantia nigra dopaminergic neurons in the rat model of Parkinson ' s disease. Iran J Basic Med Sci. 2019;22(7):722–8.

22. Kim OY, Song J. The Role of Irisin in Alzheimer's Disease. J Clin Med. 2018;7(11):407.

23. Guo P, Jin Z, Wu H, Li X, Ke J, Zhang Z, et al. Effects of irisin on the dysfunction of blood-brain barrier in rats after focal cerebral ischemia/reperfusion. Brain Behav. 2019;9(10):1–9.

24. Elhady M, Youness ER, Gafar HS, Abdel Aziz A, Mostafa RSI. Circulating irisin and chemerin levels as predictors of seizure control in children with idiopathic epilepsy. Neurol Sci. 2018;39(8):1453–8.

25. Yıldırım M, Değirmenci U, Yalın S. The role of inflammation in epilepsy. Mersin Univ J Heal Sci. 2019;12(3):525–33.

26. Xiong XQ, Geng Z, Zhou B, Zhang F, Han Y, Zhou YB, et al. FNDC5 attenuates adipose tissue inflammation and insulin resistance via AMPK-mediated macrophage polarization in obesity. Metabolism. 2018 Jun 1;83:31–41.

27. Myint PK, Ito A, Appiah MG, Obeng G, Darkwah S, Kawamoto E, et al. Irisin supports integrin-mediated cell adhesion of lymphocytes. Biochem Biophys Reports. 2021 Jul 1;26:100977.

28. Buscemi S, Corleo D, Vasto S, Buscemi C, Barile AM, Rosafio G, et al. Serum Irisin Concentrations in Severely Inflamed Patients. Horm Metab Res. 2020;52(4):246–50.

29. Mazur-Bialy AI. Irisin acts as a regulator of macrophages host defense. Life Sci. 2017 May 1;176:21–5.

30. Anand Narayanan S, Metzger CE, Bloomfield SA, Zawieja DC. Inflammation-induced lymphatic architecture and bone turnover changes are ameliorated by irisin treatment in chronic inflammatory bowel disease. FASEB J. 2018;32(9):4848–61.

31. Ergul Erkec O, Algul S, Kara M. Evaluation of ghrelin, nesfatin-1 and irisin levels of serum and brain after acute or chronic pentylenetetrazole administrations in rats using sodium valproate. Neurol Res [Internet]. 2018;40(11):923–9. Available from: https://doi.org/10.1080/01616412.2018.1503992

32. Gruhn K, Siteneski A, Camargo A, Freitas AE, Olescowicz G, Brocardo PS, et al. Physical exercise stimulates hippocampal mTORC1 and FNDC5/irisin signaling pathway in mice: Possible implication for its antidepressant effect. Behav Brain Res. 2021 Feb 26;400:1–8.

33. Huh JY, Siopi A, Mougios V, Park KH, Mantzoros CS. Irisin in Response to Exercise in Humans With and Without Metabolic Syndrome. J Clin Endocrinol Metab [Internet]. 2015 Mar 1 [cited 2021 May 5];100(3):453–7. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2014-2416

34. Palacios-González B, Vadillo-Ortega F, Polo-Oteyza E, Sánchez T, Ancira-Moreno M, Romero-Hidalgo S, et al. Irisin levels before and after physical activity among school-age children with different BMI: A direct relation with leptin. Obesity. 2015;23(4):729–32.

35. Huh JY, Mougios V, Kabasakalis A, Fatouros I, Siopi A, Douroudos II, et al. Exercise-Induced Irisin Secretion Is Independent of Age or Fitness Level and Increased Irisin May Directly Modulate Muscle Metabolism Through AMPK Activation. J Clin Endocrinol Metab [Internet]. 2014 Nov 1 [cited 2021 May 5];99(11):2154–61. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2014-1437

36. Jedrychowski MP, Wrann CD, Paulo JA, Gerber KK, Szpyt J, Robinson MM, et al. Detection and quantitation of circulating human irisin by tandem mass spectrometry. Cell Metab [Internet]. 2015 Oct 6 [cited 2021 May 5];22(4):734–40. Available from: /pmc/articles/PMC4802359/

37. Dehghan F, Zamani S, Barreiro C, Jami MS. Irisin injection mimics exercise effects on the brain proteome. Eur J Neurosci [Internet]. 2021 Nov 1 [cited 2022 Mar 7];54(10):7422–41. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/ejn.15493

38. de Lima C, Arida RM, Andersen ML, Polesel DN, de Alvarenga

TAF, Vancini RL, et al. Effects of acute physical exercise in the light phase of sleep in rats with temporal lobe epilepsy. Epilepsy Res. 2017 Oct 1;136:54–61.

39. Folbergrová J, Kunz WS. Mitochondrial dysfunction in epilepsy. Mitochondrion [Internet]. 2012 Jan [cited 2022 Mar 6];12(1):35–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1567724911001851

40. Wu Y, Chen M, Jiang J. Mitochondrial dysfunction in neurodegenerative diseases and drug targets via apoptotic signaling. Mitochondrion. 2019 Nov 1;49:35–45.

41. Zhang J, Bi J, Ren Y, Du Z, Li T, Wang T, et al. Involvement of GPX4 in irisin's protection against ischemia reperfusion-induced acute kidney injury. J Cell Physiol [Internet]. 2021 Feb 1 [cited 2022 Mar 6];236(2):931–45. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jcp.29903

42. Bi J, Zhang J, Ren Y, Du Z, Li T, Wang T, et al. Irisin reverses intestinal epithelial barrier dysfunction during intestinal injury via binding to the integrin αVβ5 receptor. J Cell Mol Med. 2020 Jan 1;24(1):996–1009.

43. Cheng Y, Cui Y, Zhai Y, Xin W, Yu Y, Liang J, et al. Neuroprotective Effects of Exogenous Irisin in Kainic Acid-Induced Status Epilepticus. Front Cell Neurosci. 2021;15:738533.

44. Momenzadeh S, Zamani S, Dehghan F, Barreiro C, Jami MS. Comparative proteome analyses highlight several exercise-like responses of mouse sciatic nerve after IP injection of irisin. Eur J Neurosci. 2021 May 1;53(10):3263–78.

45. Sepehr A, Taheri F, Heidarian S, Motaghinejad M, Safari S. Neuroprotective and neuro-survival properties of safinamide against methamphetamine-induced neurodegeneration: Hypothetic possible role of BDNF/TrkB/PGC-1α signaling pathway and mitochondrial uncoupling protein −2(UCP-2). Med Hypotheses. 2020 Oct 1;143:110094.

46. Rabiee F, Lachinani L, Ghaedi S, Nasr-Esfahani MH, Megraw TL, Ghaedi K. New insights into the cellular activities of Fndc5/Irisin and its signaling pathways. Cell Biosci [Internet]. 2020;10(1):1–10. Available from: https://doi.org/10.1186/s13578-020-00413-3

47. Dey A, Kang X, Qiu J, Du Y, Jiang J. Anti-inflammatory small molecules to treat seizures and epilepsy: from bench to bedside. Trends Pharmacol Sci [Internet]. 2016 Jun 1 [cited 2022 Mar 11];37(6):463. Available from: /pmc/articles/PMC5064857/

48. Zhao YT, Wang H, Zhang S, Du J, Zhuang S, Zhao TC. Irisin Ameliorates Hypoxia/Reoxygenation-Induced Injury through Modulation of Histone Deacetylase 4. Fan G-C, editor. PLoS One [Internet]. 2016 Nov 22 [cited 2021 May 4];11(11):e0166182. Available from: https://dx.plos.org/10.1371/journal.pone.0166182

49. Mazur-Bialy AI, Pocheć E, Zarawski M. Anti-inflammatory properties of irisin, mediator of physical activity, are connected with TLR4/Myd88 signaling pathway activation. Int J Mol Sci [Internet]. 2017 Apr 1 [cited 2021 May 4];18(4). Available from: /pmc/articles/PMC5412287/

50. Rana A, Musto AE. The role of inflammation in the development of epilepsy. J Neuroinflammation 2018 151 [Internet]. 2018 May 15 [cited 2022 Mar 11];15(1):1–12. Available from: https://link.springer.com/articles/10.1186/s12974-018-1192-7

51. Ergul Erkec O, Milanlıoğlu A, Komuroglu AU, Kara M, Huyut Z, Keskin S. Evaluation of serum ghrelin, nesfatin-1, irisin, and vasoactive intestinal peptide levels in temporal lobe epilepsy patients with and without drug resistance: a cross-sectional study. Rev Assoc Med Bras [Internet]. 2021 [cited 2022 Feb 14];67(2):207–12. Available from: https://doi.org/10.1590/1806-9282.67.02.20200521

52. Bráz JM, Sharif-Naeini R, Vogt D, Kriegstein A, Alvarez-Buylla A, Rubenstein JL, et al. Forebrain GABAergic Neuron Precursors Integrate into Adult Spinal Cord and Reduce Injury-Induced Neuropathic Pain. Neuron [Internet]. 2012 May 24 [cited 2021 May 10];74(4):663–75. Available from: /pmc/articles/PMC3361692/

53. Li C, Lei Y, Tian Y, Xu S, Shen X, Wu H, et al. The etiological contribution of GABAergic plasticity to the pathogenesis of neuropathic pain. Mol Pain [Internet]. 2019 May 1 [cited 2021 May 10];15:1–13. Available from: /pmc/articles/PMC6509976/

54. Dun SL, Lyu R-M, Chen Y-H, Chang J-K, Luo JJ, Dun NJ. Irisin-immunoreactivity in neural and non-neural cells of the rodent. Neuroscience [Internet]. 2013 Jun;240:155–62. Available from: http://dx.doi.org/10.1016/j.neuroscience.2013.02.050

55. Roberts LD, Ashmore T, McNally BD, Murfitt SA, Fernandez BO, Feelisch M, et al. Inorganic nitrate mimics exercise-stimulated muscular fiber-Type switching and myokine and g-Aminobutyric acid release. Diabetes. 2017;66(3):674–88.

56. Farzad B, Rajabi H, Gharakhanlou R, Allison DJ, Hayat P, Jameie SB. Swimming training attenuates allodynia and hyperalgesia induced by peripheral nerve injury in an adult male rat neuropathic model: Effects on irisin and GAD65. Pain Med (United States) [Internet]. 2018 Nov 1 [cited 2021 May 10];19(11):2236–45. Available from: https://academic.oup.com/painmedicine/article/19/11/2236/4791839

57. Maddock RJ, Casazza GA, Fernandez DH, Maddock MI. Acute modulation of cortical glutamate and GABA content by physical activity. J Neurosci [Internet]. 2016 Feb 24 [cited 2021 May 10];36(8):2449–57. Available from: /pmc/articles/PMC6705493/

58. Coxon JP, Cash RFH, Hendrikse JJ, Rogasch NC, Stavrinos E, Suo C, et al. GABA concentration in sensorimotor cortex following high-intensity exercise and relationship to lactate levels. J Physiol [Internet]. 2018 Feb 15 [cited 2021 May 10];596(4):691–702. Available from: /pmc/articles/PMC5813602/

59. Barzroodi pour M, Bayat M, Golab F, Eftekharzadeh M, Katebi M, Soleimani M, et al. The effect of exercise on GABA signaling pathway in the model of chemically induced seizures. Life Sci [Internet]. 2019;232:116667. Available from: https://doi.org/10.1016/j.lfs.2019.116667

60. Huang SH, Yang SM, Lo JJ, Wu SH, Tai MH. Irisin gene delivery ameliorates burn-induced sensory and motor neuropathy. Int J Mol Sci [Internet]. 2020 Oct 2 [cited 2021 May 10];21(20):1–17. Available from: www.mdpi.com/journal/ijms

61. Akdogan I, Goksin N. Experimental Epilepsy Models and Morphologic Alterations of Experimental Epilepsy Models in Brain and Hippocampus. In: Underlying Mechanisms of Epilepsy [Internet]. InTech; 2011. Available from: http://www.intechopen.com/books/underlying-mechanisms-of-epilepsy/experimental-epilepsy-models-and-morphologic-alterations-of-experimental-epilepsy-models-in-brain-an

62. Erfanparast A, Tamaddonfard E. Effects of intracortical microinjection of vitamin B12 on penicillin-induced epileptiform activity in rats. Acta Neurobiol Exp (Wars). 2015;75(2):200–7.

63. Nierenberg AA, Ghaznavi SA, Sande Mathias I, Ellard KK, Janos JA, Sylvia LG. Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1 Alpha as a Novel Target for Bipolar Disorder and Other Neuropsychiatric Disorders. Biol Psychiatry [Internet]. 2018;83(9):761–9. Available from: https://doi.org/10.1016/j.biopsych.2017.12.014

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Research Article

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Comparison of the treatment effects of extracorporeal shock wave therapy and trigger point injection in myofascial pain syndrome

Alper UYSAL[1,*], Murat GÜNTEL[2]

[1]Department of Physical Medicine and Rehabilitation, Hatay Training and Research Hospital, Hatay, Türkiye
[2]Department of Neurology, Faculty of Medicine, Hatay Mustafa Kemal University, Hatay, Türkiye

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Abstract

Myofascial pain syndrome (MPS) is a common chronic musculoskeletal disease and the lifetime prevalence is about 85%. Comparison of the effects of ESWT (extracorporeal shock wave therapy) and trigger point injection (TPI) treatments on sleep quality in myofascial pain syndrome has rarely been studied. The aim of this study was to compare the effects of TPI + Dry Needling (DN) and ESWT treatments on pain, neck disability level, and sleep quality in patients with myofascial pain syndrome. 63 patients diagnosed with myofascial pain syndrome were included in the study. The patients were divided into two groups. ESWT was given to 32 patients and TPI to 31 patients. These two treatment regimens were compared in terms of pain (VAS), sleep quality (PSQI), and neck disability (NDI). In terms of mean VAS, NDI, and PSQI values, there was no statistically significant difference between the groups in the mean of pre-treatment, post-treatment day 0 and post-treatment 1 month. A similar and significant improvement was observed in both groups in terms of the evaluated parameters and based on our results, we can recommend both treatment options to MPS patients.

Keywords: myofascial pain syndrome, ESWT, trigger point injection, trapezius, dry needling

1. Introduction

Myofascial pain syndrome (MPS) is a common chronic musculoskeletal disease and the lifetime prevalence is about 85%. MPS is characterized by the presence of palpable taut bands and trigger points in skeletal muscle (1). It is characterized by perceived local and referred pain due to the presence of trigger points. Apart from pain, patients present with muscle spasm, tenderness, regional twitching, sensory changes, and sometimes autonomic dysfunctions (2).

MPS negatively affects functionality and participation in activities of daily living. Myofascial trigger points often affect postural muscles, including trapezius, and can cause associated muscle pain, motor dysfunction, and autonomic reactions (3).

Many treatment options are available for inactivation of trigger points and relaxation of taut bands. Treatment methods include modification of relevant factors, medications, stretching exercises, massage therapy, manual therapy, acupuncture, injections, mesotherapy, transcutaneous electrical nerve stimulation (TENS), ultrasound (US), laser therapy, and biofeedback (4-6). In addition, nonsteroidal anti-inflammatory drugs (NSAIDs), muscle relaxants, tricyclic antidepressants (TCAs), and 5-hydroxytryptamine and norepinephrine reuptake inhibitors (SNRIs) are the main pharmacological treatment options (7, 8).

One of the most commonly used treatment methods in MPS is trigger point injection (TPI). TPI blocks the release of neurotransmitters from peripheral nerve endings, prevents the passage of painful stimuli reaching the dorsal horn of the spinal cord, activates the endogenous opioid system, and increases blood circulation with local vasodilation effect. TPI can be done as local anesthetic, steroid, botulinum toxin injections or dry needling (DN) to the trigger point (9). Extracorporeal shock waves (ESWT) are pressure waves that can be focused on any part of the body and used for therapeutic purposes after they are produced outside the body. The analgesic effects of ESWT have been demonstrated by many clinical studies. However, the mechanism of formation of this effect is not known exactly. Mechanisms such as neuronal membrane damage, nociceptor blockade, central control of sensory inputs, and reduction of neuropeptides can be mentioned (10). ESWT is thought to have pain-reducing effect on ischemic muscle tissue by stimulating angiogenesis and increasing blood flow (11).

The trapezius muscle is one of the muscles in which myofascial trigger point formation is most common. In patients

*Correspondence: alperuysal82@gmail.com

suffering from MPS, neck movements may be restricted due to trigger points in the trapezius muscle. However, it has been previously shown that sleep quality is impaired in patients with neck pain due to MPS. Deterioration of sleep quality negatively affects activities of daily living and the emotional state of the patient (12).

2. Materials and Methods

2.1. Study design, setting, and population

63 patients aged between 18-65 years who were diagnosed with MPS according to the diagnostic criteria of Travell and Simons, who applied to the Physical Medicine and Rehabilitation Outpatient Clinic, were included in the study. All patients had active trigger points in the upper and middle zones of the trapezius muscle. Detailed anamnesis of the patients was taken, general physical examinations, musculoskeletal and neurological examinations were performed. The patients were divided into two groups. A total of 3 sessions of radial ESWT were applied to the trigger point of the patients in Group I, at a frequency of 2 bar 10 Hz, every 3 days. Group II was injected with 1 ml of 2% prilocaine every 3 days using an insulin injector, and then dry needling was applied to the same point 8-10 times with inward and outward needle movements. Injection treatment was applied to Group II for a total of 3 sessions.

Having a serious psychiatric disease and receiving medical treatment for it, having cervical disc herniation or severe cervical osteoarthritis, radiculopathy, presence of kyphosis or scoliosis, neurological disease, inflammatory rheumatological disease, presence of cardiovascular problems, pregnancy, malignancy, infection, using anticoagulants, having received injections or physical therapy for MPS in the last 3 months, symptom duration less than 3 months, having uncontrolled endocrine diseases (thyroid or parathyroid disorders, diabetes mellitus) were accepted as exclusion criteria from the study.

All patients' age, weight, height, and body mass index were noted. The patients were evaluated with questionnaires 3 times, before the treatment, at the 0th day after the treatment, and at the 1st month after the treatment. Pain severity of the patients was evaluated with Visual Analog Scale (VAS), neck disability level was evaluated with neck disability index (NDI), and sleep quality was evaluated with Pittsburgh sleep quality index (PSQI) (13-15). Thus, the efficacy of these 2 treatment methods was compared in terms of pain, neck disability, and sleep quality.

The present study was approved by the clinical study ethics committee of Hatay Mustafa Kemal University (approval no. 14, dated June 03, 2021). Written informed consent was obtained from all participants.

2.2. Statistical analysis

SPSS (Statistical Package for Social Sciences) 22.0 program was used to evaluate the data obtained as a result of the study. Conformity of continuous variables with normal distribution was checked by the Shapiro Wilk test. All data were given as mean ± standard deviation, frequency and percentage. Statistical difference between ESWT and TPI+DN treatment groups in terms of categorical variables was determined by Pearson Chi-Square Test. The statistical difference between ESWT and TPI+DN treatment groups in terms of continuous variables was determined by Independent Sample T-Test. The differences between the ESWT and TPI+DN treatment groups in the changes of VAS, NDI, and PSQ parameters before treatment, at day 0 after treatment and at 1 month after treatment were determined by Repeated Measures Analysis of Variance. The statistical significance limit was accepted as $p<0.05$.

3. Results

There was no statistically significant difference between the groups in terms of age, gender, and mean BMI of the patients included in the study ($p>0.05$) (Table 1).

Table 1. Comparison of demographic characteristics (Age, BMI, and Gender) of ESWT and injection groups

Variables		ESWT (n= 32)	TPI+DN (n= 31)	p
Age (year) (Mean ± SD)		33.25±9.78	35.19±8.53	0.404*
BMI (kg/m²) (Mean± SD)		23.67±1.78	24.10±1.38	0.280*
Gender (n/%)	Male	27 (84.4)	25 (80.6)	0.398**
	Female	5 (15.6)	6 (19.4)	

n: Number of patients; SD: Standard deviation; BMI: Body Mass Index; ESWT: Extracorporeal Shock Wave Therapy; TPI: Trigger point injection; DN: Dry needling; *Independent Sample T-Test; ** Pearson Chi-square Test

In terms of mean VAS, NDI, and PSQI values, there was no statistically significant difference between the groups in the mean of pre-treatment, post-treatment day 0, and post-treatment 1 month ($p>0.05$). In the comparison within the group, a statistically significant difference was found in terms of VAS, NDI, and PSQI averages between the 0th day before and after the treatment and between the 1st month before and after the treatment in both groups ($p<0.001$). There was no statistically significant difference between the mean VAS, NDI, and PSQI at day 0 after treatment and at month 1 after treatment ($p>0.05$) (Table 2).

A vasovagal reaction developed in the first session of the treatment in one patient in the injection group. No side effects were observed in the ESWT group.

Table 2. Comparison of VAS, NDI, and PSQI values of both groups before treatment, at day 0 after treatment, and at 1 month after treatment

		GROUP (Mean ± SD)		p*
		ESWT (n= 32)	TPI+DN (n= 31)	
VAS	Before treatment	6.97±1.36	7.23±1.8	0.443
	Post-treatment (day 0)	3.47±1.37	3.58±1.09	0.721
	Post-treatment (1st month)	3.22±1.84	3.10±1.42	0.770
	p**	**<0.001[a]**	**<0.001[b]**	
NDI	Before treatment	25.78±8.76	26.61±7.71	0.691
	Post-treatment (day 0)	15.09±7.09	15.87±5.53	0.630
	Post-treatment (1st month)	14.59±8.37	14.71±6.77	0.952
	p**	**<0.001[a]**	**<0.001[b]**	
PSQ	Before treatment	9.22±3.60	10.13±3.35	0.303
	Post-treatment (day 0)	5.97±2.74	5.94±2.80	0.962
	Post-treatment (1st month)	5.59±2.99	5.35±2.93	0.750
	p**	**<0.001[a]**	**<0.001[b]**	

n: Number of patients; SD: Standard deviation; VAS: Visual Analog Scale; NDI: Neck disability index; PSQI: Pittsburgh sleep quality index; ESWT: Extracorporeal Shock Wave Therapy; TPI: Trigger point injection; DN: Dry needling; * Independent Sample T-Test; ** Repeated Measures Analysis of Variance

a: There is a difference between the 0th day before and after the treatment, there is a difference between the 1st month before and after the treatment

b: There is a difference between the 0th day before and after the treatment, there is a difference between the 1st month before and after the treatment

4. Discussion

MPS is one of the most common musculoskeletal disorders. Although there are a wide variety of treatment options, there is no clear consensus on which treatment to use, when and how. The basis of treatment in MPS consists of breaking the vicious circle in pain by eliminating the trigger point. Our aim in this study is to compare the effects of TPI + DN and ESWT treatments on pain, neck disability level, and sleep quality in patients with MPS. In some studies, both local anesthetic and DN were found to be effective in MPS, but the local anesthetic injection was recommended for trigger point inactivation since the discomfort that may occur during injection was significantly less in the local anesthetic group (16). Lewit preferred DN to local anesthetics (17) and systematic reviews concluded that the efficacy of LA and DN was equal (16).

Raeissadat et al. compared lidocaine injection, dry needling, and ozone therapy methods in patients with MPS and found that all three treatment methods were effective in short-term follow-up (1 month), but the groups treated with ozone and LA were slightly better in pain reduction and functional recovery compared to the group treated with dry needling (18). In a systematic review comparing TPI with local anesthetics applied to the head, neck, and shoulder areas with placebo and dry needling treatments, local anesthetics showed a significant reduction in pain compared to dry needling. However, it was stated that there was no difference between the treatment methods in terms of improvement in depression and joint range of motion, and it was also emphasized that well-organized studies involving more participants were needed to determine the superiority of the treatments over each other (19).

In one of the groups, we formed in our study, we applied the combination of TPI + DN to increase the effectiveness of the treatment and to reduce the discomfort that may occur after the injection. We applied ESWT treatment in the other group for comparison.

ESWT has also become one of the evidence-based treatment methods for MPS in recent years. The decrease in the mean VAS score after ESWT treatment has also been shown by many studies (20, 21). TPI may not always be a suitable treatment option for dispersedly located multiple taut bands. In these cases, ESWT may be a more appropriate treatment modality due to its advantages such as non-invasiveness, wider area of effect and no post-injection pain. Furia et al. in 34 patients and Hsu et al. in 36 participants examined the effects of ESWT on pain and functional improvement and found significant changes in VAS (22, 23).

Jung-Ho Lee et al. compared ESWT and TPI treatments in MPS patients and found similar efficacy (24). In another study, Jeon et al. divided the patients into ESWT and TENS + TPI groups, and the researchers found that pain decreased in both groups (25). Hong et al. compared the efficacy of ESWT and TPI in the treatment of myofascial pain syndrome in the quadratus lumborum (QL) muscle. Both treatment options showed statistically significant improvements in pain and disability scores, but they found that ESWT was more effective than TPI in terms of pain reduction. In terms of disability, they did not detect a statistically significant difference between the groups. In many studies in the literature conducted with the trapezius muscle in MPS patients, similar results were found when the efficacy of ESWT and TPI treatments were compared. The reason for the different results in the study of Hong et al. may be related to the fact that the upper trapezius muscle is more superficial, while the QL muscle is more deeply located (26). Jung-Ho Lee et al. compared the effects of proprioceptive neuromuscular facilitation (PNF), ESWT, and TPI treatment modalities on pain reduction and functional recovery in patients with myofascial pain syndrome. They found that PNF treatment was more effective than the other two methods in improving neck functions and activities of daily living, and increasing the range of motion of the shoulder joint. ESWT was found to be effective in reducing pain and functioning. TPI treatment was effective in reducing pain but

had limited effects in increasing functional activities (27).

In a study by Muñoz-Muñoz et al., it was stated that sleep quality is related to pain severity and these parameters are disability factors that affect each other (28). In addition, there are many studies in the literature showing that there is a relationship between sleep quality and pain intensity in both MPS and other chronic musculoskeletal diseases. In their research, Çağlar et al. showed that connective tissue massage provided a decrease in pain intensity and an increase in sleep and quality of life in MPS patients (29, 30).

As a result of our research, we determined that both treatment modalities were effective in reducing the severity of pain and neck disability, and also increased the sleep quality of the patients. Both treatment regimens were effective, but they were not statistically superior to each other.

Limitations of our study include: 1- Relatively small number of patients. 2- Relatively short follow-up period. 3- The possibility that individual modes of administration of treatment modalities will have variable effects.

In this study, we showed that TPI + DN and ESWT treatment modalities had similar efficacy in reducing pain, improving neck disability, and improving sleep quality in patients with MPS. At the end of this randomized controlled study, a significant improvement was observed in both groups in terms of the evaluated parameters and based on our results, we can recommend both treatment options to MPS patients. In order to determine which treatment is superior, we should say that multicenter studies with more participants and longer follow-up periods are needed.

Conflict of interest

Authors declare that there is no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.U., M.G., Design: A.U., M.G., Data Collection or Processing: A.U., Analysis or Interpretation: M.G., Literature Search: M.G., A.U., Writing: M.G., A.U.

References

1. Gerber LH, Sikdar S, Armstrong K, Diao G, Heimur J, Kopecky J, et al. A systematic comparison between subjects with no pain and pain associated with active myofascial trigger points. PM&R. 2013;5(11):931-8. https://doi.org/10.1016/j.pmrj.2013.06.006
2. Öz N, Çağlar NS, Akar N, Akar A, Aytekin E. Miyofasiyal Ağrı Sendromunda Lazer Tedavisinin Etkinliğinin Araştırılması. J Acad Res Med. 2021;11(2). https://doi.org/10.4274/jarem.galenos.2021.3981
3. Nazlıkul FGU, Dizdar D. Miyofasiyal ağrı sendromunda kuru iğnenin etkinliği: ön çalışma. Bilimsel Tamamlayıcı Tıp Regülasyon ve Nöral Terapi Dergisi.14(3):64-8.
4. Bron C, De Gast A, Dommerholt J, Stegenga B, Wensing M, Oostendorp RA. Treatment of myofascial trigger points in patients with chronic shoulder pain: a randomized, controlled trial. BMC Med. 2011;9(1):1-14. https://doi.org/10.1186/1741-7015-9-8
5. Desai MJ, Saini V, Saini S. Myofascial pain syndrome: a treatment review. Pain Ther. 2013;2(1):21-36. https://doi.org/10.1007/s40122-013-0006-y
6. Borg-Stein J, Iaccarino MA. Myofascial pain syndrome treatments. Phys Med Rehabil Clin N Am. 2014;25(2):357-74. https://doi.org/10.1016/j.pmr.2014.01.012
7. Cao Q-W, Peng B-G, Wang L, Huang Y-Q, Jia D-L, Jiang H, et al. Expert consensus on the diagnosis and treatment of myofascial pain syndrome. World J Clin Cases. 2021;9(9):2077. https://doi.org/10.12998/wjcc.v9.i9.2077
8. Galasso A, Urits I, An D, Nguyen D, Borchart M, Yazdi C, et al. A comprehensive review of the treatment and management of myofascial pain syndrome. Curr Pain Headache Rep. 2020;24(8):1-11. https://doi.org/10.1007/s11916-020-00877-5
9. Han SC, Harrison P. Myofascial pain syndrome and trigger-point management. Reg Anesth Pain Med. 1997;22(1):89-101. https://doi.org/10.1016/S1098-7339(06)80062-3
10. Ali G, İrfan K, Karagüllü H, Altindağ Ö, Madenci E, Tutoğlu A, et al. Comparison of the effectiveness of two different extracorporeal shock wave therapy regimens in the treatment of patients with myofascial pain syndrome. Arch Rheumatol. 2014;29(3):186-93. https://doi.org/10.5606/ArchRheumatol.2014.3738
11. Ji HM, Kim HJ, Han SJ. Extracorporeal shock wave therapy in myofascial pain syndrome of upper trapezius. Ann Rehabil Med. 2012;36(5):675-80. https://doi.org/10.5535/arm.2012.36.5.675
12. Ural FG. Miyofasiyal ağrı sendromu olan hastalarda obezitenin uyku kalitesi ve günlük yaşam aktiviteleri üzerine etkisi. Çukurova med j. 2018;43(3):600-4. https://doi.org/10.17826/cutf.351503
13. Langley G, Sheppeard H. The visual analogue scale: its use in pain measurement. Rheumatol Int. 1985;5(4):145-8. https://doi.org/10.1007/BF00541514
14. Buysse DJ, Reynolds III CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213. https://doi.org/10.1016/0165-1781(89)90047-4
15. Vernon H, Mior S. The Neck Disability Index: a study of reliability and validity. J Manipulative Physiol Ther. 1991.
16. Güzel R, Akkoca H, Şeydaoğlu G, Uğuz Ş, Kozanoğlu E, Sarpel T. Miyofasiyal ağrı sendromunun tedavisinde lokal anestezik enjeksiyonu ve kuru iğneleme yöntemlerinin etkinliğinin karşılaştırılması. Türk Fiz Tıp Rehab Derg. 2006;52(1):22-7.
17. Lewit K. The needle effect in the relief of myofascial pain. Pain. 1979;6(1):83-90. https://doi.org/10.1016/0304-3959(79)90142-8
18. Raeissadat SA, Rayegani SM, Sadeghi F, Rahimi-Dehgolan S. Comparison of ozone and lidocaine injection efficacy vs dry needling in myofascial pain syndrome patients. J Pain Res. 2018;11:1273. https://doi.org/10.2147/JPR.S164629
19. Nouged E, Dajani J, Ku B, Al-Eryani K, Padilla M, Enciso R. Local Anesthetic Injections for the Short-Term Treatment of Head and Neck Myofascial Pain Syndrome: A Systematic Review with Meta-Analysis. J Oral Facial Pain Headache. 2019;33(2). https://doi.org/10.11607/ofph.2277
20. Cho Y-S, Park S-J, Jang S-H, Choi Y-C, Lee J-H, Kim J-S. Effects of the combined treatment of extracorporeal shock wave therapy (ESWT) and stabilization exercises on pain and functions of patients with myofascial pain syndrome. J Phys Ther Sci.

2012;24(12):1319-23. https://doi.org/10.1589/jpts.24.1319

21. Park KD, Lee WY, Park M-h, Ahn JK, Park Y. High-versus low-energy extracorporeal shock-wave therapy for myofascial pain syndrome of upper trapezius: A prospective randomized single blinded pilot study. Medicine. 2018;97(28). https://doi.org/10.1097/MD.0000000000011432

22. Furia JP. High-energy extracorporeal shock wave therapy as a treatment for chronic noninsertional Achilles tendinopathy. Am J Sports Med. 2008;36(3):502-8. https://doi.org/10.1177/0363546507309674

23. Hsu C-J, Wang D-Y, Tseng K-F, Fong Y-C, Hsu H-C, Jim Y-F. Extracorporeal shock wave therapy for calcifying tendinitis of the shoulder. J Shoulder Elbow Surg. 2008;17(1):55-9. https://doi.org/10.1016/j.jse.2007.03.023

24. Lee J-H, Jang S-H, Cho S-H, Kim J-S. Comparison of extracorporeal shock wave therapy and trigger point injection in terms of their effects on pain and bodily functions of myofascial pain syndrome patients. J Phys Ther Sci. 2012;24(10):1069-72. https://doi.org/10.1589/jpts.24.1069

25. Jeon JH, Jung YJ, Lee JY, Choi JS, Mun JH, Park WY, et al. The effect of extracorporeal shock wave therapy on myofascial pain syndrome. Ann Rehabil Med. 2012;36(5):665. https://doi.org/10.5535/arm.2012.36.5.665

26. Hong JO, Park JS, Jeon DG, Yoon WH, Park JH. Extracorporeal shock wave therapy versus trigger point injection in the treatment of myofascial pain syndrome in the quadratus lumborum. Ann Rehabil Med. 2017;41(4):582. https://doi.org/10.5535/arm.2017.41.4.582

27. Lee J-H, Han E-Y. A comparison of the effects of PNF, ESWT, and TPI on pain and function of patients with myofascial pain syndrome. J Phys Ther Sci. 2013;25(3):341-4. https://doi.org/10.1589/jpts.25.341

28. Muñoz-Muñoz S, Muñoz-García MT, Alburquerque-Sendín F, Arroyo-Morales M, Fernández-de-las-Peñas C. Myofascial trigger points, pain, disability, and sleep quality in individuals with mechanical neck pain. J Manipulative Physiol Ther. 2012;35(8):608-13. https://doi.org/10.1016/j.jmpt.2012.09.003

29. Marin R, Cyhan T, Miklos W. Sleep disturbance in patients with chronic low back pain. Am J Phys Med Rehabil. 2006;85(5):430-5. https://doi.org/10.1097/01.phm.0000214259.06380.79

30. Çağlar A, Yüksel İ. Konnektif doku masajının miyofasyal ağrı sendromlu bireylerde ağrı, uyku ve yaşam kalitesi üzerine etkisi: randomize kontrollü çalışma. Journal of Exercise Therapy and Rehabilitation. 2019;6(1):19-24.

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Evaluation of dynamic thiol-disulfide homeostasis on HPV positive-women in progression to cervical intraepithelial lesion

Recep ERİN[1,*], Yeşim BAYOĞLU TEKİN[1], Hatice KÜÇÜK[2], Özcan EREL[3]

[1]Department of Obstetrics and Gynecology, University of Health Sciences, Trabzon Kanuni Training and Research Hospital, Trabzon, Türkiye
[2]Department of Pathology, University of Health Sciences, Trabzon Kanuni Training and Research Hospital, Trabzon, Türkiye
[3]Department of Biochemistry, Yıldırım Beyazıt University, Ankara Training and Research Hospital, Ankara, Türkiye

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Abstract

Dynamic thiol disulfide homeostasis (TDH) is critical in cervical carcinogenesis at HPV infection as a sign of antioxidant consumption native and total thiol levels decrease in progress to cervical intraepithelial lesions. TDH is the main actor in signaling pathways, apoptosis, antioxidant and detoxification reactions. In this study, we aimed to evaluate the effect of TDH intraepithelial progression of cervical precancerous lesions on HPV positive women. This was a prospective cross-sectional study. Subjects were selected from newly diagnosed high risk HPV DNA-positive patients. TDH results were calculated as the levels of disulfide, native and total thiol, the ratios of disulfide/total thiol (SS/SH+SS), disulfide/native thiol (SS/SH) and native thiol/total thiol (SH/SH+SS). A total of 146 women were included in the study. Study groups were as group one; control included 66 participants, group two; HPV DNA-positive women without preinvasive cervical lesion included 30 participants and group three; HPV DNA-positive women with preinvasive cervical lesion included 50 participants. Native and total thiol levels were elevated on HPV-positive women without preinvasive cervical lesions. There were no significant differences between groups related to the ratios of SS/SH, SS/ Total SH, SH/ Total SH levels. HPV infection related to oxidative stress has effects on oxidant/antioxidant balance and could be demonstrated in systemic circulation by TDH parameters. Consumption of thiol substances play a role in the cervical neoplastic process, replacement with antioxidants would be a treatment option for HPV infections.

Keywords: antioxidants, cervical intraepithelial lesions, human papillomavirus, oxidative stress, thiol-disulfide homeostasis

1. Introduction

Human Papilloma Virus (HPV) is a double-stranded DNA virus that involves the squamous epithelium. HPV is a sexual transmitted infection and the main cause of the cervical cancer. There are about 40 HPV types indicating anogenital involvement. High oncogenic types of HPV persistence cause cervical intraepithelial lesions and cancer (CIN) formation. More than 90% of HPV infection is cleared from the body in about two years (1-3). On the other hand, up to 10% of the infection persists and causes a cervical intraepithelial lesion.

HPV targets basal layer and metaplastic cells to create infection and reaches there through the micro abrasions formed in the stratified squamous epithelium. After transmission of the virus to the cervical epithelium, it integrates host genome and oncogenic differentiation occurs by expressing E6 and E7 oncogenes in the cell. Dysfunction at native immune response of the host, chronic inflammation and oxidative stress also have an effect on HPV persistence and carcinogenesis. Impairment at oxidant-antioxidant balance and elevated oxidative status were found to be correlated with CIN and cervical carcinoma (4, 5).

The primary target of oxygen radicals are proteins such as cysteine, methionine, glutathione called sulfides containing sulfide groups. These proteins oxidize to form reversible disulfide bonds. Structural and functional changes occur in these proteins during losing thiol groups (6, 7). Plasma and tissue levels of thiol groups decrease in the course of prevention from the destructive effects of free oxygen radicals (8). In many cellular events as signaling pathways, apoptosis, antioxidant and detoxification reactions, dynamic TDH is the main actor (9, 10).

In recent years, TDH has maintained its popularity and has been the subject of many studies. In the literature, there are a growing body of studies showing the effect of TDH in many acute and chronic disorders. In this study, we evaluated the effect of TDH intraepithelial progression of cervical precancerous lesions at HPV-positive women. This is the first

*Correspondence: erinrecep@gmail.com

study that investigates the potential impact of TDH on cervical carcinogenesis.

2. Material and Method

This prospective cross-sectional study was performed in the Gynecology and Obstetrics Department of Trabzon Kanuni Training and Research Hospital. The study was organized in accordance with the Helsinki Declaration guide and received approval from the local ethics committee of Trabzon Kanuni Training and Research Hospital (23618724-799, 2022/01). The informed consent form was subscribed by all participants after giving information about the study.

A total of 146 women were included in the study. The participants consist of three groups. Group one was control group including 66 participants (with negative HPV and pap smear test), group two was HPV DNA positive women without preinvasive cervical lesion and included 30 participants and group three was HPV DNA positive-women with preinvasive cervical lesion and included 50 participants. Age, gravidity, body mass index (BMI) were evaluated as demographic features.

Subjects were selected from newly diagnosed high-risk HPV DNA-positive patients without concomitant active sexually transmitted infections, previous history of cervical preinvasive lesions or cervical cancer. Pregnancy, lactation, smoking, any disease associated with immune deficiency, corticosteroid usage, having any genitourinary system infection were the other exclusion criteria. Age matched women who were admitted for routine gynecological check-ups were enrolled as a control group. All participants were interrogated about sexual, reproductive, medical and surgical history.

Gynecological evaluation was performed including bimanual palpation and transvaginal ultrasonographic assessment. During speculum examination cervicovaginal swaps obtained for Liquid-based (ThinPrep Pap Test, Hologic) cervicovaginal smear test and the Hybrid Capture 2 DNA test (Qiagen, Hilden, Germany) for High-Risk HPV detection. Bethesda 2001 classification system was used in the evaluation of smear tests. HPV carriers underwent to colposcopic evaluation (Leica MSV 197, Germany), 3-5% acetic acid administered for visualization of cervical intraepithelial lesions and punch biopsies were taken from suspected areas.

About 4 mL of fasting blood picked up by venopuncture from the antecubital region. The blood samples were centrifuged at 4000 rpm for 10 minutes, and the serum was decomposed and stored at -80°C until an assessment of TDH. Serum TDH was evaluated with an automated spectrophotometric measuring technique defined by Erel (11). TDH results were figured out as μmol/L.

Firstly, disulfide links were reduced by using sodium borohydride to form free functional thiol groups. For prevention of the reduction of 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) reductant sodium borohydride was consumed and removed with formaldehyde. Reduced and native thiol groups were identified after the reaction with DTNB. The dynamic disulfide amount provided from the difference between the total and native thiols and calculated by the relationship between disulfide and thiol groups. If the ratio increases in favor of thiols, it indicates oxidative stress, while an increase in favor of disulfides indicates elevation of antioxidant capacity. The parameters for calculation of dynamic TDH were disulfide/total thiol percent ratios (SS/SH+SS), disulfide/native thiol percent ratios (SS/SH), and native thiol/total thiol percent ratios (SH/SH+SS), disulfide, native and total thiol quantity.

2.1. Statistical analysis

Version 18 of SPSS was used for statistical analysis. Kolmogorov-Smirnov method was used to determine the normal distribution of data. Mean ± standard deviation was used for normally distributed data, and median ± interquartile range values were used for non-parametric data. Comparison of TDH between study groups was done with One Way ANOVA test with post hoc LSD analysis.

3. Results

Mean age of the participants was 42.22±7.16 year, gravidity 3.6±0.61 and BMI 25.56 ±3.27 kg/m^2. No significant differences were obtained amongst the groups ($p>0.05$).

TDH was measured by native thiol and disulfide values and SS/ total SH, SH/ total SH, SS/ SH ratios. Mean native thiol levels were 246.84±81.80 (μmol/L), disulfide 22.07±7.26 (μmol/L), SS/ total SH ratio was 8.21±3.81 (μmol/L), SH/ total SH ratio was 83.52±7.63 (μmol/L) and SS/ SH ratio was 10.49±7.67 (μmol/L). Comparison of TDH parameters between study groups was given in Table 1. There were no significant differences between group according to native thiol, disulfide, SS/SH, SS/ Total SH, SH/ Total SH levels. Native and total thiol levels were elevated in HPV positive women without preinvasive cervical lesions. Contrarily in cervical intraepithelial lesion group native and total thiol levels were decreased, however the results could not reach to statistical significance (Fig. 1).

Table 1. Thiol disulfide homeostasis parameters in study groups

	Group 1 (n=66)	Group 2 (n=30)	Group 3 (n=50)	P
Native Thiol (μmol/L)	249,96±74,06	273,26±68,45	233,21±82,72	0,07
Total Thiol (μmol/L)	293,96±80,14	317,86±68,17	271,08±93,52	0,05
Disulfide (μmol/L)	22,84±7,68	22,29±5,76	21,31±6,85	0,51
SS/SH (μmol/L)	10,05±5	8,90±3,69	10,75±5,76	0,29
SS/Total SH (μmol/L)	8,14±3,20	7,40±2,58	8,70±3,98	0,25
SH/Total SH (μmol/L)	83,81±6,52	85,18±5,17	82,35±8,67	0,21

Data are given mean±std; (-SH): sulfhydryl

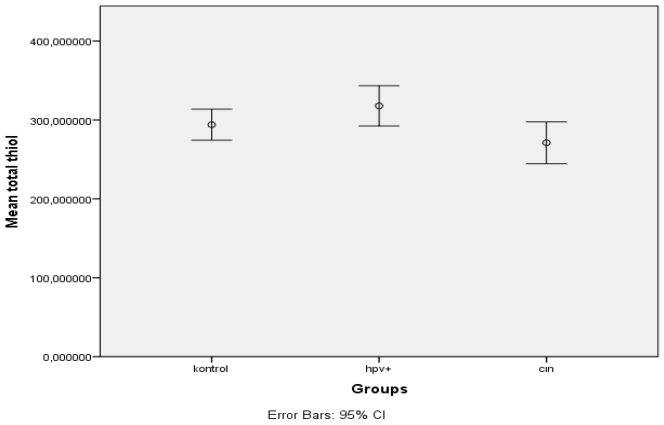

Fig. 1. The graph of total thiol levels distribution in study groups

4. Discussion

In this study, we compared the differences between TDH parameters, which are the main components of antioxidant protection, in HPV infected women without cervical intraepithelial lesions and those who developed cervical dysplasia.

Our results demonstrated that TDH parameters affected in HPV positive- women with cervical intraepithelial lesions. Evaluations from peripheral blood samples of HPV positive-women represented that total and native thiol levels decreased at cervical intraepithelial lesions. Our findings supported that HPV infection related to cellular changes activates detoxification systems and causes consumption of cellular antioxidant. However, our results were not statistically significant, differences between study groups were thought that cervical neoplastic progression effects oxidant/antioxidant status and that could be shown in systemic circulation. These findings demonstrated that HPV infection caused cervical neoplastic process is closely related with oxidant and antioxidant regulation. According to this data we concluded that replacement of antioxidants in HPV infection could be a strategy for treatment of infection and prevention from cellular changes.

Experimental and observational studies show that a large portion of HPV infection are spontaneously regressed however a small part of them progress and generate cervical cancer. Being HPV infected is not just enough for cancer development, there is still lack of information about other individual and environmental factors and their mechanisms on pathogenesis. Free oxygen radicals are important factors, those are effective in carcinogenesis by signaling pathway up regulation, cell differentiation, proliferation and change of cellular survival. HPV reproduces in infected and transformed cells and disrupts the redox balance (12-15). Past studies showed that immune response of the host to HPV infection influences oxidative stress and could be demonstrated with alteration of stress markers. Siegel et al (16) evaluated the relation between oxidant load and HPV clearance, and they determined a high oxidant status. On the contrary, an increase in oxidant levels, antioxidant enzymes have been shown to be lowered in patients with CIN and cervical carcinoma due to excessive consumption (17-19). According to an in-vivo study evaluating the effect of the Redox system on carcinogenesis, an increased oxidant environment has been shown to be effective in HPV 16 neoplastic progression, and oxidative modification of DNA and proteins in dysplastic tissues have influenced cellular differentiation, leading to neoplastic progression. In cancerous tissue, controlling oxidative damage could be provided by selective reduction of key detoxification proteins (13).

Histopathological evaluations show an increased inflammatory infiltration in severe HPV-induced lesions. In the early stages, the infection caused by the virus at basal cells is not associated with circulating immune cells therefore inflammation does not play a central role in the pathogenesis of HPV infection. Persistent infection causes chronic inflammation and triggers an imbalance between pro-oxidant and antioxidants (14).

During intracellular reactive oxygen species increase, local antioxidant capacity and numerous intracellular adaptive mechanisms upregulate to prevent the development of apoptosis and to protect the tissue. Throughout free radicals rise above physiological levels, the regulation of redox homeostasis, which was the cellular protection system of the organism, is disrupted and initiates the process of uncontrolled cell growth and carcinogenesis (20, 21). Redox homeostasis is controlled by oxidizing and reducing of free radicals and thiol-containing proteins in the cell.

Thiols are the parts of the natural antioxidant enzyme system in the organism that contain Sulfhydryl and form disulfide in antioxidant activation (22). Attachment of sulfur and hydrogen atoms to a carbon atom forms sulfhydryl and oxidation reactions form disulfide bonds between two sulfhydryl groups (23). This binding is reversible and disulfides can reduce to thiol groups to sustain homeostasis (24). This homeostasis plays a crucial role in antioxidant protection, detoxification, signal transmission, programmed cell death, organizing enzymatic reaction, transcription factors and intra and inter-cellular signaling mechanisms (25).

Many compounds like cysteine, methionine, glutathione, homocysteine, cysteinyl-glycine and glutamyl cysteine are containing thiol groups and have structural alterations under oxidative stress. These proteins oxidize to form reversible disulfide bonds. Structural and functional changes occur in these proteins during losing thiol groups (6, 7). Plasma and tissue levels of thiol groups decrease in the course of prevention from the destructive effects of free oxygen radicals (8). The transformation of thiols into disulfides is an early indicator of protein oxidation from reactive oxygen radicals. Measurement of total thiol level and determination of TDH are the mirror of excessive free oxygen radical formation in many

illnesses (26).

Disruption of this ratio acts a part in the pathogenesis of many inflammatory diseases such as diabetes mellitus, inflammatory arthritis, renal failure, cancer, Parkinson, Alzheimer, multiple sclerosis. Shifting thiol/disulfide balance to disulfide direction was seen in degenerative diseases such as diabetes, obesity, and pneumonia, to thiol direction poses risk factor neoplastic processes such as multiple myeloma, bladder, colon and kidney cancer (27).

The colorimetric method improved by Erel and Neşelioğlu (11) renders possible to provide information about oxidative stress by identifying the total plasma thiol/disulfide ratio. The easy, inexpensive and practical method is carried out with a fully automatic analyzer that does not require separation. It can be used to evaluate free radicals synthesized by many different metabolic pathways, including aerobic respiration in mitochondria (12). Before this measurement technique was developed, only low molecular weight thiol components which were cysteine, glutathione, and homocysteine could be measured. This method allows measuring the majority part of thiol and disulfides in albumin and proteins. According to the old method, thiol / disulfide measurement did not reflect true homeostasis.

In enzymes containing thiol, free radicals formed after normal metabolites or pathological processes cause structural and functional disturbance and alterations in thiol/disulfide balance. A decrease in plasma thiol concentration indicates an increase in free radical formation. A small proportion of HPV-infected cells progresses to cancer, the expression of E6 and E7 oncogenes play roles in this process. Camporeale et al. (28) studied the molecular mechanism of potential damages of the oxidative environment in HPV infected cells. They reported that carboxy-terminal of E7 oncoprotein is rich in the domain of cysteine and sensitive to ROS. Exposure to free radicals regulates the transition from the cytoplasm to the nucleus by creating disulfide bonds.

HPV infection has a local effect at cervical epithelium and evaluation of TDH parameters at cervical secretions could be most informative for detection of oxidant/ antioxidant status. This was one of the limitations of our study. The other limitation was cross-sectional design of the study, the levels of TDH parameters previous the infection and cervical lesion were not included in the study.

In conclusion, HPV infection related oxidative stress has systemic effects and could be demonstrated in the systemic circulation by TDH parameters. Consumption of thiol substances play a role in cervical neoplastic process, replacement with antioxidants would be a treatment option for HPV infections.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: Y.B.T., Design: Y.B.T., Data Collection or Processing: Y.B.T., R.E., H.T., Analysis or Interpretation: Y.B.T., Ö.E., Literature Search: Y.B.T., R.E., Writing: Y.B.T, R.E.

References

1. Myers ER, McCrory DC, Nanda K, Bastian L, Matchar DB. Mathematical model for the natural history of human papillomavirus infection and cervical carcinogenesis. Am J Epidemiol. 2000;15;151(12):1158-71. doi: 10.1093/oxfordjournals.aje.a010166.
2. Richardson H, Kelsall G, Tellier P, Voyer H, Abrahamowicz M, Ferenczy A et al. The natural history of type-specific human papillomavirus infections in female university students. Cancer Epidemiol Biomarkers Prev. 2003;12(6):485-90. PMID: 12814991.
3. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM; ALTS Group. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J Infect Dis. 2007;1;195(11):1582-9. doi: 10.1086/516784.
4. Looi ML, Mohd Dali AZ, Md Ali SA, Wan Ngah WZ, Mohd Yusof YA. Oxidative damage and antioxidant status in patients with cervical intraepithelial neoplasia and carcinoma of the cervix. Eur J Cancer Prev. 2008;17(6):555-60. doi: 10.1097/CEJ.0b013e328305a10b.
5. Kim SY, Kim JW, Ko YS, Koo JE, Chung HY, Lee-Kim YC. Changes in lipid peroxidation and antioxidant trace elements in serum of women with cervical intraepithelial neoplasia and invasive cancer. Nutr Cancer. 2003;47(2):126-30. doi: 10.1207/s15327914nc4702_3.
6. Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. Biochem J. 1997;15;324 (Pt 1)(Pt 1):1-18. doi: 10.1042/bj3240001.
7. Ziegler DM. Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation. Annu Rev Biochem. 1995;54:305-29. doi: 10.1146/annurev.bi.54.070185.001513.
8. McCord JM. Human disease, free radicals, and the oxidant/antioxidant balance. Clin Biochem. 1993; 26(5):351-7. doi: 10.1016/0009-9120(93)90111-i.
9. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol. 2006;28;71(5):551-64. doi: 10.1016/j.bcp.2005.10.044.
10. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med. 2010;15;48(6):749-62. doi: 10.1016/j.freeradbiomed.2009.12.022.
11. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014;47(18):326-32. doi: 10.1016/j.clinbiochem.2014.09.026.
12. De Marco F. Oxidative stress and HPV carcinogenesis. Viruses. 2013;12;5(2):708-31. doi: 10.3390/v5020708.
13. De Marco F, Bucaj E, Foppoli C, Fiorini A, Blarzino C, Filipi K,

et al. Oxidative stress in HPV-driven viral carcinogenesis: redox proteomics analysis of HPV-16 dysplastic and neoplastic tissues. PLoS One. 2012;7(3):e34366. doi: 10.1371/journal.pone.0034366.

14. Williams VM, Filippova M, Soto U, Duerksen-Hughes PJ. HPV-DNA integration and carcinogenesis: putative roles for inflammation and oxidative stress. Future Virol. 2011;1;6(1):45-57. doi: 10.2217/fvl.10.73.

15. Foppoli C, De Marco F, Cini C, Perluigi M. Redox control of viral carcinogenesis: The human papillomavirus paradigm. Biochim Biophys Acta. 2015;1850(8):1622-32. doi: 10.1016/j.bbagen.2014.12.016.

16. Siegel EM, Patel N, Lu B, Lee JH, Nyitray AG, Craft NE, et al. Biomarkers of oxidant load and type-specific clearance of prevalent oncogenic human papillomavirus infection: markers of immune response? Int J Cancer. 2012;1;131(1):219-28. doi: 10.1002/ijc.26363.

17. Sedjo RL, Roe DJ, Abrahamsen M, Harris RB, Craft N, Baldwin S, et al. Vitamin A, carotenoids, and risk of persistent oncogenic human papillomavirus infection. Cancer Epidemiol Biomarkers. 2002;11(9):876-84. PMID: 12223432.

18. Manju V, Kalaivani Sailaja J, Nalini N. Circulating lipid peroxidation and antioxidant status in cervical cancer patients: a case-control study. Clin Biochem. 2002;35(8):621-5. doi: 10.1016/s0009-9120(02)00376-4.

19. Looi ML, Mohd Dali AZ, Ali SA, Wan Ngah WZ, Mohd Yusof YA. Oxidative damage and antioxidant status in patients with cervical intraepithelial neoplasia and carcinoma of the cervix. Eur J Cancer Prev. 2008;17(6):555-60. doi: 10.1097/CEJ.0b013e328305a10b.

20. Burhans WC, Weinberger M. DNA replication stress, genome instability and aging. Nucleic Acids Res. 2007;35(22):7545-56. doi: 10.1093/nar/gkm1059.

21. Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Pat Inflamm Allergy Drug Discov. 2009;3(1):73-80. doi: 10.2174/187221309787158371.

22. Cremers CM, Jakob U. Oxidant sensing by reversible disulfide bond formation. J Biol Chem. 2013;13;288(37):26489-96. doi: 10.1074/jbc.R113.462929.

23. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. Free Radic Biol Med. 2009;15;47(10):1329-38. doi: 10.1016/j.freeradbiomed.2009.08.021.

24. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol. 2006;28;71(5):551-64. doi: 10.1016/j.bcp.2005.10.044.

25. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med. 2010;15;48(6):749-62. doi: 10.1016/j.freeradbiomed.2009.12.022.

26. Ozyazici S, Karateke F, Turan U, Kuvvetli A, Kilavuz H, Karakaya B, et al. A Novel Oxidative Stress Mediator in Acute Appendicitis: Thiol/Disulphide Homeostasis. Mediators Inflamm. 2016;2016:6761050. doi: 10.1155/2016/6761050.

27. Aksoy M, Çelik H. Dynamic thiol/disulphide homeostasis in vitiligo patients. Postepy Dermatol Alergol. 2018;35(5):498-501. doi: 10.5114/ada.2018.72856.

28. Camporeale G, Lorenzo JR, Thomas MG, Salvatierra E, Borkosky SS, Risso MG, et al. Degenerate cysteine patterns mediate two redox sensing mechanisms in the papillomavirus E7 oncoprotein. Redox Biol. 2017;11:38-50. doi: 10.1016/j.redox.2016.10.020.

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Comparison of diadynamic current, interferential current and transcutaneous electrical nerve stimulation therapies in patients with chronic low back pain

Alper UYSAL[1,*], Murat GÜNTEL[2]

[1]Department of Physical Medicine and Rehabilitation, Hatay Training and Research Hospital, Hatay, Türkiye
[2]Department of Neurology, Faculty of Medicine, Hatay Mustafa Kemal University, Hatay, Türkiye

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Abstract

This study aims to compare the effects of diadynamic current (DDC), interferential current (IFC), and transcutaneous electrical nerve stimulation (TENS) therapies on pain and disability levels in patients with chronic low back pain (CLBP). Patients with chronic low back pain between the ages of 18-65 were included in the study. The patients were divided into three groups. The first group received DDC, the second group IFC, and the third group TENS. The patients were evaluated in terms of pain and disability levels before the treatment, the 0th day after the treatment, and the 1st month after the treatment. Thus, these three treatment modalities were compared in terms of their effectiveness. A total of 83 patients were included in the study. There was no statistically significant difference between the groups in terms of age, gender, BMI, disease duration, pain, and disability levels of the patients before treatment. A statistically significant difference was found between the 0th day before and after the treatment and the 1st month before and after the treatment in terms of pain and disability levels in all three groups. The VAS scores of the individuals in the IFC group were significantly lower on the 0th day and 1st month after the treatment than in the DDC and TENS groups. Although there was no statistically significant difference, when looked at clinically, the RMDQ scores of the individuals in the IFC group tended to decrease more than those in the DDC and TENS groups. All three treatment modalities are effective in patients with CLBP. However, IFC seems to be superior.

Keywords: chronic low back pain, diadynamic current, interferential current, transcutaneous electrical nerve stimulation

1. Introduction

Low back pain (LBP) is defined as muscle spasms and pain in the region between the lower border of the 12th rib and the lower gluteal fold. Chronic low back pain (CLBP) is a health problem in all age groups and creates significant financial burdens on health systems. Up to 90% of the adult population will suffer from LBP at some point in their lives (1). LBP is classified according to duration as acute, subacute, and chronic (<6 weeks, 6-12 weeks, > 12 weeks). CLBP constitutes 2% to 7% of LBP (2). While pain is self-limiting in 6 weeks or less in the majority of patients, it may last six weeks or longer in 10-40% of patients (3).

Acute LBP can be triggered by physical, psychosocial, or both factors. LBP is primarily due to non-specific causes. According to a study, it was shown that approximately 4% of patients with LBP in primary health care facilities had compression fractures, 3% had spinal stenosis, 2% had visceral disease, 0.7% had tumor or metastasis, and 0.01% had an infection (1, 4).

Patient education and pharmacological and non-pharmacological approaches constitute the basis of treatment. Pharmacological treatments are essential in the treatment of both acute and chronic LBP. Acetaminophen and NSAIDs are effective in the short-term control of pain. In addition, muscle relaxants, tramadol, some types of antidepressants, and antiepileptics have also been useful in treating LBP. Non-pharmacological treatment approaches aim to reduce the patient's pain and increase their functionality, and they can be combined with pharmacological treatments. These methods include exercise, heat application, back schools, manual therapy, massage, acupuncture, spinal manipulation, EMG biofeedback, back support, and cognitive behavioral therapy. Epidural steroid injections and local injections can also be used for treatment (3, 5).

TENS is a non-pharmacological treatment modality widely used in the management of LBP. It is a safe, non-invasive, and easy-to-use form of treatment. TENS units provide electrical stimulation to the underlying peripheral nerves through electrodes placed on the intact skin surface near the maximum source of pain. The gate control theory explains the pain relief

*Correspondence: alperuysal82@gmail.com

effect of TENS. The given electrical currents prevent the transmission of pain signals from the spinal cord to the brain. According to the gate control theory, the electrical currents provided by TENS stimulate the A-beta to type sensory nerves under the skin; This reduces the transmission rate of the signals of the C-type nerve fibers carrying the pain sensation from the spinal cord to the brain. Another explanation is that it increases endorphins, the body's natural pain relievers. A-delta nerve fibers are activated to use the endorphin mechanism. In addition, A-delta nerve stimulation causes the spinal cord to release a molecule called enkephalin that suppresses pain signals (6).

IFC is a form of electrical therapy in which two medium-frequency currents are used to produce a low-frequency current. Low-frequency currents play a fundamental role in pain relief, which is one of the essential effects of interferential currents. The gate control theory explains this effect. Additionally, it removes pain-causing chemicals from the affected area through increased blood flow (7). The effect mechanism of IFC application is interesting. When two medium-frequency alternating currents are applied from the skin surface, these currents can reach deeper tissues due to their medium-frequency characteristics. As a result of the interaction of these two medium-frequency currents in deeper tissues, a low-frequency alternating current is obtained. Since IFC currents can reach deeper tissues, they create more muscle torque than low-frequency alternating currents (8).

Diadynamic currents (DDC) are single-phase sinusoidal if currents with a low frequency of up to 100 Hz. DDC consists of direct current and repetitive sinusoidal alternating currents. There are five types of DDC: Diphase fixe, Monophase fixe, Short period, Long period, and Rhythm syncope current. It has been reported that DDC may have beneficial effects in reducing pain through muscle fiber stimulation, pain masking, vasodilation, and hyperemia mechanisms (9).

In the light of all this information, we aimed to see and compare the effectiveness of DDC, IFC, and TENS treatment modalities on disability and pain in chronic low back pain patients.

2. Material and Methods

2.1. Study design, setting, and population

Eighty-three patients aged between 18-65 years with chronic low back pain who applied to the Hatay Training and Research Hospital Physical Medicine and Rehabilitation Outpatient Clinic were included in the study. To diagnose CLBP, detailed anamnesis of the patients was taken, general physical examinations, musculoskeletal and neurological examinations were performed, and hemogram, sedimentation rate, CRP, lumbar X-Ray, or lumbar MRI were taken. The patients were divided into three groups: Group 1 (DDC), Group 2 (TENS), and Group 3 (IFC). This study was carried out between May and November 2021 with the approval of the Hatay Mustafa Kemal University Ethics Committee (Decision no:01, date: 06.05.2021). A written informed consent form was obtained from all participants.

Firstly, a total of 10 minutes of DDC treatment consisting of diphasic fixed (2 min), courtes period (4 min), and long period (4 min) was applied to the patients in Group I. A total of 10 sessions of DDC treatment, five sessions per week, were applied.

A total of 10 sessions of conventional TENS were applied to the lumbar spine of the patients in Group II, with a frequency of 100 Hz and a pulse duration of 60 ms, five sessions per week. The amplitude intensity was adjusted to produce a slight tingling sensation without causing contractions or excessive discomfort. The treatment time was 30 minutes for each session with a two-channel portable TENS device.

IFC was applied to the patients in Group III for 30 minutes. Four electrodes were placed crosswise, so the pain area was in the middle. The input current frequency was set to be 4000 Hz, with a 100 Hz amplitude modulated frequency. A total of 10 sessions of IFC were applied, with five sessions per week.

A hot pack was applied to all three groups for ten sessions, each session for 30 minutes. BTL brand device was used for the application in all three groups.

The following were accepted as exclusion criteria from the study: Fracture, scoliosis, neurological disease, inflammatory rheumatologic disease, previous lumbar spine surgery, pregnancy, malignancy, infection, injection, or physical therapy for CLBP in the last three months, and symptom duration less than three months. Electrotherapy contraindication criteria such as a cardiac pacemaker, presence of dermatological problems, and epilepsy were also accepted as exclusion criteria.

The pain and disability levels of the patients were evaluated with questionnaire forms three times, before the treatment, on the 0th day after the treatment, and in the 1st month after the treatment. Patients with CLBP were asked about their age, weight, height, body mass index, how long they had low back pain complaints, and the treatments they received in the last three months. The patients' pain severity was evaluated using the VAS (Visual Analog Scale), and the disability status was assessed using the Roland-Morris Disability Questionnaire (RMDQ).

In this way, the effectiveness of the three treatment methods was compared by evaluating whether there was a change in pain and disability levels and, if there was, how much.

2.2. Statistical analysis

SPSS (Statistical Package for Social Sciences) 22.0 program was used to evaluate the data obtained from the study. The Shapiro Wilk test analyzed the normal distribution fit of continuous numerical variables. All data were given as mean ± standard deviation, median, minimum-maximum, frequency,

and percentage. Pearson Chi-Square Test determined statistical difference between groups in terms of categorical variables. The One-Way ANOVA Test determined the statistical difference between the groups in terms of continuous variables for normally distributed variables and the Kruskal Wallis Test for non-normally distributed variables. The Bonferroni correction method was used to compare repeated measurements within the same group. Analysis of Variance (ANOVA) was used for Repeated Measures. A p-value of <0.05 was considered significant according to statistical tests.

3. Results

Eighty-three patients with CLBP diagnosis were included in the study. There was no statistically significant difference between the groups in terms of age, gender, BMI, and disease duration of the patients (p>0.05) (Table 1).

Table 1. Comparison of demographic characteristics

Variables		DDT (n= 28)	TENS (n= 27)	IFT (n=28)	p
Age (year) (Mean. ± SS)		49.60 ± 10.35	46.22 ± 10.15	47.64 ± 9.68	0.460*
BMI (kg/m²) (Mean. ± SS)		27.17 ± 2.59	27.71 ± 3.65	28.10 ± 3.19	0.549*
Disease duration [Median (Min-Max)]		33 (6-180)	24 (6-180)	30 (6-120)	0.784**
Gender (n/%)	Male	10 (35.7)	9 (33.3)	10 (35.7)	0.978***
	Female	18 (64.3)	18 (66.7)	18 (64.3)	

n: Number of Patients; SD: Standard Deviation; BMI: Body Mass Index; *One-Way Anova Test; **Kruskal Wallis Test; *** Pearson Chi-Square Test

There was no statistically significant difference between the pre-treatment VAS averages of the patients in all three groups (p>0.05). In the intragroup comparison, all three groups found a statistically significant difference between the mean VAS scores on day 0 before and after treatment and the 1st month before and after treatment. A statistically significant difference was found between the mean VAS scores of the individuals in all three groups on day 0 after treatment and one month after treatment (p=0.010, p=0.028, respectively). The VAS scores of the individuals in the IFC group tended to decrease more than those in the DDC and TENS groups.

There was no statistically significant difference between the RMDQ averages of the individuals in all three groups before, on day 0 after, and one month after treatment (p>0.05). In the intragroup comparison, all three groups found a statistically significant difference between the mean RMDQ scores at day 0 before and after treatment, and at the 1st month before and after treatment (p<0.001). There was no statistically significant difference between the mean RMDQ scores on day 0 after treatment and month one after treatment (p>0.05). Although there was no statistically significant difference, clinically, the RMDQ scores of the patients in the IFC group tended to decrease more than those in the DDC and TENS groups (Table 2).

Table 2. Comparison of VAS and RMDQ scores of the groups

		GROUP (Mean± SS)			p
		DDT (n= 28)	TENS (n= 27)	IFT (n=28)	
VAS	Before treatment	7.28 ± 1.18	7.48 ± 1.01	7.35 ± 1.16	0.808*
	Post-treatment (day 0)	4.96 ± 1.50	5.00 ± 1.35	3.92 ± 1.48	**0.010***
	Post-treatment (1st month)	4.78 ± 1.81	4.66 ± 1.75	3.64 ± 1.56	**0.028***
	p**	**<0.001[a]**	**<0.001[b]**	**<0.001[c]**	
RMDQ	Before treatment	14.10 ± 4.16	14.44 ± 3.61	14.42 ± 4.26	0.940*
	Post-treatment (day 0)	9.21 ± 3.78	9.22 ± 3.41	7.17 ± 3.58	0.056*
	Post-treatment (1st month)	8.92 ± 4.11	8.81 ± 3.90	6.85 ± 3.77	0.094*
	p**	**<0.001[a]**	**<0.001[b]**	**<0.001[c]**	

n: Number of Patients; SD: Standard Deviation; * One-Way Anova Test; **Analysis of variance in repeated measurements

a: There was a difference between the 0th day before and after the treatment, there was a difference between the 1st month before and after the treatment

b: There was a difference between the 0th day before and after the treatment, there was a difference between the 1st month before and after the treatment

c: There was a difference between the 0th day before and after the treatment, there was a difference between the 1st month before and after the treatment

4. Discussion

The aim of treatment in CLBP should be to reduce pain, provide mobility, prevent physical disability, and improve quality of life and biological functions. Patients' VAS scores and RMDQ values decreased after treatment with all three treatment modalities, and this decrease was statistically significant. In other words, we determined that these three treatment modalities were effective in terms of pain and disability scores.

Many physical therapy agents are frequently used in the treatment of LBP. Various electrotherapy applications are also frequently used in this field, but their superiority over each other is still controversial. Studies conducted by Brazilian researchers have determined that TENS and IFC modalities are highly effective in the treatment of low back pain. Faci et al. compared the effects of TENS and IFC modalities in patients with non-specific CLBP. In this study, which included 150 patients, three groups were formed. TENS was given to the first group and IFC to the second group. The third group was not given any physical stimulus. The results of this study show that TENS and IFC treatment produced significant effects such as a reduction in pain level, disability, and the number of NSAIDs used compared to the control group. However, no significant

difference was observed in the TENS and IFC treatment groups (10).

In another study, although IFC and TENS treatment modalities were influential in treating pain due to lumbar discopathies, the DDC treatment modality seems ineffective (11). Another study found that both DDC and TENS modalities can relieve pain and improve functional abilities in patients with lumbar discopathy (12). In their studies, Sayıtır and Yıldızgören showed that both DDC and TENS treatments were effective on pain after one month of treatment. They also showed that the pain relief achieved with DDC in CLBP patients was as effective as that provided by TENS (9).

While some studies indicate that DDC is effective in acquiring physical functions, they also show that it is useless (11, 12). Our study found all three treatment modalities effective in terms of pain and disability scores.

Conflicting results have been obtained in studies with CLBP patients comparing the efficacy of TENS and IFC. In the study of Tella et al., positive results were obtained on pain and disability in both the IFC and TENS groups, and the effectiveness of both treatment modalities was evaluated as similar (13). Again, a systematic review emphasized that TENS and IFC modalities successfully reduced pain and that physical medicine and rehabilitation specialists could prefer both methods. In this review, it was stated that both methods reduced pain equally. Equal improvements in VAS scores were found in six of the eight studies evaluated in this review, regardless of current type and frequency (14). Some studies have shown that IFC therapy tends to be better than TENS at controlling pain and reducing pain medication intake, but it did not reach statistical significance (14). Zeng et al. evaluated the benefits of electrical stimulation and concluded that IFC was a more promising treatment for pain relief (15). Acedo et al. compared the effects of TENS and IFC treatments on upper trapezius relaxation and pain control in patients with chronic non-specific neck discomfort. They found that IFC provided upper trapezius relaxation at the end of 3 sessions, but TENS application did not change muscle tension. Both modalities successfully reduced pain, but IFC was associated with a better clinical improvement (16). In a study comparing the effectiveness of IFC, TENS, and splint therapy in patients with carpal tunnel syndrome, it was found that IFC was more effective than TENS in terms of VAS, symptom severity, and functional capacity (17).

Rajfur et al. found that DDC had poor efficacy in improving pain and function. Although TENS and high voltage are more effective treatment options, they have not been as effective as IFC in penetrating deep tissues (11).

To our knowledge, our work is the second study in the literature to compare these three treatment modalities in patients with CLBP, and it is highly significant in this respect. We found that all three treatments were effective on pain and disability levels in CLBP patients. We discovered that IFC was statistically superior to the other two treatment methods on pain score. When evaluated clinically, we found that IFC decreased more in disability scores, although not statistically. We have tried to show with the references above that there are quite contradictory results on this subject in the literature. This contradiction may be because pain or disability assessments are a complex and multidimensional process, but evaluating them with one-dimensional scales may yield different results.

The study's limitations are as follows: 1- The number of patients could have been kept higher. 2- A placebo or control group could be included. 3- Due to the complex nature of pain and disability, multiple assessment scales could have been used instead of a single scale. 4- Combined efficacy of treatment modalities could be evaluated. 5- The effects of these treatment methods on the frequency of painkiller use could be considered. 6- The differences in frequency, pulse duration, electrode size, and intensity variability on treatment efficacy were not evaluated. 7- The results were evaluated before the treatment, on the 0th day after the treatment, and on the 1st month after. A longer follow-up period and preservation of treatment efficacy were not evaluated. 8- The effectiveness of these treatment methods on specific causes of low back pain was not assessed.

CLBP is a common health problem. TENS, DDC and IFC are effective on pain and disability in the treatment of CLBP. When the literature is examined in terms of the effectiveness of these methods, it is seen that there are contradictions between the results. We found that the efficacy of the IFC treatment method is better than TENS and DDC. There is a need for larger, well-designed, and standardized studies that minimize the limitations mentioned above to determine the most effective treatment method.

Conflict of interest

Authors declare that there is no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.U., M.G., Design: A.U., M.G., Data Collection or Processing: A.U., Analysis or Interpretation: M.G., Literature Search: M.G., A.U., Writing: M.G., A.U.

References

1. Aydin E, Nazlikul FGU. Bel ağrisi olan hastalarda TENS'in ağri ve yaşam kalitesi üzerine etkinliği: ön çalişma. Bilimsel Tamamlayıcı Tıp Regülasyon ve Nöral Terapi Dergisi.15(1):5-8.
2. Andersson GB. Epidemiological features of chronic low-back pain. Lancet. 1999;354(9178):581-5. https://doi.org/10.1016/S0140-6736(99)01312-4
3. Urits I, Burshtein A, Sharma M, Testa L, Gold PA, Orhurhu V, et al. Low back pain, a comprehensive review: pathophysiology,

diagnosis, and treatment. Curr Pain Headache Rep. 2019;23(3):1-10. https://doi.org/10.1007/s11916-019-0757-1

4. Pengel LH, Herbert RD, Maher CG, Refshauge KM. Acute low back pain: systematic review of its prognosis. Bmj. https://doi.org/2003;327(7410):323

5. Koes BW, Van Tulder M, Thomas S. Diagnosis and treatment of low back pain. Bmj. 2006;332(7555):1430-4. https://doi.org/10.1136/bmj.332.7555.1430

6. Khadilkar A, Odebiyi DO, Brosseau L, Wells GA. Transcutaneous electrical nerve stimulation (TENS) versus placebo for chronic low-back pain. Cochrane Database of Systematic Reviews. 2008(4). https://doi.org/10.1002/14651858.CD003008.pub3

7. Kibar S, Konak HE, Ay S, Erdoğan BD, Evcik D. Transkutanöz elektrik sinir stimülasyonu ve interferansiyel akımın kombine tedavisinin kronik bel ağrısına etkisi: Randomize, çift kör, sham kontrollü çalışma [The Effectiveness of combined transcutaneous electrical nerve stimulation and ınterferential current therapy on chronic low back pain: a randomized, double-blind, shamcontrolled study]. Fiziksel Tıp ve Rehabilitasyon Bilimleri Dergisi. 2020;23:32-40. https://doi.org/10.31609/jpmrs.2019-71464

8. Rampazo ÉP, Liebano RE. Analgesic Effects of Interferential Current Therapy: A Narrative Review. Medicina. 2022;58(1):141. https://doi.org/10.3390/medicina58010141

9. Sayilir S, Yildizgoren MT. The medium-term effects of diadynamic currents in chronic low back pain; TENS versus diadynamic currents: A randomised, follow-up study. Complement Ther Clin Pract. 2017;29:16-9. https://doi.org/10.1016/j.ctcp.2017.07.002

10. Facci LM, Nowotny JP, Tormem F, Trevisani VFM. Effects of transcutaneous electrical nerve stimulation (TENS) and interferential currents (IFC) in patients with non-specific chronic low back pain: randomized clinical trial. Sao Paulo Med J. 2011;129:206-16. https://doi.org/10.1590/S1516-31802011000400003

11. Rajfur J, Pasternok M, Rajfur K, Walewicz K, Fras B, Bolach B, et al. Efficacy of selected electrical therapies on chronic low back pain: a comparative clinical pilot study. Medical science monitor: international medical journal of experimental and clinical research. 2017;23:85. https://doi.org/10.12659/MSM.899461

12. Ratajczak B, Hawrylak A, Demidaś A, Kuciel-Lewandowska J, Boerner E. Effectiveness of diadynamic currents and transcutaneous electrical nerve stimulation in disc disease lumbar part of spine. J Back Musculoskelet Rehabil. 2011;24(3):155-9. https://doi.org/10.3233/BMR-2011-0289

13. Tella BA, Oghumu SN, Gbiri CAO. Efficacy of transcutaneous electrical nerve stimulation and interferential current on tactile acuity of individuals with non-specific chronic low back pain. Neuromodulation: Technology at the Neural Interface. 2022. https://doi.org/10.1111/ner.13522

14. de Almeida CC, da Silva VZM, Júnior GC, Liebano RE, Durigan JLQ. Transcutaneous electrical nerve stimulation and interferential current demonstrate similar effects in relieving acute and chronic pain: a systematic review with meta-analysis. Braz J Phys Ther. 2018;22(5):347-54. https://doi.org/10.1016/j.bjpt.2017.12.005

15. Zeng C, Yang T, Deng Z-h, Yang Y, Zhang Y, Lei G-h. Electrical stimulation for pain relief in knee osteoarthritis: systematic review and network meta-analysis. Osteoarthr Cartil. 2015;23(2):189-202. https://doi.org/10.1016/j.joca.2014.11.014

16. Acedo AA, Antunes ACL, dos Santos AB, de Olveira CB, dos Santos CT, Colonezi GLT, et al. Upper trapezius relaxation induced by TENS and interferential current in computer users with chronic non-specific neck discomfort: an electromyographic analysis. J Back Musculoskelet Rehabil. 2015;28(1):19-24. https://doi.org/10.3233/BMR-140482

17. Koca I, Boyaci A, Tutoglu A, Ucar M, Kocaturk O. Assessment of the effectiveness of interferential current therapy and TENS in the management of carpal tunnel syndrome: a randomized controlled study. Rheumatol Int. 2014;34(12):1639-45. https://doi.org/10.1007/s00296-014-3005-3

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Effect of preemptive ibuprofen and dexketoprofen on postoperative pain after septorhinoplasty

Ozgur KEMAL[1], Ayse ÇEÇEN [2*], Esra KAVAZME [1], Mustafa TAŞTAN [1], Yavuz ÇEÇEN [3], Ersin KÖKSAL[3]

[1]Department of Otolaryngology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

[2]Department of Otolaryngology, Faculty of Medicine, Samsun University, Samsun, Türkiye

[3]Department of Anaesthesiology and Reanimation, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

In septorhinoplasty patients, postoperative comfort is as important as postoperative results. Intravenous non-steroidal anti-inflammatory drugs are playing an increasingly large role in analgesia, antiinflammation, and antipyresis in the hospitalized setting. The aim of this study is to assess the preemptive analgesic effects of intravenous ibuprofen and dexketoprofen on postoperative pain in patients undergoing septorhinoplasty. This study was performed with 76 patients who underwent open septorhinoplasty between 2016 and 2017. The patients were separated into two groups; Group D (n=46) received preemptive intravenous dexketoprofen (50 mg), and Group I (n=30) received preemptive intravenous ibuprofen (800 mg). A visual analogue scale (VAS) was used to assess pain at the 30 th minute, as well as the first, second, sixth, twelfth, and twenty-fourth hours after surgery. There was no statistically significant difference between the groups for sex, age, weight, height, ASA class, anesthesia, and surgery duration. VAS scores gradually decreased between 30 minutes and 24 hours in both groups and this decrease was statistically significant in both groups (Group D; $p < 0.001$, Group I; $p < 0.001$). The mean VAS scores in the same periods were lower in Group I at all times, but the difference was not statistically significant ($p > 0.05$). This study indicated that both ibuprofen and dexketoprofen had similar analgesic efficacy. Clinicians can use these two drugs interchangeably by comparing their side effects and costs.

Keywords: Analgesia, dexketoprofen, ibuprofen, pain, rhinoplasty

1. Introduction

Postoperative pain depends on the premedication applied, the type of operation and general anesthesia used, and the personal sensitivity of the patient (1). Poorly controlled pain has a negative effect on the quality of life, functions, and functional recovery and causes postoperative morbidity, complications and persistent pain and increases hospitalization and medical costs (2). 'Preemptive analgesia' means preventing or reducing pain that is likely to develop by administering analgesia before a painful stimulus. The purpose of preemptive analgesia is to prevent the occurrence of pain memory, thereby reducing the need for analgesics (3).

The postoperative pain management guideline recommendations for head and neck surgeries are not specific for surgical types, such as septorhinoplasty (4). Surgeons generally evaluate the success of septorhinoplasty based on late functional and cosmetic results (5). However, patients care about their early comfort as much as the late results. Pain, edema and bruising are factors that determine patient satisfaction and comfort in the early postoperative period.

Metamizole, paracetamol, dexketoprofen trometamol, ibuprofen, lornoxicam and opioids are used to control the pain after septorhinoplasty. Dexketoprofen and ibuprofen are non-steroidal anti-inflammatory drugs (NSAIDs) with few side effects (3, 6). The oral form of ibuprofen is one of the most commonly used NSAID for many years (5, 6). Intravenous (IV) ibuprofen has been approved in the USA since 2009 for the treatment of pain.

Limited studies have been reported about the IV ibuprofen used in postoperative pain management. The aim of this study was to evaluate and compare the pre-emptive analgesic efficacy of ibuprofen and dexketoprofen for postoperative pain control in patients who underwent septorhinoplasty.

2. Material and Methods

This study was performed with 76 patients who underwent open septorhinoplasty between January 2016 and December 2017 in a tertiary referral hospital. The patients included were those aged over 18 years with ASA score I–II, without a known systemic disease, with no history of peptic ulcer, allergy, chronic pain, routine analgesic use or analgesic use in the last 24 hours, and without sensitivity to NSAID agents. Written consent was obtained from all patients included in the study. The approval for all procedures in the study was granted by the Ondokuz Mayıs University Ethics Committee for Clinical Studies (2016/230).

*Correspondence: aysebel55@hotmail.com

The patients were randomly separated into two groups: Dexketoprofen Group (Group D) and Ibuprofen Group (Group I).

Group D (n=46) received 200 ml saline containing 50 mg dexketoprofen trometamol (Arveles 50 mg/2 mL, Menarini International, Florence, Italy) as an infusion 1 hour preoperatively. Then, 50 mg IV dexketoprofen trometamol was administered two more times at 12-hour intervals at the 12th and 24th hours. In the 6th and 18th hours, 200 ml saline was administered.

Group I (n=30) received 200 ml saline containing 800 mg ibuprofen (Intrafen 800 mg/4 mL, Gen Ilaç, Istanbul, Turkey), as an infusion for approximately 30 minutes, 1 hour before the anesthesia induction. Then, 800 mg IV ibuprofen was administered four times at 6-hour intervals in the post-operative period.

The standard general anesthesia method was applied to all cases and all surgeries were performed by the same surgeon using the open technique. The times of medication, duration of anesthesia, duration of surgery and application of nasal packing were recorded. Extubation time was accepted as postoperative 0 minute. Patients were asked to score postoperative pain using a Visual Analogue Scale (VAS) (0: no pain and 10: worst pain). All patients were informed about VAS preoperatively. In the postoperative period, the patients were asked to note the pain level they felt at the 30th min, 1, 2, 6, 12 and 24th hours. 5 mgr/kg tramadol, an atypical opioid analgesic, was applied to patients with a VAS score greater than 4 as an additional analgesic. The additional analgesic requirements of patients were recorded during the first 24 h postoperatively. The postoperative follow-up and evaluation of the cases was performed by a researcher blinded to the groups.

2.1. Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS vn. 22 for Windows software (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD) values, and frequency data as number (n) and percentage (%). The Shapiro-Wilk test was used to determine whether variables conformed to a normal distribution. Since the data did not show normal distribution, the Mann Whitney U-test was used in the comparison of two groups, and Variance Analysis-Friedman Test was used in the comparison of repeated measurements in the group. Fisher' s Exact Test was used to compare the data obtained by counting. A value of $p<0.05$ was accepted as statistically significant.

3. Results

As shown in Table 1, there was no statistically significant difference between the groups in terms of demographic characteristics such as age, gender and body mass index (BMI) ($p>0.05$). The comparisons of the groups according to VAS values and additional analgesic requirements are shown in Table 2. When the groups were evaluated within themselves, it was determined that mean VAS values gradually decreased between 30 minutes and 24 hours in both groups and this decrease was statistically significant in both groups (Group D; $p <0.001$ and Group I; $p <0.001$). In comparisons between the groups, the mean VAS values in the same periods were lower in Group I at all times, but there was no statistically significant difference ($p >0.05$). There was no significant difference between the groups in terms of additional analgesic requirement ($p>0.05$).

There was no significant difference in the rates of patients with and without nasal packing in Group D and Group I ($p>0.05$) (Table 1). It was observed that the VAS values decreased gradually from 30 minutes to 24 hours in both patients without nasal packing and patients with nasal packing in Group D and Group I, and this decrease was statistically significant in both groups (Group D; $p<0.001$ and Group I; $p<0.001$) (Table 3 and 4).

The pain scores in the same periods were lower in patients without nasal packing in Group I than Group D at all times and the difference was statistically significant in the 2nd hour ($p = 0.04$) and the 6th hour ($p = 0.03$) (Table 3).

In patients with nasal packing, although the mean VAS values in the same periods were lower in Group I at all times, there was no statistically significant difference (Table 4).

4. Discussion

As a result of this study, it was observed that both ibuprofen and dexketoprofen caused a significant decrease in VAS scores over time (Group D; $p<0.001$ and Group I; $p<0.001$), and ibuprofen caused a significant decrease in VAS at the 2nd and 6th hours compared to dexketoprofen in patients without nasal packing ($p = 0,04$, $p = 0,03$).

The International Association for the Study of Pain has defined pain as ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage'. Surgical tissue trauma leads to nociceptor activation and sensitization, increased inflammatory cytokines, decreased tissue pH and oxygen tension. Inadequate analgesia decreases quality of life, increases the length of hospitalization and healthcare costs (7).

In a study, higher VAS pain scores have been reported in patients undergoing septorhinoplasty and open septoplasty than in other otolaryngological procedures (8).

Different doses of IV ibuprofen have been used to assess the analgesic efficacy in previous studies. In one study, a single dose of 800 mg ibuprofen given intravenously 15 minutes before thyroidectomy resulted in lower opioid consumption and less postoperative pain (9). Analgesic effect of preemptive single dose iv 800 mg ibuprofen on

Table 1. Demographic Characteristics of the Patients

	Group D (n:46)	Group I (n:30)	p
Female Gender, N (%) Male	28 (65.1%) 18 (54.5%)	15 (34.9%) 15 (45.5%)	0.48*
Age, (Mean±SD (min-max))	27,8±9,4 (18-52)	27.7±10.3(18-51)	0.92#
BMI (kg/m2), (Mean±SD (min-max))	22,7 ±3,9 (16,6-35,6)	22.9 ±3.5 (18.0-34.9)	0.62#
Nasal packing, N (%)	25 (65.8%)	13 (34.2%)	0.48*

* Continuity Correction, # Mann Whitney U-test

Abbreviations: BMI, Body Mass Index; Group D, Dexketoprofen Group; Group I, Ibuprofen Group; N, number; SD, Standart Deviation.

Table 2. VAS Score, Analgesic Requirement of Patients

		Group D (n:46) Mean±SD (min-max)	Group I (n:30) Mean±SD (min-max)	p#
	30 min	5.4 ±2.8(0-10)	5.4 ±2.6(2-10)	0.86
	1h	4.8 ±2.2(1-10)	4.4 ±2.1 (1-9)	0.46
VAS (cm)	2 h	4.0 ±2.2(0-10)	3.4 ±2.0(1-8)	0.20
(Mean±SD)	6 h	3.3 ±2.4(0-9)	2.6 ±2.0(0-8)	0.19
	12 h	3.1 ±2.6 (0-10)	2.6 ±2.0(0-8)	0.10
	24 h	2.3 ±2.5 (0-10)	1.6±2.0 (0-8)	0.20
		*p:<0.001	*p:<0.001	
Additional analgesia n(%)		3 (6.5%)	2 (6.7%)	1.00

* Variance Analysis-Friedman Test in repeated measurements # Mann Whitney U-test

Abbreviations: Group D, Dexketoprofen Group; Group I, Ibuprofen Group; N, number; SD, Standard Deviation; VAS, Visual Analog Scale

Table 3. VAS Scores of Patient Without Nasal Packing

		Group D (n:21) Mean±SD (min-max)	Group I (n:17) Mean±SD (min-max)	p#
	30 min	4.19 ±2.97	3.97 ±3.59	0.70
	1 h	3.71 ±2.36	2.82 ±2.94	0.20
VAS(cm)				
	2 h	3.62 ±2.59	2.24 ±2.53	0.04
	6 h	2.90 ±2.80	1.06±1.67	0.03
(Mean.±SD)				
	12 h	2.10 ±2.44	1.00 ±1.36	0.20
	24 h	1.95 ±2.57	0.59 ±1.17	0.07
	p*	*p:□0.001	*p:□0.001	

* Variance Analysis-Friedman Test in repeated measurements #Mann Whitney U-test

Abbreviations: Group D, Dexketoprofen Group; Group I, Ibuprofen Group; N, number; SD, Standard Deviation; VAS, Visual Analog Scale

Table 4. VAS Scores of Patient with Nasal Packing

		Group D (n:25) Mean±SD (min-max)	Group I (n:13) Mean±SD (min-max)	p#
	30 mins	5.52 ±3.41	6.31 ±3.25	0.46
VAS(cm)	1 hr	5.04 ±2.73	5.62 ±2.98	0.64
	2 hrs	3.84 ±2.98	4.15 ±2.70	0.83
(Mean±SD)	6 hrs	3.20 ±2.53	4.15 ±2.91	0.36
	12 hrs	3.56 ±3.02	3.62 ±2.56	0.85
	24 hrs	2.48 ±2.63	3.38 ±3.09	0.34
	p*	*p:<0.001	*p:<0.001	

* Variance Analysis-Friedman Test in repeated measurements #Mann Whitney U-test

Abbreviations: Group D, Dexketoprofen Group; Group I, Ibuprofen Group; N, number; SD, Standard Deviation; VAS, Visual Analog Scale

septorhinoplasty was investigated in a study and they reported that pain scores and total fentanyl consumption were lower in patients using ibuprofen (5). In another study, it was reported that postoperative VAS values and morphine consumption were lower after hysterectomy in patients who were administered 50 mg IV dexketoprofen one hour before and 8 and 16 hours after surgery than in the placebo group (10). In a study, it was reported that the use of dexketoprofen decreased the pain after septorhinoplasty regardless of the administration timing (preoperative or intraoperative) (11). Supporting these studies, in the current study, only 6.7 % of the patients in Group I and Group D needed additional analgesics and the decrease in VAS scores over time was significant (Group I: p□0.001, Group D: p□0.001). However,

in contrast to these studies, we preferred to compare ibuprofen and dexketoprofen, two different analgesic drugs, rather than comparing any one of them, whose analgesic efficacy is already known, to the placebo group.

As in our study, there are also several studies that compare the effects of two different analgesic drugs. In a study, comparing the analgesic efficacy of IV ibuprofen and ketorolac, it was reported that a preemptive and a second dose of IV 800 mg ibuprofen decreases postoperative pain and opioid consumption (12). It was reported that ibuprofen had a more potent analgesic effect compared to intravenous paracetamol after septorhinoplasty (3). In a study comparing the pain and tramadol consumption in patients who underwent septorhinoplasty, it was found that the analgesic efficacy of preemptive dexketoprofen trometamol was more potent than acetaminophen (13). Although there are a few studies comparing the analgesic effects of oral forms of ibuprofen and dexketoprofen, a study comparing the effectiveness of IV forms has not been found in accessible literature. When we examine the studies comparing oral forms of ibuprofen and dexketoprofen, some reported that ibuprofen was more effective, while others reported that dexketoprofen was more effective (14, 15).

When the literature is reviewed, it seems that the use of ibuprofen and dexketoprofen alone or in combination with opioids is very effective in postoperative pain management. In the current study, when the two groups were compared, there was no difference between the ibuprofen and dexketoprofen groups in terms of pain control. Moreover, when the groups were evaluated within themselves, it was determined that mean VAS values gradually decreased between 30 minutes and 24 hours in both groups and this decrease was statistically significant in both groups (Group D; $p < 0.001$ and Group I; $p < 0.001$). Incision, intervention to the nasal roof and skeleton, nasal roof osteotomies, and nasal packing were reported to be the most common causes of postoperative pain after septoplasty and septorhinoplasty (5, 16). When comparing only patients with or without nasal packing in both groups, although the mean VAS values in the same periods were lower in Group I at all times, there was no statistically significant difference between Group D and I in patients with nasal packing. Whereas, the pain scores in the 2nd hour and 6th hour were lower in Group I than Group D in patients without nasal packing. This indicates that the ibuprofen has more potent early analgesic efficacy than dexketoprofen and further studies are needed with larger study groups.

The absence of a placebo group was one of the limitations of the present study. Although both ibuprofen and dexketoprofen were observed to cause a significant decrease in pain scores over time, this could not be compared with a control group. However, it would not have been ethically acceptable to perform the painful procedure of septorhinoplasty without medication. Furthermore, side effects of drugs, postoperative hemorrhage, medical costs and length of hospital stay were not recorded in the present study. We think that evaluating these variables in the next studies can help us choose one of these two agents with similar analgesic efficiency by making a profit-loss ratio.

Our study indicated that both ibuprofen and dexketoprofen had similar analgesic efficacy. In conclusion, we demonstrated that ibuprofen and dexketoprofen had strong and similar analgesic efficacy. Clinicians can use two drugs interchangeably in their studies by analyzing of side effects and medical costs.

References

1. Carr DB, Goudas LC. Acute pain. Lancet. 1999;353(9169):2051-8.
2. Savoia G, Alampi D, Amantea B, Ambrosio F, Arcioni R, Berti M, et al. Postoperative pain treatment SIAARTI Recommendations 2010. Short version. Minerva Anestesiol. 2010;76(8):657-67.
3. Celik EC, Kara D, Koc E, Yayik AM. The comparison of single-dose preemptive intravenous ibuprofen and paracetamol on postoperative pain scores and opioid consumption after open septorhinoplasty: a randomized controlled study. Eur Arch Otorhinolaryngol. 2018;275(9):2259-63.
4. Wittekindt D, Wittekindt C, Schneider G, Meissner W, Guntinas-Lichius O. Postoperative pain assessment after septorhinoplasty. Eur Arch Otorhinolaryngol. 2012;269(6):1613-21.
5. Gozeler MS, Sakat MS, Kilic K, Ozmen O, Can A, Ince I. Does a single-dose preemptive intravenous ibuprofen have an effect on postoperative pain relief after septorhinoplasty? Am J Otolaryngol. 2018;39(6):726-30.
6. Southworth S, Peters J, Rock A, Pavliv L. A multicenter, randomized, double-blind, placebo-controlled trial of intravenous ibuprofen 400 and 800 mg every 6 hours in the management of postoperative pain. Clin Ther. 2009;31(9):1922-35.
7. Nguyen BK, Yuhan BT, Folbe E, Eloy JA, Zuliani GF, Hsueh WD, et al. Perioperative Analgesia for Patients Undergoing Septoplasty and Rhinoplasty: An Evidence-Based Review. Laryngoscope. 2019;129(6):E200-E12.
8. Aydil U, Yilmaz M, Akyildiz I, Bayazit Y, Keseroglu K, Ceylan A. Pain and safety in otorhinolaryngologic procedures under local anesthesia. J Otolaryngol Head Neck Surg. 2008;37(6):851-5.
9. Mutlu V, Ince I. Preemptive intravenous ibuprofen application reduces pain and opioid consumption following thyroid surgery. Am J Otolaryngol. 2019;40(1):70-3.
10. Tuncer S, Reisli R, Kececioglu M, Erol A. [The effects of intravenous dexketoprofen on postoperative analgesia and morphine consumption in patients undergoing abdominal hysterectomy]. Agri. 2010;22(3):98-102.
11. Ozer AB, Erhan OL, Keles E, Demirel I, Bestas A, Gunduz G. Comparison of the effects of preoperative and intraoperative intravenous application of dexketoprofen on postoperative analgesia in septorhinoplasty patients: randomised double blind clinical trial. Eur Rev Med Pharmacol Sci. 2012;16(13):1828-33.
12. Uribe AA, Arbona FL, Flanigan DC, Kaeding CC, Palettas M, Bergese SD. Comparing the Efficacy of IV Ibuprofen and Ketorolac in the Management of Postoperative Pain Following Arthroscopic Knee Surgery. A Randomized Double-Blind Active Comparator Pilot Study. Front Surg. 2018;5:59.

13. Keskioglu I, Aktay Inal M, Ozlu O. [The effectiveness of preemptive analgesic techniques on postoperative analgesia in patients undergoing open septorhinoplasty]. Agri. 2016;28(3):135-42.

14. Jimenez-Martinez E, Gasco-Garcia C, Arrieta-Blanco JJ, Gomez del Torno J, Bartolome Villar B. Study of the analgesic efficacy of Dexketoprofen Trometamol 25mg. vs. Ibuprofen 600mg. after their administration in patients subjected to oral surgery. Med Oral. 2004;9(2):143-8, 38-43.

15. Kupeli I, Gulnahar Y. Impact of Pre-Emptive Intravenous Ibuprofen on Perioperative Analgesia in Patients Undergoing Third Molar Extraction: A Randomised Controlled Study. Turk J Anaesthesiol Reanim. 2019;47(6):480-4.

Sener M, Yilmazer C, Yilmaz I, Caliskan E, Donmez A, Arslan G. Patient-controlled analgesia with lornoxicam vs. dipyrone for acute postoperative pain relief after septorhinoplasty: a prospective, randomized, double-blind, placebo-controlled study. Eur J Anaesthesiol. 2008;25(3):177-82.

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Does endoscopic retrograde cholangiopancreatography procedures for benign pathology treatment cause biliary reflux?

Amira Ahmed OTHMAN[1,*], Amal Ahmed Zaki DWEDAR[2], Hany Mohammed ELSADEK[2], Hesham Radwan ABDELAZİZ[3] Abeer Abdulla Fikry Abdelrahman[4]

[1]Department of Internal Medicine, Faculty of Medicine, Suez University, Suez, Egypt
[2]Department of Internal Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[3]Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[4]Department of Clinical Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

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Abstract

Bile reflux is caused by the backward flow of duodenal fluid into the stomach. A retrospective cohort study was performed to estimate the prevalence and risk factors of bile reflux gastritis post-ERCP, and its endoscopic and histopathologic consequences on gastric mucosa. The study enclosed 58 patients with refractory epigastric pain and dyspeptic symptoms. They were split into two categories.; the control group (CG): 30 subjects who had never undergone any biliary interventions, and the post-ERCP group (PEG): 28 subjects who have had at least one of the following procedures for benign pathology treatment: endoscopic sphincterotomy (ES) or endoscopic stenting. In CG, the ages ranged from 25 to 68 years with a mean age ± SD of 42.1±12.42. In PEG: the ages ranged from 27 to 59 years with mean age ± SD of 39.25 ± 7.47. All participants had undergone clinical and laboratory assessment and gastroscopy for gastric aspirate analysis as well as gastric mucosa biopsy for histopathological examination. The study showed that the prevalence of bile reflux gastritis was found to be (16.7 %) in CG and (71.43 %) in PEG with a P value of 0.000. In both groups, diabetes, obesity, increased gastric bilirubin, and increased gastric pH were risk factors for bile reflux gastritis (r= 0.28, 0.42, 0.84, 0.66 respectively). However, there were no correlations between age, sex, epigastric pain, heartburn, vomiting, and the occurrence of bile reflux gastritis. In Conclusion, bile reflux gastritis is common post-ERCP being more among obese and diabetic patients.

Keywords: bile reflux, bilirubin, ERCP, endoscopy

1. Introduction

Biliary reflux, bile reflux, biliary gastropathy, duodenogastric reflux (DGR), or duodenogastroesophageal reflux (DGER) is a pathological condition in the form of the backward flow of duodenal fluid that consists of bile, pancreatic juices, and secretions of the intestinal mucosa into the stomach and esophagus (1). Stomach pH is increased when bile pours in it and its bile acids cause mucosal lesions (2). Bile acids, in combination with gastric acid, have been shown to cause bile reflux gastritis symptoms (heartburn, regurgitation, epigastric pain, etc.) (3).

Bile reflux gastritis frequently occurs after gastric surgeries that that damages the pyloric sphincter (2), and after biliary surgeries and procedures as cholecystectomy, endoscopic sphincterotomy (EST), endoscopic stenting, or choledochoduodenostomy that cause the sphincter of Oddi malfunction (secondary bile reflux gastritis) (4). Sometimes it can occur spontaneously without former surgeries. Bile gastritis is a normal physiological event in a prolonged fasting period (primary bile reflux gastritis) (5). In non-responsive individuals to PPI medication, the total prevalence of biliary reflux was 68.7%. These people have acid and bile reflux at the same time and have never had biliary surgery (6).

Endoscopic retrograde cholangiopancreatography (ERCP) is an interventional technique that combines an upper gastrointestinal endoscope (a thin, lighted flexible tube) and x-ray fluoroscopy, allowing other equipment to enter through the main duodenal papilla into the biliary and pancreatic ducts, to examine and treat diseases of the bile and pancreatic ducts (7). It represents one of the most demanding and technically challenging procedures in gastrointestinal endoscopy, which must be performed effectively and safely by operators with substantial training and experience to maximize success and safety (8).

2. Subjects and methods

1.1. Subjects:

The study started with 288 patients who were admitted to the

*Correspondence: amira.othman@med.suezuni.edu.eg

university hospitals with inclusion criteria of refractory epigastric pain and dyspeptic symptoms. Informed consent was obtained, and the Zagazig university hospital ethics committee approved this study protocol (Approval Date: 1.1.2018 and Approval Number 4238). A total of 96 patients were eliminated because of the study exclusion criteria or because they refused to participate in the study. Exclusion criteria included unstable cardiopulmonary, neurologic, or cardiovascular status, other causes of biliary diseases (CBD strictures, and hepatolithiasis), structural abnormalities of the esophagus, stomach, or small intestine, patients who underwent bariatric surgery out of the scope of the study, patients who underwent cholecystectomy, patients on long-term non-steroidal analgesics, patients on oral contraceptive drugs and antigen stool positive patient for H. Pylori. Gastroscopy was performed on 192 patients who met the study inclusion criteria and accepted to participate in the study. Another 134 patients were eliminated because of the presence of findings other than bile gastritis such as hiatus hernia, biliary dyskinesia, and psychosomatic patients. Hence, the study was performed on 58 patients who were split into two groups; the control group (CG) which included 30 patients who had never undergone any biliary interventions, and the post-ERCP group (PEG) which included 28 patients who had undergone at least one of the following procedures for the treatment of benign pathology: endoscopic sphincterotomy (ES) or endoscopic stenting. In CG, the ages ranged from 25 to 68 years with a mean age ± SD of 41.1±12.42. They were 17 Female (56.6%) and 13 Male (43.3%). In PEG: the ages ranged from 27 to 59 years with mean age ± SD of 39.25 ± 7.47. They were 15 Female (53.6%) and 13 Male (46.4%). Such cross-sectional analytical study was conducted at Internal Medicine Department, of the university hospitals.

1.2. Diagnostic techniques of bile reflux gastritis:

Gastroscopy:

Esophageal mucosa alterations as erythema, presence of bile into the esophagus, edema, GERD (Gastroesophageal reflux disease), incompetent cardia, and petechiae were recorded using the gastroscope (Olympus single-channel CLK-4).

Histopathology:

Gastric mucosa alterations as erythema, bile existence in the stomach, gastric folds thickening, erosions, and petechiae were also recorded. Multiple biopsies were taken from gastric mucosa via disposable flexible endoscopic biopsy forceps, 2 cup-shaped jaws with a central spike (Boston Scientific®) for histopathological study.

1.3. Gastric aspirate analysis:

Via Triple Lumen ERCP Cannula, 5.5 F (Boston Scientific®), immediately after insertion of the scope into the stomach, 5 mL of gastric fluid was aspirated through the suction channel of the endoscope and collected in a sterile trap placed in the suction line, to be sent for analysis. Quantitative determination of gastric aspirate total bilirubin level was performed (Gen.3® kit and Cobas 8000 analyzer). The pH monitoring of gastric aspirate was performed during the gastroscopy just after collection with a glass electrode pH meter (Adwa®).

1.4. Statistical Analysis:

The obtained data were statistically analyzed using SPSS program version 22 (IBM Corp., Armonk, NY, USA). Data were expressed as means ± standard deviation ($\bar{X}$ ± SD) in quantitative variables, and numbers and percentages for qualitative variables. Independent-Sample (T) test was used to compare quantitative variables means of two groups. Chi-Square test (X2) was used to compare qualitative variable means. The results were considered statistically significant if the P-value was <0.05. Correlation between variables was done using the Person correlation coefficient (r).

3. Results

The demographic data of the patients with bile reflux gastritis in CG ranged from 30 to 52 years with mean age ± SD of 38.83±7.55 years. Bile reflux gastritis was noted in 2 males (33.3%) and 4 females (66.7%). The demographic data of the patients with bile reflux gastritis in PEG ranged from 27 to 59 years with mean age ± SD of 39.25 ± 7.47. Bile reflux gastritis was noted in 9 males (45%) and 11 females (55%).

In CG, the endoscopic findings of esophageal mucosa included GERD in 16 cases (53.3%), incompetent cardia in 6 cases (20%), and mucosal changes in 5 cases (16.7%). While, the endoscopic findings of gastric mucosa included erythema in 15 cases (50%), presence of bile in 5 cases (16.7%), thick gastric fold in 5 cases (16.7%), erosions in 7 cases (23.3%), edema in 2 cases (6.7%), and petechiae in 4 cases (13.3%) (Table 1). In PEG, the endoscopic findings of esophageal mucosa in PEG included GERD in 21 cases (42.9%), mucosal changes in 11 cases (39.3%), and incompetent cardia in 7 cases (25%). While, the endoscopic findings of gastric mucosa included erythema in 18 cases (64.3%), presence of bile in 16 cases (57.1%), thick gastric fold in 13 cases (46.4%), petechiae in 8 cases (28.6%) erosions in 6 cases (21.4%), and edema in 5 cases (17.9%) (Table 1, Fig. 1a-c).

In our study, Bilirubin level in gastric aspirate in CG was within normal serum range in 24 patients (< 1.3 mg/dl). It ranged from 1.88 to 11.50 mg/dl (mean 7.53±3.63 mg/dl) in the remaining 6 patients. Gastric aspirate pH in the 30 cases of such group ranged from 4 to 8 (mean 6.32±1.29). Meanwhile, bilirubin level in gastric aspirate in PEG was within normal serum range in 9 patients (< 1.3 mg/dl). It ranged from 6.7 to 19.15 mg/dl (mean 10.59±3.97 mg/dl) in the remaining 19 patients. Gastric aspirate pH ranged from 5.50 to 8 (mean 7.72±0.23).

Table 1. Endoscopic findings of our study

Parameter		Control Group (CG)		Post-ERCP Group (PEG)		X^2	P
		n= 30		n= 28			
		No. of case	%	No. of case	%		
Esophageal	GERD	16	53.3	12	42.9	0.64	0.43
	incompetent cardia	6	20	8	28.6	0.58	0.45
	Mucosal changes	5	16.7	7	25	0.61	0.43
Gastric	Erythema of gastric mucosa	15	50	18	64.3	1.21	0.27
	Presence of bile	5	16.7	16	57.1	10.27	0.001
	Thick gastric fold	5	16.7	13	46.4	5.99	0.01
	Erosions	7	23.3	8	28.6	0.21	0.65
	Edema	2	6.7	6	21.4	2.65	0.11
	Petechiae	4	13.3	5	17.9	0.23	0.63

GERD; *Gastroesophageal reflux disease*

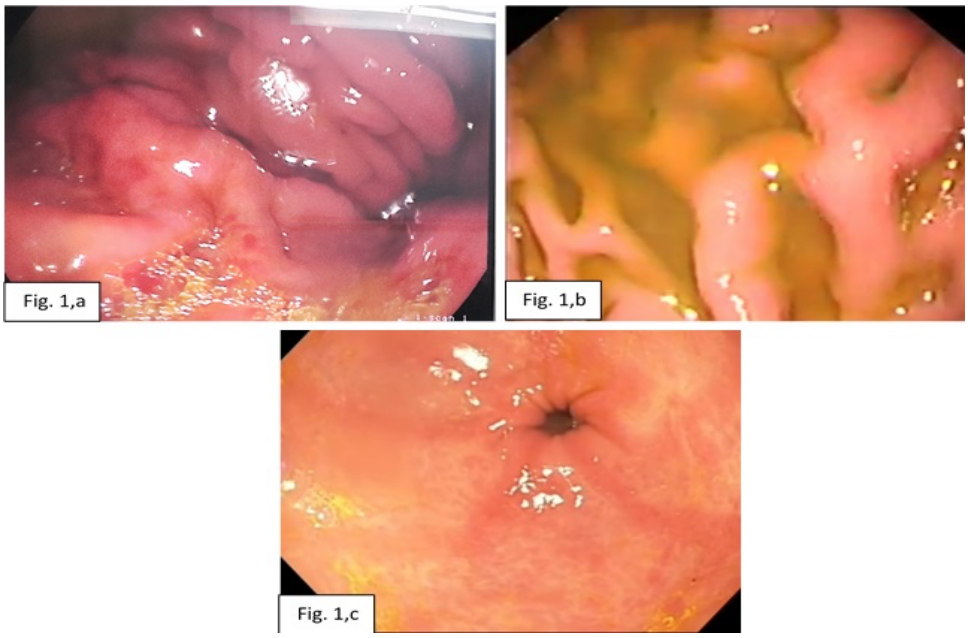

Fig. 1. Upper GIT endoscopic picture showing (a): gastric petechiae and presence of bile in the stomach, (b): thinking of gastric folds and presence of bile in the stomach, (c): antral gastritis with mucosal erythema and erosions with the presence of bile in the stomach

The histopathological finding of gastric mucosa biopsies in CG included chronic inflammation in 5 cases (16.7%), foveolar hyperplasia in 3 cases (10%), chronic Atrophic gastritis in 3 cases (10%), bile stasis in 6 cases (20%), interstitial inflammation in 7 cases (23.3%), edema in 2 cases (6.7%), intestinal metaplasia in 1 case (3.3%), and acute inflammation in 0 cases (0%) (Table 2). The histopathological finding of gastric mucosa biopsies in PEG included chronic inflammation in 18 cases (64.3%), foveolar hyperplasia in 13 cases (46.4%), bile stasis in 9 cases (32.1%), intestinal metaplasia in 8 cases (28.6%), chronic atrophic gastritis in 6 cases (21.4%), interstitial inflammation in 6 cases (21.4%), edema in 6 cases (21.4%), and acute inflammation in 2 cases (7.1%) (Table 2, Fig. 2 a-d).

As regards the presence of bile reflux gastritis, it was present in (6) cases in CG patients with a percentage of 20%. However, it was present in (20) cases in PEG patients with a percentage of 71.43 % (Table 3).

The risk factors for bile reflux gastritis in CG included increased gastric bilirubin (6 cases with a percentage of 100%), and alkaline gastric pH (6 cases with a percentage of 100%), diabetes (4 cases with a percentage of 66.6%), and obesity (5 cases with a percentage of 83.3%). However, the risk factors in PEG included increased gastric bilirubin (19 cases with a percentage of 95%), and alkaline gastric pH (19 cases with a percentage of 95%), diabetes (9 cases with a percentage of 45%), and obesity (8 cases with a percentage of 40%).

Table 2. Gastric mucosa histopathological findings of our study

Parameter	Control Group (CG) n= 30		Post-ERCP Group (PEG) n= 28		X^2	P
	No. of case	%	No. of case	%		
Chronic inflammation	5	16.7	18	64.3	13.72	0.00
Foveolar hyperplasia	3	10	13	46.4	9.62	0.002
Chronic Atrophic gastritis	3	10	9	32.1	4.33	0.15
Bile stasis	6	20	8	28.6	0.58	0.45
Interstitial inflammation	7	23.3	6	21.4	0.03	0.86
Edema	2	6.7	6	21.4	2.65	0.10
Intestinal metaplasia	1	3.3	6	21.6	4.47	0.04
Acute inflammation	0	0	2	7.1	2.22	0.14

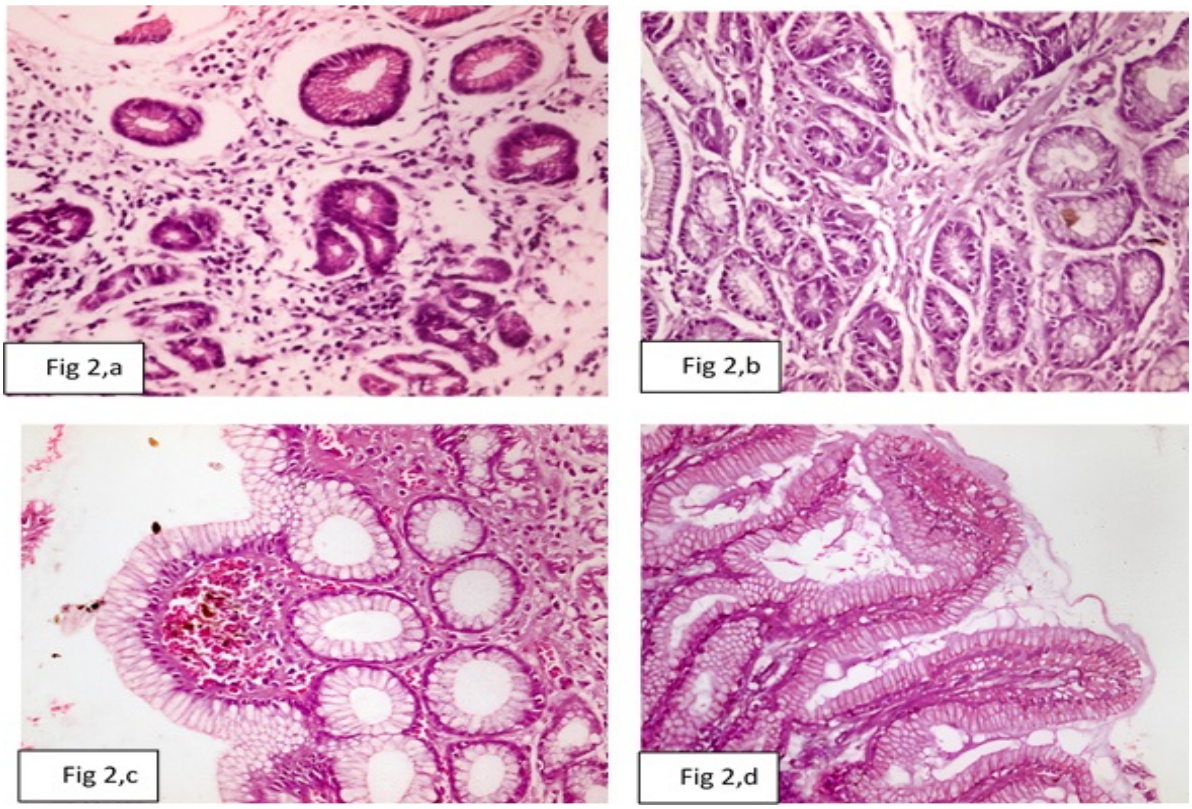

Fig. 2. Photomicrograph of (a): chronic atrophic gastritis with per glandular fibrosis, chronic inflammatory infiltrate, and mild dysplasia (H&E X400), (b): chronic gastritis with per glandular fibrosis and mild chronic inflammatory infiltrate (H&E X400), (c): chronic gastritis with focal bile stasis (H&E X400), (d): chronic gastritis with diffuse intestinal metaplasia and interstitial inflammation (H&E X40)

Table 3. Percentage of bile reflux gastritis and non- bile reflux gastritis post-ERCP

Parameter	Control Group (CG)		Post-ERCP Group (PEG)		X2	P
	N=30	%	N=28	%		
bile reflux gastritis	6	20	20	71.4	15.49	0.000
Non- bile reflux gastritis	24	80	8	28.6		

The study revealed statistically significant positive correlations between increased gastric bilirubin, increased gastric aspirate pH, diabetes, obesity, and the presence of bile reflux gastritis in both studied groups. There were no correlations between age, sex, epigastric pain, heartburn, vomiting, and the presence of bile reflux gastritis in both groups of our study (Table 4).

Table 4. Correlation between risk factors and presence of bile reflux gastritis in studied groups

	Bile Reflux Gastritis	
BMI	r	P
Gastric pH	0.42**	0.001
Gastric Bilirubin	0.66**	0.00
RBS	0.84**	0.00
Epigastric pain	0.28*	0.03
Heart burn	1.01	0.45
Vomiting	0.005	0.97

BMI: Body mass index, **RBS:** Random blood sugar**, GERD:** Gastroesophageal reflux disease. **Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

4. Discussion

Bile reflux gastritis is a disease characterized by upper abdominal pain, frequent heartburn, nausea, and vomiting of bile. Such disease appears to be caused by the backward flow of duodenal fluid into the stomach and esophagus. This fluid contains bile, pancreatic juices, and duodenal secretion (1). The diagnosis was based on clinical findings (9), pH monitoring of the aspirated gastric juice on the assumption that the bile reflux would cause an increase of pH over 7 as a result of the alkaline nature of duodenal juice (9), with the aid of endoscopic and pathologic findings (10).

In this study, the esophageal endoscopic findings were GERD, incompetent cardia, and mucosal changes. The most frequent esophageal endoscopic finding was GERD (42.9%). However, the gastric mucosa endoscopic findings were erythema, presence of bile, thick gastric fold, erosions, edema, and petechiae (Figure 1a-c). The most frequent gastric endoscopic finding was erythema of gastric mucosa with a percentage of (64.3%) (Table 1). Such findings were similar to that of former studies. Martamala and Rani (11) diagnosed bile reflux gastritis endoscopically, based on the presence of bile in the gaster, with adherence of bile on the gastric mucous membrane in the form of crusts and changes in the mucous membrane that becomes hyperemic, frail, and erosive (12). In a Romanian endoscopic study of bile reflux gastritis, the endoscopic gastric mucosa changes were erythema, bile existence in the stomach, over thickening of gastric folds, erosions, atrophic mucosa, petechiae, intestinal metaplasia, and polyp (2). Al-Bayati and Alnajjar conducted that the endoscopic findings were erythema, gastric erosion, thickening of gastric folds, and gastric atrophy. The most common gastric endoscopic finding was erythema of the gastric mucosa (50%). All the patients had bile in the stomach (12).

Shenouda et al. found that mean normal bilirubin levels in gastric aspirate was 1.3 mg/dl (13). The normal pH of gastric juice was 1.5 to 3.5 in the human stomach lumen (14). In our study, the alkaline gastric aspirate (7.72±0.23) with a high level of gastric bilirubin level (10.59±3.97) was supposed to be the cause of esophageal and gastric mucosal damage although the exact mechanisms were still unclear (15). Some studies indicated that interaction of bile acid, a component of bile; with M3 muscarinic receptor subtype expressed in chief cells might contribute to mucosal damage, manifested as active inflammation, intestinal metaplasia, glandular atrophy and focal hyperplasia, and other pathophysiological consequences of bile reflux (16). Apoptosis and redox reactions had been reported to be associated with bile acid-induced gastritis (17). Some studies reported that bile acids and other contents of the duodenum would act synergistically in the development of chronic gastritis with gastric acid and Helicobacter pylori infection (15).

The most frequent histopathological finding in our study was chronic inflammation with a percentage of (64.3%). However, the least frequent histopathological finding was acute inflammation with a percentage of (7.1%) (Table 2). Our results agreed with a previous study that proved that the histopathological changes of tissues samples were chronic gastritis, foveolar hyperplasia, intestinal metaplasia, dysplasia, acute gastritis, chronic atrophic gastritis, polyps, benign ulcers, and edema. The most frequent histopathological finding was chronic inflammation (84.06%) (2). The histopathologic changes due to bile reflux gastritis in children were characterized by chronic inflammation with foveolar hyperplasia in both the gastric corpora and antrum, vascular congestion, edema of lamina propria, and smooth muscle hyperplasia (18).

In our study, bile reflux gastritis was present in (20) cases out of the 28 cases post-ERCP. The prevalence of bile reflux gastritis was (71.43 %) post-ERCP. Previous studies have shown that the prevalence of bile reflux after therapeutic biliary procedures was 60% (19).

Our study showed that there were statistically significant positive correlations between obesity, increased gastric aspirate pH, increased gastric bilirubin, RBS, and bile reflux gastritis occurrence in the studied groups. Such findings went with that of the former studies. Obesity was a risk factor for bile reflux gastritis (20). Deenadayalu et al (21). demonstrated a significantly higher rate of post-ERCP complications in obese patients (BMI≥30 kg/m2) in comparison to overweight (BMI 25-30 kg/m2), normal-weight (BMI 18.5-25 kg/m2), and underweight (BMI<18.5 kg/m2) patients (22). Shenouda et al (13). declared that increased gastric aspirate bilirubin level and pH are confirmatory tools to diagnose biliary gastritis with a significant relationship between the level of gastric bilirubin and the degree of inflammation (14). The gastric bilirubin level above 20 mg/dL could indicate severe esophagitis, erosive gastritis, or gastroesophageal metaplastic changes. In fact, more severe biliary gastritis was associated with higher bilirubin levels in the gastric aspirates (22).

Diabetes mellitus was considered a risk factor for bile gastritis (2). Barakat et al (5). reported that diabetes mellitus might be considered a risk factor for primary biliary gastritis (5). Prevalence of type II diabetes mellitus was associated with gastroduodenal dysmotility (23). Diabetes gastroparesis

was a condition where persistent hyperglycemia, in either type 1 or type 2 diabetes, could cause Vagus nerve damage, which was responsible for proper gastric movement resulting in delayed gastric emptying without mechanical obstruction (24). Severe bile reflux gastritis was a consequence of diabetes gastroparesis (25,26).

Our study showed that there were no correlations between age, sex, epigastric pain, heartburn, vomiting, and the presence of bile reflux gastritis. A few previous research had looked into the relationship between age and biliary gastritis. Byrne et al. stated that there was no evidence of a connection between age and bile reflux gastritis (27). Bollschweiler et al.also could not prove any significant difference between the patient's age and occurrence of bile reflux gastritis (28).

In our study, histopathological examination of gastric mucosa revealed chronic atrophic gastritis showing per glandular fibrosis, chronic inflammatory infiltrate, and mild dysplasia (Figure 2a), chronic gastritis showing per glandular fibrosis and mild chronic inflammatory infiltrate (Figure 2b), chronic gastritis showing focal bile stasis (figure 2c), and chronic gastritis showing diffuse intestinal metaplasia and interstitial inflammation (figure 2d). The former studies reported histopathological alterations because of bile gastritis similar to our findings in form of chronic gastric mucosa inflammation, lamina propria edema, foveolar hyperplasia, antral atrophy, and intestinal metaplasia (29). Vere et al. observed chronic gastritis, foveolar hyperplasia, intestinal metaplasia, gastric dysplasia, acute inflammation, chronic atrophic gastritis, polyps, benign ulcers, and edema (2). The histologic alterations owing to bile reflux gastritis in form of foveolar hyperplasia, edema, smooth muscle fibers in the lamina propria, and paucity of acute or chronic inflammatory cells were similar to those seen in chemical (reactive) gastritis. The limitations of our study are that the patients total number was low because of the restrict exclusion criteria.

The prevalence of bile reflux gastritis was (20%) in CG, while it was (71.43 %) in PEG. Diabetes, obesity, increased gastric bilirubin, and increased gastric pH were risk factors for bile reflux gastritis in both groups. However, there were no correlations between age, sex, epigastric pain, heartburn, vomiting, and the presence of bile reflux gastritis in both groups.

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References

1. Eldredge TA, Myers JC, Kiroff GK, Shenfine J. Detecting Bile Reflux-the Enigma of Bariatric Surgery. Obes Surg. 2018 Feb;28(2):559-566. doi: 10.1007/s11695-017-3026-6. PMID: 29230622.
2. Vere CC, Cazacu S, Comănescu V, Mogoantă L, Rogoveanu I, Ciurea T. Endoscopical and histological features in bile reflux gastritis. Rom J Morphol Embryol. 2005;46(4):269-74. PMID: 16688361.
3. Sun D, Wang X, Gai Z, Song X, Jia X, Tian H. Bile acids but not acidic acids induce Barrett's esophagus. Int J Clin Exp Pathol. 2015 Feb 1;8(2):1384-92. PMID: 25973022; PMCID: PMC4396229.
4. Kuran S, Parlak E, Aydog G, Kacar S, Sasmaz N, Ozden A, Sahin B. Bile reflux index after therapeutic biliary procedures. BMC Gastroenterol. 2008 Feb 11;8:4. doi: 10.1186/1471-230X-8-4. PMID: 18267026; PMCID: PMC2257961.
5. Barakat EA, Abbas NF, El-Kholi NY. Primary bile reflux gastritis versus Helicobacter pylori gastritis: a comparative study. The Egypt J Int Med.2018; 30(1), 23-27.
6. Monaco L, Brillantino A, Torelli F, Schettino M, Izzo G, Cosenza A, Di Martino N. Prevalence of bile reflux in gastroesophageal reflux disease patients not responsive to proton pump inhibitors. World J Gastroenterol. 2009 Jan 21;15(3):334-8. doi: 10.3748/wjg.15.334. PMID: 19140233; PMCID: PMC2653330.
7. ASGE Standards of Practice Committee, Chandrasekhara V, Khashab MA, Muthusamy VR, Acosta RD, Agrawal D, Bruining DH, Eloubeidi MA, Fanelli RD, Faulx AL, Gurudu SR, Kothari S, Lightdale JR, Qumseya BJ, Shaukat A, Wang A, Wani SB, Yang J, DeWitt JM. Adverse events associated with ERCP. Gastrointest Endosc. 2017 Jan;85(1):32-47. doi: 10.1016/j.gie.2016.06.051. Epub 2016 Aug 18. PMID: 27546389.
8. Hadjiconstanti AC, Messaris GAT, Thomopoulos KC, Panayiotakis GS. Patient Radiation Doses in Therapeutic Endoscopic Retrograde Cholangiopancreatography in Patras and the Key Role of the Operator. Radiat Prot Dosimetry. 2017 Dec 1;177(3):243-249. doi: 10.1093/rpd/ncx037. PMID: 28419374.
9. Hyun JJ, Yeom SK, Shim E, Cha J, Choi I, Lee SH, Chung HH, Cha SH, Lee CH. Correlation Between Bile Reflux Gastritis and Biliary Excreted Contrast Media in the Stomach. J Comput Assist Tomogr. 2017 Sep/Oct;41(5):696-701. doi: 10.1097/RCT.0000000000000585. PMID: 28240637.
10. Boeckxstaens GE, Smout A. Systematic review: role of acid, weakly acidic and weakly alkaline reflux in gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 2010 Aug;32(3):334-43. doi: 10.1111/j.1365-2036.2010.04358.x. Epub 2010 May 18. PMID: 20491749.
11. Martamala R, Rani AA. The pathogenesis and diagnosis of bile reflux gastropathy. JGHE.2001;2(1):14-20.
12. Al-Bayati S, Alnajjar AS. Evaluation of the gastrointestinal clinical, endoscopic, and histological findings in patients with bile reflux diseases: A cross-sectional study. Mustansiriya Med J. 2019; 18(1):10-15.
13. Shenouda MM, Harb SE, Mikhail SAA, Mokhtar SM, Osman AMA, Wassef ATS, Rizkallah NNH, Milad NM, Anis SE, Nabil TM, Zaki NS, Halepian A. Bile Gastritis Following Laparoscopic Single Anastomosis Gastric Bypass: Pilot Study to Assess Significance of Bilirubin Level in Gastric Aspirate. Obes Surg. 2018 Feb;28(2):389-395. doi: 10.1007/s11695-017-2885-1. PMID: 28849330.
14. Marieb E., and Hoehn K. Human Anatomy & Physiology. Pearson Education. 2018; ISBN: 9780134580999.
15. Matsuhisa T, Tsukui T. Relation between reflux of bile acids into the stomach and gastric mucosal atrophy, intestinal

metaplasia in biopsy specimens. J Clin Biochem Nutr. 2012 May;50(3):217-21. doi: 10.3164/jcbn.11-90. Epub 2011 Dec 2. PMID: 22573924; PMCID: PMC3334375.

16. Park MJ, Kim KH, Kim HY, Kim K, Cheong J. Bile acid induces expression of COX-2 through the homeodomain transcription factor CDX1 and orphan nuclear receptor SHP in human gastric cancer cells. Carcinogenesis. 2008 Dec;29(12):2385-93. doi: 10.1093/carcin/bgn207. Epub 2008 Sep 4. PMID: 18775915.

17. Xu Y, Watanabe T, Tanigawa T, Machida H, Okazaki H, Yamagami H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Arakawa T. Bile acids induce cdx2 expression through the farnesoid x receptor in gastric epithelial cells. J Clin Biochem Nutr. 2010 Jan;46(1):81-6. doi: 10.3164/jcbn.09-71. Epub 2009 Dec 29. PMID: 20104269; PMCID: PMC2803137.

18. Zhang P, Cui Y, Li W, Ren G, Chu C, Wu X. Diagnostic accuracy of diffusion-weighted imaging with conventional MR imaging for differentiating complex solid and cystic ovarian tumors at 1.5T. World J Surg Oncol. 2012 Nov 9;10:237. doi: 10.1186/1477-7819-10-237. PMID: 23137333; PMCID: PMC3514117.

19. Hashimoto N. Duodenogastric Reflux after Biliary Procedure. Open Access Library Journal. 2014;1(7):1-5.

20. Maguilnik I, Neumann WL, Sonnenberg A, Genta RM. Reactive gastropathy is associated with inflammatory conditions throughout the gastrointestinal tract. Aliment Pharmacol Ther. 2012 Oct;36(8):736-43. doi: 10.1111/apt.12031. Epub 2012 Aug 28. PMID: 22928604.

21. Deenadayalu VP, Blaut U, Watkins JL, Barnett J, Freeman M, Geenen J, Ryan M, Parker H, Frakes JT, Fogel EL, Silverman WB, Dua KS, Aliperti G, Yakshe P, Uzer M, Jones W, Goff J, Temkit M, Lehman GA, Sherman S. Does obesity confer an increased risk and/or more severe course of post-ERCP pancreatitis?: a retrospective, multicenter study. J Clin Gastroenterol. 2008 Nov-Dec;42(10):1103-9. doi: 10.1097/MCG.0b013e318159cbd1. PMID: 18936645.

22. Lasheen M, Mahfouz M, Salama T, Salem HE. Biliary reflux gastritis after Mini Gastric Bypass: The effect of Bilirubin level. Arch Surg Clin Res. 2019;3:27-31.

23. McCabe ME 4th, Dilly CK. New Causes for the Old Problem of Bile Reflux Gastritis. Clin Gastroenterol Hepatol. 2018 Sep;16(9):1389-1392. doi: 10.1016/j.cgh.2018.02.034. Epub 2018 Mar 2. PMID: 29505908.

24. Petri M, Singh I, Baker C, Underkofler C, Rasouli N. Diabetic gastroparesis: An overview of pathogenesis, clinical presentation and novel therapies, with a focus on ghrelin receptor agonists. J Diabetes Complications. 2021 Feb;35(2):107733. doi: 10.1016/j.jdiacomp.2020.107733. Epub 2020 Sep 6. PMID: 32948398.

25. Roses RE, Fraker DL. Bile Reflux and Gastroparesis. Gastrointestinal Surgery. Springer.2015: p. 119-125.

26. Weston A, Menguer R, Giordani D, Cereser C. A Severe Alkaline Gastritis in Type 1 Diabetes Gastroparesis: A Case Report. J Gastrointest Dig Syst.2017; 7(540): 2.

27. Byrne JP, Romagnoli R, Bechi P, Attwood SE, Fuchs KH, Collard JM. Duodenogastric reflux of bile in health: the normal range. Physiol Meas. 1999 May;20(2):149-58. doi: 10.1088/0967-3334/20/2/304. PMID: 10390017.

28. Bollschweiler E, Wolfgarten E, Pütz B, Gutschow C, Hölscher AH. Bile reflux into the stomach and the esophagus for volunteers older than 40 years. Digestion. 2005;71(2):65-71. doi: 10.1159/000084521. Epub 2005 Mar 16. PMID: 15775673.

29. Genta RM. Differential diagnosis of reactive gastropathy. Semin Diagn Pathol. 2005 Nov;22(4):273-83. doi: 10.1053/j.semdp.2006.04.001. PMID: 16939055.

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Evaluation of sleep quality and physical activity levels of university students during the COVID-19 pandemic

Ramazan GÜNEŞER[1,*], Aydın HİM[2]

[1]Vocational School of Health, Bolu Abant İzzet Baysal University, Bolu, Türkiye
[2]Department of Physiology, Faculty of Medicine, Bolu Abant İzzet Baysal University, Bolu, Türkiye

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Abstract

Our study aimed to investigate university students' sleep quality and physical activity levels during the COVID-19 pandemic. We conducted the study on 128 volunteer students studying at Bolu Abant Izzet Baysal University, Vocational School of Health. We collected the data online through the socio-demographic form, the Pittsburgh Sleep Quality Index (PSQI), and the International Physical Activity Questionnaires (IPAQ). The average PSQI score of the students was 8.87±3.47, with 81.3% of the students having poor and 18.8% having good sleep quality. The total physical activity score was 1259.73±2183.33 (MET-Min/week). The percentage of students who had no vigorous physical activity was 80.5%, while 59.4% did not do moderate physical activity, and 5.5% did not have adequate walking activity. About 53.1% of the students were not physically active, 32% had low physical activity, and only 14.8% had adequate physical activity. COVID-19 pandemic had several adverse effects on students' sleep quality and physical activity levels. During the pandemic, we think that students had difficulty forming regular biological rhythms, especially sleep and physical activity, due to various reasons such as concerns about education, quarantine and social isolation. Necessary information should be acquired, and guidance should be given about quality sleep and adequate physical activity to reduce the adverse effects of the disease by keeping the immune system strong.

Keywords: COVID-19, sleep quality, physical activity, quarantine, circadian rhythm

1. Introduction

The COVID-19 pandemic is a global public health problem that threatens all humanity. Individuals are socially isolated to both protect themselves from the disease and prevent it from spreading. Therefore, some measures have been taken through various practices such as distance education and working from home. UNESCO reported that approximately 861.7 million students did not attend school (1). In addition, students switched from face-to-face education to online education and had to follow their education, measurement and evaluation processes from their homes. Such routine-free practices cause individuals to experience various physical, mental, and social problems. In addition to affecting physical health, the pandemic process affects the sleep patterns of the non-sick community.

Sleep is essential for maintaining individuals' physiological and psychological health (2). Çıtak ve Pekdemir (2020) reported that the COVID-19 epidemic causes individuals to experience sleep difficulties, and changes sleep habits (3). Sleep quality and duration might be adversely affected, resulting in various sleep-related diseases, as well as worsening of previous sleep problems (4). There is also a two-way relationship between sleep and the immune system; activation in the immune system changes sleep structure, while sleep helps shape the body's response to infections. We spend a large part of our lives in sleep, and it is of great importance for protecting healthy people and for the comfortable passage of the disease process of infected people (5). Colbay et al. (2007) stated that deterioration in individuals' sleep quality affects their daily activities and decreases energy levels (6).

Physical activity, which protects psychological health and regulates immune system functions, is considered one of our powerful weapons in the pandemic process (7). It can reduce the severity of infections by affecting the immune system and the inflammation process, improve common chronic conditions (cardiovascular disease, diabetes) that increase the risk of severe COVID-19, and is also a great stress management tool by reducing anxiety and depression symptoms (8). Campbell and Turner (2018) reported that a physically active lifestyle reduces the risk of contracting a range of infectious diseases, including viral and bacterial infections (9). During the covid-19 pandemic, our biological clock has difficulties forming regular rhythms because the normal rhythms of daily activities like socialization and working routine are disrupted. Güzel at al. (2020) suggested that creating routine tasks, physical activity, sleep, light

*Correspondence: rmzngnsr@gmail.com

adjustment, diet and stress control are helpful in adjusting a person's circadian rhythm (10). The importance of the circadian rhythm as another protective resource available to everyone besides social isolation and personal hygiene is mentioned in this process, and it is emphasized that the immune system will weaken if the circadian rhythm is disrupted (11). It is also emphasized that it is helpful to adjust ideally the timing of external stimuli that have a very high effect on the circadian clock, such as sleeping, body temperature, light, diet, physical exercise, to protect against disorders and let the circadian clock run more efficiently (12).

All this information suggests that a pandemic disease, which is not compatible with the natural biological rhythms of individuals, leads to various complications. This study investigated university students' sleep quality and physical activity levels during the COVID-19 pandemic.

2. Materials and Methods

We obtained ethical approval from Bolu Abant Izzet Baysal University Clinical Research Ethics Committee (decision no: 2020/201) and conducted our study on Bolu Abant Izzet Baysal University Vocational School of Health students. We designed this cross-sectional type study per the principles of the Helsinki Declaration and carried it out in June 2020. We obtained informed consent from all participants. The study included one hundred twenty-eight volunteer students who completed the forms correctly and completely. We excluded students with chronic sleep problems for any reason or illness or disability that would restrict them from doing physical activity in the study. We collected the data online (Google Docs) through "Personal Data Forms", the "Pittsburgh Sleep Quality Index (PSQI)", and "International Physical Activity Questionnaires (IPAQ) (short form)". The study's independent variables were age, gender, height, weight, and body mass index (BMI), while the dependent variables were sleep quality and physical activity levels.

The PSQI is a scale that evaluates sleep quality and sleep impairment in a recent one-month timeframe (13). Ağargün et al. (1996) determined the validity and reliability of the index in Turkey, and its Cronbach Alpha internal consistency coefficient was 0.80. The first 18 items of the scale included in the scoring are rated as poor sleep quality with a score higher than five and good sleep quality with a score lower than five (14).

Craig et al. (2003) developed the IPAQ to determine participants' physical activity levels in the 15-65 age range (15). Öztürk conducted IPAQ's validity and reliability study in Turkey (16). The criteria for each activity to be performed at least 10 minutes at a time are considered in the evaluation of all activities. By multiplying the minutes, days and MET values, a score is obtained as "MET-min/week". Physical activity levels are classified as non-physically active (<600 MET-min/week), low physical activity level (600-3000 MET-min/week) and adequate physical activity level (>3000 MET-min/Week) (15).

2.1. Statistical Analysis

While categorical variations are expressed as percentage and frequency distributions, continuous variables are expressed as mean and standard deviation values. We used the Kolmogorov Smirnov test for the normality distribution of the data and the Mann Whitney U test to compare the two groups. We compared categorical data using the chi-square test. We calculated Spearman's correlation coefficient to determine the relationship between sleep and physical activity. We considered $P<0.05$ values statistically significant and evaluated the data through the SPSS statistical package program.

3. Results

Table 1 shows the descriptive statistics of the participants. The participants were 85.2% (n=109) female and 14.8% (n=19) male. Male students' height, weight and BMI averages were higher than female students. The prevalence of smoking was significantly higher among men than women.

While male students' total physical activity (MET-min/week) scores were significantly higher than female students ($P=0.011$), there was no significant gender difference between the PSQI scores ($p=0.187$) (Table 2).

Regarding students' physical activity levels, 53.1% were physically inactive, 32,0% minimally active and 14.8% sufficiently active (Table 3). Furthermore, the scale results evinced that 80.5% did not do vigorous physical activity, 59.4% did not do moderate physical activity, and 5.5% did not have adequate walking activity.

According to the sleep quality classification of the participants, 81.3% of the students had poor while 18.8% had good sleep quality (Table 4).

Most students (64.84%) think their sleep pattern (sleep-wake cycle) has changed during the COVID-19 pandemic, while 28.13% think it was affected slightly/partially, and 7.03% think it was not affected. The majority of the students (73.44%) think there was a decrease in their level of physical activity during the COVID-19 pandemic, while 19.53% think it decreased slightly/partially and 7.03% think it was not affected (Table 5).

The correlation analysis results evinced a negative correlation between PSQI and total physical activity (MET-min/week) scores ($r=-0.163$). However, this relationship was not statistically significant.

Table 1. Descriptive statistics of variables

	Female		Male		Total		
	n=109	85.2%	n=19	14.8%	n=128	100%	
	Mean±Sd		Mean±Sd		Mean±Sd		P
Age	20.42±2.02		20.32±1.77		20.41±1.98		0.640
Height(cm)	163.66±5.74		175.16±7.37		165.37±7.25		**<0.001***
Weight(kg)	59.54±10.83		74.32±15.14		61.73±12.65		**<0.001***
BMI	20.22±3.87		24.14±4.22		22.50±3.96		**<0.044***
Smoking	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	
	17 15.6%	92 84.4%	10 52.6%	9 47.4%	27 21.1%	101 78.9%	**<0.001****

* Mann Whitney U test. ** $\chi 2$ test

Table 2. Comparison of PSQI and total physical activity scores by gender

	PSQI	Total physical activity (MET-min/week)
	Mean±Sd	Mean±Sd
Female n=109 85.2%	9.09±3.56	1259.73±2183.33
Male n=19 14.8%	7.58±2.67	3164.24±3733.05
Total n=128 100%	8.87±3.47	1542.43±2547.74
P	0.055*	0.011*

* Mann Whitney U test

Table 3. Participants' physical activity levels

Physical Activity Levels	Physically inactive		Minimally active		Sufficiently active		Total	
	n	%	n	%	n	%	n	%
Female	60	88.2	39	95.1	10	52.6	109	85.2
Male	8	11.8	2	4.9	9	47.4	19	14.8
Total	68	53.1	41	32.0	19	14.8	128	100

Table 4. Participants' PSQI levels

PSQI	Quality Sleep (PSQI score =< 5)		Poor Quality Sleep (PSQI score >5)		Total	
	n	%	n	%	n	%
Female	20	83.3	89	85.6	109	85.2
Male	4	16.7	15	16.4	19	14.8
Total	24	18.8	104	81.3	128	100

Table 5. Distribution of participants' responses on sleep and physical activity during COVID-19

	Yes		Slightly/Partially		No	
	n	%	n	%	n	%
Do you think your sleep pattern (sleep-wake cycle) has changed during the COVID-19 pandemic?	83	64.84	36	28.13	9	7.03
Do you think there was a decrease in your level of physical activity during the COVID-19 pandemic?	94	73.44	25	19.53	9	7.03

4. Discussion

Our study aimed to investigate university students' sleep quality and physical activity levels during the COVID-19 pandemic. Several measures were taken, such as quarantine and social isolation, to prevent the spread of COVID-19, which is considered a pandemic by the World Health Organization. Students had to follow lessons while others had to work from home in this process. Such measures limited the spread of the virus but brought along various difficulties. Quarantine exposes individuals to a highly stressful condition. Quarantine measures not only increase stress, anxiety and depression levels but also impair sleep quality. On the other hand, Altena et al. (2020) reported that carrying out work from home due to quarantine and social isolation, and lack of access to places where physical activities take place (e.g., gyms, parks, etc.) resulted in a significant decrease in physical activity levels (17). COVID-19 affected individuals altered daily routines, sleep and physical activity patterns (18).

Our findings showed that while there was no difference between male and female students in terms of PSQI scores, the vast majority of participants had low sleep quality. In their study, Casagrande et al. revealed that 57.1% of participants reported poor sleep quality, which indicates a link between

low sleep quality and post-traumatic stress disorder associated with COVID-19 (19). An online survey performed by Kaparounaki at al. (2020) on college students in COVID-19 quarantine showed that students' sleep amount increased by 66.3%, but sleep quality worsened by 43.0% (20). In line with the literature, the vast majority of students in our study had low sleep quality. These results suggested that routine disruptive practices, such as COVID-19 quarantine and social isolation, which expose individuals to high stress, negatively affected students forming regular biological rhythms. Two-thirds of the students thought their sleep pattern (sleep-wake cycle) changed during the COVID-19 outbreak. These results were consistent with participants' PSQI scores, which indicated that students experienced decreased sleep quality during the pandemic compared to previous times. Home quarantine significantly changed the timing of sleep. Individuals spent more time in bed before sleep and got up late, but paradoxically, they had lower sleep quality. Studies showed that the relationship between sleep difficulty and high levels of depression, anxiety and stress was strong (21, 22).

University students switched from face-to-face to online education and followed all educational processes from their homes, such as courses, measurement and evaluation. In addition to concerns about COVID-19, this process has caused students to experience stress on various topics such as participation in online courses, evaluation and graduation (23). Considering the strong relationship between stress and sleep, we believe all these stressors could be considered causes that decrease the quality of students' sleep. Pelin (2020) reported the importance of a strong immune system to combat COVID-19 (5). The deterioration in sleep quality during the COVID-19 pandemic made healthy individuals susceptible to infection while also complicating epidemic control (5). Alschuler et al (2020) emphasized that enough sleep was essential to reduce the risk of COVID-19 infection and also provided the secretion of melatonin, a molecule that may play a role in reducing coronavirus virulence (24). Regular sleep is directly associated with melatonin. Melatonin, a natural antioxidant, activates immune system cells either directly through melatonin receptors or indirectly due to changes in steroid hormones (25). Natural disasters are major sources of stress, and sleep disorders develop in response to these events. A worldwide crisis, such as COVID-19, is also likely to lead to sleep and circadian rhythm disorder (26). During the COVID -19 quarantine periods, students are subject to high stress due to concerns related to education and illness. Due to such stress, students have difficulty forming a regular biological rhythm. The rhythms affected are preceded by the sleep-wake cycle. Thus, students' circadian rhythms are negatively affected, and their sleep quality decreases accordingly.

Participants' classification according to the total physical activity score obtained from IPAQ revealed that the activity level of the majority was insufficient to maintain and improve health; only a small proportion performed adequate physical activity. During the COVID-19 pandemic, quarantine and social isolation practices for fear of being infected created an environment that caused decreased physical activity (27). Public health recommendations made to prevent the spread of COVID-19 (the stay-at-home proposal, closure of parks, gyms and fitness centers) can reduce the level of daily physical activity. Simpson and Katsanis (2020) suggested that daily exercise and physical activity could help fight diseases such as obesity, diabetes, hypertension and severe heart disease, making the immune system more susceptible to COVID-19 (28). Our results evidenced that the majority of participants did not have vigorous physical activity, approximately one third did not have moderate physical activity, and a few did not have adequate walking activity. The COVID-19 pandemic decreased physical activity levels in three-quarters of the students, showing that during COVID-19, there was a severe level of physical activity deficiency among university students. Kaya Ciddi (2019) reported that 67.85% (n=95) of the participants stated that their physical activity was negatively affected during the isolation process, while 32.6% (n=46) stated that they were not affected (29). In Kaya Ciddi's study (2019), among the participants, the highest proportion was inactive individuals with the lowest physical activity at 69.28% (N=97), followed by minimally active individuals with 20% (N=28) and sufficiently active individuals with 10.71% (n=15). Kaya Ciddi's study also revealed that most participants were inactive during this process and did not engage in regular physical activity compared to pre-isolation. Kaya Ciddi (2019) stated that the closure of gyms and parks during the quarantine process is reported to potentially contribute to the decline in physical activity (29). Ercan and Keklicek (2020) also reprted that due to the COVID-19 pandemic, students' regular physical activity rate decreased, and the overall rate of physical inactivation increased (30). In Maugeri et al.'s study, before and during the COVID-19 pandemic, the total physical activity was reported as MET-minute/week, and the scores of individuals for strenuous physical activity, moderate activity, and walking activity decreased significantly compared to the pre-quarantine period (31). Maugeri et al (2020) also noted that the proportion of students with high physical and moderate physical activity prior to COVID-19 decreased considerably during the COVID-19 pandemic (31). Gallo et al (2020) compared nutrient uptake, and physical activity levels of earlier periods (2018 and 2019) and in the early phase of the COVID-19 outbreak (March/April). The same study discovered higher nutrient consumption for women and lower physical exercise in both men and women. The findings of Gallo et al. (2020) suggested that the procedures to isolate people may negatively affect physical and psychological health with the possible long-lasting influence on food intake and physical exercise patterns (32). Due to social isolation and the long decaying of education, Chen et al. (2020) reported that the routine physical activities of tens of millions

of students in China were inevitably disrupted. Therefore, Chen et al. stressed that as schools begin to re-enter the service, all school administrators, teachers, and parents should be encouraged to ensure that all students effectively pass mandatory restrictions limiting exercise by participating in recommended physical activity levels. Continuing regular physical activities in this way is considered to help students recover from the stress and anxiety they experienced while in quarantine during the COVID-19 crisis (33). Our findings revealed that male students' total physical activity scores were higher than female students. Savcı et al. (2005) found that male students' total physical activity scores were higher than female students (34). Our results were in line with Savcı et al.'s study and indicated that female students were more exposed to the harmful effects of the pandemic process on physical activity than male students.

Circadian rhythm refers to behavioral and physiological changes involved in important biological processes such as the sleep-wake cycle that lasts approximately 24 hours (35). Body temperature, the sleep-wake cycle, melatonin and cortisol are important indicators of the circadian rhythm. Light is the most important rhythm regulator, also involved in social and physical activities (36). It is common knowledge that there is a strong link between the circadian rhythm and the immune system. During the COVID-19 pandemic, our biological clock had difficulty establishing regular biological rhythms for various reasons such as lack of social rhytms like socialization, child care and work life (10). The hormone melatonin, the best-known natural antioxidant and a major biorhythm regulator, effectively reduces inflammation and fibrosis in COVID-19 disease. In addition, melatonin reduces oxidative stress-inducing infections such as SARS-CoV. Shneider et al. (2020) emphasized that melatonin stimulates immunity impaired by anxiety and sleep deprivation (37). Sleep, a circadian behavioral reflection of the critical regulatory homeostatic mechanisms associated with immunity, has been shown to interact with host defences at the molecular and cellular levels (37). Shneider et al. also reported clear interdependencies between sleep duration and quality and immune responses to viral, bacterial and parasitic pathogens, with the latter altering sleep patterns. Therefore, improved sleep quality and duration in the population is likely to alleviate the spread and severity of the disease caused by SARS-CoV-2 infection (38). Hower et al. (2018) reported an association between circadian rhythm and exercise; exercise positively affected circadian rhythmicity (39). Hower et al. (2020) also noted that physical activity might regulate irregular circadian rhythm, possibly due to changes in skeletal muscle (39). The COVID-19 pandemic process highlights the need to do enough physical activity to increase immunity and reduce the harmful effects of inactivity and social isolation-induced stress on our immune system.

Kaya Ciddi (2019) stated that exercise would not prevent our likelihood of contracting COVID-19, but physical activity would help maintain the adverse effects of isolation and quarantine-induced stress on the immune system (29). Furthermore, evidence from other viral infections revealed that physically active people would have less severe symptoms, shorter recovery times, and a lower risk of transmission (28). According to Tunç et al. (2020) individuals who exercised during the COVID-19 pandemic had a higher quality of life. Physical activity increases the quality of life in normal times as well as during the epidemic period.

Moreover, Tunç et al. (2020) emphasized the importance of physical activity to maintain physical and mental health in extraordinary situations such as the pandemic and reported that physical exercise increases resistance to living conditions and disease (40). Alschuler et al (2020) showed that as schools improved their distance education practices, physical education should be given priority (24). According to Alschuler et al. (2020), besides sending homework plans for courses such as maths and English, it would be appropriate to send homework plans for physical activity (24).

In conclusion, the COVID-19 pandemic decreased students' sleep quality and physical activity levels. Keeping the immune system strong is important for protection from the disease in this process. The creation of regular biological rhythms is essential for a strong immune system. In this context, students should be informed about the importance of quality sleep and physical activity during the COVID-19 process. Training, seminars, etc., on the importance of sleep and physical activity, should be given through distance education and various activities. Encouraging directions can be made in terms of exercise practices that can be performed at home. Besides, the participation of the students can be ensured by adding physical education courses to the distance education curriculum.

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Conflict of interest

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References

1. Hammami A, Harrabi B, Mohr M, Krustrup P. Physical activity and coronavirus disease 2019 (COVID-19): specific recommendations for home-based physical training. Managing Sport and Leisure. 2020; 1-6. doi:10.1080/23750472.2020.1757494.
2. Şimşek E, Koç K, Özsoy D, Karakuş M. Investigation the Effect of Performing Physical Activity at Home on Sleep Quality and Life Satisfaction During Coronavirus (Covid-19) Outbreak. International Journal of Applied Exercise Physiology. 2020; 9: 2322-3537.

3. Çıtak Ş, Pekdemir Ü. An Analysis on Sleep Habits and Generalized Anxiety Levels of Individuals during the COVİD-19 Pandemic. Journal of Family Counseling and Education. 2020; 5(1): 60-73. doi: 10.32568/jfce.7420866.

4. Mayda Domaç F, Karacı R. COVID-19 ve Uyku. Uludüz D, Özge A, editor. Nörolojik Bilimler ve COVID-19. 1. Baskı, Ankara: Türkiye Klinikleri, 2020, p. 56-60.

5. Pelin Z. Uyku ve bağışıklık sistemi. Zeugma Sağlık Araştırmaları Dergisi.2020; 2(1):46-50.

6. Colbay M, Yuksel S, Fidan F, Acartürk G, Karaman O, Unlü M. Evaluation of the hemodialysis patient with Pittsburgh sleep quality index. Tüberküloz ve Toraks. 2007; 55:167-73.

7. Ayhan FF, Pervane Vural S. Fiziksel aktivite, obezite ve COVID-19. Ayhan FF, Demirbağ Kabayel D, editörler. COVID-19 Pandemisi ve Fiziksel Tıp ve Rehabilitasyon. 1. Baskı. Ankara: Türkiye Klinikleri; 2020, p.75-82.

8. Physical Activity and COVID-19 [Internet]. 2020 [cited: 2020 Jul 2] Available from: https://physio-pedia.com/Physical_Activity_and_COVID-19.

9. Campbell JP, Turner JE. Debunking the Myth of exercise-induced immuneSuppression: Redefining the impact of exercise on immunological health Across the Lifespan. Front Immunol. 2018; 16(9):648.

10. Güzel Özdemir P, Özışık M. COVİD-19 ve sirkadiyen ritim. Anatolian Journal of Psychiatry 2020; 20(3):336.

11. Beyond Sanitizing and Social Distancing – a Healthy Circadian Rhythm May Keep You Sane and Increase Resilience to Fight COVID-19 [Internet]. 2020 [cited: 2020 Jul 3] Available from: https://www.govexec.com/management/2020/04/beyond-sanitizing-and-social-distancing-healthy-circadian-rhythm-may-keep-you-sane-and-increase-resilience-fight-covid-19/164414/.

12. Ceylan Hİ, Saygın Ö, Türkcü ÜÖ. Obezite, Aerobik Egzersiz ve Sirkadiyen Ritim İlişkisi. İstanbul: Gece Akademi; 2005.

13. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Research. 1989; 28:193-213. doi:10.1016/0165-1781(89)90047-4.

14. Ağargün MY, Kara H, Anlar O. Pittsburgh Uyku Kalitesi İndeksinin Geçerliği ve Güvenirliği. Türk Psikiyatri Dergisi. 1996; 7:107-115.

15. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003; 35:1381-95.

16. Öztürk M. Üniversitede Eğitim-Öğretim Gören Öğrencilerde Uluslararası Fiziksel Aktivite Anketinin Geçerliliği ve Güvenirliği ve Fiziksel Aktivite Düzeylerinin Belirlenmesi. Ankara (TUR) Hacettepe Üniversitesi. Sağlık Bilimler Enstitüsü, Yüksek Lisans Tezi. 2005.

17. Altena E, Baglioni C, Espie CA, Ellis J, Gavriloff D, Holzinger B, et al. Dealing with sleep problems during home confinement due to the COVID-19 outbreak: Practical recommendations from a task force of the European CBT-I Academy. J Sleep Res.2020; 29:e13052. https://doi.org/10.1111/jsr.13052.

18. Ong, JL, Lau T, Massar S, Chong Z, Ng B, Koek D, et al. COVID-19 Related Mobility Reduction: Heterogenous Effects on Sleep and Physical Activity Rhythms. SLEEP J. 2020; 1–13. doi: 10.1093/sleep/zsaa179.

19. Casagrande M, Favieri F, Tambelli R, Forte G. The enemy who sealed the world: Effects quarantine due to the COVID-19 on sleep quality, anxiety, and psychological distress in the Italian population. Sleep Medicine. Sleep Med. 2020; 75:12-20. doi:10.1016/j.sleep.2020.05.011.

20. Kaparounaki CK, Patsali ME, Mousa DV, Papadopoulou EVK, Papadopoulou KKK, Fountoulakis KN. University students' mental health amidst the COVID-19 quarantine in Greece. Psychiatry Res. 2020; 290:113111. doi: 10.1016/j.psychres.2020.113111.

21. Huang Y, Zhao N. Chinese mental health burden during the COVID-19 pandemic. Asian J Psychiatr.2020; 51:102052. doi:10.1016/j.ajp.2020.102052

22. Cellini N, Canale N, Mioni G, Costa S. Changes in sleep pattern, sense of time and digital media use during COVID-19 lockdown in Italy. J Sleep Res. 2020; 15:e13074. doi: 10.1111/jsr.13074.

23. Sahu P. Closure of Universities Due to Coronavirus Disease 2019 (COVID-19): Impact on Education and Mental Health of Students and Academic Staff. Cureus, 2020; 12(4):e7541. https://doi.org/10.7759/cureus.7541.

24. Alschuler L, Weil A, Horwitz R, Stamets P, Chiasson AM, Crocker R, Maizes V. Integrative considerations during the COVID-19 pandemic. Explore (New York, N.Y.), 2020; S1550-8307(20)30113-0. https://doi.org/10.1016/j.explore.2020.03.007.

25. Çetin E. Melatonin ve bağışıklık sistemi. Erciyes Üniv Vet Fak Derg. 2005; 2(2):119-123.

26. Morin CM, Carrier J, Bastien C, Godbout R. Canadian Sleep and Circadian Network. Sleep and circadian rhythm in response to the COVID-19 pandemic. Can J Public Health. 2020; 111(5):654-657. doi:10.17269/s41997-020-00382-7.

27. Pinto A, Dunstan, D, Owen N, Bonfa, E, Gualano B. Combating physical inactivity during the COVID-19 pandemic. Nature Reviews Rheumatology. 2020; 16. 1-2. doi:10.1038/s41584-020-0427-z.

28. Simpson RJ, Katsanis E. The immunological case for staying active during the COVID-19 pandemic. Brain, Behavior, and Immunity.2020; 87: 6–7.

29. Kaya Ciddi P. Covid-19 Salgınında Sosyal İzolasyon Sırasında Fiziksel Aktivite Durumunun Yaşam Kalitesi Üzerine Etkisi. İstanbul Ticaret Üniversitesi Sosyal Bilimler Dergisi. 2019; 37: 262-279.

30. Ercan Ş, Keklicek H. Investigation of the Change in Physical Activity Levels of University Students Due to COVID-19 Pandemic. İzmir Katip Çelebi Üniversitesi Sağlık Bilimleri Fakültesi Dergisi. 2020; 5(2):69-74.

31. Maugeri G, Castrogiovanni P, Battaglia G, Pippi R, D'Agata V, Palma A, et al. The impact of physical activity on psychological health during Covid-19 pandemic in Italy. Heliyon, 2020; 6(6): e04315. https://doi.org/10.1016/j.heliyon.2020.e04315.

32. Gallo LA, Gallo TF, Young SL, Moritz KM, Akison LK. The Impact of Isolation Measures Due to COVID-19 on Energy Intake and Physical Activity Levels in Australian University Students. Nutrients.2020; 12(6):1865. doi:10.3390/nu12061865.

33. Chen P, Mao L, Nassis GP, Harmer P, Ainsworth BE, Li F. Returning Chinese school-aged children and adolescents to physical activity in the wake of COVID-19: Actions and precautions. J Sport Health Sci. 2020; 9(4):322-324. doi:10.1016/j.jshs.2020.04.003

34. Savcı M, Öztürk M, Arıkan H, İnce İ, Tokgözoğlu L. Üniversite Öğrencilerinin Fiziksel Aktivite Düzeyleri. Türk Kardiyol Derneği Araştırmaları. 2006; 34(3):166-172.

35. Serin Y, Tek NA. Effect of Circadian Rhythm on Metabolic Processes and the Regulation of Energy Balance. Ann Nutr Metab. 2019; 74:322–330.

36. Akıncı E, Orhan FÖ. Sirkadiyen Ritim Uyku Bozuklukları. Psikiyatride Güncel Yaklaşımlar. 2016; 8(2):178-189.

37. Shneider A, Kudriavtsev A, Vakhrusheva A. Can melatonin reduce the severity of COVID-19 pandemic?. International Reviews of Immunology. 2020; 39(4): 153–162. https://doi.org/10.1080/08830185.2020.1756284.

38. Meira e Cruz M, Miyazawa M, Gozal D. Putative contributions of circadian clock and sleep in the context of SARS-CoV-2 infection. Eur Respir J. 2020; 55(6):2001023.

39. Hower IM, Harper SA, Buford TW. Circadian Rhythms, Exercise, and Cardiovascular Health. Journal of Circadian Rhythms.2018; 16:7. https://doi.org/10.5334/jcr.164.

40. AÇ Tunç, E Zorba, YE Çingöz. Covid 19 Salgını Döneminde Egzersizin Yaşam Kalitesine Etkisi. Uluslararası Güncel Eğitim Araştırmaları Dergisi. 2020; 6(1):127-135.

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Dental patients' attitudes and behaviors towards and knowledge and fear of COVID-19

Ashhan AKBULUT[1,*], Kaan ORHAN[2]

[1]Department of Dentomaxillofacial Radiology, School of Dentistry, Istanbul Medipol University, İstanbul, Türkiye
[2]Department of Dentomaxillofacial Radiology, School of Dentistry, Ankara University, Ankara, Türkiye

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Abstract

COVID-19 is a pandemic that threatens public health worldwide. Dentists working at great risk play an essential role in protecting public health against coronavirus. This study aimed to evaluate the dental patients' attitudes and behaviors towards and knowledge and fear of COVID-19. We conducted a self-report questionnaire-based survey from 10.26.2020 to 01.12.2021 and included 1110 dental patients who applied at the Department of Dentomaxillofacial Radiology of Istanbul Medipol University Dental School. We divided the survey into four divisions: 1) Patient's knowledge about COVID-19, 2) attitudes, 3) behavior, 4) patient's fear of COVID-19. We calculated the Cronbach's alpha coefficient for the reliability of the COVID-19 Fear scale and evaluated significance at the $p < 0.05$ level. 84.6% of the participants believed that COVID-19 could be transmitted by aerosol. 94.1% of the participants considered COVID-19 a risk for their health and 93.3% that their social life was affected. 46.3% only wanted to have emergency dental treatment and postpone their dental care, and only 7% applied to the clinic for the aesthetic process. Women had a higher fear level of COVID-19 than men. We found the knowledge, attitudes, and behaviors of the patients who applied to the dentomaxillofacial radiology positive towards COVID-19. Dental health professionals should take extra measures in dental clinics to prevent the spread of COVID-19, and more efforts should be made to improve public knowledge, attitude, and behavior.

Keywords: Covid-19, knowledge, attitude, behavior, fear

1. Introduction

Human coronavirus belongs to the Coronaviridae family, and this virus is composed of big, single, plus-stranded RNA as its genome (1-3). Coronaviruses are spheric construction and have spiked glycoprotein on their surface that makes them appear like a crown, the reason for their name corona (3). The first human coronavirus (HCoV) was observed in the mid-1960s (4). The novel human coronavirus, recently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible now for coronavirus disease 2019 (COVID-19) cases in the whole world, arose in Wuhan, Hubei Province, China, on December 12, 2019. On March 11, 2020, the World Health Organization (WHO) announced the virus' spread across the globe as a pandemic (2-5). This is the third infection outbreak due to a coronavirus in less than 20 years. The severe acute respiratory syndrome (SARS) outbreak in 2002-2003 resulted in more than 8000 cases in 26 countries and had a mortality rate of approximately 10%, while the Middle East respiratory syndrome (MERS) outbreak spread to 27 countries and had a 34% mortality rate (6). The first COVID-19 case in Turkey was detected on March 11, 2020, by the Turkish Ministry of Health (7), while the first death due to the virus was reported on March 15, 2020 (8).

SARS-CoV-2 was considered to have a zoonotic route of transmission, which can spread from animals to humans, such as SARS-CoV and the MERS-CoV. (9, 10) Nonetheless, the SARS-CoV-2 is transmitted from human to human via droplet transmission and direct contact with oral, nasal, and eye mucous membranes (11-13). Several investigations have shown that human coronaviruses can remain viable on various inanimate surfaces from 2 hours to up to 9 days (1, 14, 15).

SARS-CoV-2 has an incubation period of 1 to 14 days (5 to 6 days on average). Primary symptoms are fever, cough, myalgia or fatigue. Abnormal chest computed tomography (CT) most showed bilateral pneumonia, with ground-glass opacity and bilateral patchy shadows image, and severe respiratory distress. Less prevalent symptoms are sputum production, headache, hemoptysis, and diarrhea (1, 9, 15, 16). Recent studies reported that loss of taste and smell could be the first and only signs of COVID-19 (11).

Dentists and dental assistants are in the highest risk group among all health care professionals, as they are in close contact with patients and exposed to spatter of patients' secretions/coughing/sneezing during dental treatment, droplets, aerosols, and saliva. Droplets and aerosols are the most important routes of transmission in dental procedures (1, 2, 6, 13, 17). Thus, dental offices may be the relevant hotspot for virus transmission, putting health professionals at high

*Correspondence: aakbulut@medipol.edu.tr

risk for COVID-19 infection and patients at risk for nosocomial infection (13). Besides, hand washing is of great importance for dental practices, as the fecal-oral transmission of COVID-19 has also been reported (10, 17). The use of high-speed handpiece and sonic/ultrasonic devices, and airflow instruments in the oral cavity creates significant amounts of droplets and aerosols that remain suspended in the air for up to 30 minutes before their deposition on surfaces or being sucked into the air conditioning system, due to their small size (17, 18). Dentistry practices include various biosafety precautions and recommendations due to the high risk of contamination during dental care. Dental procedures were limited to urgencies and emergencies or postponed in many countries (4, 12, 19). So, Meng et al. (18) were the first to report on personal protection equipment (PPE) that protected dental professionals in the course of the COVID-19 outbreak in Wuhan, China (1-3, 6, 18, 20, 21).

The diagnosis of COVID-19 can be based on the combination of epidemiologic information (e.g., a history of travel to or residence in affected region 14 days before symptom onset), clinical symptoms, CT imaging findings, and laboratory tests (e.g., reverse transcriptase-polymerase chain reaction [RT-PCR] tests on respiratory tract specimens) according to standards of the WHO (1, 14-18, 21, 22).

Researchers are conducting many studies on the diagnosis and treatment of COVID-19 worldwide. This outbreak creates serious problems for people's mental health, such as fear and anxiety (8,19,22-24). Hence, there is a need for an assessment tool to reveal the effects of COVID-19 on mental health. For this reason, Ahorsu et al. (24) improved a valid and reliable questionnaire to assess the fear of COVID-19 (8, 19, 24). Ladikli et al. (8) showed that the Turkish version of this fear scale is reliable and valid for the Turkish population (8).

The present study aimed to evaluate the attitudes and behaviors towards and knowledge (ABK) and fear of COVID-19 among patients who applied for dental care during the pandemic.

2. Materials and methods

The Ethics Research Committee approved this study of the Istanbul Medipol University (10840098-772.02-E.61626). This study utilized a self-report questionnaire-based survey. In the beginning, 100 patients participated in the survey for the pilot study who applied at the Istanbul Medipol University Dental School, Department of Dentomaxillofacial Radiology. We revised the questionnaire to ensure suitability, validity, and answers' practicability. We conducted a questionnaire study on 1110 patients who applied to the Dentomaxillofacial Radiology Department. The study took place from October 26, 2020, to January 12, 2021. After signing the consent form, the participants received the questionnaires consisting of 31 questions. We divided the survey into four divisions: 1) patient's knowledge (8 questions), 2) patient's attitudes (5 questions), 3) patient's behaviors (6 questions), and 4) patient's fear of COVID-19 (7 questions) and sociodemographic characteristics (5 questions).

2.1. Statistical Analysis

We evaluated the findings using IBM SPSS Statistics 22 for statistical analysis (SPSS IBM, Turkey) and the suitability of the parameters to the normal distribution by the Kolmogorov-Smirnov and Shapiro Wilks tests. We found that the parameters did not show a normal distribution. We evaluated the study data using descriptive statistical methods (mean, standard deviation, frequency) and the Kruskal Wallis and Mann Whitney U tests to compare the quantitative data. We compared the qualitative data using the Chi-Square, Fisher's Exact Chi-Square, Fisher Freeman Halton tests, and Continuity (Yates) Correction. We calculated the Cronbach's alpha coefficient for the reliability of the COVID-19 Fear scale and evaluated significance at the $p < 0.05$ level.

3. Results

We conducted the study with 1110 people ranging in age from 18 to 82. The average age was 35.38 ± 12.40 years. While 117 (10.5%) people had COVID-19, 993 (89.5%) did not (Table 1).

Table 1. shows the distribution of the sociodemographic characteristics of the patients

		n	%
Gender	Male	487	43.9
	Female	623	56.1
Age	18-30	432	38.9
	30-45	410	36.9
	45-60	221	19.9
	60+	47	4.2
Job	Student	188	16.9
	Unemployed	143	12.9
	Officer	190	
	Technical profession	300	27.0
	Physician	3	
	Army / police	26	
	Housewife	209	0.3
	Medical staff	51	17.1
Education status	Primary School	254	22.9
	High school	334	30.1
	University and above	522	47.0

The participants indicated their level of agreement using a five-item Likert-type scale. Answers included "strongly disagree," "disagree," "neither agree nor disagree," "agree," and "strongly agree". The minimum score possible for each question was 1, and the maximum was 5. We calculated the total score by adding up each item score. The higher the score, the greater the fear of COVID-19 (24). Table 2 shows the distributions of the answers given to the COVID-19 fear scale questions. The Cronbach's alpha coefficient of the scale was 0.873. The COVID-19 fear score ranged from 7 to 35, with an average of 16.46 ± 7.11 and a median score of 16.

Table 2. Distribution of answers to Fear of Coronavirus-19 scale questions

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
	n (%)	n (%)	n (%)	n (%)	n (%)
I am most afraid of coronavirus-19.	116 (25.8%)	59 (13.1%)	60 (13.3%)	95 (21.1%)	120 (26.7%)
It makes me uncomfortable to think about coronavirus-19.	121 (26.9%)	58 (12.9%)	46 (10.2%)	107 (23.8%)	118 (26.2%)
My hands become clammy when I think about coronavirus-19.	245 (54.4%)	106 (23.6%)	52 (11.6%)	25 (5.6%)	22 (4.9%)
I am afraid of losing my life because of coronavirus-19.	179 (39.8%)	75 (16.7%)	62 (13.8%)	72 (16%)	62 (13.8%)
When watching news and stories about coronavirus-19 on social media, I become nervous or anxious.	150 (33.3%)	74 (16.4%)	74 (16.4%)	96 (21.3%)	56 (12.4%)
I cannot sleep because I am worried about getting coronavirus-19.	274 (60.9%)	98 (21.8%)	45 (%10)	20 (4.4%)	13 (2.9%)
My heart races or palpitates when I think about getting coronavirus-19.	284 (63.1%)	90 (%20)	38 (8.4%)	17 (3.8%)	21 (4.7%)

"COVID-19 can be spread by aerosol (water particles in the air) created during dental treatment." 84.6% of the participants answered yes to his proposition. 100% of the participants have heard of the COVID-19. While the first source that 60.3% heard about the virus was television, 21.7% heard it from the internet, 13.8% from social media sites, 2.8% from the people around, and 1.4% from newspapers.

When asked about "the name of the disease that caused the new COVID-19 pandemic", 88.8% said COVID-19, 1.8% new corona and 0.8% CDC-19, while 9.1% did not know.

When asked about "the place where the first COVID-19 case was detected", 92.1% answered Wuhan.

68.8%, 27.5%, 3%, and 0.7% said COVID-19 would be transmitted from person to person, from person to person and from animal to person, from animal to human, and in different ways, respectively.

When asked about the expected symptoms of COVID-19, 74.5% said fever and cough, 55.1% difficulty breathing, 18.7% pneumonia, 10.3% abdominal pain, 0.9% heart attack, and 23.5% did not know.

When asked about the "ways of spreading COVID-19", 80.5% replied respiratory droplets, 30.3% cold-cold, 3.8% undercooked meat products, 2.9% pets, 1.3% leafy vegetables, and 14.9% all of the above (Table 3).

When asked about the "protection measures against COVID-19", 10.1% answered mouth mask, 5.2% physical distance, 3.4% hygiene, 88.9% all, and 0.5% none.

94.1% of the participants considered COVID-19 a health risk. 6.6% were afraid of consuming meat and meat products due to coronavirus. 93.3% believed COVID-19 affected social life.

When asked whether COVID-19 negatively affected the decision to undergo dental treatment, 46.3%,39.9%, and 13.8% chose the option "It is affected; I only had emergency dental treatments", "It did not affect me; I am getting my dental treatment", and "Affected, I am delaying my dental treatment", respectively (Table 4).

10.5% of the participants had COVID-19. When asked about the tests applied for diagnosis, 212 people answered; 89.2% had swab samples, 22.6% computed tomography, 23.6% blood test and 3.8% clinical findings.

While 0.6% of the participants had been abroad in the last 14 days, 13.5% of them had people diagnosed with COVID-19 in their immediate vicinity in the last 14 days.

When asked about what they cared about when applying to the dentist during the pandemic period, 83.4%, 78%, 77.8%, and 75.6% chose social distance from other patients in the waiting room, dentists using a disposable apron changed for each patient, to have disinfectants for the use of patients in the patient admission area, and dentists changing N95 surgical mask for each patient, respectively.

48.7% of the participants went to the dental hospital for restorative treatment, 23.2% gingival treatment (tartar removal), 19% endodontic treatment (root canal treatment), 18.3% Surgical treatment (tooth extraction), 14.8% Prosthetic treatment (implant, prosthesis), 11.4% Orthodontic treatment (braces treatment) and 7% aesthetic treatment (teeth whitening) (Table 5).

Women's mean COVID-19 fear score was statistically significantly higher than men's ($p: 0.001$; $p < 0.05$).

There was no statistically significant difference between age groups, occupational groups or education levels regarding COVID-19 fear scores ($p > 0.05$) (Table 6).

Table 3. Distribution of answers given to knowledge questions

		n	%
COVID-19 can be spread by aerosol (water particles in the air) created during dental treatment.	Yes	939	84.6
	No	171	15.4
Heard about the coronavirus	Yes	1110	100
The first source of coronavirus heard	Newspaper	16	1.4
	Television	669	60.3
	Internet	241	21.7
	Social media sites	153	13.8
	People around me	31	2.8
Where the first COVID-19 case was detected	Wuwei	4	0.4
	Xining	3	0.3
	Wuhan	1022	92.1
	Dingxi	5	0.5
	I do not know	76	6.8
COVID-19 transmission route	From animal to person	33	3.0
	From person to person	764	68.8
	All of the above	305	27.5
	None of the above	8	0.7
Symptoms of COVID-19	Fever and cough	827	74.5
	Heart attack	10	0.9
	Abdominal pain	114	10.3
	Pneumonia	208	18.7
	Difficulty breathing	612	55.1
	All of the above	262	23.6
Ways of COVID-19 spread	Leafy vegetables	14	1.3
	Undercooked meat products	42	3.8
	Pets	32	2.9
	Respiratory droplets	893	80.5
	Cold, common cold	336	30.3
	All of the above	165	14.9

Table 4. Distribution of the answers given to the attitude questions

		n	%
Protection measures against COVID-19	Mouth mask	112	10.1
	Physical distance	58	5.2
	Hygiene	38	3.4
	All of the above	**987**	**88.9**
	None of the above	6	0.5
COVID-19 is a health risk	Yes	1044	94.1
	No	66	5.9
Avoiding consumption of meat and meat products due to coronavirus	Yes	73	6.6
	No	1037	93.4
COVID-19 has an impact on social life	Yes	1036	93.3
	No	74	6.7
The negative impact of COVID-19 on the decision to undergo dental treatment	It did not affect me; I am getting my dental treatment	443	39.9
	Affected, I am delaying my dental treatment	153	13.8
	It is affected; I only had emergency dental treatments	514	46.3

Table 5. Distribution of responses given to behavior questions

		n	%
Having COVID-19 disease	Yes	117	10.5
	No	993	89.5
Tests for diagnosis (n=212)	Swab sample (PCR)	189	89.2
	Computed Tomography	48	22.6
	Blood test	50	23.6
	Clinical findings	8	3.8
Being abroad in the last 14 days	Yes	7	0.6
	No	1103	99.4
A person diagnosed with COVID-19 in the immediate environment in the last 14 days	Yes	150	13.5
	No	960	86.5
Important points when applying to the dentist during the pandemic period	The dentist's use of disposable gowns changed for each patient	866	78.0
	The dentist's use of N95 surgical masks changed for each patient	839	75.6
	Social distance with other patients in the waiting room	926	83.4
	Keeping a disinfectant material for the use of patients in the patient admission area	864	77.8
Reason for visiting the dental hospital for treatment	Orthodontic treatment (braces treatment)	126	11.4
	Restorative treatment (tooth decay, fillings)	541	48.7
	Endodontic treatment (root canal treatment)	211	19.0
	Aesthetic treatment (teeth whitening)	78	7.0
	Prosthetic treatment (implant, prosthesis)	164	14.8
	Periodontal treatment (tartar removal)	258	23.2
	Surgical treatment (tooth extraction)	203	18.3

Table 6. Evaluation of COVID-19 Fear score according to demographic characteristic

		COVID-19 Fear Score				
		N	Aver±SS	Median	p	
Age	18-30	186	16.77±7.16	16	10.253	
	30-45	153	16.14±6.63	16		
	45-60	93	15.82±7.48	15		
	60+	18	19.33±8.22	20.5		
Gender	Male	197	15.12±6.48	15	20.001*	
	Female	253	17.51±7.41	17		
Job	Student	86	16.12±6.43	15,5	10.111	
	Housewife/Unemployed	155	17.45±7.62	17		
	Officer	79	17.05±7.54	16		
	Technical profession	104	15.36±6.4	15		
	Medical staff / Physician	26	14.38±6.8	11.5		
Education status	Primary School	109	15.82±7.57	16	10.432	
	High school	135	16.4±7.16	16		
	University and above	206	16.84±6.83	16		

There was a statistically significant difference between the sources men and women first heard about COVID-19 (p: 0.001; p <0.05). While the rate of men hearing from the internet (27.3%) was higher than women (17.3%), that of women learning from TV (64.5%) was significantly higher than men (54.8%).

Men gave the Wuhan correct answer for where the first COVID-19 case was detected (94.3%) significantly higher than women (90.4%) (p: 0.010; p <0.05).

The ratio of women who chose from person to person as the transmission route of COVID-19 (74.5%) was significantly higher than men (61.6%) (p: 0.001; p <0.05).

Abdominal pain in women (11.9%) for symptoms of COVID-19 was significantly higher than men (8.2%) (p: 0.046; p <0.05). There was no statistically significant difference between the responses of men and women to other COVID-19 symptoms (p >0.05).

The rate of men calling the COVID-19 transmission routes as pets (4.1%) was significantly higher than that of women (1.9%) (p:0.048; p<0.05). There was no statistically significant difference between the responses of men and women to other COVID-19 transmission routes (p>0.05).

There was no statistically significant difference between the responses of men and women to other COVID-19 information questions (p >0.05) (Table 7).

There was also no statistically significant difference between the genders in terms of the answers given to the attitude questions (p >0.05) (Table 8).

Table 7. Evaluation of answers given to knowledge questions according to gender

		n (%)	n (%)	p
COVID-19 can be spread by the aerosol generated during dental treatment.	Yes	403 (82.8%)	536 (86%)	[1]0.133
	No	84 (17.2%)	87 (14%)	
The first source of coronavirus heard	Newspaper	9 (1.8%)	7 (1.1%)	[1]0.001*
	TV	267 (54.8%)	402 (64.5%)	
	Internet	133 (27.3%)	108 (17.3%)	
	Social media sites	65 (13.3%)	88 (14.1%)	
	People around me	13 (2.7%)	18 (2.9%)	
The name of the disease that caused the new coronavirus outbreak	New Corona	9 (1.8%)	5 (0.8%)	[1]0.069
	CDC-19	2 (0.4%)	7 (1.1%)	
	COVID-19	440 (90.3%)	546 (87.6%)	
	I do not know	36 (7.4%)	65 (10.4%)	
Where the first COVID-19 case was detected	Wuwei	3 (0.6%)	1 (0.2%)	[2]0.010*
	Xining	1 (0.2%)	2 (0.3%)	
	Wuhan	459 (94.3%)	563 (90.4%)	
	Pingxi	3 (0.6%)	2 (0.3%)	
	I do not know	21 (4.3%)	55 (8.8%)	
COVID-19 transmission route	From animal to person	18 (3.7%)	15 (2.4%)	[2]0.001*
	From person to person	300 (61.6%)	464 (74.5%)	
	All of the above	167 (34.3%)	138 (22.2%)	
	None of the above	2 (0.4%)	6 (%1)	
Symptoms of COVID-19	Fever and cough	361 (74.1%)	466 (74.8%)	10.799
	Heart attack	7 (1.4%)	3 (0.5%)	30.115
	Abdominal pain	40 (8.2%)	74 (11.9%)	10.046*
	Pneumonia	84 (17.2%)	124 (19.9%)	10.261
	Difficulty breathing	255 (52.4%)	357 (57.3%)	10.100
	All of the above	119 (24.4%)	143 (%23)	10.564
Ways of COVID-19 spread	Leafy vegetables	7 (1.4%)	7 (1.1%)	40.846
	Undercooked meat products	22 (4.5%)	20 (3.2%)	10.257
	Pets	20 (4.1%)	12 (1.9%)	40.048*
	Respiratory droplets	391 (80.3%)	502 (80.6%)	10.904
	Cold, common cold	150 (30.8%)	186 (29.9%)	10.734
	All of the above	76 (15.6%)	89 (14.3%)	10.540

Table 8. Evaluation of the answers given to the attitude questions according to gender

		Male	Female	
		n (%)	n (%)	p
Protection measures against COVID-19	Mouth mask	42 (8.6%)	70 (11.2%)	10.152
	Physical distance	23 (4.7%)	35 (5.6%)	10.506
	Hygiene	14 (2.9%)	24 (3.9%)	20.470
	All of the above	441 (90.6%)	546 (87.6%)	10.125
	None of the above	1 (0.2%)	5 (0.8%)	30.239
COVID-19 is a health risk	Yes	451 (92.6%)	593 (95.2%)	10.072
	No	36 (7.4%)	30 (4.8%)	
Avoiding consumption of meat and meat products due to coronavirus	Yes	29 (%6)	44 (7.1%)	10.460
	No	458 (%94)	579 (92.9%)	
COVID-19 had an impact on social life	Yes	453 (%93)	583 (93.6%)	10.710
	No	34 (%7)	40 (6.4%)	
The negative impact of COVID-19 on the decision to undergo dental treatment	It didn't affect me, I am getting my dental treatment	206 (42.3%)	237 (%38)	10.134
	Affected, I am delaying my dental treatment	72 (14.8%)	81 (%13)	
	It is affected, I only had emergency dental treatments	209 (42.9%)	305 (%49)	

4. Discussion

The ABK towards COVID-19 affects the severity of the infectious disease, the extent of its spread, and the mortality rate. It is necessary to eliminate the lack of knowledge of the society against COVID-19, which poses a serious threat to public health, to change attitudes and behaviors, and to take necessary measures. The serious increase in the number of cases and loss of life negatively affected the patients' psychology and caused fear (8,22,24). Although there were many web-based surveys on COVID-19 in the literature review, the current study is a rare hand-delivered survey. This study aimed to evaluate the level of ABK and fear of COVID-19 of patients who applied at the School of Dentistry in Istanbul Medipol University.

Dentists are at great risk because they are in close contact with the patient's oral cavity, with the aerators and micromotor instruments rotating at high speed, scattering around aerosols and droplets (3,9,10). Dental treatments can cause the virus to spread easily due to aerosols. Therefore, various preventions should be taken before and during dental treatments (9,16-18).

The majority of the participants said that COVID-19 could be spread by aerosol generated during dental treatment, from person to person was the main transmission route, the most common symptoms of the disease were fever and coughing, and breathing difficulties, and the spread route was primarily respiratory droplets (4,11,14,16,20,23).

In line with the current study, a survey conducted in Lebanon and another study in Turkey revealed that television was the first news source about COVID-19 (27). The rate of men hearing about COVID-19 from the internet and women hearing from television were higher.

In addition, the rate of women stating from person to person as the transmission route of the disease and abdominal pain as one of the symptoms of the disease was higher than men.

In this study, we examined COVID-19 fear levels of patients who applied to the dental school during the pandemic period in Turkey and determined that individuals had a moderate fear of COVID-19 in agreement with the literature (28).

Alicilar et al. (25) conducted a study on 1179 people in Turkey; 30% of the participants stated that they felt completely safe, while the majority were worried about COVID-19.

Gencer N (28) and Bakioglu et al. (29) evaluated the fear level of women and found it to be higher than men. They found no statistically significant difference between the individuals regarding age, occupation, and educational status. They also stated that those who feared COVID-19 most were between the ages of 15-20. However, the current study revealed that the level of fear did not show a significant difference in terms of age (28). Consistent with the studies of Gencer N (28), Bakioglu et al. (29), we concluded that the level of education of our participants had no effect on their fear of COVID-19 (28).

88.9 % of our respondents stated that mouth masks-social distance-hygiene should be maintained against the new COVID-19. Alicilar et al.'s (25) study in April 2020 reported that the individuals practised protective measures at a very high rate; 98.0% washed their hands with soap and water, 83.3% wore masks, and 76.9% maintained social distance in Turkey (25).

94.1% of patients perceived COVID-19 as a risk for health, and 93.3% that COVID-19 affected their social life. The literature also showed a significant reduction in the number of patients applying to dental clinics during the COVID-19 outbreak (23). Almas et al. (23) reported that

91.2% of participants reported having only urgent dental care, whereas 6.5% did not choose to have a dental procedure at all. 46.3% of the patients had only emergency dental treatment, while 13.8% postponed their dental treatment until after the pandemic (10,14,23). They also found no statistically significant difference between the genders regarding the answers given to the attitude questions.

At the COVID-19 Scientific Committee meeting held on 23.03.2020, decisions were taken regarding the emergency dental treatments to be carried out during the pandemic period. These decisions are listed below (Ministry of Health, General Directorate of Public Health, "Emergency and Mandatory Service in Dentistry" item No: 44773052).

1. Severe pain due to pulpal inflammation toothache

2. Severe pain from pericoronitis or third molar

3. Postoperatively developed osteitis or alveolitis

4. Abscess causing localized pain and swelling or bacterial infection

5. Pain or soft tissue trauma due to tooth fracture

6. Trauma-induced tooth avulsion/luxation

7. Jaw or face fractures

8. Acute and painful lesions/ulcerations of the oral mucosa

9. Life-threatening or uncontrolled bleeding

10. Intraoral/extraoral infections threatening the patient's airway patency

11. Treatment of patients who are planning to receive radiotherapy and chemotherapy or who have received an organ transplant and are planned to be transplanted

12. Patients have systemic disease requiring dental consultation

13. Suture removal, temporary restoration loss/fractures, and preventing the use of removable prosthesis non-aerosol treatment of dental caries

14. Patients with orthodontic treatment dislocation and breakage and wires

15. Newborn patients with cleft lip and palate requiring nutritional plate application

16. Temporomandibular joint luxation

17. Biopsy (in cases malignancy is suspected) (30).

In this study, 117 participants out of 1110 had SARS-CoV-2. We determined that the PCR test was primarily performed for the diagnosis. A small number was abroad in the last 14 days, and 13.5% had people diagnosed with COVID-19 in their immediate surroundings in the last 14 days. Most stated that they should maintain social distance with other patients in the waiting room (83.4%).

We observed that the patients mostly applied to the clinic for restorative dental treatment and the least visited the school of dentistry with aesthetic complaints. This situation supports the patients' views of having emergency treatment procedures first.

According to recent research, the preventive measures that dentists should take against the possibility of the spread of SARS-CoV-2 infection were defined in 3 different stages:

Primary precautions: Use of a disposable cap, disposable surgical mask, white coat, safety glasses or face shield, disposable latex or nitrile gloves.

Secondary measures: In addition to the above measures, wearing reusable isolation gowns or surgical gowns.

Although no action should be taken on an infected person, in such a situation, close contact is inevitable, and special protective clothing is required (17).

Dental professionals should prefer extraoral imaging methods such as panoramic radiography instead of intraoral radiographic techniques during this pandemic, as it may cause coughing (17).

In addition, mouth rinse before the dental process has been proved to decrease microorganisms' load in droplets and aerosols. Widely used as a mouthwash in dentistry, it is known that chlorhexidine is not effective enough to kill the COVID-19. Since SARS-CoV-2 is sensitive to oxidation, 1% hydrogen peroxide or mouthwashes containing oxidative agents such as 0.2% povidone are recommended (3,6,7,14,17,18).

Further studies with more participation and questionnaire surveys including different questions are needed to raise society's awareness against COVID-19 to improve and protect public health. Furthermore, there was no vaccine evaluation since the vaccination studies for COVID-19 had not yet been clarified at the time of this study.

Dental clinics, especially the department of dentomaxillofacial radiology, are places of intensive routine patient access and high risk for COVID-19 outbreak. Dentists and clinical staff should take necessary measures to protect the health of both the patients and their own. All dental patients should be considered suspicious for COVID-19.

As a result, we found in the current study positive attitudes and behaviors towards and knowledge of COVID-19 in a group of patients who applied to a dental school. There was a significant positive correlation in the patients' attitudes and behaviors towards and knowledge of COVID-19. There is a need for further studies to raise society's awareness of COVID-19.

With the recent developments in vaccines against COVID-19 and the decrease in the number of deaths and cases due to COVID-19, fear is gradually decreasing in patients applying to dentistry and in the Turkish society.

Finally, as long as the pandemic continues, dental health professionals should take supplement precautions in dental clinics to prevent contamination against COVID-19, and more efforts should be made to improve public knowledge, attitude, and behavior.

Conflict of interest

There was no conflict of interest to declare.

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References

1. Chen N., Zhou M., Dong X., Qu J., Gong F., Han Y., Qui Y., Wang J., Liu Y., Wei Y., Xia J., Yu T., Zhang X., & Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020; 395(10223), 507–513. doi:10.1016/S0140-6736(20)30211-7
2. Kamate S.K., Sharma S., Thakar S., Srivastava D., Sengupta K., Hadi A.J., Chaudhary A., Joshi R., & Dhanker K. Assessing knowledge, attitudes, and practices of dental practitioners regarding the COVID-19 pandemic: A multinational study. Dent Med Probl. 2020; 57(1), 7-14. doi.org/10.17219/dmp/119743
3. Kochhar A.S., Bhasin R., Kochhar GK, & Dadlani H. COVID-19 pandemic and dental practice. Int J Dent. 2020,8894794, 1-5. doi: 10.1155/2020/8894794
4. Putrino A., Raso M., Magazzino C., & Galluccio G. Coronavirus (COVID-19) in Italy: knowledge, management of patients, and clinical experience of Italian dentists during the spread of contagion. BMC Oral Health. 2020; 20:200. https://doi:10.1186/s12903-020-01187-3
5. Sirin H., Ketrez G., Ahmadi A.A., & Arslan A. Community approach towards COVID-19 in Turkey: one month after the first confirmed case. THBD. 2020; 77(4), 381-98. doi:10.5505/TurkHijyen.2020.87059
6. Stangvaltaite-Mouhat L., Uhlen M.M., Skudutyte-Rysstad R., Hovden E.A.S., Shabestari M., & Ansteinsson V.E. Dental health services response to COVID-19 in Norway. Int J Environ Res Public Health. 2020; 17(16), 5843.2-17. doi: 10.3390/ijerph17165843
7. Karayurek F., Yilmaz-Cırakoglu N., Gulses A., & Ayna M. Awareness and knowledge of SARS-CoV-2 infection among dental professionals according to the Turkish national dental guidelines. Int J Environ Res. 2021; 8,18(2), 442, 2-13. doi.org/10.3390/ijerph18020442
8. Ladikli N., Bahadır E., Yumuşak F.N., Akkuzu H., Karaman G., & Turkkan Z. [KOVİD-19 Korkusu Ölçeği'nin Türkçe güvenirlik ve geçerlik çalışması] The reilability and validity of Turkish version of Coronavirus Anxiety Scale. Int J Soc Sci. 2020; 3 (2), 71-80.
9. Moffat R.C., Yentes C.T, Crookston B.T, & West JH Patient perceptions about professional dental services during the COVID-19 pandemic. IAADR. 2021; 6(1), 15–23. doi: 10.1177/2380084420969116
10. Smail FS & Ates MM. Knowledge and awareness of Turkish population about dental treatment during COVID-19- A questionnaire-based survey. J Clin Diagnostic Res. 2020; 14(8), ZC20-ZC24. 20. doi:10.7860/JCDR/2020/45101.13917
11. Biadsee A., Biadsee A., Kassem F., Dagan O., Masarwa S., & Ormianer Z. Olfactory and oral manifestations of COVID-19: Sex-related symptoms—A potential pathway to early diagnosis. Otolaryngol Head Neck Surg. 2020; 163(4), 722–28. doi:10.1177/0194599820934380
12. Faccini M., Ferruzzi F., Mori AA., Santin GC., Oliviera R.C., de Oliviera R.C.G., Queiroz PM., Salmeron S., Pini N.I.P., Sundfeld D., & Freitas K.M.S. Dental care during COVID-19 outbreak: A web-based survey. Eur J Dent. 2020; 14 (Suppl 1), 14–9. doi: 10.1055/s-0040-1715990
13. Gurzawska-Comis K., Becker K., Brunello G., Gurzawska A., & Schwarz F. Recommendations for dental care during the COVID-19 pandemic. J Clin Med. 2020; 9(6), 1833. doi:10.3390/jcm9061833
14. Falahchai M., Hemmati Y.B., & Hasanzade M. Dental care management during the COVID-19 outbreak. Spec Care Dentist. 2020; 1-10. doi:10.1111/scd.12523
15. Wang D., Hu B., Hu C., Zhu F., Liu X., Zhang J., Wang B., Xiang H., Cheng Z. Peng Z. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–ınfected pneumonia in Wuhan, China. JAMA. 2020; 323(11),1061–69. doi:10.1001/jama.2020.1585
16. Cagetti M.G, Cairoli J.L, Senna A., & Campus G. COVID-19 outbreak in North Italy: An overview on dentistry. A questionnaire survey. Int J Environ Res. 2020; 17(11), 3835, 2-12. doi:10.3390/ijerph17113835
17. Peker I., Pamukcu U., Taka K., & Ucok O. [Diş hekimliği pratiğinde koronavirüs salgınına karşı alınması gereken önlemler] Measures to be taken against the coronavirus outbreak in dentistry practice. Turkiye Klinikleri J Dental Sci. 2021; 27(2), 294-306. doi:10.5336/dentalsci.2020-75270
18. Meng L., Hua F., & Bian Z. Coronavirus disease 2019 (COVID-19): Emerging and future challenges for dental and oral medicine. J Dental Res. 2020; 99(5), 481-487. doi:10.1177/002203452091426
19. Satici B., Gocet-Tekin E., Deniz M.E., & Satici S.A. (2020). Adaptation of the Fear of COVID-19 Scale: Its association with psychological distress and life satisfaction in Turkey. Int J Ment Health Addict. 8,1-9. doi:10.1007/s11469-020-00294-0
20. Nasser Z., Fares Y., Daoud R., & Abou-Abbas L. Assessment of knowledge and practice of dentists towards Coronavirus Disease (COVID-19): a cross-sectional survey from Lebanon. BMC Oral Health. 2020; 13,20(1),281, 1-9. doi.org/10.1186/s12903-020-01273-6
21. Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., Zhang L., Fan G., Xy J., …& Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223), 497–506. doi:10.1016/S0140-6736(20)30183-5
22. González-Olmo M.J., Delgado-Ramos B., Ortega-Martínez A.R., Romero-Maroto M., & Carillo-Diaz M. Fear of COVID-19 in Madrid. Will patients avoid dental care? Int Dent J. 2021; 2,1-7. doi:10.1016/j.identj.2021.01.013
23. Almas K., Khan A.S., Tabassum A., & Nazir M.A. Knowledge, attitudes, and clinical practices of dental professionals during the COVID-19 pandemic in Pakistan. Eur J Dent, 2020; 14(S 01), 563-69. doi.org/10.1055/s-0040-1718785
24. Ahorsu D.K., Lin C.Y., Imani V., Saffari M., Griffiths M.D., & Pakpour A.H. The Fear of COVID-19 Scale: Development and

initial validation. Int J Ment Health Addict. 2020; 27,1–9. doi:10.1007/s11469-020-00270-8

25. Alicilar H.E., Gulsen G., & Col M. [Toplumda COVID-19 Pandemisiyle ilgili farkındalık, tutum ve davranışların değerlendirilmesi] Evaluation of awareness, attıtudes and behaviors related to COVID-19 pandemic in society. ESTUDAM Halk Sağlığı Dergisi. 2020; 5,1-16. doi.org/10.35232/estudamhsd.763461

26. Ceyhan S. & Uzuntarla Y. [Akademik Personelin COVID-19'a Yönelik Bilgi, Tutum ve Davranışlarının Belirlenmesi] Determination of knowledge, attitudes and behaviors of academic staff towards COVID-19. Turkish Stud. 2020; 15(6), 259-76. doi.org/10.7827/TurkishStudies.45988

27. Ahmed M.A., Jouhar R., Adnan S., & Ahmed N. Evaluation of patient's knowledge, attitude, and practice of cross-infection control in dentistry during COVID-19 pandemic. Eur J Dent. 2020; 14(S 01), 1-6. doi.org/10.1055/s-0040-1721295

28. Gencer N. [Pandemi sürecinde bireylerin koronavirüs (KOVİD-19) korkusu: Çorum örneği] Coronavirus (COVID-19) fear of individuals during the pandemia: Çorum sample. USBAD. 2020; 4(4), 1153-1173. doi:10.47994/usbad.791577

29. Bakioğlu, F., Korkmaz, O., & Ercan, H. Fear of covid-19 and positivity: Mediating role of intolerance of uncertainty, depression, anxiety, and stress. Int J Ment Health Addict. 2021; 19(6):2369-82. doi: 10.1007/s11469-020-00331-y

30. Celik H., Celik Z.C, Yanikoglu F., Tagtekin D., & Hidiroglu S. [Yeni koronavirüs 2019 (COVID-19) salgınının ağız ve diş sağlığı çalışanları ve uygulamaları açısından önemi] The importance of novel coronavirus 2019 (COVID-19) for oral and dental professionals and treatments. Turkiye Klinikleri J Dent Sci. 2021; 27(2):318-23. doi.org/10.5336/dentalsci.2020-75465

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With metastatic colorectal cancer: A single center experience use of monoclonal antibodies (Bevacizumab, Cetuximab, and Panitumumab) in patients

İlkay GÜLTÜRK*, Gülçin ŞAHİNGÖZ ERDAL, Aykut ÖZMEN, Seher YILDIZ TACAR Mesut YILMAZ, Deniz TURAL

Department of Medical Oncology Department, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Türkiye

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Abstract

Despite all the advances in therapeutic modalities such as targeted therapies and immunotherapies that have recently been used in cancer treatment, Colorectal Cancer (CRC) continues to be the fourth most common cause of cancer-related deaths. The introduction of targeted monoclonal antibodies (mAbs) and their use in cancer treatment led to revolutionary advances in oncology. The aim of this study was to share our experiences regarding the usage rates of mAbs (Bevacizumab, Cetuximab, and Panitumumab), Overall Survival (OS) and progression-free survival (PFS) in patients with mCRC followed up in our hospital. This retrospective study included 210 patients with mCRC who were followed up in our hospital's oncology clinic between January 2010 and October 2020 and who received mAb treatment, regardless of their stage at the time of diagnosis. Fifty-two (24.8%) of the patients received a treatment regimen with Cetuximab and 46 with Panitumumab mAb. 29 patients (17.8%) received Cetuximab and Bevacizumab mAb treatment at different times, and 22 patients received Panitumumab and Bevacizumab mAb treatment, 112 of the patients received only Bevacizumab treatment. Panitumumab and Cetuximab mAb treatment was mostly taken in the 1st lines (69.6%, 76.9%, respectively). A statistically significant difference was found between the OSs of the cases according to the mAb treatment received ($p = 0.001$). Administration of Panitumumab and Cetuximab mAb in the 1st or 2nd series did not make a significant difference to PFS. When the retrospective data were evaluated, the distribution of Panitumumab and Cetuximab mAb treatments was seen to be balanced. Panitumumab and Cetuximab mAb therapy were not preferred for K-RAS mutant patients. They were preferred to give it in the first line. Patients who received anti EGFR mAb treatment had longer OS and PFS duration than those who received anti VEGF mAb only. It can be said that taking anti EGFR mAb treatment (being KRAS WT) has a positive effect on prognosis.

Keywords: Colorectal cancer, bevacizumab, cetuximab, panitumumab, prognosis

1. Introduction

Despite all the advances in therapeutic modalities such as targeted therapies and immunotherapies that have recently been used in cancer treatment, Colorectal Cancer (CRC) continues to be the fourth most common cause of cancer-related deaths after lung, stomach, and liver cancer. While the primary goal of early-stage colorectal cancer treatment is to provide a cure, the goal of stage IV CRC treatment is to reduce tumor-related symptoms and to prolong overall survival (OS) by minimizing the adverse effects of drug toxicities on patient quality of life parameters (1, 2).

In the 1990s, the primary treatment for CRC was fluoropyrimidine-based chemotherapy (5-fluorouracil [5-FU] or capecitabine), and the OS benefits of this therapy were proven (3, 4). Irinotecan and oxaliplatin are widely used in combination with 5-FU and Leucovorin (folinic acid) as first or second-line therapy for metastatic CRC (mCRC) (5, 6). While this combination has been shown to prolong survival by an average of 2–4 months, the presence of severe side effects and toxicities affecting the quality of life have also emerged (7).

The introduction of targeted monoclonal antibodies (mAbs) and their use in cancer treatment led to revolutionary advances in oncology. The abnormal over-expression of the Epidermal Growth Factor Receptor (EGFR) is associated with many human malignancies, one of the most common of which is CRC (8, 9). Drugs targeting EGFR have become a focus of interest in the treatment of mCRC. Currently, there are two anti-EGFR mAbs in clinical use, Cetuximab, and Panitumumab. These two drugs received Food and Drug Administration (FDA) approval for the treatment of mCRC in 2004 and 2007, respectively (10, 11).

Angiogenesis is a crucial stage for the development of tumors, and antiangiogenic agents inhibit the growth of new blood vessels, opening a new approach to cancer therapy (12). Bevacizumab is an angiogenic inhibitor that targets tumor vascularization and acts primarily on vascular endothelial growth factor (VEGF) or its receptors and was approved by the FDA in 2004 (13).

In this article we share our experiences regarding the

*Correspondence: gulturkilkay@gmail.com

usage rates of mAbs (Bevacizumab, Cetuximab, and Panitumumab), OS, and progression-free survival (PFS) in patients with mCRC followed up in our hospital.

2. Material and Method

This retrospective study included patients with mCRC who were followed up in our hospital's oncology clinic between January 2010 and October 2020 and who received mAb treatment, regardless of their stage at the time of diagnosis. Patients who started oncological treatment in another hospital or did not continue their treatment in our hospital were not included in the study. Clinical retrospective data were obtained from the electronic medical records, including demographic characteristics, medical history, clinical features, laboratory findings, treatments, and radiological images.

2.1. Compliance with ethical standards

This retrospective study was approved by the Ethics Committee of Bakirköy Sadi Konuk Training and Research Hospital, and the National Ethics Committee. All procedures were applied in accordance with the Helsinki Declaration and its later amendments or comparable ethical standards (No:2020/403).

2.2. Outcome measures

The primary outcome measures of the study were OS and PFS. The assessment of OS, as the time between diagnosis and death for any reason, is the most accepted method for evaluating the outcomes of cancer treatments. American and European oncology groups also agree that OS should be the primary outcome measure in clinical trials. It should be noted that PFS, the time until a disease progresses, is used as a measure to evaluate the direct effect of a treatment in patients with metastatic cancer (14).

2.3. Statistical analysis

NCSS (Number Cruncher Statistical System) program was used for statistical analysis. The Mann-Whitney U test was applied to inter-group comparisons of quantitative variables that did not show normal distribution, and the Kruskal-Wallis test and Dunn-Bonferroni test were used in the comparisons of more than two groups of quantitative variables that did not show normal distribution. The relationships between quantitative variables were evaluated with Spearman correlation analysis. Statistical significance was accepted as $p < 0.05$.

3. Results

The median age of 210 CRC patients included in the study was 63 years, and 57.6% (n = 121) of the patients were male. The prevalence of males decreased as the age decreased, and 50% of the patients in the ≤40 years age group (young patients) (n = 10) were male.

In 75.7% of the patients (n = 159), the tumor was located on the left side, and this tumor location was similar in the young patient group (70% on the left side).

The majority of the patients (64.8%) had metastatic disease at presentation, and the most common metastasis region was the liver at 87.7%, followed by the lung at 6.1%. In the young patient group, 70% had liver metastasis. Surgery was applied as the first treatment in 48.1% of the patients (n = 101), chemotherapy alone was given to 41.4%, neoadjuvant chemotherapy to 10.5%, and then surgical resection was performed.

The median OS in the whole patient population was found to be 23.05 months. In the subgroup analysis of patients aged ≤40 years (n = 10), the median OS was found to be significantly lower at 17.2 months. ($p < 0.001$). Mortality developed in 57.1% of the patients (n = 120) due to disease progression, and 90 patients are still alive and receiving treatment. Thirty-one patients (14.8%) presented with intestinal obstruction and 14 with intestinal perforation. Approximately half of the patients (45.2%) had lymphovascular invasion, while 37.1% had perineural invasion. During the first surgical treatment, a total of 27 patients underwent metastasectomy. The time to median metastasis in patients was found to be 6.65 months. (Table 1).

Table 1. Clinical characteristics of patients

Age *year*	*Median (Range)*	63 (26-89)
		N (%)
Gender	**Female**	89 (42.4)
	Male	121 (57.6)
Gender of 40 years and under	**Female**	5 (50)
	Male	5 (50)
Location of tumor	**Right**	51 (24.3)
	Left	159 (75.7)
Initial stage	**Metastatic**	136 (64.8)
	Limited	74 (35.2)
Site of metastasis	**Liver**	186 (87.7)
	Lung	13 (6.1)
	Intraperitoneal local	6 (2.9)
	Brain	3 (1,4)
	Bone	2 (0.9)
	Bladder	1 (0.5)
	Gastric	1 (0.5)
First type of treatment	**Surgical resection**	101 (48.1)
	Chemotherapy	87 (41.4)
	Neoadjuvant chemotherapy	22 (10.5)
Overall survival *months*	*Median (Range)*	23.05 (1-99)
OS in the ≤ 40 years age group *months*	*Median (Range)*	17.02 (8-32)
Survival	**Alive**	90 (42.9)
	Dead	120 (57.1)
Obstruction	*Median (Range)*	31 (14.8)
Perforation		14 (6.7)
Lymphovascular invasion presence		95 (45.2)
Perineural invasion presence		78 (37.1)
Metastasectomy		27 (12.9)
Metastasis free survival *months*		6.65 (1-62)

Patients most frequently received 1 and 2 lines of treatments (41.4%, 32.9%, respectively), fifty-two (24.8%) patients received a treatment regimen with Cetuximab and 46 with Panitumumab mAb. Twenty-nine patients (17.8%) received Cetuximab and Bevacizumab mAb treatment at different times, and 22 patients received Panitumumab and Bevacizumab mAb treatment. One hundred twelve patients received only Bevacizumab treatment. Panitumumab and Cetuximab mAb treatment was mostly taken in the 1st lines (69.6%, 76.9%, respectively). The distribution of the treatments is shown in Table 2.

Table 2: Distribution of treatments received and side-effects, n (%)

Number of treatment lines received	**1**	87 (41.4)
	2	69 (32.9)
	3	38 (18.1)
	4	16 (7.6)
Treatment	**Bevacizumab**	112 (53.3)
	Cetuximab	52 (24.8)
	Panitumumab	46 (21.9)
Bevacizumab	**Bevacizumab**	112 (68.7)
	Cetuximab+ Bevacizumab	29 (17.8)
	Panitumumab+ Bevacizumab	22 (13.5)
Panitumumab	**1st line**	32 (69.6)
	2nd line	13 (28.3)
	3rd line	1 (2.2)
Cetuximab	**1st line**	40 (76.9)
	2nd line	10 (19.2)
	3rd line	2 (3.8)
Rash/Dermatitis acneiform	**No**	191 (91)
	Yes	19 (9)
Grade of Rash/ Dermatitis acneiform	**2**	17 (89.5)

In 9% of the patients (n = 19), rash/dermatitis was observed during the treatment, which was mostly (89.5%) grade 2 severity (Table 2). The treatments and mAbs taken by the cases in the 1st, 2nd, 3rd, and 4th lines are shown in Table 3. The response rates of the cases distributed according to the lines are shown in Table 4. The frequency of progressive disease in the 1st, 2nd and 3rd lines of treatments was seen to be similar at 78.6%, 78.9%, and 74.1%, respectively.

Table 3. Preferred treatments in 1st, 2nd, 3rd, and 4th series

• 1st line treatment	**FOLFOX**	101 (48.1)
	FOLFIRI	71 (33.8)
	XELOX	35 (16.7)
	Capecitabine	3 (1.4)
	Monoclonal Antibodies	
	Bevacizumab	80 (52.6)
	Combination with Cetuximab	40 (26.3)
	Combination with Panitumumab	32 (21)
• 2nd line treatment	**FOLFIRI**	69 (57.5)
	FOLFOX	37 (30.8)
	XELOX	8 (.7)
	Capecitabine	6(5)
	Monoclonal Antibodies	
	Bevacizumab	60 (67.4)
	Combination with Panitumumab	14 (15.7)
	Combination with Cetuximab	12 (13.4)
	Regorafenib	3 (3.4)
• 3rd line treatment	**Regorafenib**	28 (51.8)
	FOLFIRI	13 (24.1)
	FOLFOX	10 (18.5)
	Capecitabine	3 (5.5)
	Monoclonal Antibodies	
	Bevacizumab	12 (60)
	Combination with Panitumumab	4 (20)
	Combination with Cetuximab	4 (20)
•4th line treatment	**Regorafenib**	6 (37.5)
	FOLFOX	3 (18.7)
	FOLFIRI	3 (18.7)
	Capecitabine	3 (18.7)
	XELOX	1 (6.2)
	Monoclonal Antibodies	
	Bevacizumab	3 (60)
	Combination with Cetuximab	1 (20)
	Combination with Panitumumab	1 (20)

FOLFIRI= Fluorouracil, Leucovorin plus Irinotecan, FOLFOX= 5-Fluorouracil plus Oxaliplatin, XELOX= Capecitabine plus Oxaliplatin

Table 4. Response to Treatment and Progression-free survival (PFS) results

Treatment	**Response**	**n(%)**
1st line	**stable**	45 (21.5)
	progression	165 (78.6)
2nd line	**stable**	26 (21.1)
	progression	97 (78.9)
3rd line	**stable**	14 (25.9)
	progression	40 (74.1)
4th line	**stable**	6 (37.5)
	progression	10 (62.5)
1st line PFS *months*	*Median (Range)*	12.92 (1-130)
2nd line PFS *months*	*Median (Range)*	6.94 (1-57)
3rd line PFS *months*	*Median (Range)*	6.89 (1-38)
4th line PFS *months*	*Median (Range)*	7.83 (2-28)

The 1st line median PFS was 12.92 months, which was longer than the 2nd, 3rd, and 4th series PFS duration (6.94, 6.89, 7.83, respectively) (Table 4). There was no significant difference in OS according to gender and location (right,

left) of the tumor (p> 0.05). OS was found to be significantly lower in patients with metastatic disease at the time of diagnosis (p = 0.001). A statistically significant difference was found between the OS of the cases according to the first treatment method. The OS of the patients who received only chemotherapy treatment (patients without surgical resection) was found to be significantly lower than those who underwent surgical resection after diagnosis or underwent surgical resection after receiving neoadjuvant chemotherapy treatment (p = 0.001; p = 0.001; p <0.01). A statistically significant difference was found between the OSs of the cases according to the mAb treatment received (p = 0.001; p <0.01). According to the results of the paired comparisons made to determine the difference, the OS of the patients who received only Bevacizumab mAb treatment was found to be significantly lower than those who received Panitumumab and Cetuximab mAb (p = 0.004; p = 0.011; p <0.05). The number of treatment lines received made a significant difference to OS. (p = 0.001; p <0.01). According to the results of the paired comparisons made to determine the difference, the survival time of patients who received 1 line of treatments was found to be significantly lower than those who received 3 and 4 lines of treatments (p = 0.008; p = 0.001; p <0.01). The survival time of the patients who received 2 lines of treatments was found to be significantly lower than those who received 4 lines of treatments (p = 0.001; p <0.01). The administration of Panitumumab and Cetuximab mAb on the 1st or 2nd line did not have a statistically significant effect on OS durations (p> 0.05).

The OS of patients who developed rash/dermatitis during treatment was found to be higher than those without (p = 0.020; p <0.05). While the OS of the cases presenting with obstruction was found to be statistically significantly lower (p = 0.044; p <0.05), the presence of perforation did not have a significant effect on OS (p> 0.05). The OS of cases with lymphovascular invasion was found to be statistically significantly lower than cases without (p = 0.001; p <0.01), but the presence of perineural invasion did not have a significant effect on OS (p> 0.05). There was no significant difference in OS of the patients with and without metastasectomy (p> 0.05) (Table 5).

Table 5. Overall survival and comparison of parameters

		Overall survival *months*		
		Range	Median	*p*
Gender	**Female (n=89)**	2-99	25.9	[a]***0.971***
	Male (n=121)	1-90	26.8	
Location of tumor	**Right (n=51)**	2-66	22.3	[a]***0.079***
	Left (n=159)	1-99	27.7	
Initial stage	**Metastatic (n=136)**	1-71	20.5	[a]***0.001****
	Limited (n=74)	7-99	37.2	
First type of treatment	**Neoadjuvant chemotherapy (n=22)**	12-77	34.7	[b]***0.001****
	Surgical resection (n=101)	6-99	31.6	
	Chemotherapy (n=87)	1-66	18.2	
Treatment	**Bevacizumab (n=112)**	1-99	22.5	[b]***0.001****
	Panitumumab (n=46)	3-90	32.6	
	Cetuximab (n=52)	8-71	29.4	
Number of treatment lines received	**1 (n=87)**	1-90	22.1	[b]***0.001****
	2 (n=69)	2-99	26.3	
	3 (n=38)	9-66	30.1	
	4 (n=16)	22.8-65	41.1	
Panitumumab	**1st line treatment (n=32)**	3-90	29.5	[a]***0.244***
	2nd line treatment (n=13)	11-77	37.7	
	Φ 3rd line treatment (n=1)	63	63	
Cetuximab	**1st line treatment (n=40)**	8-71	29.1	[a]***0.467***
	2nd line treatment (n=10)	15-59	32.7	
	Φ 3rd line treatment (n=2)	18-20	18.8	
Rash/Dermatitis acneiform	**No (n=191)**	1-99	25.6	[a]***0.020****
	Yes (n=19)	12-69	34.9	
Obstruction	**No (n=179)**	1-99	27.5	[a]***0.044****
	Yes (n=31)	6-50	20.1	
Perforation	**No (n=196)**	1-99	26.2	[a]***0.522***
	Yes (n=14)	9-71	28.6	
LVI presence	**No (n=115)**	6-84	30.2	[a]***0.001****
	Yes (n=95)	1-99.63	23.3	
PNI presence	**No (n=132)**	1-99	25.3	[a]***0,090***
	Yes (n=78)	2-66	22.3	
Metastasectomy	**No (n=183)**	1-99	27.7	[a]***0.082***
	Yes (n=27)	1-71	20.5	

[a]Mann Whitney U Test, [b]Kruskal Wallis Test, *p<0.05, **p<0.01, included in comparison due to insufficient number of patients, LVI=Lymphovascular invasion, PNI= Perineural invasion

There was no statistically significant relationship between the age of the patients and OS (p> 0.05), but OS was significantly shorter in the ≤40 years age group, the young patients. A moderate negative correlation was observed between the carcinoembryonic antigen (CEA) levels at the time of diagnosis and OS (OS decreased with increasing CEA value at the time of diagnosis) (r = -0.411; p = 0.001; p <0.01). A moderate positive correlation (OS increased with increasing PFS) between PFS and OS was observed (r = 0.509; p = 0.001; p <0.01). (Spearman's Correlation analysis) (Table 6).

Table 6. Relationship between overall survival and age, initial CEA, and Progression-Free Survival

		Overall survival
Age	**r**	0.112
	p	***0.105***
Initial CEA ng/ml	**r**	-0.411
	p	***0.001****
PFS	**r**	0.509
	p	***0.001****

r=Spearman's correlation coefficient, **p<0,01 PFS=Progression-free survival, CEA: carcinoembryonic antigen

Administration of Panitumumab and Cetuximab mAb in the 1st or 2nd series did not make a significant difference to PFS (p> 0.05) (Table 7).

There was no statistically significant difference in OS and PFS according to the disease location (right / left) of the patients who received Bevacizumab mAb treatment (p> 0.05) (Table 8).

A significant difference was found in OS and PFS when Bevacizumab mAb was administered single or sequentially with other mAbs (p = 0.001; p <0.01). According to the results of the pairwise comparisons, the OS of patients who received only Bevacizumab mAb treatment was found to be significantly lower than that of patients with Bevacizumab and Panitumumab mAb at different times (p = 0.001; p <001). The PFS of the patients who received Bevacizumab and Panitumumab mAb treatment at different times was significantly higher than those who received Bevacizumab alone and those who received Bevacizumab and Cetuximab mAb at different times (p = 0.004; p = 0.005; p <001) (Table 9).

Table 7. Comparisons related to Progression-free Survival

		Progression-free survival (month)		
		Range	**Median**	***p***
Panitumumab	**1st line treatment (n=32)**	1-62	8.9	***[a]0.412***
	2nd line treatment (n=13)	1-26	8.7	
	ϕ 3rd line treatment (n=1)	15	15.5	
Cetuximab	**1st line treatment (n=40)**	1-39	5.6	***[a]0.186***
	2nd line treatment (n=10)	1-44	14	
	ϕ 3rd line treatment (n=2)	1	1	

[a]Mann Whitney U Test *p<0,05, ϕNot included in comparison due to insufficient number of patients

Table 8. Overall survival and progression-free survival in Bevacizumab treated patients by tumor location

		Location of tumor		
		Right (n=29)	**Left (n=83)**	***p***
OS (months)	*Median (Range)*	20.3 (2-66)	23.2 (1-99)	***[a]0.347***
PFS (months)	*Median (Range)*	3.9 (1-31)	5.6 (1-64)	***[a]0.845***

[a]Mann Whitney U Test OS=Overall survival, PFS= Progression-free survival

Table 9. Overall survival and progression-free survival by monoclonal antibodies treatment

		Treatment			
		Beva (n=112)	**Beva + Pan (n=22)**	**Beva + Cet (n=29)**	***p***
OS (months)	*Range*	1-99	11-77	8-50	***[b]0.001****
	Median	22.5	38	25.9	
PFS (months)	*Range*	1-63	1-46	1-32	***[b]0.002****
	Median	5.2	11.8	4.3	

[b]Kruskal Wallis Test **p<0,01 OS=Overall survival, PFS= Progression-free survival

4. Discussion

The annual incidence of CRC worldwide is higher in males than in females, with reported rates of just over was equal. Most studies have shown that the incidence of CRC diagnosed at a young age is more common, mainly in the distal colon and rectum, and is at an advanced stage at diagnosis. Similarly, 70% of the currently studied young patients were determined to have CRC originating from the left colon. Although this issue is controversial, it is thought that CRC in young patients has 1 million for males and 79,500 for females (15). In the current study patient population, the frequency of male patients was higher in the general group, while the ratio of female and male patients aged 40 years and younger a more aggressive biological behavior and worse prognosis (16-18). Also supporting this view, the median OS was significantly lower in the ≤ 40 years age group of this study.

In CRC, the most common and generally first metastasis site is the liver, and liver metastasis is one of the most important factors determining survival (19). Similarly, in the current study patient population, the liver was the most common metastasis site in both the general group and the young patients, and the median OS was found to be longer in patients who underwent metastasectomy compared to those who did not. As expected, patients who underwent surgical resection at the time of initial diagnosis or after

receiving neoadjuvant chemotherapy had a longer median OS than those who had no surgical resection. It can also be said that operability affects overall survival. Patients with metastases that could not be resected at the time of diagnosis were treated with systemic chemotherapy only.

There was a negative correlation between the CEA level at the time of diagnosis and the median OS in the current study patients. Although studies have been carried out on the availability of new methods such as new parameters, personalized analysis, and mutation analysis to predict OS, CEA still continues to provide an idea about OS. Other advantages of CEA are that the levels change with treatment, it provides guidance in response to treatment, and it is relatively inexpensive compared to new parameters (20).

Bevacizumab, which acts as an anti-VEGF, inhibits VEGF function in vascular endothelial cells and inhibits tumor angiogenesis and has been shown to result in a significant increase in OS and PFS when co-administered with chemotherapy in most randomized controlled mCRC studies (21). The current study patient group consisted of CRC patients who received only mAb treatment, and the most common mAb treatment administered in the study was Bevacizumab. Anti-EGFR mAb (Panitumumab and Cetuximab) treatment was given to patients with wild-type Kirsten Rat Sarcoma viral oncogene mutation (WT K-RAS), and combination therapies with Bevacizumab mAb were given in progressive series to patients with progression. The administration of Bevacizumab mAb to patients who could not be given anti-EGFR mAb treatment (such as being K-RAS mutant or the lack of reimbursement of anti-EGFR treatment by health insurances in the early 2000s) may have had an effect on Bevacizumab being the leading treatment (22).

Biological and clinical evidence supports that carcinogenesis follows different molecular pathways in proximal (right side) and distal (left side) CRCs and may have different expression profiles due to their different embryonic origins (23-26). Nevertheless, the results obtained in studies evaluating the effect of primary tumor location on OS in mCRC are complicated due to the heterogeneity in molecular and pathological features and treatments received (27-29). Although different levels of efficacy of Bevacizumab mAb have been reported in cancers located in the right and left colon, in the current study, no relationship was found between tumor location and OS in patients who received Bevacizumab mAb.

An important factor for adding Cetuximab or Panitumumab to conventional therapy in mCRC patients is the K-RAS mutation status. Mutation in the K-RAS gene is a negative predictor of response to Cetuximab and/or Panitumumab, and in a meta-analysis, the response to anti-EGFR mAb therapy in K-RAS mutant patients was reported to be significantly lower than in those with WT K-RAS (30). In the current study patient group, only WT K-RAS patients received anti-EGFR mAb treatment. The fact that only this group is reimbursed by the national health insurance system was also influential in this choice.

In most clinical trials, anti-EGFR mAbs have been used in the treatment protocol in patients with mCRC resistant to conventional chemotherapy. In this regard, anti-EGFR mAbs are generally used in second or third line therapy for treatment and often in combination with some chemotherapeutic agents. However, in some studies, anti-EGFR mAbs have been used as monotherapy due to chemotherapy failure or intolerable toxicity (21). In the current study, anti-EGFR mAbs were given more frequently to mCRC patients in the first lines. Although there were patients who underwent dose reduction due to chemotherapy toxicity, none of the patients were given anti-EGFR mAb therapy as a monotherapy. It was seen that the lines in which anti-EGFR mAb was given did not affect PFS, which was observed to be similar when anti-EGFR mAb was given in the 1st or 2nd lines.

In general, targeted agents and monoclonal antibodies do not induce many of the systemic side effects that are typically associated with conventional cytotoxic agents and are difficult to tolerate. However, a number of specific toxicities of these agents have been reported, which can be severe and impair quality of life (31). A wide range of skin-related side effects can occur, ranging from mildly dry skin to widespread and life-threatening rashes, which can sometimes seriously affect patients' physical, psychological, and social well-being (32, 33). In the current study, patients who developed grade 2 and 3 rash/dermatitis were recorded, and OS was found to be better in patients with skin toxicity. In a previous trial conducted on mCRC patients receiving anti-EGFR mAb treatment, there was determined to be a relationship between the skin inflammatory response associated with the development of skin rash and the efficacy of the treatment (34).

A series of meta-analyses have shown that Panitumumab and Cetuximab mAb therapy in mCRC patients have similar efficacy in terms of OS and PFS, and even the side-effect profiles were similar (35). In the current study, OS was similar in mCRC patients who received Panitumumab and Cetuximab mAb treatment. However, a detail that drew attention was that the OS and PFS of those who received Panitumumab and Bevacizumab mAb treatment at different times were significantly longer than those who received only Cetuximab or Cetuximab and Bevacizumab mAb at different times.

In conclusion, our patients' treatments are planned considering the OS advantage obtained by adding mAb treatments to conventional chemotherapy in mCRC patients that have been followed up in our clinic for the last ten

years. When the retrospective data were evaluated, the distribution of Panitumumab and Cetuximab mAb treatments was seen to be balanced.

Panitumumab and Cetuximab mAb therapy was not preferred for K-RAS mutant patients because of its low contribution to OS and the lack of reimbursement from health insurance. It was noticed that there was no significant difference in terms of efficacy when anti-EGFR mAb therapy was given in the 1st or 2nd lines for mCRC patients. Generally, it was preferred to give it in the first line. Patients who received anti-EGFR mAb treatment had longer OS and PFS duration than those who received anti-VEGF mAb only. It can be said that taking anti-EGFR mAb treatment (being KRAS WT) has a positive effect on prognosis.

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Authors' contributions

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References

1. American Cancer Society. Global Cancer Facts & Figures 4th Edition American Cancer Society, 2018. Accessible at https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/global-cancer-facts-and-figures/global-cancer-facts-and-figures-4th-edition.pdf. [Google Scholar]
2. American Cancer Society. Cancer Facts & Figures 2019. American Cancer Society, 2019. Accessible at https://www.cancer.org/latest-news/facts-and-figures-2019.html. [Google Scholar]
3. Mitry E, Fields ALA, Bleiberg H, Labianca R, Portier G, Tu D, et al. Adjuvant chemotherapy after potentially curative resection of metastases from colorectal cancer: a pooled analysis of two randomized trials. J Clin Oncol [Internet]. 2008;26(30):4906–11. Available from: http://dx.doi.org/10.1200/JCO.2008.17.3781.
4. Rougier P, Mitry E. Cancers colorectaux avant et après les biothérapies : une révolution dans la prise en charge des patients ? Gastroentérologie Clinique et Biologique [Internet]. 2009;33(8–9):672–80. Available from: http://dx.doi.org/10.1016/j. gcb.2009.07.019.].
5. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol [Internet]. 2000;18(16):2938–47.
6. Yamaguchi T, Iwasa S, Nagashima K, Ikezawa N, Hamaguchi T, Shoji H, et al. Comparison of panitumumab plus irinotecan and cetuximab plus irinotecan for KRAS wild-type metastatic colorectal cancer. Anticancer Res.].
7. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol [Internet]. 2000;18(16):2938–47.
8. Gullick WJ. Prevalence of aberrant expression of the epidermal growth factor receptor in human cancers. Br Med Bull 1991;47:87-98.
9. Herbst RS, Shin DM. Monoclonal antibodies to target epidermal growth factor receptor-positive tumors: a new paradigm for cancer therapy. Cancer 2002;94:1593-1611.
10. US Food and Drug Administration. FDA approves Erbitux for colorectal cancer. Available at: www.Fda.Gov [Last accessed 2004].
11. US Food and Drug Administration. FDA approves Vectibix (Panitumumab) to treat metastatic colorectal carcinoma. Available at: www.Fda.Gov [Last accessed 2007].
12. Chou T, Finn RS. Brivanib: A review of development. Future Oncol 2012;8:1083.)
13. Ferrara, N. et al. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nature Rev. Drug Discov. 3, 391–400 (2004).
14. Ellis LM, Bernstein DS, Voest EE, Berlin JD, Sargent D, Cortazar P, et al. American Society of Clinical Oncology perspective: raising the bar for clinical trials by defining clinically meaningful outcomes. J Clin Oncol [Internet]. 2014;32(12):1277–80. Available from: http://dx.doi.org/10.1200/jco.2013.53.8009
15. Allemani C, Matsuda T, Di Carlo V et al. Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 2018; 391: 1023– 75.
16. Fancher TT, Palesty JA, Rashidi L, Dudrick SJ. Is gender related to the stage of colorectal cancer at initial presentation in young patients? J Surg Res. 2011;165:15-18.
17. Al-Barrak J, Gill S. Presentation and outcomes of patients aged 30 years and younger with colorectal cancer: a 20-year retrospective review. Med Oncol. 2011;28:1058–1061.
18. da Fonseca LM, da Luz MM, Lacerda-Filho A, Cabral MM, da Silva RG. Colorectal carcinoma in different age groups: a histopathological analysis. Int J Colorectal Dis. 2012;27:249–255. [PubMed] [Google Scholar]
19. Engstrand, J., Nilsson, H., Strömberg, C. et al. Colorectal cancer liver metastases – a population-based study on incidence, management and survival. BMC Cancer 18, 78 (2018). https://doi.org/10.1186/s12885-017-3925-x
20. Vishal Das, Jatin Kalita, Mintu Pal, Predictive and prognostic biomarkers in colorectal cancer: A systematic review of recent advances and challenges, Biomedicine & Pharmacotherapy, Volume 87, 2017, Pages 8-19, ISSN 0753-3322, https://doi.org/10.1016/j.biopha.2016.12.064.
21. Yazdi, M. H., Faramarzi, M. A., Nikfar, S., & Abdollahi, M. (2015). A Comprehensive Review of Clinical Trials on EGFR Inhibitors Such as Cetuximab and Panitumumab as Monotherapy and in Combination for Treatment of Metastatic Colorectal Cancer. Avicenna journal of medical biotechnology, 7(4), 134–144.
22. He, W. Z., Liao, F. X., Jiang, C., Kong, P. F., Yin, C. X., Yang, Q., Qiu, H. J., Zhang, B., & Xia, L. P. (2017). Primary Tumor Location as a Predictive Factor for First-line Bevacizumab Effectiveness in Metastatic Colorectal Cancer

Patients. Journal of Cancer, 8(3), 388–394. https://doi.org/10.7150/jca.16804

23. Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. Ann Intern Med. 1990;113(10):779–788.

24. Distler P, Holt PR. Are right- and left-sided colon neoplasms distinct tumors? Dig Dis. 1997;15(4–5):302–311.

25. Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer. 2002;101(5):403–408 Glebov OK, Rodriguez LM, Nakahara K, et al. Distinguishing right from left colon by the pattern of gene expression. Cancer Epidemiol Biomarkers Prev. 2003;12(8):755–762.

26. Birkenkamp-Demtroder K, Olesen SH, Sørensen FB, et al. Differential gene expression in colon cancer of the caecum versus the sigmoid and rectosigmoid. Gut. 2005;54(3):374–384.

27. Meguid RA, Slidell MB, Wolfgang CL, Chang DC, Ahuja N. Is there a difference in survival between right- versus left-sided colon cancers? Ann Surg Oncol. 2008;15(9):2388–2394.

28. Benedix F, Kube R, Meyer F, et al. Colon/Rectum Carcinomas (Primary Tumor) Study Group. Comparison of 17,641 patients with right- and left-sided colon cancer: differences in epidemiology, perioperative course, histology, and survival. Dis Colon Rectum. 2010;53(1):57–64.

29. Weiss JM, Pfau PR, O'Connor ES, et al. Mortality by stage for right- versus left-sided colon cancer: analysis of surveillance, epidemiology, and end results—Medicare data. J Clin Oncol. 2011;29(33):4401–4409.

30. Petrelli, F., Borgonovo, K., Cabiddu, M. et al. Cetuximab and Panitumumab in KRAS wild-type colorectal cancer: a meta-analysis. Int J Colorectal Dis 26, 823–833 (2011). https://doi.org/10.1007/s00384-011-1149-0

31. Fornasier G, Taborelli M, Francescon S, et al. Targeted therapies and adverse drug reactions in oncology: the role of clinical pharmacist in pharmacovigilance. Int J Clin Pharm. 2018;40(4):795–802

32. Fabbrocini G, Romano MC, Cameli N, et al. "Il corpo ritrovato": dermocosmetological skin care project for the oncologic patient. ISRN Oncol. 2011;2011:650482.

33. Pinto C, Barone CA, Girolomoni G, et al. Management of skin toxicity associated with cetuximab treatment in combination with chemotherapy or radiotherapy. Oncologist. 2011;16(2):228–38.

34. Tougeron, D., Emambux, S., Favot, L., Lecomte, T., Wierzbicka-Hainaut, E., Samimi, M., et al. (2020). Skin inflammatory response and efficacy of anti-epidermal growth factor receptor therapy in metastatic colorectal cancer (CUTACETUX). OncoImmunology, 9(1), 1848058. doi:10.1080/2162402x.2020.1848058

35. Timothy J Price, Marc Peeters, Tae Won Kim, Jin Li, Stefano Cascinu, Paul Ruff, et al., Panitumumab versus Cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study, The Lancet Oncology, Volume 15, Issue 6, 2014, Pages 569-579, ISSN 1470-2045, https://doi.org/10.1016/S1470-2045(14)70118-4. (http://www.sciencedirect.com/science/article/pii/S1470204514701184)

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Effect of lead exposure during perinatal period on kidney of adult offspring in rat: A stereological study

Malihe Saghebray SHİRAZI [1], Javad SADEGHİNEZHAD[1*], Hassan MOROVVATI [1], Sara SHOKRPOOR[2] Ehsan ROOMİANI[1], Sareh Najaf ASAADI[1], Mahdi AGHABALAZADEH [1], Sana GHOLİPOUR[1] , Ali BAYAT[1]

[1]Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2]Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

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Abstract

Lead is the most common toxic metal in nature, and its adverse effects on various organs, including the kidneys. As a complicated process, kidney development is influenced by various environmental variables. Although lead toxicity can occur at any age, it is important in pregnant mothers and infants. Therefore, the present study investigated how the low dose of lead administration could affect kidney offspring in rat model. To this aim, a stereology technique was used. A randomized sampling was used to assign animals to five groups. The first one (i.e., Group 1 as the control) was provided with ordinary drinking water plus glacial acetic acid (0.5 ml/liter) 0 as a lead acetate solvent. Animals in Group 2 were administrated 0.2% of lead acetate in drinking water for 30 days prior mating. Rats in Group 3 received drinking water with 0.2% of lead for 21 days during pregnancy. Animals in Group 4 consumed 0.2% of lead acetate in their water for 21 days within their lactation. Group 5 was provided with 0.2 % lead acetate in water in their pre-pregnancy (30 days), pregnancy (21 days) and lactation (21 days) periods. The left kidney was removed from male offspring 60 days after birth. The volume of the kidney, cortex, medulla, proximal convoluted tubules (PCT) as well as distal convoluted tubules (DCT), and also the length of PCT and DCT, were analyzed by means of stereology. The findings revealed a reduction in the volume and length of the DCT as well as some pathological effects in experimental groups, compared to the control group. Due to the ameliorating effect of lead in perinatal period even in low doses on offspring kidneys, cautiousness is needed in this period.

Keywords: kidney, lead, stereology, perinatal period

1. Introduction

Lead is known as the most prevalent poisonous metal existing in nature (1). As a result of human activities, the amount of lead that exists in nature during the past three centuries is estimated as 1000 times (2) Consuming contaminated food and drink is the basis of lead poisoning across communities. In addition, toxicity through inhaling contaminated dust and gases is prevalent (3). Toxicological studies have shown that lead affects central nervous system (4-6), peripheral nervous system (7, 8), cardiovascular system (9, 10), endocrine system (11, 12), immune system (13, 14), digestive system (15, 16), male and female reproduction system (17-20) and also urinary system (21, 22). The nephropathy induced by lead causes nephron malfunctioning, metabolic disorders and more protein excretion in urine (23, 24). Lead exposure is accompanied by pathological side effects such as the blockage of tubes, nucleus hetero-chromatization, increased diameter of renal tubules (25-29).

The kidney development is complex and progresses gradually followed differentiation into pronephrosis, mesonephrosis and metanephrosis (30). Many factors like environmental contamination can influence renal development result in malfunctions of the kidney. In this way, although prevalence of lead is considered as an ecological threat for all regardless of age (31) but pregnant women and young children are at the highest risk. A wide range of research showed that the level of lead in newborns exceeded 10 microgram / deciliter in blood and exceeded a standard limit (32).

The present research aimed to explore the toxic effect of a lower dose of lead during perinatal period on the morphometrical properties of the kidney of offsprings in rat model via an unbiased designed stereology. At present, stereology is a method of making unbiased and precise quantitative estimation of kidney structure (33). The structural features such as the number, size, length or nephron distribution are correlated with the renal function (34).

Assessing kidney volume, volume fraction of cortex and medulla, glomerular volume, length and the volume of proximal (PCT) as well as distal convoluted tubules (DCT) in kidney following lead toxicity during perinatal period (Pre-pregnancy, pregnancy and lactation) may contribute to knowledge for protections of mothers from lead toxicity and

*Correspondence: sadeghinezhad@ut.ac.ir

its adverse effects on neonates' kidney.

2. Materials and methods

2.1. Experimental animals and treatment

The male and female adult Wistar rats were purchased from the Pasteur Institute, Tehran, Iran. The rats were allowed to mate overnight. Those animals with vaginal plug were considered pregnant at gestation day 0. The rats were maintained in a temperature of 23–25°C with a 12-h light/12 h dark cycle provided with food and ad libitum. The experiment abided by the standard guide for the care and use of laboratory animals. The rats were divided randomly to five groups each with five animals:

Group 1 (control): This group was provided with ordinary drinking water plus glacial acetic acid (0.5 ml/liter) as a lead acetate solvent throughout the study.

Group 2 (pre-pregnancy): This group received lead acetate (0.2%) in drinking water for 30 days in advance to mating. After that, this group was provided with ordinary drinking water till the end of the treatment.

Group 3 (Pregnancy): This group received drinking water with lead (0.2%) for 21 days during pregnancy.

Group 4 (lactation): This group received lead acetate (0.2%) in drinking water for 21 days during lactation.

Group 5 (pre-pregnancy, pregnancy and lactation): This group was provided with lead acetate (0.2%) in their drinking water during pre-pregnancy (30 days), pregnancy (21 days) and lactation (21 days) periods.

Lead acetate (0.2%) was freshly added to distilled water 0plus glacial acetic acid (0.5 ml/liter) to impede lead acetate sedimentation (35, 36).

After the postnatal day (PND) 21, neonates were separated from mothers in each group and maintained in separate cages and they received ordinary food and water.

2.2. Tissue sampling and stereological methods

Five male offspring in each group (one offspring from each mother) were selected randomly and were then anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) on PND 65. Animals were perfused with neutral formalin 10 % intracardially. The left kidneys were removed and weighted using a digital scale. Then, each kidney was immersed in the same fixative for more fixation.

After that each kidney was embedded in agar 6 % and isotropic, uniform samples were randomly taken via the orientator method (33). Each kidney was sectioned into parallel slabs in 2 mm thickness using a tissue slicer. A routine processing of slabs followed for a light microscopy. They were embedded in paraffin, cut into 5 µm-thick sections, and stained with hematoxylin-eosin and Periodic acid-Schiff. Geometrical probes were created using ImageJ package (https://imagej.nih.gov/ij/).

2.3. Estimation of the volume

The total volume of each kidney was calculated by changing its weight to a volume with the help of the specific kidney tissue density:

$$V(kid) = W(kid) / \rho,$$

in which ρ stands for the weight to volume ratio of kidney tissue (1.04 g/cm3).

The sections were scanned using a slide scanner (Optic lab H850, Plustek, China) and test point system was covered on each image (Fig. 1). Then, the fractional volume (Vv) of renal cortex and medulla was calculated using the following formula (37):

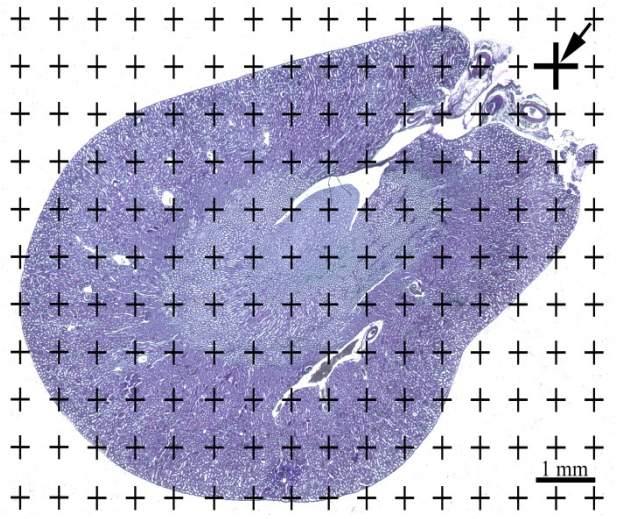

Fig. 1. Volume estimation. A point grid was superimposed on the sections. If tissue was found in the right upper side of a point of test (arrow), it was counted

$$Vv\ (\text{cortex or medulla}) =$$

in which ΣP cortex or medulla represents the total number of points that hit the cortex or medulla in kidney; ΣP (kidney) represents the total number of points that hit the entire kidney.

The total volume of renal cortex and medulla were obtained as:

$$V\ (\text{cortex or medulla}) = Vv\ (\text{cortex or medulla}) \cdot V\ (\text{kidney})$$

In order to estimate the volume of the glomeruli, PCT and DCT, we captured the systematic uniform random fields of view and moved the microscope stage in the same step lengths along the x and y directions for each section by a microscope (CX40, Olympus) linked with a digital camera (MB-2250, Germany). Then, the volume fraction was calculated by counting points hitting the glomeruli, PCT or DCT and reference section (Fig. 2a). Next, the total volume of glomeruli, PCT and DCT was estimated as the volume density multiplied by the total volume of kidney.

2.4. Estimation of the renal tubules length

For Estimation of length of PCT and DCT, unbiased counting frame were superimposed on chosen microscopic fields of

views. The number of profiles which is in the counting frame and does not reach the exclusion borders of the frame were counted (Fig. 2b) and the length density was estimated as:

Lv (tubules)=

in which ΣQ(tubules) stands for the total number of the tubule profiles sampled for the counting frame; a frame is the area related to a counting frame; P (kidney) represents the total number of points that hit the kidney. PCT and DCT total lengths were estimated as the length density multiplied by the kidney total volume (33).

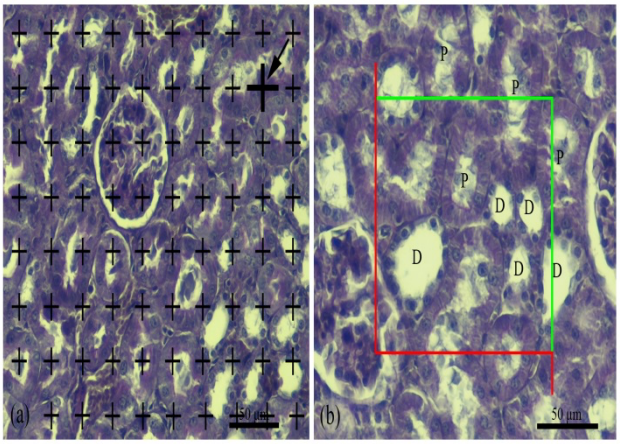

Fig. 2. Stereological estimation of volume fraction of glomeruli, proximal and distal convoluted tubules via the point counting method. The arrow shows the point on the right upper corner of the cross (a). Estimation of length of proximal and distal convoluted tubules using an unbiased counting frame with inclusion (green) and exclusion (red) lines (b). The tubular profiles inside the counting frame or touching the inclusion lines were counted versus those outside the counting frame or touching the exclusion lines which were ignored. Here four proximal (P) and five distal (D) convoluted tubule profiles were counted

2.5. Statistical Analysis

Kolmogorov-Smirnov test was used to check the data normality. Kruskal-Wallis and Mann-Whitney test and ANOVA followed by Tukey's post-hoc test were run to compare data between different groups in with abnormal and normal distribution, respectively. A $p<0.05$ was considered significant.

2.6. Ethical consideration

The present research project was performed based on the guidelines of the Ethics Committee at Tehran University of Medical Sciences (# 91-01-159- 18022). All the experiments were done in adherence to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

3. Results

3.1. Total kidney volume

The total kidney volume was estimated at 0.895± .108 cm3 in group 1, and 0.919 ± 0.560 cm3 in the group 2, 0.990± 0.148 cm3 in group 3, 0.829 ± 0.051 cm3 in group 4 and 0.930 ± 0.063 cm3 in group 5. Statistical analysis revealed no statistically significant difference in total kidney volume between different groups (Fig. 3).

3.2. Cortex and medulla volume

The cortex volume of the cortex is 0.510± .045 cm3 in group 1, 0.560 ± .031 cm3 in group 2, 0.606± .137 cm3 in group 3, 0.467± .061 cm3 in group 4, and 0.551 ± 0.064 cm3 in group 5. No significant difference was observed between the groups (Figure 1). The fractional volume of the cortex was calculated as is 57.28 ± 4.89 % in group 61.1 ± 4.38 % in group 2, 60.74 ± 7.1 % in group 3, 56.34 ± 5.06 % in group 4 and 59.27 ± 4.83% in group 5.

The total volume of the medulla was 0.384± .080 cm3 in group 1, 0.358 ± .059 cm3 in group 2, 0.386± .078 cm3 in group 3, 0.361± .041 cm3 in group 4 and 0.378 ± .044 cm3 in group 5. The fractional volume of the medulla was 42.69 ± 4.93 % in group 1, 38.86 ± 4.39 % in group 2, 39.260 ± 7.10% in group 3, 43.66 ± 5.06 % in group 4 and 40.72 ± 4.83% in group 5. The groups did not differ significantly in either the cortex or the medulla (Fig. 3, 4).

3.3. PCT and DCT volume

The total volume of the PCT was found to be 0.529± .091 cm3 in group 1, 0.511 ± .044 cm3 in group 2, 0.610± .089 cm3 in group 3, 0.511± .054 cm3 in group 4 and 0.515 ± .043 cm3 in group 5. The fractional volume of the PCT was 60.19 ± 14.27 % in group 1, 55.68 ± 3.38 % in group 2, 61.60 ± 2.02% in group 3, 61.56 ± 2.84 % in group 4 and 55.25 ± 1.13% in group 5. No significant divergence was found between the different groups in terms of the PCT volume.

DCT total volume was estimated to be 0.108± .010 cm3 in group 1, 0.106 ± .006 cm3 in group 2, 0.127± .005 cm3 in group 3, 0.115± .016 cm3 in group 4 and 0.144 ± .012 cm3 in group 5. The results indicated that the total volume of the DCT in the group 5 increased significantly in comparison to groups 1, 2 and 4 ($p<0.05$). The fractional volume of the DCT was 11.68 ± 0.950% in group 1, 11.82 ± 0.162 % in group 2, 11.54 ± .732% in group 3, 13.75 ± 1.320 % in group 4 and 15.53 ± 0.458% in group 5. The fractional volume of the DCT in group 5 indicated a significant increase in comparison with the other groups ($p<0.05$) (Fig. 3, 4).

3.4. Glomerulus volume

The total volume of the glomeruli was 0.019± .005 cm3 in group 1, 0.019 ± .001 cm3 in group 2, 0.022± .003 cm3 in group 3, 0.017± .002 cm3 in group 4 and 0.022 ± .005 cm3 in group 5. The fractional volume of the glomerular was estimated as 2.154 ± .778 % in group 1, 2.066 ± .159 % in group 2, 2.180 ± .240% in group 3, 2.034 ± .213 % in group 4 and 2.370 ± .494% in group 5. There was not significant difference between groups in glomeruli volumes (Fig. 3, 4).

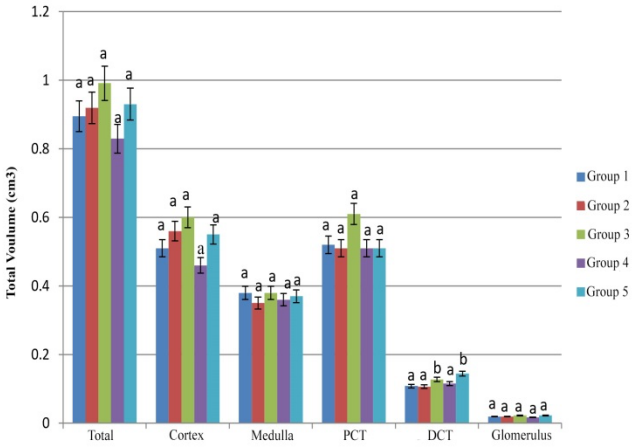

Fig. 3. Comparison of total kidney volume, total volume of the cortex, medulla, proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and the glomeruli between group 1 (control), group 2 (pre-pregnancy), group 3 (pregnancy), group 4 (lactation), group 5 (pre-pregnancy-pregnancy-lactation). Different letters represent statistically significant divergences ($p < 0.05$) between groups

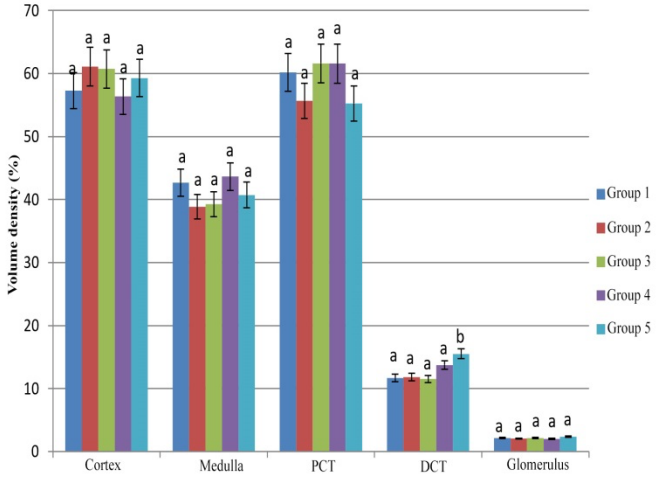

Fig. 4. Comparison of fractional volume of the cortex, medulla, proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and glomeruli between group 1 (control), group 2 (pre-pregnancy), group 3 (pregnancy), group 4 (lactation), group 5 (pre-pregnancy-pregnancy-lactation). Different letters point to a significant difference ($p < 0.05$) between groups

3.5. PCT and DCT length

The PCT length was estimated as 133.83± 30.78 m in group 1, 131.98 ± 21.66 m in group 2, 182.60± 32.91 m in group 3, 126.64± 23.18 m in group 4 and 154.01 ± 2.882 m in group 5. The between-group differences were not statistically significant.

The DCT length was 129.78 ± 14.75 m in group 1, 79.94 ± 14.57 m in group 2, 99.02± 20.87 m in group 3, 50.61± 5.25 m in group 4 and 98.220 ± 11.32 m in group 5. The DCT was shorter in all the groups receiving a treatment than the control (group 1), which was significant in groups 2 and 4 ($p < 0.05$) (Fig. 5).

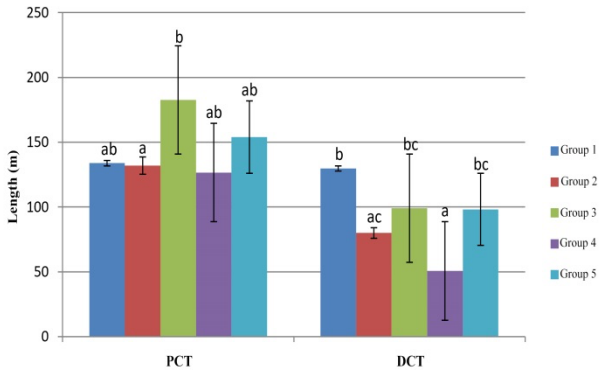

Fig. 5. Comparison of proximal (PCT) and distal (DCT) convoluted tubules length between group 1 (control), group 2 (pre-pregnancy), group 3 (pregnancy), group 4 (lactation), group 5 (pre-pregnancy-pregnancy-lactation). Different letters represent a significant difference ($p < 0.05$) between groups

3.6. Pathological findings

Histological examination revealed hemorrhage (Fig. 6a) and multifocal lymphoplasmacytic nephritis (Fig. 6.b) in the kidneys in lactation group. Microscopically, in some renal tubules of PPL group, acute cell swelling (hydropic degeneration) was observed in the tubular epithelial cells (Fig. 6c). Epithelial cell detachment and proteinuria were also seen in the sections in lactation group (Fig. 6d).

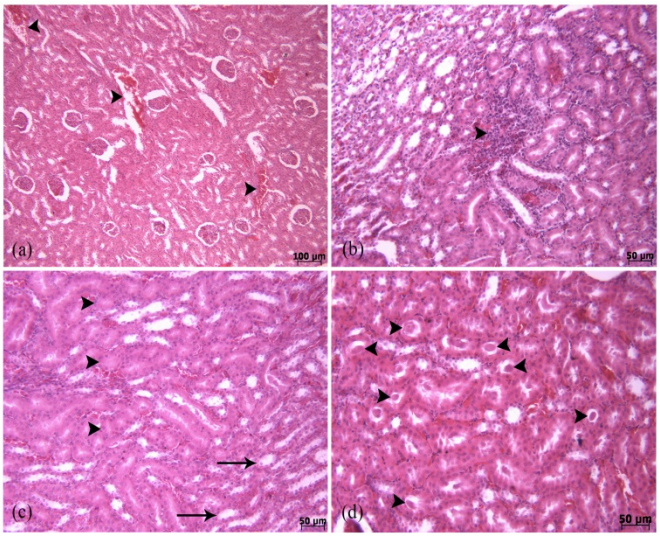

Fig. 6. Pathological examination revealed hemorrhage (a) and multifocal lymphoplasmacytic nephritis (b) in the kidneys of lactation group. Microscopically, in some renal tubules, acute cell swelling (Hydropic degeneration) was observed in the tubular epithelial cells of PPL group (c). Epithelial cell detachment and proteinuria were also seen in the sections of lactation group (d)

4. Discussion

Despite the negative effects of lead, it is almost impossible to avoid lead toxic exposure. Besides, the presence of asymptomatic lead in women who are pregnant leads to changes in the fetus that remain unknown. This study was conducted to assess the structure of kidney in offspring following lead poisoning in mothers during the perinatal period using unbiased designed based stereology.

In this study, no significant difference was found in the total volume or fractional volumes of the cortex or medulla between the experimental and control groups. However, Heidari et al. (38), showed that the mean total volume of kidney in 0.13% lead acetate groups, increased compared to the control group in adult male rat. It was significant in groups that received lead acetate in their drinking water for 8 and 12 weeks and not for 4 weeks. In addition, cortex and medulla volumes has been reported increased significantly in 12 weeks lead acetate administration compared with control group. It was assumed that renal hypertrophy is due to protein synthesis and proximal tubular epithelial cell hypertrophy (39). In contrary, Skröder et al. (40), showed that increased lead exposure at late gestation reduced kidney volume in children. It seems that the differences in the impact of lead on volume parameters in kidneys is related to the dose used and the duration of treatment. It was shown that kidney volume is related with glomerular filtration rate (41) and any change in cortex and medulla or renal volume can point to a renal pathology (42).

The present findings revealed that the distal tube total volume in the PPL group increased significantly in comparison to the control group. Consistently, the related literature confirmed an increase in the volume of renal tubules due to heavy metal poisoning (27). In a study by Karimfar et al. (26), DCT, PCT, and collecting ducts were dilated in rabbits following prolonged exposure to lead acetate. Lead toxicity causes mitochondrial swelling in renal tubular cells and impaired the production of energy, which may be another reason for increased tubular volume (43). Higher doses of lead also impair the transport of salts and amino acids and atrophy of the renal tubules (27). It is important to note that tubular modifications happen sooner than glomerulus and interstitial tissue (44). Similarly, in this research, despite changes in the tubular volume, there was no change in the volume of the kidney, cortex or medulla.

In this study, no major change in glomerular volume was found between control and experimental groups. In a study, following the exposure to 0.5% lead acetate for periods longer than three months, no divergence was found in glomerular diameter between control and experimental groups with different periods of administration (39). This study also showed that the amount of glomerular filtration rate (GFR) increases from the third month onwards, along with kidney weight, which indicates renal hypertrophy (39).

However, renal hypertrophy is not associated with hypertrophy of the glomeruli, and the rise of kidney weight and volume is due to hypertrophy of the proximal tubules (39), which is due to protein synthesis in these areas (38). Goyer et al. (45) showed no changes in glomeruli, while signs of changes in glomerular cells became apparent 6 weeks after lead administration in rat.

Glomerular status following lead exposure differs according to factors such as differences in dose, duration of exposure, path of administration (oral or respiratory) and period of exposure or age (pre-pregnancy, pregnancy, postpartum, lactation, puberty) and gender (46-48).

Overall, glomerular volume changes usually occur during long periods of exposure. Unlike changes in other kidney tissues, the lack of changes in glomerular volume can be due to different physiological and histological conditions of glomeruli compared to other kidney tissues, which increases its resistance to toxins compared to other tissues. Glomeruli have a defense barrier due to their special structure (49).

In our results, the length of the distal tube in all experimental groups (pre-pregnancy, pregnancy, lactation and PPL) showed a significant decrease in comparison to the control in the pre-pregnancy and lactation groups. In general, the length of PCT and DCT in heavy metals decreased compared to control group. According to previous studies, lead acetate is a peroxidating agent and by damaging cell membrane lipids and membrane permeability leads to destruction and necrosis of renal tubular tissue (25, 50, 51).

Adverse effects of lead on the fetus varied according to gestational age. Lead crosses the placental blood barrier and is able to delay fetal development and has teratogenic effects on it. Maternal lead contamination through nutrition can also be transmitted to infants through milk (52, 53). Regarding shorter length of DCT of pre-pregnancy group in comparison with control group, it has been pinpointed that exposure to lead before pregnancy, even after the removal of lead during pregnancy, can still have effects on the fetus due to the very slow excretion of lead from the body (54).

Among the tissues, lead accumulates at the highest level in the kidney and causes marked pathobiological changes in the kidney structure and function (55). In the present study, multifocal lymphoplasmic hemorrhage and nephritis of the kidney were observed in the lactation group. In previous studies, exposure to leadwas observed hyperemia in interstitial tissue, renal arteries, and mild focal inflammation (56). Damage to kidney cells indicates impairment in mesangial tissue in the cell vascular pole (26). Lead has an inhibitory effect on several enzymes in the heme production pathway, reducing the life of erythrocytes by increasing membrane fragility and resulting in hemolysis. Finally, due to spasm and narrowing of cutaneous arteries, it causes bleeding and inflammation of renal arteries (57, 58). In pathological analysis some renal tubules of PPL group showed acute cell swelling (hydropic degeneration) in tubular epithelial cells. Epithelial cell detachment and proteinuria were also seen in the lactation group. These pathological findings are early signs of acute tubular necrosis, and damage to renal vascular endothelial cells causes ischemia and structural changes such as loss of the brush border, disruption of tight cell connections, and detachment of tubular epithelial cells in kidneys and the swelling of tubes (59, 60). In general, protein

kinase c activates a wide range of kinases and phosphatases that affect the process of cell division, proliferation and cell communication, and lead acts by disrupting the protein kinase c receptor system and causing tissue damage and changes in the kidney (55).

Knowing that lead passes through placental blood barrier and milk, this study confirms the effect of lead at low doses on offspring kidney following perinatal period. The results of this study can be useful in quantifying the changes in kidneys following lead poisoning and adopting appropriate methods to protect mothers from lead poisoning and its effects on all births.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

References

1. Elgawish RAR, Abdelrazek HM. Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. Toxicol Rep. 2014;1:795-801. doi: 10.1016/j.toxrep.2014.10.010.
2. Abadin H, Ashizawa A, Stevens YW, Llados F, Diamond G, Sage G, et al. Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles. Toxicological Profile for Lead. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2007. PMID: 24049859.
3. Ghane T, Zamani N, Hassanian-Moghaddam H, Beyrami A, Noroozi A. Lead poisoning outbreak among opium users in the Islamic Republic of Iran, 2016–2017. Bull World Health Organ. 2018;96(3):165. doi: 10.2471/BLT.17.196287.
4. Hou S, Yuan L, Jin P, Ding B, Qin N, Li L, Liu X, et al. A clinical study of the effects of lead poisoning on the intelligence and neurobehavioral abilities of children. Theor Biol Med Model. 2013;10(1):1-9. doi: 10.1186/1742-4682-10-13.
5. Gąssowska M, Baranowska-Bosiacka I, Moczydłowska J, Frontczak-Baniewicz M, Gewartowska M, Strużyńska L, et al. Perinatal exposure to lead (Pb) induces ultrastructural and molecular alterations in synapses of rat offspring. Toxicology. 2016;373:13-29. doi: 10.1016/j.tox.2016.10.014.
6. Schottlaender LV, Abeti R, Jaunmuktane Z, Macmillan C, Chelban V, O'callaghan B, et al. Bi-allelic JAM2 variants lead to early-onset recessive primary familial brain calcification. Am J Hum Genet. 2020;106(3):412-21. doi: 10.1016/j.ajhg.2020.02.007.
7. Murata K, Araki S, Aono H. Effects of lead, zinc and copper absorption on peripheral nerve conduction in metal workers. Int Arch Occup Environ Health. 1987;59(1):11-20. doi: 10.1007/BF00377674.
8. Kumar A, Pandey R, Sharma B. Modulation of superoxide dismutase activity by mercury, lead, and arsenic. Biol Trace Elem Res. 2020;196(2):654-61. doi: 10.1007/s12011-019-01957-3.
9. Machoń-Grecka A, Dobrakowski M, Kasperczyk A, Birkner E, Kasperczyk S. Angiogenesis and lead (Pb): is there a connection? Drug Chem Toxicol. 2020:1-5. doi: 10.1080/01480545.2020.1734607.
10. Wildemann TM, Mirhosseini N, Siciliano SD, Weber LP. Cardiovascular responses to lead are biphasic, while methylmercury, but not inorganic mercury, monotonically increases blood pressure in rats. Toxicology. 2015;328:1-11. doi: 10.1016/j.tox.2014.11.009.
11. Kelainy EG, Ibrahim Laila IM, Ibrahim SR. The effect of ferulic acid against lead-induced oxidative stress and DNA damage in kidney and testes of rats. Environ Sci Pollut Res Int. 2019;26(31):31675-84. doi: 10.1007/s11356-019-06099-6.
12. Yang Q, Liu X, Chen J, Wen Y, Liu H, Peng Z, et al. Lead-mediated inhibition of lysine acetylation and succinylation causes reproductive injury of the mouse testis during development. Toxicol Lett. 2020;318:30-43. doi: 10.1016/j.toxlet.2019.10.012.
13. Metryka E, Chibowska K, Gutowska I, Falkowska A, Kupnicka P, Barczak K, et al. Lead (Pb) exposure enhances expression of factors associated with inflammation. Int J Mol Sci. 2018;19(6):1813. doi: 10.3390/ijms19061813.
14. Lee J-W, Choi H, Hwang U-K, Kang J-C, Kang YJ, Kim KI, et al. Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. Environ Toxicol Pharmacol. 2019;68:101-8. doi: 10.1016/j.etap.2019.03.010.
15. Zhou J-P, Wang F, Wang X-Y, Jiang Y-S, Yi X-Q. Effects of embryonic lead exposure on motor function and balance ability in offspring rats and possible mechanisms. Zhongguo Dang dai er ke za zhi= Chinese J Contem Pediatrics. 2017;19(3):361-7. doi: 10.7499/j.issn.1008-8830.2017.03.022.
16. Ollson CJ, Smith E, Herde P, Juhasz AL. Influence of co-contaminant exposure on the absorption of arsenic, cadmium and lead. Chemosphere. 2017;168:658-66. doi: 10.1016/j.chemosphere.2016.11.010.
17. Łuszczek-Trojnar E, Drąg-Kozak E, Szczerbik P, Socha M, Popek W. Effect of long-term dietary lead exposure on some maturation and reproductive parameters of a female Prussian carp (Carassius gibelio B.). Environ Sci Pollut Res Int. 2014;21(4):2465-78. doi: 10.1007/s11356-013-2184-x.
18. Rzymski P, Tomczyk K, Poniedzialek B, Opala T, Wilczak M. Impact of heavy metals on the female reproductive system. Ann Agric Environ Med. 2015;22(2). doi: 10.5604/12321966.1152077.
19. Xie J, Yu J, Fan Y, Zhao X, Su J, Meng Y, et al. Low dose lead exposure at the onset of puberty disrupts spermatogenesis-related gene expression and causes abnormal spermatogenesis in mouse. Toxicol Appl Pharmacol. 2020;393:114942. doi: 10.1016/j.taap.2020.114942.
20. Soleimanzadeh A, Kian M, Moradi S, Mahmoudi S. Carob (Ceratonia siliqua L.) fruit hydro-alcoholic extract alleviates reproductive toxicity of lead in male mice: Evidence on sperm parameters, sex hormones, oxidative stress biomarkers and expression of Nrf2 and iNOS. Avicenna J Phytomed. 2020;10(1):35. PMCID: PMC6941692.
21. Kalahasthi R, Barman T. Assessment of lead exposure and urinary-δ-aminolevulinic acid levels in male lead acid battery workers in Tamil Nadu, India. J Health Pollut. 2018;8(17):6-13. doi: 10.5696/2156-9614-8.17.6.
22. Wang Z, Xu X, He B, Guo J, Zhao B, Zhang Y, et al. The impact of chronic environmental metal and benzene exposure on human urinary metabolome among Chinese children and the elderly population. Ecotoxicol Environ Saf. 2019;169:232-9. doi: 10.1016/j.ecoenv.2018.11.016.
23. Farag MR, Alagawany M, Abd El-Hack ME, El-Sayed SA, Ahmed SY, Samak DH. Yucca schidigera extract modulates the

lead-induced oxidative damage, nephropathy and altered inflammatory response and glucose homeostasis in Japanese quails. Ecotoxicol Environ Saf. 2018;156:311-21. doi: 10.1016/j.ecoenv.2018.03.010.

24. Riaz MA, Nisa ZU, Mehmood A, Anjum MS, Shahzad K. Metal-induced nephrotoxicity to diabetic and non-diabetic Wistar rats. Environ Sci Pollut Res Int. 2019;26(30):31111-8. doi: 10.1007/s11356-019-06022-z.

25. Vyskocil A, Pancl J, Tusl M, Ettlerova E, Semecky V, Kašparová L, et al. Dose-related proximal tubular dysfunction in male rats chronically exposed to lead. J Appl Toxicol. 1989;9(6):395-9. doi: 10.1002/jat.2550090605.

26. Karimfar MH, Bargahi A, Moshtaghi D, Farzadinia P. Long-term exposure of lead acetate on rabbit renal tissue. Iran Red Crescent Med J. 2016;18(2). doi: 10.5812/ircmj.22157.

27. Orr SE, Bridges CC. Chronic kidney disease and exposure to nephrotoxic metals. Int J Mol Sci. 2017;18(5):1039. doi: 10.3390/ijms18051039.

28. Sanders AP, Svensson K, Gennings C, Burris HH, Oken E, Amarasiriwardena C, et al. Prenatal lead exposure modifies the effect of shorter gestation on increased blood pressure in children. Environ Int. 2018;120:464-71. doi: 10.1016/j.envint.2018.08.038.

29. Qin J, Ning H, Zhou Y, Hu Y, Huang B, Wu Y, et al. LncRNA Uc. 173 is a key molecule for the regulation of lead-induced renal tubular epithelial cell apoptosis. Biomed Pharmacother. 2018;100:101-7. doi: 10.1016/j.biopha.2018.01.112.

30. McGeady TA, Quinn PJ, Fitzpatrick ES, Ryan MT, Kilroy D, Lonergan P. Veterinary embryology: John Wiley & Sons; 2017.

31. Gavaghan H. Lead, unsafe at any level. Bull World Health Organ. 2002;80:82-. PMCID: PMC2567638.

32. Al-Shimali H, Al-Musaileem A, Rao M, Khan K. Low-dose exposure to lead during pregnancy affects spatial learning, memory and neurogenesis in hippocampus of young rats. J Neurol Neurosci. 2016;7(3).

33. Nyengaard JR. Stereologic methods and their application in kidney research. J Am Soc Nephrol. 1999;10(5):1100-23. doi: 10.1681/ASN.V1051100.

34. Keller C, Katz R, Cushman M, Fried LF, Shlipak M. Association of kidney function with inflammatory and procoagulant markers in a diverse cohort: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis (MESA). BMC nephrol. 2008;9(1):1-8. doi: 10.1186/1471-2369-9-9.

35. Heidmets L-T, Zharkovsky T, Jurgenson M, Jaako-Movits K, Zharkovsky A. Early post-natal, low-level lead exposure increases the number of PSA-NCAM expressing cells in the dentate gyrus of adult rat hippocampus. Neurotoxicology. 2006;27(1):39-43. doi: 10.1016/j.neuro.2005.05.015.

36. Jaako-Movits K, Zharkovsky T, Romantchik O, Jurgenson M, Merisalu E, Heidmets L-T, et al. Developmental lead exposure impairs contextual fear conditioning and reduces adult hippocampal neurogenesis in the rat brain. Int J Dev Neurosci. 2005;23(7):627-35. doi: 10.1016/j.ijdevneu.2005.07.005.

37. Gundersen HJ, Jensen E. The efficiency of systematic sampling in stereology and its prediction. J Microsc. 1987;147(3):229-63. doi: 10.1111/j.1365-2818.1987.tb02837.x.

38. Heidari Z, Mahmoudzadeh SH, Rafighdoust H, Moien A. Analysis of Gross Anatomical Parameters of Kidney in Male Rats Following Lead Poisoning by Using a Stereological Method. Zahedan Journal Of Research In Medical Sciences (Tabib-E-Shargh). 2003; 5(2)

39. Khalil-Manesh F, Gonick HC, Cohen AH, Alinovi R, Bergamaschi E, Mutti A, et al. Experimental model of lead nephropathy. I. Continuous high-dose lead administration. Kidney Int. 1992;41(5):1192-203. doi: 10.1038/ki.1992.181.

40. Skröder H, Hawkesworth S, Moore SE, Wagatsuma Y, Kippler M, Vahter M. Prenatal lead exposure and childhood blood pressure and kidney function. Environ Res. 2016;151:628-34. doi: 10.1016/j.envres.2016.08.028.

41. Cibulskyte D, Pedersen M, Hjelm-Poulsen J, Hansen HE, Madsen M, Mortensen J. The pharmacokinetics and acute renal effects of oral microemulsion ciclosporin A in normal pigs. Int Immunopharmacol. 2006;6(4):627-34. doi: 10.1016/j.intimp.2005.09.013.

42. Padigala KK, Hartle JE, Kirchner HL, Schultz MF. Renal cortical thickness as a predictor of renal function and blood pressure status post renal artery stenting. Angiology. 2009;60(6):719-24. doi: 10.1177/0003319709339587.

43. Coleman WB, Tsongalis GJ. Essential concepts in molecular pathology: Academic Press; 2010.

44. Jarrar BM. Histological and histochemical alterations in the kidney induced by lead. Ann Saudi Med. 2003;23(1-2):10-5. doi: 10.5144/0256-4947.2003.10.

45. Goyer RA. Results of lead research: prenatal exposure and neurological consequences. Environ Health Perspect. 1996;104(10):1050-4. doi: 10.1289/ehp.961041050.

46. Mitra P, Sharma S, Purohit P, Sharma P. Clinical and molecular aspects of lead toxicity: An update. Crit Rev Clin Lab Sci. 2017;54(7-8):506-28. doi: 10.1080/10408363.2017.1408562.

47. Korach KS. Reproductive and developmental toxicology: CRC Press; 1998.

48. Shahsavari A, Yazdi FT, Moosavi Z, Heidari A, Sardari P. A study on the concentration of heavy metals and histopathological changes in Persian jirds (Mammals; Rodentia), affected by mining activities in an iron ore mine in Iran. Environ Sci Pollut Res Int. 2019;26(12):12590-604. doi: 10.1007/s11356-019-04646-9.

49. Daehn IS, Duffield JS. The glomerular filtration barrier: a structural target for novel kidney therapies. Nat Rev Drug Discov. 2021;20(10):770-88. doi: 10.1038/s41573-021-00242-0.

50. Vyskocil A, Cizkova M, Tejnorova I. Effect of prenatal and postnatal exposure to lead on kidney function in male and female rats. J Appl Toxicol. 1995;15(4):327-8. doi: 10.1002/jat.2550150416.

51. Afshari AT, Shirpoor A, Farshid A, Saadatian R, Rasmi Y, Saboory E, et al. The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. Food chem. 2007;101(1):148-53. doi.org/10.1016/j.foodchem.2006.01.013

52. Antonio-García MT, Massó-Gonzalez EL. Toxic effects of perinatal lead exposure on the brain of rats: involvement of oxidative stress and the beneficial role of antioxidants. Food Chem Toxicol. 2008;46(6):2089-95. doi: 10.1016/j.fct.2008.01.053.

53. Patriarca M, Menditto A, Rossi B, Lyon T, Fell G. Environmental exposure to metals of newborns, infants and young children. Microchemical J. 2000;67(1-3):351-61. doi.org/10.1016/S0026-265X(00)00088-6

54. Kaji M, Nishi Y. Lead and growth. Clin Pediatr Endocrinol. 2006;15(4):123-8. doi: 10.1297/cpe.15.123.

55. Azarnia M, Mirabolghasemi G, Hamauatkhah V, editors. The effect of lead acetate on histological structure of the kidney of 1-

day-old rats. Toxicology; 2001: Elsevier Sci Ireland Ltd Customer Relations Manager, Bay 15, Shannon Industrial Estate Co, Clare, Ireland: Elsevier Sci Ireland Ltd.

56. Mohammadi S, Khakbaz M, Marzieh S. Effects of different doses of manganese on lead poisoning in the kidney of adult male mice. koomesh. 2016;18.

57. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates. Interdiscip Toxicol. 2012;5(2):47. doi: 10.2478/v10102-012-0009-2.

58. Gundacker C, Pietschnig B, Wittmann KJ, Lischka A, Salzer H, Hohenauer L, et al. Lead and mercury in breast milk. Pediatrics. 2002;110(5):873-8. doi: 10.1542/peds.110.5.873.

59. Hanif MO, Bali A, Ramphul K. Acute renal tubular necrosis. StatPearls [Internet]. 2021. PMID: 29939592.

60. Breshears MA, Confer AW. The urinary system. Pathologic basis of veterinary disease. 2017:617. 10.1016/B978-0-323-35775-3.00011-4

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Why do we do EEG? Experience of two years from a new pediatric neurology center

Emine TEKİN *, Betül DİLER DURGUT

Department of Pediatric Neurology, Faculty of Medicine, Giresun University, Giresun, Türkiye

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Abstract

Electroencephalography (EEG) is very important for pediatric neurologists. The request reasons and results of EEGs from a newly established center were enrolled. Total of 2021 (1299 sleep + awake, 652 sleep, 70 awake) EEGs evaluated. Patients included 1005 girls and 1016 boys. 65% of the EEGs were normal and 30% was epileptic. Electroencephalography was performed due to epilepsy, fainting, first afebrile seizure, febrile seizure, speech retardation, dizziness, headache, movement disorders, gaze abnormalities, behavioral disorders, tremor, sleep disorders, tic, autism spectrum disorders, infantile spasm, encephalopathy, vision loss and abdominal pain in decreasing order. Significantly more common EEGs were performed due to tics (p:0,006), autism spectrum disorders (p:0,04) and speech retardation(p:<0,001) in boys and due to syncope (p:0,001) and dizziness(p:0,038) in girls. When EEG requests were examined by age groups, statistical significance was found. The EEG requests were parallel to the distribution of epileptic and non-epileptic events seen in that age group.

Keywords: electroencephalography, nonepileptic events, seizure

1. Introduction

Electroencephalography (EEG) is an indispensable tool in pediatric neurology practice. Electroencephalography is used to differentiate epileptic and non-epileptic activity, to define epilepsy type and epileptic syndromes, to follow up patients using anti convulsive drugs, and to decide on drug discontinuation1. It may be useful for prediction of long-term outcome or recurrence.

Electroencephalography is a noninvasive, readily available and inexpensive investigation to study the neuronal dysfunction and abnormal cortical excitability in children who present with seizures (1).

Electroencephalography must not be used to exclude epilepsy since epilepsy is a clinical condition or to determine the efficacy of the anti convulsive medication (1).

Who made the EEG request, may cause changes in the reasons of EEG request. As pediatric neurologists working in a newly established center, we wanted to evaluate the reasons and results of the EEGs performed in 2 years.

2. Material and methods

Electroencephalographies taken at the Maternity and Children Hospital between June 2018 and June 2020 were evaluated according to the reasons for request, age, gender and EEG results.

Electroencephalographies were recorded with an 18-channel EEG device (Neurofax QP-112AK ver.07-21 Nihon Kohden Corporation). Electrodes were placed according to the 10-20 international system (Bipolar and reference montage). Sleep, wakefulness and sleep + wakefulness EEG recordings were taken after sleep deprivation. Activation methods of hyperventilation and intermittent photic stimulation were done routinely. For both sleep and wakefulness records of at least 20 minutes were taken. The families were given a written document on what to do before the EEG procedure, and their consents were obtained for the EEG procedure.

2.1 Ethics

Informed consent were taken from the parents/guardians of the patients. The study was conducted in concordance with the Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. Ethical approval was obtained from the local Clinical Research Ethics Committee for the study (Date: 09.11.2020, Number: 04).

2.2 Statistical analysis

All analyses were performed using IBM SPSS (v22.0) package program. Data are expressed as percentiles, frequencies, mean, standard deviation (SD), median, minimum–maximum values. Pearson chi-square test was used for between-group comparisons. Significance level (p value) was determined to be at the ≤0.05 level.

3. Results

A total of 2021 EEG records were evaluated in the study.

*Correspondence: dreminetekin@yahoo.com

1299 (64.3%) sleep + awake EEG 652 (32.3%) sleep EEG and 70 (3.5%) awake EEG were performed. Of the patients, 1005 (49.7%) were female and 1016 (50.3%) were male. Electroencephalography results were interpreted as: normal 1321 (65.4%), epileptic 598 (29.6%) and uncertain (suspicious) 102 (5.0%).

Electroencephalographies in which the EEG recording was not completely normal but sharp wave activity not evaluated as epileptic or background slowing was accepted as suspicious EEG and control EEG was requested. There were also repeated EEG records: 2 times for 243 patients, 3 times for 64 patients, 4 times for 13 and 6 times for 3 patients (totally 748 EEG). We found that patients with 3 or more EEG records were patients with refractory epilepsy, status epilepticus, epileptic encephalopathy, and status epilepticus in early sleep (ESES). Only 1.8% of the patients were under the age of 2, the other 20% were under 2 years old, 50% were between 2-12 years old, and 30% were >12 years old (12-18 years).

Electroencephalography was performed most frequently due to epilepsy. Other causes of EEG request and EEG results are given in Table 1. There was a significant difference between the reasons for requesting EEG according to gender and age categories.

Table 1. The indications for EEG request and the EEG results

	No of EEG		EEG results		
	n	%	Epileptic N, (%)	Normal N, (%)	Suspicious N, (%)
Epilepsy	**803**	**39.7**	382 (47.60%)	388 (48.30%)	33 (4.1%)
Syncope	**205**	**10.1**	33 (16.10%)	158 (77.10%)	14 (6.8%)
First afebrile seizure	**190**	**9.4**	42 (22.10%)	135 (71.10%)	13 (6.8%)
Febrile seizure	**143**	**7.1**	25 (17.50%)	111 (77.60%)	7 (4.9%)
Language impairment	**95**	**4.7**	14 (14.70%)	72 (75.80%)	9 (9.5%)
Vertigo	**93**	**4.6**	16 (17.20%)	75 (80.60%)	2 (2.2%)
Breath holding spell	**76**	**3.8**	5 (6.60%)	68 (89.50%)	3 (3.9%)
Headache	**67**	**3.3**	13 (19.40%)	51 (76.10%)	3 (4.5%)
Movement disorder	**49**	**2.4**	7 (14.30%)	41 (83.70%)	1 (2.0%)
Newborn seizure	**48**	**2.4**	8 (16.70%)	38 (79.20%)	2 (4.2%)
Abnormal eye movement	**44**	**2.2**	6 (13.60%)	34 (77.30%)	4 (9.1%)
Behavioural disorder	**35**	**1.7**	9 (25.70%)	22 (62.90%)	4 (11.4%)
Tremor	**34**	**1.7**	8 (23.50%)	24 (70.60%)	2 (5.9%)
Sleep disorder	**32**	**1.6**	3 (9.40%)	29 (90.60%)	0
Tic	**30**	**1.5**	2 (6.70%)	27 (90.00%)	1 (3.3%)
Autism spectrum disorder	**24**	**1.2**	4 (16.70%)	17 (70.80%)	3 (12.5%)
Other	**15**	**0.7**	8 (53.30%)	6 (40.00%)	1 (6.7%)
Infantile spazm	**14**	**0.7**	10 (71.40%)	4 (28.60%)	0
Encephalopathy	**8**	**0.4**	1 (12.50%)	7 (87.50%)	0
Vision loss	**8**	**0.4**	1(12.50%)	7 (87.50%)	0
Masturbation	**4**	**0.2**	0	4 (100.00%)	0
Abdominal pain	**4**	**0.2**	1(25.00%)	3 (75.00%)	0
Total	**2021**	**100%**	598 (29.60%)	1321 (65.40%)	102 (5%)

Table 2. Statistically significant EEG request reasons according to gender

	Total	Male (n, %)	Female (n, %)	P*
Tic	30	23 (76.7%)	7 (23.3%)	0.006
Autism spectrum disorder	24	17 (70.8%)	7 (29.2%)	0.043
Language impairment	95	65 (68.4%)	30 (31.6%)	<0.001
Syncope	205	80 (39%)	125 (61%)	0.001
Vertigo	93	37 (39.8%)	56 (60.2%)	0.038

*Pearson chi-square

Table 3. Statistically significant EEG request reasons according to age categories

	Newborn	1-24 months	2-6 years	6-12 years	12-18 years	P*
Epilepsy	10	92	130	326	244	<0.001
First afebrile seizure	6	49	35	52	48	0.012
Febrile seizure	0	58	49	31	5	<0.001
Breath holding spell	2	46	12	10	6	<0.001
Movement disorder	0	20	7	12	10	0.001
Syncope	0	7	14	60	124	<0.001
Language impairment	0	13	54	20	8	<0.001
Headache	0	1	7	25	34	<0.001
Vertigo	0	1	4	30	58	<0.001
Newborn seizure	16	25	2	4	1	<0.001
Behavioural disorder	0	0	3	15	17	0.008

*Pearson chi-square

4. Discussion

In this article, we aimed to find out the EEGs taken in our center, to whom and why we had EEG and what our rate of epileptic activity.

Epilepsy is a clinical diagnosis. Although EEG is not necessary for the diagnosis of epilepsy, it is useful for classification of epilepsy, determination of the severity of epilepsy and differentiation from non-epileptic paroxysmal events. Epileptiform discharges may be found in EEGs of the healthy people but this does not mean they are epileptic. Epileptiform discharges are found in 5.6% of normal healthy children and 0.5% of adults without any event of seizure (2).

Among children with new onset seizures, 18-56% display epileptiform discharges on initial EEG and 15% will never show abnormal findings (3).

In our study, epileptic activity was detected in 30% of all EEGs and 48% of patients with epilepsy. The rate of epileptic EEGs taken with the suspicion of the first afebrile seizure remained at 22%. In a study from Turkey where EEG recording has been performed for a long time, 38% of 2045 EEGs were found to be epileptic. EEG was epileptic in the 54% of patients with epilepsy and 29% of first afebrile seizures (4).

We determined that our epileptic rates were slightly lower, but there was no significant difference. In another retrospective study from Turkey; in which 1000 patients aged 5-18 years with seizures and seizure-like complaints were examined; 14% of all EEGs was epileptic and this ratio was 39% in the patients who were thought to have seizures (5). We thought the difference was due to study design.

Considering the distribution of EEGs by age group, the group with the highest incidence of epilepsy was school age and the second was adolescents.

The number of EEGs taken on suspicion of seizures was similar in all age groups except newborn period. EEG scan for FS was most common in infants and up to 6 years of age. Most of the EEGs taken for the breath holding spells were infants. Sleep disturbance was distributed to all age groups except newborn. Tic was most common reason for request in the 6-12 age group, tremor was most common in the 12-18 age group. Movement disorders were most common in the infant group and least common in the toddler. Shuddering attack in infancy, migraine equivalent syndromes like benign paroxysmal vertigo and torticollis, Sandifer syndrome and many paroxysmal non-epileptic conditions were in this group. Syncope was most common reason in the adolescent with increasing rate according to ages. Speech retardation was the most common reason for EEG request in the 2-6 age group. Headache, dizziness, disorientation were found to be the reasons for EEG evident in school age children and adolescence. The reason for the statistical difference according to age groups was that the mentioned events were more common in that age group. We did not encounter a different result than expected here.

According to gender, we found that syncope was a statistically significant reason for more EEG requests in girls. Dizziness/vertigo was also higher in girls, but it was not statistically significant. Tic, speech retardation and autism spectrum disorders (ASD) were the causes of EEG requests at a significantly higher rate in males. Since these disorders are seen more frequently in male, it was expected.

In studies on EEG duration and time, it is seen that detection rate of epileptic activity increases as the duration of EEG recording increases (6-8).

In the first 20 minutes, the epileptic activity can be detected by 45-48%. The detection rate increases by 19% after 30 minutes of the interictal EEG8. Ambulatory EEG records of 20-30 minutes seems to be appropriate for the outpatient clinic conditions. Our sleep+wake EEG recordings were taken as minumum 40 minutes (20 minutes for each).

All of our sleep EEGs were taken after sleep deprivation (all night or less depending on the age of the patient (9). In case of difficulty in sleeping due to co-morbidities such as ASD, attention deficit hyperactivity disorder (ADHD), cerebral palsy, mental retardation, chloralhydrate was given to patients for sedation. Many drugs are used for EEG recordings to supply sedation and sleep, chloralhydrate is one of the best known, it is effective and safe (10).

In the study of Orgun et al. (4), when the EEG was evaluated according to the reasons for the request, it was found to be epileptic at a rate of 8.5% in FS, 16% in tic, 15.7% in speech disorder, 44% in learning disability, 20% in sleep disorder, 11% in night terror, 10% in breath holding spells, 8.4% in syncope, and 11.6% in headache.

In the study of Kamaşak et al. (5), EEG abnormalities were found in 8% of speech disorder, 4% of headache, 7% of syncope, 6% of sleep disorder, 10% of movement disorder and 13% of learning disability.

EEG was requested under different headings in different studies for similar patients who applied. For example, sleep disorder can be taken as a single heading, or it can be named as night terror and sleep disorder. Even involuntary movements in sleep can be classified as sleep disorder or movement disorder.

In different clinics, patient distribution may vary and affect these rates. EEG requests are made only by child neurologists in some clinics, and additionally by child and child psychiatry specialists in some clinics. The branch of the requester (child neurologist or pediatrician) may affect these rates. We think that only EEG is requested by a pediatric neurologist in our clinic increases this rate. In the other two studies conducted in Turkey, the rate of finding epileptic in headache is very different from each other, such as 11.6% and

4% (4, 5). In our study, a higher result of 19% was found. We can attribute this not to all headaches, but to EEG request in patients selected by a pediatric neurologist or to the presence of findings suggestive of seizures in the medical history.

From the point of view of speech retardation, things get even more complicated. In a meta-analysis study 33.5% of children with language impairment but without epilepsy were found to have isolated epileptiform activity in sleep EEGs. This corresponded to 6 times greater than for typically developing children. The overall pooled prevalence of epileptiform activity was 27.3%. A wide variation between the prevalence estimates was, to a certain degree, explained by type of impairment such as 8.1% in speech impairments, 25.8% in language impairments, and 51.5% in language regression (11).

In a prospective study in which 24 children with speech disorders were evaluated by sleep EEG, epileptiform activity was found in 7 children and abnormal EEG in 5 children (12). In our study, 14.7% (14/95) of the children had epilepsy, and 9.5% (9/95) had suspicious epileptic activity. The rates were found similar. In another study, 7/54 (13%) of cases with developmental speech-language disorder had an epileptic EEG, compared with 3/45 (6.7%) of healthy controls, yielding an odds ratio of 2.1. They reported a weak association between epileptic EEGs and speech-language disorder but significant association between EEG status and Performance IQ (13). In another recently published study 55 children with developmental language disorders were enrolled, 33 (%61.1) of them also had motor coordination disorders and 39 (70.9%) of them had diagnosis of ADHD. Awake EEG examinations showed epileptiform discharges in 36.4% and nocturnal EEG and polysomnography (PSG) recordings enhanced epileptiform discharges in up to 55.6% of the children (14). The rates are found very high in this study. The comorbidities of the children (ADHD, mental retardation, motor developmental problems) might be the cause of epileptiform discharges. Epileptic activity is observed with a rate of 52% in ASD and 42% in ADHD (15). This issue is still not fully clear (16).

As it can be understood from the publications we mentioned about speech retardation, it is necessary to examine specific groups homogeneously in order to talk about EEG. The differences between the publications are also due to the heterogenous groups. However, our study was found to be compatible with the literature on most subjects.

EEG was performed in 143 patients with febrile seizures, and 17.5% were found to be epileptic. There are no RCTs related to the time of EEG in complicated FS. Electroencephalography was performed to the patients with complicated FS and recurrent FS due to panic states of the families, recurrent FS, and family history of epilepsy in some patients (17).

It has been reported that EEG characteristics in FS can predict the development of epilepsy in the later stages (18). Kanemura et al. (19, 20) stated that epileptic activity in the frontal region had a significantly higher risk for the development of epilepsy than those with focal paroxysms in other regions.

In two studies (18, 20) investigating the development of paroxysmal activity on EEG and epilepsy in FS, 16.8% and 21.8% of epileptic paroxysmal abnormalities detected, respectively, and epilepsy occurred at a rate of 6.7 and 7.6 (18, 20). In our study, epileptic activity was found at a rate of 17.5 %, similar to the literature. We cannot comment on epilepsy since long-term follow-ups have not been performed yet.

Electroencephalography is often misused to justify the need for AED among children with clear history of paroxysmal non-epileptic events, headache, simple febrile seizures and head trauma. An abnormal EEG report should always be interpreted in clinical context.

In the study of Park et al. (21), the patients who underwent video EEG monitorisation (VEM) evatuated and overmedication rate was 27%. Children present age-specific non-epileptic paroxysmal patterns, recognition of the clinical aspects of them according to age plays a key role in diagnose. They recommend long-term VEM in differentiating epileptic from nonepileptic events to prevent overmedication and guide proper treatment. We found that there are differences in the EEG requests according to age and also gender. We used video assisted EEG but unfortunately we were unable to record for long time.

Today, studies are carried out on the EEG maturation and the use of EEG in terms of academic success (22, 23). The effect of anesthesia on the child's brain is another field of research (24). Electroencephalography can be used in patients with hearing loss (25), analysis in perceptional decision making can be made by using EEG (26). Since this study was retrospective, there was no EEG recording for research purposes.

The major limitation of this study is the lack of knowledge about the detailed results of the EEG recordings such as the background activity, hemispheric asymmetry, localization of the epileptic activity. Instead, normal, epileptiform discharges (spike and sharp waves) and suspicious was used.

Another limitation of the study was its retrospective design and lack of homogeneous groups.

Patterns of children in EEG can be mistakenly defined as epilepsy. It is very important to evaluate the EEG by the specialist, knowing the patient's age and clinic. Suspicious abnormalities in EEG can be diagnosed as epilepsy (27, 28).

Common reasons for misinterpretation of EEG include poor expertise, lack of good quality recording, inappropriate

indication, and absence of clinical correlation (29).

In our study, all EEGs were requested and evaluated by two pediatric neurologists. In order to minimize false readings, in cases where clinical information was insufficient or unclear, the expression suspicious was used in EEGs without definite epileptiform anomaly, and patients were referred for re-examination of EEG.

Electroencephalography was also performed for non-epileptic events that could be diagnosed clinically because it is a newly established center and the number of EEG recordings is low. We presented our own EEG request reasons and results by comparing them with the literature.

The answer to the question of why we take the EEG seems to be getting harder. For whatever reason, clinical information is required for EEG recording. Every paroxysmal activity on EEG should not be considered as epilepsy. Sleep EEG recording and activation methods are very valuable in children.

Although EEG is a very valuable auxiliary technique, we think that it would not be very useful to evaluate the results independently from the clinical evaluation. Also pediatric neurologist decision to order an EEG and reporting would lead to better consequences.

References

1. Kaushik JS, Farmania R. Electroencephalography in Pediatric Epilepsy. Indian Pediatr. 2018;55(10):893-901.
2. So EL. Interictal epileptiform discharges in persons without a history of seizures: what do they mean? J Clin Neurophysiol. 2010;27(4):229-38. doi:10.1097/WNP.0b013e3181ea42a4.
3. Wirrell EC. Prognostic significance of interictal epileptiform discharges in newly diagnosed seizure disorders. J Clin Neurophysiol. 2010;27(4):239-48. doi:10.1097/WNP.0b013e3181ea4288.
4. Tekin Orgun L, Arhan E, Aydın K, Rzayeva T, Hırfanoğlu T, Serdaroğlu A. What has changed in the utility of pediatric EEG over the last decade? Turk J Med Sci. 2018;48(4):786-93. doi:10.3906/sag-1712-188.
5. Tülay Kamaşak, Durgut BD, Arslan EA, Şahin S, Dilber B, Kurt T et al. Adolesanlarda Epileptik Nonepileptik Olayların Ayrımında Elektroensefalografinin Yeri: Bir Pediatrik Nöroloji Merkezinin Üç Yıllık Deneyimi. Güncel Pediatri. 2018;16(2):19-30.
6. Badry R. Latency to the first epileptiform activity in the EEG of epileptic patients. Int J Neurosci. 2013;123(9):646-9. doi:10.3109/00207454.2013.785543.
7. Lee CH, Lim SN, Lien F, Wu T. Duration of electroencephalographic recordings in patients with epilepsy. Seizure. 2013;22(6):438-42. doi:10.1016/j.seizure.2013.02.016.
8. Nickels KC. Routine Versus Extended Outpatient EEG: Too Short, Too Long, or Just Right? Epilepsy Curr. 2016;16(6):382-3. doi:10.5698/1535-7511-16.6.382.
9. Theitler J, Dassa D, Heyman E, Lahat E, Gandelman-Marton R. Feasibility of sleep-deprived EEG in children. Eur J Paediatr Neurol. 2016;20(2):218-21. doi:10.1016/j.ejpn.2015.12.012.
10. Olson DM, Sheehan MG, Thompson W, Hall PT, Hahn J. Sedation of children for electroencephalograms. Pediatrics. 2001;108(1):163-5. doi:10.1542/peds.108.1.163.
11. Systad S, Bjørnvold M, Sørensen C, Lyster SH. The Value of Electroencephalogram in Assessing Children With Speech and Language Impairments. J Speech Lang Hear Res. 2019;62(1):153-68. doi:10.1044/2018_jslhr-l-17-0087.
12. Billard C, Hassairi I, Delteil F. [Specific language impairment and electroencephalogram: which recommendations in clinical practice? A cohort of 24 children]. Arch Pediatr. 2010;17(4):350-8. doi:10.1016/j.arcped.2010.01.012.
13. Parry-Fielder B, Collins K, Fisher J, Keir E, Anderson V, Jacobs R et al. Electroencephalographic abnormalities during sleep in children with developmental speech-language disorders: a case-control study. Dev Med Child Neurol. 2009;51(3):228-34. doi:10.1111/j.1469-8749.2008.03163.x.
14. Dlouha O, Prihodova I, Skibova J, Nevsimalova S. Developmental Language Disorder: Wake and Sleep Epileptiform Discharges and Co-morbid Neurodevelopmental Disorders. Brain Sci. 2020;10(12). doi:10.3390/brainsci10120910.
15. Kawatani M, Hiratani M, Kometani H, Nakai A, Tsukahara H, Tomoda A et al. Focal EEG abnormalities might reflect neuropathological characteristics of pervasive developmental disorder and attention-deficit/hyperactivity disorder. Brain Dev. 2012;34(9):723-30. doi:10.1016/j.braindev.2011.11.009.
16. Laasonen M, Smolander S, Lahti-Nuuttila P, Leminen M, Lajunen HR, Heinonen K et al. Understanding developmental language disorder - the Helsinki longitudinal SLI study (HelSLI): a study protocol. BMC Psychol. 2018;6(1):24. doi:10.1186/s40359-018-0222-7.
17. Shah PB, James S, Elayaraja S. EEG for children with complex febrile seizures. Cochrane Database Syst Rev. 2020;4(4):Cd009196. doi:10.1002/14651858.CD009196.pub5.
18. Gradisnik P, Zagradisnik B, Palfy M, Kokalj-Vokac N, Marcun-Varda N. Predictive value of paroxysmal EEG abnormalities for future epilepsy in focal febrile seizures. Brain Dev. 2015;37(9):868-73. doi:10.1016/j.braindev.2015.02.005.
19. Kanemura H, Sano F, Ohyama T, Mizorogi S, Sugita K, Aihara M. EEG characteristics predict subsequent epilepsy in children with their first unprovoked seizure. Epilepsy Res. 2015;115:58-62. doi:10.1016/j.eplepsyres.2015.05.011.
20. Kanemura H, Mizorogi S, Aoyagi K, Sugita K, Aihara M. EEG characteristics predict subsequent epilepsy in children with febrile seizure. Brain Dev. 2012;34(4):302-7. doi:10.1016/j.braindev.2011.07.007.
21. Park EG, Lee J, Lee BL, Lee M, Lee J. Paroxysmal nonepileptic events in pediatric patients. Epilepsy Behav. 2015;48:83-7. doi:10.1016/j.yebeh.2015.05.029.
22. Whedon M, Perry NB, Bell MA. Relations between frontal EEG maturation and inhibitory control in preschool in the prediction of children's early academic skills. Brain Cogn. 2020;146:105636. doi:10.1016/j.bandc.2020.105636.
23. Whedon M, Perry NB, Calkins SD, Bell MA. Changes in frontal EEG coherence across infancy predict cognitive abilities at age 3: The mediating role of attentional control. Dev Psychol. 2016;52(9):1341-52. doi:10.1037/dev0000149.
24. Brandt SP, Walsh EC, Cornelissen L, Lee JM, Berde C, Shank ES et al. Case Studies Using the Electroencephalogram to Monitor Anesthesia-Induced Brain States in Children. Anesth Analg. 2020;131(4):1043-56. doi:10.1213/ane.0000000000004817.

25. Cartocci G, Scorpecci A, Borghini G, Maglione AG, Inguscio BMS, Giannantonio S et al. EEG rhythms lateralization patterns in children with unilateral hearing loss are different from the patterns of normal hearing controls during speech-in-noise listening. Hear Res. 2019;379:31-42. doi:10.1016/j.heares.2019.04.011.

26. Manning C, Wagenmakers EJ, Norcia AM, Scerif G, Boehm U. Perceptual Decision-Making in Children: Age-Related Differences and EEG Correlates. Comput Brain Behav. 2021;4(1):53-69. doi:10.1007/s42113-020-00087-7.

27. Sheth RD. Patterns Specific to Pediatric EEG. J Clin Neurophysiol. 2019;36(4):289-93. doi:10.1097/wnp.0000000000000600.

28. Amin U, Benbadis SR. The Role of EEG in the Erroneous Diagnosis of Epilepsy. J Clin Neurophysiol. 2019;36(4):294-7. doi:10.1097/wnp.0000000000000572.

29. Benbadis SR. The tragedy of over-read EEGs and wrong diagnoses of epilepsy. Expert Rev Neurother. 2010;10(3):343. doi:10.1586/ern.09.157.

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Evaluation of demographic characteristics and laboratory results of patients with Covid-19 treated in the intensive care unit

Nevin AYDIN [1,*], Osman ESEN [2], Umut KARAYALÇIN [3]

[1]Department of Anaesthesiology and Reanimation, Istanbul Kanuni Sultan Suleyman Training and Research Hospital, Istanbul, Türkiye
[2]Department of Anaesthesiology and Reanimation İstinye University Vocational High School, İstanbul, Türkiye
[3]Department of emergency Dr.Sadi Konuk Training and Research Hospital, Istanbul, Türkiye

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Abstract

We aimed to investigate the clinical features, hemodynamic and respiratory profiles as well as prognostic outcomes of critically sick COVID-19 patients admitted to intensive care units (ICUs). This retrospective study was performed using data derived from 99 adult patients treated in the ICU. Demographic and clinical data as well as hemodynamic and respiratory profiles, therapeutic outcomes were recorded. The relationship between these features and ICU stay was sought. The average age was 65.94 ± 14.93 years (24 to 96), and 73 patients (73.7%) had comorbidities. Smokers constituted 13.1% of the Covid-19 patient population (n=13) in ICU. Thirty-one cases (31.3%) had received at least one dose of Covid-19 vaccine and 63 patients (63.6%) died in the ICU after their initial hospitalization. Blood products were utilized in 29 patients (29.3%) and delta mutation was detected in 23 (23.2%) of ICU patients. The mean duration of ICU stay was 16.90 ± 11.41 days (1 to 60). The duration of ICU stay was remarkably different between groups receiving different antibiotic regimens ($p<0.001$). There was no significant relationship between the duration of ICU stay and blood groups ($p=0.052$), systolic ($p=0.572$) and diastolic blood pressure ($p=0.098$) and initial arterial oxygen saturation ($p=0.223$). We detected a high mortality rate in our series with severe COVID-19 infection treated in ICU. These data are critical for understanding the impact of COVID-19 on our hospitals, identifying areas for clinical management improvement, and allowing for continuous international and temporal comparisons of COVID-19 patient outcomes.

Keywords: COVID-19, treatment, intensive care unit, demographic, laboratory

1. Introduction

The novel Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) disease (COVID-19) first appeared in Wuhan, China, and quickly spread throughout the world. Almost 30 million people were affected, and 1 million people have died as a result (1). Although the majority of patients enter with minor illnesses and recover, life-threatening illnesses can occur, necessitating admission in an Intensive Care Unit (ICU). Acute Respiratory Distress Syndrome (ARDS), sepsis, multi-system organ failure, hyperinflammation, neurological and extrapulmonary signs, and thromboembolic illness are all symptoms of severe COVID-19 (2). Old age, the prevalence of comorbidities such as hypertension, diabetes mellitus (DM), morbid obesity, chronic lung illness, coronary artery disease, chronic renal disease, and malignancies were all linked to a poor prognosis. Lymphocytopenia and elevated levels of inflammatory biomarkers such as C-reactive protein, lactate dehydrogenase, and interleukin-6, among others, were found to be associated with a bad prognosis (3).

The COVID-19 pandemic is still a major public health concern around the world. Despite the fact that scientific knowledge of COVID-19 is growing by the day, there is a scarcity of data on the presenting symptoms and outcomes of patients who need to be admitted to critical care units (ICUs). As a result, the current study looked at the clinical features and risk variables of critically sick COVID-19 patients admitted to ICUs.

Acute respiratory distress syndrome is present in nearly all COVID-19 patients who require mechanical ventilation (ARDS). ARDS is a life-threatening, progressive inflammatory lung disease marked by diffuse alveolar destruction and fast clinical deterioration. COVID-19 patients, on the other hand, have a distinct clinical trajectory than most other ARDS patients, according to specialists around the world. A number of patient features have been linked to a higher probability of a severe disease course (4, 5).

COVID-19 patients have a different illness trajectory than most other ARDS patients, according to specialists around the world (6). COVID-19 infection causes respiratory failure in 25-70 percent of hospitalized patients, necessitating invasive mechanical ventilation (IMV) and treatment in the intensive care unit (ICU) (7). Given the different capacities to prevent, test for, and treat COVID-19, a better understanding of the variables connected to mortality in patients requiring critical

*Correspondence: nevinaydin4334@gmail.com

care and mechanical ventilation is necessary (8).

The COVID-19 pandemic is still a major public health concern around the world. Despite the fact that scientific knowledge of COVID-19 is growing by the day, there is a scarcity of data on the characteristics and outcomes of patients who need to be admitted to ICUs. As a result, the current study investigated the clinical features, hemodynamic and respiratory profiles as well as prognostic outcomes of critically sick COVID-19 patients admitted to ICUs.

2. Materials and methods

This retrospective, single-center study was performed using data derived from the electronic hospital database of a tertiary care center (Prof. Dr.Murat Dilmener Emergency Hospital) after receiving permission from the Institutional Review Board. A total of 99 adult COVID-19 patients (aged 18 or older) who have been diagnosed with COVID-19 and admitted to the hospital's ICUs, between April 2021 and June 2021 were included in this study. The approval of the local institutional review board had been obtained before the study (11/11/2021-296 Health Sciences University Kanuni Sultan Süleyman Training and Research Hospital Clinical Resesarch Ethics Committee.) and adherence to the principles announced in the Helsinki Declaration was provided.

In all patients, reverse transcriptase-polymerase chain reaction (RT-PCR) was employed to confirm the diagnosis of COVID-19 disease in addition to particular computed thoracic tomography findings. Applicable data included age, body-mass index (BMI), comorbidities, blood group, smoking habit, history of vaccination for COVID-19, antibiotic treatment, culture results, prognostic outcomes, and duration of ICU stay.

Chronic comorbidities were chosen using pre-existing International Classification of Diseases classifications based on previously reported data (ICD-10). Cardiovascular illness, pulmonary disease, hypertension, diabetes, diabetes mellitus, renal disease, liver disease, and a history of a solid malignant tumor were among the conditions.

The Turkish Ministry of Health classified our hospital as a pandemic institution. Only moderate and severe COVID-19 patients needing an ICU stay were examined in this study. Patients under the age of 18, pregnant women, patients with terminal cancer, and patients with one or more hematological illnesses were excluded from the study.

The research was carried out per the Declaration of Helsinki's Good Clinical Practice principles. Since the study was retrospective, informed consent was not required. The data on baseline demographic parameters, comorbidities, interventions administered, and hospital outcomes were gathered on admission and on hospital discharge. Patients were treated according to local standards of medical care. By the time the data was analyzed, and the study conclusions were published, all the patients had either been discharged alive from the ICU or had died. Patients who were mechanically ventilated through endotracheal intubation and admitted to the ICU for hypoxemic respiratory failure were selected.

2.1. Statistical analysis

Data were analyzed using Statistical Package for Social Sciences program version 21.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as means (standard deviations) and categorical variables were demonstrated as frequencies and percentages. Missing data were not imputed. Independent Samples and Kruskal-Wallis tests were used to compare variables between groups.

2.2. Outcome parameters

Baseline descriptives under investigation include age, body-mass index (kg/m2), comorbidities, blood group, smoking habit, history of vaccine, antibiotic treatment regimen, culture results, prognostic outcome, and the duration of ICU stay were extracted from the hospital database.

3. Results

In this study, we enrolled 99 patients on mechanical ventilation who tested positive for COVID-19. Baseline demographics, patient comorbidities, data about disease progression, and treatment interventions are summarized in Table 1. The average age was 65.94 ± 14.93 years (24 to 96), and the average BMI was 29.83 ± 6.05 kg/m2 (17.65 to 57.53).

Table 1. Baseline descriptives and clinical characteristics in our series (n=99)

Variable		n	%
Comorbidity	No	20	20.2
	Yes	73	73.7
	Missing data	6	6.1
Blood group	A	31	31.3
	B	14	14.1
	0	27	27.3
	AB	2	2.0
	Missing data	25	25.3
Rh factor	No	10	10.1
	Yes	64	64.6
	Missing data	25	25.3
Smoking	No	70	70.7
	Yes	13	13.1
	Missing data	16	16.2
Vaccine	No	54	54.5
	Yes	31	31.3
	Missing data	14	14.1
Antibiotic regimen	Tazocin	18	18.2
	Ceftriaxone	3	3.0
	Combined	69	69.7
	Missing data	9	9.1
Culture results	Negative	35	35.4
	Positive	48	48.5
	Missing data	16	16.1
Delta variant	Negative	46	46.5
	Positive	23	23.2
	Missing data	30	30.3
Use of blood products	No	56	56.6
	Yes	29	29.3
	Missing data	14	14.1

In this series, 73 patients (73.7%) had comorbidities while 20 cases (20.2%) did not have any systemic diseases. The number and percentage of patients with blood groups A, B, AB, and 0 were 31 (31.3%), 14 (14.1%), 2 (2.0%), and 27 (27.3%), respectively. Rh factor was positive in 64 patients (64.6%), and negative in 10 (10.1%), respectively. Smokers constituted 13.1% of the Covid-19 patient population (n=13) in ICU. Thirty-one cases (31.3%) had received at least one dose of Covid-19 vaccine. The antibiotics administered included a combined regimen (n=69, 69.7%), ceftriaxone (n=3, 3%), and tazocin (n=18, 18.2%). Culture results were positive in 35 (35.4%) cases.

In terms of prognostic outcome, 63 patients (63.6%) died in the ICU after their initial hospitalization, while 24 patients (24.2%) were discharged from ICU. The mean duration of ICU stay was 16.87 ± 11.41 days (1 to 60). Blood products were utilized in 29 patients (29.3%) and delta mutation was detected in 23 (23.2%) of ICU patients. The modes of ventilatory support were continuous positive airway pressure (CPAP) (n=37, 37.4%), high flow nasal oxygen (HFNO) (n=23, 23.2%), CPAP and HFNO (n=2, 2.0%), and nasal oxygen (n=4, 4.0%), respectively. The mean duration of ICU stay was 16.90 ± 11.41 days (1 to 60). Table 2 outlines the characteristics and distribution of patients in ICU per clinical, hemodynamic, and respiratory variables under investigation. There was no significant relationship between the duration of ICU stay and blood groups (p=0.052), systolic (p=0.572) and diastolic blood pressure (p=0.098) at admission as well as initial arterial oxygen saturation (p=0.223). The duration of ICU stay was remarkably different between groups per antibiotic regimen (p<0.001), whereas there was no difference between antibiotic treatment groups as for systolic (p=0.366), diastolic (p=0.895), and arterial oxygen saturation at admission (p=0.110).

Table 3 The results of measurements at initial admission and at discharge or before mortality were compared. Our data yielded that serum levels of BUN (p<0.001), procalcitonin (p=0.001), pro-brain natriuretic peptide (BNP) (p<0.001), sodium (p=0.008), lactate dehydrogenase (p<0.001), lactate (p=0.001), INR (p=0.001), fibrinogen (p=0.007), ferritin (p=0.001), D-dimer (p=0.014), CRP (p<0.001), creatinine (p=0.006), basophil count (p=0.003), AST (p=0.013), and APTT (p<0.001) were significantly higher in Covid-19 patients ending up with mortality. On the other hand, monocyte (p=0.002), platelet (p<0.001), and basophil (p=0.003) counts as well as hemoglobin (p=0.003) and pH levels (p<0.001) were higher in patients who were discharged from ICU after treatment.

4. Discussion

There is a need for a national clinical characterization data infrastructure for hospital-admitted patients that is quickly accessible for clinicians, researchers, public health officials, and policymakers to inform understanding of baseline characteristics, treatment regimens, and hospital use, as well as to benchmark disease severity across waves of outbreaks and across different causative agents. This approach would be useful in both pre-and post-pandemic situations. Thus, we present our experience with adult COVID-19 patients who were treated in ICU in our tertiary care center and describe the demographic, clinical, and therapeutic features of our series are described.

Namendys-Silva et al. reported that most COVID-19 patients admitted to ICUs were males over 57 years old with hypertension and diabetes, and 6% were healthcare workers (9). More than 60% of patients with critical COVID-18 were men, according to prior investigations (2). Patients admitted to the ICU on average were 57 years old and patients with hypertension and diabetes mellitus had significantly worse survival rates, although neither of these comorbidities was an independent predictor of death. Invasive mechanical ventilation was administered to all patients, and virtually all of them received vasopressors (2). In previous research, these individuals' mortality rates ranged from 35.2%-72% (2, 10, 11).

Our mortality was consistent with these publications indicating a remarkably high risk of fatality in patients with critical COVID-19. Patients treated with severe COVID-19 in the fall had better outcomes than those admitted in the summer, with lower mortality and shorter ICU stays (12). This is most likely owing to a better understanding of COVID-19 and advancements in therapeutic options.

Age, malignancy, insurance status, and ethnicity were all linked to a higher 30-day death rate in mechanically ventilated COVID-19 patients. These findings support our hypothesis that specific patient features are linked to an increased risk of death in our patient population (8). The capacity to prevent and treat COVID-19 has varied over the World (8). Our findings add to the growing body of knowledge about the COVID-19 pandemic's critical care consequences.

COVID-19-infected patients' overall mortality may be reduced if preventive measures are improved in the elderly (13). More research is needed, however, to determine which specific protective strategies should be recommended for at-risk populations like the elderly, as well as the extent to which such treatments reduce COVID-19-related mortality (14).

The most common presenting symptoms were fever and cough, while the most common comorbidities were hypertension, diabetes, and chronic heart disease (15). There has been confusion about the best modalities of oxygenation and ventilation support for severely sick COVID-19 patients, which has likely contributed to their low utilization in this population.

Table 2. An overview of distribution of hemodynamic and respiratory variables across clinical feature groups

Variable			N	Mean ± SD	Minimum	Maximum
Comorbidity	No data	ICU stay	2	10.00 ± 8.48	4	16
		Systolic BP	3	132.00 ± 23.06	110	156
		Diastolic BP	3	83.67 ± 13.87	72	99
		Pulse rate	3	90.67 ± 34.60	65	130
		Respiratory rate	3	33.67 ± 9.07	24	42
		Arterial oxygen saturation	3	84.33 ± 10.17	74	94
	No	ICU stay	19	18.63 ± 14.00	4	16
		Systolic BP	19	134.32 ± 19.94	95	165
		Diastolic BP	19	71.21 ± 11.40	51	95
		Pulse rate	19	93.21 ± 16.85	56	115
		Respiratory rate	18	28.67 ± 7.80	18	42
		Arterial oxygen saturation	19	87.74 ± 5.57	73	94
	Yes	ICU stay	64	16.56 ± 10.68	1	50
		Systolic BP	62	142.69 ± 30.80	60	240
		Diastolic BP	62	74.65 ± 16.74	30	160
		Pulse rate	62	96.47 ± 21.30	56	160
		Respiratory rate	62	30.84 ± 8.17	16	51
		Arterial oxygen saturation	65	84.98 ± 9.26	56	96
Blood group	No data	ICU stay	14	9.79 ± 5.16	4	23
		Systolic BP	18	141.67 ± 33.23	100	240
		Diastolic BP	18	79.11 ± 23.42	60	160
		Pulse rate	18	100.72 ± 23.42	72	160
		Respiratory rate	18	28.17 ± 9.04	16	50
		Arterial oxygen saturation	18	86.39 ± 6.96	70	96
	0	ICU stay	27	19.85 ± 13.02	4	50
		Systolic BP	25	140.40 ± 35.54	60	230
		Diastolic BP	25	71.56 ± 15.30	30	110
		Pulse rate	25	97.08 ± 18.73	63	130
		Respiratory rate	25	31.36 ±7.58	18	47
		Arterial oxygen saturation	26	84.04 ± 9.15	61	93
	A	ICU stay	29	15.76 ± 8.82	4	35
		Systolic BP	27	136.70 ± 21.22	95	190
		Diastolic BP	27	70.70 ± 10.70	47	90
		Pulse rate	27	94.41 ± 18.75	56	126
		Respiratory rate	26	29.46 ± 7.66	17	42
		Arterial oxygen saturation	28	87.54 ± 7.69	60	96
	B	ICU stay	13	22.92 ± 13.41	5	60
		Systolic BP	12	147.83 ± 22.11	110	190
		Diastolic BP	12	80.25 ± 9.32	70	96
		Pulse rate	12	87.62 ± 24.17	56	130
		Respiratory rate	12	32.25 ± 6.86	22	44
		Arterial oxygen saturation	13	84.85 ± 9.32	56	92
	AB	ICU stay	2	3.00 ± 2.83	1	5
		Systolic BP	2	135.00 ± 7.07	130	140
		Diastolic BP	2	73.50 ± 4.95	70	77
		Pulse rate	2	93.00 ± 26.87	74	112
		Respiratory rate	2	42.50 ± 12.02	34	51
		Arterial oxygen saturation	2	75.00 ±19.80	61	89
Rh factor	No data	ICU stay	14	9.79 ± 5.16	4	23
		Systolic BP	18	141.67 ± 33.22	100	240
		Diastolic BP	18	79.11 ± 23.42	60	160
		Pulse rate	18	100.72 ± 23.42	72	160
		Respiratory rate	18	28.17 ± 9.04	16	50
		Arterial oxygen saturation	18	86.39 ± 6.96	70	96
	Negative	ICU stay	8	22.13 ± 13.67	5	50
		Systolic BP	8	152.13 ± 28.13	121	190
		Diastolic BP	8	76.63 ± 9.41	64	96
		Pulse rate	8	90.50 ± 25.65	56	130
		Respiratory rate	7	29.14 ± 8.42	21	44
		Arterial oxygen saturation	8	87.00 ± 6.61	72	93
	Positive	ICU stay	63	17.78 ± 11.58	1	60
		Systolic BP	58	138.41 ± 27.04	60	230
		Diastolic BP	58	72.33 ± 13.01	30	110
		Pulse rate	58	94.60 ± 19.08	56	130
		Respiratory rate	58	31.34 ± 7.73	17	51

		Arterial oxygen saturation	61	85.13 ± 9.30	56	96
Smoking	No data	ICU stay	14	12.00 ± 6.63	4	23
		Systolic BP	13	129.54 ± 21.97	87	160
		Diastolic BP	13	73.08 ± 13.21	47	99
		Pulse rate	13	92.15 ± 21.92	64	130
		Respiratory rate	14	30.43 ± 6.73	19	44
		Arterial oxygen saturation	15	84.73 ± 9.62	56	94
	No	ICU stay	60	17.65 ± 11.80	4	60
		Systolic BP	59	140.66 ± 27.93	60	240
		Diastolic BP	59	73.59 ± 16.45	30	160
		Pulse rate	59	96.12 ± 20.63	56	160
		Respiratory rate	59	29.49 ± 7.87	16	50
		Arterial oxygen saturation	57	86.20 ± 7.66	60	96
	Yes	ICU stay	11	18.82 ± 3.22	1	50
		Systolic BP	12	151.00 ± 34.93	95	230
		Diastolic BP	12	78.33 ± 14.26	51	110
		Pulse rate	12	96.25 ± 20.85	56	120
		Respiratory rate	12	35.17 ± 9.48	17	51
		Arterial oxygen saturation	12	83.42 ± 11.73	61	96
Vaccine	No data	ICU stay	11	10.09 ± 6.70	4	23
		Systolic BP	11	140.82 ± 40.02	87	240
		Diastolic BP	11	82.27 ± 29.18	43	160
		Pulse rate	11	103.73 ± 31.53	64	160
		Respiratory rate	12	33.33 ± 8.09	23	50
		Arterial oxygen saturation	12	82.08 ± 10.93	56	94
	No	ICU stay	49	18.22 ± 12.64	1	60
		Systolic BP	48	139.46 ± 25.23	95	230
		Diastolic BP	48	74.58 ± 11.42	51	110
		Pulse rate	48	92.00 ± 18.39	56	120
		Respiratory rate	47	29.70 ± 7.58	18	51
		Arterial oxygen saturation	50	86.66 ± 7.73	61	96
	Yes	ICU stay	25	17.20 ± 9.64	4	50
		Systolic BP	25	142.08 ± 29.78	60	190
		Diastolic BP	25	69.88 ± 13.61	30	96
		Pulse rate	25	98.68 ± 18.23	65	130
		Respiratory rate	24	30.54 ± 9.04	16	47
		Arterial oxygen saturation	25	85.04 ± 8.91	60	96
Antibiotic regimen	No data	ICU stay	3	7.33 ± 4.16	4	12
		Systolic BP	5	143.20 ± 18.86	110	156
		Diastolic BP	5	78.80 ± 17.17	60	99
		Pulse rate	5	108.80 ±20.85	74	130
		Respiratory rate	5	31.80 ± 9.52	17	40
		Arterial oxygen saturation	5	88.60 ± 6.54	80	96
	Tazocin	ICU stay	16	11.19 ± 10.48	4	46
		Systolic BP	18	137.06 ± 32.40	100	230
		Diastolic BP	18	75.00 ± 12.69	60	110
		Pulse rate	18	87.89 ± 17.43	64	137
		Respiratory rate	18	25.56 ± 5.48	18	34
		Arterial oxygen saturation	17	89.41 ± 2.72	84	94
	Ceftriaxone	ICU stay	3	5.33 ± 4.51	1	10
		Systolic BP	3	130.00 ± 10.00	120	140
		Diastolic BP	3	74.67 ± 2.52	72	77
		Pulse rate	3	97.67 ± 15.63	81	112
		Respiratory rate	3	35.33 ± 14.64	22	51
		Arterial oxygen saturation	3	79.67 ± 16.29	61	91
	Combined	ICU stay	63	19.32 ± 11.11	4	60
		Systolic BP	58	141.76 ± 28.77	60	240
		Diastolic BP	58	73.52 ± 16.86	30	160
		Pulse rate	58	96.64 ± 21.35	56	160
		Respiratory rate	57	31.65 ± 7.87	16	50
		Arterial oxygen saturation	62	84.55 ± 9.12	56	96
Culture results	No data	ICU stay	2	23.50 ± 27.58	4	43
		Systolic BP	6	127.67 ± 21.18	110	156
		Diastolic BP	6	73.33 ± 12.96	64	99
		Pulse rate	6	107.67 ± 24.37	77	137
		Respiratory rate	6	25.00 ± 6.54	17	35
		Arterial oxygen saturation	6	87.67 ± 8.64	72	96

	Negative	ICU stay	35	14.06 ± 11.57	4	60
		Systolic BP	34	144.47 ±32.88	60	240
		Diastolic BP	34	76.00 ±19.66	30	160
		Pulse rate	34	99.32 ± 20.45	64	160
		Respiratory rate	35	29.80 ± 7.98	16	50
		Arterial oxygen saturation	35	86.20 ± 8.06	61	94
	Positive	ICU stay	48	18.65 ± 10.43	1	50
		Systolic BP	44	139.02 ± 25.42	87	230
		Diastolic BP	44	72.91 ± 12.29	47	110
		Pulse rate	44	90.93 ± 19.49	56	126
		Respiratory rate	42	31.81 ± 8.14	17	51
		Arterial oxygen saturation	46	84.80 ± 9.08	56	96
Delta variant	No data	ICU stay	17	19.53 ± 15.32	4	50
		Systolic BP	18	142.72 ± 23.53	110	180
		Diastolic BP	18	75.00 ± 10.80	60	99
		Pulse rate	18	103.33 ± 21.12	56	137
		Respiratory rate	18	29.61 ± 8.11	17	44
		Arterial oxygen saturation	19	86.53 ± 9.19	61	96
	No	ICU stay	46	14.52 ± 10.48	1	60
		Systolic BP	45	142.58 ± 33.91	60	240
		Diastolic BP	45	74.80 ± 19.13	30	160
		Pulse rate	45	96.67 ± 20.73	63	160
		Respiratory rate	45	31.77 ± 8.30	16	51
		Arterial oxygen saturation	44	85.56 ± 7.90	61	94
	Yes	ICU stay	22	19.73 ± 8.98	8	46
		Systolic BP	21	133.81 ± 17.45	100	172
		Diastolic BP	21	72.19 ± 10.19	47	90
		Pulse rate	21	86.38 ± 17.32	56	126
		Respiratory rate	21	28.48 ± 7.47	17	44
		Arterial oxygen saturation	23	84.78 ± 9.68	56	94
Use of blood products	No data	ICU stay	2	4.00 ± 0.00	4	4
		Systolic BP	5	131.20 ± 21.62	110	156
		Diastolic BP	5	74.00 ± 14.37	64	99
		Pulse rate	5	107.20 ± 27.22	77	137
		Respiratory rate	5	24.60 ± 7.23	17	35
		Arterial oxygen saturation	6	87.00 ± 8.48	72	96
	No	ICU stay	56	12.72 ± 7.25	1	32
		Systolic BP	53	144.28 ± 28.98	87	240
		Diastolic BP	53	75.89 ± 16.92	47	160
		Pulse rate	53	95.11 ± 19.57	56	160
		Respiratory rate	52	30.73 ± 7.80	16	51
		Arterial oxygen saturation	54	85.96 ± 7.00	61	94
	Yes	ICU stay	27	26.44 ± 12.83	9	60
		Systolic BP	26	134.41 ± 28.00	60	180
		Diastolic BP	26	70.77 ± 12.81	30	90
		Pulse rate	26	94.12 ± 21.71	63	130
		Respiratory rate	26	31.08 ± 8.65	17	47
		Arterial oxygen saturation	27	84.44 ± 11.31	56	96

SD: standard deviation; BP: blood pressure; ICU: intensive care unit

Table 3. A comparative overview of laboratory parameters in Covid-19 patients in ICU

Variable	Prognostic outcome	Time	Minimum	Maximum	Mean	Standard deviation	p-value
WBC count	Discharged	Initial	2.50	22.90	10.50	5.77	0.277
		Final	1.24	28.20	10.47	5.01	
	Mortality	Initial	2.86	96.80	14.58	12.24	
		Final	0.46	163.17	17.16	21.81	
BUN	Discharged	Initial	14.7	81.9	43.45	17.20	<0.001*
		Final	11.9	147.5	41.26	29.81	
	Mortality	Initial	17.1	248.0	69.82	46.93	
		Final	23.7	380.0	138.03	76.49	
Troponin T	Discharged	Initial	0.0007	0.1340	0.214	0.035	0.662
		Final	0.0040	28.30	1.92	6.23	
	Mortality	Initial	0.0060	36.52	3.156	8.00	
		Final	0.0030	101.10	2.128	14.14	
spO_2	Discharged	Initial	42.2	98.30	80.83	19.08	0.482
		Final	33.9	99.60	86.65	16.16	
	Mortality	Initial	29.40	100.0	84.62	15.94	

		Final	51.20	99.40	86.62	11.73	
Procalcitonin	Discharged	Initial	0.02	4.01	0.41	0.84	0.001*
		Final	0.02	1.56	0.18	0.32	
	Mortality	Initial	0.02	26.70	2.02	5.10	
		Final	0.17	100.00	12.25	18.30	
ProBNP	Discharged	Initial	21.64	8307.00	1070.22	1791.55	<0.001*
		Final	10.00	4347.00	634.33	1010.68	
	Mortality	Initial	26.57	35000	3783.43	7246.43	
		Final	26.30	35000	15407.78	14968.06	
pO_2	Discharged	Initial	29.0	116.0	60.86	25.57	0.872
		Final	27.2	151.0	73.78	35.06	
	Mortality	Initial	25.3	215.0	68.65	37.45	
		Final	38.2	169.0	81.15	31.93	
Platelet count	Discharged	Initial	88000	359000	243960	77602	<0.001*
		Final	120000	600000	320820	115171	
	Mortality	Initial	84000	581000	252620	107557	
		Final	12000	571000	153520	117922	
pH	Discharged	Initial	7.30	7.63	7.43	0.065	<0.001*
		Final	7.38	7.51	7.45	0.032	
	Mortality	Initial	7.13	7.90	7.42	0.113	
		Final	6.83	7.56	7.18	0.179	
pCO_2	Discharged	Initial	20.8	70.7	42.329	10.468	0.062
		Final	32.0	83.3	45.530	12.019	
	Mortality	Initial	24.0	105.0	42.56	13.533	
		Final	28.1	97.7	54.68	16.418	
Neutrophil count	Discharged	Initial	1.83	21.80	9.41	5.56	0.321
		Final	3.72	23.45	8.33	3.97	
	Mortality	Initial	2.47	28.05	12.03	5.77	
		Final	0.36	44.30	12.83	9.39	
Sodium	Discharged	Initial	133.0	158.0	137.91	5.00	0.008*
		Final	128.0	143.0	136.30	3.43	
	Mortality	Initial	124.0	153.0	137.25	5.36	
		Final	126.0	161.0	141.74	7.76	
Potassium	Discharged	Initial	2.98	5.27	4.31	0.69	0.424
		Final	3.38	6.36	4.38	0.65	
	Mortality	Initial	3.47	5.96	4.38	0.58	
		Final	2.86	7.94	4.97	1.31	
Monocyte count	Discharged	Initial	0.03	1.09	0.37	0.25	0.002*
		Final	0.22	1.32	0.74	0.33	
	Mortality	Initial	0.02	1.72	0.40	0.31	
		Final	0.00	1.47	0.40	0.38	
Lymphocyte count	Discharged	Initial	0.14	1.85	0.78	0.46	0.776
		Final	0.61	3.45	1.75	0.83	
	Mortality	Initial	0.15	1.81	0.62	0.32	
		Final	0.08	8.65	1.49	1.52	
LDH	Discharged	Initial	65.3	1444.0	487.55	279.64	<0.001*
		Final	193.0	569.0	324.48	83.50	
	Mortality	Initial	202.0	1075.0	471.48	190.24	
		Final	230.0	5660.0	990.71	1134.91	
Lactate	Discharged	Initial	0.7	5.5	2.12	1.13	0.001*
		Final	0.7	4.1	1.98	0.97	
	Mortality	Initial	0.8	8.6	2.36	1.20	
		Final	0.6	21.0	5.66	4.07	
INR	Discharged	Initial	0.9	1.87	1.13	0.21	0.001*
		Final	0.9	1.66	1.10	0.18	
	Mortality	Initial	0.9	4.87	1.25	0.54	
		Final	0.9	4.24	1.65	0.64	
Hemoglobin	Discharged	Initial	8.2	15.3	12.82	1.80	0.003*
		Final	9.6	15.8	12.19	1.80	
	Mortality	Initial	6.6	16.6	11.86	2.04	
		Final	6.7	16.7	9.64	1.88	
Glucose	Discharged	Initial	91.2	592.00	187.28	103.50	0.816
		Final	71.5	332.00	140.23	73.68	
	Mortality	Initial	28.4	709.00	194.98	96.88	
		Final	36.3	328.80	164.22	78.06	
Fibrinogen	Discharged	Initial	260	923	600.83	158.77	0.007*

		Final	212	950	501.05	178.81	
	Mortality	Initial	148	1200	626.54	209.64	
		Final	311	1200	769.49	279.24	
Ferritin	Discharged	Initial	62.95	4738	999.63	964.07	0.001*
		Final	57.70	1868	653.84	424.13	
	Mortality	Initial	31.14	2935	943.63	721.97	
		Final	20.00	20000	4459.15	5092.95	
Eosinophil count	Discharged	Initial	0.00	0.17	0.117	0.034	0.036*
		Final	0.00	1.06	0.144	0.243	
	Mortality	Initial	0.00	0.16	0.013	0.028	
		Final	0.00	0.78	0.056	0.150	
D-dimer	Discharged	Initial	0.46	5.90	1.48	1.40	0.014*
		Final	0.54	5.90	1.44	1.25	
	Mortality	Initial	0.35	18.96	2.27	2.79	
		Final	0.56	6.95	3.45	1.87	
CRP	Discharged	Initial	2.90	294.00	110.36	77.71	<0.001*
		Final	0.96	152.18	27.91	35.09	
	Mortality	Initial	1.44	412.00	115.23	84.65	
		Final	6.69	516.23	220.32	135.58	
Creatinine	Discharged	Initial	0.40	1.63	0.79	0.27	0.006*
		Final	0.36	1.88	0.71	0.38	
	Mortality	Initial	0.30	4.06	1.08	0.76	
		Final	0.36	6.80	2.07	1.43	
Basophil count	Discharged	Initial	0.01	0.16	0.043	0.038	0.003*
		Final	0.004	0.10	0.030	0.023	
	Mortality	Initial	0.00	0.20	0.034	0.031	
		Final	0.00	0.62	0.079	0.104	
AST	Discharged	Initial	16.2	118.0	40.85	23.80	0.013*
		Final	12.3	108.9	28.90	19.06	
	Mortality	Initial	12.0	356.9	46.01	57.31	
		Final	0.0	2498.6	321.06	538.89	
ALT	Discharged	Initial	8.9	128.9	41.33	27.58	0.066
		Final	7.9	161.5	53.71	41.46	
	Mortality	Initial	4.8	288.7	42.00	50.82	
		Final	1.9	730.0	106.17	138.35	
APTT	Discharged	Initial	22.4	35.9	29.52	3.87	<0.001*
		Final	20.0	40.2	29.56	4.95	
	Mortality	Initial	20.3	53.1	30.52	7.05	
		Final	25.2	117.7	51.10	20.00	

*: statistically significant; WBC: White blood cell count; BUN: blood urea nitrogen; BNP: brain natriuretic peptide; LDH: lactate dehydrogenase; INR: international normalized ratio; CRP: C-reactive protein; AST: aspartate transaminase; ALT: alanine transaminase; APTT: activated partial thromboplastin time

Concerns about aerosolization and nosocomial amplification of COVID-19 transmission have centered on the timing of intubation and mechanical ventilation, as well as the possible risk to health care workers in adopting non-invasive ventilation and high-flow nasal oxygen (16). Due to the impossibility to fully account for confounding, eternal time, and treatment indication bias in observational studies like this one, we have not explored the connection of specific pharmaceutical or breathing treatments with clinical outcomes (15).

In hospitalized COVID-19 patients, sociodemographics, co-morbidities, and inpatient characteristics have been demonstrated to influence outcomes (12). Older age, numerous co-morbid diseases, hypertension, and obesity with a BMI of less than 35 kg/m2 were all shown to be substantially linked with an elevated risk of death in our analysis, which is consistent with the current literature (17).

COVID-19 infection can spread quickly, especially in people who have a lot of risk factors (18). Advanced age and male gender related to enhanced mortality rates in COVID-19 patients in a recent meta-analysis assessing the participants admitted to the ICU due to COVID-19 infection in Italy (19).

COVID-19 poses a significant threat to health-care systems and ICUs, a large number of patients with the same condition require simultaneous access to intense therapies. Supportive care is the mainstay of treatment for critically sick patients until effective and targeted medicines become available. All health-care systems face a problem in providing this care at a high-quality level for the large number of people they must treat (11). Our results are useful for everyday clinical practice. Due to the excess number of patients in the current pandemic, patients with ARDS are admitted to the ICU. The rate of mortality in ICU was 63.6% in our series. Thus, based on the study findings, we have concluded that recognition of clinical, hemodynamic, and respiratory characteristics may provide useful clues in the management of Covid-19 patients in ICU.

The current study has several limitations. First, our

findings may be limited in their generalizability due to the small sample size. Second, the study's observational character is a drawback, and some unaccounted confounders may be present. Finally, we lacked data on survivors' long-term outcomes or quality of life. More research is needed to extrapolate our findings in bigger groups. Further prospective, multicentric, controlled trials on larger series are warranted to achieve more reliable results.

To conclude, we present the clinical features and outcomes of 99 COVID-19 patients admitted to ICU. Our study demonstrated a high ICU mortality rate in a retrosepctive cohort of mechanically ventilated patients with severe COVID-19 infection treated in a tertiary care center. These data are critical for understanding the impact of COVID-19 on our hospitals during future pandemic waves, identifying areas for clinical management improvements, and allowing for continuous international and temporal comparisons of COVID-19 patient outcomes. Improved protective measures in individuals under higher risk may reduce total COVID-19 mortality, but further randomized controlled trials are needed to validate this link.

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References

1. Alharthy A, Aletreby W, Faqihi F, Balhamar A, Alaklobi F, Alanezi K, et al. Clinical Characteristics and Predictors of 28-Day Mortality in 352 Critically Ill Patients with COVID-19: A Retrospective Study. J Epidemiol Glob Health. 2021; 11: 98-104.
2. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. JAMA 2020; 323; 1574–81.
3. Du RH, Liang LR, Yang CQ, Wang W, Cao TZ, Li M, et al. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. Eur Respir J 2020; 55; 2000524.
4. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al.; LICORN and the Lille COVID-19 and Obesity study group. High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. Obesity (Silver Spring). 2020; 28: 1195-99. Erratum in: Obesity (Silver Spring). 2020; 28: 1994.
5. Lighter J, Phillips M, Hochman S, Sterling S, Johnson D, Francois F, et al. Obesity in patients younger than 60 years is a risk factor for COVID-19 hospital admission. Clin Infect Dis. 2020; 71: 896-7.
6. Gattinoni L, Chiumello D, Rossi S. COVID-19 pneumonia: ARDS or not? Crit Care. 2020; 24: 154.
7. Vahidy FS, Drews AL, Masud FN, Schwartz RL, Askary BB, Boom ML, et al. Characteristics and Outcomes of COVID-19 Patients During Initial Peak and Resurgence in the Houston Metropolitan Area. JAMA 2020; 324: 998-1000.
8. Krause M, Douin DJ, Kim KK, Fernandez-Bustamante A, Bartels K. Characteristics and Outcomes of Mechanically Ventilated COVID-19 Patients-An Observational Cohort Study. J Intensive Care Med 2021; 36: 271-6.
9. Ñamendys-Silva SA, Alvarado-Ávila PE, Domínguez-Cherit G, Rivero-Sigarroa E, Sánchez-Hurtado LA, Gutiérrez-Villaseñor A, et al.; Mexico COVID-19 Critical Care Collaborative Group. Outcomes of patients with COVID-19 in the intensive care unit in Mexico: A multicenter observational study. Heart Lung. 2021; 50: 28-32.
10. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020; 8: 475-81. Erratum in: Lancet Respir Med. 2020; 8: e26.
11. Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G, et al.; COVID-19 Lombardy ICU Network. Risk Factors Associated With Mortality Among Patients With COVID-19 in Intensive Care Units in Lombardy, Italy. JAMA Intern Med. 2020; 180: 1345-55.
12. Olanipekun T, Abe TA, Effoe VS, Musonge-Effoe JE, Chuks A, Kwara E, et al. Intensive care unit hospitalizations and outcomes in patients with severe COVID-19 during summer and fall surges in Georgia. World J Crit Care Med. 2021; 10: 369-76.
13. Pan A, Liu L, Wang C, Guo H, Hao X, Wang Q, et al. Association of public health interventions with the epidemiology of the COVID-19 outbreak in Wuhan, China. JAMA. 2020; 323: 1-9.
14. Koo JR, Cook AR, Park M, Sun Y, Sun H, Lim JT, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a modelling study. Lancet Infect Dis. 2020; 20: 678-88.
15. Murthy S, Archambault PM, Atique A, Carrier FM, Cheng MP, Codan C, et al; SPRINT-SARI Canada Investigators and the Canadian Critical Care Trials Group. Characteristics and outcomes of patients with COVID-19 admitted to hospital and intensive care in the first phase of the pandemic in Canada: a national cohort study. CMAJ Open 2021; 9: E181-E188.
16. Agarwal A, Basmaji J, Muttalib F, Granton D, Chaudhuri D, Chetan D, et al. High-flow nasal cannula for acute hypoxemic respiratory failure in patients with COVID-19: systematic reviews of effectiveness and its risks of aerosolization, dispersion, and infection transmission. Can J Anaesth 2020; 67: 1217-48.
17. Auld SC, Caridi-Scheible M, Blum JM, Robichaux C, Kraft C, Jacob JT, et al.; and the Emory COVID-19 Quality and Clinical Research Collaborative. ICU and Ventilator Mortality Among Critically Ill Adults With Coronavirus Disease 2019. Crit Care Med. 2020; 48: e799-e804.
18. Guney BC, Hayiroglu M, Senocak D, Cİcek V, Cinar T, Kaplan M. Evaluation of N/LP Ratio as a Predictor of Disease Progression and Mortality in COVID-19 Patients Admitted to the Intensive Care Unit. Medeniyet Med J 2021; 36: 241-8.
19. Pijls BG, Jolani S, Atherley A, Derckx RT, Dijkstra JIR, Franssen GHL, et al. Demographic risk factors for COVID-19 infection, severity, ICU admission and death: a meta-analysis of 59 studies. BMJ Open. 2021;11:e044640.

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Comparison of the effects of acupressure and ıce massage in primary dysmenorrhea: A randomized controlled trial

Naimeh POURRAMEZANI[1], Maryam FIROUZABADI [2], Fatemeh ALAVI [2], Mohadeseh BALVARDI[3] Moazameh Sadat Razavi NASAB [4*]

[1]Department of Nursing, Sirjan School of Medical Sciences, Sirjan, Iran
[2]Department of Midwifery, Sirjan School of Medical Sciences, Sirjan, Iran
[3] Instructor of Biostatistics, Sirjan School of Medical Sciences, Sirjan, Iran
[4]Sirjan School of Medical Sciences, Sirjan, Iran

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Abstract

The aim of the study was to compare the effectiveness of acupressure and ice massage treatment methods in the treatment of primary dysmenorrhea. The 210 female students were randomly divided into three groups: acupressure, ice massage and control. In the acupressure group, pressure was performed at the Hugo point by applying medium pressure for 10 minutes of massage. The procedure was repeated five times. In the ice massage group, 2 cm diameter circular ice pieces were used. Massage with ice was carried out rotationally for 10 minutes. Like the acupressure, the procedure was repeated five times. In the control group, glass marbles were applied at the Hugo point with no pressure and massage for 10 minutes. The intensity of pain was measured prior to the intervention, during the intervention and following the intervention using a visual analogue scale. In the beginning, the mean VAS scores were 7.41 ± 1.82, 6.74 ± 2.23, and 7.03 ± 1.72 in the participants in groups control, acupressure and ice massage, respectively (P = 0.13). After the intervention, the mean pain scores were significantly lower at all of the time points in groups acupressure and ice massage than in group control participants (P < 0.001). Although the pain scores showed a more decreasing trend after the intervention in group ice massage than that in group acupressure, the difference between the two groups was not statistically significant (P = 0.97). It was revealed that treatment with acupressure and ice massage could also be recommended as a complementary medicine treatment for the treatment of primary dysmenorrhea with no reported side effects.

Keywords: acupressure, ice massage, Hugo point, dysmenorrhea, pain

1. Introduction

Dysmenorrhea is the most prevalent gynecologic ailment affecting women and is characterized by uncomfortable menstruation. Because of the many definitions and approaches utilized, determining the exact prevalence rate of dysmenorrhea is challenging. The prevalence of the disease has been reported to range from 17% to 90% (1). In adolescents and young adults, the prevalence of dysmenorrhea is estimated to be between 34% and 94% (2).

A recent comprehensive analysis of persistent pelvic discomfort shows that dysmenorrhea affects 17 per cent to 80 per cent of women (3). According to research done in various locations using various approaches, more than 70% of Iranian females suffer from dysmenorrhea (4, 5). Some women have minor discomfort during their menses, while others have major limitations in their ability to operate. Lower abdomen and back pain are the signs most toughly related to absences from or impaired effectiveness at work and school among all menstrual-related complaints.

Up to 15% of women with dysmenorrhea have symptoms severe enough to keep them from going to work, school, or other activities (6, 7). Flexibility in hours or the option to work from home may help to alleviate this issue. Still, even for females who do not miss work or school due to menstrual-related symptoms, the diminished attention and productivity that comes with them significantly impact performance (8). The presence or absence of an underlying cause determines whether dysmenorrhea is classed as primary or secondary (9). Primary dysmenorrhea is discomfort associated with menstruation with no underlying cause, and endometriosis leiomyomas, pelvic inflammatory disease, and interstitial cystitis are all examples of secondary dysmenorrhea (10).

The majority of unpleasant menses in ovulatory women are caused by primary dysmenorrhea. The discomfort associated with primary dysmenorrhea might be minor or severe. It is generally stronger on the first day of menstruation and progressively fades. The pathogenesis of primary

*Correspondence: razavinasab12@chmail.ir

dysmenorrhea is unknown; however, one of the most widely acknowledged explanations is the production of prostaglandins and other inflammatory substances from the endometrium during menstruation, such as Leukotriene (11-13).

Today, a variety of treatments for controlling menstruation pain are employed, mostly grouped into two categories: medical and non-medical procedures. Although medications have immediate effects, long-term usage might result in nausea, digestive issues, peptic ulcers, and diarrhea. Furthermore, these medications are ineffective in 10-20% of patients (12). As a result, non-medical treatments known as complementary therapies have recently gained popularity among patients. These procedures are easy, low-cost, and non-invasive, with no adverse side effects (14, 15).

Acupressure is a well-known complementary and alternative medicine technique used worldwide. It is based on stimulating acupoints along meridians as a basic premise. Acupoint activation is aided by using fingers or a variety of readily accessible hand-held acupressure devices (16). Muscle tension is relieved with acupressure by applying pressure to specific acupoints with the hand or with the thumbs on particular points or by applying pressure to acupoints to balance the flow of physiological energy (17, 18). Acupressure demands applying physical pressure on trigger points/acupoints/specific pressure points located along the meridians. Meridians are the channels within the human body that aid in the maintenance of Qi and, as a result, the stability of one's health. Acupressure is a non-invasive, needle-free, cost-effective, and non-pharmacological curative method that is performed manually. Acupressure's biochemical process (Fig. 1) includes the stimulation of acupoints, which results in complicated neuro-hormonal reactions (19). It results from a negative feedback loop between the hypothalamus and pituitary-adrenocortical axis, which causes cortisol overproduction and relaxation response (20). It also affects physiological responses by enhancing endorphin and serotonin transmission through nerves and meridians to the brain and particular organs (21).

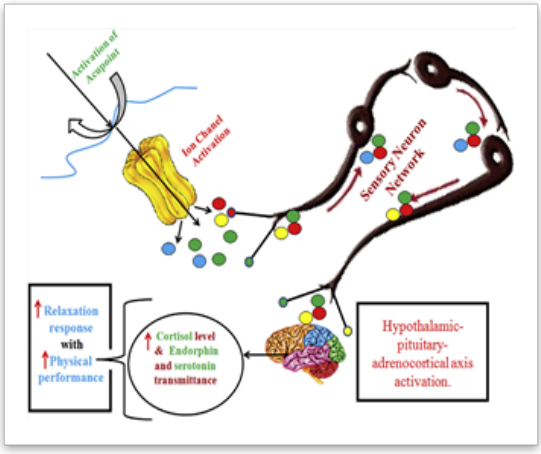

Fig. 1. Biochemical mechanism of acupressure

The large intestine or Hugo point (LI4) is the body's greatest significant pain reliever point (22). Hugo point is the center of the angle between the bones of the first and second fingers of the hand between the thumb and the pointer on the back of the hand, which is the subject of this research. The energy flow is closer to the skin surface at this location, which may be easily triggered by pressure, needles, or intense cold (23). Figure 2 depicts the exact location of Hugo point.

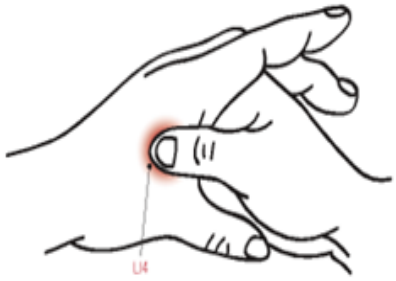

Fig. 2. position of Hugo point (Li4)

Numerous researches have revealed different acupressure effects; among them, the impact of acupressure on dysmenorrhea is the most usually quoted one (24). Furthermore, data shows that acupressure has aided in the relief of pain in a variety of situations. According to Chang and Hang's investigation findings, acupressure at the Hugo point can reduce pain instantly, half an hour later, and an hour afterwards (25). Hugo Point massage was also employed in the Sultanpour et al. research to reduce discomfort produced by inserting a needle into the artery-venous fistula in hemodialysis patients (25). Furthermore, the study proposes that cold efficiently slows nerve conduction rate and blocks nerve impulses and communication in the sensory fibres (26). Hugo point ice massage may be a simple and cost-effective pain-relieving alternative for patients since it is simple to administer by nurses and has few adverse effects (27).

Furthermore, studies have shown that Hugo point ice massage helps relieve pain (28). Shahdadi et al. found that ice massage may be used to reduce primary menstruation pain since it is a non-invasive, easy, and economical approach with no side effects that can be taught in the minimum amount of time (29). Both acupressure and ice massage treatments decreased pain severity, labour stage length, and anxiety level in primipara women, according to research by Kaviani et al.; however, the benefit of ice massage was more significant (30).

Melzak investigated the ice massage effect at the LI4 point on toothache in forty outpatients referred to a dental clinic in Montreal and found that both massage and ice massage on the LI4 point significantly reduced the intensity of toothache in the participants; however, the ice massage group experienced a greater reduction in pain intensity than the massage group (31).

Given the potential effect of dysmenorrhea and its signs

on young women's academic performance at a critical stage in their lives, and because ice massage and acupressure are simple and low-priced approaches, as well as the restricted number of studies performed in this area, the current study compared the effects of acupressure and ice massage on Hugo point (LI4) pain to relieve dysmenorrhea pain.

2. Material and Methods

2.1. Study design and sample size

This study was a prospective, single-blind randomized clinical trial conducted on female students at Sirjan University of Medical Sciences from February 2021 to April 2021. The study population was the entirety of female students who fulfilled all inclusion criteria. The inclusion criteria were age between 18-32; willingness to cooperate; experience of painful menstruation in previous cycles; regular menstrual cycle; not suffering from diseases such as hormonal disorders, heart disease, thyroid, diabetes, asthma, kidney disease, pregnancy, polycystic ovary; no history of taking drugs such as corticosteroids, painkillers, contraceptives. Also, exclusion criteria were interval between menarche (first menstruation in women) less than six months, history of coagulation disorders, cognitive disorders, and mental illness. The sample size was determined using convenience sampling. A pilot study with 48 people was performed using permutated block randomization method to determine the sample size. Individuals were randomly divided into three groups: control, acupressure and ice therapy (8 blocks of 6). Considering the mean and standard deviation (SD) of pain intensity scores before, during and after the intervention in two groups of acupressure and ice therapy (dBefore = 0.86 [SD = 1.78], dNow=1.20 [SD=1.87], dAfter=0.60 [SD=1.53]), the final sample size was calculated using NCSS software at a significance level of 0.05 and a power 80% of 210 people (70 people in each group).

2.2. Data collection and instruments

After receiving informed consent to participate in the study, the admitted patients who met the study's inclusion criteria were considered the study's sample. Prior to signing the written consent form, all participants were assured of the confidentiality of their information and the ability to leave the study at any time with no repercussions. To prevent discrepancies, the researchers ensured that all treatments were executed correctly and that all individuals received equivalent pressure at the Hugo point. The researchers also performed the required intervention while adhering to all health procedures.

A demographic questionnaire was one of the data gathering tools comprised of age, length of menstrual period, the field of study, duration of the first menstrual period, degree of birth, etc.

The visual analogue scale was used to determine the severity of the pain (VAS), which is a commonly used pain measuring tool. A patient is asked to estimate the severity of her pain along a horizontal line of 100 mm (most typically), and the rating is then measured from the left edge (=VAS score). Although the VAS score strongly correlates with acute pain levels, it is 20 mm inaccurate (32). The tool's reliability and validity have been endorsed by Shaban et al. in Iran (33). Bijour et al. declared a correlation coefficient of 0.97 with a confidence range of 0.96 to 0.98 in a research to assess the reliability of visual acuity instruments of patients sent to the Emergency unit that were appropriately reliable to measure the pain (34).

In the current study, an acupuncturist offered crucial commands on finding the pressure point, applying pressure, the quantity of pressure, and other related issues. We maintained emotional, verbal, and nonverbal communication with the subjects throughout the study and provided psychological support. The acupressure group received a 5-minute regular and rotational massage. Initially, a 60-second massage was given without regard to contractions (30 seconds clockwise and 30 seconds counterclockwise). After that, the patient had a 10-second break. This method was repeated until the 5-minute massage time was up. The pressure exerted by the research assistant's fingers was assessed using a digital weighing scale with a 1 g accuracy before the intervention. The point was subjected to a mean pressure of 1.5 to 3.5 kg. To administer ice massage, the researcher utilized circular ice chunks with a diameter of 2 cm inside plastic bags and wrapped with a thin gauze to avoid moisture transfer and direct ice contact with the skin. Massage with ice was done rotationally for one minute (30 seconds clockwise and 30 seconds counterclockwise). Like the acupressure, the procedure was repeated five times with a time-lapse of 10 seconds. No intervention was done in the control group; the patients received routine care, and the ice ball spun outside the Hugo point. The pain severity was recorded before, immediately, and one hour after intervention in the three groups.

2.3. Statistical analysis

Categorical and continuous variables were presented as the numbers (per cent), median (interquartile range) and mean (standard deviation), respectively. The independent t-test, one-way ANOVA, and repeated measures ANOVA were used to analyze the data in SPSS V. 19, with the significance level set at 0.05 in all tests.

2.4. Protocol to perform validation of electronic visual analog scale

Before beginning the procedure, participants were given a quick interview to check if they fulfilled the selection criteria and to enquire about their personal information. The researcher explained the procedure, and participants signed the informed consent form after reading the information page. For the application of pressure, participants were directed to draw a short vertical line on the paper's horizontal line to record their discomfort, with the left end representing no pain and the right end being the most severe suffering conceivable.

To improve dependability, the procedure (paper) was repeated twice, with a minimum of 5 minutes between tries. To establish a more uniform and reproducible system that could be easily employed in a clinical context, the authors elected not to randomize the initial device for evaluating pain; furthermore, the possibility of a sequence effect was previously confirmed using panel data regression in a random sample of similarly sequenced individuals, but no such effect was observed. Any earlier recordings were erased to avoid biasing the patient's pointing. Participants were not allowed to view the paper while pointing to the next one and were not notified of their results until the procedure was completed.

3. Results

This study was performed on 210 participants in three groups of 70 individuals with control, acupressure, and ice massage. The mean (SD) age of subjects was 23.0 (3.2), in ranges of 18-32 years. Most participants were single (75.7%) and with an Associate s' degree (53.4%). There was no significant difference between these three groups in terms of their demographic characteristics using one-way ANOVA, Chi-Square, or Fisher's Exact Test ($p > 0.05$) (Table 1).

Table 1. Respondents' demographics and characteristics

Variable		Control (n=70)	Acupressure (n=70)	Ice massage (n=70)	*P*-value*
Age, Mean ± SD		22.0 ± 2.4	22.3 ± 2.9	22.7 ± 2.8	**0.73**
BMI, Mean ± SD		21.3 ± 2.03	22.7 ± 3.5	22.0 ± 2.6	**0.58**
Marriage, n (%)	Single	58 (82.9)	49 (70.0)	53 (75.7)	**0.20**
	Married	12 (17.1)	21 (30.0)	17 (24.3)	
Education, n (%)	Associate	39 (58.2)	32 (47.1)	39 (58.2)	**0.32**
	Bachelor s' degree	28 (41.8)	36 (52.9)	28 (41.8)	
Underlying disease in student, n/N (%)	Kidney	-	1/10 (10.0)	-	**0.98**
	Liver	-	-	1/13 (7.7)	
	Thyroid	5/9 (55.6)	4/10 (40.0)	8/13 (61.5)	
	Mental	1/9 (11.1)	2/10 (20.0)	1/13 (7.7)	
	Migraine	2/9 (22.2)	2/10 (20.0)	2/13 (15.4)	
	Respiratory	1/9 (11.1)	1/10 (10.0)	1/13 (7.7)	
Obesity in relatives, n/N (%)	Grade1	22/26 (84.6)	21/29 (72.4)	22/27 (81.5)	**0.51**
	Grade2	4/26 (15.4)	4/26 (15.4)	5/27 (18.5)	
History of underlying disease in relatives, n/N (%)	Grade1	22/26 (84.6)	22/26 (84.6)	20/23 (87.0)	**0.97**
	Grade2	4/26 (15.4)	4/26 (15.4)	3/23 (13.0)	
Activity, n (%)	Normal	30 (42.9)	29 (41.4)	28 (40.0)	**0.57**
	Light	33 (47.1)	28 (40.0)	34 (48.6)	
	Professional	7 (10.0)	13 (18.6)	8 (11.4)	
Nutrition, n/N (%)	Low Salt	3/5 (60.0)	11/16 (68.7)	3/5 (60.0)	**0.99**
	Vegetable	2/5 (40.0)	5/16 (31.3)	2/5 (40.0)	
Disorder, n/N (%)	Depress	47/67 (70.1)	43/67 (64.2)	42/68 (61.8)	**0.57**
	Angry	20/67 (29.9)	24/67 (35.8)	26/68 (38.2)	
To take action, n (%)	Drug	45(64.3)	37 (52.8)	43 (61.4)	**0.79**
	Imam	8 (11.4)	9 (12.9)	8 (11.4)	
	Rest	13 (18.6)	15 (21.4)	13 (18.6)	
	No work	4 (5.7)	9 (12.9)	6 (8.6)	

*P-Values were calculated by One-way ANOVA, χ^2 or Fisher's Exact Test.

Table 2 presents the mean (SD) of pain intensity scores before, during and after the intervention for the control group, acupressure and ice massage. In all dimensions, the score reduction in the acupressure and ice massage groups was greater than in the control group. However, One-way ANOVA demonstrated that the difference between the mean scores of the three groups was significant only in post-test ($p < 0.001$) and follow-up ($p < 0.001$).

According to Repeated Measure ANOVA, the mean scores of pain intensity in the two groups of acupressure ($p < 0.001$) and ice massage ($p < 0.001$) had significant changes over time.

In the Acupressure group and based on Bonferroni Post Hoc, this difference was significant between all times, before and during ($p < 0.001$), before and follow-up ($p < 0.001$), and during and follow-up ($p < 0.001$).

In the Ice massage group and based on Bonferroni Post Hoc, this difference was observed between all times before and during ($p < 0.001$), before and follow-up ($p < 0.001$), and during and follow-up ($p = 0.012$). However, in the control group, the mean scores changed very little over time ($p = 0.220$). The Repeated Measure ANOVA test results also showed significant changes in the mean pain intensity reduction in the three groups during the study ($p < 0.001$). The results of repeated measures analysis of variance also showed that the mean pain intensity scores differed in the three control groups, acupressure and ice massage ($F = 21.503$, $p < 0.001$). Tukey test showed this difference significantly only between control and acupressure groups ($p < 0.001$) and control groups and ice massage ($p < 0.001$). It is north worthy that this difference was not significant between the two groups of acupressure and ice massage ($p = 0.97$).

Table 2. Comparison of mean pain intensity score in three groups of control, acupressure and ice therapy over time

Pain intensity	Total n=210 Mean ± SD Median (IQR[a])	Control n=70 Mean ± SD Median (IQR)	Acupressure n=70 Mean ± SD Median (IQR)	Ice massage n=70 Mean ± SD Median (IQR)	Comparison of three groups (Between groups) (One-way ANOVA)
Pre test	7.06 ±1.95 7 (6. 9)	7.41 ± 1.82 8 (6.9)	6.74 ± 2.23 7 (5. 8)	7.03 ± 1.72 7 (6.8)	**F = 2.06, p = 0.13**
Post test	6.31 ± 2.05 6 (5. 8)	7.58 ± 1.67 8 (7. 9)	5.74 ± 2.09 6 (4, 7.5)	5.61 ± 1.75 5 (4.7)	**F = 19.32, p < 0.001**
Follow-up	6.06 ± 2.19 6 (4. 8)	7.60 ± 1.65 8 (7. 10)	5.17 ± 2.10 5 (4. 7)	5.21 ± 1.69 5 (4. 6)	**F = 32.01, p < 0.001**
Compare the before, now and after (Within groups) (Repeated Measure ANOVA test)		F = 1.51 p = 0.22	F = 43.15 p < 0.001	F = 60.34 p < 0.001	
Time × Group*	F = 36.96, p < 0.001				

[a]Inter quartile range*Repeated Measure ANOVA test.

4. Discussion

Primary dysmenorrhea is pain that occurs during the menstrual cycle without a known reason. It's one of the main reasons for pelvic pain in females, and it can make daily tasks difficult (1). There are a variety of therapeutic options available, many of which have acceptable efficiency and safety profiles.

Different methods are used to control menstrual pain today, which are mainly divided into two categories of medical and non-medical techniques. The recommendations reflect a balance between the available evidence and an assessment of benefit, harm, and cost. Nonsteroidal anti-inflammatory drugs, oral hormonal medications and other analgesics are among the medical treatments (35). Although these drugs have quick effects, their long-term use can cause problems such as nausea, digestive problems, peptic ulcer and diarrhea (36). Also, these drugs are not helpful in 10-20% of patients (37).

Effective dysmenorrhea treatment necessitates a discussion of risks, advantages, and alternatives, with a particular emphasis on finding a treatment method that matches the patient's goals in the context of her lifestyle and other medical problems. In recent years, patients have become more interested in non-medical treatments, sometimes known as complementary therapies. These methods are easy, low-cost, and non-invasive, with no adverse side effects (15, 38).

Regarding non-pharmacological treatment options for dysmenorrhea, by conducting a review of physical therapy treatments in dysmenorrhea, a study pointed out that thermotherapy, either by cold or heat, is a systematic treatment for that dysfunction. The authors added that both could eliminate or reduce pain practically and economically (39).

The goal of this study was to compare the effects of two types of acupressure and ice massage on the degree of dysmenorrhea pain at the Hugo point. Our findings demonstrated that the acupressure and ice massage groups experienced higher pain reduction than the control group. Furthermore, the findings revealed that the difference in mean scores between the three groups was significant only in the post-test and follow-up periods. The outcomes of the current study are in line with those of other studies in this field. For example, Wahdi et al. (40) revealed that applying acupressure to the Hugo point can reduce the severity of menstruation pain (41). The efficacy of different acupuncture times for primary dysmenorrhea was explored, and it was concluded that acupuncture has a favorable effect on alleviating primary dysmenorrhea pain (30). Both acupressure and ice massage approaches were found to lower pain severity, labour stage duration, and anxiety in primipara women. Furthermore, Bastani et al., when looking at the effect of acupressure on the Hugo point on the pain induced by the removal of a chest tube in cardiac patients, discovered that acupressure lessened the agony (42).

These outcomes are consistent with the results of Rostami et al., that investigated the effect of acupressure at the LI4 point on labour satisfaction in women and found that the intervention and control groups' satisfaction levels were significantly different, with participants in the intervention group scoring the greatest in terms of treatment satisfaction (27). This finding is consistent with the findings of other investigations. For example, the results of the Khalili-Shomia et al. demonstrated that cold massage at Hugo site alleviated post-appendectomy pain in patients, which is consistent with the findings of this study (25).

Rakhshekhorshid et al. compared the massage and ice massage impact on SP6 on primary dysmenorrhea's duration and severity and found that using acupressure at SP6 with or without ice significantly decreased the duration and severity of primary dysmenorrhea in the intervention group in comparison to the control group (43). A study by Barani et al. revealed that ice massage could be used to reduce primary menstrual pain as a non-invasive, simple, and economical method with no side effects, considering the feasibility of training it in the shortest time, which is consistent with the findings of this study (29).

The results of Aeen et al. revealed that the mean pain intensity in the Hugo point massage with ice group and the control group was considerably lower than in the Hugo point massage without ice group (44). After the intervention, the Hugo point massage with ice group had a considerably lower mean anxiety score than in the ice-free massage group ($p = 0.040$) and in the control group. The results of Al-Najjar et al. indicated that adding ice massage to a short-term treatment reduces pain, improves pain threshold and function, and increases side bending of the cervical area in participants with mechanical neck pain and activated trigger points in the upper trapezius muscle, with no statistically significant difference (45).

Supporting the results of the present study, Howatson et al. demonstrated that cold massage was unsuccessful in lowering the indirect indicators linked with an exercise-induced muscle injury, according to the study (46). Furthermore, MacAuley et al., in a comprehensive assessment of the evidence for cryotherapy in acute soft tissue injuries treatment, discovered that ice with exercise was the most beneficial after an ankle sprain and following surgery (47).

Based on the findings, it can be concluded that acupressure and ice massage can be effective therapy for treating primary dysmenorrhea that is simple to administer and does not require any specific equipment. These approaches are also advised for persons who find that taking analgesics is damaging to them, those who suffer from these medications' adverse effects, and those who are hesitant to use the drugs. As a result, acupressure and cold massage are indicated in individuals with primary dysmenorrhea. Furthermore, there were no unfavourable side effects associated with the administration. These data suggest that acupressure and cold massage can be used as a safe therapy for primary dysmenorrhea, particularly in severe instances. The application of cold massage and acupressure at Hugo point considerably decreased the degree of discomfort in primary dysmenorrhea, according to the findings of this study.

This study has a number of potential limits that should be considered. Pain perception, pain threshold, financial and psychological status, and social and cultural backgrounds may have varied among our participants. All patients were chosen from the same geographic location to control the confounding influence of social and cultural variety in our sample. To ensure that all patients received the same level of care, they were all admitted to the same ward and cared for by the same nursing team. However, we need to replicate our findings.

Ethics Committee Approval

The study was conducted with permission from the deputy of medical research and approval from the ethics board of Sirjan University of Medical Sciences (code number: IR.SIRUMS.REC.1399.029). This study is also listed in the Iranian Clinical Trials Registry (code number: IRCT20200624047911N1) on 2021.02.05.

The authors are responsible for all aspects of the work, including ensuring that any questions about the work's accuracy or integrity are thoroughly explored and resolved.

Authors' contributions

The conception and design of the study: Naimeh Pourramezani; Acquisition of data: Moazameh Sadat Razavi-Nasab; Analysis and interpretation of data: Mohadeseh Balvardi; writing the article or revising it for significant intellectual content: Fatemeh Alavi-Arjas, Maryam Firouzabadi, Moazameh Sadat Razavi-Nasab; Final approval of the version to be submitted: Naimeh Pourramezani.

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Conflict of interest

There are no conflicts of interest.

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References

1. Ferries-Rowe E, Corey E, Archer JSJO, Gynecology. Primary dysmenorrhea: diagnosis and therapy. 2020;136(5):1047-58.
2. De Sanctis V, Soliman AT, Elsedfy H, Soliman NA, Elalaily R, El Kholy MJAB. Dysmenorrhea in adolescents and young adults: A review in different countries. 2016;87(3):233-46.
3. Latthe P, Latthe M, Say L, Gülmezoglu M, Khan KSJBph. WHO systematic review of prevalence of chronic pelvic pain: a neglected reproductive health morbidity. 2006;6(1):1-7.
4. Kabirian M, Abedian Z, Mazlom SR, Mahram B, Jalalian MJLSJ. Self-management in primary dysmenorrhea: toward evidence-based education. 2011;8(2):13-8.
5. Afshari P, Pazandeh F, Sadeghi SJEJP. Frequency of dysmenorrhea and treatment methods in virgin students (19-22) in Ahvaz University. 2006;1(10):17-20.
6. Ju H, Jones M, Mishra GJEr. The prevalence and risk factors of dysmenorrhea. 2014;36(1):104-13.
7. Ullah A, Fayyaz K, Javed U, Usman M, Malik R, Arif N, et al. Prevalence of dysmenorrhea and determinants of pain intensity among university age women. 2021.
8. Schoep ME, Adang EMM, Maas JWM, De Bie B, Aarts JWM, Nieboer TE. Productivity loss due to menstruation-related symptoms: a nationwide cross-sectional survey among 32 748 women. BMJ Open. 2019;9(6):e026186.
9. Latthe PM, Champaneria R. Dysmenorrhoea. BMJ clinical evidence. 2014;2014.
10. Guimarães I, Póvoa AMJRBdGeO. Primary dysmenorrhea: assessment and treatment. 2020;42:501-7.
11. Burkman RTJJ. Berek & Novak's gynecology. 2012;308(5):516-7.
12. Kashefi F, Ziyadlou S, FADAEI A, Ashraf A, Jafari P. The effect of SP6 acupressure on primary dysmenorrhea in medical

univesity students in Birjand, 2006-2007. 2009.

13. Dawood MYJO, Gynecology. Primary dysmenorrhea: advances in pathogenesis and management. 2006;108(2):428-41.

14. Lindquist R, Tracy MF, Snyder M. Complementary and alternative therapies in nursing: Springer Publishing Company; 2018.

15. Balouchi A, Mahmoudirad G, Hastings-Tolsma M, Shorofi SA, Shahdadi H, Abdollahimohammad AJCticp. Knowledge, attitude and use of complementary and alternative medicine among nurses: A systematic review. 2018;31:146-57.

16. Mejías-Gil E, Garrido-Ardila EM, Montanero-Fernández J, Jiménez-Palomares M, Rodríguez-Mansilla J, González López-Arza MVJJopm. Kinesio Taping vs. Auricular Acupressure for the Personalised Treatment of Primary Dysmenorrhoea: A Pilot Randomized Controlled Trial. 2021;11(8):809.

17. Tournaire M, Theau-Yonneau AJE-bc, medicine a. Complementary and alternative approaches to pain relief during labor. 2007;4(4):409-17.

18. Dorland W. Dorland's Pocket medical dictionary. 1982.

19. Moyer CA, Seefeldt L, Mann ES, Jackley LMJJob, therapies m. Does massage therapy reduce cortisol? A comprehensive quantitative review. 2011;15(1):3-14.

20. Remington R. Calming music and hand massage with agitated elderly: University of Massachusetts Amherst; 1999.

21. Pan B, Castro-Lopes J, Coimbra AJBrb. Activation of anterior lobe corticotrophs by electroacupuncture or noxious stimulation in the anaesthetized rat, as shown by colocalization of fos protein with ACTH and β-endorphin and increased hormone release. 1996;40(3):175-82.

22. Frost H, Stewart-Brown S. Acupressure for low back pain. BMJ. 2006;332(7543):680-1.

23. Pouraboli B, Abazari F, Rostami M, Jahani YJJoPN. Comparison the effect of two methods of acupressure and massage with ice on Huko point on pain intensity during IV insertion in pediatrics with thalassemia. 2015;2(2):20-7.

24. Bastani F, Sobhani M, Bozorgnejad M, Shamsikhani S, Haghani HJCMJ. Effect of acupressure on severity of pain in women with multiple sclerosis (MS). 2012;2(1):75-84.

25. Khalili-Shomia S, Nasab S, Davarinia Motlagh Quchan AJIJoAM. Comparison of the effect of two methods of acupressure and ice massage on the Hugo point on the severity of pain after appendectomy surgery. 2019;10(2):203-6.

26. Fareed M, Abd El-Hay AH, El-Shikh AAJJoE, Practice. Cutaneous stimulation: Its effect on pain relieving among hemodialysis patients. 2014;5(1).

27. ROSTAMI M, ABAZARI F, POURABOLI B, JAHANI Y, SHIRZADI F. The effects of huko point massage with ice on intensity of pain due of phlebotomy in children with thalassemia in kerman samen alhojaj medical center, iran. 2014.

28. Dabiri F, Shahi AJOmj. The effect of LI4 acupressure on labor pain intensity and duration of labor: a randomized controlled trial. 2014;29(6):425.

29. Barani ZY, Shahraki MJMS. Comparison of the effect of ice massage in the LI4 and SP6 on Primary Dysmenorrhea: A blinded clinical trial. 2018;22:544-8.

30. Kaviani M, Ashoori M, Azima S, Rajaei Fard A, Hadian Fard MJSJ. Comparing the effect of two methods of acupressure and ice massage on the pain, anxiety levels and labor length in the point LI-4. 2012;20(2):220-28.

31. Melzack R, Guite S, Gonshor AJCMAJ. Relief of dental pain by ice massage of the hand. 1980;122(2):189.

32. Kaviani M, Ashoori M, Azima S, Rajaei Fard A, Hadian Fard MJSJ. Comparing the effect of two methods of acupressure and ice massage on the pain, anxiety levels and labor length in the point LI-4. 2012;20(2):220-28.

33. Chao A-S, Chao A, Wang T-H, Chang Y-C, Peng H-H, Chang S-D, et al. Pain relief by applying transcutaneous electrical nerve stimulation (TENS) on acupuncture points during the first stage of labor: a randomized double-blind placebo-controlled trial. 2007;127(3):214-20.

34. Qu F, Zhou JJE-BC, Medicine A. Electro-acupuncture in relieving labor pain. 2007;4(1):125-30.

35. Parsanezhad M, Alborzi S, Namavar Jahromi B. Menstrual Abnormalities and Pain after Five Tubal Sterilization Methods: A Randomized Controlled Trial. Iranian Journal of Medical Sciences. 2015;28(2):51-6.

36. Payne LA, Rapkin AJ, Seidman LC, Zeltzer LK, Tsao JC. Experimental and procedural pain responses in primary dysmenorrhea: a systematic review. Journal of Pain Research. 2017;10:2233.

37. Nasri M, Barati A, Ramezani A. The effects of aerobic training and pelvic floor muscle exercise on primary dysmenorrhea in adolescent girls. Journal of Clinical Nursing and Midwifery. 2017;5(3).

38. Lindquist R, Tracy MF, Snyder M. Complementary and Alternative Therapies in Nursing Eighth Edition. Springer Publishing Company; 2018.

39. Gerzson L, Padilha J, Braz M, Gasparetto A. Physiotherapy in primary dysmenorrhea: literature review. Revista Dor. 2014;15.

40. Wahdi A, Pratiwi TF, Susanti N, Kai MW, Puspitosari DRJJK. The Acupressure Effect on Hegu Points and Sanyinjio Points on Reducing the Level of Menstruation Pain. 2021;13(1):75-82.

41. Xue X, Huang Y, Liu X, Yue Z-h. Clinical studies on different acupuncture time for primary dysmenorrhea. Journal of Acupuncture and Tuina Science. 2016;14(6):416-9.

42. Bastani F, Momenabadi H, Safdari M-T, Haghani HJDMB, Journal CR. The effect of acupressure on pain severity of chest tube removal in patients with open heart surgery in intensive care unit. 2012;20(4):1-12.

43. Rakhshekhorshid M, Foadoddini M, Saadatjoo SAJJoBUoMS. Comparison between the effects of applying massage and ice massage to SP6 (SPLEEN6) point on severity and length of primary dysmenorrhea. 2013;20(01).

44. Aeen s, Esmaelzadeh F, Sadeghi T, Davoudi N. Comparison the Effect of two methods of Hogu point massage with ice and without ice on pain intensity, anxiety and success rate on Arterial blood sampling in patients admitted to Imam Reza hospital. %J Evidence Based Care. 2021:-.

45. Al-Najjar HMM, Mohammed AH, Mosaad DMJBoFoPT. Effect of ice massage with integrated neuromuscular inhibition technique on pain and function in subjects with mechanical neck pain: randomized controlled trial. 2020;25(1):1-7.

46. Howatson G, Gaze D, Van Someren KJSjom, sports si. The efficacy of ice massage in the treatment of exercise-induced muscle damage. 2005;15(6):416-22.

47. MacAuley DJTAJoSM. The use of ice in the treatment of acute soft-tissue injury. 2004;32(1).

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The effects of methanolic plant extract on the cardiovascular system of induced preeclamptic Wistar rats

Kenneth ATOE[1,2,3,*], Macdonald IDU[1]

[1]Phytomedicine and Drug Discovery Research Group, Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria
[2]Department of Chemical Pathology, Edo State University Uzairue, Edo State, Nigeria
[3]Applied Environmental Biosciences and Public Health Research Group, Department of Microbiology, University of Benin, Benin City, Nigeria

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Abstract

This work investigated the effects of methanolic plant extract on the cardiovascular system of induced preeclampticWistar rats. Preeclampsia is associated with increase in high blood pressure and proteinuria. Studies have shown the only medically acceptable treatment is the removal of the fetus from the mother, however there is considerable lack of studies done to show the use of herbal medicine in the treatment and management of preeclampsia. This study reveals the use of plant extract and its effect on cardiovascular system. Three plants extract; *Jatropha curcas, Alchornea cordifolia and Secamone afzelii* were used for the study. Results showed that there were no observable lesion or abnormally in the cardiovascular system and the use of all three plant extracts significantly reduced blood pressure and proteinuria, thus indicating a reduction of preeclampsia from severe to mild.

Keywords: preeclampsia, highblood pressure, proteinuria, herbal medicine, *Jatropha curcas, Alchornea cordifolia and Secamone afzelii*

1. Introduction

Preeclampsia is a condition that causes an unexpected rise in blood pressure and edema, particularly in the face, hands, and feet, during pregnancy (1). The exact causes of preeclampsia are unknown, however blood vessels in the placenta are thought to be involved. Although preeclampsia may not cause any symptoms, early warning signs include protein in the urine (proteinuria) and increased blood pressure (hypertension). Some pregnant women have elevated blood pressure, but this does not necessarily indicate preeclampsia; the presence of protein in the urine is the most noticeable indication (1). The most prevalent pregnancy-related illness is preeclampsia. It commonly appears in the second trimester and affects one out of every twenty pregnancies.

Preeclampsia causes the heart to become overworked and inefficient in pumping blood because it is unable to relax between contractions. Preeclampsia has been linked to an increased risk of heart failure, heart attack, and stroke among mothers who have recovered from it. It can lead to blood clotting issues, pulmonary edema, seizures, and, in severe cases or if left untreated, death of the mother and newborn (2-6).

The most likely method of treatment for this condition is delivery. Women with this condition are at a higher risk of seizures, placental abruption, stroke, and possibly major bleeding until their blood pressure lowers. If high blood pressure or hypertension occurs too early in pregnancy, delivery may not be the best decision for the baby (3).

Aspirin is now the only medication having strong evidence to support its use in reducing the risk of heart attack and stroke (2, 6). Nutritional supplements and pharmacological therapies are examples of other interventions medicines, as well as dietary and lifestyle modifications, have been studied for their ability to protect against preeclampsia, with varied degrees of success. Vitamin D insufficiency has been linked to an increased risk of preeclampsia (4, 5, 7-10). Vitamin D supplementation has been shown to help reduce preeclampsia risk (11, 5). While supplementation is frequently advised in clinical practice, strong randomized controlled trial (RCT) data is still needed to prove its effectiveness (8, 12). High-dose folic acid does not appear to be useful in preventing repeated preeclampsia (13), while some evidence suggests that supplementing with 5-methyl-tetrahydrofolate, a more accessible form of folic acid, may be effective in preventing recurrent preeclampsia (14).

*Correspondence: atoe.kenneth@edouniversity.edu.ng

Herbal medications are commonly perceived as a safe, natural alternative to conventional drugs, and are frequently used to improve the welfare of many pregnant women, or treat non-life-threatening diseases. Herbal medicine has become increasingly popular over time. Ginger, cranberry, valerian, raspberry leaf, chamomile, peppermint, thyme, fenugreek, green tea, sage, anise, garlic, and bitter kola are the most widely utilized herbs. The usage of herbal medication during pregnancy is linked to a woman's educational status, household economic level, and age. Herbal medications were used to relieve nausea and vomiting, lower the risk of preeclampsia, speed up labor, and treat the common cold and urinary tract infection during pregnancy (15). Though studies have shown that the use of herbal medicine goes a long way in reducing the chances of infections, however, it is best to consult a doctor and a pharmacist before using any herbal therapy during pregnancy to ensure that the herbs are appropriate and safe to use (15,16, 17, 18). The usage of herbal medicine during pregnancy varies a lot depending ethnicity, cultural customs, and socioeconomic level (18). Herbal medication use during pregnancy is quite frequent in Sub-Saharan Africa, according to previous study (16, 17). Study by Gharoro and Igbafe (19) in Benin City, Edo State showed that the use of native medication during pregnancies. Many these patients believed in herbal treatments' efficacy and had found them to be a cost-effective and accessible alternative treatment.

In the present study, extracts from 3 commonly used plants in Benin City are used in the management of preeclampsia in animal models. These are *Jatropha curcas, Alchornea cordifolia and Secamone afzelii.* These plants have been previously reported to have a number of therapeutic properties, including antibacterial, anti-cancer, with capacities to treat coughs, infertility, bacterial infections, inflammation, fever, and bronchial problems. Ugbogu and Chukwuma (20) reported the use of some of these herbs in the management of high blood pressure; but no study has reported use of any herbal preparation in the management of preeclampsia. The aim of the study therefore for to investigate the use of these plant extracts in the management of preeclampsia and associated cardiovascular complications.

2. Materials and Methods

2.1. Collection and preparation of plant samples

Plant samples (leaves) were collected from First Generation Farms Ltd in Iguosula, Uhunmwonde Local Government Area, Edo State. They were identified and verified at the University of Benin's Department of Plant Biology and Biotechnology's Phytomedicine Unit in Benin City. The plant samples were cleaned many times with distilled water, air-dried for two weeks, then processed into powder with a Panasonic® medium kitchen blender, model MX-GX1021WTZ. After soaking 100g of each powder sample in 200ml of methanol for 12 hours, the extracts were filtered using Whatman Filter Paper No 42. (125mm).

2.2. Study design

Females Wistar rats of comparable age (3 days) weighing 237 g (mean) were utilized in the study. The animals were housed in well-ventilated metabolic cages. The animals were given unrestricted access to a standard diet (0.35 g NaCl, 20 g protein, and 1.17 g arginine per 100 g food) as well as ad libitum tap water (pH range 6.8 – 7.2). They were given a one-week acclimation period before the trial began.

In this experiment, the Wistar rats were randomly divided into 15 groups, each with 10 rats. Whereas Group 1 served as the positive control, Groups 2, 3, and 4 served as the negative controls. Other groups are as presented below (Table 1).

Table 1. Designation of experimental groups

Group	Description
Group 1	Control
Group 2	Administered with Ext-JC (No induced Preeclampsia)
Group 3	Administered with Ext-AC (No induced Preeclampsia)
Group 4	Administered with Ext-SA (No induced Preeclampsia)
Group 5	Induced Preeclampsia, no treatment provided
Group 6	Induced Preeclampsia + 100 mg/kg Standard drug
Group 7	Induced Preeclampsia + 50 mg/kg Ext-JC
Group 8	Induced Preeclampsia + 100 mg/kg Ext-JC
Group 9	Induced Preeclampsia + 200 mg/kg Ext-JC
Group 10	Induced Preeclampsia + 50 mg/kg Ext-AC
Group 11	Induced Preeclampsia + 100 mg/kg Ext-AC
Group 12	Induced Preeclampsia + 200 mg/kg Ext-AC
Group 13	Induced Preeclampsia + 50 mg/kg Ext-SA
Group 14	Induced Preeclampsia + 100 mg/kg Ext-SA
Group 15	Induced Preeclampsia + 200 mg/kg Ext-SA

Ext-JC, Metholic leaf extract of Jatrophacacus; Ext-AC, Metholic leaf extract of Alchonneacordifolia; Ext-SA, Metholic leaf extract of Secamone afzelii.Standard drug was methyl DOPA (Aldomet®)

2.3. Induction of preeclampsia

In this work, the Adriamycin Model developed by Podjarny et al (21) was utilized to induce preeclampsia. Adriamycin (Adriablastina, Abic) was delivered into rats under light ether anesthesia at 3.5 mg/kg IV through a superficial caudal vein. Two weeks later, the rats were mated for four days with a fertile male.

2.4. Management of experimental animals

The animals were cared for and used in compliance with international guidelines for laboratory animal care and use (22).

2.5. Determination of blood pressure

Determination of blood pressure of the Wistar rat were by the non-invasive methods described by Feng and DiPetrillo (23), using the CODA® High Throughput System with 2 Activated Channels (CODA-HT2) by Kent Scientific Corporation, USA.

2.6. Determination of ECG

Determination of ECG was according to the methods of Pereira-Junior et al. (24). Prior to ECG recordings, the animals were conditioned for 7 days in a Plexi glass restrainer for 20 minutes each day. All subsequent recordings were made in the morning in a controlled environment (0700-0900h). The animal was restrained in a Plexi glass restrainer with

ventilation holes on the front and other surfaces, and electrodes were connected to a differential A/C amplifier (A-M Systems, USA), with signals digitized by a 16 bit A/D interface converser (Axon 1322-A, USA), and sampled at 10 KHz by software Axoscope 9.0. (USA: Axon Instruments). The ECG recording began 10 minutes after the animal was placed into the Plexi glass restrainer and lasted 5 minutes. The steadiest 180s continuous section from each record was chosen for HRV analysis.

2.7. Determination of proteinuria

This was carried out using the dipstick (combi2) method (25).

2.8. Sacrifice of experimental animal

The animals were anaesthetized with chloroform and humanely slaughtered twenty-four (24) hours after the last dosage of the standard medicine and various treatment extracts were administered to the relevant groups (26). Following that, the hearts were histologically examined using the procedure of Molh et al. (27).

2.9. Statistical analysis

Data collected were analyzed using SPSS version 20 and GraphPad Prism 5. Results were presented in Tables and Quantitative variables were expressed as mean $\pm$ standard deviation

2.10. Ethical issues

The Research and Ethics Committee of the Faculty of Life Sciences, University of Benin, Benin City, granted ethical permission with reference LS19017, dated March 7, 2019.

3. Results

When the methanolic extracts of the test plants were administered to the Wistar rats for the first 15 days, no mortalility was recorded (Fig. 1.) for the highest concentration (5000 mg/kg) for this reason, the extracts were used.

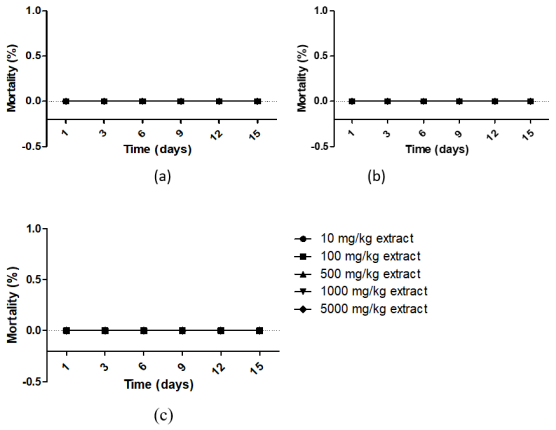


Fig. 1. Percentage mortality of rats used during acute toxicity study for 15 days (a) *Jatropha curcas*(b) *Alchornea cordifolia and* (c) *Secamone afzelii*

Table 2 shows the blood pressure of preeclampsia in rats. In the control, blood pressure during 3rd trimester was 124/98 mmHg. However, when plant extracts were administered, without the inducement of preeclampsia, bp was119/85 for administration of methanolic extracts of *Jatropha curcas*. Blood pressure of the preeclamptic group was 177/121 mmHg during the 3rd trimester. However, upon administration of extracts (100 mg/kg) of *Jatropha curcas*, blood pressure significantly dropped to 118/86 mmHg. Blood pressure in Wistar rats administered with 200 mg/kg of extract of *Alchornea cordifolia* was 127/87 mg/kg during the third trimester. Similarly, although at post-partum, for control group, blood pressure was 121/96 mmHg; however blood pressure of the preeclamptic group was 160/125 mmHg. Blood pressure in Wistar rats administered with 100mg/kg of extract of Secamone afzelii was 141/101 at third trimester and 116/86 at post-partum. Significant reductions in blood pressure were achieved upon administration of methanolic extracts of the testy plants (Table 2).

Table 2. Blood pressure measurement of preeclamptic and control groups after administration of extracts of test plants

Treatments	(n)	Systolic	Diastolic	(n)	Systolic	Diastolic
	3rd trimester			**Post-partum**		
Control	14	124	98	8	121	96
Only Ext-A (No induced PreEc) (200 mg/kg)	15	119	85	12	110	85*
Only Ext-B (No induced PreEc) (200 mg/kg)	19	132	101	13	122	94
Only Ext-C (No induced PreEc) (200 mg/kg)	19	131	95	12	123	91
Induced PreEc, no treatment	26	177*	121*	12	160*	125*
Induced PreEc + 100 mg/kg StdD	36	141	103	10	135	104
Induced PreEc + 50 mg/kg Ext-A	16	143	99	7	117	93
Induced PreEc + 100 mg/kg Ext-A	12	118	86	4	129	101
Induced PreEc + 200 mg/kg Ext-A	12	145	100	3	119	99
Induced PreEc + 50 mg/kg Ext-B	14	143	97	4	113	90
Induced PreEc + 100 mg/kg Ext-B	10	136	96	5	113	87
Induced PreEc + 200 mg/kg Ext-B	19	127	87	10	127	94
Induced PreEc + 50 mg/kg Ext-C	11	140	98	6	136	102
Induced PreEc + 100 mg/kg Ext-C	16	141	101	6	116	86
Induced PreEc + 200 mg/kg Ext-C	13	150*	92	8	121	106
LSD(0.05)		22	14		16	11
F-test		5.913	7.795		4.332	4.241
p-value		<0.001	<0.001		<0.001	<0.001

* Means are significantly different from the Control ($p<0.05$). Values presented are to the nearest integer

Proteinuria was negative in the control group as expected. Similarly, as evidence that the extracts did not cause any damage, proteinuria was also negative in upon administration of 200 mg/kg of the extracts (Table 3). During 3rd trimester, proteinuria was 3+ in the preeclamptic group but reduced to 1+ at postpartum. During the 3rd trimester also, there was at least 33% reduction in level of proteinuria upon administration of plant extracts. Administration of 50mg/kg of methanolic extracts of *Jatropha curcas* reduced proteinuria by over 60% (i.e 1+) compared to 3+ in the preeclamptic group. At postpartum, significant reduction in proteinuria was also recorded upon administration of plant extracts.

Table 3. Level of proteinuria in both preeclamptic groups after administration of extracts of test plants

Group	Baseline	At Third trimester	At post-partum
Control	Negative	Negative	Negative
Only Ext-A (No induced PreEc) (200 mg/kg)	Negative	Negative	Negative
Only Ext-B (No induced PreEc) (200 mg/kg)	Negative	Negative	Negative
Only Ext-C (No induced PreEc) (200 mg/kg)	Negative	Negative	Negative
Induced PreEc, no treatment provided	+++	+++	+
Induced PreEc + 100 mg/kg StdD	NA	+	trace
Induced PreEc + 50 mg/kg Ext-A	NA	+	trace
Induced PreEc + 100 mg/kg Ext-A	NA	++	+
Induced PreEc + 200 mg/kg Ext-A	NA	++	trace
Induced PreEc + 50 mg/kg Ext-B	NA	++	trace
Induced PreEc + 100 mg/kg Ext-B	NA	++	trace
Induced PreEc + 200 mg/kg Ext-B	NA	++	trace
Induced PreEc + 50 mg/kg Ext-C	NA	++	+
Induced PreEc + 100 mg/kg Ext-C	NA	++	+
Induced PreEc + 200 mg/kg Ext-C	NA	++	trace

Present + (The number of "+" indicates level of severity); NA not applicable

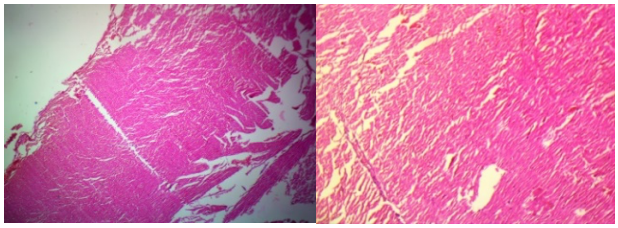
The severity of preeclampsia is shown in Table 4 above. In the third trimester, the percentage increase in blood pressure relative to the control group was 42.7 percent for systolic blood pressure and 23.5 percent for diastolic blood pressure, respectively. Significant decreases in these increases were observed when plant extracts were used in the third trimester and afterward. Similarly, the severity of preeclampsia in the untreated preeclamptic group decreased to mild in the third trimester and postpartum.

Table 4. Severity of preeclampsia in the Wistar rats before and after administration of plant extracts

Group	3rd trimester				Post-partum			
	Percentage rise (%)				Percentage rise (%)			
	Systolic BP	Diastolic BP	Proteinuria	Preeclampsia	Systolic BP	Diastolic BP	Proteinuria	Preeclampsia
Induced PreEc, no treatment provided	42.7	23.5	+++	Severe	32.2	30.2	+	Severe
Induced PreEc + 100 mg/kg StdD	13.7	5.1	+	Mild	11.6	8.3	trace	Mild
Induced PreEc + 50 mg/kg Ext-A	15.3	1	+	Mild	-3.3	-3.1	trace	Mild
Induced PreEc + 100 mg/kg Ext-A	-4.8	-12.2	++	Mild	6.6	5.2	+	Mild
Induced PreEc + 200 mg/kg Ext-A	16.9	2	++	Severe	-1.7	3.1	trace	Mild
Induced PreEc + 50 mg/kg Ext-B	17.7	-1	++	Severe	-6.6	-6.3	trace	Mild
Induced PreEc + 100 mg/kg Ext-B	9.7	-2	++	Mild	-6.6	-9.4	trace	Mild
Induced PreEc + 200 mg/kg Ext-B	2.4	-11.2	++	Mild	5	-2.1	trace	Mild
Induced PreEc + 50 mg/kg Ext-C	12.9	0	++	Mild	12.4	6.3	+	Mild
Induced PreEc + 100 mg/kg Ext-C	13.7	3.1	++	Mild	-4.1	-10.4	+	Mild
Induced PreEc + 200 mg/kg Ext-C	21	-6.1	++	Severe	0	11.5	trace	Mild

1 - 15% rise in BP (compared to control) plus significant Proteinuria – Mild preeclampsia
> 15% rise in BP (compared to control) plus significant Proteinuria – Severe preeclampsia
*Incidences herein reported in the Table above are modal presentation of symptoms by the model animals

There were no significant changes in heart rates and other ECG parameters measured during both 3rd trimester and postpartum (Table 5). Perhaps this insignificant impact on the heart parameters as presented in Table 5 mayhave resulted especially because the increase in blood pressure was acute.This was also evident in the histological findings (Table 6, Fig. 2.).



(a) Normal Heart muscle x4 (b) Normal Heart muscle x10

Fig. 2. Histological slide of a normal heart

Table 5. ECG in both preeclamptic groups after administration of extracts of test plants

Treatments	Heart Rate (/min)	Pdur (ms)	PR-Interval (ms)	QRS (ms)	QT (ms)	QTc (ms)	R amplitude (mV)
Basal reading	203.0	22.3	52.3	18.7	101.0	185.0	0.5
3rd trimester							
Control	276.7	20.7	53.0	11.0	82.3	155.3	0.5
Only Ext-A (No induced PreEc)	275.0	24.7	48.3	13.0	101.0	207.0	0.6
Only Ext-B (No induced PreEc)	272.3	22.0	43.0	13.0	116.0	211.3	0.4
Only Ext-C (No induced PreEc)	260.0	23.0	41.0	15.3	102.7	218.7	0.7
Induced PreEc, no treatment provided	238.3	26.3	41.3	14.7	119.7	225.3	0.9
Induced PreEc + 100 mg/kg StdD	251.7	25.0	51.0	14.7	95.0	189.3	0.6
Induced PreEc + 50 mg/kg Ext-A	274.8	21.8	50.4	22.8	105.2	224.6	0.6
Induced PreEc + 100 mg/kg Ext-A	276.8	31.8	52.0	15.8	83.2	177.2	0.4
Induced PreEc + 200 mg/kg Ext-A	294.6	20.2	47.8	12.8	84.6	187.2	0.5
Induced PreEc + 50 mg/kg Ext-B	275.0	20.0	45.5	15.3	92.5	197.3	0.4
Induced PreEc + 100 mg/kg Ext-B	276.2	27.2	50.4	11.6	107.8	230.6	0.4
Induced PreEc + 200 mg/kg Ext-B	294.8	24.0	44.5	15.3	99.8	219.8	0.6
Induced PreEc + 50 mg/kg Ext-C	291.2	23.6	45.8	15.2	84.6	185.4	0.7
Induced PreEc + 100 mg/kg Ext-C	266.0	24.5	48.5	16.5	93.8	197.3	0.5
Induced PreEc + 200 mg/kg Ext-C	241.0	25.3	48.0	20.0	95.7	190.7	0.4
F-test	4.762	0.859	1.106	1.793	1.993	1.529	2.465
LSD (0.05)	52.4	12.7	24.7	21.7	21.7	43.4	0.3
p-value	<0.001	0.611	0.379	0.066	0.038	0.135	0.010
Post-partum							
Control	262.3	18.7	49.0	10.3	76.0	153.0	0.5
Only Ext-A (No induced PreEc)	254.0	23.3	44.3	13.0	93.3	209.3	0.6
Only Ext-B (No induced PreEc)	258.0	20.0	41.0	13.0	114.0	195.3	0.4
Only Ext-C (No induced PreEc)	246.0	21.0	39.7	14.3	100.7	202.0	0.7
Induced PreEc, no treatment provided	220.0	24.3	41.0	13.7	110.3	208.0	0.9
Induced PreEc + 100 mg/kg StdD	249.0	23.0	47.0	13.7	87.7	175.0	0.6
Induced PreEc + 50 mg/kg Ext-A	221.3	28.0	54.7	19.3	94.0	182.0	0.6
Induced PreEc + 100 mg/kg Ext-A	219.3	23.3	56.3	18.7	96.0	172.0	0.5
Induced PreEc + 200 mg/kg Ext-A	217.3	29.7	57.7	14.3	104.3	192.0	0.5
Induced PreEc + 50 mg/kg Ext-B	209.7	21.3	43.3	15.3	106.3	198.7	0.4
Induced PreEc + 100 mg/kg Ext-B	227.7	24.0	53.3	13.0	87.0	168.7	0.4
Induced PreEc + 200 mg/kg Ext-B	223.0	23.8	55.8	14.3	106.3	204.5	0.5
Induced PreEc + 50 mg/kg Ext-C	268.0	23.9	47.6	15.0	90.4	189.0	0.6
Induced PreEc + 100 mg/kg Ext-C	250.0	23.9	50.1	16.4	92.6	188.4	0.5
Induced PreEc + 200 mg/kg Ext-C	215.2	24.5	49.8	17.3	96.8	182.2	0.4
LSD (0.05)	26.8	12.1	11.9	2.3	23.1	43.2	0.3
F-test	2.209	0.981	1.949	2.947	2.557	1.178	2.188
p-value	0.021	0.490	0.043	0.003	0.008	0.323	0.022

4. Discussion

Preeclampsia is associated with high blood pressure and proteinuria. According to research, the only medically acknowledged treatment for preeclampsia is the removal of the fetus from the mother. However, there are just a few researches that suggest herbal medicine can be used to treat and manage preeclampsia. This study looked at the effects of methanolic plant extract on the cardiovascular system of induced preeclamptic Wistar rats. When 50mg/kg of methanolic extracts of *Jatropha curcas* were given, proteinuria was reduced by roughly 60% (i.e., 1+).

Although all plant extracts caused significant reductions in blood pressure; this was more significant with the use of extracts of *Jatropha curcas*. *Jatropha curcas* L. (Euphorbiaceae) has been previously used to treat a variety of disorders, including skin, cancer, digestive, respiratory, and infectious infections and has also been shown to have anti-inflammatory, antioxidant, antimicrobial, antiviral, anticancer, antidiabetic, anticoagulant, hepatoprotective, analgesic, and abortifacient capacities. This plant has been reported to have Angiotensin I-converting enzyme (ACE)-inhibiting activity (28). Angiotensin I-converting enzyme (ACE) plays an important role in regulating blood pressure and hypertension.

Jatropha curcas contains lignans, which have a steroid-like

chemical structure and are classified as phytoestrogens. Traditionally, lignans have been associated with reduced risk of heart disease, menopausal symptoms, osteoporosis, and breast cancer as Consumption of lignan-rich foods could help to avoid chronic illnesses including cancer and cardiovascular disease (29). *Jatropha curcas* also contains coumaris. Coumarin (2H-1-benzopyran-2-one) is an anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, and neuroprotective plant-derived natural substance.

Table 6. Histological findings of the heart in both preeclamptic groups after administration of extracts of test plants

Groups	Hearth	
	3rd trimester	post-partum
Control	There is no observable lesion	Normal cardiac muscles, no observable lesion seen
Only Ext-A (No induced PreEc)		
Only Ext-B (No induced PreEc)		
Only Ext-C (No induced PreEc)		
Induced PreEc, no treatment		
Induced PreEc + 100 mg/kg StdD		
Induced PreEc + 50 mg/kg Ext-A		
Induced PreEc + 100 mg/kg Ext-A		
Induced PreEc + 200 mg/kg Ext-A		
Induced PreEc + 50 mg/kg Ext-B		
Induced PreEc + 100 mg/kg Ext-B		
Induced PreEc + 200 mg/kg Ext-B		
Induced PreEc + 50 mg/kg Ext-C		
Induced PreEc + 100 mg/kg Ext-C		
Induced PreEc + 200 mg/kg Ext-C		

Results showed from the study, *Alchornea cordifolia* was able to reduce the blood pressure in preeclamptic Wister rats. The history of the use of herbs in the management of diseases dates back to the time of the early man (30, 31). In herbal medicine, herbs/plants are being used in their unaltered form for the treatment of disease. *Alchornea cordifolia* is a plant widely used in Africa alone or in association with other plants to solve many health problems (32). *A. cordifolia* has been very valuable locally in some ethnic groups in Nigeria for the management of haemorrhoids and high blood pressure. It has been found to have anti-inflammatory, antibacterial and analgesic properties (32, 33). Study by Eliakim-Ikechukwu and Riman (34) revealed that *A. corifolia* is capable of inducing elastogensis in the aorta; this attribute of the herb may be beneficial in increasing elastic recoil of the aortic wall and may reduce blood pressure. The phytochemical includes alchorneine, anthranilic acid, gentisinic acid, isoalchorneine, yohimbine and alkaloids (35).

Results from this study also revealed that *Secamone afzelii* had the ability to reduce blood pressure and proteinuria in preeclamtic Wister rats. *Secamone afzelii* is used in traditional medicine for stomach problems, diabetes, colic, dysentery and also for kidney problems. *Secamone afzelii* have also been reported to have anti-inflammatory and antioxidant properties due to flavonoids, triterpenoids, diterpenoids and caffeic acid derivatives (36, 37, 38).

The findings of this study revealed that the administration of *Jatropha curcas* was found to be responsible for lowering severe preeclampsia to mild. There has been no prior research to back this up, but this study has shown that *Jatropha curcas, Alchornea cordifolia and Secamone afzelii* can reduce blood pressure and proteinuria, both of which are symptoms of preeclampsia in the Wistar rats.The incidence of preeclampsia had no significant effect on the heart presentations as shown in the ECG and histology reports. Moreover, the administration of the plant extract did not cause any observable lesion or abnormally in the heart.

Conflict of interest
None to declare.
Funding
None to declare.

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None to declare.

Authors' contributions
Concept: K.A., M.I., Design: K.A., M.I., Data Collection or Processing: K.A., Analysis or Interpretation: K.A., M.I., Literature Search: K.A., Writing: K.A.

References

1. Felman A. Everything you need to know about preeclampsia. Medical news. 2017.
2. Asuk AA, Willie AJ. The phytochemical proximate mineral compositions of the leaf stem bark root of Jatropha curcas. 2015; https: //doiorg/101016/japjtb
3. Adinma ED. Maternal perinatal outcome of eclampsia in tertiary health institution in Southeast Nigeria. J Matern Fetal. 2013; 26(2): 211-4 doi: 103109/147670582012722708
4. Wei S-Q, Qi H-P, Luo Z-C, Fraser WD. Maternal Vitamin D Status Adverse Pregnancy Outcomes: A Systematic Review Meta-Analysis. J Matern Fetal Neonatal Med. 2013; 26: 889–899
5. Arain N, Mirza WA, Aslam M. Review-Vitamin D the Prevention of Preeclampsia: A Systematic Review. Pak J Pharm Sci. 2015; 28:1015–1021

6. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-Eclampsia. Lancet 2016; 387: 999–1011.

7. Mirzakhani H, Litonjua AA, McElrath TF, O'Connor G, Lee-Parritz A, Iverson R et al. Early Pregnancy Vitamin D Status Risk of Preeclampsia. J Clin Investig. 2016; 126: 4702–4715

8. Purswani JM, Gala P, Dwarkanath P, Larkin HM, Kurpad A, Mehta S 2017The Role of Vitamin D in Pre-Eclampsia: A Systematic Review. BMC Pregnancy Childbirth 2010; 17: 231

9. Akbari S, Khodadadi B, Ahmadi SAY, Abbaszadeh S, Shahsavar F. Association of Vitamin D Level and Vitamin D Deficiency with Risk of Preeclampsia: A Systematic Review Updated Meta-Analysis. Taiwan J Obstet Gynecol. 2018; 57: 241–247

10. Ali AM, Alobaid A, Malhis TN, Khattab AF. Effect of Vitamin D3 Supplementation in Pregnancy on Risk of Pre-Eclampsia—Randomized Controlled Trial. Clin Nutr. 2019; 38: 557–563

11. Nassar N, Halligan GH, Roberts CL, Morris JM, Ashton AW Systematic Review of First-Trimester Vitamin D Normative Levels and Outcomes of Pregnancy. Am J Obstet Gynecol 2011; 205e1–e7

12. Roth DE, Leung M, Mesfin E, Qamar H, Watterworth J, Papp E. Vitamin D Supplementation during Pregnancy: State of the Evidence from a Systematic Review of Randomised Trials. BMJ 2017; 35 j5237

13. Wen SW, White RR, Rybak N, Gaudet LM, Robson S, Hague W et al. Effect of High Dose Folic Acid Supplementation in Pregnancy on Pre-Eclampsia (FACT): Double Blind Phase III Randomised Controlled International Multicentre Trial. BMJ. 2018; 362 k3478

14. Saccone G, Sarno L, Roman A, Donadono V, Maruotti GM, Martinelli P. 5-Methyl-Tetrahydrofolate in Prevention of Recurrent Preeclampsia. J Matern Neonatal Med. 2016; 29: 916–920

15. Laelago T. Herbal Medicine Use during Pregnancy: Benefits Untoward Effects, in Herbal Medicine. Ed. P. Builders. London: Intech Open. 2018.

16. Ahmed M, Hwang JH, Hasan MA, Han D. Herbal medicine use by pregnant women in Bangladesh: a cross-sectional study. BMC Complement Altern Med. 2018a; 18 (1): 333 doi: 101186/s12906-018-2399-y

17. Ahmed SM, Nordeng H, Sundby J, Aragaw Y A, de Boer HJ. The use of medicinal plants by pregnant women in Africa: A systematic review. J Ethnopharmacol. 2018b; 224: 297–313 doi: 101016/jjep201805032

18. Illamola SM, Amaeze OU, Krepkova LV, Birnbaum AK, Karanam A, Job KM, et al. Use of Herbal Medicine by Pregnant Women: WhatPhysicians Need to Know. Front Pharmacol. 2020; 10: 483–1483 doi: 103389/fphar201901483

19. Gharoro EP, Igbafe AA. Pattern of drug useamong antenatal patients in Benin City, Nigeria. Med Sci Monit. 2000; 6(1): 84 - 87

20. Ugbogu OA Chukwuma EC. Ethnobotany of Okomu Forest Reserve Edo State Nigeria. J Appl Sci Environ Manage. 2019; 23 (7): 1391-1401

21. Podjarny E, Bernheim JL, Rathaus M, Pomeranz A, Tobvin D, Shapira J, Bernheim J. Adriamycin nephropathy: a model to study the effects of pregnancy on renal disease in rat. Am J Physiol. 1992; 263 (4 Pt 2):F711-715.

22. National Research Council (NRC) Guide for the care and use of laboratory animals (8th Edition). The National Academic Press, Washington, D.C., 2011; 1-246

23. Feng M, DiPetrillo K. Non-invasive Blood Pressure Measurement in Mice. Methods Mol Biol. 2009; 573: 45 – 55 doi: 101007/978-1-60761-247-6_3

24. Pereira-Junior PP, Marocolo M, Rodrigues FP, Medei M Nascimento JHM. Noninvasive method for electrocardiogram recording in conscious rats: feasibility for heart rate variability analysis. Anais da Academia Brasileira de Ciências 2010; 82(2): 431-437

25. Stefańska K, Zieliński M, Zamkowska D, Adamski P, Jassem-Bobowicz J, Piekarska K et al. Comparisons of Dipstick Test Urine Protein-to-Creatine Ratio Total Protein Measurement for the Diagnosis of Preeclampsia. International Journal of Environmental Research Public Health 2020; 17(12): 4195 https: //doiorg/103390/ijerph17124195

26. Rowell TE. Reproductive cycle of the talapoin monkey (Miopithecus talapoin) Folia Primatol. 1977; 28:188-202.

27. Molh AK, Ting LC, Khan J, Al-Jashamy K, Jaafar H, Islam MN. Histopathological studies of cardiac lesions after an acute high dose administration of methamphetamine The Malaysian journal of medical sciences (MJMS). 2008; 15(1): 23–30

28. Segura-Campos MR, Peralta-González F, Castellanos-Ruelas A, Chel-Guerrero LA, Betancur-Ancona DA. Effect of Jatropha curcas peptide fractions on the angiotensin I-converting enzyme inhibitory activity. BioMed Research International 2013; ID 541947 https: //doiorg/101155/2013/541947

29. Rodríguez-García C, Sánchez-Quesada C, Toledo E, Delgado-Rodríguez M, Gaforio JJ. Naturally Lignan-Rich Foods: A Dietary Tool for Health Promotion? Molecules. 2019 Mar 6;24(5):917. doi: 10.3390/molecules24050917. PMID: 30845651; PMCID: PMC6429205.

30. Sofowora EA. Medicinal plants traditional medicine in Africa. John Wiley sons Ltd, Hoboken. 1982; 64p

31. Kafaru E. Immense help from nature's workshop Nigeria. Elikat Health Services Limited. 1994; P32.

32. Olaleye MT, Adegboye OO Akindahunsi AA. Alchorne acordifoli extract protects Wistar albino rats against acetaminophen-induced liver damage. Afr J Biotech. 2006; 5: 2439-2445

33. Cesario A. Screening of plants used in Argentine folk medicine for antimicrobial activity. Journal of Ethnopharamacology 1993; 139: 119-128

34. Eliakim-Ikechukwu C F Riman E B. The effect of aqueous ethanolic extract of Alchornea cordifolia leaf on the histology of the aorta of Wister rats. Niger J Physiol Sci. 2009; 24(2):149-151

35. Duke J Vasquez R 1994 Amazonian Ethnobotanical Dictionary, Florida, CRC Press Incorporated. 122: 101-102

36. Mensah AY, Houghton PJ, Akyirem GNA et al. Evaluation of the antioxidant free radical scavenging properties of Secamone afzelii Rhoem. Phytotherapy Research 2004; 18(12): 1031-1032 2004

37. Houghton PJ, Hylands PJ, Mensah AY, Hensel A, Deters AM. In vitro tests ethnopharmacological investigations: wound healing as an example. Journal of Ethnopharmacology 2005; 100(1-2): 100–107

38. Zabri HKodjo C, Benie A, Bekro JM, Bekro YA. Phytochemical screening determination of flavonoids in Secamone afzelii (Asclepiadaceae): extracts. African Journal of Pure Applied Chemistry 2008; 2(8): 80–82

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Early predictors of return of spontaneous circulation in patients undergoing cardiopulmonary resuscitation

Ercan NALBANT[1,*], Mehmet ALTUNTAŞ [2], Ali ÇELİK[2], Özcan YAVAŞİ [2] Gökhan ERSUNAN[2], Özlem BİLİR [2], Gürkan ALTUNTAŞ[2]

[1]Department of Emergency Medicine, Rize State Hospital, Rize, Türkiye
[2]Department of Emergency Medicine, Recep Tayyip Erdoğan University, Faculty of Medicine, Rize, Türkiye

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Abstract

Decisions about when to start, how long to continue and how to end cardiopulmonary resuscitation (CPR) are important in the management of these critically ill patients. The aim of our study is to determine the factors that can help in the early prediction of patients in whom spontaneous circulation can be restored during CPR. Patients who had arrest due to nontraumatic causes were included in the study. The patients whose spontaneous circulation (ROSC) was restored in the emergency service and who were hospitalized in intensive care were included in ROSC (+) group, while the patients who did not have ROSC and who became exitus were included in ROSC (-) group. Patients' demographic characteristics, chronic diseases, places of arrest, admission laboratory values and possible causes of death were compared between ROSC groups. This study was conducted with the retrospective examination of 309, 118 (38.2%) female and 191 (61.8%) male, cardiopulmonary arrest cases. ROSC was achieved in 94 (30.4%) of the patients who underwent CPR. It was found that a 0,1 unit increase in pH value increased the probability of ROSC by 38% [OR: 1,38 (95% CI: 1.22-1.55), p<0.0001], while multivariate logistic regression analysis showed that it could be an independent predictor of ROSC and increased the probability of survival by 0,43 times [Corrected OR: 1.43 (95% CI: 1.13-1.83), p=0.0033]. It was also found with multivariate logistic regression analysis that respiratory causes could be an independent predictor of ROSC and increased the probability of survival by 2.76 times [Corrected OR: 3,76 (95% CI=1,499.53), p=0.0052]. In patients who undergo CPR, pH value in blood gas analysis and the presence of respiratory system diseases as the cause of arrest are important parameters in determining the probability of ROSC.

Keywords: Return of spontaneous circulation, cardiopulmonary resuscitation, emergency medicine, early prediction

1. Introduction

Effective resuscitation for cardiac arrest (CA) patients, which includes early defibrillation appropriate post-arrest care, results in survival and improvement in neurological outcomes (1).

In more than 300.000 cardiac arrest cases that occur each year in the United States of America, survival rates are typically lower than 10% for out-of-hospital cardiac arrests (OHCA) and lower than 20% for in-hospital cardiac arrest (IHCA) (2, 3). In addition, studies have shown that survival is reduced by 10-15% for each minute of cardiac arrest without cardiopulmonary resuscitation (CPR) (4, 5).

Despite many advances in CPR, mortality and morbidity rates are very high after CA. The course of patients after CPR ranges from mild or moderate symptoms to persistent vegetative state or death. Decisions about when to start CPR, how long to apply CPR, how to end CPR and predetermined patient conditions after CPR are important in the management of these critical patients (6). Therefore, there is a need for reliable parameters that can be used as the early predictors of recovery after CA.

The aim of our study is to determine the factors that can enable early prediction of patients in whom spontaneous circulation can be restored during CPR.

2. Materials and Methods

This study was conducted with retrospective examination of patients who underwent CPR in the emergency service of a tertiary hospital between 01.01.2018 and 31.12.2019. Approval was obtained from the local ethics committee for this study (Date: 20.02.2020, Decision number: 2020/25).

2.1. Patient selection

Patients older than 18 years of age who had arrest due to non-major trauma causes and who underwent CPR in the emergency service were included in the study. The patients who had ROSC in the emergency service with CPR and who were hospitalized in intensive care were included in ROSC (+) group, while the patients who did not have ROSC and who became exitus were included in ROSC (-) group.

*Correspondence: dr.nalbant_ercan@hotmail.com

2.2. Data collection

Computer based hospital information management system (HBYS), in which patient records are kept), was used to collect the data required for the study. Demographic features of the patients, their chronic diseases and information about initial laboratory values before arrest were obtained from consultation and epicrisis information obtained from HBYS program. In addition, information about the initial examination and possible causes of death was obtained from patient detailed examination records kept for patients and from patient files and emergency service archive.

2.3. Statistical analysis

Mean, standard deviation (SD), median, interquartile range (ICA), frequency and ratio values were used in the descriptive statistics of data. Distribution of the variables was evaluated with histogram, Q-Q graphs and Shapiro Wilk Test. Mann-Whitney U test was used in the analysis of non-parametric qualitative independent variables, while Student T test was used in the analysis of parametric data. Pearson X^2 or Fisher Exact Test was used in the analysis of qualitative independent data. Factors which were thought to determine survival and the variables which were thought to be important in univariate analysis were included in multivariate logistic regression analysis. Multicorrelation was checked with correlation analysis before and after multiple regression analysis. In univariate and multivariate analyses, probability rates (OR= odds ratio) were calculated with 95% confidence intervals (95% CI). $p<0.05$ was considered as statistically significant in all statistical analysis. Jamovi (version 1.1.6.0; https://jamovi.org) statistical program was used in analyses.

3. Results

This study was conducted with retrospective examination of 309, 118 (38.2%) female and 191 (61.8%) male, cardiopulmonary arrest cases. ROSC was achieved in 94 (30.4%) of the patients who underwent CPR. 39 (41.5%) of the patients who had ROSC were female, while 55 (58.5%) were male (p=0.4296). Statistically significant difference was found in the mean age of patients who underwent CPR in terms of gender (p<0.0001). Table 1 shows the comparison of ROSC states by gender and mean age.

Table 1. Distribution of patients who underwent CPR in terms of gender and mean age

	ROSC			p
	+	-	Total	
Female, n (%)	39 (41.5)	79 (36.7)	118 (38.2)	0.4296*
Age (female), *median (IQR)*	80 (67-85)	80 (70-85)	80 (69-85)	0.8623†
Age (male), *median (IQR)*	68 (60-77)	67 (59-78)	68 (59-77)	0.9566†
Age (total), *median (IQR)*	71 (68-81)	72 (61-68)	72 (61- 81)	0.8986†
	Gender			**p**
	Male	**Female**	**Total**	
Age, *median (IQR)*	68 (59-77)	80 (69-85)	72 (61- 81)	**<0.0001**†

*Pearson's X^2 Test, † Mann-Whitney U Test, CPR; Cardiopulmonary resuscitation, ROSC (+); return of spontaneous circulation, ROSC (-); no return of spontaneous circulation, n; number, %; percentage, m; median age, IQR; interquartile range

It was found that ROSC occurred in 22.6% (n=212) of the patients who had OHCA and in 47.4% (n=97) of the patients who had IHCA (p<0.0001). No statistically significant difference was found between airway applications performed in out-of-hospital environments (p=0.6989). It was found that 36.3% (n=193) of the patients who were intubated in the hospital had ROSC (p=0.039). When CPR duration was compared in terms of the state of having ROSC, it was found that the difference was statistically significant (IH-CPR/min; p<0.0001 OH-CPR/min; p=0.0004). Table 1 shows the comparison of the place of arrest, airway practices, arrest rhythms and CPR durations of patients who underwent CPR in terms of ROSC states.

Table 2. Distribution of place of arrest, airway practices, arrest rhythms and CPR durations of patients who underwent CPR

		ROSC		Total	p
		+	-		
Place of arrest, n (%)	**OH**	48 (22.6)	164 (77.4)	212 (100)	**<0.0001***
	IH	46 (47.4)	51(52.6)	97 (100)	
Airway-OH, n (%)	**ETI**	24 (20.7)	92 (79.3)	116 (100)	0.6989*
	BVM	24 (25.0)	72 (75.0)	96 (100)	
Airway-IH, n (%)	**ETI**	70 (36.3)	123 (63.7)	193 (100)	**0.0039***
Arrest rhythms, n (%)	**Cannot receive shock**	71 (28.5)	178 (71.5)	249 (100)	0.2776†
	Can receive shock	23 (38.3)	37 (61.7)	60 (100)	0.1378†
CPR duration (IQR)	**OH-CPR/min.**	10 (10-20)	20 (10-30)	15 (10-30)	**0.0004** †
	IH- CPR/min.	10 (10-15)	40 (30-45)	35 (20-45)	**<0.0001**†

CPR; Cardiopulmonary resuscitation, ROSC (+); Return of spontaneous circulation, ROSC (-); No return of spontaneous circulation, OH; Out-of- hospital, IH; In hospital, BVM; Bag valve mask, ETI; Endotracheal intubation, m; median, IQR; interquartile range, *Pearson's X2 Test, †Mann-Whitney U Test

When the patients' laboratory parameters were examined, it was found that the differences between pH, pCO_2, pO_2, $SatO_2$, HCO_3, BE, Lactate, Na^+ and K^+ values were statistically significant in terms of ROSC. Table 3 shows the comparison of mean laboratory values of patients who underwent CPR in terms of their ROSC states.

Table 3. Mean laboratory values of patients who underwent CPR

Laboratory values, m (IQR)	ROSC		CPR	p†
	+	-	Total	
pH	7.20 (7.03-7.35)	7.02 (6.86-7.19)	7.09 (6.89-7.25)	**<0.0001**
pCO_2 (mmHg)	45.9 (37.3-61.1)	59.8 (41.5-77.7)	52.9 (39.4-72.3)	**<0.0004**
pO_2 (mmHg)	55.4 (28.5-104.8)	36.8 (21-60.5)	42.7 (27-69.3)	**<0.0001**
$SatO_2$ (%)	80.7 (38.8-95.3)	42.1 (13.2-76.2)	52.5 (16.8-84.4)	**<0.0001**
HCO_3 (mEq/L)	15.9 (11.7-22.8)	11.1 (7.7-16.3)	12.6 (8.3-17.8)	**<0.0001**
BE (mmol/L)	8.2 (0.8-15.3)	14 (7.1-19.2)	12.6 (5.3-18.6)	**<0.0001**
Lactate (mmol/L)	6.8 (3-11.4)	11.7 (8.3-16)	10.6 (5.9-14.9)	**<0.0001**
Glucose (mg/dL)	196 (129-269)	202 (129-294)	198 (129-290)	0.5555
BUN (mg/dL)	56 (40.8-91.3)	55 (36.5-94.5)	55 (37-93)	0.8245
Creatinine (mg/dL)	1.4 (0.93-1.97)	1.5 (1.09-1.99)	1.4 (1.01-1.99)	0.4827
Na^+ (mg/L)	137 (135-140)	139 (135-142)	139 (135-142)	**0.0368**
K^+ (mg/L)	4.6 (3.7-5.4)	5.3 (4.5-6.4)	5 (4.3-6.2)	**<0.0001**
AST (U/L)	40 (26-115)	56 (26-129)	50 (46.3-57)	0.1934
ALT (U/L)	36 (18.6-76)	38 (18-102)	38 (18-87.5)	0.5006
WBC (10^3/uL)	12.4 (9.6-15.4)	11.8 (8.6-15.2)	11.9 (8.8-15.3)	0.3812
HGB (g/dL)	11.7 (9.8-13.6)	12.2 (9.8-13.9)	12 (9.8-3.8)	0.5279
HCT (%)	36.8 (31.1-41.1)	38.5 (31.9-43.9)	37.7 (31.8-43)	0.2491
PLT (10^3/uL)	205 (134-249)	179 (121-234)	194 (124-238)	0.1512

BE; Base extract, CPR; Cardiopulmonary resuscitation, ROSC (+); Return of spontaneous circulation, ROSC (-); No return of spontaneous circulation, m; median, IQR; Interquartile range, †Mann-Whitney U Test

More successful ROSC results were found in patients with a history of CHF and/or CPTD diagnosis (CHF; p=0.0369, COPD; p=0.0069). Of the patients who were considered to have respiratory system diseases as a cause of arrest, 40 (42.6%) were in the group that had ROSC, while 55 (25.6%) were in the group that did not have ROSC (p=0.029). Table 4 shows the comparison of comorbidities and possible arrest causes in patients who underwent CPR in terms of the state of ROSC.

It was found that each one minute increase in out-of-hospital CPR decreased ROSC by 5% [OR: 0.95 (95% CI: 0.92-0.98), p=0.0031]. However, multivariate regression analysis showed that it was not a predictor for ROSC [Corrected OR: 0.98 (95% CI: 0.93-1.02), p=0,2873]. In the IH-ETI group, it was found that the probability of ROSC was 118% higher when compared with patients who were intubated out of the hospital [OR: 2.18 (95% CI: 1.28-3.73), p=0.0044]. Although IH – ETI increased the probability of by ROSC 1.18 times in univariate regression analysis, it was understood in multivariate logistic regression analysis that IH – ETI [corrected OR: OR: 1.01 (95% CI: 0.43-2.35), p=0.9838] was not an independent factor of survival [Corrected 1.01 (95% CI: 0.43-2.35), p=0.9838].

While low and moderate level of correlation was found between pH level in blood gas and Na+ and K+ values measured in biochemistry and out-of-hospital CPR duration (spearman r=-0.19; spearman r=-0.42; spearman r=-0.37; p=0.0014, p<0.001, p<0.001, respectively), strong correlation was found between pCO2, BE, Lactate and HCO3 values in blood level (spearman r=-0.60, p<0.0001; spearman r=0.81, p<0.0001; spearman r=-0.77, p<0.0001; spearman r=0.90, p<0.0001, respectively). pCO_2, BE, Lactate and HCO_3 measurements were not included in multivariate logistic regression model. Since blood gas intake could not be separated as arterial, venous and mixed, PaO_2 and saturation measurements were also not included in the model. 0.1 unit increase in pH values increased the ROSC success rate by 38% [OR: 1.38 (95% CI: 1.22-1.55), p<0.0001]. Similarly, in multivariate regression analysis, it was also found to be an independent predictor of ROSC and it was found to increase the probability of survival by 0.43 times [corrected OR: 1.43 (95% CI: 1.13-1.83), p=0.0033]. An increase of 1 unit in pCO_2 value decreases survival rate by 2% [OR: 0.98 (95% CI: 0.97-0.99), p=0.0006]. An increase of 1 unit in pO_2 value increases survival rate by 1% [OR: 1.01 (95% CI: 1.003-1.01), p=0.0006]. An increase of 1 unit in SatO2 value increases survival rate by 2% [OR: 1.02 (95 CI%: 1.01-1.03), p<0.0001]. An increase of 1 unit in HCO3 increases survival rate by 11% [OR: 1.11 (95% CI: 1.06-1.15), p<0.0001]. An increase of 1 unit in BE increases survival rate by 7% [OR: 1.07 (95% CI: 1.04-1.1), p<0.0001].

It was found that the probability of ROSC in patients who had arrest due to respiratory causes was 15% higher in patients who had arrest due to non-respiratory causes [OR: 2.15 (95% CI: 1.29-3.59), p=0.0032]. Similarly, it was found to be an independent indicator of ROSC in multivariate logistic regression analysis and it was found to increase the probability of survival 2.76 times [corrected OR: 3.76 (95% CI=1.49-9.53), p=0.0052].

The results of univariate and multivariate logistic

regression analysis according to paired comparison tests of ROSC groups (OR, 95% CI) are shown in Table 5.

Table 4. Comorbidities and possible arrest causes of patients who received CPR

	ROSC		CPR	p*
	+	-	Total	
Comorbidities	**n (%)**	**n (%)**	**n (%)**	
HT	65 (69.1)	151 (70.2)	216 (69.9)	0.8485
DM	32 (34)	58 (27)	90 (29)	0.2085
CAD	29 (30.9)	57 (26.5)	86 (28)	0.4336
AF	18 (19.1)	32 (14.9)	50 (16)	0.3489
CHF	20 (21.3)	26 (12.1)	46 (15)	**0.0369**
CVD	14 (14.9)	31 (14.4)	45 (15)	0.9133
Malignity	7 (7.4)	32 (14.9)	39 (13)	0.0701
Alzheimer	10 (10.6)	23 (10.7)	33 (11)	0.9876
COPD	16 (17)	15 (7)	31 (10)	**0.0069**
CRF	7 (7.4)	22 (10.2)	29 (9)	0.4398
VTE	1 (1.1)	7 (3.3)	8 (3)	0.4427 FE
Other	9 (9.5)	25 (11.6)	34 (12)	0.6532
Possible causes of arrest				
Circulatory system diseases	47 (50)	113 (52.6)	160 (51.8)	0.6789
Respiratory system diseases	40 (42.6)	55 (25.6)	95 (30.7)	**0.029**
Metabolic Causes	1 (1.1)	8 (3.7)	9 (2.9)	-
Nervous sensory diseases	1 (1.1)	-	1 (0.3)	-
Other causes	5 (5.3)	3 (1.4)	8 (2.6)	-

CPR; Cardiopulmonary resuscitation, ROSC (+); Return of spontaneous circulation, ROSC (-); No return of spontaneous circulation, HT; Hypertension, DM; Diabetes mellitus, CAD: Coronary artery disease, AF; Atrial fibrillation, CHF; Congestive heart failure, CVD; cerebrovascular disease, COPD; Chronic obstructive pulmonary disease CRF; Chronic renal failure, VTE; Venous thromboembolism, *Pearson's X^2

Table 5. Logistic regression analyses of CPR variables

INDICATORS	Odds Ratio (OR)	p	Corrected OR	p
Respiratory Arrest	2.15 (1.29-3.59)	**0.0032**	3.76 (1.49-9.53)	**0.0052**
COPD	2.74 (1.29-5.8)	**0.0087**	3.09 (0.65-14.64)	0.1559
CHF	1.96 (1.03-3.73)	**0.0392**	1.75 (0.49-6.21)	0.3862
pH*10 (mmHg, 0.1 unit)	1.38 (1.22-1.55)	**<0.0001**	1.43 (1.13-1.83)	**0.0033**
pH_{mmHg} (1 unit of change)	25.09 (7.49-84.08)	**<0.0001**	36.89 (3.3-410)	**0.0033**
pCO_2	0.98 (0.97-0.99)	0.0006	-	-
paO_2	1.01 (1.003-1.01)	0.0006	-	-
$SatO_2$	1.02 (1.01-1.03)	<0.0001	-	-
HCO_3	1.11 (1.06-1.15)	<0.0001	-	-
BE	1.07 (1.04-1.10)	<0.0001	-	-
Lactate	0.89 (0.85-0.93)	<0.0001	-	-
Na^+	0.96 (0.92-0.99)	0.0190	0.95 (0.89-1.02)	0.1641
K^+	0.93 (0.8-1.07)	0.2989	0.75 (0.54-1.03)	0.0721

CHF; Congestive heart failure, COPD; Chronic obstructive pulmonary disease, BE; Base extract, CPR; Cardiopulmonary resuscitation

4. Discussion

Cardiopulmonary resuscitation, early defibrillation and early appropriate resuscitation lead to survival and improved neurological results in arrest patients. Targeted training can improve survival rates in cardiac arrest.

In a cross-sectional study they evaluated the factors affecting CPR success rates, Hajzargarbashi E. al evaluated a total of 190 patients who had undergone CPR, 75 (39.5%) female and 115 (60.5%) male, with a mean age of 69.4 (±17.7). Success (initial success) CPR was reported in 55 (28.9%) patients, while unsuccessful CPR was reported in 135 (71.1%) patients. Only 10 (5.3%) of the 190 patients examined were discharged from the hospital. No significant difference was found between the success of CPR and age (p=0.969) and gender (p=0.062) (7). In a prospective study Herlitz J. et al. examined the demographic characteristics affecting mortality in out-of-hospital cardiac arrests, 19791 patients were included in the study. Mean age of general arrest population was reported as 69 (±14) and 30% of this population was reported to be females. ROSC was reported in 17.3% of the patients (8). In a study Winther-Jensen M. et al. compared out-of-hospital cardiac arrest according to gender, they reported that cardiac arrest developed more in women when compared with men (p=0.04) and female gender was correlated with higher death rate in university analysis (p=0.02). Mean age of female patients (n=761) was found as 66, while mean age of male patients (n=178) was found as 65 and statistically significant difference was found between them (p=0.66) (9). In a study conducted by Torres E. et al., ROSC was reported in 717

(58.1%) of 1234 male arrest cases and in 190 (59.75%) of 318 female arrest cases and no statistically significant correlation was in occurrence of ROSC between genders (p=0.596) (10). The mean age of 136 arrest cases examined by Kim Y. Et al. was 67.5 (53-77.8) and median age was 68 (53-78) in cases who had ROSC and 67 (52.5-77.5) in cases who did not have ROSC and no statistical difference was found between them (p=0.817). In terms of gender, ROSC was reported in 38 (56.7%) of 90 (66.2%) male cases and in 29 (43.3%) of 46 (33.8%) female patients and statistically significant difference was found (11). In our study, 38.2% of the patients were female, while 60.8% were male. ROSC occurred in 94 (30.4%) of the patients who were applied CPR (n=309). Of the patients who were reported to have ROSC, 39 (41.5%) were female, while 55 (58.5%) were male (p=0.4296). The mean age of the female patients who were applied CPR was 80, while the mean age of the male patients who were applied CPR was 68 and the difference between them was statistically significant (p<0.0001). No statistically significant difference was found between the mean ages of the patients who were reported to have ROSC and those who were not (p=0.8986).

In a retrospective study in which Kim Y. et al. examined the role of blood gas analysis during resuscitation in out-of-hospital arrests, median pH was 6.96 (6.8-7.07) for the group that was reported to have ROSC and pH 6.85 (6.8-6.99) for the group that was not reported to have ROSC and statistically significant difference was found (p=0.009). When pCO_2 was examined, median value was found as 74 (55.5-91) mmHg in those who were reported to have spontaneous circulation and as 89.5 (73-112.3) mmHg in those who were not (p<0.001). In the study, according to multivariant regression analysis, the only predictive value was found as pCO_2 in predicting ROSC (OR 0.979; 95% CI 0.960-0.997; p=0.025) and the probability of ROSC was found to be 3.3 times higher when pCO2<75 mmHg (OR 0.302; 95% CI 0.146-0.627; p=0.001) (11). In a prospective study Corral Torres E. et al. examined the relationship between blood gas, ROSC and neurological survival in out-of-hospital non-traumatic arrests, median pH value of patients who had ROSC was found as 7.13, while median pH value of patients who did not have ROSC was found as 7.11 and the difference between was found to be statistically significant (p=0.020). In the multivariate analysis, it was found that low pH level [corrected OR 0.03 (0.002-0.59), p=0.020], high pCO_2 level [corrected OR 1.03 (1.01-1.05), p=0.008] and high K+ level [corrected OR=2.28 (1.43-3.61), p=0.008] were found to decrease ROSC success. Similarly, it was found that low pH level [corrected OR 0.06 (0.02-0.18), p<0.001], high pCO_2 level [corrected OR 1.05 (1.03-1.08), p<0.001], low HCO_3 level [corrected OR 0.97 (0.94-0.999), p=0.044], low BE level [corrected OR=0.96 (0.93-0.98), p<0.001] and high K+ level had a negative effect on neurological recovery (10). In a prospective study by Çalbay A. et al., the relationship between blood gas parameters and end-tidal carbon dioxide prognostic values and ROSC and neurological survival was examined in out-of-hospital cardiac arrests. Median initial pH value was 6.97 in patients who had ROSC and 6.99 in patients who did not have ROSC and the difference was found to be statistically significant (p=0.721). Median pCO_2 was 54.55 mmHg in patients who had ROSC and 68.5 mmHg in patients who died and statistically significant was found (p=0.0012). Median pO_2 value was 51.5 mmHg in patients who had ROSC and 26.85 mmHg in patients who did not have ROSC and the difference was found to be statistically significant (p=0.0006) (12). Similarly, in our arrest population with 309 individuals we included in the study, when blood gas parameters were compared, median pH was 7.20 (7.3-7.35) in patients who had ROSC and 7.02 (6.86-7.19) in patients who did not have ROSC and the difference between was found to be significant (p<0.0001). When pCO_2, pO_2, $SatO_2$, HCO_3, BE blood gas parameters and lactate median values were examined separately for patients who had ROSC and for patients who did not have ROSC, statistically significant difference was found and median and p values were found as 45.9-59.8 mmHg (p<0.0004) for pCO_2, 55.4-36.8 mmHg (p<0.0001) for pO_2, 80.7-42.1% (p<0.0001) for $SatO_2$, 15.9-11.1 mmol/L (p<0.0001) for HCO_3, 8.2-14 mmol/L (p<0.0001) for BE and 6.8-11.7 mmol/L (p<0.0001) for lactate, respectively. Only pH value was found as an independent indicator of spontaneous resuscitation in multivariate logistic regression analysis and it was found to increase survival rate by 0.43 times [corrected OR: 1.43 (95% CI: 1.13-1.83), p=0.0033].

In a study conducted by Hajzargarbashi E. et al., the cause of arrest was reported as internal causes in 51.1% of the patients (7), while the cause of arrest was reported as cardiac problems in 70% of the patients in a study by Herlitz J. et al (8). In a study by Kim Y. et al., when the effects of arrest etiology on ROSC was examined, 37 (55.2%) of 78 (57.4%) patients who had arrest due to cardiogenic causes had ROSC, while 17 (25.4%) of 30 (22.1%) patients who had arrest due to respiratory causes had ROSC and no statistically significant correlation was found between them (p=0.690) (11). In a retrospective, multi-centred, observational study by Orban J., while ROSC success rate was 42% in arrests due to cardiac pathologies, it was found as 19% in arrests due to respiratory pathologies (p<0.001) (13). In our study, while ROSC success rate was 29.4% in arrests due to circulatory system diseases, this rate was found as 42.1% in arrests due to respiratory system diseases. Statistically significant difference was found in respiratory system diseases in terms of ROSC (p=0.029). According to univariate regression analysis, the probability of return of spontaneous circulation will be 115% higher in patients who have arrest due to respiratory causes when compared with the patients who die [OR: 2.15 (95% CI: 1.29-3.59), p=0.0032]. Similarly, it was found as an independent indicator of ROSC in multivariate logistic regression analysis and the probability of survival was found to increase 2.76 times [corrected OR: 3.76 (95% CI: 1.49-9.53), p=0.0052].

Our study has some limitations. Our sample includes both

in-hospital and out-of-hospital cardiac arrest patients. The time between arrest and intervention was not accessed due to the retrospective design of the study. In addition, non-emergency outcome of the patients was not also included in the study, only ROSC success was evaluated within emergency service. Although pH value and especially arrests with respiratory cause are leading in terms of extending or ending CPR duration, different results can be found in different study groups. Although these differences include differences such as geography, ethnicity and demographic characteristics, it is thought that they result from the fact that the patient population covers all of the in-hospital and out-of-hospital arrests.

In this study, pH value, which is the first blood gas parameter evaluated in patients who were applied CPR, was found as the independent indicator of ROSC and it was found to increase the probability of survival 0.43 times. Similarly, respiratory causes were also found as the independent indicator of ROSC and they were found to increase the probability of survival 2.76 times.

Conflict of interest

The author(s) declare no conflicts of interest.

Acknowledgments

None to declare.

Authors' contributions

Concept: E.N., M.A., A.Ç., Design: E.N., M.A., Data Collection or Processing: E.N., M.A., G.E., Ö.Y., G.A., Analysis or Interpretation: E.N., M.A., A.Ç., Literature Search: E.N., M.A., Ö.B., G.E., G.A., Writing: E.N., M.A.

References

1. Lick CJ, Aufderheide TP, Niskanen RA, Steinkamp JE, Davis SP, Nygaard SD et al. Take Heart America: A comprehensive, community-wide, systems-based approach to the treatment of cardiac arrest. Critical care medicine. 2011;39(1):26-33. doi:10.1097/CCM.0b013e3181fa7ce4

2. Nolan JP. Cardiac arrest and cardiopulmonary resuscitation. Seminars in Neurology. 2017;37(1):5-12. doi:10.1055/s-0036-15978327

3. Baldi E, Caputo ML, Savastano S, Burkart R, Klersy C, Benvenuti C et al. An Utstein-based model score to predict survival to hospital admission: The UB-ROSC score. International Journal of Cardiology. 2020;308:84-89. doi:10.1016/j.ijcard.2020.01.032

4. Sedig K, Seaton MB, Drennan IR, Cheskes S, Dainty KN. "Drones are a great idea! What is an AED?" novel insights from a qualitative study on public perception of using drones to deliver automatic external defibrillators. Resuscitation Plus. 2020;4(August 2020):100033. doi:10.1016/j.resplu.2020.100033

5. Olasveengen TM, Mancini ME, Perkins GD, Avis S, Brooks S, Castrén M et al. Adult Basic Life Support: International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Resuscitation. 2020;156:A35-A79. doi:10.1016/j.resuscitation.2020.09.010

6. Cakmak S, Sogut O, Albayrak L, Yildiz A. Serum Copeptin Levels Predict the Return of Spontaneous Circulation and the Short-Term Prognosis of Patients with Out-of-Hospital Cardiac Arrest: A Randomized Control Study. Prehospital and Disaster Medicine. 2020;35(2):120-127. doi:10.1017/S1049023X2000014X

7. Hajzargarbashi E, Omidi E, Esmailian M. Correlation of Patients' Baseline Characteristics with Success Rate of Cardiopulmonary Resuscitation; a Cross-Sectional Study. Advanced Journal of Emergency Medicine. 2018;3(1):1-5. doi:10.22114/AJEM.v0i0.115

8. Herlitz J, Engdahl J, Svensson L, Young M, Angquist KA, Holmberg S. Changes in demographic factors and mortality after out-of-hospital cardiac arrest in Sweden. Coronary artery disease. 2005;16(1):51-57. doi:10.1097/00019501-200502000-00010

9. Winther-Jensen M, Kjaergaard J, Wanscher M, Nielsen N, Wetterslev J, Cronberg T et al. No difference in mortality between men and women after out-of-hospital cardiac arrest. Resuscitation. 2015 Nov;96:78-84. doi: 10.1016/j.resuscitation.2015.06.030. Epub 2015 Jul 26. PMID: 26215480.

10. Corral Torres E, Hernández-Tejedor A, Suárez Bustamante R, de Elías Hernández R, Casado Flórez I, San Juan Linares A. Prognostic value of venous blood analysis at the start of CPR in non-traumatic out-of-hospital cardiac arrest: association with ROSC and the neurological outcome. Critical care (London, England). 2020;24(1):60. doi:10.1186/s13054-020-2762-5

11. Kim YJ, Lee YJ, Ryoo SM, Sohnl CH, Ahn S, Seo DW et al. Role of blood gas analysis during cardiopulmonary resuscitation in out-of-hospital cardiac arrest patients. Medicine. 2016;95(25):e3960. doi:10.1097/MD.0000000000003960

12. Çalbay A, Çakır Z, Bayramoğlu A. Prognostic value of blood gas parameters and end-tidal carbon dioxide values in out-of-hospital cardiopulmonary arrest patients. Turkish journal of medical sciences. 2019;49(5):1298-1302. doi:10.3906/sag-1812-156

13. Orban JC, Truc M, Kerever S, Novain M, Cattet F, Plattier R et al. Comparison of presumed cardiac and respiratory causes of out-of-hospital cardiac arrest. Resuscitation.2018;129:24 doi:10.1016/j.resuscitation.2018.05.033

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The prevalence of *TPH1* and *TPH2* genetic polymorphisms susceptible to irritable bowel syndrome among unrelated, healthy Malays in Malaysia

Rasmaizatul Akma ROSDI[1], Nurfadhlina MUSA[2], Zalina ZAHARI[3,*], Mohd Khairi ZAHRI JOHARI[4], Mulham ALFATAMA[3], Boon Yin KHOO[5]

[1]Biomedicine Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia
[2]Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia
[3]Faculty of Pharmacy, Universiti Sultan Zainal Abidin, Besut Campus, Terengganu, Malaysia
[4]Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Gong Badak Campus, Terengganu, Malaysia
[5]Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Penang, Malaysia

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Abstract

Tryptophan hydroxylase (TPH) gene which encodes the first rate-limiting enzyme in the serotonin biosynthesis pathway is one of the leading candidate genes in the etiology of the most common gastrointestinal (GI) disease, the irritable bowel syndrome (IBS). SNPs in the gene would distract the serotonergic function which led to the susceptibility to the syndrome. This study is aimed to determine the genotype distributions and allele frequencies of the three SNPs from two TPH genes, TPH1 and TPH2 genes among healthy, unrelated Malays in Malaysia. Nested-multiplex-allele specific PCR (NMAS-PCR) was subjected to 404 archived Malays' DNA to genotype rs211105, rs4537731 and rs4570625 variants following the validation of the obtained genotyping results through the direct Sanger sequencing. Results showed that the genotype frequencies of AA in rs211105 and rs4537731 among Malays were 59.2 and 51.5%. The heterozygous of GT was found to be slightly higher than GG with 47.5 to 43.3% in rs4570625. Meanwhile, the mutant allele frequencies of rs211105, G and rs4537731, T were relatively comparable with 30.3 to 33.0% accordingly. Concurrently, no departure of HWE was detected except in rs4537731. This study described low frequencies of *TPH1* and *TPH2* SNPs mutant variants associated with the IBS among unrelated, healthy Malays. Data generated from this study is important to enhance our knowledge of the association between IBS pharmacogenetic profiles and ethnic differences. Future studies on Malaysian IBS patients are recommended to determine the influence of rs211105, rs4537731 and rs4570625 on the syndrome locally.

Keywords: irritable bowel syndrome, rs211105, rs4537731, rs4570625, Malay, Malaysia

1. Introduction

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles in which the least common allele has a frequency of about 1% or greater. A genetic polymorphism would inscribe a difference in the DNA sequence among individuals, groups or populations. Understanding the functions of single nucleotide polymorphism (SNP) will significantly help to comprehend the variations of human phenotype in the genetic basis of complex human health and diseases (1). Therefore, local data on the types and frequencies of genetic polymorphisms of the putative genes among individuals or ethnicities is important as it can serve as a baseline for future studies on the associated health problems.

Serotonin (5-hydroxy tryptophan; 5-HT) is one of the most abundant neurotransmitter molecules in the gastrointestinal (GI) tract and has been considered an important enteric nervous system with essential roles in the gut (2). The biosynthetic pathway of serotonin is firstly underlying by the rate-limiting tryptophan hydroxylase enzyme (TPH) prior to its secretion in the GI motility and sensation (3). Due to that, any predisposition in the serotonergic system caused by the disruption of the enzyme will lead to the pathophysiology of the GI disorders including the most common, the irritable bowel syndrome (IBS) (4,5).

There are two isoforms of the TPH enzyme identified which associated with the clinical manifestations of IBS namely TPH1 and TPH2, encoded by the two different genes, *TPH1* and *TPH2*. The TPH1 gene is located on the chromosome 11p15.1, spanning more than 24 kbp, consisting of 10 exons and mainly expressed in the gut and peripheral organs. Meanwhile, the *TPH2* is located on the chromosome 12q21.1, spanning more than 247 kbp which composed of 13 exons and expressed mainly in the central nervous system and peripheral neurons. The genetic polymorphisms facilitating in the genes could alter the expression of the enzyme which cause to the susceptibility of the IBS in the individual (2,6). The

*Correspondence: zalinazahari@unisza.edu.my

SNPs of *TPH1* and *TPH2* genes such as rs211105, rs4537731 and rs4570625 are among the most extensively studied which relates to the IBS (7,8,9). The *TPH1* rs211105 polymorphism (known as 11:g.18033757T>G) which located within intron 3 showed some associations with the daily reporting of gastrointestinal symptoms including diarrhea, bloating and loose stools among women with the IBS (7) while rs4537731 polymorphism (known as −6526A/G, 11:g.18047335T>C) located at the upstream region (−6.5 kbp) where the homozygous minor allele (CC) carriers in the IBS patients were reported to experience severe diarrhea, bloating and a trend of more watery stool (9). These findings indicated that the SNPs might disrupt the function and level of 5-HT biosynthesis. On the other hand, previous studies also showed possible associations between a *TPH2* gene SNP in the promoter region, rs4570625 and the stool characteristics (7). The homozygous genotype of the minor allele (TT) of rs4570625 carriers experienced longer period of either with very hard or watery stools compared to the other genotype groups; GG and GT (7).

Even though the IBS may not be a life-threatening issue, the consequences have impacted the quality of life and become an economic burden to the societies (10,11). Among Malaysians, the prevalence of *TPH* gene variations of rs211105, rs4537731 and rs4570625 have not yet been studied. Therefore, this study will determine the types and frequencies of rs211105, rs4537731 and rs4570625 among unrelated healthy Malays of Malaysia. The findings from this study can be used as a database for future genetic association research on the IBS and other significant TPH-related medical diseases that involve with population- or geographic-specific analyses.

2. Materials and Methods

2.1. Ethics and general information

This study is an observational and comparative genotyping population research involving the largest major ethnic in Malaysia, the Malay. A total of 404 archived blood samples of healthy, unrelated Malays from a previous study; Development of Ethno-pharmacogenetics Relatedness and Personalised (Grant no. 1001/PSK/8620013), were used to investigate the SNPs of rs211105, rs4537731 and rs4570625. The subjects were categorized as healthy according to the blood donor's criteria. History of symptoms related to the IBS was not recorded in the study. The human ethical approvals were obtained from the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM), Kelantan, Malaysia (Reference number: USM/JEPeM/19020149) and the Universiti Sultan Zainal Abidin (UniSZA) Human Research Ethics Committee (UHREC), Terengganu, Malaysia (Reference number: UniSZA.C/2/UHREC/628-2/73).

2.2. Genotyping

Genomic DNA was isolated from 200µL of -20ºC EDTA blood using QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. A two-steps polymerase chain reaction (PCR); PCR1 and PCR2, combining the nested, multiplex and allele-specific techniques, was performed to determine the SNPs. The use of NMAS-PCR would provide 100% sensitivity, robust and reproducible genotyping result of the SNPs. Primers used in the study are listed in the Table 1 while the PCR1 and 2 thermocycler settings are listed in the Table 2. The DNA yield and PCR products were stained using a non-mutagenic DNA staining reagent and were electrophoresed in 1.5% agarose gel before examined under the UV light. The gel image was analyzed using a digital imaging and analysis system (Alphaimager, CA). Sanger sequencing was performed on a few samples for genotyping result validation using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, USA) according to the manufacturer's recommendations.

Table 1. List of the primers used to genotype rs211105, rs4537731 and rs4570625 through NMAS-PCR. For ease of reference, primers have been appended with 'FW' and 'RV', referring to forward and reverse sequences for particular gene/SNP. Each set of PCR2 primers consisted of a PCR1 primer sequence and a newly designed primer to exhibit a specific allele variant either wild or variant type (bold)

SNP	PCR1		PCR2	
	Primer sequence	Product size (bp)	Primer sequence	Product size (bp)
rs211105	*TPH1a_FW* AAC CAA GGA ACA GTT TCC ATA CCT *TPH1a_RV* AAA CAG AAG GGT AGG GTG GG	571	*TPH1a_FW* AAC CAA GGA ACA GTT TCC ATA CCT *rs211105A_RV (Wild type)* GAT TTC TAA GAT CTT TTC CAT CGG C**A** *rs211105C_RV (Variant type)* GAT TTC TAA GAT CTT TTC CAT CGG C**C**	430
rs4537731	*TPH1b_FW* AGG ACT GTA CAC ATA ACG AAG TAT *TPH1b_RV* TGA AAG GTC TCT CCC TGA CCA	664	*rs4537731T_FW (Wild type)* TGG ATG TAC TTT AAA GCT CAG GA**T** *rs4537731C_FW (Variant type)* TGG ATG TAC TTT AAA GCT CAG GA**C** *TPH1b_RV* TGA AAG GTC TCT CCC TGA CCA	251
rs4570625	*TPH2_FW* GCT TTC TCC TCA CCA CAT AAC G *TPH2_RV* CTG GCA AGT TAA CCT CAG TCT	801	*TPH2_FW* GCT TTC TCC TCA CCA CAT AAC G *rs4570625C_RV (Wild type)* AGC TTT TTC TGA CTT GAC ATA TT**C** *rs4570625A_RV (Variant type)* AGC TTT TTC TGA CTT GAC ATA TT**A**	390

Table 2. Thermal cycling conditions for PCR1 and 2. The amplifications product of PCR1 were used as template in the PCR2. PCR2A was used to detect rs211105 variants and PCR2B for the detection of rs4537731 and rs4570625 variants concurrently. The lid of the thermal cycler was set at 105°C

PCR profile	PCR1			PCR2					
				A			B		
	Temp. (°C)	Time	Cycles	Temp. (°C)	Time	Cycles	Temp. (°C)	Time	Cycles
Pre-denaturation	95	5 min	1	95	2 min	1	95	2 min	1
Denaturation	95	30 sec	34	95	30 sec	20	95	30 sec	20
Annealing	65	30 sec		68	30 sec		62	30 sec	
Extension	72	1 min		72	30 sec		72	30 sec	
Final extension	72	5 min	1	72	5 min	1	72	5 min	1
Hold	12	∞	-	12	∞	-	12	∞	-

2.3. Statistical analysis

The data of the SNPs derived from this study were analyzed using descriptive statistics. The observed genotype distributions were expressed in count and percentage and were used to calculate the percentage of the allele frequencies. Possible deviation from Hardy–Weinberg equilibrium (HWE) was tested for statistical significance by comparing the observed and expected genotypes frequency using the chi-square (χ^2) test with one degree of freedom. *P*-value of <0.05 was considered statistically significant. All statistical calculations were performed using SPSS ver. 20 Windows (SPSS Inc., Chicago, IL).

3. Results

This study employed a total of 404 DNA samples from an unrelated, healthy Malay ethnic of Malaysia. The male subjects were 328 while the females were 76. Their ages were between 19 and 55 years with the average being 29 years old. All SNPs were successfully genotyped in all DNA samples. Table 3 shows the genotype distributions and allelic frequencies of *TPH1* (rs211105 and rs4537731) and *TPH2* (rs4570625) polymorphisms among Malays in Malaysia. All variants of the three SNPs; rs211105 (AA, AC, CC), rs4537731 (AA, AG, GG) and rs4570625 (GG, GT, TT) were presented in the Malay subjects of study. However, it only demonstrated considerably low frequencies of allele mutant variants associated with the IBS; rs211105 (C- 24.0%), rs4537731 (G- 30.3%) and rs4570625 (T- 33.0%). The carriers of the mutant variant among Malays were also low with 7.1% in rs211105, 12.1% in rs4537731 and 9.2% in rs4570625. The genotype frequencies of AA in rs211105 and rs4537731 in Malays were 59.2 and 51.5% accordingly. Meanwhile, in rs4570625, the heterozygous GT was found to be slightly higher than GG with 47.5 to 43.3%. No departure of Hardy-Weinberg equilibrium was detected suggesting no unexpected genetic drift or sampling bias occurred except for the rs4537731 ($\chi^2 = 7.79$; $P < 0.005$). To the best of our knowledge, the current study was the first to publish on the polymorphisms of rs211105, rs4537731 and rs4570625 among Malaysian ethnics.

Table 3. Genotype distributions and allele frequencies of *TPH1* and *TPH2* polymorphisms (rs211105, rs4537731 and rs4570625) among Malays

SNP	Observed genotypes		Predicted genotypes		χ^2 test	*P*-value	Allele frequencies (%)	
	n	%	*n*	%				
rs211105								
TT	239	59.2	233	57.7			A	76.0
TG	136	33.7	148	36.5	2.42	0.119	C	24.0
GG	29	7.1	23	5.8				
rs4537731								
TT	208	51.5	196	48.6			A	69.7
TC	147	36.4	171	42.3	7.79	0.005	G	30.3
CC	49	12.1	37	9.2				
rs4570625								
GG	175	43.3	182	45.0			G	67.0
GT	192	47.5	178	44.1	2.33	0.126	T	33.0
TT	37	9.2	44	10.9				

Abbreviation: *n* – number of samples

4. Discussion

Tables 4, 5 and 6 exhibit the comparisons of the rs211105, rs4537731 and rs4570625 variants between the Malay from this study and other ethnics around the world. The ethnics tabulated in the Tables 4-6 were depicted from the healthy, control group of comparatives, case-controlled studies related to the SNPs. According to the Table 4, the rs211105 TT genotype was the most frequent genotype detected in all ethnicities; 46.5-71.9%, with the allele frequencies were ranging between 68.5 to 85.9%. Japanese was detected to derive the least of the G variant allele frequency with 14.1% (8). Apparently, the findings from the table were in concordance with the genotype distributions obtained from 1000 Genomes Project Phase 3 (www.internationalgenome.org) data where AA is also the major genotype in many ethnics worldwide for instances, among African Caribbean (91.7%), Pakistani (69.8%), Mexican (53.1%) and Vietnamese Kinh (50.5%). Meanwhile,

an epidemiology study showed that the IBS occurred less frequently among African Americans than the Whites (12). The finding was in agreement with the 1000 Genomes Project Phase 3 data where the frequency of the G minor allele was very low (less than 5%) among Africans compared to the Europeans; above 20%. This suggests that the rs211105 variant may a common genetic cause or pathophysiology of the TPH-related diseases including the IBS. More studies should be performed to solidify the findings.

Table 4. Comparisons of genotype distributions and allele frequencies of rs211105 in Malays and other reported ethnics worldwide

Ethnic	*n* (*M/F*)	Mean age	Observed genotypes, *n* (%)			Allele frequencies (%)		Reference
			TT	TG	GG	T	G	
Malay	404 *(328/76)*	29	239 (59.2)	136 (33.7)	29 (7.1)	76.0	24.0	Present study
Caucasian	79 (*0/79*)	36	48 (60.8)	28 (35.4)	3 (3.8)	78.5	21.5	(7)
Japanese	64 *(nr)*	nr	46 (71.9)	18 (28.1)	0 (0)	85.9	14.1	(8)
Han Taiwanese	84 *(0/84)*	29.7	39 (46.5)	38 (45.2)	7 (8.3)	68.5	31.5	(19)
Caucasian (Olsztyn)	91 (*66/25*)	44.2	54 (59.3)	34 (7.1)	3 (3.3)	78.0	22.0	(21)
Scandinavian	1473 (*812/661*)	44.1	839 (57.4)	520 (35.6)	102 (7.0)	75.2	24.8	(22)
Swedish	132 (*78/54*)	27	76 (58.0)	50 (38.2)	5 (3.8)	77.0	23.0	(27)

Abbreviations: *n* – number of samples, M – male, F – female, nr – not reported.

With respect to the rs4537731 (Table 5), Caucasians of the Toronto were noted to possess higher allele frequency of C, almost two-fold than the T's (35%) in the population (13). On the other hand, the substitution of T to C of the SNP only appeared in heterozygous genotypes where no GG carrier was detected among Japanese population (8). Data of rs4537731 allele frequency derived from the Allele Frequency Aggregator (ALFA) project (https://www.ncbi.nlm.nih.gov/snp) also recorded multiple trends among different ethnicities and geographical. The GG genotype carriers were the dominant among Africans of Kenya, Nigeria, Gambia and Sierra Leone; 68.1-73.2%. While among East Asians, for instances Chinese, Vietnamese and Japanese, AA carriers are the biggest proportion in the populations with the average frequency was 77.3% (12). According to a study conducted in Caucasian females of IBS patients, the homozygous of the minor allele C carriers experienced more severe diarrhea symptoms than the other two genotype groups of the patients (7). However, Katsumata et al. (8) have found no link between the *TPH1* rs4537731 SNP and the GI symptoms among Japanese people.

Table 5. Comparisons of genotype distributions and allele frequencies of rs4537731 in Malays and other reported ethnics worldwide

Ethnic	*n* (*M/F*)	Mean age	Observed genotypes, *n* (%)			Allele frequencies (%)		Reference
			TT	TC	CC	T	C	
Malay	404 (*328/76*)	29	208 (51.5)	147 (36.4)	49 (12.1)	69.7	30.3	Present study
Caucasian	79 (*0/79*)	36	29 (36.7)	41 (51.9)	9 (11.4)	62.7	37.3	(7)
Japanese	66 *(nr)*	nr	39 (59.0)	27 (41.0)	0 (0)	79.5	20.5	(8)
Caucasian (Toronto)	30 (*0/30*)	nr	4 (13.3)	13 (43.3)	13 (43.3)	35.0	65.0	(13)
Swedish	132 (*78/54*)	27	49 (37.1)	68 (51.5)	15 (11.4)	62.9	37.1	(20)
Scandinavian	1473 (*812/661*)	44.1	470 (32.0)	725 (49.3)	275 (18.7)	56.6	43.4	(22)

Abbreviations: *n* – number of samples, M – male, F – female, nr – not reported.

In the Table 6, the allele frequencies of the SNP from chromosome 12, rs4570625, seemed to have unique patterns in the observed genotype distributions among ethnicities. Korean exhibited equal carriers of G and T variants in the population according to the studies of Han et al. (14) and Serretti et al. (15), but Kim et al. (16) manifested that the ethnic derived higher minor allele carriers of T, 57.5% in the population. In contrast, Germany demonstrated the observable vice versa genotype distributions in two different findings where Baehne et al. (17) reported the population consisted of high homozygous G carriers, 67.9% while Reuter et al. (18) proved that Germany was high as homozygous T carriers, 57.4%. Interestingly, it was found that the East Asian communities were the only populations who have T allele frequencies of more than 50% while the rest of the populations in the world for examples American, African, South Asian and European, exhibited of less than 35% of the allele according to the 1000 Genomes database. Overall, based on the presented findings in

this study, studies cited above and the databases, it is understandable that the rs211105, rs4537731 and rs4570625 polymorphisms are commonly found in the individual or ethnics.

Table 6. Comparisons of genotype distributions and allele frequencies of rs4570625 in Malays and other reported ethnics worldwide

Ethnic	*n* (*M/F*)	Mean age	Observed genotypes, *n* (%)			Allele frequencies (%)		Reference
			GG	GT	TT	G	T	
Malay	404 (*328/76*)	29	175 (43.3)	192 (47.5)	37 (9.2)	67.0	33.0	Present study
Caucasian	79 (*0/79*)	36	46 (58.2)	25 (31.6)	8 (10.1)	74.0	26.0	(7)
Korean	86 (*59/27*)	nr	30 (34.9)	40 (46.5)	16 (18.6)	58.1	41.9	(14)
Korean	170 (*105/65*)	38.8	57 (33.5)	77 (45.3)	36 (21.2)	56.2	43.8	(15)
Korean	247 (*125/122*)	39.4	45 (18.2)	120 (48.6)	82 (33.2)	42.5	57.5	(16)
Germany	84 (*44/40*)	34.8	57 (67.9)	24 (28.6)	3 (3.6)	82.1	17.9	(17)
Germany	404 *(113/291)*	23.7	19 (4.7)	153 (37.9)	232 (57.4)	23.6	76.4	(18)
Polish	230 (*114/116*)	53.2	48 (20.9)	179 (77.8)	3 (1.3)	59.8	40.2	(28)
Chinese Han	244 (*126/118*)	38.5	97 (39.7)	110 (45.1)	37 (15.2)	62.3	37.7	(29)

Abbreviations: *n* – number of samples, M – male, F – female, nr – not reported.

The polymorphisms of rs211105, rs4537731 and rs4570625 also were documented to have significant correlations with other etiology of pathophysiology and clinical manifestations of depression and anxiety (19), psychosis (20), acute pancreatitis (21), schizophrenia (22) and many more. The SNPs implicate the alterations of protein expression of the TPH enzyme that can cause serotonin deficiency and distract the serotonergic functions (23,24). The lack of serotonin will lead to many problems including abdominal symptoms, visceral hypersensitivity, functional dyspepsia, unpredictable or violent behaviors and other neurological disorders (25,26). Eventually, this would lead to a higher susceptibility to exhibit the IBS and other related health problems. Therefore, research on the prevalence of the SNPs such as from this study is crucial in order to enhance a better understanding of the ethnic differences to the most common functional GI disorders, the IBS and provide some clues for the other health problems.

The investigation of the SNPs in the populations provides wider insight and some keys to the human phenotype variations individually or ethnically, especially when it relates to the health issues and diseases. This study described the prevalence of *TPH1* and *TPH2* SNPs susceptible to the IBS among unrelated, healthy Malays in Malaysia. The study proved only low frequencies of the mutant alleles of the SNPs among the Malays. The diverse trends observed in the distributions of genotypes and alleles frequencies of rs211105, rs4537731 and rs4570625 in different ethnic groups worldwide would play a decisive role in the IBS cohorts. Data generated from this study is beneficial to enhance our knowledge of the association between IBS pharmacogenetic profiles and the ethnic differences. While many researchers continue to investigate various possible factors to overcome and combat the symptoms of the IBS, data reported in the present study has provided a genetic hint of the *TPH* polymorphisms' roles on the pathophysiology. Future studies on Malaysian IBS patients are recommended to determine the susceptibility of rs211105, rs4537731 and rs4570625 to the IBS syndrome locally.

Conflict of interest

All authors declared having no conflict of interest.

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Authors' contributions

Concept: Z.Z., K.B.Y., M.A., Design: Z.Z., M.K.Z.J., Data Collection or Processing: N.M., Z.Z., M.K.Z.J., Analysis or Interpretation: N.M., R.A.R., Literature Search: R.A.R., M.A., Writing: R.A.R., N.M.

References

1. Relling MV, Evans WE. Pharmacogenomics in the clinic. Nature. 2015; 526(7573): 343-350.
2. Zhu S, Wang B, Jia Q, Duan L. Candidate single nucleotide polymorphisms of irritable bowel syndrome: A systemic review and meta-analysis. BMC Gastroenterol. 2019; 19(165): 1-10.
3. Davies A, Rodriguez-Vicente AE, Austin G, Loaiza S, Foroni L, Clark RE, et al. Serotonin re-uptake transporter gene polymorphisms are associated with imatinib-induced diarrhoea in chronic myeloid leukaemia patients. Sci Rep. 2020; 10(8394): 1-9.

4. De Bortoli N, Tolone S, Frazzoni M, Martinucci I, Albano E, Ceccarelli L, Stasi C, et al. Gastroesophageal reflux disease, functional dyspepsia and irritable bowel syndrome: Common overlapping gastrointestinal disorders. Ann Gastroenterol. 2018; 31: 639-648.

5. Talley NJ. What Causes Functional Gastrointestinal Disorders? A Proposed Disease Model. Am J Gastroenterol. 2020; 115(1): 41-48.

6. Xiao QY, Fang XC, Li XQ, Fei GJ. Ethnic differences in genetic polymorphism associated with irritable bowel syndrome. World J Gastroenterol. 2020; 26(17): 2049-2063.

7. Jun S, Kohen R, Cain KC, Jarrett ME, Heitkemper MM. Associations of tryptophan hydroxylase gene polymorphisms with irritable bowel syndrome. Neurogastroenterol Motil. 2011; 23(3): 233-240.

8. Katsumata R, Shiotani A, Murao T, Ishii M, Matsumoto H, et al. The TPH1 rs211105 gene polymorphism affects abdominal symptoms and quality of life of diarrhea-predominant irritable bowel syndrome. J Clin Biochem Nutr. 2018; 62(3): 270-276.

9. Shiotani A, Kusunoki H, Ishii M, Imamura H. Manabe N, Hata J, et al. Pilot study of Biomarkers for predicting effectiveness of ramosetron in diarrhea-predominant irritable bowel syndrome: Expression of S100A10 and polymorphisms of *TPH1*. Neurogastroenterol Motil. 2015; 27: 81-91.

10. Sperber AD, Bangdiwala SI, Drossman DA, Ghoshal UC, Simren M, Tack J, et al. Worldwide Prevalence and Burden of Functional Gastrointestinal Disorders, Results of Rome Foundation Global Study. Gastroenterology. 2021; 160: 99-117.

11. Yeo A, Boyd P, Lumsden S, Saunders T, Handley A, Stubbins M, et al. Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. Gut. 2004; 53: 1452-1458.

12. Wigington WC, Johnson WD, Minocha A. Epidemiology of irritable bowel syndrome among African Americans as compared with Whites: A Population-Based Study. Clin Gastroenterol Hepatol. 2005; 3(7): 647-653.

13. Ruocco AC, Rodrigo AH, Dean C. Tryptophan Hydroxylase 1 gene polymorphisms alter prefrontal cortex activation during response inhibition. Neuropsychology. 2016; 30(1): 18-27.

14. Han KM, Won E, Kang J, Kim A, Yoon H-K, Chang HS, et al. Local gyrification index in patients with major depressive disorder and its association with Tryptophan hydroxylase-2 (*TPH2*) polymorphism. Hum Brain Mapp. 2017; 38: 1299-1310.

15. Serretti A, Chiesa A, Porcelli S, Han C, Patkar AA, Lee S-J, et al. Influence of *TPH2* variants on diagnosis and response to treatment in patients with major depression, bipolar disorder and schizophrenia. Psychiatry Res. 2011; 189: 26-32.

16. Kim YK, Lee HJ, Yang JC, Hwang JA, Yoon HK. A tryptophan hydroxylase 2 gene polymorphism is associated with panic disorder. Behav Genet. 2009; 39(2): 170-175.

17. Baehne CG, Ehlis AC, Plichta MM, Conzelmen A, Jacob C, Gutknecht L, et al. Tph2 gene variants modulate response control processes in adult ADHD patients and healthy individuals. Mol Psychiatry. 2009; 14(11): 1032-1039.

18. Reuter M, Kuepper Y, Hennig J. Association between a polymorphism in the promoter region of the TPH2 gene and the personality trait of harm avoidance. Int J Neuropsychopharmacol. 2007; 10: 401-404.

19. Sun HS, Tsai HW, Ko HC, Chang FM, Yeh TL. Association of tryptophan hydroxylase gene polymorphism with depression, anxiety and comorbid depression and anxiety in a population-based sample of postpartum Taiwanese women. Genes, Brain Behav. 2004; 3: 328-336.

20. Andreou D, Söderman E, Axelsson T, Sedvall GC, Terenius L, Agartz I, et al. Polymorphisms in genes implicated in dopamine, serotonin and noradrenalin metabolism suggest association with cerebrospinal fluid monoamine metabolite concentrations in psychosis. Behav Brain Funct. 2014; 10(26): 1-10.

21. Snarska J, Fiewdorowicz E, Rozmus D, Wronski K, Latacz M, Kordulewska N, et al. TPH1 gene polymorphism rs211105 influences serotonin and tryptophan hydroxylase 1 concentrations in acute pancreatitis patients. Res Sq. Published online 2021: 1-21.

22. Saetre P, Lundmark P, Wang A, Hansen T, Rasmussen HB, Djurovic S, et al. The Tryptophan Hydroxylase 1 (TPH1) gene, schizophrenia susceptibility, and suicidal behavior: A multi-centre case-control study and meta-analysis. Am J Med Genet Part B. 2010; 153(B): 387-396.

23. Liu X, Li H, Qin W, He G, Li D, Shen Y, et al. Association of *TPH1* with suicidal behaviour and psychiatric disorders in the Chinese population. J Med Genet. 2006; 43(e4): 1-6.

24. Tao S, Chattun MR, Yan R, Geng J, Zhu R, Shao J, et al. Tph-2 gene polymorphism in major depressive disorder patients with early-wakening symptom. Front Neurosci. 2018; 12(NOV): 1-10.

25. Camilleri M. Implications of Pharmacogenomics to the Management of IBS. Clin Gastroenterol Hepatol. 2019; 17(4): 584-594.

26. Narayanan SP, Anderson B, Bharucha AE. Sex- and Gender-Related Differences in Common Functional Gastroenterologic Disorders. Mayo Clin Proc. 2021; 96(4): 1071-1089.

27. Andreou D, Saetre P, Werge T, Andreassen OA, Agartz I, Sedvall GC, et al. Tryptophan hydroxylase gene 1 (*TPH1*) variants associated with cerebrospinal fluid 5-hydroxyindole acetic acid and homovanillic acid concentrations in healthy volunteers. Psychiatry Res. 2010; 180(2-3): 63-67.

28. Wigner P, Czarny P, Synowiec E, Bijak M, Bialek K, Talarowska M, et al. Association between single nucleotide polymorphisms of TPH1 and TPH2 genes, and depressive disorders. J Cell Mol Med. 2018; 22(3): 1778-1791.

29. Xu XM, Ding M, Pang H, Wang BJ. TPH2 gene polymorphisms in the regulatory region are associated with paranoid schizophrenia in Northern Han Chinese. Genet Mol Res. 2014; 13(1): 1497-1507.

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Sex-related differences in anxiety in experimental functional dyspepsia induced by chronic stress

Osman SİNEN*

Department of Physiology, Faculty of Medicine, Akdeniz University, Antalya, Türkiye

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Abstract

Functional dyspepsia (FD) is associated with gastric sensorimotor dysfunction (including delayed gastric emptying (GE)) and psychosocial comorbidities. Anxiety is among the many psychiatric disorders that are related to FD. The aim of this study was to compare the effectiveness of chronic sequential stress to create an experimental FD model in male and female rats. The FD model was established by neonatal maternal separation (MS) early in life and repeated homotypic stress (RHS) in adulthood. Newborn pups from postnatal day 1 to day-21 underwent MS for 3 h. In adulthood, the control or maternally separated rats were loaded with RHS for 7 days comprised of 90-min restraint stress. The anxiety-like behaviors were evaluated by the open field (OF) and elevated plus maze (EPM) tests. To validate the experimental FD model, body weight and solid GE were measured in rats after the behavioral experiments. Compared with control males, body weight and GE were significantly ($p<0.05$) decreased in stressed rats, but not in females. Exposed to chronic stress male rats appeared to exhibit more anxiety-like behavior than control male rats on the OF and EPM. In contrast to the males, no significant differences were found in female groups. Unlike female rats, the male rats appear to be highly suitable to create an experimental FD model under chronic sequential stressed conditions. Therefore, anxious behaviors may not be observed in females due to the absence of dyspeptic symptoms.

Keywords: anxiety, gender, functional dyspepsia, chronic stress

1. Introduction

Exposure to acute or chronic stress is a major risk factor for various functional gastrointestinal disorders (FGID) such as irritable bowel syndrome and functional dyspepsia (FD) (1-3). Adverse events in early life are one of the risk factors for developing FD in adulthood (4, 5). FD is characterized by upper gastrointestinal symptoms such as bloating, early postprandial satiety, epigastric pain, and burning (6, 7). Moreover, delayed gastric emptying (GE), body weight loss, and anxiety are considered to be pathophysiological features of FD (8, 9). The detection rate of anxiety symptoms in FD is 25.5% (10).

Recently, different experimental FD models have offered insights into understanding the FD process, its, pathology, etiology, and molecular mechanisms (11-13). The decision on which model should be chosen as a basis for researching FD varies depending on the study aims and questions of interest. Compound animal models with multiple factors contributing to the development of FD have come to the fore in recently published studies (14-17). It is thought that FD seen in adulthood may be caused by early stress exposure and re-exposure to stress later in life may aggravate the situation. Therefore, in this study, the experimental FD model was established by neonatal maternal separation (MS) early in life and repeated homotypic stress (RHS) in adulthood.

The purpose of this study is to examine sex-related differences in anxiety in chronically stressed rats. Especially, the suitability of the chronic sequential stress model, which is known to be effective in male rats, was tested for female rats.

2. Material and Methods

2.1. Animals

Adult male and female Wistar rats weighing 250-300 g were maintained on a 12 h:12 h dark-light cycle (starting at 6:00 AM) with access to food and water ad libitum. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethical Committee of Akdeniz University (with unique authorization number B.30.2.AKD.0.05.07.00/138).

2.2. Chronic sequential stress model for functional dyspepsia (FD)

For the experimental FD model, chronic sequential stress was loaded with MS early in life and RHS in adulthood, as described elsewhere (14, 15). Newborn pups initially underwent neonatal MS for 3 h from postnatal day (PND)-1 to day-21. The control (non-stressed) pups were non-handled and kept with their dams. On PND 21, animals were weaned and

*Correspondence: osmansinen@akdeniz.edu.tr

sexed. Male control and MS pups were kept with their littermates until the experiments performed at their 12-week-old age. Adult MS rats were exposed to RHS comprised of 90-min restraint stress for 7 consecutive days. To confirm that body weight loss and delayed gastric emptying (GE) in our model, body weight was recorded and solid GE was measured in overnight fasted rats exposed to chronic stress or non-stressed animals after the behavioral experiments. The experimental procedures are schematically summarized in Fig.1.

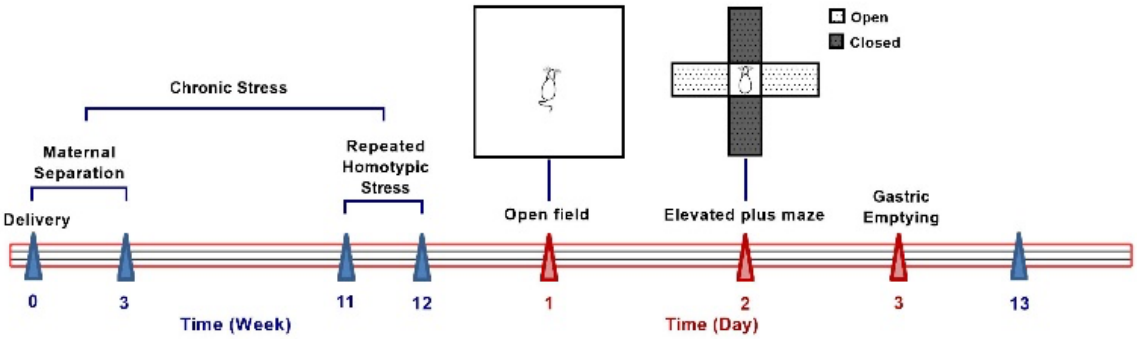

Fig. 1. Representative flow chart of the experimental design. The experiments were performed after the chronic stress loading

2.3. Measurement of gastric emptying (GE)

After the behavioral experiments, animals underwent overnight fasting, as previously reported (18, 19). Overnight fasting rats were given pre-weighed pellets (1.6 g). Immediately after the completion of feeding, rats were euthanized by urethane (1.25 g·kg-1, i.p) and the stomachs were removed and emptied thoroughly. The gastric contents were collected and dried at room temperature. After weighing, GE was calculated according to the following formula:

%GE=1-(weight of the dried content/weight of the pellet) ×100

2.4. Measurement of anxiety-like behaviors

The open field (OF) test and elevated plus maze (EPM) test were used to FD-induced anxiety-related behaviors. The OF and EPM were performed as described in previous studies (20-22). Briefly, the rats were gently placed in the center of an open field chamber. A more anxious animal would present more time at the periphery than in the center. For EPM, each animal was placed onto the central platform facing an open arm. The increase in open arm activity reflects less anxiety-like behavior. All rats were allowed to freely explore the maze for 5 min. Video-tracking system (Ethovision XT, Noldus Information Technology, Netherlands) was used to record and analyze the behavioral tests. The anxiety-related behaviors were evaluated by measuring the time spent in closed/open arms (s), the number of open arm entries, time in center/periphery (s), and entries in the center. An increase in periphery or close arm duration reflects anxiety-related behavior. All areas were cleaned with 70% ethanol solution after each test.

2.5. Statistics

All statistical analyses were performed using Prism 9 software (GraphPad Software, Inc, La Jolla, CA). All data are presented as mean ± standard error of the mean (SEM). Shapiro-Wilk test was used to determine whether the data were normally distributed. The non-parametric Mann Whitney-U test was used to assess the significance. A p value <0.05 was considered as statistically significant.

3. Results

3.1. Validation of functional dyspepsia model

In order to validate the experimental FD model, body weight and solid GE were measured. The body weight of male rats exposed to chronic stress was significantly decreased (258.6±15.3 g, $p<0.05$, n=6) compared with the control male rats (341.4±5.3 g, n=6). Unlike males, chronic stress did not affect the body weight of female rats (194±5.4 g, n=6) compared to that in control females (188.3±6.2 g, n=6), (Fig. 2A)

The measured GE in control male rats was 66.2±5.6 (n=6), however, it was significantly delayed in male rats exposed to chronic stress (37.6±5, $p<0.05$, n=6). In contrast to the males, chronic stress had no significant effect on females (control: 54.1±4.2%; chronic stress: 60.2±5.4%, n=6), (Fig. 2B)

Taken together, these results suggest that the experimental FD model was established successfully in male rats, however, it is ineffective in female rats.

3.2. Sex-related differences in anxiety

To investigate the effect of chronic stress on anxiety-like behavior, we performed the OF and EPM tests. Exposed to chronic stress male rats appeared to exhibit more anxiety-like behavior than control male rats on the OF, as indicated by greater total time spent in the periphery (control: 283.7±2 s; chronic stress: 293.2±2.3 s, $p<0.05$, n=6) and lower spent in center (control: 11.7±0.9 s; chronic stress: 1.2±0.3 s, $p<0.05$, n=6) of the maze. Similarly, the number of entries to the center zone of the OF was also significantly less in stressed male rats (5.3±0.4, $p<0.05$, n=6) compared to control males (15.4±2.2,

n=6), but not in females. Unlike males, no differences were found in time spent in the center/periphery of the OF between the stressed and non-stressed female groups (Fig.3).

In the EPM test, chronic stress-treated male rats spent more time in closed arms (254.9±16.8 s, $p<0.05$, n=6) than control counterparts (165.9±27 s, n=6), and stressed males also spent less time in open arms (230±4.9 s, $p<0.01$, n=6) than non-stressed males (114.2±13.2 s, n=6), which are the common indexes of anxiety-like behavior. In contrast to the males, no differences were found in time spent in open/closed arms and the number of open arms entries between female groups (Fig.4).

These results suggest that chronic stressed male rats demonstrated typical anxiety-like behaviors, but not in females.

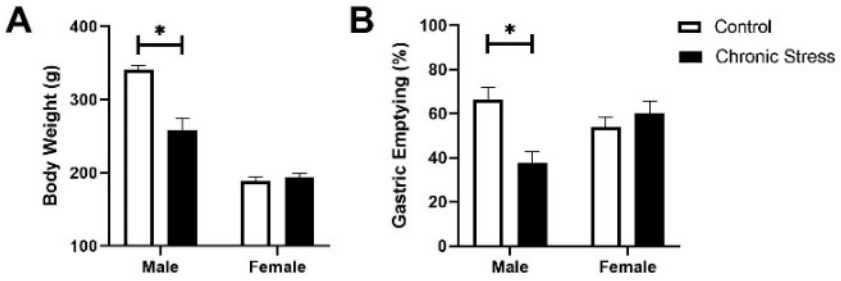

Fig. 2. The effect of chronic stress on body weight (A) and gastric emptying (B) in male and female rats. Chronic stress decreased body weight and gastric emptying in males. *p<0.05, control vs chronic stress. Mann Whitney-U test was used to carry out statistical comparisons, n=6 rats per group. All values are means ± SEM

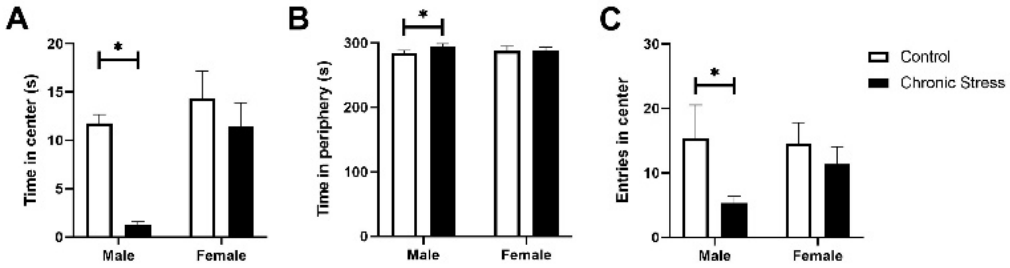

Fig. 3. The effect of chronic stress on the behavior of male and female rats in the open field test. Chronic stress increased anxiety-related behaviors in males. *p<0.05, control vs chronic stress. Mann Whitney-U test was used to carry out statistical comparisons, n=6 rats per group. All values are means ± SEM

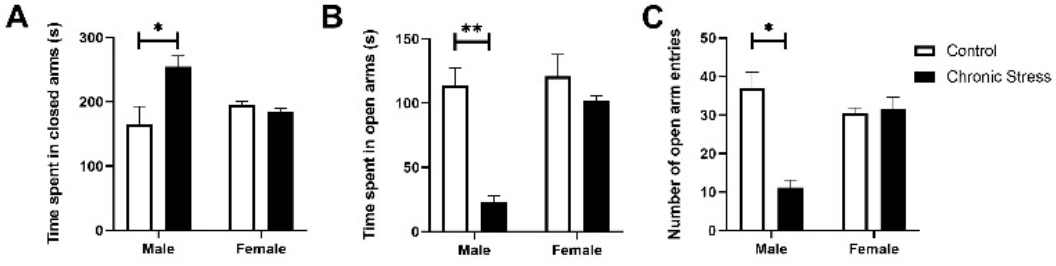

Fig. 4. The effect of chronic stress on the behavior of male and female rats in the elevated plus maze. Chronic stress increased anxiety-related behaviors in males. *p<0.05, **p<0.01, control vs chronic stress. Mann Whitney-U test was used to carry out statistical comparisons, n=6 rats per group. All values are means ± SEM

4. Discussion

The findings of this study indicate that exposure to chronic sequential stress could lead to an experimental FD model with decreased body weight, GE, and increased anxiety-like behavior in male rats, but not in females. A summary of the all results is shown in Fig.5.

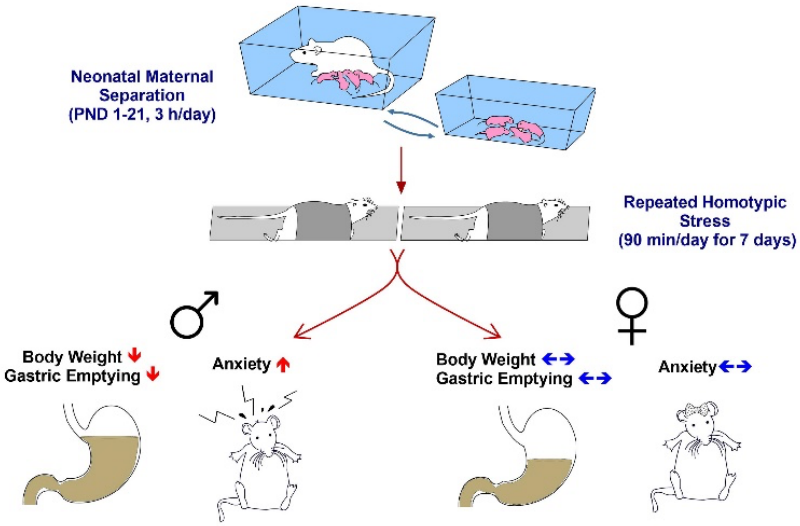

Fig. 5. A schematic summary of the results.

Stress contributes to the onset and exacerbation of symptoms in the majority of FGID (1). Thus, the different stress models are widely used as an experiment for the investigation of FGID or FD (14-16). In recent preclinical studies, a compound animal model that includes two or more stressors has been preferred for FD development (14-17). However, no sex differences have also been reported in these studies. Thus, the efficacy of this compound model in female rats is still unknown. Consequently, in this study, the experimental FD model was established by neonatal MS followed by adult RHS in male and female rats to observe sex-related differences in anxiety.

Pathophysiologic mechanisms of FD include slowed gastric emptying, impaired gastric accommodation, visceral hypersensitivity, duodenal inflammation, and psychosocial factors (6, 23). Evidence from epidemiologic studies suggests dyspeptic patients experience early satiety and weight loss due to gastric accommodation disorder (9, 24). Thus, in this study, body weight and GE were measured to validate the experimental FD model. Our present data showed that the body weight decreased only in chronically stressed male rats compared to non-stressed rats. In rodent studies, FD-induced delayed GE has been found in several measures (15, 25, 26). In this study, dyspeptic symptoms expected to occur in animals in the FD group were evaluated by the measurement of solid GE. Similar to previous studies, delayed GE has been detected in male stressed rats. On the other hand, no significant effect of chronic stress was observed in females. These results suggest that the experimental FD model was established successfully in male rats as previously reported in studies using chronic sequential stress, however, it is ineffective in female rats. Importantly, it was shown that the acute stress-induced gastrointestinal motor dysfunction were completely recovered following 5-day RHS loading (27, 28). In response to a chronic sequential stressor, adaptation may have occurred in female rats.

Anxiety, one of the most common symptoms among FD patients, has a high (>30%) incidence clinically (29). In this study, anxiety was evaluated with EPM and OF tests, which are frequently used in the literature to assess anxiety-related behaviors (30, 31). Consistent with the literature, exposed to chronic sequential stress male rats presented distinctive anxiety-like behavior, but not in females. Considering that even MS alone causes anxiety-like behaviors (32, 33), possible adaptation mechanisms in female rats due to RHS-loading may be in question. The discrepancies may also relate to the estrous cycle. Multiple studies have demonstrated the anxiolytic effects of both estradiol and progesterone (34-36). Future experiments about gender differences in stress responses are needed to confirm such a possibility.

The efficacy of this chronic sequential stress model in female Wistar rats is unknown. The effectiveness of this chronic sequential stress on female rats was first demonstrated in this study. Taken together, the male Wistar rats are more suitable to create an experimental FD model under chronic sequential stressed conditions compared to the females. It is important to note that the males seem to be vulnerable to chronic sequential stress.

Conflict of interest

No conflicts of interest exist

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None to declare.

Authors' contributions

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References

1. Monnikes H, Tebbe JJ, Hildebrandt M, Arck P, Osmanoglou E, Rose M, et al. Role of stress in functional gastrointestinal disorders - Evidence for stress-induced alterations in gastrointestinal motility and sensitivity. Digest Dis. 2001;19(3):201-11.
2. Duan C, Chen C, Ouyang Z, Duan C, Zhang S, Shang H, et al. Association of stress and functional gastrointestinal disorders in high school graduates. J Affect Disord. 2021;292:305-10.
3. Richter JE. Stress and psychologic and environmental factors in functional dyspepsia. Scand J Gastroenterol Suppl. 1991;182:40-6.
4. Bonilla S, Saps M. Early life events predispose the onset of childhood functional gastrointestinal disorders. Rev Gastroenterol Mex. 2013;78(2):82-91.
5. Winston JH, Sarna SK. Enhanced sympathetic nerve activity induced by neonatal colon inflammation induces gastric hypersensitivity and anxiety-like behavior in adult rats. Am J Physiol Gastrointest Liver Physiol. 2016;311(1):G32-9.
6. Enck P, Azpiroz F, Boeckxstaens G, Elsenbruch S, Feinle-Bisset C, Holtmann G, et al. Functional dyspepsia. Nat Rev Dis Primers. 2017;3:17081.
7. Talley NJ, Goodsall T, Potter M. Functional dyspepsia. Aust Prescr. 2017;40(6):209-13.
8. Asano H, Tomita T, Nakamura K, Yamasaki T, Okugawa T, Kondo T, et al. Prevalence of Gastric Motility Disorders in Patients with Functional Dyspepsia. J Neurogastroenterol Motil. 2017;23(3):392-9.
9. Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology. 1998;115(6):1346-52.
10. Zhang AZ, Wang QC, Huang KM, Huang JG, Zhou CH, Sun FQ, et al. Prevalence of depression and anxiety in patients with chronic digestive system diseases: A multicenter epidemiological study. World journal of gastroenterology. 2016;22(42):9437-44.
11. Ye Y, Wang XR, Zheng Y, Yang JW, Yang NN, Shi GX, et al. Choosing an Animal Model for the Study of Functional Dyspepsia. Can J Gastroenterol Hepatol. 2018;2018:1531958.
12. Liu LS, Winston JH, Shenoy MM, Song GQ, Chen JD, Pasricha PJ. A rat model of chronic gastric sensorimotor dysfunction resulting from transient neonatal gastric irritation. Gastroenterology. 2008;134(7):2070-9.
13. Li S, Chen JD. Down-regulation of A-type potassium channel in gastric-specific DRG neurons in a rat model of functional dyspepsia. Neurogastroenterol Motil. 2014;26(7):962-70.
14. Abdel-Aziz H, Wadie W, Zaki HF, Muller J, Kelber O, Efferth T, et al. Novel sequential stress model for functional dyspepsia: Efficacy of the herbal preparation STW5. Phytomedicine : international journal of phytotherapy and phytopharmacology. 2015;22(5):588-95.
15. Jing FC, Zhang J, Feng C, Nian YY, Wang JH, Hu H, et al. Potential rat model of anxiety-like gastric hypersensitivity induced by sequential stress. World journal of gastroenterology. 2017;23(42):7594-608.
16. Tominaga K, Fujikawa Y, Tanaka F, Kamata N, Yamagami H, Tanigawa T, et al. Structural changes in gastric glial cells and delayed gastric emptying as responses to early life stress and acute adulthood stress in rats. Life Sci. 2016;148:254-9.
17. Winston JH, Aguirre JE, Shi XZ, Sarna SK. Impaired Interoception in a Preclinical Model of Functional Dyspepsia. Dig Dis Sci. 2017;62(9):2327-37.
18. Sinen O, Bulbul M. The role of autonomic pathways in peripheral apelin-induced gastrointestinal dysmotility: involvement of the circumventricular organs. Exp Physiol. 2021;106(2):475-85.
19. Sinen O, Ozkan A, Agar A, Bulbul M. Neuropeptide-S prevents 6-OHDA-induced gastric dysmotility in rats. Brain Res. 2021;1762:147442.
20. Myers B, Greenwood-Van Meerveld B. Divergent effects of amygdala glucocorticoid and mineralocorticoid receptors in the regulation of visceral and somatic pain. Am J Physiol Gastrointest Liver Physiol. 2010;298(2):G295-303.
21. Schrader AJ, Taylor RM, Lowery-Gionta EG, Moore NLT. Repeated elevated plus maze trials as a measure for tracking within-subjects behavioral performance in rats (Rattus norvegicus). Plos One. 2018;13(11).
22. Galeano P, Martino Adami PV, Do Carmo S, Blanco E, Rotondaro C, Capani F, et al. Longitudinal analysis of the behavioral phenotype in a novel transgenic rat model of early stages of Alzheimer's disease. Front Behav Neurosci. 2014;8:321.
23. Choung RS, Talley NJ. Novel mechanisms in functional dyspepsia. World journal of gastroenterology. 2006;12(5):673-7.
24. Kindt S, Tack J. Impaired gastric accommodation and its role in dyspepsia. Gut. 2006;55(12):1685-91.
25. Tang L, Zeng Y, Li L, Wang J, Peng D, Zhang T, et al. Electroacupuncture Upregulated Ghrelin in Rats with Functional Dyspepsia via AMPK/TSC2/Rheb-Mediated mTOR Inhibition. Dig Dis Sci. 2020;65(6):1689-99.
26. Ji E, Wang T, Guo F, Zhang Y, Tang C, Tang D, et al. Xiaoerfupi alleviates the symptoms of functional dyspepsia by regulating the HTR3A and c-FOS. Biomed Pharmacother. 2019;120:109442.
27. Babygirija R, Bulbul M, Yoshimoto S, Ludwig K, Takahashi T. Central and peripheral release of oxytocin following chronic homotypic stress in rats. Auton Neurosci. 2012;167(1-2):56-60.
28. Yoshimoto S, Cerjak D, Babygirija R, Bulbul M, Ludwig K, Takahashi T. Hypothalamic circuit regulating colonic transit following chronic stress in rats. Stress. 2012;15(2):227-36.
29. Jing F, Zhang J. Metabolic kinetics of 5-hydroxytryptamine and the research targets of functional gastrointestinal disorders. Dig Dis Sci. 2014;59(11):2642-8.
30. Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav Brain Res. 2002;134(1-2):49-57.
31. Otsuka T, Nishii A, Amemiya S, Kubota N, Nishijima T, Kita I. Effects of acute treadmill running at different intensities on activities of serotonin and corticotropin-releasing factor neurons, and anxiety- and depressive-like behaviors in rats. Behavioural Brain Research. 2016;298:44-51.
32. Bolukbas I, Mundorf A, Freund N. Maternal separation in rats induces neurobiological and behavioral changes on the maternal side. Sci Rep. 2020;10(1):22431.
33. Daniels WM, Pietersen CY, Carstens ME, Stein DJ. Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to

a subsequent stressor. Metab Brain Dis. 2004;19(1-2):3-14.

34. Frye CA, Walf AA. Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. Horm Behav. 2002;41(3):306-15.

35. Koss WA, Gehlert DR, Shekhar A. Different effects of subchronic doses of 17-beta estradiol in two ethologically based models of anxiety utilizing female rats. Horm Behav. 2004;46(2):158-64.

36. Bowman RE, Ferguson D, Luine VN. Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. Neuroscience. 2002;113(2):401-10.

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Values of intensive care scores in predicting morbidity and mortality in patients treated for COVID-19 pneumonia

Hale KEFELİ ÇELİK[1,*], Zahide DOĞANAY[2], Ramazan BURAK FERLİ[1]

[1] Department of Anesthesiology and Reanimation, Samsun Education and Research Hospital, Samsun, Türkiye
[2] Department of Anesthesiology and Reanimation, Medical School, Kastamonu University, Kastamonu, Türkiye

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Abstract

Some of the patients with COVID-19 pneumonia are followed up in intensive care units (ICU). This study aimed to determine the success of intensive care scores used in patients followed up in the ICU with the diagnosis of COVID-19 pneumonia in predicting morbidity and mortality. This retrospective study included patients treated for COVID-19 pneumonia in the ICUs of Samsun Training and Research Hospital. We used the patients' demographic characteristics, vital signs, arterial blood gas values, radiological imaging, and laboratory data by using the hospital database and patient files. Group I was composed of alive patients, while Group II was of dead ones. A total of 75 patients were included in the study, of which 34 (45.3%) were female and 41 (54.7%) were male. The median length of intensive care stay was 8 (5-15) days in Group I patients and 5 (2-8) days in Group II patients, which was higher in alive patients (p=0.004). Radiological involvement was present in 93.3% (n=70) of the patients, and involvement was observed in both lungs in 77.3% (n=58). We observed complications in 54.7% (n=41) of the patients, whereas the incidence of complications was 20% in Group I and 72% in Group II, which was statistically significant (p<0.001). APACHE II, PSI, SOFA, qSOFA, SMART-COP, CURB65, A-DROP and NEWS2 scores were statistically significantly higher in patients who died, whereas APACHE II, SOFA, qSOFA, and SMART-COP scores were more successful in predicting morbidity. It is vital to predict the mortality risk early in patients with COVID-19 pneumonia followed up in intensive care units. Among the scoring systems, APACHE II, PSI, SOFA, qSOFA, SMART-COP, CURB65, A-DROP, and NEWS2 can be used safely to predict mortality.

Keywords: COVID-19, intensive care unit, mortality, pneumonia, score

1. Introduction

The novel coronavirus disease 19 (COVID-19), also known as novel coronavirus pneumonia, first appeared in Wuhan, China, at the beginning of December and spread almost worldwide within two months, leading to the pandemic. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It can increase up to 50% in some populations even if the overall mortality rate of the disease is around 2%, and the most important reason for this is virus-induced pneumonia. 80% of COVID-19 patients have mild disease, 20% require hospitalization, and some need to be followed up in the intensive care unit (ICU). Patients with severe pneumonia require ICU follow-up and invasive or non-invasive respiratory support in an acute respiratory distress syndrome clinic (1,2).

Today, many scoring systems are used to estimate the mortality of patients followed up in ICUs. Recently, especially NEWS2 and SOFA are considered to be recommended scoring systems for predicting the prognosis of severe COVID-19 disease. Pneumonia severity index (PSI) is also reported as a scoring system that can be used in COVID-19 pneumonia as it questions additional diseases and radiological results (3,4). It is yet unclear which scoring system is more useful in patients with severe COVID-19 pneumonia, even though many scoring systems are used in ICUs (5). This study compared the existing intensive care scoring systems used to predict morbidity and mortality in patients who are followed up in the ICU due to COVID-19 pneumonia and determined which test is more sensitive and specific.

2. Material and Methods

2.1. Study design and patients

This retrospective study included patients treated for pneumonia between April 1, 2020, and November 1, 2020, in the ICUs of Samsun Training and Research Training and Research Hospital after obtaining the permission of the local ethics committee (GOKA/2021/1/10) following the approval of the Republic of Türkiye Ministry of Health on December 5, 2020. We used the patients' demographic characteristics, clinical and radiological characteristics, initial blood gas values, vital signs, and laboratory data at the time of admission to the intensive care unit by using the hospital

*Correspondence: ck_hale@hotmail.com

database and patient files. We divided the patients into two groups, alive patients (Group I) and dead patients (Group II), and accordingly tried to determine the values of the scoring systems in predicting morbidity and mortality.

We used the Acute Physiology and Chronic Health Evaluation II (APACHE II), Pneumonia Severity Index (PSI), Sequential Organ Failure Assessment (SOFA), Quick Sequential Organ Failure Assessment (qSOFA), SMART-COP (acronym for Systolic blood pressure, Multilobar infiltrates, Albumin, Respiratory rate, Tachycardia, Confusion, Oxygen, and pH), MuLBSTA (Score for Viral Pneumonia Mortality), CURB65 (Confusion, Urea, Respiratory rate, Blood pressure, Age>65), A-DROP (Age, Dehydration, Respiratory failure, Orientation disturbance, blood Pressure), and National Early Warning Score (NEWS) 2 as the scoring systems.

We used the following internet address for APACHE II calculation; https://www.mdcalc.com/apache-ii-score, for PSI; https://www.mdcalc.com/psi-port-score-pneumonia-severity-index-cap, for SOFA; https://www.mdcalc.com/sequential-organ-failure-assessment-sofa-score, for qSOFA; https://www.mdcalc.com/qsofa-quick-sofa-score-sepsis, for SMART-COP; https://www.mdcalc.com/smart-cop-score-pneumonia-severity, for MuLBSTA; https://www.mdcalc.com/mulbsta-score-viral-pneumonia-mortality, for CURB65; https://www.mdcalc.com/curb-65-score-pneumonia-severity, and for NEWS2; https://www.mdcalc.com/national-early-warning-score-news-2.

APACHE II

The APACHE II score was first used in 1985. This score was developed to identify and classify the risk of critical patients in ICUs, including surgery and trauma patients. It is known to be useful in predicting mortality in critical trauma patients, transplant patients, and sepsis patients. The score includes 12 physiological variables, ranging from 0 to 71, based on age and underlying health status. APACHE II sections are a) 12 acute physiological parameters (acute physiological score), b) patient age, and c) chronic diseases and surgical interventions (6).

PSI

PSI was developed by the Pneumonia Patient Outcomes Research Team (PORT) in 1997 to estimate short-term mortality in patients with community-acquired pneumonia. It is a comprehensive scoring system calculated based on the patient's demographic information, accompanying comorbidities, physical examination results, laboratory values, and radiological results. It is successful in predicting mortality in cases of pneumonia requiring intensive care and is widely used (7).

SOFA

The SOFA scoring system was developed by an international group of experts in 1996. SOFA describes multiple organ dysfunction with the following parameters, oxygenation index (arterial oxygen tension [PaO2]/fraction of inspiration oxygen [FiO2]), mean blood pressure, Glasgow Coma Scale (GCS), BUN and creatine value, bilirubin, and platelet value. The function of each organ system is scored between 0 and 4, and then separate SOFA scores are summed up to a total score from 0 to 24 (8).

qSOFA

qSOFA score was defined in the Third International Consensus Definitions for Sepsis and Septic Shock and recommended to be used to evaluate organ dysfunction in patients with suspected sepsis. However, many recent studies have found its effectiveness in predicting mortality in patients with different diseases (9). Three clinical variables are each scored with a score of variable mental status, systolic blood pressure ≤100 mmHg, and respiratory rate ≥22/min. The clinician should direct the patient to investigate organ dysfunction, initiate or increase treatment, consider increased follow-up, or refer to an ICU if the qSOFA total score is two and above (10).

SMART-COP

SMART-COP is one of the latest models in pneumonia scoring and has been defined by Australian researchers. SMART-COP was created to find patients with pneumonia who needed intensive care unit or vasopressor support and included systolic blood pressure <90 mmHg-2 scores, multiple lobe involvement-1 score on chest X-ray, albumin value <3.5 g/dL-1 scores, respiratory rate >30 N/min-1 scores, heart rate >125 beats/min-1 score, confusion (acute)-1 score, low oxygen saturation (SpO2) <90%-2 scores, and pH value <7.35–2 scores. 0-2 points: low risk, 3-4 points: medium risk, 5-6 points: high risk and >7 points are defined as very high risk for the need for vasopressor support (11,12).

MuLBSTA

MuLBSTA is a scoring system developed to predict 90-day mortality in patients with viral pneumonia. This score uses the following data: multilobular infiltration, lymphopenia, bacterial co-infection, smoking history, hypertension, and age. Clinical access to all parameters defined in this score is easy and is used in the risk classification of hospitalized patients with viral pneumonia. Mortality rates for each class are classified as follows: MuLBSTA 0–11 (‘low-risk’, mortality=5.07%); MuLBSTA 12–22 (‘high-risk’, mortality=33.92%) (13,14).

CURB65

This classification system is fairly simple and can be easily applied in daily practice and was defined in 2003. The CURB65 score consists of confusion, urea >7 mmol/L,

respiratory rate ≥30 breaths/min, blood pressure (systolic <90 mmHg or diastolic ≤60 mmHg), and age ≥65 years. The risk of mortality in patients with a CURB65 score of 0-1 is <3%, and these patients can be monitored for outpatient care. The risk of mortality in patients with a score of 2 is around 9%, and short-term hospitalization is recommended for these patients. Those with a CURB65 score of 3-5 have a mortality risk of 15-40% and should be monitored at the hospital (15).

A-DROP

The A-DROP score is a modified version of the CURB65 score recommended by the Japanese Respiratory Society in 2006. Criteria are as follows: men aged ≥70 years or women aged ≥75 years, blood urea nitrogen ≥21 mg/dL or dehydration, oxyhemoglobin saturation measured with pulse oximetry <90% or PaO2 <60 mmHg, confusion and systolic blood pressure ≤90 mmHg (4).

NEWS2

The Royal College of Physicians of London released NEWS2, making a few changes to its NEWS score in December 2017. NEWS2 is a standard clinical scoring system developed to improve the detection of worsening in acute patients. It is based on the total scoring of six physiological parameters: respiratory rate, oxygen saturation, systolic blood pressure, pulse rate, level of consciousness, or consciousness and temperature. In addition, two points are added for patients in need of oxygen support. The NEWS2 score of 5 or 6 is considered a key threshold that may indicate clinical deterioration and should be evaluated urgently by a clinician or team competent in treating the patient (10).

Exclusion criteria:

- Patients diagnosed with pneumonia other than COVID-19 pneumonia

- Negative RT-PCR test from a throat swab sample

- Patients without blood gas and laboratory values in the patient file

- Patients without radiological images in the hospital database

- Patients not followed up in the ICU due to respiratory failure

2.2. Statistical analysis

The Kolmogorov-Smirnov test examined the suitability of the data for normal distribution. We used the student's t-test to compare normally distributed values in two independent groups and the Mann-Whitney U test to compare non-normally distributed values in two independent groups. We used the Exact and Pearson's Chi-square tests to analyze the relationship of categorical variables. We first analyzed age, gender, some clinical characteristics, and laboratory and treatment methods by the Univariate LR (Logistic Regression) method and then analyzed the variables found to be significant by the Stepwise Multivariate Enter LR method. We determined the cut-off value by ROC analysis over mortality and complication using variables such as APACHE II, PSI, SOFA, qSOFA, SMART-COP, MuLBSTA, CURB65, A-DROP, and NEWS2. We gave median and quarterly values for numerical variables and number (n) and % values for categorical variables as descriptive statistics. We used SPSS windows version 23.0 package software for statistical analysis and considered $p<0.05$ statistically significant.

3. Results

A total of 75 patients were included in the study, of which 34 (45.3%) were female and 41 (54.7%) were male. The median age was 75 (IQR 65-83) years, and there was no difference between the groups (p=0.706). There was no significant difference between the groups except for BUN, creatine, lactate and base excess values in arterial blood gas, total bilirubin, CK-MB, and troponin values. BUN (p=0.004) and creatine (p=0.002) values were statistically significantly higher in patients who died than in those who were alive. Similarly, lactate, base excess, total bilirubin, CK-MB, and troponin values were statistically significantly higher in patients who died ($p<0.05$) (Table 1).

The median length of ICU stay was 8 (IQR 5-15) days in Group I patients and 5 (IQR 2-8) days in Group II patients, which was statistically significantly longer in alive patients (p=0.004). The rate of consciousness was significantly higher in alive patients (p=0.006). Radiological involvement was present in 93.3% (n=70) of the patients, and this involvement was present in both lungs in 77.3% (n=58). The most common radiological feature was ground-glass opacity, with a rate of 80% (n=60), and the rate of parenchymal consolidation was 46.7% (n=35). The incidence of parenchymal consolidation in both lungs was 36% higher in patients who died, and this was statistically significant, whereas the incidence of parenchymal consolidation was 60% higher in patients experiencing parenchymal consolidation (p=0.024). Complications were observed in 54.7% (n=41) of the patients. The most common ones were acute renal failure (n=18), septic shock (n=9), ARDS (n=6), MODS (n=5), respectively. The incidence of complications was 20% in Group I and 72% in Group II, which was statistically significant ($p<0.001$) (Table 2).

APACHE II (p=0.004), SOFA (p=0.001), qSOFA (p=0.036), SMART-COP (p=0.032), and NEWS2 (p=0.010) scores were statistically significantly higher in patients with complications whereas there was no difference in PSI (p=0.492), MuLBSTA (p=0.374), CURB65 (p=0.119), and A-DROP (p=0.078) scores when the complication status was evaluated (Table 3).

APACHE II (p=0.001), PSI (p=0.006), SOFA (p=0.001), qSOFA (p=0.017), SMART-COP (p=0.001), CURB65 (p=0.001), A-DROP (p=0.001), and NEWS2 (p=0.001) scores

were statistically significantly higher in patients who died whereas there was no difference between the groups in MuLBSTA scoring when the mortality status was evaluated (p=0.896) (Table 4).

Table 1. Vital signs and laboratory findings

	Group I (n=25)			Group II (n=50)			Total (n=75)			
	Median	Min	Max	Median	Min	Max	Median	Min	Max	p
Age, years	77	68	81	74.5	65	84	75	65	84	0.706
SBP, mmHg	115	105	143	110.5	86	130	112	86	143	0.041
DBP, mmHg	66	63	78	64.5	50	79	65	50	79	0.231
Respiratory rate, min^{-1}	26	24	30	29.5	20	32	28	20	32	0.731
Heart rate, min^{-1}	100	88	122	108	92	128	105	88	128	0.532
Temperature, °C	36.6	36.5	36.8	36.6	36.5	36.7	36.6	36.5	36.8	0.102
Hemoglobin, g/l	12.1	11.1	12.9	11.9	10.3	13.3	11.9	10.3	13.3	0.870
Hematocrit, %	35	33.1	37.2	34.8	31.3	39.4	34.9	31.3	39.4	0.736
WBC count, 109/l	10.8	7.4	13.3	11.6	7.5	18.3	11.4	7.4	18.3	0.406
Lymphocyte count, 10^9/l	0.9	0.5	2	1.1	0.6	2.3	1.1	0.5	2.3	0.229
Lymphocyte, %	8	5.7	13.7	9.5	4.5	15.2	9	4.5	15.2	0.818
Neutrophil count, 10^9/l	9.9	7.8	12.7	9.9	6.4	15.6	9.9	6.4	15.6	0.549
Neutrophil, %	85.3	78.1	88.9	83.2	76.1	90.6	84	76.1	90.6	0.802
Patelet, 10^9/l	264	174	351	244	181	325	246	174	351	0.529
Glucose, mmol/l	154	124	213	158	106	244	157	106	244	0.897
Sodium, mmol/l	136	134	141	137	133	143	137	133	143	0.405
Potassium, mmol/l	4.3	3.8	4.8	4.3	3.8	4.9	4.3	3.8	4.9	0.477
Urea, mmol/l	47	36	69	89	50	137	71	36	137	0.004
Creatine, mmol/l	1.1	0.8	1.3	1.5	1	2.3	1.3	0.8	2.3	0.002
Arterial pH	7.4	7.4	7.4	7.4	7.2	7.4	7.4	7.2	7.4	0.200
Saturation, %	93	90	96	90	81.7	94	92	81.7	96	0.126
$PaCO_2$, mmHg	38.2	34.6	40.7	39.3	33.5	47.3	39	33.5	47.3	0.381
PaO_2, mmHg	69	50	90	66	46	78	67.6	46	90	0.34
Arterial HCO_3, mmol/l	24.3	21.8	26.1	21.3	18.6	24.2	22.1	18.6	26.1	0.064
Arterial lactate, mmol/l	1.6	1	2.7	2.3	1.7	4.1	2.2	1	4.1	0.006
BE	1.4	-3.1	4.2	-2.8	-6.7	1.6	-1.8	-6.7	4.2	0.026
CRP, mg/l	95.6	46.5	140	107	59.1	182.6	98.9	46.5	182.6	0.310
Procalcitonin, µg/l	0.2	0.1	3.4	1.4	0.3	5.7	1	0.1	5.7	0.068
D-Dimers, mg/dl	1.3	0.9	4	2.2	1	4.1	2.1	0.9	4.1	0.588
PT, sec	13.7	13.4	15.2	13.9	12.9	16.1	13.9	12.9	16.1	0.499
INR	1.2	1.1	1.4	1.2	1.1	1.4	1.2	1.1	1.4	0.771
AST, u/l	29	24	48	49	29	73	40	24	73	0.048
ALT, u/l	16	13	24	25	15	44	20	13	44	0.086
Albumin, g/l	3	2.7	3.3	2.9	2.5	3.1	2.9	2.5	3.3	0.212
Total bilirubin, µmol/l	0.5	0.4	0.7	0.6	0.5	1.3	0.6	0.4	1.3	0.030
CK-MB, u/l	1.6	1.2	3	4.4	1.9	9.3	3.2	1.2	9.3	0.001
Troponin, ng/l	0	0	0.1	0.4	0	1.9	0.1	0	1.9	0.001

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BE: base excess, CK-MB: creatine kinase-MB, CRP: C-reactive protein, DBP: diastolic blood pressure, HCO_3: bicarbonate, INR: International Normalized Ratio, GKS: Glasgow Coma Score, WBC: white blood cell, PT: Prothrombin time, PaO_2: arterial oxygen pressure, $PaCO_2$: arterial carbon dioxide pressure, SBP: systolic blood pressure

Table 2. Comparison of the patients' state of consciousness, radiological features and complications

	Group I (n=25)		Group II (n=50)		Total (n=75)		p
Sex (n,%)							0.189
Female	14	56	20	40	34	45.3	
Male	11	44	30	60	41	54.7	
Consciousness (n,%)							**0.006**
Awake	14	56	8	16	22	29.3	
Confusion	4	16	8	16	12	16.0	
Delirium	0	0	2	4	2	2.7	
Stupor	4	16	22	44	26	34.7	
Coma	3	12	10	20	13	17.3	
Thorax BT (n,%)							0.233
Unilateral involvement	5	20	7	14	12	16.0	
Bilateral involvement	20	80	38	76	58	77.3	
None	0	0	5	10	5	6.7	
Ground glass opacity (n,%)							0.540
Yes	21	84	39	78	60	80.0	
No	4	16	11	22	15	20.0	
GGO involvement (n,%)							0.575
Unilateral	3	12	5	10	8	10.7	
Bilateral	19	76	34	68	53	70.7	
None	3	12	11	22	14	18.7	
Consolidation (n,%)							0.102
Yes	15	60	20	40	35	46.7	
No	10	40	30	60	40	53.3	
Consolidation involvement (n,%)							**0.024**
Unilateral	6	24	2	4	8	10.7	
Bilateral	9	36	18	36	27	36.0	
None	10	40	30	60	40	53.3	
Complication (n,%)							**<0.001**
No	20	80	14	28	34	45.3	
Yes	5	20	36	72	41	54.7	

Table 3. Comparison of the complication status in terms of scoring systems

	COMPLICATION									
	YES			NO			TOTAL			p
	Median	Min	Max	Median	Min	Max	Median	Min	Max	
APACHE II	26	21	33	21	17	27	25	17	33	**0.004**
PSI	150	125	185	150.5	120	174	150	120	185	0.492
SOFA	8	5	11	5.5	4	7	7	4	11	**0.001**
qSOFA	2.5	2	3	2	1	2	2	1	3	**0.036**
SMART-COP	7	5	8	5.5	4	7	6	4	8	**0.032**
MuLBSTA	13	11	15	12.5	11	13	13	11	15	0.374
CURB65	4	3	4	3	2	4	3	2	4	0.119
A-DROP	3.5	3	4	3	2	4	3	2	4	0.078
NEWS2	12	9	13	10	8	12	11	8	13	**0.010**

Table 4. Comparison of mortality in terms of scoring systems

	Group I			Group II			Total			p
	Median	Min	Max	Median	Min	Max	Median	Min	Max	
APACHE II	20	14	25	26	21	32	25	14	32	**0.001**
PSI	128	112	159	164	131	190	150	112	190	**0.006**
SOFA	5	3	7	8	5	10	7	3	10	**0.001**
qSOFA	2	1	2	2.5	2	2	2	1	2.5	**0.017**
SMART-COP	5	4	6	7	5	8	6	4	8	**0.001**
MuLBSTA	13	11	17	13	11	15	13	11	17	0.896
CURB65	3	2	3	4	3	4	3	2	4	**0.001**
A-DROP	3	2	3	3.5	3	4	3	2	4	**0.001**
NEWS2	9	8	11	12	10	13	11	8	13	**0.001**

ROC analysis for complication showed that all scoring systems except PSI, qSOFA, MuLBSTA, CURB65, and A-DROP scores were statistically significant in terms of AUC values ($p<0.05$). ROC analysis determined an APACHE II score above 23 to increase the risk of complications (sen:0.71, spe:0.65). Similarly, the SOFA score of 7.5 (sen:0.56, spe:0.82), the SMART-COP score of 7.5 (sen:0.37, spe:0.85), and the NEWS2 score of 11.5 (sen:0.54, spe:0.74) were statistically significant in increasing the risk of complications by ROC analysis. The highest value was observed in the APACHE II score in terms of sensitivity value in the parameters examined through complication estimation (sen:0.71). In other words, we observed that this score correctly predicted the occurrence of complications by 71% in patients with an APACHE II score above 23. We found that the scoring system that makes the most accurate estimation in terms of specificity value was SMART-COP in the parameters examined through complication estimation (spe:0.85). In other words, we estimated with an accuracy rate of 85% that patients with a SMART-COP score below 7.5 would not experience any complications (Table 5, Fig. 1).

Table 5. ROC analysis for complication

	Cut-off	Area (95%CI)	Std Error	Sensitivity	Specificity	p
APACHE II	>23	0.693 (0.575 0.811)	0.060	0.71	0.65	**0.004**
PSI	>184.5	0.546 (0.415 0.677)	0.067	0.29	0.85	0.492
SOFA	>7.5	0.718 (0.603 0.834)	0.059	0.56	0.82	**<0.001**
qSOFA	>2.5	0.629 (0.503 0.754)	0.064	0.27	0.94	0.056
SMART-COP	>7.5	0.643 (0.519 0.767)	0.063	0.37	0.85	**0.034**
MuLBSTA	>13.5	0.559 (0.427 0.692)	0.068	0.39	0.77	0.380
CURB65	>4.5	0.600 (0.473 0.728)	0.065	0.20	0.99	0.136
A-DROP	>3.5	0.612 (0.484 0.740)	0.065	0.44	0.71	0.097
NEWS2	>11.5	0.672 (0.551 0.793)	0.062	0.54	0.74	**0.011**

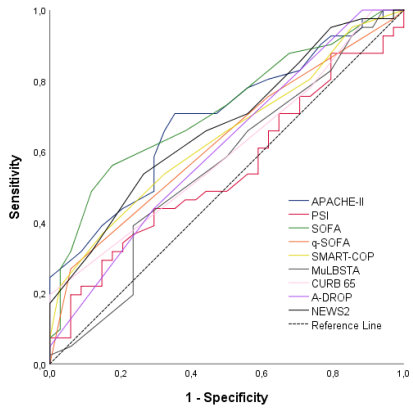

Fig. 1. Complication ROC curve

It was determined that an APACHE II score above 23 posed a risk for mortality; similarly, a PSI score of 140, SOFA score of 7.5, qSOFA score of 1.5, SMART-COP score of 6.5, CURB65 score of 3.5, A-DROP score of 3.5, and NEWS2 score of 9.5 were found to pose a significant risk for mortality when ROC analysis was performed for mortality. MuLBSTA score was not significant in predicting mortality in terms of AUC values ($p=0.897$). The highest value was observed in the qSOFA score in terms of sensitivity value in the tests examined through mortality estimation (sen:0.78), in other words, it was seen that this score correctly predicted mortality by 78% in patients with a qSOFA score above 1.5. On the other hand, we observed that the scoring system that makes the most accurate estimation in terms of specificity value was the SOFA score (spe:0.88). In other words, we estimated with an accuracy rate of 88% that patients with a SOFA score below 7.5 would survive (Table 6, Fig. 2).

Table 6. ROC analysis for mortality

	Cut-off	Area (95%CI)	Std Error	Sensitivity	Specificity	p
APACHE II	>23	0.729 (0.606 0.852)	0.063	0.68	0.72	**0.001**
PSI	>140	0.697 (0.675 0.820)	0.063	0.72	0.64	**0.006**
SOFA	>7.5	0.782 (0.677 0.866)	0.053	0.52	0.88	**<0.001**
qSOFA	>1.5	0.656 (0.524 0.787)	0.067	0.78	0.48	**0.029**
SMART-COP	>6.5	0.759 (0.651 0.868)	0.055	0.58	0.84	**<0.001**
MuLBSTA	>8.5	0.509 (0.363 0.655)	0.074	0.92	0.84	0.897
CURB65	>3.5	0.720 (0.602 0.838)	0.060	0.62	0.76	**0.002**
A-DROP	>3.5	0.737 (0.616 0.858)	0.062	0.48	0.84	**0.001**
NEWS2	>9.5	0.738 (0.622 0.854)	0.059	0.76	0.60	**0.001**

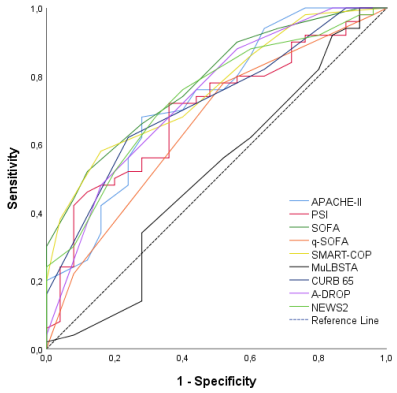

Fig. 2. Mortality ROC curve

4. Discussion

The COVID-19 pandemic has led to healthcare system lockdown in many countries worldwide and even its collapse in some countries. Appropriate criteria should be established for the hospitalization of patients with severe illnesses, and medical resources should be used as accurately as possible if this happens. Intensive care scoring systems help select ICU inpatients at this point (14). In addition to reports from the US and China, European surveillance data suggest that approximately 15-20% of hospitalized patients with COVID-19 have died or developed severe illnesses requiring intensive care. In this respect, using scoring systems by emergency or intensive care physicians is essential in identifying severe COVID-19 patients and evaluating treatment.

NEWS2, qSOFA, and CRB65 are the most commonly used clinical risk scoring systems, but no study has shown precisely which should be used in COVID-19 patients so far. Another option is to revise the existing scoring systems used to predict mortality in patients with severe COVID-19 (10,11). This study aimed to determine the power of existing scoring systems used in ICUs in predicting morbidity and mortality in COVID-19 patients.

Advanced age and pre-existing diseases are considered risk factors for patients with severe COVID-19. In addition, many studies have shown that the severity of the disease is associated with the severity of patients' thoracic CT scans and many laboratory test parameters, including various enzyme levels, coagulation factors, inflammatory markers, and absolute immune cell count in peripheral blood (16,17). We found BUN, creatine, total bilirubin, CK-MB, troponin, lactate, and base deficit values in arterial blood gas to be higher in the group of patients who died and closely followed these prognostic parameters in our patients followed up in ICUs in our study, as indicated in the literature.

Evaluation of disease severity is critical in guiding therapeutic options such as hospitalization or the need for ICU hospitalization in evaluating and managing pneumonia (3). The pneumonia severity index allows the classification of patient groups according to mortality risks and characteristics. A variable-based score needs to be calculated in PSI and may therefore not be practical for routine practice in intensive hospital emergency departments or primary care centers but can be easily used in ICUs. The CURB65 score accurately predicts clinical outcomes in viral-induced community-acquired pneumonia. The use of the CURB65 score is much simpler than PSI, but the sensitivity to predict mortality in pneumonia is reported to be lower than PSI (18,19). We found that PSI and CURB65 were insufficient in predicting morbidity and sufficient in predicting mortality in our study. Accordingly, a PSI score of 140 and a CURB65 score of 3.5 posed a significant risk for mortality.

The SOFA score, first developed in 1994, is used to estimate the results of patients in the ICU. The SOFA score assesses organ dysfunction in six different systems using a 5-point scale. A higher SOFA score has been reported to be associated with an increased mortality rate in hospitalized COVID-19 patients (3). Liu et al. determined that the SOFA score was above three and the qSOFA score was above 1 in critical COVID-19 patients. In addition, they stated in this study that the SOFA score was a highly sensitive indicator of in-hospital mortality in COVID-19 patients and prognostically superior to qSOFA (11). The median SOFA score was reported as 3 in another study examining 109 patients who died due to complications associated with COVID-19 pneumonia (20). Another study conducted on patients with COVID-19 pneumonia reported that the median SOFA score of the patients at the time of the first admission

was three, and the median SOFA score of 3 patients who died was 5 (2). We found in our study that SOFA and qSOFA scoring systems were sufficient in predicting both morbidity and mortality. Accordingly, a SOFA score of 7.5 and a qSOFA score of 1.5 posed a significant risk for mortality. In fact, the sensitivity value for qSOFA was 78%, and the specificity value for SOFA was 88%, which were the highest values among the scoring systems in our study. Accordingly, we determined with an accuracy rate of 88% that patients with a SOFA score below 7.5 would survive and that we could correctly predict mortality rate by 78% in patients with a qSOFA score above 1.5.

The MuLBSTA score is used to predict mortality in viral pneumonia, and 5 to 11 scores are reported to be reliable accordingly. The value 11 is a cut-off value indicating that the disease will worsen and the patient should be referred to the ICU (14). MuLBSTA scoring systems include markers such as multilobular infiltration, lymphopenia, and the presence of bacterial co-infection, which play an essential role in predicting disease progression and worsening. However, it cannot predict the progression and worsening of the disease significantly more accurately than the patient's risk score. Therefore, Iijima et al. stated that CRP value, known as one of the primary mechanisms that show high inflammatory status and explain the worsening of COVID-19 and related to complex cytokine storm, should also be considered. They also reported that they would predict the worsening of the disease more accurately than the MuLBSTA score with this scoring system modified in their study (14). We found in our study that the MuLBSTA score was insufficient to predict morbidity (p=0.380) or mortality (p=0.897) in critical COVID-19 patients followed up in the ICU compared to other scoring systems.

The A-DROP score is a modified version of the CURB65 score and provides predictive power similar to the CURB65 score. Fan et al. examined the accuracy of their various scores to predict mortality in 654 COVID-19 patients admitted to the hospital and reported that A-DROP was the best scoring system for predicting mortality in patients with critical COVID-19 pneumonia with a value of 0.87 AUC (95% CI 0.84–0.90), sensitivity value was 80%, and specificity value was 86% (1). They also stated that PSI might be inadequate in COVID-19 pneumonia since more emphasis is placed on the underlying disease than on respiratory function in PSI than A-DROP (1). Even though A-DROP was insufficient to predict complications and morbidity (p=0.097), it showed 48% sensitivity and 84% specificity with 0.73 AUC (95% CI 0.61-0.85) and the highest specificity value after SOFA in predicting mortality in our study.

NEWS2 scoring system evaluates respiratory rate, oxygen saturation, systolic blood pressure, heart rate, temperature, and level of consciousness and is easy to use in the emergency department. It proved to be a valid tool for identifying acutely ill patients with infection (1). Myrstad et al. reported that the NEWS2 score at the time of admission was superior to qSOFA and other commonly used clinical risk scores in predicting severe COVID-19 disease and hospital mortality. One advantage of NEWS2 compared to other scores is that it uses both hypoxemia and supportive oxygen therapy as scoring parameters. However, the increased oxygen requirement may not be fully reflected in the NEWS2 score, where oxygen supplementation is evaluated only as a binary variable (yes/no). However, the authors stated that they detected severe disease with 80% sensitivity and 84% specificity in patients with a NEWS2 score above 6 (10). Jang et al. reported that the NEWS2 score predicted clinical deterioration such as ARDS, septic shock development, and intensive care needs in critical COVID-19 patients and also predicted 28-day mortality and clinical outcomes as accurately as SIRS and qSOFA (21). We found that the NEWS2 score was sufficient to predict both morbidity (p=0.011) and mortality (p=0.001) in our study and that it was even the most successful scoring system after qSOFA with a 76% sensitivity value in predicting mortality, and the cut-off value was 9.5.

In conclusion, we found that many of the scoring systems used in ICUs were sufficient in predicting morbidity and mortality in patients with severe COVID-19 pneumonia. We found that APACHE II and SMART-COP were superior to others in predicting morbidity, and SOFA and qSOFA were superior to others in predicting mortality among these scoring systems.

The limitations of our study were as follows: First, our study is a single-center retrospective study with a small number of participants. However, our results will help determine the criteria for admission to the hospital and the criteria for admission to the ICU to prevent the collapse of the healthcare system. Second, we only included patients who were discharged or died and excluded those still hospitalized. Third, selection bias cannot be avoided. We did not use data from a large population, and the severity of COVID-19 may differ among hospitals and around Turkey. However, the strength of our study is that the patients included in the study consisted of patients followed up by a single intensive care team in the same hospital and applied the same treatment protocol.

Conflict of interest

The authors declared no conflicts of interest with respect to this article's authorship and/or publication.

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Authors' contributions

Concept: H.K.Ç., Design: H.K.Ç., Z.D., Data Collection or Processing: H.K.Ç., R.B.F., Analysis or Interpretation: H.K.Ç., M.K., Literature Search: H.K.Ç., Z.D., R.B.F., Writing: H.K.Ç.

References

1. Fan G, Tu C, Zhou F, Liu Z, Wang Y, Song B, et al. Comparison of severity scores for COVID-19 patients with pneumonia: a retrospective study. Eur Respir J. 2020;56(3):2002113.
2. Erden A, Ozdemir B, Karakas O, Mutlu NM, Izdes S, Kalem AK, et al. Evaluation of 17 patients with COVID-19 pneumonia treated with anakinra according to HScore, SOFA, MuLBSTA, and Brescia-COVID respiratory severity scale (BCRSS) scoring systems. J Med Virol. 2021;93(3):1532-7.
3. Guo J, Zhou B, Zhu M, Yuan Y, Wang Q, Zhou E, et al. CURB-65 may serve as a useful prognostic marker in COVID-19 patients within Wuhan, China: a retrospective cohort study. Epidemiol Infect. 2020;148:e241.
4. Miyashita N, Matsushima T, Oka M, Japanese Respiratory Society. The JRS guidelines for the management of community-acquired pneumonia in adults: an update and new recommendations. Intern Med. 2006;45(7):419-28.
5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.
6. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985;13(10):818-29.
7. Diwakar TN, Ravish KS, Zakir HG, Kulkarni A. Comparative study of pneumonia severity index and curb65 in assesing the severity of community acquired pneumonia. J Evol Med Dent Sci. 2013;2(8):836-50.
8. Vincent JL, Moreno R, Takala J, Willatts S, Mendonca A, Bruining H. The SOFA (Sepsis related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-related Problems of the European Society of Intensive Care Medicine. Intensive Care Med. 1996;22(7):707–10.
9. Jiang Jianjun, Yang Jin, Mei Jing, Jin Yongmei, Lu Youjin. Head-to-head comparison of qSOFA and SIRS criteria in predicting the mortality of infected patients in the emergency department: a meta-analysis. Scand J Trauma Resusc Emerg Med. 2018;26(1):56.
10. Myrstad M, Ihle-Hansen H, Tveita AA, Andersen EL, Nygård S, Tveit A, et al. National Early Warning Score 2 (NEWS2) on admission predicts severe disease and in-hospital mortality from Covid-19 - a prospective cohort study. Scand J Trauma Resusc Emerg Med. 2020;28(1):66.
11. Liu S, Yao N, Qiu Y, He C. Predictive performance of SOFA and qSOFA for in-hospital mortality in severe novel coronavirus disease. Am J Emerg Med. 2020;38(10):2074-80.
12. Charles PG, Wolfe R, Whitby M, Fine MJ, Fuller AJ, Stirling R, et al. Australian Community-Acquired Pneumonia Study Collaboration, Grayson ML. SMART-COP: a tool for predicting the need for intensive respiratory or vasopressor support in community-acquired pneumonia. Clin Infect Dis. 2008;47(3):375-84.
13. Guo L, Wei D, Zhang X, Wu Y, Li Q, Zhou M, et al. Clinical features predicting mortality risk in patients with viral pneumonia: The MuLBSTA Score. Front Microbiol. 2019;10:2752.
14. Iijima Y, Okamoto T, Shirai T, Mitsumura T, Sakakibara R, Honda T, et al. MuLBSTA score is a useful tool for predicting COVID-19 disease behavior. J Infect Chemother. 2021;27(2):284-90.
15. Lim WS, van der Eerden MM, Laing R, Boersma WG, Karalus N, Lewis SA, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax. 2003;58(5):377-82.
16. Dong Y, Zhou H, Li M, Zhang Z, Guo W, Yu T, et al. A novel simple scoring model for predicting severity of patients with SARS-CoV-2 infection. Transbound Emerg Dis. 2020;67(6):2823-29.
17. Zhao Z, Chen A, Hou W, Graham JM, Li H, Richman PS, et al. Prediction model and risk scores of ICU admission and mortality in COVID-19. PLoS One. 2020;15(7):e0236618.
18. Singanayagam A, Chalmers JD. Severity assessment scores to guide empirical use of antibiotics in community acquired pneumonia. Lancet Respir Med. 2013;1(8):653-662.
19. Zhou F, Wang Y, Liu Y, Liu X, Gu L, Zhang X, et al.; CAP-China Network. Disease severity and clinical outcomes of community-acquired pneumonia caused by non-influenza respiratory viruses in adults: a multicentre prospective registry study from the CAP-China Network. Eur Respir J. 2019;54(2):1802406.
20. Du RH, Liu LM, Yin W, Wang W, Guan LL, Yuan ML, et al. hospitalization and critical care of 109 decedents with COVID-19 pneumonia in Wuhan, China. Ann Am Thorac Soc. 2020;17(7):839-46.
21. Jang JG, Hur J, Hong KS, Lee W, Ahn JH. Prognostic accuracy of the SIRS, qSOFA, and NEWS for early detection of clinical deterioration in SARS-CoV-2 infected patients. J Korean Med Sci. 2020;35(25):e234.

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Significance of tissue oxygenation in patients connected to a mechanical ventilator

Fulya KÖSE[1,*], Bülent GÜNGÖRER [2], Hanifi ARSLAN [3], Abdullah Sadık GİRİŞGİN [4] Zerrin Defne DÜNDAR [4], Merve GÜVEN [5]

[1]Department of Emergency Medicine, Karaman Education and Research Hospital, University of Karamanoğlu Mehmet Bey, Karaman, Türkiye
[2]Department of Emergency Medicine, University of Health Sciences, Ankara City Hospital, Ankara, Türkiye
[3]Department of Emergency Medicine, Şanlıurfa Education and Research Hospital, Şanlıurfa, Türkiye
[4] Department of Emergency Medicine, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye
[5]Department of Emergency Medicine, Vezirköprü State Hospital, Samsun, Türkiye

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Abstract

The aim of this study is to investigate whether the follow-up of patients on a mechanical ventilator using a tissue oxygenation device is superior to that using an O2 saturation probe. Our study was conducted at the Necmettin Erbakan University Meram Medical School Emergency Medicine Critical Care Unit between 01/04/2016 and 01/06/2016. Patients over the age of 18, non-pregnant, and followed up on mechanical ventilators were prospectively recruited, and the saturations of the patients with tissue oxygenation devices and pulse oximetry were evaluated and compared. SPSS (ver. 19.0) was used for the statistical analysis of the collected data, and the descriptive measures of all the obtained variables were calculated. The collected data revealed a statistically significant positive correlation between oxygen saturation measured by pulse oximetry and tissue oxygen saturation (StO2) in the patient group (n = 53), in which deceased and discharged patients were evaluated together. Both the hemoglobin levels and StO2 were low in the sepsis patients. It is important to follow tissue perfusion in intensive care patients, and this can be done with a noninvasive method. The results of our study reveal that perfusion should be followed with tissue oxygenation in patients on mechanical ventilators because low tissue oxygenation indicates increased patient mortality.

Keywords: saturation, tissue oxygenation, mechanical ventilator, hypoperfusion

1. Introduction

Monitoring tissue oxygenation adequacy is important for ensuring organ functions in the follow-up and treatment of critically ill patients (1). An important factor affecting morbidity and mortality in sepsis and critically ill patients is tissue hypoxia, which is caused by an imbalance between oxygen delivery and tissue oxygen utilization (2). A tissue oxygenation device is used alongside infrared spectroscopy to measure the local oxygen saturation of hemoglobin and the total hemoglobin index in the tissue (StO_2). With the rotating light absorption spectrum of a tissue sample, the concentrations of oxyhemoglobin and deoxyhemoglobin vary substantially. The percentage of StO_2 is determined by hemoglobin oxygen saturation, which is limited by the tissue value in the blood.

The most important technological progress in oxygenation monitoring is the introduction of pulse oximeters (PO). Oximeters are used for the frequent monitoring of SO_2 owing to the regular occurrence of hypoxemia in ICU patients, the necessity of adjusting the O_2 concentration frequently given to avoid inadequate treatment or O_2 toxicity, and the inability to clinically detect mild hypoxemia (3). PO was developed based on the pulsation of arterial blood flow and the absorption of light of two different wavelengths by oxyhemoglobin and reduced hemoglobin (4). It mathematically calculates arterial SO_2 from the hemoglobin saturation it measures during systole and diastole. Although the measurements made with PO are quite reliable, it is necessary to consider some factors to check this reliability. The fact that the peripheral pulse or ECG rhythm and the oximeter pulse wave are identical suggests that the measurement is reliable. If the SO_2 is between 70% and 92%, there is a ±4% variation range; if it is below 70%, its reliability decreases (5); If it is over 92%, it is generally accepted as correct and parallel with PaO_2 (6). Therefore, it is necessary to keep SO_2 above 92% in monitoring oxygen therapy.

The aim of this study was to investigate whether follow-up with a device that measures the tissue oxygenation of patients on a mechanical ventilator is superior to the O_2 saturation probe.

*Correspondence: drfulyakose@hotmail.com

2. Materials and Methods

The study was carried out with the permission of University of Necmettin Erbakan Meram Faculty of Medicine Ethichs Comite (Date 30.03.2016, Decision no: 2016/124). All procuderes were carried out in accordance with the etical rules and the principles of the Declaration of Helsinki.

2.1. Study design

Our study was conducted in the Necmettin Erbakan University Meram Emergency Medicine Critical Intensive Care Unit between 01/04/2016 and 01/06/2016. Patients over the age of 18, who were not pregnant and on mechanical ventilators, were recruited prospectively. A voluntary consent form was signed by the relatives of the patients, and the patients who would participate in the study were determined after their consent was obtained.

2.2. Study population

The conditions for inclusion in the study were that the patients should be connected to a mechanical ventilator and be women over the age of 18 who were not pregnant. Men and women were included in the study regardless of gender. Patients were included in the study with permission from their relatives.

2.3. Data collection

The names, ages, genders, and file numbers of the patients included in the study were recorded. The blood gases of the patients were collected using a tissue oxygenation device, and measurements were made with pulse oximetry. Measurements were made with a tissue oxygenation device in the right and then in the left hands of the patients, a total of two times. The patients' blood pressure, heart rate, and whether they took any vasodilator or vasoconstrictor drugs at the time of measurement were included in the study. In addition, it was determined whether the diseases in the patients' histories and smoking affected the tissue oxygenation. The pH, $_{PaO2,}$ and saturation measured by pulse oximetry were determined from the blood gases taken from the patients using a tissue oxygenation device. Based on the user manual of the tissue oxygenation device, the device is used alongside infrared spectroscopy to measure the local oxygen saturation of the tissue hemoglobin and StO_2. The light absorption spectrum of a tissue sample varied mainly according to the concentration of oxyhemoglobin and deoxyhemoglobin. The percentage of StO_2 was measured by hemoglobin oxygen saturation, which is limited by the tissue value in the blood. The Inspectra StO_2 Spot Checker (Hutchinson Technology Inc., United States) has a non-invasive imaging system that measures the percentage of the estimated value of hemoglobin oxygen saturation in thenar skeletal muscle tissue (StO_2). We determined whether the tissue oxygenation of the patients affected their length of stay in the hospital and its effects on the patients' discharge and exit. Although the diagnoses of the patients participating in this study were followed up, they were not taken into account for inclusion in this study.

2.4. Statistical analysis

Our study was designed in a prospective manner, with a total of 61 patients admitted to the emergency department. The dataset comprised data on tissue oxygen saturation and various vital signs from the patients, and SPSS (ver. 19.0) was used for the statistical analysis of the data. The descriptive measures of all the obtained variables were calculated, and the categorical variables were frequency and percentage. Additionally, the proportional scale numerical variables were presented as mean ± SD or (median, min, max) in Table 1. The Kolmogorov–Smirnov test was used to analyze whether the continuous numerical values among the proportional scale variables conformed to the normal distribution. The StO_2, blood pressure, pulse, and hemoglobin values followed the normal distribution, whereas the SO_2 values did not conform to the normal distribution. Hence, parametric comparison methods were used in cases where there were sufficient observation values in the group numbers, and non-parametric tests were used for group comparisons in other cases. Moreover, the Student's t-test was used in the case of two independent groups, and a one-way analysis of variance was used in multiple groups. For non-parametric cases, Mann–Whitney U tests were preferred in the case of the two groups, whereas Kruskal–Wallis tests were preferred for multiple groups. Pearson's or Spearman's correlation analysis was used to determine the relationship between the proportional scale variables. The relationship between the categorical variables was determined using the Monte Carlo-corrected chi-square analysis method. Significant results of pairwise comparisons are shown in Fig. 1 and presented in Table 2 with the same lowercase letters. In the study, the Type-I error value was taken as 5%, and the result was considered statistically significant at $p < 0.05$.

3. Results

In this study, 50.8% of the 61 patients admitted to the emergency department were males, whereas 49.2% were females. Meanwhile, more than half of the patients (54.1%; n=33) died. Among the patients who died, chest disease had the highest rate (37.7%; n=23). Sepsis also had a high rate (26.2%), as well as neurological problems and intracranial hemorrhage (Table 1).

Two-thirds of the patients were non-smokers, and 54.1% of 33 patients used noradrenaline. Meanwhile, the number of patients using dopamine was lower (11.5%; n = 7). Additionally, no patient used dobutamine and esmolol, and only one patient used nitroglycerin. Because the final status of the patients transferred to other centers was not known, eight patients were excluded, and a recomparison was made. In this case, the heart rate and StO_2 value were significantly different between the discharged and excluded patients ($p = 0.041$). While the pulse rate was high in patients with Ex, the StO_2 value was higher in discharged patients (Table 2).

The patients were grouped into middle and advanced age categories based on the baseline age of 40 years, and the difference in the mean StO_2 ($p = 0.043$) between the groups was significant. The measurement results showed that the values decreased in patients of advanced age. While the mean PaO_2 had a higher value in middle-aged patients, no significant difference was found between the groups (Table 3).

Table 1. Descriptive measures of patients according to their discharge status (including those who were referred)

Vital measurements **Average ±SS**	Units	Discharge n=20	Exitus n=33	To transfer n=8	P
AGE	Year	62.45±22.33	67.09±18.26	71.5±21.23	*0.106*
Systolic blood pressure	mmHg	117.4±24.46	103.73±33.34	110.5±21.33	*0.233*
Diastolic blood pressure	mmHg	57.5±11.62	55.67±15.09	63.13±13.31	*0.368*
MAP	mmHg	77.46±12.60	71.68±18.52	78.91±14.53	*0.412*
Pulse	beats/min	90.65±19.12[a,b]	114.48±24.98[a]	117.5±33.54[b]	*0.004**
Blood sugar	*Mg/dl*	161.5±72.98	154.67±72.74	165±62.81	*0.814*
Hemoglobin	*G/L*	12.3±2.13	11.26±2.48	13.05±2.65	*0.095*
Pulse O_2	*%*	95.75±2.31	93.61±9.82	97.13±3.09	*0.120*
StO_2	*%*	82.97±8.19	76.21±11.65	76.00±7.47	*0.084*
Ph		7.36±0.09	7.33±0.11[a]	7.49±0.15[a]	*0.014**
PaO_2	*%*	106.11±34.85	113.34±36.53	126.05±33.04	*0.390*
Length of hospital stay	day	22.5±16.45[a]	17.55±20.47	9.13±13.91[a]	*0.041**

Table.2. Descriptive measures according to the discharge status of the patients

Vital measurements **Average ±SS**	Units	Discharge n=20	Exitus n=33	P
Age	Year	62.45±22.33	67.09±18.26	*0.509*
systolic blood pressure	mmHg	117.4±24.46	103.73±33.34	*0.106*
Diastolic blood pressure	mmHg	57.5±11.62	55.67±15.09	*0.876*
MAP	mmHg	77.46±12.60	71.68±18.52	*0.393*
Pulse	beats/min	90.65±19.12	114.48±24.98	*0.001**
Blood sugar	*Mg/dl*	161.5±72.98	154.67±72.74	*0.700*
Hemoglobin	*G/L*	12.3±2.13	11.26±2.48	*0.095*
SO_2	*%*	95.75±2.31	93.61±9.82	*0.677*
StO_2	*%*	82.97±8.19	76.21±11.65	*0.041**
Ph		7.36±0.09	7.33±0.11	*0.633*
PaO_2	*%*	106.11±34.85	113.34±36.53	*0.419*
Length of hospital stay	day	22.5±16.45	17.55±20.47	*0.046**

Table 3. Descriptive measures of patients according to middle and advanced age

Vital measurements **Average ±SS**	Units	Age 40 and below (n=9)	Age over 40 (n=52)	P
Age	Year	26.22±8.33	73.06±11.24	*<0.001**
systolic blood pressure	mmHg	108.38±18.29	109.29±31.24	*0.846*
Diastolic blood pressure	mmHg	61.00±10.41	56.65±14.33	*0.281*
MAP	mmHg	76.66±11.92	74.16±17.08	*0.614*
Pulse	beats/min	101.89±28.85	107.95±26.53	*0.943*
Blood sugar	*Mg/dl*	165.11±71.04	157.08±71.16	*0.839*
Hemoglobin	*G/L*	12.17±2.71	11.77±2.42	*0.684*
SO_2	*%*	97.22±1.64	94.35±8.01	*0.098*
StO_2	*%*	84.55±8.16	77.33±10.57	*0.043**
Ph		7.34±0.09	7.36±0.12	*0.569*
PaO_2	*%*	125.22±49.18	110.47±32.76	*0.259*
Length of hospital stay	day	9.22±6.85	19.60±19.68	*0.210*

Noradrenaline drug use had an effect on blood pressure and heart rate. The systolic and diastolic blood pressures of the patients who used the drug were lower, whereas their heart rates were higher. The use of noradrenaline significantly decreased the StO_2 ($p = 0.012$) and SO_2 measurements (Table 4). Dopamine use was effective only on systolic blood pressure ($p = 0.036$) and hospital stay ($p = 0.028$). Furthermore, StO_2 or SO_2 measurements had lower values in patients using dopamine, but no difference was detected between the groups.

The StO_2 measurements taken from the right hand had a positive and significant correlation ($R = 0.344$; $p = 0.007$) with systolic blood pressure and a negative and significant correlation with pulse rate ($R = -0.388$; $p = 0.002$). A higher positive correlation was found with SO_2 values ($R = 0.498$; $p < 0.001$). While the StO2 rates were concentrated between 80% and 90%, the SO_2 values were concentrated between 90%–99%. No significant correlation was found between diastolic blood pressure, blood glucose, hemoglobin, PaO_2, and StO_2.

Approximately 50% positive correlation was calculated between the StO_2 measurements taken from the left hand and the systolic, diastolic, and SO_2 values.

Table 4. Descriptive measures according to noradrenaline use status

Vital measurements **Average ±SS**	Units	YES (n=33)	NO (n=28)	P
Age	Year	68.03±15.08	63.93±24.57	*0.813*
Systolic blood pressure	mmHg	97.24±27.43	123.07±26.02	*0.001**
Diastolic blood pressure	mmHg	53.03±13.78	62.21±12.32	*0.010**
MAP	mmHg	67.76±15.80	82.50±13.31	*0.112*
Pulse	beats/min	114.91±27.11	97.82±23.51	*0.012**
Blood sugar	*Mg/dl*	161.3±70.7	154.68±71.63	*0.487*
Hemoglobin	*G/L*	11.42±2.24	12.32±2.62	*0.134*
SO_2	*%*	93.42±9.73	96.36±2.72	*0.047**
StO_2	*%*	75.48±11.10	81.83±8.77	*0.012**
Ph		7.34±0.13	7.39±0.1	*0.100*
PaO_2	*%*	106.95±31.49	119.34±39.29	*0.284*
Length of hospital stay	day	17.03±18.6	19.29±19.05	*0.256*

Furthermore, the StO_2 values had a positive and significant correlation (R = 0.269; p = 0.036) with systolic blood pressure and a negative and significant correlation with pulse rate (R = −0.324; p = 0.011). There was a significant correlation between StO_2 and mean arterial pressure (MAP) (R = 0.507; p < 0.001). Meanwhile, there was no significant correlation between SO_2, diastolic blood pressure, blood glucose, hemoglobin, PaO_2, and StO_2. Moreover, StO_2 did not change with the length of hospital stay. When the referred patients were excluded, there was no significant correlation between StO_2, SO_2, and MAP among the discharged patients, whereas a significant and positive correlation was found between all three variables in the ex-patient group (R MAP − StO_2 = 0.529; p = 0.002). When both the Ex and discharged patients were considered (n = 53), the correlation between pulse O_2 and StO_2 was positive and significant (R = 0.597; p < 0.001) (Table 5).

Table.5. Relationship between SpO2 measurement values and vital signs

Sto2 (n=61)	AGE (YEAR)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Pulse (beats/min)	Blood sugar *(Mg/dl)*	Hb (g/L)
R	-0.165	0.269	0.020	-0.324	-0.010	0.197
P	*0.203*	*0.036**	*0.880*	*0.011**	*0.940*	*0.128*
	MAP (mmhg)	Pulse O2 (%)	Ph	PaO_2 (%)	Length of hospital stay(day)	
R	*0.507*	0.054	0.024	0.066	0.188	
P	*<0.001**	*0.678*	*0.852*	*0.614*	*0.146*	

4. Discussion

In our study, it was aimed to investigate whether the follow-up with a device that measures tissue oxygenation of patients followed on mechanical ventilator is superior to the O_2 saturation probe. The StO_2 measurements taken from the right hand had a positive and significant correlation (R = 0.344; p = 0.007) with systolic blood pressure and a negative and significant correlation with pulse rate (R = −0.388; p = 0.002). A higher positive correlation was found with SO_2 values (R = 0.498; p < 0.001). While the StO_2 rates were concentrated between 80% and 90%, the SO_2 values were concentrated between 90% and 99%. Meanwhile, no significant correlation was found between diastolic blood pressure, blood glucose, hemoglobin, PaO_2, and StO_2. Approximately 50% positive correlation was found between the StO_2 measurements taken from the left hand and the systolic, diastolic, and SO_2 values, and no significant correlation was found between blood sugar, hemoglobin, PaO_2, and StO_2. However, no correlation was found between the mean StO_2 value and SO_2. When both the Ex and discharged patients were considered (n = 53), the correlation between pulse O_2 and StO_2 values was positive and significant.

Kır et al. (7) found that mixed and central venous oxygen saturation are useful for determining disease severity and evaluating the response to treatment in various critically ill conditions in which the cardiovascular system is affected. In line with current knowledge, SvO_2, and $ScvO_2$ are considered useful tools for evaluating and managing tissue perfusion in critically ill patients (7). However, these are invasive procedures for tissue perfusion measurement. The tissue oxygenation device we used in our study is a non-invasive tool with no contraindications. With this device, we have also provided information about tissue perfusion without any harm to the patients. In a clinical study by Lima et al., the normal range of StO_2 was 75%–91%. StO_2 measurements below 75% can consistently be considered an important indicator of a patient's hypoperfusion. High StO_2 levels (> 91%) were found to be measurable when oxygen delivery was well above use (8). In our study, the StO_2 value was found to be 79%, and it was lower in patients with impaired tissue perfusion and ex. In the studies of Myers et al. (9) and Gomer et al. (10), while StO_2

was used for the resuscitation of peripheral organs, it emerged as a covert detector of hypoperfusion. This result prompted the researchers to focus on the usefulness of StO_2 measured from skeletal muscle in critically ill patients. StO_2 measurements can be made from various muscles in the intensive care unit, and it has been thought that StO_2 measurements may be affected by local edema and adipose tissue. In our study, the StO_2 value was found to be higher in men than in women. The high StO_2 value in men was not associated with any of the measured values, such as Hemoglobin (Hb), blood sugar, blood pressure, and pulse. From this point of view, since tissue oxygenation is related to tissue perfusion and muscle mass is greater in men than in women, StO_2 measurement should be taken from the thenar region because women have more adipose tissue than men and StO_2 may be affected by muscle mass and adipose tissue.

Because perfusion is known to be impaired in sepsis, the work of Lima et al. supports our study. In the studies of Colin et al., Leone et al., and Shapiro et al., the StO_2 value was also commonly investigated in patients with severe sepsis and septic shock. Although StO_2 values have robust prognostic implications in trauma patients, they appear to be more complex in septic conditions (11–12). In a study by Creteur et al., the StO_2 value was found to be lower in septic patients (13). In our study, when all patients were considered, the lowest StO_2 ratio was found in sepsis patients. Tissue perfusion was impaired in sepsis patients, and StO_2 is low, which confirmed this fact. The StO_2 value was low in patients with low systolic blood pressure and high in patients with high systolic blood pressure, confirming that hypoperfusion has an effect on tissue oxygenation.

In our study, the StO_2 and SO_2 values were low in patients using noradrenaline and dopamine. Thus, noradrenaline and dopamine disrupted tissue perfusion, thereby decreasing the StO_2 value. This highlights the importance of measurements taken with the tissue oxygenation device for monitoring the general condition and tissue perfusion of patients. In addition, while the StO_2 value was high for patients under the age of 40, it was low for patients over the age of 40. As age progresses, tissue perfusion deteriorates; hence, tissue oxygenation was lower in patients over the age of 40. Additionally, StO_2 was higher in discharged patients. Hence, it was determined that patients with high StO_2 values are less likely to die because their tissue perfusion is not impaired.

Although our study supports the idea that tissue oxygenation is an indicator of perfusion, its shortcoming is that we did not consider the ejection fraction (EF) of our patients. In this study, EF could be determined in patients with heart failure, and its effects on tissue oxygenation could be evaluated. While our perfusion evaluation of the patients was adequate, a secondary evaluation was needed because we could not follow up our referral patients for a long time.

It is important to follow tissue perfusion in intensive care patients, and this can be done with a noninvasive method. The results of our study reveal that perfusion should be followed with tissue oxygenation in patients on mechanical ventilators because low tissue oxygenation indicates increased patient mortality.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: F.K., A.S.G., Design: F.K., Data Collection or Processing: F.K., B.G., H.A., M.G., Analysis or Interpretation: F.K., Literature Search: F.K., Writing: F.K., A.S.G., Z.D.D.

References

1. Annat G, Viale JP, Percival C, Froment M, Motin J. Oxygen delivery and uptake in the adult respiratory distress syndrome. Lack of relationship when measured independently in patients with normal blood lactate concentrations. Am Rev Respir Dis. 1986 Jun;133(6):999-1001. doi: 10.1164/arrd.1986.133.6.999. PMID: 3521420.

2. Gilbert EM, Haupt MT, Mandanas RY, Huaringa AJ, Carlson RW. The effect of fluid loading, blood transfusion, and catecholamine infusion on oxygen delivery and consumption in patients with sepsis. Am Rev Respir Dis. 1986 Nov;134(5):873-8. doi: 10.1164/arrd.1986.134.5.873. PMID: 3777684.

3. Jubran A, Tobin MJ. Monitoring during mechanical ventilation. Clin Chest Med. 1996 Sep;17(3):453-73. doi: 10.1016/s0272-5231(05)70327-0. PMID: 8875007.

4. Tremper KK, Barker SJ. Pulse oximetry. Anesthesiology. 1989 Jan;70(1):98-108. doi: 10.1097/00000542-198901000-00019. PMID: 2643368.

5. Androgue HJ, Tobin MJ. Respiratory Failure. 1st ed. USA: Black well Science Ltd, 1997.

6. Jubran A. Pulse oximetry. In: Tobin MJ (ed). Principles and Practice of Intensive Care Monitoring. New York: Mc GrawHill, 1998:261-87.

7. Kacmarek RM. Indications for ventilatory support. In: Raoof S, Khan FA (eds). Mechanical Ventilation Manuel. 1st ed. Philadelphia: Versa Press, 1998:3.

8. Lima A, van Bommel J, Jansen TC, Ince C, Bakker J. Low tissue oxygen saturation at the end of early goal-directed therapy is associated with worse outcome in critically ill patients. Crit Care. 2009;13 Suppl 5(Suppl 5):S13. doi: 10.1186/cc8011. Epub 2009 Nov 30. PMID: 19951385; PMCID: PMC2786115.

9. Leone M, Blidi S, Antonini F, Meyssignac B, Bordon S, Garcin F, et al. Oxygen tissue saturation is lower in nonsurvivors than in survivors after early resuscitation of septic shock. Anesthesiology. 2009 Aug;111(2):366-71. doi: 10.1097/ALN.0b013e3181aae72d. PMID: 19602965.2009

10. Shapiro NI, Arnold R, Sherwin R, O'Connor J, Najarro G, Singh S, et al. Emergency Medicine Shock Research Network (EMShockNet). The association of near-infrared spectroscopy-derived tissue oxygenation measurements with sepsis syndromes, organ dysfunction and mortality in emergency department patients with sepsis. Crit Care. 2011;15(5):R223. doi: 10.1186/cc10463.

Epub 2011 Sep 22. PMID: 21939529; PMCID: PMC3334769.

11. Raoof S. Objectives of mechanical ventilation. In: Raoof S, Khan FA (eds). Mechanical Ventilation Manuel. 1st ed. Philadelphia: Versa Press, 1998:4-5.8

12. Kır B., Bilgili B., Cinel İ., Mixed venous oxygen saturation and clinical significance. Journal of Anestesthesia 2014; 22 (4): 181 – 185

13. Colin G, Nardi O, Polito A, Aboab J, Maxime V, Clair B, et al. Masseter tissue oxygen saturation predicts normal central venous oxygen saturation during early goal-directed therapy and predicts mortality in patients with severe sepsis. Crit Care Med. 2012 Feb;40(2):435-40. doi: 10.1097/CCM.0b013e3182329645. PMID: 22020233.

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Lung ultrasound in the follow-up of stable idiopathic pulmonary fibrosis

Oya BAYDAR TOPRAK*, Ezgi ÖZYILMAZ, İsmail HANTA, Sedat KULECİ Efraim GÜZEL

Department of Chest Diseases, Faculty of Medicine, Çukurova University, Adana, Türkiye

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Abstract

Idiopathic pulmonary fibrosis is the most common and severe form of idiopathic interstitial pneumonia and is responsible for 20% of interstitial lung disease (ILD) cases. In this study, it was planned to evaluate the relationship of these two methods in detecting lung changes in IPF using a 12-zone lung ultrasound protocol with the current standard evaluation method, high-resolution computed tomography. 22 patients diagnosed with idiopathic pulmonary fibrosis by multidisciplinary evaluation were included in the study, and HRCT and pulmonary function tests and LUS protocol of 12 lung regions were used. The mean age ± SD of the patients was 69.0 ± 7.59 years. 21 (95.5%) were male. While 17 (77.3%) of the patients included in the study were diagnosed with radiological evidence, the diagnosis of the rest was confirmed histopathologically. While 5 of the patients (22.7%) did not receive any special treatment, 13 of the remaining patients were taking pirfenidone, and 4 were taking nintedanib. When the HRCT total fibrotic score was evaluated with the total LUS score, a correlation coefficient of 0.702 (P:0.000) was obtained. In stable idiopathic pulmonary fibrosis, lung ultrasonography can be a readily accessible, non-irradiating, short-term, and rapidly informative monitoring technique that can be utilised at the bedside or during consultation instead of high-resolution thorax computerized tomography.

Keywords: idiopathic pulmonary fibrosis, lung ultrasonography, thoracic tomography, pulmonary function test, gap

1. Introduction

The most severe form of idiopathic interstitial pneumonia, idiopathic pulmonary fibrosis (IPF), has a dismal prognosis and primarily affects older persons, demonstrating a close correlation between the fibrosis process and ageing. In all ILD examinations, high-resolution computed tomography (HRCT) of the chest is presently regarded as the primary standard diagnosis, not only for the first assessment but also for disease monitoring and treatment effectiveness prediction (1). In cases of respiratory function impairment and during yearly follow-ups, HRCT is typically necessary. Tomography has some drawbacks, including repetitive radiation exposure, expense, accessibility, and occasionally challenging supine positioning. It is essential to be aware of the radio shielding issue since the cumulative dosage for each exam is 7 mSv, which is equivalent to 2 years of exposure to natural light (2). Alternative diagnostic techniques are required due to the radiation danger, even though HRCT is now the preferred approach for the assessment of IPF (3). This seems to be a promising application for lung ultrasonography (LUS). The key benefits of the LUS examination are that it doesn't involve radiation exposure and is affordable, repeatable, convenient, bearable, and non-invasive (4). There are still specific gaps in this area that are particular to illnesses like IPF, despite the fact that several studies have proven that lung ultrasound results are now connected with HRCT scores in various disease categories (5-7). The purpose of this study is to clearly demonstrate the association between radiological results and lung ultrasound findings in stable period IPF follow-up.

2. Materials and Methods

2.1. Hypothesis

Lung ultrasound (LUS) may be a suitable method for the follow-up of patients with idiopathic pulmonary fibrosis.

2.2. Primary endpoint

Evaluation of the efficacy and safety of LUS in stable IPF

2.3. Excepted benefits

- Reducing the total radiation dosage that IPF patients get through HRCT in light of the accurate information that will be gathered.
- Reducing Health expenditures if it is determined that the LUS examination is sufficient for the clinical care of IPF

2.4. Study design and population

Twenty-two individuals with a confirmed diagnosis of IPF from a multidisciplinary perspective were included in the study between 1.1.2020 and 1.5.2020 sequentially. The trial excluded patients who had symptoms of aggravation in the previous 4 weeks. After each patient signed the informed consent form, they were all enrolled in the research. The Cukurova University Non-Interventional Ethics Committee (96/2020) approved this

*Correspondence: oyabaydarr@yahoo.com.tr

cross-sectional study.

At the time of the visit, included patients completed a clinical evaluation that comprised a thoracic ultrasound, pulmonary functional tests, and mMRC score. The most recent thorax CT conducted within three months of enrollment and those completed while the patient was enrolled were assessed. During subsequent reassessments, the presence, location, and severity of ultrasound abnormalities were noted for each patient and compared to the development of clinical, functional, and CT scans.

2.5. Lung ultrasound (LUS)

A GE Logic e R7 pro, USA, equipped with a 2-5 MHz curve array (C5-2) and a 4-12 MHz linear array (L12-4), was used for all LUS studies. Imaging parameters were adjusted manually to ensure maximum contrast between the examined structures. The "12-lung regions" LUS protocol was used. The LUS protocol provided an equal assessment of the anterior, lateral, and posterior lung regions on both sides. LUS was performed in a sitting position with the arms raised above the head while breathing normally to evaluate the lateral chest wall.

The transducer was placed perpendicular to the chest wall to provide a short-axis view of the intercostal space. During the LUS protocol, the number of B lines was re-registered in each preset IC. B-lines were defined as vertical hyperechoic reverberation artifacts originating from the pleural line and extending to the edges of the screen. In order to determine the degree of IPF severity, the total number of B lines per patient was scored by adding B lines from each of the 12 lung regions.

2.6. Thorax HRCT

Thoracic HRCT scans were evaluated in all patients in the last three months prior to participation in the radiological evaluation. Radiological images were assessed and graded by a single specialist physician in Çukurova University Faculty of Medicine, Department of Radiology. In the computed tomography that has been taken contrast in the early arterial phase, the section thickness is 1 mm.

Images were acquired while the patient was supine position with full inspiration covering the entire chest area. Additional sections were made in the prone decubitus position to exclude changes due to gravity. An intravenous contrast agent was not administered. In the early arterial phase, the section thickness is 1 mm. Major HRCT images have been described in international standard terminology defined by the Fleischner Society dictionary and in the peer-reviewed literature on viral pneumonia using terms such as ground-glass opacities (GGO), crazy-paving pattern, and consolidation (8). Image analysis was evaluated by expert radiologists in our institution using the institutional digital database system (HBYS Mergentech PACS, version v3.22.03.1-20220314).

Fibrotic changes were scored using a semi-quantitative technique. An HRCT fibrotic index was obtained by counting the presence and extension of reticulation and honeycomb for each lobe (9):

0—no reticulation,

1—reticulation without honeycombing,

2—septal reticulation with honeycomb in <25% of a lobe

3— septal reticulation with honeycomb in 2-49% of a lobe

4— septal reticulation with honeycomb in 50-75% of one lobe

5— septal reticulation with honeycomb in >75% of one lobe

Radiological involvements are divided into three;

Mild: scores ≤6

Medium: scores 7-13

Severe: scores ≥14

Gender-Age-Physiology (GAP) model – The most widely validated clinical prediction model is the GAP model, which incorporates age, sex, FVC, and DLCO into a simple score-score index and staging system that predicts one-, two-, and three-year mortality (10). The severity of the disease was determined according to this model.

2.7. Pulmonary function tests (PFTs)

PFTs were performed with a calibrated Sensor Medics V-Max 20 Spirometer (Jaeger MS-PFT Analyzer Unit, Wiasys Healthcare GmbH, Höchberg, Germany) in accordance with the ATS guideline. Basal forced expiratory volume for 1 second (FEV1) and forced vital capacity (FVC) were measured three times, and the best values were recorded. Total lung capacity was measured with the helium dilution technique (Jaeger MS-PFT Analyzer Unit), and Transfer Factor for Carbon Monoxide (TLCO) was measured with the single breath method. It was measured with a single breath technique in which 10% helium and 0.3% carbon monoxide were rapidly inhaled, held for 10 seconds, and then exhaled by measuring the remaining carbon monoxide (11). Test results are presented as a percentage of predicted values. The results of pulmonary function tests were interpreted according to the ATS/ERS recommendations (12).

3. Results

3.1. The characteristics of the participants

The mean age ± SD of the patients was 69.0 ± 7.59 years (range 58 to 81 years). 21 (95.5%) were male. Five patients were non-smokers, and 17 (77.2%) were active smokers or had a smoking history. While 17 (77.3%) of the patients included in the study were diagnosed with radiological evidence, the diagnosis of the rest was confirmed histopathologically. It was observed that hypoxemia developed at rest in 8 (36.4%) of the patients.

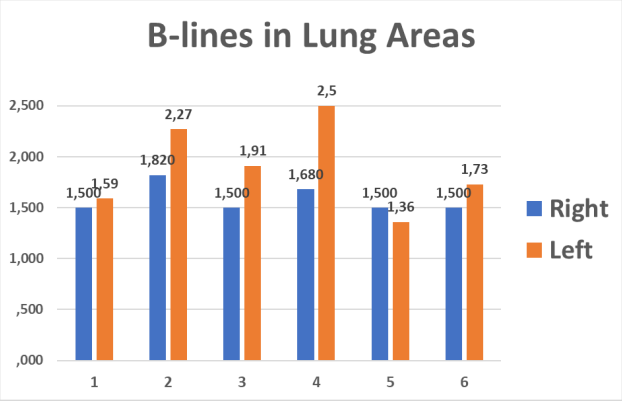

Fig. 1: Distribution of B-Lines in Lung Ultrasound Fields

The GAP stage was 1 in 10 (45.5%) of the participants, 2 in 10 (45.5%) and 3 in 2 (9.1%). While 5 of the patients (22.7%) did not receive any special treatment, 13 of the remaining patients were taking pirfenidone, and 4 were taking nintedanib. Table 1 summarizes the clinical characteristics of individuals.

Table 1. Sociodemographic And Clinical Caharcateristics of The Participants

	n	%
Gender		
Female	1	4.5
Male	21	95.5
Age (mean±*SD*)	69.0+7.59	
Radiological Pattern		
UIP	15	68.2
Probable IPF	4	18.2
Indeterminate IPF	3	13.6
mMRC Score		
1	4	18.2
2	9	40.9
3	6	27.3
4	3	13.6
GAP Score		
2	2	9.1
3	8	36.4
4	4	18.2
5	6	27.3
6	1	4.5
7	1	4.5
FEV1L	1.98±0.47	
FEV1%	79.3±21.1	
FVCL	2.48±0.69	
FVC%	77.2±22.9	
FEV1/FVC	81.3±10.4	
DLCO%	40.5±22.1	
DLCO/VA%	63.6±29.6	

3.2. HRCT and LUS Scores

In the present study group, none of the participants had mild IPF according to the HRCT severity score, while 8 (36.4%) had moderate and 14 (63.6%) had severe IPF. The distribution of B-lines in both lungs is shown in Fig.1. When the HRCT total fibrotic score was evaluated with the total LUS score, a correlation coefficient of 0.702 (P:0.000) was obtained (Fig. 2.).

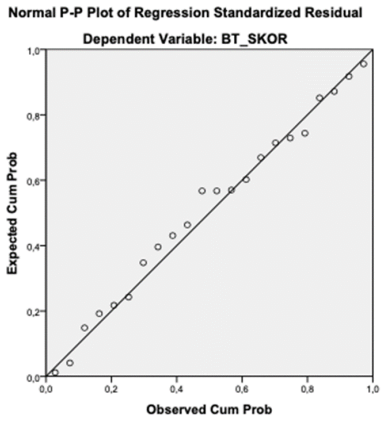

Fig. 2. Linear correlation of HRCT severity score and LUS fibrotic score

4. Discussion

The presence of many B lines with diverse distribution in both lungs is the key characteristic in the LUS assessment of fibrotic interstitial involvement. In stable IPF, the current investigation showed a substantial correlation between thoracic HRCT and LUS.

Today, with the rapid increase in the use of antifibrotic drugs, the use of HRCT has expanded not only for initial evaluation but also for monitoring the course of the disease and possible response to treatment. The main HRCT changes in a UIP pattern include reticulation and honeycombing, but evidence of ground glass changes is less common and has limited predictive value for the diagnosis of IPF (13). When dealing with radiation exposure, especially the biological effect of the cumulative dosage, attention should be used since people with IPF have a higher chance of developing lung cancer (14). Although HRCT is the reference standard diagnostic technique, the increasing role of LUS as a nonradiative tool in lung assessment has emerged to avoid radiation risk. In LUS, first in patients with interstitial lung disease associated with scleroderma; the presence of multiple B lines has been shown to be an indicator of pulmonary diffuse interstitial disorder and a significant linear correlation between the total number of B lines and the tomographic score, laying the groundwork for future research.[5] Studies of LUS are increasingly focusing on various interstitial lung illnesses other than IPF. IPF patients have been a part of several pieces of research, yet there is still a shortage of information on how to assess IPF patients (6,15). This study contributes to the literature showing a positive correlation between LUS fibrotic scores and HRCT severity scores. With this data and further studies to support it, the early detection of ultrasound signs of worsening interstitial changes will provide an additional argument for earlier evaluation with an HRCT scan or even initiation of treatment.

The first restriction of the presented study is the short sample size. Because IPF is an uncommon disease with a median survival duration of 2 to 5 years following diagnosis, enrolling on this population may be challenging. More extensive

investigations are required to corroborate the experimental and encouraging results. However, despite the fact that it was single-centre research and hence had a smaller patient population, it allowed a single skilled practitioner to complete all of the ultrasonographic examinations. The consistency of the data and a better understanding of the role of ultrasonography in the follow-up may be gained if follow-up research employing a comparative assessment can be carried out.

In conclusion; LUS can be an additional and reliable tool for IPF, which is a rapidly progressing disease that requires a multidisciplinary approach, that can be used at the bedside or during a consultation, is easily accessible, does not emit radiation, can be reached in a short time and is instantly informative.

Conflict of interest

The authors declare that they have no potential conflict of interest, including any financial, personal or other relationships with the other people or organisations that could inappropriately influence or be perceived to influence the presented work. The authors have no relevant financial or non-financial interests to disclose.

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Informed consent was obtained from all individual participants included in the study. Informed consent was obtained from legal guardians. All participants signed the document stating they participated in the study.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval was granted by the Ethics Committee of Cukurova University (96/2020).

Authors' contributions

Concept: O.B.T, E.Ö., İ.H., S.K., E.G. Design: O.B.T, E.Ö., İ.H., S.K., E.G. Data Collection or Processing: O.B.T, E.Ö., İ.H., S.K., E.G., Analysis or Interpretation: O.B.T, E.Ö., İ.H., S.K., E.G., Literature Search: O.B.T, Writing: O.B.T, E.Ö., İ.H., S.K., E.G.

References

1. Devaraj A. Imaging: how to recognise idiopathic pulmonary fibrosis. Eur Respir Rev. 2014;23(132):215-219. doi:10.1183/09059180.00001514
2. European Commission, Directorate-General for Energy, Chateil, J. et al. Referral guidelines for medical imaging: availability and use in the European Union, Publications Office, 2014, https://data.europa.eu/doi/10.2833/18118
3. Manolescu D, Davidescu L, Traila D, Oancea C, Tudorache V. The reliability of lung ultrasound in assessment of idiopathic pulmonary fibrosis. Clin Interv Aging. 2018;13:437-449. Published 2018 Mar 22. doi:10.2147/CIA.S156615
4. Lichtenstein D, Hulot JS, Rabiller A, Tostivint I, Mezière G. Feasibility and safety of ultrasound-aided thoracentesis in mechanically ventilated patients. Intensive Care Med. 1999;25(9):955-958. doi:10.1007/s001340050988
5. Gargani L, Doveri M, D'Errico L, Frassi F, Bazzichi ML, Sedie A D, et al. Ultrasound lung comets in systemic sclerosis: a chest sonography hallmark of pulmonary interstitial fibrosis. Rheumatology (Oxford). 2009;48(11):1382-1387. doi:10.1093/rheumatology/kep263
6. Sperandeo M, Varriale A, Sperandeo G, Filabozzi P, Piattelli ML, Carnevale V, et al. Transthoracic ultrasound in the evaluation of pulmonary fibrosis: our experience. Ultrasound Med Biol. 2009;35(5):723-729. doi:10.1016/j.ultrasmedbio.2008.10.009
7. Sperandeo M, De Cata A, Molinaro F, Trovato FM, Catalano D, Simeone A, et al. Ultrasound signs of pulmonary fibrosis in systemic sclerosis as timely indicators for chest computed tomography. Scand J Rheumatol. 2015;44(5):389-398. doi:10.3109/03009742.2015.1011228
8. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Muller N L, Remy J, et al. Fleischner Society: glossary of terms for thoracic imaging. Radiology. 2008;246(3):697-722. doi:10.1148/radiol.2462070712
9. Kazerooni EA, Martinez FJ, Flint A, Jamadar DA, Gross BH, Spizarny DL, et al. Thin-section CT obtained at 10-mm increments versus limited three-level thin-section CT for idiopathic pulmonary fibrosis: correlation with pathologic scoring. AJR Am J Roentgenol. 1997;169(4):977-983. doi:10.2214/ajr.169.4.9308447
10. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med. 2018;198(5):e44-e68. doi:10.1164/rccm.201807-1255ST
11. Ranu H, Wilde M, Madden B. Pulmonary function tests. Ulster Med J. 2011;80(2):84-90.
12. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardisation of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med. 2019;200(8):e70-e88. doi:10.1164/rccm.201908-1590ST
13. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183(6):788-824. doi:10.1164/rccm.2009-040GL
14. Tomassetti S, Gurioli C, Ryu JH, Decker PA, Ravaglia C, Tantalocco P, et al. The impact of lung cancer on survival of idiopathic pulmonary fibrosis. Chest. 2015;147(1):157-164. doi:10.1378/chest.14-0359
15. Hasan AA, Makhlouf HA. B-lines: Transthoracic chest ultrasound signs useful in assessment of interstitial lung diseases. Ann Thorac Med. 2014;9(2):99-103. doi:10.4103/1817-1737.128856

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Comparison of the pregnant and non-pregnant women of reproductive age hospitalized due to COVID-19 infection

Özgür KILIÇ[1,*], Mehmet POLAT[2], Nazmiye TİBEL TUNA[3], Eda KÖPRÜ[4] Muzaffer ELMALI[5], İlkay BOZKURT[6], Davut GÜVEN[7], Melda DİLEK[8]

[1]Division of Intensive Care Medicine, Department of Internal Medicine, Faculty of Medicine, Ondokuz Mayıs University Samsun, Türkiye
[2]Division of Nephrology, Department of Internal Medicine, Nevsehir State Hospital, Nevsehir, Türkiye
[3]Department of Pulmonary Medicine, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[4]School of Medicine, Ondokuz Mayıs UniversitySamsun, Türkiye
[5]Department of Radiology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[6]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[7]Department of Obstetrics and Gynecology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[8] Division of Nephrology, Department of Internal Medicine, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

COVID-19 affects pregnant women more severely than nonpregnant women of reproductive age. However, the rate of critical illness and fatality reported in other studies varied in a wide range in both groups. The study aims to investigate the clinical outcomes of COVID-19 in the pregnant and nonpregnant matched control patients admitted to the hospital. Pregnant and nonpregnant patients of reproductive age (18-45 years) infected with COVID-19 who were admitted to Ondokuz Mayıs University Hospital, Samsun, Turkey, from March 11 to December 11, 2020, were enrolled in the study. The clinical, radiological, and laboratory data of the patients were analyzed retrospectively. A total of 153 patients were investigated; 123 were nonpregnant, and 30 were pregnant. Emergency delivery occurred in 5 (17%) pregnant women due to acute respiratory failure associated with COVID-19 and 1 (3%) pregnant woman due to obstetric reasons. Four premature births, one perinatal death, and no stillbirth or miscarriage were reported. The rate of admission to the intensive care unit (ICU) [7/30 (23.3%) vs 3/123 (2.4%), p<0.001] and the need for invasive mechanical ventilation (IMV) [5/30 (17.0%) vs 2/123 (1.6%), p=0.003] were significantly higher in pregnant than in non-pregnant patients. However, hospital length of stay (HLOS) and mortality did not differ between groups: HLOS was median 4 vs 5 days, p=0.68, and the mortality rate was 1/123 (0.8%) vs 0/30 (0%), p=0.62 in nonpregnant and pregnant patients respectively. We observed that COVID-19 has a more severe course in pregnant women versus the nonpregnant control group, but no difference was noted in terms of hospital length of stay and mortality. The overall case fatality rate of COVID-19 in hospitalized pregnant or nonpregnant women of reproductive age was found to be much lower than the general hospitalized population worldwide.

Keywords: COVID-19 infection, pregnancy, nonpregnant patient, mortality, critical illness

1. Introduction

Coronavirus disease of 2019 (COVID-19) caused by novel coronavirus SARS-CoV-2 may be asymptomatic or symptomatic in pregnant women. While more than 90% of pregnant women infected with SARS-CoV-2 may recover without hospitalization, the rest may develop rapid clinical worsening, and symptomatic pregnant women are at higher risk for severe illness and death compared to nonpregnant women of reproductive age (1-5). Current evidence suggests that pregnancy does not increase susceptibility to COVID-19 compared to young nonpregnant women but does increase the severity of illness, including increased need for intensive care and mechanical ventilators or respiratory support and an increased risk of death (1, 6-14). In addition, pregnant patients with COVID-19 may be at higher risk of preterm birth compared to uninfected pregnant women. However, COVID-19 does not seem to increase the risk of miscarriage or congenital anomalies, and neonatal outcomes appear to be good. Physiological changes during pregnancy, such as decreased functional residual capacity, diaphragm elevation, edema in the respiratory mucosa, and impaired cellular immunity, may cause the viral disease to be more severe as they make them susceptible to viral infection and hypoxia (15). Another possible explanation may be the overexpression of ACE2 receptors in pregnancy compared to nonpregnant; the uterus and placenta are the main sources (16). Since the ACE2 receptor serves as a binding site for SARS-CoV-2, after the viral invasion, the ACE2 receptor is down-regulated, decreasing the metabolism of angiotensin II. Elevated levels of

*Correspondence: ozgur.kilic@omu.edu.tr

angiotensin II promote vasoconstriction, inflammation, and a procoagulopathic environment that occurs in COVID-19 (17). However, some data do not support an increased risk of severe COVID-19 or mortality in pregnancy compared with nonpregnant female patients of reproductive age (18-20). Consequently, despite information about the virus and COVID-19 continues to accrue, the effects of COVID-19 infection on pregnancy are not entirely resolved. In this comparative study, we report the clinical features of the pregnant and nonpregnant women of reproductive age admitted to the hospital with confirmed COVID-19 infection.

2. Materials and Methods

2.1. Study design

Pregnant and nonpregnant women of reproductive age (18-45 years) who were admitted to the hospital with the diagnosis of COVID-19 between March 11 2020 and December 11, 2020, were included in the study. We retrospectively reviewed the patients' clinical, radiological, and laboratory data through their electronic files. Since the onset of the pandemic, our hospital has been serving as a reference university hospital, particularly for severe or critical COVID-19 pregnant women. Exclusion criteria were defined as age below 18 or above 45 years, patients hospitalized with high clinical suspicion of COVID-19 but whose polymerase chain reaction (PCR) was detected negative, and who had no typical findings on chest computed tomography (CT) as well as no history of close exposure, preoperative patients who had been routinely tested for COVID-19 PCR but had a negative result, and patients with advanced cancer at terminal stage.

2.2. Diagnosis and severity of illness

The diagnosis of COVID-19 was confirmed by a PCR positivity specimen obtained from a nasopharyngeal swab. For patients with PCR negative, either antibody positivity alone or a combination of household contact history and typical findings on chest CT were considered sufficient to confirm COVID-19 diagnosis. Typical chest CT findings of COVID-19 were accepted as ground-glass opacities, crazy paving patterns, and/or consolidation. The severity of the illness was categorized into three groups based on the clinical spectrum of SARS-CoV-2 infection defined by the National Institute of Health (21). Asymptomatic to mild infection covers patients with no sign or symptom to any symptom or sign except shortness of breath, dyspnea, or abnormal chest imaging.

Moderate infection refers to patients with any sign or symptom of lower respiratory disease on clinical assessment or imaging as well as oxygen saturation (SpO2) ≥94 on ambient air at sea level.

Severe to critical infections include patients with SpO2 <94% on room air at sea level, respiratory rate >30 breaths/min, PaO2/FiO2<300, lung infiltrates >50%, respiratory failure requiring oxygen therapy and/or respiratory support, septic shock and /or multiorgan failure. Pulmonary involvement was assessed only in patients who were undertaken chest CT.

Supplemental oxygen and respiratory support were given algorithmically according to the oxygen need in the following order as appropriate; nasal cannula, simple face mask, reservoir bag, high flow nasal cannula (HFNC), noninvasive mechanical ventilation (NIV), invasive mechanical ventilation (IMV). Patients with septic shock and multiorgan failure were triaged according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis 3) (22).

2.3. Scanning protocol

Chest CT was performed with a multidetector scanner (Aquillon Prime SP, Toshiba Medical Systems Corporation, Japan) using the following parameters: 72 kW generator, 0.35 second rotation time, 78 cm gantry bore, and automatic tube current modulation. The CT scans were obtained with the patient placed in the supine position at the end inspiring period without using contrast media. Images were acquired and reconstructed as axial sections with 1 mm thickness and dose reduction protocol. The scans were interpreted and reported by ME, a thoracic radiologist with 20 years of experience. Informed consent had been obtained from all pregnant patients before CT scanning.

2.4. Assessment of pulmonary involvement in CT

The severity level of COVID-19 disease according to the radiological involvement on CT was determined based on a semi-quantitative scoring system (23). A visual score between 0 and 5 was given to the percentage of the area of radiological involvement for each lung lobe. The scoring was as follows: 0 points for no involvement, 1 point for <5% involvement, 2 points for 5-25% involvement, 3 points for 26-49% involvement, 4 points for 50-75 % involvement, and 5 points for >75% involvement. The total score obtained by summing the points calculated for 5 lobes, including the upper, middle, and lower lobes of the right lung and the upper and lower lobes of the left lung, was defined as the CT severity score (CT-SS). The CT-SS of each patient was qualitatively classified as mild (score 1-5), moderate (score 6-14), or severe (15-25) (24). Examples of scoring some CT sections are given in Fig. 1.

2.5. Statistical analysis

The data were analyzed with the SPSS program, version 21.0. Categorical parameters were expressed as percentage and frequency. Continuous data were expressed as mean with standard deviation (SD) and as median with interquartile range (IQR) for normal and non-normal distributed data, respectively. Comparison between categorical variables was made using the X2 test or Fisher's exact test. Continuous variables were compared with each other using parametric test Student T or nonparametric test Mann Whitney U, where appropriate. P values less than 0.05 were considered statistically significant.

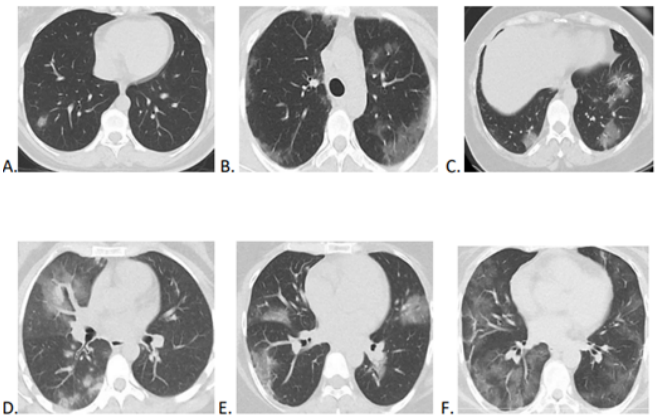

Fig. 1. Examples of CT-SS calculated on CT sections of some patients. A) CT-SS is 1 points; for area of radiologic involvement <5% in the right lung. B) CT-SS is 4 points; 2 points for radiologic involvement of 5-25% in each lung. C) CT-SS is 4 points; 1 points for area of radiologic involvement <5% in the right lung and 3 points for radiologic involvement of 26-49% in the left lung. D) CT-SS is 5 points; 4 points for radiologic involvement of 50-75% in the right lung and 1 point for radiologic involvement in the left lung. E) CT-SS is 5 points; 3 points for radiologic involvement of 26-49% in the right lung and 2 points for radiologic involvement of 5-25% in the left lung. F) CT-SS is 10 points; 5 points for radiologic involvement >75% in each lung. CT-SS: computed tomography severity score

3. Results

A total of 284 patients were screened, and 153 met the inclusion criteria. All the 30 pregnant patients tested positive for PCR, and 15 of 123 nonpregnant patients tested negative for PCR. The demographic and clinical characteristics of the patients are listed in table 1. The mean age was higher in the nonpregnant group compared to pregnant women; 32.8 (7.5) vs 30.1 (5.4) years, p=0.03. Pregnant patients were admitted at a median of 33.5 (26.8-38.0) weeks, half of them delivered during the hospital stay. Of the 15 deliveries, six were performed with the urgent cesarean section, five of which were due to respiratory distress, and one was due to an obstetric problem. Four preterm births (PTB) were recorded in the study, 3 out of 4 were<34 weeks, and 1 out of 4 was <37 weeks of pregnancy. Among newborns, only one baby born at 26 weeks of gestation died.

The rate of asymptomatic patients was higher among pregnant women, though not statistically significant; 10% (3/30) vs 3.2% (4/123), p=0.37. The cough was the most common symptom in all patients. Other symptoms, including muscle or body aches, headache, fever or chills, and diarrhea were significantly more frequent in nonpregnant than in pregnant patients. Asthma was the most common pre-existing condition, with a similar proportion of nonpregnant and pregnant patients being 8.1% (10/123) and 6.7% (2/30), respectively. Autoimmune disease (9/123 vs 1/30), diabetes (5/123 vs 1/30), and solid organ transplant (4/123 vs 1/30) were more common in nonpregnant women compared to pregnant women. History of active smoking (9/123), hypertension (3/123), and obesity (3/123) were recorded only among nonpregnant women.

CT was performed on 126 patients. Pulmonary involvement was detected in 83 of them. The CT-SS of 80 patients could be calculated as three CTs were reported as indeterminately. Semi-quantitative SS-CT was a median of 2 (0-6) points for the nonpregnant group, while 9 (0.8-17.3) points for the pregnant group (p=0.022). CT-SS was compatible with severe disease in 4.2% of nonpregnant patients and 44.0% of pregnant women.

Patient distribution rates between groups were significantly different according to disease severity (p=0.004). Patients with asymptomatic or mild illness were 35% (43/123) vs 56% (17/30), moderate illness was 50% (62/123) vs 16% (5/30), and severe or critical illness was 15% (18/123) vs 26% (8/30) in nonpregnant and pregnant cases respectively. There was no difference in terms of oxygen requirement (non-pregnant, 17.1 % vs pregnant, 30.0%; p=0.11). However, the need for intensive care and IMV was significantly higher in pregnant patients than in nonpregnant patients at 23.3% (7/30) and 17% (5/30) versus 2.4% (3/123) and 1.6% (2/123), respectively (p<0.001 and p=0.003).

Among the patients admitted to the ICU, 3 out of 7 pregnant women were referred from other hospitals, but none of the nonpregnant patients had a referral history. Of the 153 patients, only 10 (6.5%) were admitted to ICU, and one patient among them died who was a nonpregnant case with restrictive lung disease due to scoliosis. Thus, the overall case fatality rate corresponded to 0.7% (1/153) in the study population. The length of hospital stay was similar in both groups: median 4 days (2.5-7.0) and 5 days (2.0-10.0) for nonpregnant and pregnant patients, respectively (Table 1).

A comparison of laboratory parameters is summarized in table 2. Inflammation markers and D-dimer levels were found to be significantly elevated in the pregnant group. Median values for C-reactive protein (CRP) was 40.5 vs 6.4 mg/L (p<0.001), erythrocyte sedimentation rate (ESR) was 64.0 vs 24.0 mm/h (p=0.001) and D-dimer was 1070.5 vs 289.0 ng/mL (p<0.001) in pregnant and non-pregnant patients respectively. While lymphocyte (L) count was lower (0.9 vs 1.2 x103/uL; p=0.039), neutrophil (N) and neutrophil-lymphocyte ratio (NLR) was higher (5.9 vs 3.2x103/uL; p<0.001 and 5.8 vs 2.3; p<0.001 respectively) in pregnant compared to non-pregnant population. Hemoglobin (Hb), procalcitonin (ProCT), prothrombin time (PT), alkaline phosphatase (ALP), and total bilirubin showed a statistically significant difference between the groups but are of uncertain clinical significance.

Characteristics of critically ill patients are given in table 3. The median age was 33 years, with IQR of 26.8 and 36. Of these patients, only three had a pre-existing disease, two had obesity and one had scoliosis, and all were nonpregnant. Laboratory parameters were markedly different from the study population. The median values for L were 0.65 vs

1.20x103/uL, NLR was 9.60 vs 2.90, CRP was 162.5 vs 9.9 mg/L, and D-Dimer was 1039.5 vs 391 ng/mL, and ferritin was 212 vs 48.3 ng/mL in critical and overall patients respectively. CT-SS of the critical patients was higher than the overall value median of 13.5 vs 2.0 points. Nine out of ten patients were admitted with acute respiratory distress syndrome (ARDS) having moderate to severe lung involvement on chest CT. One patient was admitted for postoperative respiratory failure and underwent cesarean delivery. Sequential organ failure assessment (SOFA) score and PaO2/FiO2 at ICU admission were median of 2.5 points (2.0-4.8) and 143.5 mmHg (117.8-167.0), respectively. Of 10 patients admitted to ICU, one patient recovered with a reservoir bag, two patients with HFNC, and seven patients with IMV. Two patients developed septic shock, and one of them died. Length of stay in ICU (LOS-ICU) was median 9 days (7.0-28.0).

Table 1. Demographic and clinical characteristics of the study population

Variables	All patients N=153	Nonpregnants N=123	Pregnants N=30	P value
Age, mean (SD)	32.3 (7.2)	32.8 (7.5)	30.1 (5.4)	**0.029**
Pregnancy week, median (IQR)			33.5(26.8-38.0)	NA
Delivery during hospital stay, N (%)			15(50)	NA
Urgent delivery, N (%)			6 (40)	NA
Preterm birth, N (%)			4 (26)	NA
Perinatal death, N (%)			1(7)	NA
Presence of symptom, (N%)				0.137
Yes	7 (4.5)	4 (3.2)	3 (10.0)	
No	146 (95.5)	119 (96.8)	27 (90.0)	
Symptom, N (%)				
Cough	96 (62.7)	78 (63.4)	18 (60)	0.729
Muscle or body aches	75 (49)	67 (54.5)	8 (26.7)	**0.006**
Fatigue	74 (48.4)	63 (51.2)	11 (36.7)	0.153
Headache	68 (44.4)	61 (49.6)	7 (23.3)	**0.009**
Shortness of breath	61 (39.9)	51 (41.5)	10 (33.3)	0.415
Fever or chills	55 (35.9)	50 (40.7)	5 (16.7)	**0.014**
Sore throat	54 (35.3)	47 (38.2)	7 (23.3)	0.126
New loss of state or smell	35 (22.9)	31 (25.2)	4 (13.3)	0.159
Chest pain	30 (19.6)	26 (21.1)	4 (13.3)	0.334
Diarrhea	26 (17)	25 (20.3)	1 (3.3)	**0.026**
Runny nose	18 (11.8)	12 (9.8)	6 (20.0)	0.125
PCR, N (%)				NA
Positive	138 (90.2)	108 (87.8)	30 (100)	
Negative	15 (9.8)	15 (12.2)	0 (0)	
Preexisting conditions, N (%)				NA
Asthma	12 (7.8)	10 (8.1)	2 (6.7)	
Autoimmune disease	10 (6.5)	9 (7.3)	1(3.3)	
Diabetes	6 (3.9)	5 (4.1)	1(3.3)	
Hypertension	4 (2.6)	3 (2.4)	-	
Obesity	3 (2.0)	3 (2.4)	-	
Solid organ transplant	4 (2.6)	4 (3.3)	1 (3.3)	
Current smoking	9 (5.9)	9 (7.3)	-	
Severity of disease, N (%)				**0.004**
Asymptomatic or mild	60 (39.2)	43 (35.0)	17 (56.0)	
Moderate	67 (43.8)	62 (50.0)	5 (16.0)	
Severe or critical	26 (17.0)	18 (15.0)	8 (26.0)	
Semiquantative CT-SS median (IQR)	2 (0-7)	2 (0-6)	9 (0.8-17.3)	**0.022**
Qualitative CT-SS, N (%)				NA
Mild	44 (55.0)	42 (59.2)	2 (22.2)	
Moderate	29 (36.3)	26 (36.6)	3 (33.3)	
Severe	7 (8.8)	3 (4.2)	4 (44.4)	
Oxygen requirement, N (%)				0.110
Yes	30 (19.6)	21 (17.1)	9 (30.0)	
No	123 (80.4)	102 (82.9)	21 (70.0)	
IMV requirement, N (%)				**0.003**
Yes	7 (4.6)	2(1.6)	5 (17)	
No	3 (1.9)	1 (0.8)	2 (6.7)	
ICU requirement, N (%)				**<0.001**
Yes	10 (6.5)	3 (2.4)	7 (23.3)	
No	143 (93.5)	120 (97.6)	23 (76.7)	
HLOS, median (IQR)	4.0 (2.0-7.0)	4.0 (2.5-7.0)	5.0 (2.0-10.0)	0.680
Outcome, N (%)				0.620
Discharge	152 (99.3)	122 (99.2)	30 (100)	
Death	1 (0.7)	1 (0.8)	0 (0)	

*Antiviral medications include Favipravir, Lopinavir, Ritonavir, Remdesivir. † Heparin include either unfractionated or low molecular weight heparin PCR: Polymerase chain reaction, CT-SS: Computed tomography severity score, IMV: Invasive mechanical ventilation, ICU: Intensive care unit, HLOS: Hospital length of stay, NA: Not applicable, SD: Standard deviation, IQR: Interquartile ratio, N: Number of patients

Table 2. Laboratory characteristics of the study population

Variable*	All patients N=153	Nonpregnants N=123	Pregnants N=30	P value
Hb, g/dL	11.9 (1.6)	12.1 (1.6)	11.1 (1.0)	**0.001**
PLT, x10^3/uL	217.4 (78.4)	221 (73.5)	202.7 (96.0)	0.253
N, x10^3/uL	3.8 (2.5-5.3)	3.2 (2.4-4.7)	5.9 (4.7-7.0)	**<0.001**
L, x10^3/uL	1.2 (0.8-1.7)	1.2 (0.9-1.9)	0.9 (0.7-1.3)	**0.039**
NLR	2.9 (1.7-5.1)	2.3 (1.5-4.3)	5.8 (3.9-8.5)	**<0.001**
ESR, mm/h	30.0 (17-60)	24.0(16.0-54.0)	64.0(45.8-77.3)	**0.001**
CRP, mg/L	9.9 (3.1-40.3)	6.4 (3.1-31.9)	40.5 (9.9-114.5)	**<0.001**
D-Dimer, ng/mL	341.0(223.0-706.0)	289.(200.0-448.0)	1070.5 (686.3-2393.3)	**<0.001**
Fibrinogen, mg/dL	349.0 (249-485)	321.5 (207.8-456.5)	445 (378.5-611.0)	0.129
Ferritin, ng/mL	48.3 (27.4-124.2)	44.7 (23.9-117.5)	61.2 (35.8-136.3)	0.355
ProCT, ng/mL	0.05 (0.03-0.08)	0.04 (0.03-0.06)	0.08 (0.05-0.16)	**<0.001**
PT, sec	11.8 (11.2-12.5)	12.0 (11.4-12.7)	11.0 (10.6-11.7)	**<0.001**
PTT, sec	27.8 (25.5-30.9)	28.4 (25.5-30.9)	27.2 (24.8-30.9)	0.385
ALT, IU/L	15.6 (11.0-25.5)	16.0 (11.0-26.0)	13.0 (9.9-24.3)	0.133
AST, IU/L	21.0 (17.0-30.5)	21.4 (17.0-31.0)	19.8 (16.8-29.5)	0.379
ALP, IU/L	69.0 (51.0-99.5)	61.0 (47.0-83.3)	104.0 (92.0-130.5)	**<0.001**
GGT, IU/L	18.5 (10.0-36.0)	19.2 (10.0-41.0)	15 (7.0-27.0)	0.140
LDH, IU/L	217.0(182.0-283.0)	220.0 (181.3-270.8)	213.0 (186-337)	0.744
T.Bil, mg/dL	0.2(0.3-0.5)	0.3 (0.2-0.4)	0.4 (0.2-0.6)	**0.006**

*Hb and PLT are presented as mean (SD) and the rest of the variables are presented as median (IQR). Hb: Hemoglobin, PLT: Platelet, N: Neutrophile, L: Lymphocyte, NLR: Neutrophile lymphocyte ratio, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ProCT: Procalcitonin, PT: Prothrombin time, PTT: Partial thromboplastin time, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transaminase, LDH: Lactate dehydrogenase, T. Bil: Total bilirubine, SD: Standard deviation, SD: Standard deviation, IQR: Interquartile ratio.

Table 3. Characteristics of patients admitted to ICU

Variable	Critical patients N=10
Age, year, median (IQR)	33 (26.8-36.0)
Laboratory parameters, median (IQR)	
L, x10^3/uL	0.65 (0.48-1.40)
NLR	9.60 (5.20-17.60)
CRP, mg/L	162.5 (112.3-181.5)
D-Dimer, ng/mL	1039.5 (839.3-2163.8)
Ferritin, ng/mL	212.0 (79.6- 349.0)
Semiquantative CT-SS, median (IQR)	13.5 (8.5-19.8)
Qualitative CT-SS, n/N	
Mild	1/10
Moderate	4/10
Severe	5/10
SOFA score, median (IQR)	2.5 (2.0-4.8)
PaO_2/FiO_2 at ICU admission, mmHg, median (IQR)	143.5 (117.8-167.0)
Respiratory support, n/N	
Reservoir bag	1/10
HFNC	2/10
IMV	7/10
Days on IMV, median (IQR)	7 (1.8-21.8)
Septic shock, n/N	2/10
LOS in ICU, day, median (IQR)	9 (7-28)

L: Lymphocyte, NLR: Neutrophile lymphocyte ratio, CRP: C-reactive protein, CT-SS: Computed tomography severity score, SOFA: Sequential organ failure assessment, HFNC: High flow nasal canula, IMV: Invasive mechanical ventilation, LOS: Length of stay, ICU: Intensive care unit.

4. Discussion

In this cohort, the vast majority of pregnant cases were admitted in the third trimester, consistent with the previous data on COVID-19 and other respiratory viral infections during pregnancy. Urgent deliveries in our cases were almost due to worsening of the respiratory condition rather than primary obstetric indications. Although the pregnant women infected with COVID-19 had a worse clinical course than the nonpregnant matched control group, the mortality was low and similar in both groups. Our neonatal outcomes were also not as bad as expected.

All-cause early neonatal death rates of confirmed or suspected COVID-19 pregnant patients in 12 countries were found to be 0.2 to 0.3 percent. This rate is not higher than expected according to pre-COVID-19 national data (25).

In addition, a systematic review reported that the incidence of neonatal death in SARS-CoV-2 positive and negative pregnant women was similar (26). We reported one neonatal

death but no stillbirth or miscarriage among four premature births in 15 deliveries, which supports the current data. Nevertheless, severe maternal respiratory failure and hypoxia can disrupt placental blood flow, leading to preterm birth or miscarriage (27). Based on this knowledge, it is crucial to strictly implement infection control measures against viral respiratory illnesses in pregnant women.

The most common symptom at admission was cough in both groups, as in the CDC (Centers for Disease Control and Prevention) data (28). In other studies, fever or chills have been reported as the predominant symptom (15, 29, 30). Most notably, although the proportion of patients with either respiratory or non-respiratory symptoms and comorbidity was higher in the nonpregnant group, inversely, CT-SS was higher (9 points vs 2 points) and prognostic laboratory parameters including lymphocytopenia, NLR, CRP, ESR, and D-dimer were worse in the pregnant patients. We don't know the reason underlying this discrepancy; however, it could be attributed to the immune-compromised status of pregnancy and physiologic changes providing clinical vulnerability to severe viral lung infections and intolerance to hypoxia during pregnancy. Overexpression of ACE 2 receptor in pregnant women may also play a role in the severity of the disease, which is a hypothetical mechanism.

Based on large datasets, COVID-19 in pregnancy appears to be more severe in terms of morbidity than in nonpregnant women of reproductive age with COVID-19 (6, 7, 31). According to CDC data, the ICU admission rate was 10.5 vs 3.9, need for IMV was 2.9 vs 1.1 per 1000 cases in pregnant patients compared to nonpregnant cases (7). A prospective cohort study reported a propensity score-matched risks as 9.9 vs 6.4 percent for pneumonia and 13 vs 6.9 percent for ICU admission in pregnant and nonpregnant women with COVID-19, respectively (31). In this study, patients requiring mechanical ventilators (17% vs 1.6%) and ICU admission (23.3% vs 2.4%) were proportionally ten times higher in pregnant patients versus nonpregnant patients. A similar proportion was found between patients with high CT-SS as 44.0% vs 4.2% in pregnant patients and nonpregnant patients, respectively, indicating a crude correlation between CT severity and critical illness. Like other viral infections, early symptoms of COVID-19 may mimic physiologic dyspnea in pregnancy, which could cause a delay in diagnosis. In addition to above mentioned pregnancy-related factors, delayed diagnosis may also result in more severe disease (17, 32).

Current data suggest that mortality in either pregnant or nonpregnant women infected with COVID-19 is similar, between 0.8 and 1.5 percent (6, 7, 31). While we observed no mortality among the pregnant patients with COVID-19, the case fatality rate in nonpregnant patients of reproductive age was less than 1%. The case fatality rate of COVID-19 worldwide differs by country, age group, and setting and is changing over time. It has been reported to range from 0.5 to 10 % and higher than 20% in hospitalized patients (33, 34). Older age (≥35 years), obesity and pre-existing medical comorbidities (especially hypertension and diabetes or more than one comorbidity) are suggested to be the main risk factors for severe disease and death in pregnant women with COVID-19 (5, 35, 36). The absence of mortality in pregnant women in this cohort may be due to the younger age of mothers and lack of comorbidity. Also, there is a substantial amount of research showing that the

COVID-19 pandemic affects men more heavily in terms of disease incidence, hospitalization and death rates. The overall low mortality rates of COVID-19 infection in either pregnant or nonpregnant women of reproductive age may be due to the high progesterone level in women, particularly in pregnant women. There is preclinical evidence regarding the ability of progesterone, an immunomodulatory hormone with a steroid structure, to repair lung damage in respiratory viral infections (37). Nevertheless, this is an area of uncertainty that merits further investigation.

A recent metanalysis on prognostic factors for mortality and severity of COVID-19 disease showed that a high SOFA score defined as more than 2 points is related to a 7.3% increase in mortality and a 63% increase in severe disease with moderate and low certainty of evidence respectively (38). In this study, the SOFA score was median of 2.5 points, which was predominantly obtained from PaO2/FiO2, indicating that organ failure is mostly confined to the respiratory system rather than the multiorgan involvement. However, there was a remarkable deviation in lymphocytopenia, CRP, D-dimer, NLR, and ferritin parameters in patients admitted to the ICU, which are related to a worse prognosis (39). PaO2/FiO2 ratio was median 143 mmHg, consistent with moderate ARDS according to Berlin definition (40). The only patient in the study who died was nonpregnant and had restrictive lung disease due to scoliosis, and the SOFA score at admission was 12 points.

The factors limiting the study are the retrospective method and single-center data. Besides, the sample size of the study is not large enough to extrapolate the data on mortality and morbidity to the general population. However, it contributes to the accumulation of new data for literature on COVID-19 during pregnancy. Due to missing data, we could not make a correlation analysis between CT-SS and clinical or laboratory parameters.

Pregnant patients infected with COVID-19 seem at higher risk for severe or critical illness than nonpregnant control patients with COVID-19. Due to increased morbidity, pregnant women should be approached more alertly regarding screening, isolation, and treatment. Studies designed with a larger sample size and higher quality are necessary to obtain more conclusive data on the prognosis of COVID-19 in these patient groups.

Conflict of interest
All authors state that there is no potential conflict of interest.

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Authors' contributions
Concept: O.K., M.P., N.T.T., E.K., M.D., M.E., İ.B., D.G. Design: O.K., M.P., N.T.T., E.K., M.D., M.E., İ.B., D.G., Data Collection or Processing: M.P., N.T.T., E.K., Analysis or Interpretation: O.K., M.P., Literature Search: O.K., M.P., N.T.T., Writing: O.K.

References

1. Metz TD, Clifton RG, Hughes BL, Sandoval G, Saade GR, Grobman WA, et al. Disease Severity and Perinatal Outcomes of Pregnant Patients With Coronavirus Disease 2019 (COVID-19). Obstet Gynecol. 2021 Apr 1;137(4):571-580.
2. Mullins E, Hudak ML, Banerjee J, Getzlaff T, Townson J, Barnette K, et al. Pregnancy and neonatal outcomes of COVID-19: coreporting of common outcomes from PAN-COVID and AAP-SONPM registries. Ultrasound Obstet Gynecol. 2021 Apr;57(4):573-581.
3. Huntley BJF, Mulder IA, Di Mascio D, Vintzileos WS, Vintzileos AM, Berghella V, et al. Adverse Pregnancy Outcomes Among Individuals With and Without Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): A Systematic Review and Meta-analysis. Obstet Gynecol. 2021 Apr 1;137(4):585-596.
4. Chinn J, Sedighim S, Kirby KA, Hohmann S, Hameed AB, Jolley J, et al. Characteristics and Outcomes of Women With COVID-19 Giving Birth at US Academic Centers During the COVID-19 Pandemic. JAMA Netw Open. 2021 Aug 2;4(8):e2120456.
5. Kasehagen L, Byers P, Taylor K, Kittle T, Roberts C, Collier C, et al. COVID-19-Associated Deaths After SARS-CoV-2 Infection During Pregnancy - Mississippi, March 1, 2020-October 6, 2021. MMWR Morb Mortal Wkly Rep. 2021 Nov 26;70(47):1646-1648.
6. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. BMJ. 2020 Sep 1;370:m3320.
7. Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, et al. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020 Nov 6;69(44):1641-1647.
8. Badr DA, Mattern J, Carlin A, Cordier AG, Maillart E, El Hachem L, et al. Are clinical outcomes worse for pregnant women at ≥20 weeks' gestation infected with coronavirus disease 2019? A multicenter case-control study with propensity score matching. Am J Obstet Gynecol. 2020 Nov;223(5):764-768.
9. Qeadan F, Mensah NA, Tingey B, Stanford JB. The risk of clinical complications and death among pregnant women with COVID-19 in the Cerner COVID-19 cohort: a retrospective analysis. BMC Pregnancy Childbirth. 2021 Apr 16;21(1):305.
10. DeBolt CA, Bianco A, Limaye MA, Silverstein J, Penfield CA, Roman AS, et al. Pregnant women with severe or critical coronavirus disease 2019 have increased composite morbidity compared with nonpregnant matched controls. Am J Obstet Gynecol. 2021 May;224(5):510.e1-510.e12.
11. Lokken EM, Huebner EM, Taylor GG, Hendrickson S, Vanderhoeven J, Kachikis A, et al. Disease severity, pregnancy outcomes, and maternal deaths among pregnant patients with severe acute respiratory syndrome coronavirus 2 infection in Washington State. Am J Obstet Gynecol. 2021 Jul;225(1):77.e1-77.e14.
12. Dawood FS, Varner M, Tita A, Newes-Adeyi G, Gyamfi-Bannerman C, Battarbee A et al. Incidence, Clinical Characteristics, and Risk Factors of SARS-CoV-2 Infection among Pregnant Individuals in the United States. Clin Infect Dis. 2021 Aug 19:ciab713.
13. Gulersen M, Rochelson B, Shan W, Wetcher CS, Nimaroff M, Blitz MJ. Severe maternal morbidity in pregnant patients with SARS-CoV-2 infection. Am J Obstet Gynecol MFM. 2022 Jul;4(4):100636.
14. McClymont E, Albert AY, Alton GD, Boucoiran I, Castillo E, Fell DB, et al. Association of SARS-CoV-2 Infection During Pregnancy With Maternal and Perinatal Outcomes. JAMA. 2022 May 24;327(20):1983-1991.
15. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet. 2020 Mar 7;395(10226):809-815.
16. Tamanna S, Clifton VL, Rae K, van Helden DF, Lumbers ER, Pringle KG. Angiotensin Converting Enzyme 2 (ACE2) in Pregnancy: Preeclampsia and Small for Gestational Age. Front Physiol. 2020 Sep 30;11:590787.
17. Narang K, Enninga EAL, Gunaratne MDSK, Ibirogba ER, Trad ATA, Elrefaei A, et al. SARS-CoV-2 Infection and COVID-19 During Pregnancy: A Multidisciplinary Review. Mayo Clin Proc. 2020 Aug;95(8):1750-1765.
18. Pineles BL, Goodman KE, Pineles L, O'Hara LM, Nadimpalli G, Magder LS, et al. In-Hospital Mortality in a Cohort of Hospitalized Pregnant and Nonpregnant Patients With COVID-19. Ann Intern Med. 2021 Aug;174(8):1186-1188.
19. Leung C, de Paiva KM. Is pregnancy a risk factor for in-hospital mortality in reproductive-aged women with SARS-CoV-2 infection? A nationwide retrospective observational cohort study. Int J Gynaecol Obstet. 2022 Apr;157(1):121-129.
20. Hsu AL, Ohler AM, Goldstein A, Truong S, Tang CY, Wan XF et al. Coronavirus Disease 2019 (COVID-19) Disease Severity: Pregnant vs Nonpregnant Women at 82 Facilities. Clin Infect Dis. 2022 Feb 11;74(3):467-471.
21. National Institutes of Health. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. Available from: https://www.covid19treatmentguidelines.nih.gov/
22. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-810.
23. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, et al. Time Course of Lung Changes at Chest CT during Recovery from Coronavirus Disease 2019 (COVID-19). Radiology. 2020;295(3):715-721.

24. Tabatabaei SMH, Rajebi H, Moghaddas F, Ghasemiadl M, Talari H. Chest CT in COVID-19 pneumonia: what are the findings in mid-term follow-up? Emerg Radiol. 2020;27(6):711-719.

25. Mullins E, Hudak ML, Banerjee J, Getzlaff T, Townson J, Barnette K et al. Pregnancy and neonatal outcomes of COVID-19: coreporting of common outcomes from PAN-COVID and AAP-SONPM registries. Ultrasound Obstet Gynecol. 2021 Apr;57(4):573-581.

26. Huntley BJF, Mulder IA, Di Mascio D, Vintzileos WS, Vintzileos AM, Berghella V et al. Adverse Pregnancy Outcomes Among Individuals With and Without Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): A Systematic Review and Meta-analysis. Obstet Gynecol. 2021 Apr 1;137(4):585-596.

27. Wong SF, Chow KM, Leung TN, Ng WF, Ng TK, Shek CC, et al. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. Am J Obstet Gynecol. 2004;191(1):292-297.

28. Ellington S, Strid P, Tong VT, Woodworth K, Galang RR, Zambrano LD, et al. Characteristics of Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-June 7, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(25):769-775.

29. Di Mascio D, Khalil A, Saccone G, Rizzo G, Buca D, Liberati M, et al. Outcome of coronavirus spectrum infections (SARS, MERS, COVID-19) during pregnancy: a systematic review and meta-analysis. Am J Obstet Gynecol MFM. 2020;2(2):100107.

30. Liu D, Li L, Wu X, Zheng D, Wang J, Yang L, et al. Pregnancy and Perinatal Outcomes of Women With Coronavirus Disease (COVID-19) Pneumonia: A Preliminary Analysis . AJR Am J Roentgenol. 2020;215(1):127-132.

31. Martinez-Portilla RJ, Sotiriadis A, Chatzakis C, Torres-Torres J, Espino Y Sosa S, Sandoval-Mandujano K, et al.Pregnant women with SARS-CoV-2 infection are at higher risk of death and pneumonia: propensity score matched analysis of a nationwide prospective cohort (COV19Mx). Ultrasound Obstet Gynecol. 2021 Feb;57(2):224-231.

32. Society for Maternal-Fetal Medicine. Management considerations for pregnant patients with COVID-19. Available from: https://s3.amazonaws.com/cdn.smfm.org/media/2334/SMFM_COVID_Management_of_COVID_pos_preg_patients_4-29-20_final.pdf.

33. Centre for Evidence-Based Medicine. Global COVID-19 Case Fatality Rates. Available from: https://www.cebm.net/covid-19/global-covid-19-case-fatality-rates/

34. International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC). Available from: https://media.tghn.org/medialibrary/2020/05/ISARIC_Data_Platform_COVID- 19_Report_19MAY20.pdf.

35. Karimi L, Makvandi S, Vahedian-Azimi A, Sathyapalan T, Sahebkar A. Effect of COVID-19 on Mortality of Pregnant and Postpartum Women: A Systematic Review and Meta-Analysis. J Pregnancy. 2021;2021:8870129. Published 2021 Mar 5.

36. Galang RR, Newton SM, Woodworth KR, Griffin I, Oduyebo T, Sancken CL et al. Risk Factors for Illness Severity Among Pregnant Women With Confirmed Severe Acute Respiratory Syndrome Coronavirus 2 Infection-Surveillance for Emerging Threats to Mothers and Babies Network, 22 State, Local, and Territorial Health Departments, 29 March 2020-5 March 2021. Clin Infect Dis. 2021 Jul 15;73(Suppl 1):S17-S23.

37. Wastnedge EAN, Reynolds RM, van Boeckel SR, Stock SJ, Denison FC, Maybin JA, et al. Pregnancy and COVID-19. Physiol Rev. 2020;1;101(1):303-318.

38. Izcovich A, Ragusa MA, Tortosa F, Lavena Marzio MA, Agnoletti C, Bengolea A, et al. Prognostic factors for severity and mortality in patients infected with COVID-19: A systematic review. PLoS One. 2020;15(11):e0241955.

39. Pierce-Williams RAM, Burd J, Felder L, Khoury R, Bernstein PS, Avila K, et al. Clinical course of severe and critical coronavirus disease 2019 in hospitalized pregnancies: a United States cohort study. Am J Obstet Gynecol MFM. 2020;2(3):100134.

40. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012;307(23):2526-2533.

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Plasma netrin-1 levels in Familial Mediterranean fever: A potential biomarker?

Ebru ATALAR [1,*], Kevser GÖK [1], Esra FIRAT OĞUZ [2], Ahmet KOR [1], Yüksel MARAŞ [1]

[1]Department of Rheumatology, Ankara City Hospital, Ankara, Türkiye
[2]Department of Biochemistry Ankara City Hospital, Ankara, Türkiye

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Abstract

Netrins are primarily defined as being of guidance in axonal cell migration in the embryonic period. Cell migration has an important role in the occurrence of organogenesis and inflammation. In the etiopathogenesis of autoimmune diseases, there is said to be tissue destruction associated with uncontrolled activation of the immune system and uncontrolled migration of inflammatory cells to tissues. We aimed to determine whether netrin-1 has a role in the etiopathogenesis of the FMF disease. This study included 42 patients with Familial Mediterranean Fever (FMF) and 44 healthy control subjects. The plasma netrin 1 levels were measured, and relationships were examined between the netrin levels and demographic data, clinical findings and laboratory test results in both groups. No significant difference was determined between the groups in respect of the plasma netrin levels. The lack of difference between the two groups in this study in respect of plasma netrin levels could suggest that netrin-1 does not have an important role in the etiopathogenesis of FMF. There is a need for further in vivo and in vitro studies to examine netrin-1 and receptors in inflammation tissue samples and in normal tissue.

Keywords: amilial mediterranean fever, netrin 1 protein, human; inflammation; etiology

1. Introduction

Cellular networks play an important role in the formation of tissues in the human body and in their ability to perform specific functions. Netrins, which play an active role in the functioning of these networks, are one of the key molecules in glycoprotein structure. The word netrin is derived from the Sanskrit word meaning “one who guides” (1). Netrins are laminin-like proteins, which were first identified as axonal guiding clues during embryonic development. Two membrane-bound forms (glycosylphosphatidylinositol-linked netrins, netrin G1 and G2) and five secreted forms of netrin (Netrin1-5) have been reported in vertebrates (2). Receptors play a role in the functioning of netrins. The most well-known netrin receptors are Deleted in colorectal cancer (DCC), neogenin, Uncoordinated family member 5 (UNC5A), and the Down syndrome cell adhesion molecule (DSCAM-1) (3, 4). DCC and neogenin receptors attract while the UNC receptors have the effect of repulsion (5, 6). Mediated by the receptors, growing axons move towards or away from the region of intense netrin concentration.

Previous studies have shown that netrin-1 plays a role in several events such as angiogenesis, inflammation, osteoarthritis, osteoporosis, atherosclerosis, and tumour formation (2, 7). Netrin-1 also acts as a guide to important osteocytes in the formation of bone micro-architecture, and has been shown in vitro to have an active role in the differentiation of osteoclasts (8, 9). By taking a role in the formation of new vessels in some cancer types, Netrin-1 has been reported to cause a more aggressive course of the tumour (10, 11).

Initially found to be effective in axonal and neuronal migration, it was then shown that netrin-1 had a role in embryogenic angiogenesis, and was found in heavily vascularized organs and those with more vascular endothelium as well as in immune tissue (12, 14). However, the up-regulation and down-regulation mechanisms of the receptors that induce the effect of netrin-1 have not yet been fully understood (4).

Cell migration plays an important role in both organogenesis and the etiopathogenesis of diseases seen with inflammation, including autoimmune diseases (5). It has been reported that in synovial fibroblasts of rheumatoid arthritis patients, Netrin 1 and its potential inducer of IL-17 are higher than in osteoarthritis patients (2). Schubert et al examined joint synovial samples in osteoarthritis (OA), rheumatoid arthritis (RA) and a control group, and reported that there was greater expression of UNC5B and UNC6C receptors, which have repulsion properties, in the synovial samples of RA and OA patients. When recombinant netrin-1 was given to synovial fibroblast cells, the migration of synovial fibroblasts was seen to be reduced in the RA and OA patient groups and not to change in the control group (5).

As they are found in vascular endothelium and have a role in inflammation, netrin-1 and its receptors are thought to have

*Correspondence: atalarebrudr@yahoo.com

a role in the regulation of the immune system and in the etiopathogenesis of autoimmune diseases. Familial Mediterranean Fever (FMF) is a systemic inflammatory disease which is seen with autoimmune vasculitis (15). The aim of this study was to examine the plasma netrin-1 levels of FMF patients and a control group and to determine whether or not netrin-1 has a role in the etiopathogenesis of the disease. Thus, it was aimed to answer the question of whether netrin-1 could be used as a biomarker in diagnosis of the disease or as a potential treatment agent.

2. Materials and Methods

The study protocol was approved by the Local Research Ethics Committee (Number; E1-21-1589). The study was conducted in accordance with the ethical principles as described by the declaration of Helsinki. All participants provided their signed informed consent.

The study included 43 patients diagnosed with FMF, with a mean age of 37.04±11.27 years, who were in an attack-free period, and 44 healthy control subjects with a mean age of 36.20±10.54 years. The plasma netrin-1 levels were measured in both groups and compared. Correlations were examined between the netrin-1 levels and age, height, weight, body mass index, gender, smoking status (current smokers, non-smokers, ex-smokers; those who have not smoked for the past 3 months), and laboratory findings. In addition, correlations were examined in the patient group between netrin levels and family history, peritonitis, pleuritis, arthralgia, erysipelas, fever, attack frequency, age at diagnosis, fibrinogen level, colchicine dose, and duration of disease. Patients with malignancy, active infection and other concomitant inflammatory diseases such as Ankylosing spondylitis and Crohn's disease were not included in the study. Informed consent was obtained from all individual participants included in the study.

2.1. Determination of plasma netrin-1 amount by ELISA

Ten mL of venous blood samples were collected into vacutainer tubes and centrifuged at 1300 × g for 10 minutes. Separated sera were aliquoted into Eppendorf tubes and stored at –80 °C until analysis. Netrin-1 levels were measured with an ELISA kit (Elabscience, Texas, USA; catalog no: E-EL-H2328; lot no: GZWTKZ5SWK) using a quantitative sandwich enzyme immunoassay technique.

Netrin-1 standard and serum samples were added to the antibody-coated 96-well plate and incubated for 90 minutes at 37°C, followed by addition of biotinylated detection antibody specific for netrin-1 and incubation for an additional 1 hour at 37°C. The plate was then washed three times and incubated with avidin-horseradish peroxidase conjugate for 30 minutes at 37°C. After the five times washing step, in order to develop the color tetramethylbenzidine substrate was added and incubated for 15 minutes at 37°C, and the enzyme-substrate reaction was terminated by adding stop solution. The optical density (OD) is measured spectrophotometrically using a microplate reader at a wavelength of 450 nm. The OD value is proportional to the concentration of human Netrin-1. The detection range of the assay was 31.25–2000 pg/mL. Intra- and interassay precision were all <10% for low, medium and high levels of Netrin-1.

2.2. Statistical analysis

Data obtained in the study were analyzed statistically using SPSS vn. 25 software. In the comparisons between the patient and control groups, the t-test was applied to continuous data that met parametric conditions, and the Mann Whitney U-test or Kruskall Wallis variance analysis to data that was not parametric. The Chi-square test was used in the comparisons of categorical data. In the evaluation of the netrin level within the groups in respect of demographic and clinical data, the Spearman correlation test was used for continuous data and the Mann Whitney U-test or Kruskal Wallis variance analysis for categorical data. A value of $p<0.05$ was accepted as statistically significant.

3. Results

When the relationships were examined between the netrin level and clinical and demographic data within groups, a negative correlation was determined between height and netrin level in the patient group (Table 1, Fig. 1). No significant difference was determined between the patient group and the control group in respect of demographic data, clinical characteristics and laboratory findings (Table 2). A statistically significant difference was determined between the patient and control groups in respect of the CRP values ($p<0.01$).

Table 1. Relationships between the netrin level and variables in the patient group

Variables	p value
Age	0.73*
Height	**0.005***
Body weight	0.52*
BMI	0.37*
Gender	0.43**
Smoking	0.18***
Familial history	0.36**
Clinical findings	
Peritonitis	0.40**
Pleuritis	0.33**
Arthralgia	0.69**
Erysipelas	0.08**
Fever	0.11**
Attack frequency	0.15***
Laboratory tests	
CRP	0.90*
Sedimentation rate	0.73*
WBC	0.59*
Neutrophil	0.75*
Lymphocyte	0.97*
Neutrophil / Lymphocyte ratio	0.64*
Hgb	0.39*
Platelet	0.17*
Creatinin	0.79*
ALT	0.12*
AST	0.35*
GGT	0.34*
Fibrinogen	0.63*
Age of diagnosis	0.36*
Disease duration	0.74*
Colchicine dosage	0.49*

Table 2. The demographic data, clinical characteristics and laboratory findings of the patient group and control group

	Total (n=87)	Groups		p
		Patient	Control	
Number of subjects	87	43	44	
Age	36.6±10.8 (19-62)	37.04±11.27	36.20±10.54	0.72*
Height (cm)	169.01±10.09 (148-190)	168.1±8.9	169.8±11.1	0.44*
Body weight (kg)	75.03±12.76 (50-106)	74.3±14.2	75.6±11.3	0.63*
BMI	26.2±3.61 (19-34)	26.1±4	26.2±3.2	0.94*
Gender				
Male	42	21	21	0.91**
Female	45	22	23	
Smoking				
Non-smokers	57	27	30	0.37**
Current smokers	20	9	11	
Ex-smokers	10	7	3	
Laboratory tests				
Netrin-1 level (pg/ml)	256.56±88.80	241.9±76.3	270.8±98.2	0.13*
CRP [mg/l]	2.69±3.63	3.8±4.7	1.5±1.4	0.03*
Sedimentation rate [mm/h]	9.42±6.33	10.6±7.4	8.2±4.8	0.08*
WBC [10^9 / L]	7.154±1.608	7.284±1.624	7.027±1.600	0.45*
Neutrophil [10^9 / L]	4.294±1.516	4.538±1.551	4.055±1.460	0.13*
Lymphocyte [10^9 / L]	2.170±0.614	2.087±0.489	2.252±0.711	0.21*
Neutrophil/lymphocyte ratio	2.15±0.99	2.3±1	1.9±0.8	0.12*
Hgb [gr/dl]	14.2±1.5	13.9±1.2	14.5±1.8	0.07*
Platelet [1/mm3]	265942±51133	273186±49372	258863±52389	0.19*
Creatinine [mg/dL]	0.78±0.13	0.78±0.13	0.78±0.13	0.85*
ALT [U/l]	27.7±14.3	27.6±13.9	27.8±14.9	0.95*
AST [U/l]	20.4±10.6	20.9±8.6	20±12.3	0.70*
GGT [U/l]	21.5±11.4	23.5±13.9	19.5±7.9	0.10*
Fibrinogen [gr/l]		2.92±0.75		
Age of diagnosis		25.4±11.9 (5-62)		
Disease duration (year)		11.6±6.5 (1-26)		
Colchicine dosage (mg)		1.45±0.44 (0.5-2)		

*t-test, ** Chi-square test, BMI: body mass index, Hgb: haemoglobin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, CRP: c-reactive protein, ESR: erythrocyte sedimentation rate, WBC: white blood count

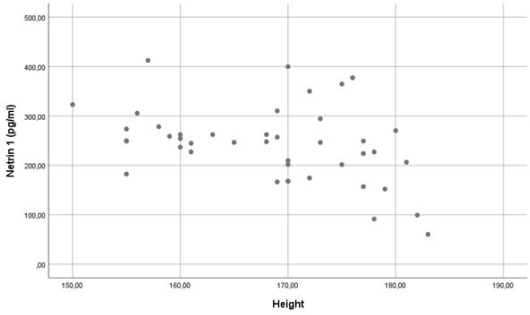

Fig. 1. The correlation between height (cm) and netrin level in the patient group

4. Discussion

That the Deleted in Colorectal Cancer (DCC) and Uncoordinated-5 (UNC5) receptors of netrin-1 have been identified in cells other than neurons strengthens the hypothesis that this protein could have roles other than in the central nervous system (1). The ability of the guidance molecule netrin-1 to repulse or eliminate the attraction of neuronal cells expressing the UNC5b receptor makes it an attractive candidate for the regulation of inflammatory cell migration.12 Cell migration is known to have a role in organogenesis in the embryonic stage, and then in inflammation and tissue homeostasis in subsequent periods (4, 16). Netrin-1 has been shown to be a regulator of leukocyte migration during inflammation (12). It is thought that diseases may start associated with inflammation when there is inappropriate migration to cells. In addition to stability of the vascular endothelium, the balance of attraction and repulsion signals between netrin-1 and its receptors has an effect on the inflammation process (12).

However, whether netrin-1 is protective against the formation of inflammation, or whether it triggers inflammation remains a matter of debate. The results of this study showed that the mean netrin level was lower in the patient group than in the control group, but the difference was not statistically significant. Miralkaj et al demonstrated that netrin-1 was reduced in pulmonary and peritoneal inflammation.17,18 In an experimental model, the application of netrin-1 was observed to reduce renal ischaemia reperfusion damage and associated inflammation (19).

Netrin-1 expressed from the vascular endothelium shows an anti-inflammatory effect by preventing migration to inflammatory cells (12). In addition, the anti-inflammatory effect of Netrin-1 as a potential treatment agent has been shown in experimental models (20, 21). With the application of netrin-1 in animal studies, neutrophil infiltration and the induction of proinflammatory cytokines have been shown to be reduced and thereby peritoneal inflammation was suppressed (18). In another animal study, netrin-1 application reduced inflammation associated with hypoxia (22). Netrin-1 prevents inflammatory cells penetrating the vascular endothelium. Inflammation becomes evident with leukocyte migration in conditions such as infection where the endothelial barrier is damaged. In such cases, the return of the netrin-1 level to normal prevents excessive tissue destruction by taking leukocyte migration under control (12).

In an experimental model, netrin-1 expression was shown to be suppressed by cytokines such as TNF and IFN, which play an active role in inflammation, and thus inflammation was facilitated (12). In that study, in inflammation associated with infection created with Staph. Aures in the lungs of mice, netrin-1 expression was rapidly down-regulated in the lungs, and the netrin-1 had an inhibitory effect on monocyte, leukocyte and granulocyte migration. Blocking the UNC5b receptor, which provided this effect, eliminated the effect of netrin-1 suppressing the migration of inflammatory cells (12).

There are also studies reporting that netrin-1 could have an inflammation triggering effect. In samples taken from patients applied with revision surgery due to prosthesis loosening after arthroplasty, the netrin-1 expression was compared with netrin-1 expression in samples taken during primary arthroplasty operations. The netrin-1 expression was determined to be significantly greater in the samples taken from the revision operations. Taking the effect of osteoclastic activity into consideration, it was reported that netrin-1 could have a key role in inflammatory osteolysis that develops following arthroplasty operations (23). The same authors reported that in an animal model, the blockage of netrin-1 reduced wear particle-induced inflammation seen after arthroplasty operations. Consistent with that study, there are studies that have reported that by inhibiting macrophage movement netrin-1 increased inflammatory events as a result of atherosclerosis and insulin resistance (3,24). Similarly, it has been reported that when netrin-1 was removed from cells obtained from bone marrow in mice, the dimensions of atherosclerotic lesions reduced significantly, and netrin-1 had an active role in the development of atherosclerosis by preventing the migration of cholesterol-loaded macrophages to the lymphatic system (24,25). In another animal model study of diet induced obesity, it was shown that the reduced migratory capacity of macrophages could be restored by blocking netrin-1. It was demonstrated that hematopoietic deletion of netrin-1 reduced inflammation and improved insulin sensitivity, so it was concluded that netrin-1 promotes chronic inflammation and insulin resistance (26). Paradisi et al determined increased netrin-1 levels in the mucosa of patients with inflammatory intestinal disease (Crohn's disease and ulcerative colitis), especially in the inflammation region, compared to normal colonic mucosa (27). According to that study, netrin-1 up-regulation in inflammatory bowel disease is necessary for progression to colorectal cancer, although a change in netrin-1 levels does not affect inflammation (27). In addition, netrin-1 may show a dose-dependent effect. From an in vitro study it was reported that while the application of netrin-1 at a low concentration stimulated angiogenesis on the CD-146 adhesion molecule, a high concentration of netrin-1 inhibited angiogenesis on the UNC5B receptor (28).

The above-mentioned studies were conducted on tissue samples. Due to the difficulties of obtaining tissue samples from the patients and control group in the current study, and as netrin is expressed by a protein and therefore found in the peripheral circulation, the plasma levels of netrin-1 were investigated in this study (4). The conflicting results related to netrin-1 that have been reported in literature can be considered to be the result of interaction of different receptors with chemoattractive and chemorepulsant mediators of netrin-1.

As a result of the current study, a significant inverse relationship was determined between the plasma netrin level in the patient group and height. No similar correlation was found in the control group. The mechanism of this cannot be explained in the scope of this study, but there may be a similar relationship in other autoinflammatory diseases. The reliability of this finding should be questioned because the number of patients is relatively small and it is a cross-sectional study. There is a need for prospective long-term follow-up studies on this subject with a greater number of patients and including other autoinflammatory diseases. That the CRP level of the patient group was higher than that of the control group was an expected finding. However, no correlation was found between CRP and the netrin level.

The most important limitations of this study were that netrin-1 and receptors were not examined in tissues, and that plasma netrin levels were not examined during attack periods. Another limitation of our study is that we did not examine the plasma netrin-1 levels in patients in the attack period.

The results of this study demonstrated no difference between the plasma netrin-1 levels of the patient and control groups. There was also no difference found between the clinical and laboratory findings and the netrin levels of the patient group. These findings suggest that netrin-1 does not have a significant role in the etiopathogenesis of FMF disease. It would be useful for further studies to examine the netrin-1 and receptor levels in tissue samples obtained from patients and healthy control subjects, and to evaluate the effects on inflammation of the application of recombinant netrin-1 to cell cultures of FMF patients to be able to establish a relationship between netrin-1 and FMF.

Conflict of interest
The authors declared no conflict of interest.

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Authors' contributions
Concept: E.A., Design: Y.M., Data Collection or Processing: A.K., Analysis or Interpretation: E.F.O., Literature Search: K.G., Writing: E.A., Y.M.

References

1. Rajasekharan S, Kennedy TE. The netrin protein family. Genome Biol. 2009; 10:239.
2. Maruyama K, Takemura N, Martino MM, Kondo T, Akira S. Netrins as prophylactic targets in skeletal diseases: A double-edged sword? Pharmacol Res. 2017; 122:46–52.
3. Ly A, Nikolaev A, Suresh G, Zheng Y, Tessier-Lavigne M, Stein E. DSCAM is a netrin receptor that collaborates with DCC in mediating turning responses to netrin-1. Cell. 2008; 133:1241–1254.
4. Ramesh G. Role of Netrin-1 Beyond the Brain: From Biomarker of Tissue Injury to Therapy for Inflammatory Diseases. Recent Pat Biomark. 2012; 2:202–208.
5. Schubert T, Denk A, Mägdefrau U, Kaufmann S, Bastone P, Lowin T, et al. Role of the netrin system of repellent factors on synovial fibroblasts in rheumatoid arthritis and osteoarthritis. Int J Immunopathol Pharmacol. 2009; 22:715–722.
6. Finci LI, Krüger N, Sun X, Zhang J, Chegkazi M, Wu Y, et al. The crystal structure of netrin-1 in complex with DCC reveals the bifunctionality of netrin-1 as a guidance cue. Neuron. 2014; 83:839–849.
7. Moore KJ, Fisher EA. Macrophages, atherosclerosis and the potential of netrin-1 as a novel target for future therapeutic intervention. Future Cardiol. 2012; 8:349–352.
8. Mediero A, Ramkhelawon B, Perez-Aso M, Moore KJ, Cronstein BN. Netrin-1 is a critical autocrine/paracrine factor for osteoclast differentiation. J Bone Miner Res. 2015; 30:837–854.
9. Matsugaki A, Yamazaki D, Nakano T. Selective patterning of netrin-1 as a novel guiding cue for anisotropic dendrogenesis in osteocytes. Mater Sci Eng C Mater Biol Appl. 2020; 108:110391. Epub 2019.11.05.
10. Shimizu A, Nakayama H, Wang P, König C, Akino T, Sandlund J, et al. Netrin-1 promotes glioblastoma cell invasiveness and angiogenesis by multiple pathways including activation of RhoA, cathepsin B, and cAMP-response element-binding protein. J Biol Chem. 2013, 288:2210–2222.
11. Akino T, Han X, Nakayama H, McNeish B, Zurakowski D, Mammoto A, et al. Netrin-1 promotes medulloblastoma cell invasiveness and angiogenesis, and demonstrates elevated expression in tumor tissue and urine of patients with pediatric medulloblastoma. Cancer Res. 2014; 74:3716–3726.
12. Ly NP, Komatsuzaki K, Fraser IP, Tseng AA, Prodhan P, Moore KJ, et al. Netrin-1 inhibits leukocyte migration in vitro and in vivo. Proc Natl Acad Sci U S A. 2005; 102:14729–14734.
13. Dalvin S, Anselmo MA, Prodhan P, Komatsuzaki K, Schnitzer JJ, Kinane TB. Expression of Netrin-1 and its two receptors DCC and UNC5H2 in the developing mouse lung. Gene Expr Patterns. 2003; 3:279–283.
14. Lu X, Le Noble F, Yuan L, Jiang Q, De Lafarge B, Sugiyama D, et al. The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. Nature. 2004; 432:179–186.
15. Yıldız M, Haşlak F, Adrovic A, Barut K, Kasapçopur Ö. Autoinflammatory Diseases in Childhood. Balkan Med J. 2020 Aug 11;37(5):236-246.
16. Claro V, Ferro A. Netrin-1: Focus on its role in cardiovascular physiology and atherosclerosis. JRSM Cardiovasc Dis. 2020; 9:2048004020959574.Epub 2020.11.25.
17. Mirakaj V, Thix CA, Laucher S, Henes J, Unertl KE, Köhler D, Rosenberger P, et al. Netrin-1 dampens pulmonary inflammation during acute lung injury. Am J Respir Crit Care Med. 2010; 181:815–824.
18. Mirakaj V, Gatidou D, Pötzsch C, König K, Rosenberger P. Netrin-1 signaling dampens inflammatory peritonitis. J Immunol. 2011; 186:549–555.
19. Tadagavadi RK, Wang W, Ramesh G. Netrin-1 regulates Th1/Th2/Th17 cytokine production and inflammation through UNC5B receptor and protects kidney against ischemia-reperfusion injury. J Immunol. 2010; 185:3750–3758.
20. Chen J, Cai Q-P, Shen P-J, Yan RL, Wang CM, Yang DJ, et al. Netrin-1 protects against L-Arginine-induced acute pancreatitis in mice. PLoS One. 2012; 7:e46201. Epub 2012.09.27
21. Aherne CM, Collins CB, Masterson JC, Tizzano M, Boyle TA, Westrich JA, et al. Neuronal guidance molecule netrin-1 attenuates inflammatory cell trafficking during acute experimental colitis. Gut. 2012; 61:695–705.
22. Rosenberger P, Schwab JM, Mirakaj V, Masekowsky E, Mager A, Morote-Garcia JC, Unertl K, et al. Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia. Nat Immunol 2009; 10:195–202.
23. Mediero A, Ramkhelawon B, Wilder T, Purdue PE, Goldring SR, Dewan MZ, et al. Netrin-1 is highly expressed and required in inflammatory infiltrates in wear particle-induced osteolysis. Ann Rheum Dis. 2016; 75:1706–1713.
24. van Gils JM, Derby MC, Fernandes LR, Ramkhelawon B, Ramkhelawon B, Ray TD, Rayner KJ, et al. The neuroimmune guidance cue netrin-1 promotes atherosclerosis by inhibiting the emigration of macrophages from plaques. Nat Immunol. 2012; 13:136–143.
25. Joseph BB, Quan PD. The neuroimmune guidance cue netrin-1: a new therapeutic target in cardiovascular disease. Am J Cardiovasc Dis. 2013; 3:129–134.
26. Ramkhelawon B, Hennessy EJ, Ménager M, Ray TD, Sheedy FJ, Hutchison S, et al. Netrin-1 promotes adipose tissue macrophage retention and insulin resistance in obesity. Nat Med. 2014; 20:377–384.
27. Paradisi A, Maisse C, Coissieux M-M, et al. Netrin-1 up-regulation in inflammatory bowel diseases is required for colorectal cancer progression. Proc Natl Acad Sci U S A. 2009; 106:17146–17151.
28. Tu T, Zhang C, Yan H, Luo Y, Kong R, Wen P, et al. CD146 acts as a novel receptor for netrin-1 in promoting angiogenesis and vascular development. Cell Res. 2015; 25:275–287.

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The role of alveolo-arterial oxygen gradient and pneumonia severity index in predicting mortality in patients with COVID-19 pneumonia

Hale KEFELİ ÇELİK [1,*], Zahide DOĞANAY [2], Tuğçehan SEZER AKMAN [1]

[1]Department of Anesthesiology and Reanimation, Samsun Education and Research Hospital, Samsun, Türkiye
[2]Department of Anesthesiology and Reanimation, Medical School, Kastamonu University, Kastamonu, Türkiye

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Abstract

Purpose of the study is to determine the success rates of alveolo-arterial oxygen gradient (AaDO2) and the pneumonia severity index (PSI) in predicting mortality for the patients diagnosed with COVID-19 pneumonia. This retrospective study included patients who were treated with the diagnosis of COVID-19 pneumonia in the ICUs. Demographic characteristics, arterial blood gas values, radiological images and laboratory data of the patients were used through the hospital database and patient files. Group I patients consist of alive and Group II patients consist of deceased persons. 150 of 263 patients included in this study are in Group I and 113 are in Group II. RT-PCR test was positive in 20.9% of the patients. The most common symptom was dyspnea with 76.5% and the most common additional disease was hypertension with 58.1%.65% of patients had radiological involvement in both lungs, and the most common finding was the ground-glass opacity at 71.5%. In predicting mortality, PSI value was 135 in group I and 174 in group II ($p<0.001$);AaDO2 value was 154.88 mmHg in group I, 177.13 mmHg in group II ($p<0.001$), and this rate was different between the groups. Sensitivity is found at 84.1% and specificity at 67.3% for PSI, whereas sensitivity is found at 49.6% and specificity at 82.7% for the AaDO2 variable. It is important to estimate the mortality risk earlier for the patients with COVID-19 pneumonia who are also followed up in intensive care units. PSI is beneficial in detecting mortality risk whereas AaDO2 is valuable in determining the surviving patients.

Keywords: alveolo-arterial oxygen gradient, COVID-19, mortality, pneumonia severity index

1. Introduction

The new coronavirus disease-19 (COVID-19), also known as the new coronavirus pneumonia, first appeared in Wuhan Province of China during early December and spread almost all over the world within two months which also caused a pandemic. COVID-19 disease is caused by the virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). 80% of COVID-19 patients have mild illness whereas 20% require hospitalization. Some of these cases need to be followed under the intensive care units (ICU). The cases that need to be followed up in intensive care refer to the patients with severe pneumonia and acute respiratory distress syndrome (ARDS) requiring invasive or non-invasive respiratory support. This rate is between 5% and 10% for the patients requiring hospitalization (1-3).

In general, pneumonia is ranked 6th among all causes of death in the United Kingdom and the USA. Pneumonia is also ranked 1st among the deaths caused by infections. The mortality rate ranges between 1-5% for the ambulatory patients diagnosed with pneumonia whereas it reaches up to 12% for the hospitalized patients and 40% for the patients in the ICUs. In our country, the mortality rate ranges between 30% and 87% for the hospital-acquired pneumonia (4). Several scoring systems are used to predict mortality in pneumonia. The most popular ones are the pneumonia severity index (PSI) and CURB-65. The patient's demographic data, concomitant diseases, physical examination findings and laboratory values are used in calculating PSI (5-7).

The fatal disease incidents appear as severe pneumonia and ARDS in COVID-19 patients. The PaO2/FiO2 (arterial oxygen pressure/fraction of inspired oxygen) and the alveolar-arterial oxygen gradient (AaDO2) are the indicators of oxygenation status in critical ill patients and stand as the diagnostic criteria for ARDS in adults. Low PaO2/FiO2 value has been associated with the increased mortality and hospitalization period for the patients admitted to the ICUs. The PaO2/FiO2 rate provides quick and easy data on the oxygenation status of critical ill

*Correspondence: ck_hale@hotmail.com

patients and it is widely used in the ICUs (8). The AaDO2 refers to the difference between the alveolar oxygen pressure (PAO2) and arterial oxygen pressure (PaO2) whereas it enables the evaluation of ventilation/perfusion abnormalities in the lung and its calculation is fast, easy and practical on less variables in comparison to the PSI (5,9).

As end of December, the number of patients with positive COVID-19 real-time reverse transcriptase polymerase chain reaction (RT-PCR) tests in our country exceeded 2 million and the number of deaths exceeded 20.000. The purpose of this study conducted on the patients diagnosed with COVID-19 pneumonia and treated under the ICUs of Samsun Education and Research Hospital is to determine the success rate of alveolo-arterial oxygen gradient and the pneumonia severity index in predicting mortality.

2. Material and Methods

2.1. Ethics approval

Ethics approval was obtained through the Turkish Ministry of Health Scientific Research Platform with June 15, 2020 date and the ethics board of the Samsun Education and Research Hospital June 30, 2020 date and with 2020/10 number. The study adhered to Declaration of Helsinki.

2.2. Study Design and Patients

This single-center retrospective cohort study enrolled 263 patients with moderate to critical COVID-19 associated pneumonia hospitalized in Samsun Education and Research Hospital. The cohort was composed to include all patients who were admitted to the ICU during the period 01 April 2020-01 November 2020 with a primary admitting diagnosis of COVID-19 pneumonia and had both PSI and AaDO2 calculated at the time of admission. Patients were divided in to two groups based on the clinical outcomes: group I (discharge patients) and group II (deceased patients).

National Health Committee of the Republic of Turkey recommendations for diagnosis of COVID-19 associated pneumonia was used. Patients included in this study met the following criteria: confirmed COVID-19 infection based on RT-PCR testing from a throat swab sample; objective evidence of new-onset pneumonia from chest computed tomography (CT); typical symptoms of COVID-19 pneumonia, i.e., fever, cough, dyspnea (10).

Exclusion criteria

- Patients diagnosed with non-COVID-19 pneumonia
- Patients whose blood gas and laboratory values cannot be reached
- Patients without radiological images in the hospital database
- Patients who are not followed up in the ICU due to respiratory failure

2.3. Data collection

Demographic characteristics, comorbidities, presenting symptoms, vital signs (including fever, blood pressure, respiratory rate, oxygen saturation, heart rate), state of consciousness, initial laboratory parameters, and time to death were collected from electronic medical records. Clinical characteristics of all enrolled patients were recorded: gender, age, underlying disease, and smoking status. Baseline biochemical data, arterial blood gas, and complete blood count were also recorded. CRP, procalcitonin, troponin I and D-dimer tests were performed; radial arterial blood gases with arterial puncture were obtained in the first hour of hospital admission.

Calculation of alveolar–arterial oxygen gradient

Arterial blood gases, including arterial partial pressure of oxygen (PaO2), arterial partial pressure of carbon dioxide (PaCO2) and arterial oxygen saturation (SaO2), were measured on admission to the emergency room. The alveolar-arterial oxygen gradient was calculated as follows (9): AaDO2 [mmHg] = 150-1.25(PaCO2 – PaO2)

Calculation of pneumonia severity index

The PSI was calculated based on the description given by Fine et al. (7). Using the following parameters: age, gender, comorbidities (renal disease, liver disease, congestive heart failure, cerebrovascular disease and neoplasia), nursing home resident, physical examination (altered mental status, systolic blood pressure <90 mmHg, temperature <35 or ≥39.9°C, respiratory rate ≥30 breaths/min and heart rate ≥125 b.p.m.), laboratory data (pH <7.35, arterial oxygen tension <60 mmHg, serum Na <130 mEq/L, haematocrit <30%, serum glucose ≥250 mg/dL and blood urea nitrogen ≥30 mg/dL) and a radiological parameter, namely the presence of a pleural effusion. The normal value of PSI is between 8 and 90; 91-130 points indicate moderate risk, 130 points and above indicate high mortality risk. An on-line calculator is available to easily compute PSI: https://www.mdcalc.com/psi-port-score-pneumonia-severity-index-cap

2.4. Outcomes

The primary outcome was to compare the relationship between the AaDO2 and PSI outcome measures, namely LOS and survival. The secondary outcome was to assess the association between each of the two. The pneumonia diagnosis was determined by symptoms, physical examination findings, and radiological findings. After the first evaluation and radiological examination, arterial blood gases are derived from the patients.

2.5. Statistical Analysis

The compliance of relevant data with normal distribution was tested by the Shaphiro-Wilk test. The Student-test was used to compare normally distributed features in groups I and II, and the Mann-Whitney U test was used to compare non-normally distributed features in groups I and II. The relationship analysis of categorical variables observed in group I and II were analyzed by Exact or Pearson Chi-square tests. In this study, the age, gender and smoking variables as well as some clinical characteristics, laboratory and treatment methods were

analyzed firstly with the Univariate LR (Logistic Regression) method, and then the variables found as significant were analyzed with the Stepwise Multivariate Enter LR method. By means of the PSI, alveolar oxygen pressure, AaDO2, PaCO2 and PaO2 variables, the ROC graph was drawn over the mortality rate through the relevant cut-off rates. The minimum-maximum and median values are given as descriptive statistics for the numerical variables whereas the quantity and % rates are given for the categorical variables. SPSS Windows version 23.0 software was used for statistical analysis and $p<0.05$ was considered as statistically significant.

3. Results

Hundred fifty of 263 patients included in this study were evaluated in group I and 113 patients are evaluated in group II. The mortality rate was found at 42.9%. 55.9% (n=147) of the patients were male and 44.1% (n=116) were female, and the mean age was 72.05±12.2 (21-96) years. 75.7% of the patients were over 65 years old and 95.8% had comorbidity. The most common additional diseases in the patients are; hypertension at 58.1% (n=153), cardiac diseases at 50.2% (n=132), diabetes at 35% (n=92), neurological diseases at 24.3% (n=64), chronic obstructive pulmonary diseases at 23.9% (n=63) and chronic renal failure at 16.3% (n=43). The RT-PCR test was resulted positive only in 20.9% (n=55) of the patients included in this study. The remaining patients were diagnosed clinically and/or radiologically. The most common symptoms in the patients are dyspnea at 76.5% (n=202), fever at 28.8% (n=76) and cough at 26.1% (n=69). While the state of consciousness was normal in 31.9% of our patients who were under intensive care and 14.4% were in a state of coma (Table 1).

Table 1. Demographic, clinical characteristics and comorbidities of patients

	Group I n=150		Group II n=113		Total n=263		
	n	%	n	%	n	%	p
RT-PCR test							**<0.001**
Positive	16	10.7	39	34.5	55	20.9	
Negative	134	89.3	74	65.5	208	79.1	
Age (year)							0.468
<65	39	26.0	25	22.1	64	24.3	
>65	111	74.0	88	77.9	199	75.7	
Gender							0.143
Female	72	48.0	44	38.9	116	44.1	
Male	78	52.0	69	61.1	147	55.9	
Smoking status							0.195
Yes	45	30.4	43	38.1	88	33.7	
No	103	69.6	70	61.9	173	66.3	
COVID-19 Diagnosis							**0.013**
RT-PCR	21	14.0	34	30.1	55	20.9	
Radiological	41	27.3	21	18.6	62	23.6	
Clinical	42	28.0	27	23.9	69	26.2	
Clinical/Radiological	46	30.7	31	27.4	77	29.3	
Comorbidity							0.428
Yes	145	96.7	107	94.7	252	95.8	
No	5	3.3	6	5.3	11	4.2	
Symptoms							
Dyspnea	113	75.3	89	78.7	202	76.5	0.484
Fever	49	32.7	27	23.9	76	28.8	0.121
Cough	38	25.3	31	27.4	69	26.1	0.707
Medical condition abnormality	15	10.0	14	12.4	29	11.0	0.541
Nausea/vomiting	10	6.7	8	7.1	18	6.2	0.896
Chest pain	11	7.3	5	4.4	16	6.1	0.329
Fatigue	8	5.3	8	7.1	16	6.1	0.557
Diarrhea	4	2.7	6	5.3	10	3.8	0.268
Consciousness							**<0.001**
Awake	59	39.4	25	22.1	84	31.9	
Confusion	45	30.0	24	21.2	69	26.3	
Delirium	12	8.0	9	8.0	21	8.0	
Stupor	23	15.3	28	24.8	51	19.4	
Coma	11	7.3	27	23.9	38	14.4	

It was observed that the rate of positive observation of COVID-19 RT-PCR test in group II (34.5%) was statistically significant and it is higher than the rate of positive observation of COVID-19 RT-PCR test (10.7%) in the individuals under group I ($p<0.001$). In group II, the rate of determining the diagnosis of COVID-19 by RT-PCR test (30.1%) was statistically significant and it is higher in comparison to the rate of diagnosis of COVID-19 observed with RT-PCR test (14%) in the individuals under group I ($p=0.013$). Furthermore, it was observed that the probability of the patients diagnosed

radiologically and clinically to be living individuals was significantly higher. When the groups were compared in terms of the level of consciousness, it was observed that the rate of awareness was significantly higher in group I patients (39.3%) compared to group II patients (22.1%) ($p<0.001$) (Table 1).

In the patients included in this study, systolic blood pressure and diastolic blood pressure in group I were found to be statistically significant and higher than the group II patients ($p<0.05$). On the other hand, respiratory rate and heart rate were significantly higher in group II patients ($p<0.05$). While BUN, creatine, lactate, CRP, procalcitonin, D-Dimer, PT, INR, AST, total bilirubin, CK-MB and troponin values were statistically higher in group II patients, it was observed that values such as lymphocyte percentage value, pH, oxygen saturation, PaCO2, PaO2, bicarbonate, base excess and albumin were statistically significantly lower in group II patients ($p<0.05$) (Table 2).

Table 2. Vital signs and laboratory findings

	Group I		Group II		Total		
	min-max	median	min-max	median	min-max	median	p
Vital signs							
SBP, mmHg	60-214	127.5	50-194	110.0	50-214	120	**<0.001**
DBP, mmHg	40-130	74.0	18-127	66.0	18-130	70	**<0.001**
Respiratory rate, min^{-1}	14-47	23.0	10-52	25.0	10-52	24	**0.003**
Heart rate, min^{-1}	12-157	93.5	30-153	105.0	12-157	98	**<0.001**
Temperature, °C	36-40.1	36.6	34.5-39.6	36.6	34.5-40.1	36.6	0.284
GKS	5-15	14.0	3-15	12.0	3-15	13	**<0.001**
Laboratory findings							
Hemoglobin, g/l	4-18	12.1	5-16	11.5	4-18	11.7	0.212
Hematocrit, %	13-54	35.55	15-47	34.7	13-54	35.1	0.170
WBC count, 10^9/l	1-39	10.4	0-166	11.5	0-166	10.8	0.381
Lymphocyte count, 10^9/l	0-19	1.2	0-45	1.0	0-45	1.1	0.348
Lymphocyte, %	1-86	12.0	1-67	8.5	1-86	9.8	**0.004**
Neutrophil count, 10^9/l	2-21	8.8	0-30	9.8	0-30	9.45	0.155
Neutrophil, %	12-96	81.7	9-78	84.45	9-78	82.6	0.080
Patelet, 10^9/l	12-750	25.1	23-555	23.1	12-750	24.5	0.373
Glucose, mmol/l	56-593	150.0	40-435	148.0	40-593	149.0	0.760
Sodium, mmol/l	113-147	138.0	116-255	136.0	113-147	137.0	0.063
Potassium, mmol/l	3-8	4.3	3-8	4.5	3-8	4.36	0.056
Urea, mmol/l	3-290	52.5	12-407	95.0	3-407	68.0	**<0.001**
Creatine, mmol/l	0-7	1.0	0-10	1.7	0-10	1.2	**<0.001**
Arterial pH	7-8	7.38	7-8	7.36	7-8	7.37	**0.045**
Saturation, %	70-100	93.0	50-100	90.0	50-100	92.0	**<0.001**
$PaCO_2$, mmHg	23-98	43.0	24-99	39.5	23-99	41.6	**0.003**
PaO_2, mmHg	23-150	67.5	25-191	60.0	23-191	64.0	**0.006**
Arterial HCO_3, mmol/l	2-70	24.8	5-42	21.85	2-70	23.7	**<0.001**
Arterial lactate, mmol/l	0-11	1.65	1-12	2.2	0-12	1.9	**<0.001**
BE	-15-(-50)	0.7	-22-(-19)	-2.2	-22-(-50)	-0.4	**0.002**
CRP, mg/l	0-480	49.0	1-567	115.23	0-567	73.78	**<0.001**
Procalcitonin, µg/l	0-110	0.17	0-120	1.12	0-120	0.3	**<0.001**
D-Dimers, mg/dl	0-50	1.15	0-36	2.36	0-50	1.43	**<0.001**
PT, sec	0-81	12.8	11-146	14.1	0-146	13.35	**<0.001**
INR	1-11	1.14	1-11	1.23	1-11	1.17	**<0.001**
AST, u/l	9-1105	27.0	9-9551	41.0	9-9551	31.0	**<0.001**
ALT, u/l	2-515	17.0	4-3918	22.0	2-3918	19.0	0.051
Albumin, g/l	2-30	3.1	1-4	2.7	1-30	2.9	**<0.001**
Total bilirubin, µmol/l	0-2	0.5	0-11	0.7	0-11	0.6	**<0.001**
CK-MB, u/l	0-380	2.61	0-64	3.49	0-380	2.84	**0.014**
Troponin, ng/l	0-2500	0.10	0-25000	0.12	0-25000	0.1	**0.006**

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BE: base excess, CK-MB: creatine kinase-MB, CRP: C-reactive protein, DBP: diastolic blood pressure, GKS: Glaskow Coma Score, HCO3: bicarbonate, INR: International Normalized Ratio, PT: Prothrombin time, PaO2: arterial oxygen pressure, PaCO2: arterial carbon dioxide pressure, SBP: systolic blood pressure, WBC: white blood cell.

The chest computed tomography was performed in all patients included in this study. Accordingly, the radiological findings were present in a total of 84.4%, bilateral in 65% and unilateral in 19.4%. While 71.5% of these findings were ground glass opacity (GGO), 43.7% were parenchymal consolidation. GGO was detected at 69.3% for the group I patients, at 74.3% for the group II patients. Parenchyma consolidation in group I patients is at 40.7% and group II patients is scored at 47.8%. There was no statistical difference between group I and II in terms of radiological findings (Table 3). Among the patients included in the study, 9.1% (n=24) required non-invasive mechanical ventilation (NIMV) and 37.2% (n=86) required invasive mechanical ventilation (MV) support. The duration of NIMV application was 0.4 (IQR 1-13)

days, while the duration of MV application was 3.4 (IQR 1-83) days. Complications developed in 38.2% of all patients (n=102), while this rate was significantly lower in group I (18%; n=27) than group II (66.4%; n=75) (p<0.001). The most common complications are; acute renal failure in 12.5% (n=33) of patients, sepsis in 11% (n=29), MODS in 9.8% (n=26) and cardiac dysfunction in 7.9% (n=21) of the patients. The length of stay was 6 (IQR 1-95) days in all patients included in the study, 7 (IQR 1-83) days in group I patients and 4 (IQR 1-95) days in group II patients, and this was statistically significant (p<0.001).

Table 3. Radiological findings

	Group I n=150		Group II n=113		Total n=263		p
	n	%	n	%	n	%	
Chest CT							0.232
Unilateral involvement	33	22.0	18	15.9	51	19.4	
Bilateral involvement	91	60.7	80	70.8	171	65.0	
None	26	17.3	15	13.3	41	15.6	
Ground glass opacity							0.399
Yes	104	69.3	84	74.3	188	71.5	
No	46	30.7	29	25.6	75	28.5	
GGO involvement							0.169
Unilateral	26	17.3	13	11.5	39	14.8	
Bilateral	78	52.0	71	62.8	149	56.7	
None	46	30.7	29	25.7	75	28.5	
Consolidation							0.249
Yes	61	40.7	54	47.8	115	43.7	
No	89	59.3	59	52.2	148	56.3	
Consolidation involvement							0.334
Unilateral	18	12.0	12	10.6	30	11.4	
Bilateral	43	28.7	42	37.2	85	32.3	
None	89	59.3	59	52.2	148	56.3	

Table 4. Comparison of the groups in terms of PSI, PaO_2/FiO_2, alveolar oxygen pressure, alveolo-arterial oxygen gradient

	Group I		Group II		Total		
	min-max	median	min-max	median	min-max	median	p
PSI	44-210	135.0	107-242	174.0	44-242	150.0	**<0.001**
PaO_2/FiO_2	68-402	168.75	53-412	150.0	53-412	162.0	**<0.001**
PAO_2	59-350	221.19	62-385	235.88	59-385	226.25	**<0.001**
$AaDO_2$	28-300	154.88	60-343	177.13	28-342	161.63	**<0.001**

AaDO2: alveolar arterial oxygen gradient, FiO_2: fraction of inspired oxygen, PSI: pneumonia severity index, PaO_2: arterial oxygen pressure, PAO_2: alveolar oxygen pressure.

While PSI, alveolar oxygen pressure, alveolo-arterial oxygen gradient were significantly higher in group II patients, PaO2/FiO2 rate was statistically significantly higher in group I patients (p<0.05) (Table 4).

According to Stepwise logistic regression results; patients diagnosed with COVID-19 radiologically were more protective against mortality compared to those diagnosed with the RT-PCR test (OR: 0.103: p=0.014), in other words, it was observed that the mortality rate was lower in patients diagnosed with COVID-19 by radiological or clinical methods. Again, the patients with high albumin levels are more protective against mortality (OR: 0.053: p<0.001), but the patients with high total bilirubin levels are at a 3.72 times under the risk of mortality in each unit in comparison to the patients with lower levels (OR: 3.722: p<0.001). It was found that the patients who had complications during COVID-19 treatment were at 39.3 times under the risk of mortality in comparison to other patients (OR: 39.370: p<0.001) (Table 5).

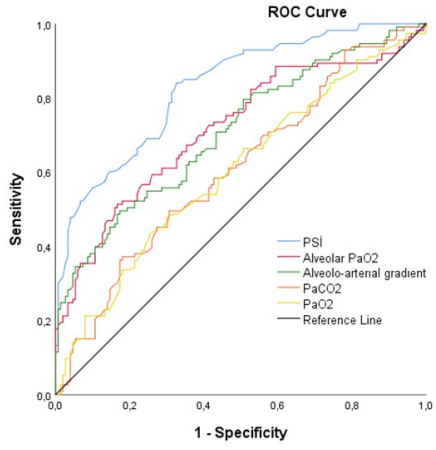

Fig 1. Mortality ROC curve

Table 5. Univariate and Stepwise Multivariate LR Analysis of Prediction of COVID-19 mortality

	Univariate LR		*Stepwise LR*	
RT-PCR (ref: positive)	***Odds Ratio (95% CI)***	***p***	***Odds Ratio (95% CI)***	***p***
Age (>65)	1.237 (0.696 2.197)	0.469		
Male	1.448 (0.882 2.376)	0.144		
Smoking status	1.406 (0.839 2.357)	0.196		
COVID-19 diagnosis (ref: test)		**0.001**	0.069	
Radiological	0.295 (0.134 0.652)	**0.003**	**0.103 (0.017 0.626)**	**0.014**
Clinical	0.351 (0.162 0.760)	**0.008**	0.739 (0.129 4.219)	0.734
Clinical/Radiological	0.387 (0.182 0.825)	**0.014**	1.007 (0.976 1.039)	0.671
Comorbity	1.626 (0.484 5.469)	0.432		
SBP	0.981 (0.972 0.992)	**<0.001**	1.007 (0.976 1.039)	0.671
DBP	0.975 (0.960 0.989)	**<0.001**	0.964 (0.916 1.014)	0.157
Respiratory rate	1.056 (1.020 1.093)	**0.002**	1.051 (0.960 1.150)	0.285
Heart rate	1.019 (1.007 1.031)	**0.001**	1.008 (0.977 1.039)	0.633
Temperature	0.867 (0.568 1.322)	0.507		
GKS	0.996 (0.970 1.023)	0.783		
Consciousness (ref: awake)		**0.001**	0.287	
Confusion	1.259 (0.637 2.488)	0.508	0.878 (0.154 4.996)	0.883
Delirium	1.770 (0.663 4.729)	0.255	1.703 (0.212 13.645)	0.616
Stupor	2.873 (1.394 5.921)	**0.004**	0.454 (0.071 2.900)	0.404
Coma	5.793 (2.494 13.455)	**<0.001**	4.559 (0.798 26.039)	0.088
Laboratory				
Hemoglobin	0.935 (0.840 1.039)	0.212		
Hematocrit	0.975 (0.939 1.011)	0.171		
Leukocyte	1.026 (0.993 1.060)	0.119		
Lymphocyte	1.077 (0.986 1.117)	0.100		
Neutrophil	1.057 (0.995 1.122)	0.071		
Platelet	1.000 (0.999 1.000)	0.205		
Glucose	0.999 (0.996 1.002)	0.999		
Sodium	0.997 (0.983 1.011)	0.625		
Potassium	1.371 (1.017 1.848)	**0.038**	1.041 (0.463 2.341)	0.923
Urea	1.011 (1.006 1.016)	**<0.001**	1.005 (0.990 1.020)	0.515
Creatine	1.446 (1.196 1.750)	**<0.001**	0.716 (0.368 1.396)	0.327
pH	0.110 (0.014 0.893)	**0.039**	326.025(0.019 550.100)	0.244
Saturation	0.923 (0.891 0.957)	**<0.001**	1.004 (0.885 1.139)	0.952
$PaCO_2$	0.967 (0.944 0.990)	**0.005**	1.049 (0.964 1.140)	0.266
PaO_2	0.990 (0.980 0.999)	**0.043**	0.998 (0.969 1.028)	0.873
Bicarbonate	0.920 (0.881 0.961)	**<0.001**	0.992 (0.857 1.148)	0.917
Lactate	1.384 (1.176 1.629)	**<0.001**	1.311 (0.821 2.092)	0.256
BE	0.940 (0.907 0.975)	**0.001**	0.971 (0.853 1.105)	0.653
CRP	1.004 (1.002 1.006)	**0.001**	1.001 (0.996 1.007)	0.652
Procalcitonin	1.024 (1.004 1.045)	**0.020**	1.001 (0,956 1.048)	0.966
D-dimer	1.058 (1.008 1.110)	**0.021**	0.991 (0.913 1.076)	0.833
PT	1.033 (0.999 1.068)	0.061		
INR	1.132 (0.891 1.439)	0.311		
AST	1.001 (0.999 1.003)	0.227		
ALT	1.002 (0.999 1.006)	0.173		
Albumin	0.164 (0.088 0.306)	**<0.001**	0.053 (0.012 0.242)	**<0.001**
Total Bilirubin	3.180 (1.667 6.067)	**<0.001**	3.722 (1.120 12.376)	**<0.001**
CK-MB	0.997 (0.988 1.006)	0.527		
Troponin	1.000 (0.999 1.000)	0.478		
Radiological involvement (ref: none)		0.756		
Unilateral	0.945 (0.401 2.227)	0.898		
Bilateral	1.524 (0.755 3.078)	0.240		
Ground glass opacity	1.262 (0.734 2.170)	0.399		
Ground glass opacity (ref: none)		0.145		
Unilateral	0.793 (0.352 1.786)	0.576		
Bilateral	1.463 (0.831 2.575)	0.188		
Consolidation	0.749 (0.458 1.225)	0.250		
Consolidation localization (ref: none)		0.645		
Unilateral	1.108 (0.505 2.433)	0.798		
Bilateral	1.499 (0.875 2.569)	0.141		
Complication	1.332 (1.167 1.522)	**<0.001**	39.370 (7.504 206.557)	**<0.001**
LOS	0.986 (0.964 1.009)	0.227		

Table 6. ROC analyses for mortality

	Cut-off	AUC	Std Eror	Sensitivity	Specificity	p
$PaCO_2$	<36.15	0.607	0.035	0.373	0.820	**0.003**
PaO_2	<56.85	0.601	0.036	0.434	0.747	**0.006**
PSI	>144.50	0.830	0.025	0.841	0.673	**<0.001**
PAO_2	>234.75	0.717	0.033	0.513	0.833	**<0.001**
$AaDO_2$	>177.89	0.710	0.032	0.496	0.827	**<0.001**

According to the arterial blood gas analysis in our study; the PaCO2 level below 36.15 mmHg posed a risk for mortality and similarly the PaO2 value below 56.85 mmHg posed a significant risk to mortality by ROC analysis. Observation of PSI value above 144.50, alveolar oxygen pressure above 234.75 mmHg and AaDO2 above 177.89 mmHg emerges as a significant risk factor for mortality. A statistical significance was observed in terms of AUC values of all these variables ($p < 0.05$). In predicting mortality, the sensitivity for the PSI variable was 84.1%, specificity 67.3%, and 49.6% specificity 82.7% for the AaDO2 variable (Table 6, Figure 1).

4. Discussion

This study evaluated the risk factors and the PSI and AaDO2 rates for mortality for the patients with moderate to severe COVID-19 pneumonia and it also provides information about the success of predicting mortality. In compliance with previous reports, the available data confirm that some biochemical markers, the presence of complications during treatment, and higher PSI rate are associated with mortality. It is also found that the AaDO2 rate stands as the valuable data in detecting the living patients as a result of this study. PSI and AaDO2 in the patients with critical COVID-19 pneumonia treated in the ICU should be used together.

Most of the patients with COVID-19 that spread from the Wuhan Province of China and caused a worldwide pandemic have been recovered within two weeks. The pneumonia occurs 15% to 20% of such patients (11). In the literature, it has been reported that 5-20% of patients diagnosed with COVID-19 had critical disease, especially acute respiratory distress syndrome, and the mortality rate is reported as 16-78% for the patients with ARDS and requiring invasive mechanical ventilation support in ICUs (12-15). Singer et al. concluded that 9 out of every 100 individuals admitted to the hospital would require ICU, invasive mechanical ventilation, or both whereas 12 out of 100 people would require ICU admission or invasive mechanical ventilation within 2 to 3 days and they reported that the duration of median mechanical ventilation is 1 week (16).

Pneumonia is an inflammatory disease of the lung alveoli caused by viruses, bacteria, and fungi. Guidelines, algorithms and scoring systems are the mechanisms that facilitate and support physicians' decision making and have the impact in reducing variability among the physicians. Many scoring systems are used to predict mortality risk in pneumonia, but the most popular ones are PSI and CURB65 (6,7,17). The PSI is the most sensitive test, with a low false negative rate, thus giving clinicians' confidence in identifying patients who do not require hospitalization. PSI includes a detailed history, physical examination, venous blood sampling, arterial blood gas measurements, and chest X-ray, so it is calculated by summing up a total of 12 parameters from history and examination and 7 parameters from further studies (7,17).

The most important pathophysiology of pneumonia is ventilation-perfusion mismatch. AaDO2 shows the integrity of the alveolocapillary membrane and is used as a gas exchange index. The affecting factors are diffusion gradient, ventilation-perfusion imbalance, and true shunt (9). AaDO2 can distinguish whether hypoxemia is caused by alveolar hypoventilation or ventilation/perfusion mismatch, but this parameter can be misleading when the patient receives additional oxygen support (18). In our study, the arterial blood gas values obtained during admission to the emergency room were taken into account. However, some patients received oxygen therapy because their oxygen saturation values were below 90% during admission and the arterial blood gas study was conducted accordingly. Therefore, the success of PSI, which is also among the aims of our study, in predicting mortality was investigated.

The most common diagnostic tool for COVID-19 disease is RT-PCR, which is considered the reference gold standard. Recent studies have emphasized the importance of chest CT in COVID-19 pneumonia with false negative RT-PCR results and reported CT sensitivity as 98% (19-22). In the diagnosis of COVID-19 when RT-PCR is accepted as the reference standard, the specificity, PPV, NPV and accuracy rates of chest CT are reported as 21.6%, 61.9%, 73.3%, and 63.3%, respectively. The most common and typical CT findings of COVID-19 are bilateral multi-lobe involvement, peripheral localized, irregular shape and ground-glass opacity (19,20). In our study, it was found that the diagnosis of COVID-19 pneumonia both radiologically and clinically in the patients with negative RT-PCR test was reliable. The most common radiological finding was the GGO observed in both lungs. We believe that the parenchymal consolidation was detected less frequently because the patients were admitted to the hospital as soon as relevant symptoms began.

Although the initial chest CT is normal in some COVID-19 patients, new lung lesions may develop during treatment. This period is reported as an average of 5.8 days. Therefore, a new chest CT is recommended especially for the patients who worsen or develop new symptoms during treatment even if the initial chest CT is negative (23). In our study, chest CT imaging was performed in all patients during admission, and no

radiological involvement caused by COVID-19 pneumonia was detected in only 15.6%. Fever with a rate of 85% and cough with a rate of 70% are the most common major symptoms in COVID-19 pneumonia (2,3). In our study, the most common presenting symptom was dyspnea at 76.5%. We attribute this case to the reason that our patients had a moderate-severe course of COVID-19 pneumonia and were in need of intensive care.

Pan et al. stated that the average time between the onset of symptoms and the admission to hospital was 11 days, and that 73.4% of patients developed ARDS within a median of 7 (IQR 4-11) days after admission to the hospital. Again, in the evaluation made during the application was reported that detection of oxygen saturation ≤89%, lymphocyte ≤0.64×109/L, CRP > 77.35 mg/L, procalcitonin >0.20 μg/L and LDH >481 U/L is a risk factor for mortality, and these values should be closely monitored in critical COVID-19 patients (11). Most of the data reported in the literature in our patients were followed closely, and it was observed that blood gas values, CRP, procalcitonin, D-dimer, albumin and total protein, especially in univariate analysis, and albumin and total bilirubin in multivariate analysis were found to be associated with high mortality. The complication rate seen in the deceased patient group was found to be significant with 66% in multivariate analysis.

In a multi-center cohort study conducted in the USA, the mortality rate of a 28-day intensive care was reported at 35.4% for the patients with critical COVID-19 disease. Again, in this study, it was reported that the risk of mortality increased in the presence of advanced age, male gender, high BMI, hypoxemia at the time of admission to hospital, and comorbidities such as malignancy, coronary artery disease and renal failure. In this study, they reported the most common cause of death in patients followed up in the ICUs as respiratory failure with 92.7%, septic shock with 39.7% and renal failure with 36.7%. They also reported that 37.2% of patients treated in ICUs could be discharged, the average length of stay in the intensive care unit was 9 (IQR, 5-14) days, and the average hospital stay was 16 (IQR, 11-22) days (13). Similarly Cummings et al. reported that the intensive care mortality rate was 39% and 37% of the patients were still treated in the hospital. Similar to previous data, advanced age, cardiopulmonary comorbidities, and high D-dimer concentration were reported as poor prognostic factors (14). Graselli et al. stated that the length of stay in the ICU was 10 (IQR, 5-16) days in the patients who were deceased, and 15 (IQR, 8-24) days in the patients who were discharged, and that the presence of hypertension as a comorbidity was associated with short survival (15).

The mortality rate in our study was 42.9%, and the most common cause of death was found to be renal failure with 12.5%. According to the multivariate analysis, it was seen that low albumin and high total bilirubin levels and the development of complications posed a risk for mortality. The length of stay was shorter in deceased patients, which means that patients with critical COVID-19 pneumonia were lost in the early period, and the study starting from the pandemic period when the treatment methods to be applied fail to settle properly.

Garcia et al. reported lack of oxygenation, renal and microvascular dysfunction, and coagulation activation as risk factors for mortality in critically ill patients, and they recommended that they be followed creatine, D-dimer, lactate, potassium and AaDO2 closely (3). Esteve et al. low PaO2/FiO2 rate was associated with increased mortality and prolonged length of stay in the patients admitted to the ICU (8).

One of the main findings of our study is that in group II patients, PaO2/FiO2 rate, i.e. low PaO2/FiO2 rate was associated with high mortality. Again, AaDO2 as an indicator of bad oxygenation and it was significantly higher in the deceased patients. Although it is calculated in patients receiving oxygen therapy in our study, it was observed that the specificity value of AaDO2 was high. In other words, it was valuable in determining the surviving patients. We attribute the low sensitivity to the arterial blood gas values obtained while the patients are under oxygen therapy. We found that PSI, which evaluates more than one data, was more powerful in predicting mortality with a sensitivity value of 84.1%.

In conclusion, early estimation of mortality risk and taking precautions accordingly are important for hospitalization and close follow-up of patients with critical COVID-19 pneumonia. We think that pneumonia severity index is reliable in predicting mortality and alveolo-arterial oxygen gradient in determining the surviving patient, especially in the patients receiving oxygen therapy under intensive care units. We recommend that both rates should be used together in the follow-up of patients with critical COVID-19 pneumonia in intensive care units.

The main limitation on our study is that it is conducted as a retrospective and a single center study with a relatively limited number of patients. The results should be confirmed with the studies involving more patients. Secondly, the number of patients diagnosed with COVID-19 pneumonia based on clinical and radiological evaluation is higher than the number of RT-PCR positive patients. Since the rate of unconfirmed cases is consistent with the reported sensitivity data of the RT-PCR test for COVID-19, we assume these are misleading negative tests.

Conflict of interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Authors' contributions

Concept: H.K.Ç., Design: H.K.Ç., Z.D., Data Collection or Processing: H.K.Ç., T.S.A., Analysis or Interpretation: H.K.Ç., M.K., Literature Search: H.K.Ç., Z.D., T.S.A., Writing: H.K.Ç.

References

1. Carlino MV, Valenti N, Cesaro F, Costanzo A, Cristiano G, et al. Predictors of intensive care unit admission in patients with coronavirus disease 2019 (COVID-19). Monaldi Arch Chest Dis. 2020;90(3):10.4081/monaldi.2020.1410.
2. Mahase E. Covid-19: death rate is 0.66% and increases with age, study estimates. BMJ. 2020;369:m1327.
3. Wendel Garcia PD, Fumeaux T, Guerci P, Heuberger DM, Montomoli J, Roche-Campo F, Schuepbach RA, Hilty MP; RISC-19-ICU Investigators. Prognostic factors associated with mortality risk and disease progression in 639 critically ill patients with COVID-19 in Europe: Initial report of the international RISC-19-ICU prospective observational cohort. EClinicalMedicine. 2020 Aug;25:100449.
4. Özlü T, Bülbül Y, Alataş F, Arseven O, Coşkun AŞ, et al. Türk Toraks Derneği Erişkinlerde Toplumda Gelişen Pnömoni Tanı ve Tedavi Uzlaşı Raporu. Türk Toraks Derg. 2009;10(Suppl9):3-16.
5. Loke YK, Kwok CS, Niruban A, Myint PK. Value of severity scales in predicting mortality from community-acquired pneumonia: systematic review and meta-analysis. Thorax. 2010;65(10):884-90.
6. Lim WS, van der Eerden MM, Laing R, Boersma WG, Karalus N, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax. 2003;58(5):377-82.
7. Fine MJ, Auble TE, Yealy DM, Hanusa B, Weissfeld LA, et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med. 1997;336(4):243–50.
8. Esteve F, Lopez-Delgado JC, Javierre C, Skaltsa K, Carrio ML, et al. Evaluation of the PaO2/FiO2 ratio after cardiac surgery as a predictor of outcome during hospital stay. BMC Anesthesiol. 2014;14:83.
9. Hsu, JT, Chu CM, Chang ST, Cheng HW, Cheng NJ, Ho WC, et al. Prognostic role of alveolar-arterial oxygen pressure difference in acute pulmonary embolism. Circ J. 2006;70(12):1611–6.
10. Scientific Committee Study. Guide to COVID-19 (SARS-CoV-2 infection). TR. Ministry of Health, General Directorate of Public Health (2 April 2020) Ankara. 2020. https://covid19rehberi.com/wpcontent/uploads/2020/04/COVID19_Eriskin_Hasta_Tedavisi_02042020.pdf.
11. Pan F, Yang L, Li Y, Liang B, Li L, et al. Factors associated with death outcome in patients with severe coronavirus disease-19 (COVID-19): a case-control study. Int J Med Sci. 2020;17(9):1281-92.
12. Xie J, Covassin N, Fan Z, Singh P, Gao W, et al. Association between hypoxemia and mortality in patients with COVID-19. Mayo Clin Proc. 2020;95(6):1138-47.
13. Gupta S, Hayek SS, Wang W, Chan L, Mathews KS, et al; STOP-COVID Investigators. Factors associated with death in critically ill patients with coronavirus disease 2019 in the US. JAMA Intern Med. 2020;180(11):1–12.
14. Cummings MJ, Baldwin MR, Abrams D, Jacobson SD, Meyer BJ, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. MedRxiv. 2020 Apr 20:2020.04.15.20067157.
15. Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, et al; COVID-19 Lombardy ICU Network. Risk Factors Associated With Mortality Among Patients With COVID-19 in Intensive Care Units in Lombardy, Italy. JAMA Intern Med. 2020;180(10):1345-55.
16. Singer AJ, Morley EJ, Meyers K, Fernandes R, Rowe AL, et al. Cohort of four thousand four hundred four persons under investigation for COVID-19 in a New York hospital and predictors of ICU care and ventilation. Ann Emerg Med. 2020;76(4):394-404.
17. Demirel B. Lactate levels and pneumonia severity index are good predictors of in-hospital mortality in pneumonia. Clin Respir J. 2018;12(3):991-5.
18. Carlino MV, Guarino M, Izzo A, Carbone D, Arnone MI, et al. Arterial blood gas analysis utility in predicting lung injury in blunt chest trauma. Respir Physiol Neurobiol. 2020;274:103363.
19. Awulachew E, Diriba K, Anja A, Getu E, Belayneh F. Computed Tomography (CT) ımaging features of patients with COVID-19: systematic review and meta-analysis. Radiol Res Pract. 2020;2020:1023506.
20. Chen HJ, Qiu J, Wu B, Huang T, Gao Y, et al. Early chest CT features of patients with 2019 novel coronavirus (COVID-19) pneumonia: relationship to diagnosis and prognosis. Eur Radiol. 2020;30(11):6178-85.
21. Kuzan TY, Murzoğlu Altıntoprak K, Çiftçi HÖ, Ergül U, Ünal Özdemir NB, et al. A comparison of clinical, laboratory and chest CT findings of laboratory-confirmed and clinically diagnosed COVID-19 patients at first admission. Diagn Interv Radiol. 2021;27(3):336-43.
22. Lv M, Wang M, Yang N, Luo X, Li W, et al. Chest computed tomography for the diagnosis of patients with coronavirus disease 2019 (COVID-19): a rapid review and meta-analysis. Ann Transl Med. 2020;8(10):622.
23. Fu B, Hu L, Lv F, Huang J, Li W, et al. Follow-up CT results of COVID-19 patients with initial negative chest CT. Infect Drug Resist. 2020;13:2681-87.

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Sex and age-dependent changes of the cerebral cortex in young adult Sudanese: A brain segmentation study

Wegdan AHMED [1,*], Tahir OSMAN [2], Bünyamin ŞAHİN [3], Samy AHMED [4] Amani ELFAKI[4]

[1]Department of Anatomy, Faculty of Medicine, National University, Khartoum, Sudan
[2]Faculty of Postgraduate Studies and Scientific Research, Ribat University, Khartoum, Sudan
[3] Rector, Tokat Gaziosmanpasa University, Tokat, Türkiye
[4] Department of Anatomy, Faculty of Medicine, Alzaiem Al-Azhari University, Khartoum, Sudan

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Abstract

The present study aimed to measure the cortical structures of cerebral hemispheres, including cortical thickness (CT), grey and white matter volume (GMV and WMV) and volume fraction (GMVF and WMVF), in addition to the volume (V) and cortical area pial (CAP) in normal young adult Sudanese and to investigate the effect of sex and age on these cortical structures. The study included 139 healthy young adult Sudanese subjects (80 males and 59 females). Participants' ages ranged from 20-40 years. T1-weighted MR brain images with a thickness of 1 mm were obtained. MR images of the subjects were analyzed using the automatic segmentation software (BrainSuite). Cortical structures of the cerebral hemispheres were estimated using the output data of the process of the software. Sex differences were noticed in the GMV (325.76±38.74cm^3), WMV (182.09±26.70cm^3), V (507.85±62.20cm^3), and CAP (1263.22±127.42cm^2) of the cerebral hemisphere ($P<0.05$). However, there were no differences between genders in CT (3.81±0.17mm), GMVF (64.20±1.98%), and WMVF (35.80±1.98%) of the cerebral hemisphere ($P>0.05$). Changes with age were seen in the GMV, GMVF, WMVF, and CAP of the cerebral hemisphere ($P<0.05$). However, there were no changes with age in CT, WMV, and V of the cerebral hemisphere ($P>0.05$). Sex and age are important factors to consider when evaluating the cortical structures of the cerebral hemisphere. Sex affects GMV, WMV, V, and CAP, but not on the CT, WMVF, and GMVF. Age affects GMV, CAP, WMVF, and GMVF, but not on the CT, V, and WMV.

Keywords: cortical thickness, volume fractions, cortical area, cerebral hemispheres, BrainSuite

1. Introduction

The cerebrum is the largest part of the brain, which consists of two hemispheres separated by a longitudinal fissure; the corpus callosum, which connects the two hemispheres, is located at the base of this fissure (1). The right and left hemispheres do tend to have specific specializations, with the left hemisphere, in particular, being devoted to language and memory and the interaction of language and memory. The right hemisphere is heavily involved in spatial cognition, which is our understanding of space around us; it also allows us to process music (2).

The cerebrum has an outer cortex consisting of grey matter (GM) and an inner core of white matter. The cortical layer is located between the pial or outer surface at the GM/CSF (cerebrospinal fluid) interface and the inner surface at the GM/WM (white matter) junction (3). Each surface unit of the cerebral cortex can be defined as topologically equivalent to a 3D sphere that assists in measuring its cortical structures such as thickness, volume, grey and white matter volume and cortical area pial (3).

It has been shown that various factors such as sex and age may influence these cortical structures in the normal human cerebrum. Knowledge and understanding of sex impact on the cortical structures are of significant importance to adequately address the sex differences in managing risk factors, symptoms, course of disease and treatment (4). Furthermore, age-associated changes in cortical structures in healthy adults have greater importance because the determinations of normal age-specific values in the brain have a role in evaluating both clinical-pathologic conditions and normal aging processes.

To our knowledge, no studies have been done to assess the effect of sex and age on all these cortical structures in healthy young adult subjects with no history of psychiatric and neurological diseases. These cortical structures were measured from T1 weighted magnetic resonance (MR) imagining by using automatic segmentation software called BrainSuite.

*Correspondence: weg.anatomist@gmail.com

2. Material and Methods

2.1. Subjects

139 Sudanese young adults in good health, between the ages of 20 and 40, participated in the current study. Age (males and females were 28.5.72 and 28.6.00 years old, respectively) and body mass index (BMI) (males and females were 23.933.6 and 24.895.07kg/cm^2, respectively) were matched between the sexes.

Participants who used drugs suffered from head trauma, neurological disorders, psychiatric disorders, or had a congenital brain deformity were excluded.

The Ethical Committee of the National Ribat University approved the study.

2.2. Magnetic resonance imaging

Three-dimensional T1-weighted MR brain pictures were acquired using a 1.5 Tesla Philips scanner, Version: 3.2.1, in 5 minutes and 18 seconds, with a slice distance of 1.0mm, a field of view of 250 read, 192mm phase, TR=1657ms, TE=2.95ms, bandwidth 180Hz/pixel, flip angle 15°, and ECHO spacing=7.5ms.

2.3. MR images analysis

BrainSuite software tools, which automatically process the MR image of the brain, were used to examine the MR images.

Surface and volume registration and cortical surface extraction sequence (CSE) are the two processes used by BrainSuite to evaluate MR images. These procedures take about three hours to complete.

2.4. Cortical surface extraction sequence (CSE)

The skull and scalp are stripped from the MR picture during this 30-minute stage, followed by correcting an error that occurred during the removal of the skull. Following a classification of the brain's tissue into white matter, grey matter, and cerebrospinal fluid, the program then divides the brain into its three main regions: the cerebrum, cerebellum, and brainstem. The mistake is fixed by removing the cortex from the affected brain hemisphere. Finally, the pial surface is generated, and each hemisphere's cortical surface is displayed in a separate hue (Fig.1.).

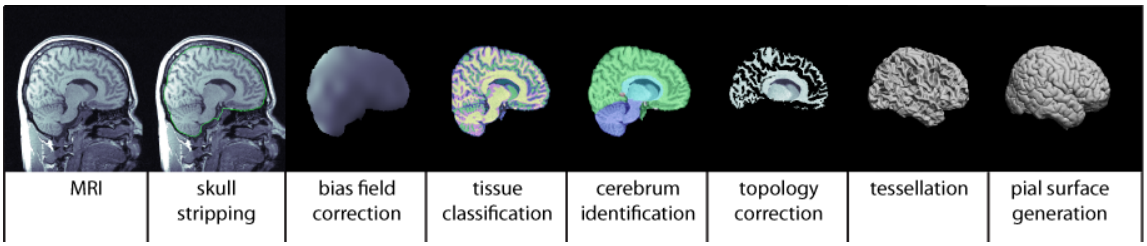

Fig. 1. Cortical surface extraction sequence (7)

2.5. Surface and volume registration (SVReg)

During this step, the cortical and subcortical structures are labelled, and the surfaces and volumes are registered to the brain atlas.

The output of BrainSuite in an excel sheet containing the measurement of each brain gyri such as grey matter volume, white matter volume, CSF volume, cortical area pial and midal and inner and cortical thickness (Fig. 2.).

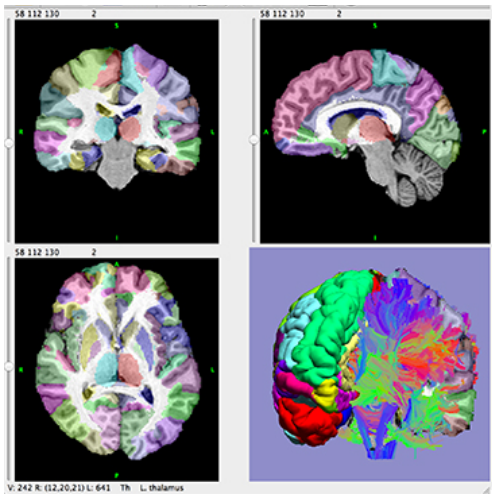

Fig. 2. MR image analyzed by BrainSuite (7)

BrainSuite automatically calculates cortical structures of the gyri of the cerebral hemispheres. The following formulas (1-5) were used in a Microsoft Excel worksheet to calculate cortical structures of the cerebral hemisphere.

2.6. Statistical analysis

Data were analyzed using Statistical Package of Social Science (SPSS) version 21.0. An Independent sample t.test was performed to compare mean values of grey and white matter between males and females. Bivariate correlation was used to evaluate the relationship between grey and white matter and age. P.value equal to or less than 0.05 was considered statistically different.

3. Results

There were no differences between genders in thickness, white and grey matter volume fraction of the right, left, and total cerebral hemispheres, and white matter volume of the left cerebral hemisphere. Conversely, gender differences were seen in the white matter volumes of the right and total cerebral hemispheres; grey matter volumes, volumes, and cortical areas pial of the right, left, and total cerebral hemispheres, which were larger in males than females ($P<0.05$).

The data details of the structures of the cerebral hemispheres are given in Table 1.

Table 1. Comparisons of structures of cerebral hemispheres between genders

	Right hemisphere		Left hemisphere		Total hemisphere	
Structures	Males	Females	Males	Females	Males	Females
Thickness (mm)	3.83±0.15	3.79±0.20	3.82±0.19	3.79±0.14	3.82±0.18	3.80±0.14
White Matter V (cm^3)	192.54±24.57	166.46±20.70*	186.32±28.76	177.81±24.20	664.69±82.76	633.68±66.30*
Grey Matter V (cm^3)	343.40±37.02	301.21±27.02*	332.84±40.88	316.81±33.16*	371.57±56.13	354.15±47.69*
Total V (cm^3)	535.93±57.96	467.68±44.02*	519.16±65.73	494.63±54.72*	1036.26±131.77	987.83±108.82*
Cortical AP (cm^2)	1327.86±118.25	1177.68±87.89*	1284.13±133.88	1232.74±107.69*	2570.57±272.64	2466.60±215.82*
White Matter VF (%)	35.89±1.93	35.55±1.94	35.82±2.21	35.88±1.79	35.80±2.08	35.79±1.75
Grey Matter VF (%)	64.11±1.93	64.45±1.94	64.18±2.21	64.12±1.79	64.20±2.08	64.21±1.75

(Mean±SD), V: volume, AP: area pial, VF: volume fraction, *: P≤0.05

3.1. Correlation between age and structures of the cerebral hemispheres

There was a negative correlation between age and cortical area pial, grey matter volume and volume fraction of the right cerebral hemisphere in males and females (Fig. 3, 4, 5) (P<0.05). Conversely, cortical areas pial, grey matter volumes and volume fractions of the left and total cerebral hemispheres were not correlated with age in males and females (P>0.05).

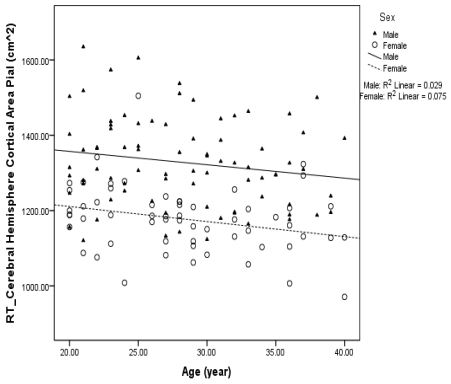

Fig. 3: Correlation between age and cortical area pial of right (RT) cerebral hemisphere

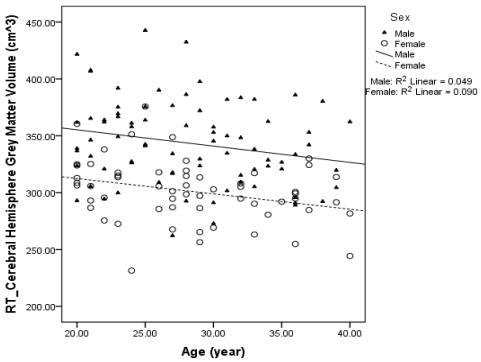

Fig. 4. Correlation between age and grey matter volume of right (RT) cerebral hemisphere

There was a positive correlation between age and white matter volume fraction of the right cerebral hemisphere in males and females (Fig. 6.) (P<0.05). Conversely, white matter volume fractions of the left and total cerebral hemispheres were not correlated with age in males and females (P>0.05).

There was no correlation between age and cortical thicknesses, white matter volumes, and volumes of the right, left, and total cerebral hemispheres in males and females (P>0.05).

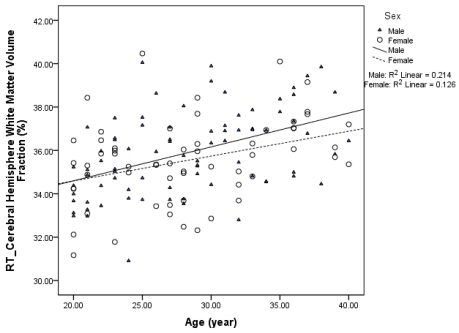

Fig. 5. Correlation between age and white matter volume fraction of right (RT) cerebral hemisphere

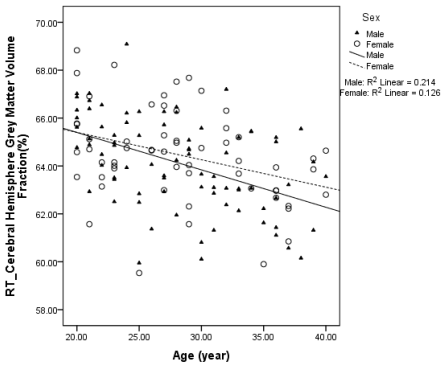

Fig. 6. Correlation between age and grey matter volume fraction of right (RT) cerebral hemisphere

4. Discussion

The present study's findings revealed no significant differences between genders in thickness, and grey and white matter volume fraction of the right, left, and total cerebral hemispheres.

The results about the cortical thickness confirmed the findings of other studies (6-8) and found that cortical thickness of the cerebral hemisphere is similar in males and females.

Results about sex differences in grey and white matter volume fractions of the cerebral hemisphere are inconsistent. The present findings are consistent with other studies which failed to detect significant sex differences in GM and WM volume fraction (9); and in contrast with previous studies, which detected higher GM volume fractions in females (10);

and others which revealed higher WM volume fraction in males (11). These conflicting results may be due to differences in methods used for obtaining tissue measurements.

The results of the present study revealed that after controlling age and BMI between genders, males had significantly larger white matter volumes of the right and total cerebral hemispheres; grey matter volumes, cortical volumes, and cortical areas pial of the right, left, and total cerebral hemispheres.

The present study's findings supported the findings of other studies, which found larger grey and white matter volumes in males (9, 12). In contrast, early studies initially found sex differences in white matter but not grey matter volumes (13, 14). The possible reasons for sex differences in grey and white matter volumes may be due to the impact of sex hormones. Recent findings have shown that there may be a connection between grey and whiter matter volumes and hormone levels of testosterone, estrogen, or progesterone (15). However, it is not yet fully understood how sex hormones lead to these differences (16).

Regarding the results of cortical volume, our results confirmed the previous studies and found larger volumes of the cerebral hemispheres in males (12, 17). An interesting result is a difference between males and females concerning the relationship between body size (height and weight) and cerebrum volumes. The current study found a significant strong positive correlation between males' weight/ height and cerebrum volume, but not for females. The present study outcomes suggest that in females, the factors that govern overall body growth (weight and height) are not closely related to or regulated by the factors that determine cerebrum growth. On the other hand, for males, there may be a modest relationship between overall body size (weight and height) and cerebrum size.

Is there any functional significance in the difference in the volume of the hemisphere between genders? Many scientists have thought over this question, but until now, there is no clear answer. Some scientists recognized a modest positive correlation between brain size and intelligence and reported that males are more intelligent than females (6, 18). Likewise, the relationship between intelligence and brain size cannot be defined as the bigger, the better; autism patients have larger brains when compared to healthy subjects (19).

Cortical volume is the product of grey and white matter volumes, so the observed differences in the volume between sexes are due to the sex differences in grey and white matter volumes. This study found a strong positive correlation between brain volume and grey and white matter volume in males and females.

Literature about sex differences in cortical area pial is not widely available. The previous studies confirmed our study and found that males had a larger cerebral hemisphere cortical surface area than females (20, 21). Sex differences in cortical volume could explain the sex differences in cortical area pial; the current study found a strong significant positive correlation between cortical volume and cortical area pial of the cerebral hemisphere. This result is consistent with a post-mortem study showing that a large cortex volume is mainly caused by a real expansion of cortical surface area (22).

The effects of aging on neuroanatomical structures have been studied using volumetric techniques such as region of interest (ROI) and voxel-based morphometric (VBM) analysis, and more recently through surface-based measures such as thickness and surface area. By applying an automatic segmentation tool (BrainSuite), the present study examined age-related structural changes upon several specific morphometric measures.

The present study found no correlation between age and cortical thickness, white matter volume and volume of the right, left and total cerebral hemispheres in males and females.

These results tally with the post-mortem and MRI studies which found that cortical volume of the cerebral hemisphere is stable between 20-50 years (23).

In contrast to the results of cortical thickness and volume, other MR imaging comprising 106 subjects ranging in age from 19 to 93 years found a negative correlation between age and thickness and volume of the right and left hemispheres (8). The possible reason for this inconsistency between the current and previous results may be related to the wide age range examined in the compared study. In contrast to the results of the white matter volume, other in-vivo studies have demonstrated that white matter volume increases until approximately the fifth decade of life and declines afterwards (24, 25).

The cortical structures which significantly change with age were cortical area pial, grey matter volume and volume fraction, and white matter volume fraction of the right hemisphere in males and females.

The finding of grey matter volume is confirmed by the in vivo studies that have shown that grey matter volume reduction begins in early adulthood and continues approximately linearly throughout adulthood (26, 27). The decrease in grey matter volume is due to degenerative changes that include loss of neurons (28) and dendritic arborization (29); and brain maturation changes which consists of synaptic pruning and myelination (30, 31).

The finding about cortical area pial is in agreement with the study that found reduction with age in cortical area pial of the cerebral hemisphere (32). A second study demonstrated that cortical surface area expanded until approximately 12.7 years and then shrank at a relatively modest rate until surface contraction became more pronounced after the age of 45 (33). The extent to which analogous cellular changes drive cortical area reduction in adulthood is unclear. Dendritic size and

complexity loss could explain this reduction (34).

The present result also revealed that the grey matter volume fraction of the right cerebral hemisphere decreased with age, while the white matter volume fraction of the right cerebral hemisphere increased with age and coincidence to a decrease in grey matter volume fraction; these combination changes explain why cerebral volume stable between 20-40 years.

The strengths of the current study are the inclusion of a large sample and measuring the cortical structures by applying an automatic segmentation tool. A limitation of the present study is a cross-sectional study, which describes differences between subjects of different ages rather than describing changes correlated with age over time in individual subjects.

Age and gender both impact the cerebral hemisphere's structural makeup. The volume, grey and white matter volumes, and cortical area pial of the hemispheres were all considerably greater in males than in females, but the thickness and grey and white matter volume fractions were not statistically different. Only the right hemisphere's cortical area pial, white matter volume fraction, and grey matter volume and volume fraction all significantly changed with age; however, white matter thickness and volume and volume did not.

This study marks the first to offer baseline information on cerebral hemisphere cortical architecture. Compared to other normative studies or illness states, it can be utilized to identify considerable deviation from normal structural values.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: W.A., T.O., A.E., B.Ş. Design: W.A., T.O., A.E., B.Ş. Data Collection or Processing: W.A., A.E., Analysis or Interpretation: W.A., A.E., S.A., Literature Search: W.A., Writing: W.A., T.O.,S.A.

References

1. Valerie C. Scanlon TS (2007). The Nervous System: Essentials of anatomy and physiology. Philadelphia: F. A. Davis Company. 5: 177-90.
2. Schwartz BL (2010). Memory and Brain: Memory: Foundations and Applications. SAGE Publications. 39.
3. Fischl B (2012). FreeSurfer: NeuroImage. 62(2):774-81.
4. Sacher J, Neumann J, Okon-Singer H, Gotowiec S, Villringer A (2013). Sexual dimorphism in the human brain: evidence from neuroimaging: Magnetic resonance imaging. 31(3):366-75.
5. www.Brainsuite.org.
6. Nopoulos P, Flaum M, O'Leary D, Andreasen NC (2000). Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging: Psychiatry research. 98(1):1-13.
7. Rabinowicz T, Dean DE, Petetot JM, de Courten-Myers GM (1999). Gender differences in the human cerebral cortex: more neurons in males; more processes in females: Journal of child neurology. 14(2):98-107.
8. Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RS, Busa E, et al. (2004). Thinning of the cerebral cortex in aging: Cerebral cortex. 14(7):721-30.
9. Gur RC, Turetsky BI, Matsui M, Yan M, Bilker W, Hughett P, et al. (1999). Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance: The Journal of neuroscience : the official journal of the Society for Neuroscience. 19(10):4065-72.
10. Good CD, Johnsrude I, Ashburner J, Henson RN, Friston KJ, Frackowiak RS (2001). Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains: NeuroImage. 14(3):685-700.
11. Schlaepfer TE, Harris GJ, Tien AY, Peng L, Lee S, Pearlson GD (1995). Structural differences in the cerebral cortex of healthy female and male subjects: a magnetic resonance imaging study: Psychiatry research. 61(3):129-35.
12. Allen JS, Damasio H, Grabowski TJ, Bruss J, Zhang W (2003). Sexual dimorphism and asymmetries in the gray–white composition of the human cerebrum: NeuroImage. 18(4):880-94.
13. Filipek PA, Richelme C, Kennedy DN, Caviness VS, Jr. (1994). The young adult human brain: an MRI-based morphometric analysis: Cerebral cortex. 4(4):344-60.
14. Passe TJ, Rajagopalan P, Tupler LA, Byrum CE, MacFall JR, Krishnan KR (1997). Age and sex effects on brain morphology: Progress in neuro-psychopharmacology & biological psychiatry. 21(8):1231-7.
15. Witte AV, Savli M, Holik A, Kasper S, Lanzenberger R (2010). Regional sex differences in grey matter volume are associated with sex hormones in the young adult human brain: NeuroImage. 49(2):1205-12.
16. van Amelsvoort T, Compton J, Murphy D (2001). In vivo assessment of the effects of estrogen on human brain: Trends in endocrinology and metabolism: TEM. 12(6):273-6.
17. Carne RP, Vogrin S, Litewka L, Cook MJ (2006). Cerebral cortex: An MRI-based study of volume and variance with age and sex: Journal of Clinical Neuroscience. 13(1):60-72.
18. Reiss AL, Abrams MT, Singer HS, Ross JL, Denckla MB (1996). Brain development, gender and IQ in children. A volumetric imaging study: Brain : a journal of neurology. 119 1763-74.
19. Piven J, Arndt S, Bailey J, Havercamp S, Andreasen NC, Palmer P (1995). An MRI study of brain size in autism: The American journal of psychiatry. 152(8):1145-9.
20. Barta P, Dazzan P (2003). Hemispheric surface area: sex, laterality and age effects: Cerebral cortex. 13(4):364-70.
21. Henery CC, Mayhew TM (1989). The cerebrum and cerebellum of the fixed human brain: efficient and unbiased estimates of volumes and cortical surface areas: Journal of anatomy. 167:167-80.
22. Pakkenberg B, Gundersen HJ (1997). Neocortical neuron number in humans: effect of sex and age: The Journal of comparative neurology. 384(2):312-20.
23. Miller AK AR, Corsellis JA. (1980). Variation with age in the volumes of grey and white matter in the cerebral hemispheres of man: measurements with an image analyser: Neuropathol Appl Neurobiol. 2:119-32.

24. Salat DH, Kaye JA, Janowsky JS (1999). Prefrontal gray and white matter volumes in healthy aging and Alzheimer disease: Archives of neurology. 56(3):338-44.

25. Walhovd KB, Fjell AM, Reinvang I, Lundervold A, Dale AM, Eilertsen DE, et al. (2005). Effects of age on volumes of cortex, white matter and subcortical structures: Neurobiology of aging. 26(9):1261-70; discussion 75-8.

26. Ge Y, Grossman RI, Babb JS, Rabin ML, Mannon LJ, Kolson DL (2002). Age-related total gray matter and white matter changes in normal adult brain. Part I: volumetric MR imaging analysis: AJNR American journal of neuroradiology. 23(8):1327-33.

27. Lehmbeck JT, Brassen S, Weber-Fahr W, Braus DF (2006). Combining voxel-based morphometry and diffusion tensor imaging to detect age-related brain changes: Neuroreport. 17(5):467-70.

28. Terry RD, DeTeresa R, Hansen LA (1987). Neocortical cell counts in normal human adult aging: Annals of neurology. 21(6):530-9.

29. Jacobs B, Driscoll L, Schall M (1997). Life-span dendritic and spine changes in areas 10 and 18 of human cortex: a quantitative Golgi study: The Journal of comparative neurology. 386(4):661-80.

30. Bartzokis G, Beckson M, Lu PH, Nuechterlein KH, Edwards N, Mintz J (2001). Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study: Archives of general psychiatry. 58(5):461-5.

31. Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW (2003). Mapping cortical change across the human life span: Nature neuroscience. 6(3):309-15.

32. Ostby Y, Tamnes CK, Fjell AM, Westlye LT, Due-Tonnessen P, Walhovd KB (2009). Heterogeneity in subcortical brain development: A structural magnetic resonance imaging study of brain maturation from 8 to 30 years: The Journal of neuroscience : the official journal of the Society for Neuroscience. 29(38):11772-82.

33. Schnack HG, van Haren NE, Brouwer RM, Evans A, Durston S, Boomsma DI, et al. (2014). Changes in Thickness and Surface Area of the Human Cortex and Their Relationship with Intelligence: Cerebral cortex.

34. Feldman ML, Dowd C (1975). Loss of dendritic spines in aging cerebral cortex: Anatomy and embryology. 148(3):279-301.

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Risk factors associated with the development of trocar site hernia after laparoscopic bariatric-metabolic surgery

Muzaffer AL

Department of Surgery, Faculty of Medicine, Near East University, Nicosia, Cyprus

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Abstract

The aim of this study is to investigate the effects of three laparoscopic bariatric-metabolic surgery (BMS) techniques [sleeve gastrectomy (SG), SG with transit bipartition (SG-TB), one anastomosis gastric by-pass (OAGB)] and patients' sociodemographic and clinical features on trocar site hernia (TSH) development after laparoscopic BMS. This was a retrospective study conducted between January 2015 and February 2022. A total of 54 obese patients, 18 who developed TSH and 36 who did not develop TSH during follow-up after laparoscopic BMS, were included in the study. The mean age was 55.28 ± 7.98 years in the TSH group and 40.58 ± 11.47 years in the non-TSH group ($p < 0.001$). Seventeen (94.4%) of the TSH group and 23 (63.9%) of the non-TSH group were females ($p = 0.021$). TSH developed in 1 patient who underwent SG (5.6%), 1 patient who underwent OAGB (5.6%), and 16 patients who underwent SG-TB (88.9%). Multiple logistic regression analysis revealed that high age ($p=0.018$) and undergoing SG-TB ($p=0.018$) were independently associated with TSH development. In order to reduce TSH risk and to increase the chance of early diagnosis in patients with advanced age or SG-TB recipients, we believe that taking additional intraoperative precautions and performing closer follow-up will be necessary.

Keywords: trocar site hernia, sleeve gastrectomy, sleeve gastrectomy with transit bipartition, one anastomosis gastric by-pass, bariatric-metabolic surgery, rick factors

1. Introduction

Bariatric-metabolic surgery (BMS) is being performed with increasing frequency in the treatment of obesity and metabolic disorders (1-3). Although BMS seems to be the most effective and permanent treatment option for obesity, it is associated with a number of perioperative complications. These include anastomotic bleeding, leakage, myocardial infarction, pulmonary embolism, and even mortality, while delayed complications, such as dumping syndrome, malnutrition due to malabsorption, inadequate weight loss, and marginal ulceration, may also be encountered relatively frequently (2, 4, 5).

Besides the common complications of laparoscopic BMS, trocar site hernia (TSH) is a problem whose true incidence is difficult to detect and its likelihood is often ignored by many surgeons, especially because TSH usually occurs long after the primary surgery, is asymptomatic at onset, and remains difficult to diagnose when weight loss after surgery is limited (6, 7). Current estimates of TSH incidence after BMS report a range of 0–39.3%, although it is notable that these values are dependent upon the method of TSH diagnosis and follow-up time (6, 8). It has been shown that higher TSH risk is observed in patients with diabetes mellitus, postoperative malnutrition, higher intraabdominal pressure, difficulty in full-thickness closure of the trocar site, thicker peritoneum, and larger preperitoneal space (5, 9, 10). Moreover, it is difficult to diagnose TSH by inspection and palpation in obese patients because of excess subcutaneous fat tissue (3, 11). There are various studies which have used imaging methods for the detection of postoperative abdominal wall defects in obese patients, and it is evident that the presented incidence of TSH is much higher in these studies (6, 8, 12, 13). Considering the difficulty of diagnosis and the delayed nature of this complication, we believe that the risk of TSH after laparoscopic BMS should be taken into account and risk factors that facilitate the development of TSH must be identified. In a study investigating the effects of many factors, including BMS procedures, only excessive weight loss was identified as a risk factor for TSH (14). Furthermore, although there are many studies assessing TSH frequency and risk factors in non-BMS laparoscopic procedures (7, 15-17), there are very few which have investigated risk factors specific to BMS (9, 14, 18). In fact, a recent systematic review described TSH development following BMS as "an underestimated issue" (6).

In this study, we aimed to investigate the effects of three laparoscopic BMS techniques and some other sociodemographic and clinical features of patients with regard to their influence on TSH development after laparoscopic BMS.

*Correspondence: drmuzaffer61@hotmail.com

2. Material and Methods

This retrospective case-control study was carried out by including patients who underwent BMS from January 2015 to February 2022 in our bariatric surgery Center of Exellence, Department of General Surgery, Büyük Anadolu Hospital, Samsun, Turkey. The study was approved by the Ethics Committee of Büyük Anadolu Hospital (date: 28.07.2021, no: 04). The study was conducted according to the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Due to the study's retrospective nature, written informed consent was deemed unnecessary by the ethics committee. All data were recorded anonymously.

2.1. Study population

A total of 54 obese patients, 18 who developed TSH and 36 who did not develop TSH in their follow-up after laparoscopic BMS, were included in the study. Participants in the control group were randomly assigned in a 2:1 ratio for patients undergoing BMS who met inclusion criteria, using an internet-based random number generator (https://www.random.org). The exclusion criteria were determined as follows for both groups: being younger than 18 or older than 65 years old, having undergone revision BMS, being diagnosed with a postoperative complication related to the trocar incision site (e.g., wound infection), presence of complications necessitating revision surgery after primary surgery, history of inguinal, femoral, umbilical or epigastric hernia, history of a previous abdominal surgery (e.g., cesarean section etc.), severe mineral or vitamin malnutrition after surgery, known wound healing disorder, steroid therapy, renal insufficiency or cancer. Patients who did not attend follow-up or had missing relevant data were also excluded from the study.

2.2. Data collection

Demographic information (age, sex, etc.), anthropometric data [height, weight, and body-mass index (BMI)], occupation, smoking status, number of transvaginal births among female participants, comorbidities and drug use, type of BMS procedure applied, use of protein supplementation, and necessary follow-up information were obtained from hospital records.

2.3. Operative technique and post-operative follow up

Patients with a BMI of ≥40 or a comorbidity with a BMI of ≥35–40 was considered eligible for BMS (19). The most appropriate BMS procedure was determined according to the preferences of the patients after necessary information was provided. All three surgical techniques [sleeve gastrectomy (SG), SG with transit bipartition (SG-TB), one anastomosis gastric by-pass (OAGB)] were performed under general anesthesia and with standard laparoscopical surgical procedures.

Five trocars for SG (20) and OAGB (21) were placed as recommended in the literature. In patients who underwent SG+TB, an 11-mm optic trocar was inserted one and a half hands from the xiphoid process, in addition to a 5-mm trocar from the right lateral, a 15-mm trocar from the left lateral, a 5-mm trocar from the left lower quadrant, and a 5-mm trocar from the xiphoid for the liver retractor. For the first stapler application in both SG and SG-TB, an 11 mm optical trocar was routinely removed and replaced with a 15 mm trocar. Sleeve material was taken out of the abdomen through a 15-mm umbilical trocar site in SG, and from the left lateral 15-mm trocar site in SG-TB.

Stapling devices and stapler materials (Endo GIA™; Covidien™; USA) in different sizes were used in all operations for basic anastomosis and stomach resection. In all operational procedures, trocar sites (except for the 5-mm fascial defect) were closed using 1/0 absorbable polyglactin (Vicryl™; Ethicon) sutures with the help of a trocar site closure device (Endo Close™ Auto Suture™; Covidien™ ; USA).

While no protein supplement was given to any patient in the SG-TB group postoperatively, it was given to the majority of patients in the SG and OAGB groups. The patients requiring protein supplementation were identified by an expert dietitian. Standard postoperative follow-up included clinical visits at 1 week, 1 month, 3 months, 6 months, 1 year, and once a year thereafter. TSH in symptomatic patients was confirmed by ultrasonography. Hernias developing in the 5-mm trocar sites were repaired by open surgery using polypropylene mesh. The remaining hernias were repaired with laparoscopic dual mesh and all the patients were discharged without any complications.

2.4. Statistical Analysis

All analyses were performed on IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA), with a significance threshold of $p<0.05$. For the normality check of the continuous variables, the Shapiro-Wilk test was used. Normally distributed continuous variables were analyzed with the independent samples t-test. Non-normally distributed continuous variables were analyzed with the Mann-Whitney U test. Categorical variables were analyzed with the chi-square tests or the Fisher's exact or Fisher-Freeman-Halton tests. Multiple logistic regression analysis (forward conditional method) was performed to determine significant risk factors of the trocar site hernia. The model included all parameters with univariate significance.

3. Results

The mean age of all participants was 45.48 ± 12.50 years. The TSH group had a mean age of 55.28 ± 7.98, while the non-TSH group had a mean age of 40.58 ± 11.47 years. Seventeen (94.4%) of the TSH patients and 23 (63.9%) of the non-TSH patients were female. There was a significant difference between the groups in terms of age ($p<0.001$) and sex ($p = 0.021$). While there was a significant difference between the groups in terms of height ($p = 0.037$) and weight ($p = 0.010$) in favor of the non-TSH group, there was no significant difference in terms of BMI ($p = 0.152$). In the TSH group, the percentage of housewives ($p = 0.021$), median number of births (among women) ($p<0.001$), frequencies of hypertension ($p =$

0.002) and type 2 diabetes mellitus (T2DM) (p<0.001), duration of T2DM (p<0.001), oral antidiabetic use (p<0.001) and the percentage of patients who underwent SG+TB (p<0.001) were significantly higher. All sociodemographic, anthropometric, and clinical characteristics and differences between the groups are summarized in Table 1.

Table 1. Summary of patients' characteristics with regard to trocar site hernia

		Trocar site hernia		
	Total (n=54)	Yes (n=18)	No (n=36)	p
Age	45.48 ± 12.50	55.28 ± 7.98	40.58 ± 11.47	**<0.001**
Sex				
Female	40 (74.1%)	17 (94.4%)	23 (63.9%)	**0.021**
Male	14 (25.9%)	1 (5.6%)	13 (36.1%)	
Height, cm	163.54 ± 9.80	160.11 ± 6.86	165.25 ± 10.65	**0.037**
Weight, kg	108.4 (96.6 - 124.3)	97.45 (92.1 - 113.0)	113.05 (99.8 - 131.6)	**0.010**
Body mass index, kg/m2	40.41 (36.43 - 46.14)	38.60 (35.30 - 42.74)	40.86 (37.62 - 46.69)	0.152
Occupation				
Student	4 (7.4%)	0 (0.0%)	4 (11.1%)	**0.021**
Housewife	23 (42.6%)	13 (72.2%)	10 (27.8%)	
Desk job	12 (22.2%)	2 (11.1%)	10 (27.8%)	
Heavy job	15 (27.8%)	3 (16.7%)	12 (33.3%)	
Smoking	24 (44.4%)	7 (38.9%)	17 (47.2%)	0.771
Number of birth	2 (0 - 3)	3 (2 - 4)	0 (0 - 2)	**<0.001**
Hypertension	22 (40.7%)	13 (72.2%)	9 (25.0%)	**0.002**
Coronary artery disease	6 (11.1%)	1 (5.6%)	5 (13.9%)	0.651
Type II Diabetes mellitus	27 (50.0%)	16 (88.9%)	11 (30.6%)	**<0.001**
Duration of diabetes mellitus, years	1 (0 - 10)	9 (5 - 13)	0 (0 - 5)	**<0.001**
Drug use				
None	27 (50.0%)	2 (11.1%)	25 (69.4%)	**<0.001**
Oral antidiabetics	3 (5.6%)	3 (16.7%)	0 (0.0%)	
Insulin	9 (16.7%)	5 (27.8%)	4 (11.1%)	
Oral antidiabetics + Insulin	15 (27.8%)	8 (44.4%)	7 (19.4%)	
Operation				
Sleeve gastrectomy	14 (25.9%)	1 (5.6%)	13 (36.1%)	**<0.001**
Sleeve gastrectomy + Transit bipartition	26 (48.1%)	16 (88.9%)	10 (27.8%)	
One anastomosis gastric by-pass	14 (25.9%)	1 (5.6%)	13 (36.1%)	
Protein supplement	14 (26.4%)	2 (11.1%)	12 (34.3%)	0.102
Time between operation and hernia, months	8.5 (7 - 10)	8.5 (7 - 10)	-	N/A
Follow-up time, months	29 (20 - 65)	20 (12 - 65)	32.5 (23.5 - 65.5)	0.060

Data are given as mean ± standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. Abbreviations: N/A: Not applicable

Multiple logistic regression analysis revealed that high age (p=0.018) and SG-TB (p=0.018) were the significant factors independently associated with TSH development. Patients who underwent SG-TB operation had 8.600-fold higher risk for TSH than other types of surgery (OR: 8.600, 95% CI: 1.446 - 51.152) (Table 2). Other variables included in the analysis, sex (p=0.168), height (p=0.923), weight (p=0.129), occupation (p=0.799), number of births (p=0.191), hypertension (p=0.854), T2DM (p=0.600), duration of T2DM (p=0.097) and drug use (p=0.624) were found to be non-significant (Table 2, Fig. 1., Fig. 2.).

Table 2. Significant risk factors of the trocar site hernia, multiple logistic regression analysis

	β coefficient	Standard error	p	Exp(β)	95.0% CI for Exp(β)	
Age	0.101	0.043	**0.018**	1.106	1.017	1.203
Sleeve gastrectomy + Transit bipartition	2.152	0.910	**0.018**	8.600	1.446	51.152
Constant	-6.954	2.201	0.002	0.001		
CI: Confidence Interval, Nagelkerke R^2=0.535						

No postoperative early complications occurred in any of the patients included in the study. The patients were followed up for a median of 29 (IQR: 20 - 65) months. TSH developed in 4 patients at the umblical 15-mm trocar site, in 11 patients at the 15-mm left lateral trocar site and in 1 patient at the right lateral 5-mm trocar site after SG-TB. After SG, TSH developed in 1 patient at the umblical 15-mm trocar site. After OAGB, TSH developed in 1 patient at the right 15-mm trocar site. The most common symptoms in 18 patients with TSH were pain while moving and swelling when lifting something heavy. Of note, the patient who developed TSH from the 5-mm incision on the right lateral side after SG-TB had been treated with hook cautery for bleeding from the trocar site. The median hospital stay after TSH repair was 2 days (IQR: 1-3).

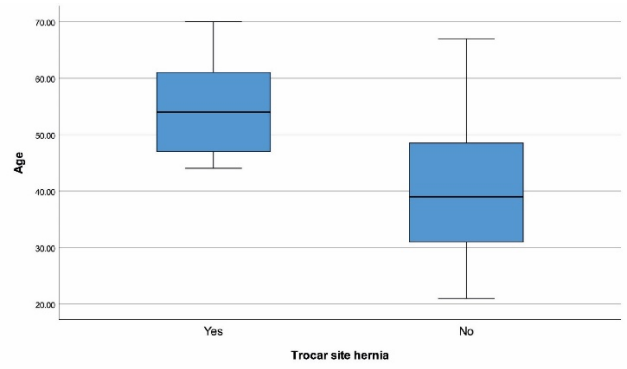

Fig. 1. Age with regard to trocar site hernia

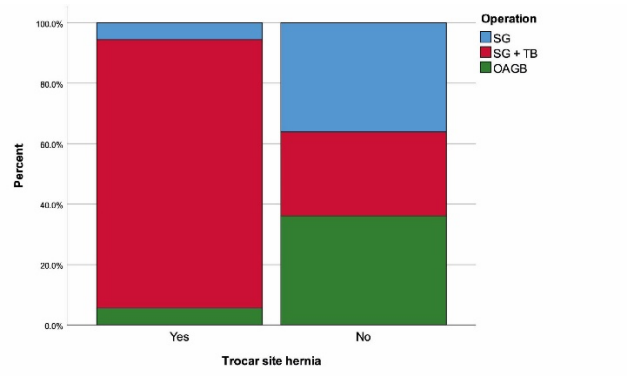

Fig. 2. Type of operation with regard to trocar site hernia

4. Discussion

Trocar site hernia is a neglected problem which is rarely diagnosed when only symptoms and physical examination are considered after BMS; however, various studies have shown that TSH frequency can reach 40% when imaging methods are utilized (6, 8, 14). As a result of the multiple logistic regression analysis of the present study, the most important risk factors for the development of TSH after BMS were found to be SG-TB and higher age.

Obesity stands out as a prominent risk factor for TSH development in a number of studies involving various patient groups and laparoscopic operations/ Obesity has been identified as a significant risk factor for TSH development in a number of studies involving different patient groups and laparoscopic procedures (9, 10). In this study, we sought to determine factors that increased TSH likelihood among patients who had undergone BMS for obesity treatment, and found that advanced age and SG-TB procedure were the risk factors independently associated with TSH development in this group of patients. Various risk factors (in addition to obesity) for TSH after laparoscopic surgeries have been described in the literature. The most common ones among these are size of the trocars, trocar site, long operating time, sex, advanced age, and smoking status (22-25). There are also some limited studies which have addressed the specific risk factors for TSH development after BMS (9, 14, 18, 26). In the multivariable analysis of a recent study, age, sex, BMI at baseline, BMI at follow-up, excessive weight loss, type of surgery, T2DM, smoking history, ASA score, abdominoplasty reconstruction, length of surgery, and length of hospital stay were evaluated to detect potential risk factors related to the development of TSH after BMS. The only factor that was related to a higher risk for TSH was found to be excessive weight loss (14). In another study investigating the risk factors for TSH in patients who underwent SG, TSH development was not found to be associated with sex, age, preoperative BMI, T2DM, comorbidities (hypertension, dyslipidemia, obstructive sleep apnea, metabolic syndrome), operation time, or weight regain (9). In another study examining similar risk factors, TSH was only found to be associated with the 12-mm trocar site closure after SG (18). Despite a lack of conclusive evidence on this matter, it appears that advanced age is a reasonable risk factor for TSH development, especially due to its relationship with fascia weakening, decreased abdominal muscle volume, and delayed wound healing (13, 22).

Various procedures have been described for BMS, and they have multiple advantages and disadvantages compared to each other. Almost all of them can be performed as open procedures as well as laparoscopic procedures. The laparoscopic approach may reduce the risk of incisional hernia, but it does not completely eliminate the risk (11, 21, 25). In an OAGB series of 407 patients, it was reported that none of the patients developed TSH during follow-up. However, in this study, the follow-up period was limited to an average of 160 days (21). Raziel et al. detected TSH in only 1 (0.3%) of 337 OAGB cases (27). In a study from Turkey, the incidence of TSH after SG was reported as 0.36% (28). In another study, 3.1% TSH was reported after laparoscopic SG-TB (29). We rarely come across studies that include SG-TB and compare TSH rates between procedures (14, 26, 30). In the present study, TSH results of three laparoscopic surgical procedures were compared, and the incidence of TSH after SG-TB was found to be significantly higher, and that after SG and OAGB was found to be significantly lower. According to the multiple regression analysis, SG-TB was identified as a risk factor for TSH. Coblijn et al. found no significant difference in TSH rates after laparoscopic Roux-en-Y gastric bypass (RYGB), laparoscopic adjustable gastric band and laparoscopic SG (26). In other similar studies, no significant differences were found in terms of TSH frequency after laparoscopic RYGB and laparoscopic SG (14, 30). A higher incidence of TSH can be expected after laparoscopic SG and SG-plus procedure because the sleeve material is usually taken out of the abdomen through the umbilical trocar site, and therefore, the incision of this site is further widened due to excessive manipulation (25). This hypothesis is supported by the results of studies stating that most TSH cases are seen in the umbilical region and that excessive manipulation of the trocar site to remove samples may be an important risk factor for TSH (31, 32).

Santoro et al. introduced the transit bipartition approach as a metabolic complement to standard SG and stated that they obtained successful results (29). The resulting SG-TB method may provide extra metabolic benefits, but it would not be

erroneous to expect a heightened risk for complications as it involves intestinal intervention and anastomosis and increases surgical duration. Although TSH was observed in only 1 patient each after SG and OAGB in this study, lower TSH probability may be expected from the other two procedures, since no specimens were removed from the trocar incisions in OAGB. In the current study, although the sleeve material was removed from the umbilical trocar site in all SG patients, TSH occurred in only 1 patient (umbilical trocar site, SG group). In the SG+TB group, trocar hernia was observed in the umbilical region in 4 patients, although all sleeve materials were removed from the 15-mm left trocar site. Moreover, the incidence of TSH after only SG was found to be quite low compared to the incidence of TSH after SG-TB. All of these contradict the hypothesis that enlargement of the trocar site while removing the sleeve material facilitates TSH development. According to the results of this study, it can be thought that the removal of the sleeve material and related manipulations do not have a significant effect on TSH development. This requires the consideration that other factors contribute to TSH after SG-TB. Age, as another independent variable, may be an effective factor in this situation. In addition, increased duration of surgery, another possible factor not investigated in this study, and more excessive manipulation as a requirement of SG-TB surgery can be considered as other possible causes. Moreover, the fact that all patients who underwent SG-TB had T2DM (and were not administered post-operative protein replacement), and that the number of females and the median number of births were higher in the group with TSH, may suggest that these factors may have influenced TSH likelihood, despite non-significant results in multiple regression analysis. Notably, diabetes mellitus and protein deficiency are factors that negatively affect wound healing (33). Also, the increase in the number of births may facilitate the development of TSH due to the weakness of the abdominal fascia (7). However, more comprehensive studies are needed to clarify factors that have a definite effect on TSH development.

Another issue to consider in a study assessing TSH after BMS is trocar site wound and/or facia closure. In laparoscopic approaches other than BMS, suturing the fascia for trocar incisions larger than 10 mm is recommended, but TSH risk can be seen even in smaller-sized lesions (≤5 mm) (15, 16). In the present study, a significant majority of TSHs (94.4%) occurred at the 15-mm trocar site ve? /whereas? TSH developed in only a single 5-mm trocar site. At this site, it was revealed that cauterization was performed due to bleeding during the operation. Karampinis et al. reported that in their department (where they did not perform fascia closure in trocar sites smaller than 10 mm) overall hernia prevalence was 2.5% in the 5-mm trocar site, 10.6% in the 12-mm site, and 22.4% in the 12-mm trocar site used for stomach extraction. Also in this study, suturing the fascia with interrupted, absorbable sutures did not appear to yield fewer TSHs than leaving the fascia open (3). However, in another study, it was reported that the frequency of TSH was significantly lower when the fascia was closed at the 12-mm epigastric trocar site using a protected port closure device (18). Aly and Lee (34), criticized the statement that the closure of trocar site wound can be omitted because the TSH incidence is 0.5% even when the trocar site wound is not closed as a conclusion of the study by Coblijn et al. (26), and stated that even this rate was rather high. They also stated that trocar site wounds of 10 mm and above should be closed and suggested that the two methods they introduced (Surgicel® and omental plug without fascial closure) could be vital in preventing TSH (30, 34). In general, it can be said that the risk of TSH increases as the size of the trocar and trocar incision increases. In addition, according to our results, we recommend routine fascial closure of trocar sites larger than 5 mm when they are involved in the removal of sleeve material. In addition, we think that fascia closure at the 5 mm trocar site is only necessary if cauterization (or other excessive manipulation) is performed during the operation.

Some limitations of the present study should be taken into account when interpreting the results. The generalizability of the results is limited, as it is a single-center study and the number of participants is small. The small number of participants may also have limited the specific comparisons between the types of surgeries compared. The retrospective nature also carries possible biases concerning patient inclusion/exclusion due to possible problems in data records. In addition, we could not assess additional possible factors such as anemia, ASA score, COPD, etc., which can cause wound healing disorders. The fact that routine imaging methods were not used for TSH screening may have resulted in an underestimation of TSH; however, the frequency reported in this study already demonstrates that TSH is not as rare as suggested by previous short-term studies.

In conclusion, SG-TB and advanced age were found to be the most important risk factors for TSH development after BMS. In order to reduce the risk of TSH development and to increase the chance of early diagnosis of TSH in patients with advanced age or SG-TB recipients, we think that it would be beneficial to take additional pre-and perioperative measures. Also, performing follow-up not only clinically but also with additional imaging methods may be necessary in these patients. We also recommend fascia closure if intraoperative cauterization or excessive manipulation has been performed in the trocar site, regardless of size. However, there is still a need for comprehensive prospective studies in which TSH screening is performed with imaging methods to precisely determine the real-world incidence of TSH after BMS and to identify risk factors specific to BMS procedures.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions
Concept: M.A., Design: M.A., Data Collection or Processing: M.A., Analysis or Interpretation: M.A., Literature Search: M.A., Writing: M.A.

References

1. Welbourn R, Pournaras DJ, Dixon J, Higa K, Kinsman R, Ottosson J, et al. Bariatric Surgery Worldwide: Baseline Demographic Description and One-Year Outcomes from the Second IFSO Global Registry Report 2013-2015. Obes Surg. 2018; 28(2): 313-22.

2. Bjørklund G, Semenova Y, Pivina L, Costea D-O. Follow-up after bariatric surgery: A review. Nutrition. 2020; 78: 110831.

3. Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Nanni G, et al. Bariatric-metabolic surgery versus conventional medical treatment in obese patients with type 2 diabetes: 5 year follow-up of an open-label, single-centre, randomised controlled trial. Lancet. 2015; 386(9997): 964-73.

4. Chang SH, Freeman NLB, Lee JA, Stoll CRT, Calhoun AJ, Eagon JC, et al. Early major complications after bariatric surgery in the USA, 2003-2014: a systematic review and meta-analysis. Obes Rev. 2018; 19(4): 529-37.

5. Livingston EH. Complications of bariatric surgery. Surgical Clinics. 2005; 85(4): 853-68.

6. Karampinis I, Lion E, Grilli M, Hetjens S, Weiss C, Vassilev G, et al. Trocar Site Hernias in Bariatric Surgery-an Underestimated Issue: a Qualitative Systematic Review and Meta-Analysis. Obes Surg. 2019; 29(3): 1049-57.

7. Üstünyurt E, Taşgöz FN, Tiğrak S. Asymptomatic trocar site hernias: An underestimated complication of laparoscopy. Turk J Obstet Gynecol. 2020; 17(3): 202-8.

8. Scozzari G, Zanini M, Cravero F, Passera R, Rebecchi F, Morino M. High incidence of trocar site hernia after laparoscopic or robotic Roux-en-Y gastric bypass. Surg Endosc. 2014; 28(10): 2890-8.

9. Rebibo L, Dhahri A, Chivot C, Cyril C, Yzet T, Regimbeau J-M. Trocar site hernia after laparoscopic sleeve gastrectomy using a specific open laparoscopy technique. Surg Obes Relat Dis. 2015; 11(4): 791-6.

10. Pilone V, Di Micco R, Hasani A, Celentano G, Monda A, Vitiello A, et al. Trocar site hernia after bariatric surgery: our experience without fascial closure. Int J Surg. 2014; 12: S83-S6.

11. Ahlqvist S, Björk D, Weisby L, Israelsson LA, Cengiz Y. Trocar Site Hernia After Gastric Bypass. Surg Technol Int. 2017; 30: 170-4.

12. Ahlqvist S, Björk D, Weisby L, Israelsson LA, Cengiz Y. Trocar Site Hernia After Gastric Bypass. Surg Technol Int. 2017; 30: 170-4.

13. Ciscar A, Badia JM, Novell F, Bolívar S, Mans E. Incidence and risk factors for trocar-site incisional hernia detected by clinical and ultrasound examination: a prospective observational study. BMC Surg. 2020; 20(1): 330.

14. Karampinis I, Lion E, Hetjens S, Vassilev G, Galata C, Reissfelder C, et al. Trocar Site HERnias After Bariatric Laparoscopic Surgery (HERBALS): A Prospective Cohort Study. Obes Surg. 2020; 30(5): 1820-6.

15. Bergemann JL, Hibbert ML, Harkins G, Narvaez J, Asato A. Omental herniation through a 3-mm umbilical trocar site: unmasking a hidden umbilical hernia. J Laparoendosc Adv Surg Tech A. 2001; 11(3): 171-3.

16. Buffone A, Lo Bianco S, Cavallaro D, Caglià P, Basile G, Cannizzaro MA. Epigastrium: unusual site of incisional hernia from a 5 mm trocar. Ann Ital Chir. 2017; 6: S2239253X17027074.

17. Ciscar A, Badia JM, Novell F, Bolívar S, Mans E. Incidence and risk factors for trocar-site incisional hernia detected by clinical and ultrasound examination: a prospective observational study. BMC Surg. 2020; 20(1): 1-7.

18. Rebibo L, Demouron M, Dembinski J, Dhahri A, Yzet T, Regimbeau JM. Impact of routine 12 mm epigastric trocar site closure on ıncisional hernia after sleeve gastrectomy: A prospective before/after study. Obes Surg. 2019; 29(11): 3500-7.

19. De Santé HA. Obésité: prise en charge chirurgicale chez l'adulte. Recommandations de bonne pratique Saint-Denis La Plaine: HAS. 2009.

20. Iranmanesh P, Rivera AR, Bajwa KS, Alibhai M, Snyder BE, Wilson TD, et al. Trocar site closure with a novel anchor-based (neoClose(®)) system versus standard suture closure: a prospective randomized controlled trial. Surg Endosc. 2020; 34(3): 1270-6.

21. Lessing Y, Pencovich N, Khatib M, Meron-Eldar S, Koriansky J, Abu-Abeid S. One-Anastomosis Gastric Bypass: First 407 Patients in 1 year. Obes Surg. 2017; 27(10): 2583-9.

22. Swank HA, Mulder IM, La Chapelle CF, Reitsma JB, Lange JF, Bemelman WA. Systematic review of trocar-site hernia. Br J Surg. 2012; 99(3): 315-23.

23. Helgstrand F, Rosenberg J, Bisgaard T. Trocar site hernia after laparoscopic surgery: A qualitative systematic review. Hernia. 2011; 15(2): 113-21.

24. Chang W-T, Yu F-J, Hsieh M-Y, Huang M-C, Lee K-T, Chen J-S, et al. Laparoscopic cholecystectomy in aged patients. Hepatogastroenterology. 2009; 56(93): 950-5.

25. Ahlqvist S, Edling A, Alm M, Dackhammar JB, Nordin P, Cengiz Y. Trocar site hernia after gastric sleeve. Surg Endosc. 2022; 36(6): 4386-91.

26. Coblijn UK, De Raaff CA, Van Wagensveld BA, Van Tets WF, De Castro SM. Trocar port hernias after bariatric surgery. Obes Surg. 2016; 26(3): 546-51.

27. Raziel A, Goitein D, Sakran N, editors. Safety and Effectiveness of One Anastomosis Gastric Bypass. Obes Surg; 2018: Springer 233 Spring St, New York, NY 10013 USA.

28. Durmus A, Mutlu T, editors. Should Trocar-Site Be Closed Routinely After Laparoscopic Sleeve Gastrectomy; Teaching Of 10 Years' Experience. Obes Surg; 2018: Springer 233 Spring St, New York, NY 10013 USA.

29. Santoro S, Klajner S, Sampaio R. Sleeve gastrectomy and transit bipartition. Obesity and Diabetes: Springer; 2015, p. 89-110.

30. Chiu C-C, Lee W-J, Wang W, Wei P-L, Huang M-T. Prevention of trocar-wound hernia in laparoscopic bariatric operations. Obes Surg. 2006; 16(7): 913-8.

31. Zhu Y-P, Liang S, Zhu L, Sun Z-J, Lang J-H. Trocar-site hernia after gynecological laparoscopic surgery: a 20-year, single-center experience. Chin Med J. 2019; 132(22): 2677-83.

32. Comajuncosas J, Hermoso J, Gris P, Jimeno J, Orbeal R, Vallverdú H, et al. Risk factors for umbilical trocar site incisional hernia in laparoscopic cholecystectomy: a prospective 3-year follow-up study. Am J Surg. 2014; 207(1): 1-6.

33. Beyene RT, Derryberry SL, Barbul A. The effect of comorbidities on wound healing. Surg Clin North Am. 2020; 100(4): 695-705.

34. Mohammed Y, Aly F, Lee W-J. trocar site hernia prevention in laparoscopic bariatric surgery. Obes Surg. 2016; 26(9): 2227.

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An evaluation of IGF-1 and IGFBP-3 levels in patients receiving growth hormone therapy and these parameters therapeutic efficacy

Abdulvahit AŞIK[1,*], Semih BOLU[2]

[1] Department of Pediatrics, Adıyaman University Education and Research Hospital, Adıyaman, Türkiye
[2] Department of Pediatric Endocrinology, Adıyaman University Education and Research Hospital, Adıyaman, Türkiye

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Abstract

Serum insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-binding protein-3 (IGFBP-3) levels in healthy children reflect endogenous growth hormone (GH) levels, and their daily variations are very low. This study investigated the relationship between growth response and serum IGF-1 and IGFBP-3 levels before and on the first year of treatment in children with GH deficiency (GHD) started on GH therapy. The records of 44 patients diagnosed with GHD, under follow-up at the Adıyaman Education and Research Hospital Pediatric Endocrinology Clinic, and receiving GH therapy for at least one year were examined retrospectively. Patients' ages, pubertal development stages, peak GH-responses to GH stimulation tests and height standard deviation scores (SDS) measured before and at the first year of treatment, and IGF-1 and IGFBP-3 levels were recorded. Girls represented 27 (61.4%) of the cases in the study and boys 17 (3.6%), with ages ranging between 0.5 and 16.6 years (mean=11.5±3.3). Partial GHD was present in 33 patients (75%) and complete GHD in 11 (25%). Basal height SDS, IGF-1 and IGFBP-3 values were compared with those after one year of treatment. Patients with basal IGF-1 levels < -2 SDS exhibited significantly higher growth responses ($p < .05$). All three parameters' mean values were significantly higher after one year compared to baseline. Δ IGF-1 and Δ IGFBP-3 values investigated in the first year of treatment together with basal IGF-1 levels can be a useful diagnostic tool in showing growth response in children with GHD started on GH therapy.

Keywords: growth hormone, growth hormone therapy, insulin-like growth factor-1, insulin-like growth factor-binding protein-3

1. Introduction

Growth hormone deficiency (GHD) is a severe endocrine disorder leading to short stature and is seen in approximately one in 4000 live births (1). Since growth hormone (GH) release exhibits diurnal variation, basal GH level measurement is not meaningful in diagnosing GHD. GH stimulation tests are therefore needed in cases requiring GH level investigation (2).

Insulin-like growth factors (IGFs) are GH-dependent peptide factors that mediate the effects of GHs (3). In healthy children, serum IGF-1 and insulin-like growth factor-binding protein-3 (IGFBP-3) levels reflect endogenous GH secretion. Diurnal variations in IGF-1 and IGFBP-3 levels are insignificant (4). Therefore, both serum IGF-1 and IGFBP-3 levels have been reported to be capable of use as screening tests in diagnosing GHD (5, 6).

Prompt diagnosis and early commencement of treatment of GHD are of great importance in children's reaching target heights (5, 7, 8). Patients started on GH therapy must be followed up in terms of efficacy (9). Growth velocity and IGF-1 and IGFBP-3 levels can be evaluated in terms of the effectiveness of treatment in these cases (10).

This study aimed to investigate the relationship between growth response and serum IGF-1 and IGFBP-3 levels before and during the first year of treatment in children with GHD who started GH therapy.

2. Materials and Methods

2.1. Study design

This study was performed using data from 44 patients followed up with diagnoses of GHD at the Adıyaman University Education and Research Hospital Pediatric Endocrinology Clinic, Turkey, between August 2016 and February 2021 and receiving GH therapy for at least one year. We took approval for the study from the Adıyaman University Non-Interventional Research Ethical Committee (decision no. 2020/7-7 dated 21/07/2021). We recorded the patients' ages, pubertal development stages, height standard deviation scores (SDS), basal IGF-1 SDS, basal IGFBP-3 SDS, and peak GH response values to clonidine and L-Dopa stimulation tests. Inclusion criteria were the following: 1) The patient's height at the time of presentation <-2 Standard

*Correspondence: dr.serdar55@hotmail.com

Deviation Score (SDS) for age and gender or <3rd percentile, 2) annual growth velocity <25th percentile, 3) bone age two years or more behind calendar age in prepubertal children, 4) exclusion of pathological causes of short stature other than GHD, such as systemic diseases, Turner syndrome, and skeletal dysplasias, and 5) an insufficient GH response to at least two GH stimulation tests (peak GH response to GH stimulation test <10 ng/mL) (11,12). We divided the patients into two groups based on the maximum GH response to the clonidine and L-Dopa GH stimulation tests, partial GHD (stimulated maximum GH response 5.1-10 ng/ml) and complete GHD (stimulated maximum GH response ≤ 5 ng/ml). We calculated the difference (Δ) in the laboratory and clinical data investigated before and one year after commencement of GH therapy as the change in the two values. We further divided the patients into two groups: Those with basal IGF-1 and IGFBP-3 levels deviating less than -2 Standard Deviation (SD) and those with deviations more than -2 SD. We calculated IGF-1 and IGFBP-3 SDS values based on age- and gender-specific reference values for healthy Turkish children (13).

We excluded patients with regular follow-up times of less than one year, those who started GH therapy in another center, those not adhering to GH therapy, or those with incomplete data in the patient files.

A children's nurse working in the pediatric endocrine clinic measured the heights of patients under two years in the supine position and those over two years standing barefoot, with their heads erect, hips and shoulders against a wall, and heels together. Measurements were taken using a SECA 216 stadiometer with 1 mm graduations. We calculated the target height of the patient's using the formula (father's height -13 + mother's height) / 2 for girls and (father's height + mother's height +13) / 2 for boys. We calculated estimated adult heights using the Bayley-Pinneau method (15). We determined the pubertal stages of the patients according to the Tanner-Marshall system. We accepted the onset of puberty as breast development in girls and testicular volume ≥4 cc in boys. We measured testicular volumes using a Prader orchidometer. We determined the bone ages of the patients with the left hand/wrist radiograph. The hand-wrist radiographs were evaluated by the same pediatric endocrinologist using the Greulich-Pyle radiology atlas (16).

2.2. Statistical analysis

We performed all statistical analyses on SPSS 25 software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA) and expressed descriptive statistics as frequency, rate, mean, and standard deviation for different variables. We presented percentage and % values for categorical variables and mean plus standard deviation values for continuous variables in the tables. We evaluated the research values in terms of normality of distribution and assessed them using skewness and histograms. We performed two-way comparisons between independent groups using the t-test and reported the results as mean ± standard deviation. We applied the non-parametric Mann-Whitney U test when the difference between the numbers of individuals in groups was significant and expressed the results as rank average and median values. We applied chi-square analysis to examine differences in distributions of different variables by groups and added cross tables. We regarded p levels <0.05 as significant for all results.

3. Results

Twenty-seven (61.4) of the 44 cases were girls, and 17 (38.6) were boys. Ages ranged between 0.1 and 16.6 years (11.5±3.3). Based on the Tanner-Marshall puberty staging system, 24 (54.5) patients were stage 1, nine (20.5%) were stage 2, three (13.6%) were stage 3, and five (11.4%) were stage 4. Partial GHD was present in 33 (75%) patients and complete GHD in 11 (25%). The mean height SDS before treatment was -3.3±0.8, and the mean target height SDS was -1.5±0.9. We determined maximum GH responses by the L-Dopa stimulation test in 43 cases, the clonidine stimulation test in 44, and the glucagon stimulation test in one. The mean maximum stimulated GH response was 6.1±2.8 ng/ml.

According to the results obtained, the rate of those with basal IGF-1 levels SDS <-2 is significantly lower than the rate of those with higher than basal IGF-1 levels SDS > -2 in the group with partial GH deficiency (χ^2 (1) = 12.61, p < 001) (Table-1). No significant difference was present in terms of IGFBP-3 distributions based on SDS in the cases with complete and partial GHD (p>0.05) (Table 2).

Table 1. Distribution of IGF-1 SDS by Growth Hormone Stimulation Test Results

GH Response		Deviation in IGF-1		Total
		SD <-2	SD >-2	
Partial GH Deficiency	n	1	32	33
	%	3	97	100
Komplet GH Deficiency	n	5	6	11
	%	45.5	54.5	100
Total	n	6	38	44
	%	13.6	86.4	100

IGF-1:Insulin-like growth Factor-1, GH: Growth Hormone, SDS: Standart Deviation Scores

Table 2. Distribution of IGF BP-3 SDS by Growth Hormone Stimulation Test Results

GH Response		Deviation in IGF BP-3		Total
		SD <-2	SD >-2	
Partial GH Deficiency	n	2	31	33
	%	6.1	93.9	100
Komplet GH Deficiency	n	2	9	11
	%	18.2	81.8	100
Total	n	4	40	44
	%	9.1	90.9	100

IGF BP-3:Insulin growth Factor Binding Protein-3, GH: Growth Hormone, SDS: Standart Deviation Scores

A comparison of the growth responses of patients with basal IGF-1 SDS values lower and higher than -2SD revealed that the mean rank responses of patients with severe deviations in basal IGF-1 (<-2 SDS) were significantly higher ($p<0.05$). However, there was no significant difference between the subjects with severe deviations in basal IGFBP-3 and those without in terms of growth responses ($p >0.05$) (Table 3).

Table 3. Comparison of growth response rank means according to the level of deviation in IGF-1 and IGFBP-3 values

Variables	Groups	n	Rank Average	Median	*z*	*p*
IGF -1	SD >-2	6	33.25	.94	-2.21	.025
	SD < -2	38	20.80	.37		
IGF BP-3	SD >-2	4	10.75	-.15	-1.91	.056
	SD < -2	40	23.60	.42		

IGF-1:Insulin-like growth Factor-1, IGF BP-3:Insulin growth Factor Binding Protein-3, SD: Standart Deviation

Examination of whether patients' pre-treatment height SDS, IGF-1 SDS, and IGFBP-3 SDS differed significantly from the values obtained after one year revealed that the mean values for all three parameters were significantly higher compared to the baseline ($p < 0.01$) (Table 4).

Table 4. Changes in the height SDS, IGF-1 SDS and IGF BP-3 SDS values of the cases

Variables	Period	mean	S	% 95 Confidence Interval		*t*	*p*
				Lower Limit	Upper Limit		
Height SDS	Basal	-3.39	.81	-0.77	-.32	-4.96	**.000**
	One year later	-2.83	.72				
IGF-1 SDS	Basal	-1.35	.82	-1.80	-.96	-6.56	**.000**
	One year later	.026	1.58				
IGF-BP-3 SDS	Basal	-.78	.75	-1.06	-.58	-6.81	**.000**
	One year later	.041	.93				

IGF-1:Insulin-like growth Factor-1, IGF BP-3:Insulin growth Factor Binding Protein-3, SDS: Standart Deviation Scores

Analysis of the relationship between the criteria employed in the study and growth response revealed a significant negative correlation between delta height SDS and maximum stimulated GH response, with growth response increasing as stimulated maximum GH response decreased (Table 5).

Table 5. Relationships between growth response and other metrics

Variables		Basal IG-1 SDS	Basal IGF BP-3 SDS	Peak GH Response	Target height SDS	Height SDS
Δ Height SDS	r	-.185	-.150	**-.407****	.028	-.267
	p	.230	.330	**.006**	.855	.080

*IGF-1:Insulin-like growth Factor-1, IGF BP-3:Insulin growth Factor Binding Protein-3, GH: Growth Hormone, SDS: Standart Deviation Scores **p <0 .01*

4. Discussion

GHD is one of the treatable causes of short stature in children. Due to the pulsatile nature of GH release, GH stimulation tests are required in cases of suspected GHD (17). GH secretion can be affected by factors such as age, gender, puberty, insufficient nutrition, and obesity. The pharmacological stimulus is not physiological, and the diagnostic threshold varies among different centers (18). Researchers have also emphasized the need for simpler methods for reasons such as difficulty in application, limited repeatability, side effects, cost, and the invasive nature of the procedure (19). Serum IGF-1 and IGFBP-3 levels reflect GH secretion in healthy children, and diurnal changes are very low (4). Evaluating IGF-1 and IGFBP-3 levels in children with short stature can therefore be employed as an auxiliary method for avoiding unnecessary GH stimulation tests or as a complementary tool in diagnosing GHD (20, 21). Studies have shown that IGF-1 levels lower than -2 SDS require powerful consideration of GHD (19, 22). Similarly, the present study revealed severe deviation in IGF-1 values as a significant variable in showing GHD. However, the study findings did not support those previous studies describing baseline IGFBP-3 levels as a useful diagnostic tool for showing GHD.

This study also investigated the relationship between IGF-1 and IGFBP-3 levels and growth response following GH therapy in cases of GHD. The first-year growth response after GH therapy in children with GHD is known to be one of the best indicators of long-term growth (1, 23), while GH is the primary factor determining IGF-1 levels in circulation (10). In that context, it has been assumed that the positive effects of GH therapy on growth will be parallel to the increase in IGF-1 levels (24, 25). In addition, Kim et al. (10) showed a weak correlation between IGF-1 levels and growth response in children with GHD who started GH therapy, with this relationship being observed mainly in the group with severe GHD. On the other hand, Cutfield et al. (26) determined that patients with GHD with low IGF-1 levels before GH therapy exhibited better growth responses in the first year of treatment. Kim et al. (27) showed that serum Δ IGF-I levels measured in the first year of treatment in prepubertal cases with GHD started on GH therapy and for whom GH treatment was initiated can be used as a marker to predict the growth response. However, there are also opposing views. Lanes et al. (28) found that the increase in IGF-1 SDS during GH therapy was not consistent with the increase in height and suggested that IGF-1 follow-up during treatment would be more useful in terms of safety and adherence to treatment than GH dose adjustment. In the present study, the growth responses obtained in the first year of GH therapy were significantly higher in patients with severe deviations in basal IGF-1 levels compared to the other group, the growth responses increasing in line with delta IGF-1 levels. This finding supports those studies suggesting that IGF-1 levels exhibiting severe negative deviation before GH therapy and delta IGF-1 levels evaluated in the first year of treatment may be useful in predicting the response to that treatment.

The GH-IGF-1 axis is an essential component of the endocrine system that controls linear growth in childhood

(29). IGF-1 and IGFBP-3 bind to 90% of IGF-1 in the circulation, and the acid-labile subunit are in a triple complex (30), and serum IGF-1 and IGFBP-3 levels are associated with GH levels under normal conditions (31). However, the usefulness of observing IGFBP-3 levels during GH therapy in assessing the response to treatment is controversial. IGFBP-3 has been reported to play a role in apoptosis and growth inhibition of cancer cells and be broken down by proteases after their secretion during inflammation (32,33). Functional studies have investigated the relationship between genetic polymorphism in IGFBP-3 and the response to GH therapy. Patients with GHD with the -202 A allele IGFBP3 genotype exhibited better average growth velocity and higher IGFBP-3 levels in the first year of GH therapy than patients with the -202 AC or CC IGFBP3 genotypes (34). These factors cast doubt on the use of IGFBP-3 as a diagnostic tool in evaluating the efficacy of GH therapy. Despite these disadvantages, some researchers have reported that changes in IGFBP-3 in children receiving GH therapy are significantly associated with growth response and have even suggested that IGFBP-3 may be a more useful marker than delta IGF-1 in predicting growth response (27). In the present study, we found that the increase in growth velocity in the first year in cases of GHD started on GH therapy was associated with increased IGFBP-3 levels. However, we did not observe the significant growth response seen in cases with severe deviations in basal IGF-1 levels in cases with severe deviations in basal IGFBP-3 levels.

There are several limitations to this study. The first is its single-centre nature and the low patient number. Second, we did not perform priming with sex steroids before the GH stimulation test in peripubertal children. Third, the evaluation period after GH therapy was limited to one year. And fourth, we did not apply the same GH stimulation test in all cases. We applied two separate tests, clonidine and L-Dopa, with glucagon used as the second test in only one case. Another limitation is that not all cases were in the same puberty stage.

Severe deviation in basal IGF-1 levels should strongly suggest GHD. Delta IGF-1 and delta IGFBP-3 levels evaluated in the first year of treatment, together with basal IGF-1 levels, can be a useful diagnostic tool in demonstrating growth response in children with GHD receiving GH therapy.

Conflict of interest

Authors declared that there is no conflict of interest.

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Authors' contributions

AA, SB: conceptualized the study; AA, SB: managed the field conduct and logistics; AA, SB: drafted the manuscript; SB: analyzed the data. All authors contributed to the critical revision of the manuscript, and its final approval.

References

1. Murray PG, Dattani MT, Clayton PE. Controversies in the diagnosis and management of growth hormone deficiency in childhood and adolescence. Arch Dis Child. 2016;101(1):96-100.
2. Ghigo E, Bellone J, Aimaretti G, Bellone S, Loche S, Cappa M, et al. Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. J Clin Endocrinol Metab. 1996;81:3323-3327
3. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Daughaday WH, Rotwein P. Endocr Rev. 1989;10:68–91
4. Juul A, Skakkebaek NE. Prediction of the outcome of growth hormone provocative testing in short children by measurement of serum levels of insulin-like growth factor I and insulin-like growth factor binding protein 3. J Pediatr. 1997 Feb;130(2):197-204.
5. Lifshitz F. Growth and Growth disorders. Edby Liftshitz F. Pediatric Endocrinology (5th ed) New York: İnforma Health Care Inc. 2007, pp 1-40, pp 65-90, pp 113-145
6. Reiter EO and Rosenfeld RG. Normal and aberrant growth In: Wilson JD, Foster DW, Kronenberg HM and Larsen PR (eds). Williams Textbook of Endocrinology (9th ed). Philadelphia: W.B. Saunders Co 1998, pp 1427-1505.
7. Günöz H. Büyüme bozuklukları, Pediatrik Endokrinoloji. Editörler Günöz H, Öçal G, Yordam N, Kutoğlu S: Pediatrik Endokrinoloji ve Oksoloji yayınları 1, Ankara: Kalkan Matbacılık, 2003, ss 43-62, ss 66-131.
8. Cinaz P, Güran T, Bereket A. Boy kısalığı olan çocuğa yaklaşım. Güncel Çocuk Sağlığı Pediatrik Endokrinoloji Dergisi, 2008, 3: 2-9.
9. Bang P, Ahmed SF, Argente J, Backeljauw P, Bettendorf M, Bona G, et al. Identification and management of poor response to growth-promoting therapy in children with short stature. Clin Endocrinol (Oxf) 2012;77:169-181
10. Kim JH, Kim SJ, Lee J, Shin CH, Seo JY. Factors affecting IGF-I level and correlation with growth response during growth hormone treatment in LG Growth Study. PLoS One. 2021 Jul 19;16(7):e0252283.
11. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. J Clin Endocrinol Metab. 2000;85(11):3990-93.
12. Wilson TA, Rose SR, Cohen P, Rogol DA, Backeljauw P, Brown R et al. Update of guidelines for the use of growth hormone in children: the Lawson Wilkins Pediatric Endocrinology Society Drug and Therapeutics Committee. J Pediatr. 2003;143(4):415-21.
13. Guven B, Can M, Mungan G, Acikgoz S. Reference values for serum levels of insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) in the West Black Sea region of Turkey. Scand J Clin Lab Invest. 2013 Mar;73(2):135-40.
14. Neyzi O, Günöz H, Furman A, Bundak R, Gökçay G, Darendeliler F, ve ark. Türk çocuklarında vücut ağırlığı, boy uzunluğu, baş çevresi ve vücut kitle indeksi referans değerleri. Çocuk Sağlığı ve Hastalıkları Derğisi;2008, 51:1-14.
15. Bayley N, Pinneau SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich- Pyle hand standards. J Pediatr XL 1952; 432-441.
16. Greulich WW, Pyle SI. Radiographic atlas of skeleral

development of the hand and wrist, second edition. Standford, CA: Standford University Pres, 1959.

17. Cattoni A, Molinari S, Medici F, De Lorenzo P, Valsecchi MG, Masera N, et al. Dexamethasone Stimulation Test in the DiagnosticWork-Up of Growth Hormone Deficiency in Childhood: Clinical Value and Comparison With Insulin-Induced Hypoglycemia. Front Endocrinol (Lausanne). 2020 Dec 9;11:599302. doi: 10.3389/fendo.2020.599302. PMID: 33362716; PMCID: PMC7757782

18. Clemmons DR. Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. Clinical Chemistry 2011 57 555–559. , Schilbach K & Bidlingmaier M. Laboratory investigations in the diagnosis and follow-up of GH-related disorders. Archives of Endocrinology and Metabolism 2019 63 618–629.

19. Ibba A, Corrias F, Guzzetti C, Casula L, Salerno M, di Iorgi N, et al. IGF1 for the diagnosis of growth hormone deficiency in children and adolescents: a reappraisal. Endocr Connect. 2020 Nov;9(11):1095-1102.

20. Shen Y, Zhang J, Zhao Y, Yan Y, Liu Y, Cai J. Diagnostic value of serum IGF-1 and IGFBP-3 in growth hormone deficiency: a systematic review with meta-analysis. Eur J Pediatr. 2015 Apr;174(4):419-27.

21. Berberoğlu M, Sıklar Z, Darendeliler F, Poyrazoğlu S, Darcan S, Işgüven P, ve ark. Evaluation of permanent growth hormone deficiency (GHD) in young adults with childhood onset GHD: a multicenter study. J Clin Res Pediatr Endocrinol. 2008;1(1):30-7.

22. Federico G, Cianfarani S. Usefulness of serum insulin-like growth factor I assessment in the diagnosis of childhood-onset growth hormone deficiency. Horm Res Paediatr. 2010;74(2):145-8.

23. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al. Drug and Therapeutics Committee and Ethics Committee of the Pediatric Endocrine Society: Guidelines for growth hormone and insulin-like growth factor-I treatment in children and adolescents: growth hormone deficiency, idiopathic short stature, and primary insulin-like growth factor-I deficiency. Horm Res Paediatr. 2016; 86:361–397.

24. Ranke MB, Schweizer R, Elmlinger MW, Weber K, Binder G, Schwarze CP, et al. Relevance of IGF-I, IGFBP-3, and IGFBP-2 measurements during GH treatment of GH-deficient and non-GH-deficient children and adolescents. Horm Res. 2001;55(3):115-24.

25. Choi YJ, Lee YJ, Lee NY, Lee SH, Kim SK, Ahn MB, et al. Discriminatory performance of insulin-like growth factor 1 and insulin-like growth factor binding protein-3 by correlating values to chronological age, bone age, and pubertal status for diagnosis of isolated growth hormone deficiency. Ann Pediatr Endocrinol Metab. 2020 Dec;25(4):240-247.

26. Cutfield WS, Lundgren F. Insulin-like growth factor I and growth responses during the first year of growth hormone treatment in KIGS patients with idiopathic growth hormone deficiency, acquired growth hormone deficiency, turner syndrome and born small for gestational age. Horm Res. 2009 Jan;71 Suppl 1:39-45.

27. Kim M, Kim EY, Kim EY, So CH, Kim CJ. Investigating whether serum IGF-1 and IGFBP-3 levels reflect the height outcome in prepubertal children upon rhGH therapy: LG growth study database. PLoS One. 2021 Nov 1;16(11):e0259287.

28. Lanes R, Jakubowicz S. Is insulin-like growth factor-1 monitoring useful in assessing the response to growth hormone of growth hormone-deficient children? J Pediatr. 2002 Nov;141(5):606-10.

29. David A, Hwa V, Metherell LA, Netchine I, Camacho-Hübner C, Clark AJ, et al. Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. Endocr Rev. 2011 Aug;32(4):472-97.

30. Frystyk J. Utility of free IGF-I measurements. Pituitary. 2007;10(2):181-7.

31. Polidori N, Castorani V, Mohn A, Chiarelli F. Deciphering short stature in children. Ann Pediatr Endocrinol Metab. 2020 Jun;25(2):69-79.

32. Johannsson G, Bidlingmaier M, Biller BMK, Boguszewski M, Casanueva FF, Chanson P, et al. Growth Hormone Research Society perspective on biomarkers of GH action in children and adults. Endocr Connect. 2018; 7:R126–R134.

33. Mohanraj L, Kim HS, Li W, Cai Q, Kim KE, Shin HJ, et al. IGFBP-3 inhibits cytokine-induced insulin resistance and early manifestations of athelosclerosis. Plos One. 2013; 8:e55084.

34. Costalonga EF, Antonini SR, Guerra-Junior G, Mendonca BB, Arnhold IJ, Jorge AA. The -202 A allele of insulin-like growth factor binding protein-3 (IGFBP3) promoter polymorphism is associated with higher IGFBP-3 serum levels and better growth response to growth hormone treatment in patients with severe growth hormone deficiency. J Clin Endocrinol Metab. 2009 Feb;94(2):588-95.

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Association of body mass index & android obesity with uterine leiomyoma among premenopausal women: A case–control study

Zeynab KARAMIHAMIDABAD[1,*], Sara AZIMA[2], Fatemeh NAJIB[3], Marzieh AKBARZADEH[4]

[1]Department of midwifery, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of midwifery, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of obstetrics and gynecology, School of medicine, infertility research center, Shiraz University of Medical Sciences, Shiraz, Iran
[4]Maternal –fetal medicine Research Center, Department of Midwifery, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran

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Abstract

Uterine leiomyomas (ULs) or uterine fibroids are the primary genital tumors in women of reproductive age. Obesity and increased visceral fat, and the production of inflammatory mediators might be risk factors for ULs. This study aimed to assess the correlation between ULs and body fat distribution among Iranian women. This case-control study involved 280 women of reproductive age with ULs and without myoma in 2020. They referred to three women's clinics in Shiraz (Shahid Faghihi, Zeinabieh, and Motahhari clinics). The sample size for each group consisted of at least 140 subjects selected via consumptive sampling methods. A gynecologist pre-diagnosed ULs based on the findings of vaginal ultrasound. We recorded and compared both groups' demographic information, fertility and ULs histories, body mass index (BMI), and android obesity. We used chi-square and t-tests to analyze the data. Most patients with ULs (64 people, 45.7%) were 41-50 years old. Most of them (126, 90%) were housewives, and the level of education of most patients (74 people, 52.9%) was under high school diploma. The mean BMI in women with myoma was 26.05±5.32 and 25.81 ±4.38 in women without myoma. There was no significant difference in the mean scores of BMI between the two groups, but the Android fat obesity was higher in patients with ULs. Few studies have attempted to identify specific risk factors for this tumor. Preventing weight gain and obesity and lifestyle modification can prevent Uterine leiomyoma.

Keywords: gynecology, premenopausal, uterine leiomyoma, women, body mass index, android obesity

1. Introduction

Uterine leiomyomas (ULs), or uterine fibroids, of the most common myometrial muscle cell tumors of benign pelvic origin (1), are benign steroid monoclonal tumors that stimulate the smooth muscle (myometrium) of the uterus (2). It is estimated that up to 77% (25-77%) of all women suffer from ULs during their life, and 15-30% suffer from significant symptoms (3-5). ULs are a common cause of menstrual irregularities, pelvic discomfort, menorrhagia, dysmenorrhea, anemia, recurrent pregnancy loss, preterm labor, incontinence, and infertility (4). Ekin et al. (2014) reported that the frequency of genital symptoms, urinary incontinence, including stress urinary incontinence, urgency and frequency of urination, and painful intercourse were higher in women with ULs. Women with ULs greater than 5 cm had more urinary incontinence than other women during physical activity and travel (6).

These seemingly benign tumors can be associated with abnormalities in preterm labor and infertility and recurrent miscarriages. The prevalence of abortion in such women is also twice as high as in other myoma-free pregnancies (7, 8). ULs are also the most common significant cause of uterine resection, which causes several complications for the patient (5, 9). The exact causes of ULs remain unknown, but two hypotheses propose genetics and hormones to be the cause (10, 11). Risk factors for ULs' development are obesity, reproductive factors such as nulliparity, young age in the first pregnancy, premature menarche, menstrual cycle length of more than 30 days and bleeding duration of more than six days, diabetes, and hypertension (12-16). A case-control study by Giri et al. (2017) (539 cases and 794 controls) entitled "African genetic ancestry interacts with body mass index to modify risk for uterine fibroids." reported that race, especially African race, and obesity are important risk factors for ULs and create suitable conditions for ULs growth (17). Obesity decreases the 2-hydroxylation of estrone to catechol estrogens and an increase in 16-alpha-hydroxylation of

*Correspondence: marzieh.akhbar@gmail.com

estrone to estriol. Eventually, it produces estrogens with more uterotropic activity (18, 19). Other studies in a white population had contradictory results regarding the association between BMI and uterine leiomyoma (20-24). Some studies showed a positive correlation (20, 22, 23) or an inverse correlation (21, 24), while others showed no correlation between BMI and uterine leiomyoma (5, 25, 26). There are different conclusions about the effect of obesity on UL incidence, some of which may be related to disease definitions and climatic and geographical differences. Prompt diagnosis and early commencement of treatment of GHD are of great importance in children's reaching target heights (5, 7, 8). Patients started on GH therapy must be followed up in terms of efficacy (9). Growth velocity and IGF-1 and IGFBP-3 levels can be evaluated in terms of the effectiveness of treatment in these cases (10).

We conducted this case-control study to investigate the BMI and Android obesity in women with and without ULs among the population referred to the gynecological clinics of Shiraz University of Medical Sciences. With the knowledge of these predictive risk factors, appropriate strategies can be taken to determine the etiology of this tumor and design preventive measures.

2. Materials and Methods

2.1. Study design

This case-control study involved 280 women of reproductive age with ULs (symptomatic and asymptomatic) and without Uls referred to Shiraz Women's Clinics (Shahid Faghihi, Zeinabieh and Motahhari Clinics) in 2020.

2.2. Study size, setting and search strategy

We determined the sample size by a statistical expert using the Medcalc software according to a similar previous study (27) considering 90% power, first type error of 5%, the mean difference of 0.19 and the standard deviation of 0.46 and 0.52 for the two groups. Using the convenience sampling method based on inclusion criteria, we involved at least 140 people in the study.

2.3. Participants

The case group included people with symptomatic or asymptomatic ULs who were pre-menopausal, and the control group also included all women referred to clinics to receive periodic care for ULs and Endometriosis for other reasons.

2.4. Inclusion and exclusion criteria

Inclusion criteria: Married women with Iranian citizenship, pre-menopausal age and myoma (from the age of 15 until menopause, when the average age of menopause is 51 years), any hormonal drug affecting the size of the myoma from the beginning to one month after completion, signing the informer written consent form, not attending any classes or programs that affect the quality of life and lifestyle such as relaxation, yoga, meditation, and no history of endometriosis in the myoma group.

Exclusion criteria: Not using special diets during the study, taking any drug that affects the quality of life and lifestyle from three months before the study, and patients' unwillingness to continue the study.

2.6. Research tools

Research tools: Demographic information questionnaire including demographic characteristics of subjects such as age, weight, height, abdominal circumference, blood pressure, history of hypertension, education, age of marriage, economic status, number of pregnancies, number of abortions and stillbirths, age of onset of the first menstrual period, the history of myoma and the impact of myoma on quality of life, the history of myoma in the first-degree family, menstrual order and bleeding rate, smoking or alcohol consumption, and contraception. BMI is determined by dividing weight by the square of height and classified into lean (less than 18.5), normal (18.5 to 24.9), overweight (25 to 29.9), obese (30 to 39.9), and very obese (greater than or equal to 40) (95). Android obesity is calculated by dividing abdominal circumference by hip circumference and interpreted as low risk (equal to or less than 0.8) and moderate risk (0.81 to 0.89), and high risk (equal to or more than 0.9). We recorded the weight of all individuals in a fixed state using a digital scale (Camry, I.R. Iran) with an accuracy of 100 grams. We measured the heights with an accuracy of 0.5 cm using a tape measure in a standing position with the head was straight, the legs together, the knees straight, and the four points of the heel, buttocks and shoulders attached to the wall without any footwear or headwear.

2.7. Statistical tests

It was done by SPSS statistical software v.22. We used the independent t-test to evaluate the mean scores of body mass index and Android obesity in the two groups with and without myoma. We used a Chi-square test to compare the frequency of Android obesity in the two groups with and without myoma.

2.8. Ethical considerations

The Ethics Committee of Shiraz University of Medical Sciences confirmed the research (grant No: 20735-98, ethics code: (18120-98, IR.SMS-TEC1398-989). All participants signed written informed consent. We designed the protocol per the Helsinki principles of ethics.

3. Results

3.1. Clinical characteristics of the study subjects

Out of the 140 people in the ULs group, 53 (37.9%) were from Motahhari Hospital and 87 (62.1%) from Zeinabieh Hospital. Sixty-four (45.7%) were in the 41-50 years age range, 126 (90%) were housewives, and 74 (52.9%) had an educational level under high school diploma (Table 1).

Table 1. Frequency distribution and comparison of the studied variables in two groups with myoma and without myoma

Variable		Group Myoma N (%)	Without Myoma N (%)	P-value
Age	20-30	10 (7.1)	24 (17.1)	0.054†
	31-40	60 (42.9)	61 (43.6)	
	41-50	64 (45.7)	50 (35.7)	
	>51	6 (4.3)	5 (3.6)	
Job	housewife	126 (90)	124 (88.6)	0.722*
	Employee	12 (8.6)	12 (8.6)	
	Free	2 (1.4)	4 (2.9)	
Education	Less than a diploma	74 (52.9)	60 (42.9)	0.083†
	Diploma	44 (31.4)	54 (38.6)	
	Associate Degree	18 (12.9)	15 (10.7)	
	Bachelor and higher	4 (2.9)	11 (7.9)	
Number of Pregnant	once	14 (11)	32 (26.7)	0.057†
	Twice	39 (30.7)	42 (35)	
	three times	34 (26.8)	23 (19.1)	
	Four times	27 (21.25)	14 (11.7)	
	Five times and above	13 (10.25)	9 (7. 5)	
Number of Abortion	once	35 (25.8)	25 (19.25)	0.055†
	Twice	3 (2.2)	12 (9.25)	
	Three times and more	2 (1.5)	5 (3.9)	
	Have not	96 (70.5)	88 (67.6)	
Number of child	once	21 (17)	35 (29.4)	0.059†
	Twice	42 (33.8)	49 (41.2)	
	three times	35 (28.2)	20 (16.8)	
	Four times	18 (14.5)	8 (6.7)	
	Five times and above	8 (6.5)	7 (5.9)	

†:Chi-Square Test, *: Fisher's Exact Test

3.2. Obesity and UL correlation

We used an independent t-test to evaluate the mean scores of BMI in with and without ULs. Comparing the means, the calculated T-statistic value was 0.412 with a degree of freedom of 278, and considering that the significance level was 0.681, there was no significant difference between the mean scores of BMI between the two groups (Table 2).

Table 2. Comparison of mean body mass index between myoma and non-myoma groups

Variable	Group	N	Mean ± SD	Test statistics	P-value *
BMI	Myoma	140	26.05 ± 5.32	0.42	0.675
	Without Myoma	140	25.81 ± 4.38		

**Independent T-test*

We used a Chi-square test to compare the frequency of Android obesity in the two groups with and without ULs. Considering the significance level of the Chi-square test of 0.05, which was less than the assumed error in this study, there was a statistically significant difference in the observed frequencies of Android obesity between participants with and without ULs (Table 3). We used an independent t-test to evaluate the mean scores of Android obesity in the two groups. Comparing the means, the value of the T statistic was -5.88 with a degree of freedom of 278, and according to the value of the significance level of 0.001, there was a significant difference in the mean scores of Android obesity between the two groups, and Android obesity was higher in the non-myomas group (Table 4).

Table 3. Descriptive results of Android obesity scores in two groups with and without myoma

		Android Obesity			total
		Low	Moderate	High	
Group	Myoma	21	23	96	140
	Without Myoma	33	36	71	140
Total		54	59	167	280

Table 4. Comparison of mean scores of Android obesity in case group with control

Variable	Group	N	Mean ± SD	Test statistics	P-value*
Android Obesity	Myoma	140	0.98 ± 0.16	-5.88	0.001
	Without Myoma	140	0.91 ± 0.16		

*Independent t-test

4. Discussion

Our results showed that the mean scores of BMI were not significantly different between the two groups with and without ULs. Takeda et al.'s study (16), which compared 213 women with and 159 without ULs, reported that ULs was significantly associated with obesity and HTN and the presence of several risk factors associated with ULs increased the risk of metabolic syndrome in patients. In a prospective study in the US, Marshall et al. (27) found that the risk of ULs increases with BMI and ULs are associated with weight gain. Sharmi et al. (2009) examined the risk factors associated with uterine fibroids in a case-control study on 990 women of reproductive age in Rasht, Iran. They asked about myoma family history, fertility history, BMI and the contraceptive method in their questionnaire. The results showed a statistically significant relationship between uterine fibroids and BMI higher than 5 (28). Their results were inconsistent with the present study, which can be due to the type of information collected, the face-to-face interview method, or the large size of their study population (990 people). He et al. (2013) also showed that BMI significantly increased the risk of uterine myoma in pre-menopausal women (29). Their obtained results were inconsistent with the present study. This could be due to the difference in the study population and the racial differences between Iran and China. They studied pre-menopausal women with a mean age of 45-555, while our study involved women 31-40 years old. Another debatable point is that the mean BMI of patients with myoma and non-patients was 26.05 and 25.81, respectively, indicating that the study population was only slightly overweight. In contrast, several reported studies showed no association between UL incidence and obesity (24, 26), consistent with the present study.

In our study, the mean scores of Android obesity in the case group were significantly greater than that of the control group. In a case study, Chen et al. (2001) reported that BMI, hip circumference, waist-to-height ratio, waist circumference (WC), body fat content, and body fat percentage were

positively associated with uterine fibroids. Women with high BMI and waist-to-thigh ratio had the most significant uterine fibroids risk. Also, women with a body fat percentage of higher than 30% were comparatively prone to uterine fibroids, and uterine fibroids could be associated with overweight and central obesity (5), which is consistent with some of the findings of the present study. In a study by Tak et al., the WC and body fat were significantly greater in the ULs group. These outcomes are consistent with previous reports showing a positive correlation between obesity and the occurrence of ULs (15). In another study, visceral fat area (VFA), BMI, WC, body fat percentage, waist-to-height ratio, and waist-to-pelvic ratio were positively associated with uterine fibroids (30). Boclin et al. (2015) examined the association between adult weight gain and uterine myomas among Brazilian women. The results showed no significant relationship in the presence of uterine myomas among people with weight gain (31). Their results were inconsistent with our study. This difference might be due to the study population and the racial differences between Iran and Brazil. They studied 1560 Brazilian women whose weight gain had been continuously studied.

Studies show that the estrone to estradiol conversion in uterine fibroids is significantly lesser than the normal muscle tissue, and the estrogen receptors' concentration in fibroids is obviously greater than in peripheral muscle tissue. Thus, the pathogenesis of uterine fibroids might be related to the level of sex hormones (32). Obesity can lead to metabolic disorders, leading to local tissues creating an unusually great estrogen environment. This mechanism contains the following: 1) Androstenedione secreted through the adrenal glands could be converted to estrone via aromatase in adipose tissue, and plasma estrone levels increase with increased adipose tissue, hence causing a continuous effect of estrogen. 2) Obesity causes a periodic lack of regulation of progesterone; therefore, the endometrium is over-stimulated in an environment where no progesterone has an estrogen antagonist (33, 34). Uterine fibroids may develop in an abnormal environment with high estrogen. Therefore, obesity can be a risk factor for uterine fibroids.

Evidence shows that the levels of SHBG are lower in women with central obesity, and they have altered estrogen metabolism and hyperinsulinemia that are anticipated to stimulate the growth of UL (14, 23). However, a clear link between obesity and UL is connected to the hormonal effects associated with obesity. For instance, obesity increases with increased circulating adrenal androgens to estrones conversion due to adipose tissue accumulation (15). In addition, hepatic SHBR production is reduced, leading to more unrestricted physiologically active estrogen (35), which can cause a comparatively hyperestrogenic state. Previous studies have shown increased levels of estrogen and adipokines due to extreme fat accumulation and raised systemic inflammatory cytokines levels that may raise the tumorigenesis risk (36, 37).

Considering the above issues, it can be concluded that various factors are involved in the development of fibroids, and its true etiology remains unknown but understanding the risk factors associated with fibroids can be effective in providing preventive measures in the development of the disease. Preventing weight gain during fertility and lifestyle modification can be one of the preventative measures.

In conclusion, there was no significant difference in the mean scores of BMI between the case and control groups, but the Android fat index was higher in patients with myoma. Preventing weight gain during the reproductive period and improving lifestyle can be one of the preventive ways. Providing nutritional tips, changing diets, and exercising are essential steps to preventing uterine fibroids.

One of the study's limitations was that it was conducted during the COVID-19 pandemic in hospitals and public centers. As a result, a series of protocols were observed during the presence of participants, and completing the questionnaires, including maintaining the physical distance, necessitated a longer time than stated in the proposal. Furthermore, because only Iranian women participated in the study, our findings could not be generalized to other ethnicities or geographies.

Conflict of interest

All authors have no financial or personal conflict of interest.

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Authors' contributions

MA and ZK prepared the first draft of the manuscript, and MA &SA made critical revisions to the paper and responded to the reviewers. FN helped the Surge Articles and Clinical Research.

References

1. Walker CL, Stewart EA. Uterine fibroids: the elephant in the room. Science. 2005;308(5728):1589-92.
2. Hoffman PJ, Milliken DB, Gregg LC, Davis RR, Gregg JP. Molecular characterization of uterine fibroids and its implication for underlying mechanisms of pathogenesis. Fertil Steril. 2004;82(3):639-49.
3. Catherino WH, Parrott E, Segars J. Proceedings from theNational Institute of Child Health and Human Development conference on the Uterine Fibroid Research Update Workshop. Fertil Steril. 2011;95(1):9-12.
4. Bulun SE. Uterine fibroids. N Engl J Med. 2013;369(14):1344-55.

5. Chen CR, Buck GM, Courey NG, Perez KM, Wactawski-Wende J. Risk factors for uterine fibroids among women undergoing tubal sterilization. Am J Epidemiol. 2001;153(1):20-6.

6. Ekin M, Cengiz H, Ozturk E, Kaya C, Yasar L, Savan K. Genitourinary symptoms and their effects on quality of life in women with uterine myomas. Int Urogynecol J. 2014;25(6):807-10.

7. Gambadauro P. Dealing with uterine fibroids in reproductive medicine. J Obstet Gynaecol. 2012;32(3):210-6.

8. Cook H, Ezzati M, Segars JH, McCarthy K. The impact of uterine leiomyomas on reproductive outcomes. Minerva Ginecol. 2010;62(3):225-36.

9. Farquhar CM, Steiner CA. Hysterectomy rates in the United States 1990-1997. Obstet Gynecol. 2002;99(2):229-34.

10. Hillard J. Benign disease of female reproductive tract symptoms and sign. In: Berek J, editor. Novaks gynecology 13th ed. Philadelphia: Lippincott William & willkins; 2002. p. 351-420.

11. Medikare V, Kandukuri LR, Ananthapur V, Deenadayal M, Nallari P. The genetic bases of uterine fibroids; a review. J Reprod Infertil. 2011;12(3):181-91.

12. Bharathi C, Anupama D, Pratibha N, Venkateshwari A. Impact of Genetic Variants in Estrogen Receptor-beta Gene in the Etiology of Uterine Leiomyomas. J Reprod Infertil. 2019;20(3):151-60.

13. Wise LA, Palmer JR, Harlow BL, Spiegelman D, Stewart EA, Adams-Campbell LL, et al. Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in African-American women: a prospective study. Am J Epidemiol. 2004;159(2):113-23.

14. Wise LA, Palmer JR, Spiegelman D, Harlow BL, Stewart EA, Adams-Campbell LL, et al. Influence of body size and body fat distribution on risk of uterine leiomyomata in U.S. black women. Epidemiology. 2005;16(3):346-54.

15. Tak YJ, Lee SY, Park SK, Kim YJ, Lee JG, Jeong DW, et al. Association between uterine leiomyoma and metabolic syndrome in parous pre-menopausal women: A case-control study. Medicine (Baltimore). 2016;95(46):e5325.

16. Takeda T, Sakata M, Isobe A, Miyake A, Nishimoto F, Ota Y, et al. Relationship between metabolic syndrome and uterine leiomyomas: a case-control study. Gynecol Obstet Invest. 2008;66(1):14-7.

17. Giri A, Edwards TL, Hartmann KE, Torstenson ES, Wellons M, Schreiner PJ, et al. African genetic ancestry interacts with body mass index to modify risk for uterine fibroids. PLoS Genet. 2017;13(7):e1006871.

18. Fishman J, Boyar RM, Hellman L. Influence of body weight on estradiol metabolism in young women. J Clin Endocrinol Metab. 1975;41(5):989-91.

19. Terry KL, De Vivo I, Hankinson SE, Missmer SA. Reproductive characteristics and risk of uterine leiomyomata. Fertil Steril. 2010;94(7):2703-7.

20. Orsini G, Laricchia L, Fanelli M. [Low-dose combination oral contraceptives use in women with uterine leiomyomas]. Minerva Ginecol. 2002;54(3):253-61.

21. Harmon QE, Baird DD. Use of depot medroxyprogesterone acetate and prevalent leiomyoma in young African American women. Hum Reprod. 2015;30(6):1499-504.

22. Wise LA, Palmer JR, Harlow BL, Spiegelman D, Stewart EA, Adams-Campbell LL, et al. Risk of uterine leiomyomata in relation to tobacco, alcohol and caffeine consumption in the Black Women's Health Study. Hum Reprod. 2004;19(8):1746-54.

23. Faerstein E, Szklo M, Rosenshein N. Risk factors for uterine leiomyoma: a practice-based case-control study. I. African-American heritage, reproductive history, body size, and smoking. Am J Epidemiol. 2001;153(1):1-10.

24. Parazzini F, Negri E, La Vecchia C, Chatenoud L, Ricci E, Guarnerio P. Reproductive factors and risk of uterine fibroids. Epidemiology. 1996;7(4):440-2.

25. Romieu I, Walker A, Jick S. Determinants of uterine fibroids. 1991.

26. Samadi AR, Lee NC, Flanders WD, Boring JR, 3rd, Parris EB. Risk factors for self-reported uterine fibroids: a case-control study. Am J Public Health. 1996;86(6):858-62.

27. Marshall LM, Spiegelman D, Manson JE, Goldman MB, Barbieri RL, Stampfer MJ, et al. Risk of uterine leiomyomata among pre-menopausal women in relation to body size and cigarette smoking. Epidemiology. 1998;9(5):511-7.

28. Sharami S, Zahiri Z, Ghanaie M. Risk Factors of Uterine Leiomyoma in Women of Reproductive Ages. Journal of Babol University of Medical Sciences. 2009;11(4):54-61.

29. He Y, Zeng Q, Dong S, Qin L, Li G, Wang P. Associations between uterine fibroids and lifestyles including diet, physical activity and stress: a case-control study in China. Asia Pac J Clin Nutr. 2013;22(1):109-17.

30. Sun K, Xie Y, Zhao N, Li Z. A case-control study of the relationship between visceral fat and development of uterine fibroids. Exp Ther Med. 2019;18(1):404-10.

31. Boclin Kde L, Torres FP, Faerstein E. Body weight gain during adulthood and uterine myomas: Pro-Saude Study. Rev Saude Publica. 2015;49.

32. Wong JY, Gold EB, Johnson WO, Lee JS. Circulating Sex Hormones and Risk of Uterine Fibroids: Study of Women's Health Across the Nation (SWAN). J Clin Endocrinol Metab. 2016;101(1):123-30.

33. Zimmermann A, Bernuit D, Gerlinger C, Schaefers M, Geppert K. Prevalence, symptoms and management of uterine fibroids: an international internet-based survey of 21,746 women. BMC Womens Health. 2012;12:6.

34. Curti ML, Jacob P, Borges MC, Rogero MM, Ferreira SR. Studies of gene variants related to inflammation, oxidative stress, dyslipidemia, and obesity: implications for a nutrigenetic approach. J Obes. 2011;2011:497401.

35. Sparic R, Mirkovic L, Malvasi A, Tinelli A. Epidemiology of Uterine Myomas: A Review. Int J Fertil Steril. 2016;9(4):424-35.

36. Flood A, Strayer L, Schairer C, Schatzkin A. Diabetes and risk of incident colorectal cancer in a prospective cohort of women. Cancer Causes Control. 2010;21(8):1277-84.

37. Baker R, Dauner JG, Rodriguez AC, Williams MC, Kemp TJ, Hildesheim A, et al. Increased plasma levels of adipokines and inflammatory markers in older women with persistent HPV infection. Cytokine. 2011;53(3):282-5.

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Clinical toxicology of propranolol and metoprolol overdose in adults

Figen ÖZCAN[1], Mehmet ALTUNTAŞ[1,*], Celal KATI[2]

[1]Department of Emergency of Medicine, Recep Tayyip Erdoğan University, Research and Training Hospital, Rize, Türkiye
[2]Department of Emergency of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

Beta-adrenergic receptor antagonists potentially risk causing fatal poisoning when taken over the daily recommended doses. The aim of this study is to investigate the differences and potential dose-related effects of propranolol and metoprolol toxicity depending on their selectivity. This 7-year-long retrospective cohort study was conducted among on 43 adult patients who received overdose propranolol (n= 22) and metoprolol (n= 21). Patients were divided into groups, with a daily overdose ≥ 240 mg/day for propranolol, ≥ 200 mg/day for metoprolol, and toxic dose ≥ 400 mg/day for both drugs. The groups were compared in terms of admission symptoms, heart rate, blood pressure, electrocardiography findings, cardiovascular effects, toxicity severity scores, treatment, follow-up times, and outcomes. Thirty-four (79.1%) of the patients who exceeded the daily dose were female, and there were no statistically significant differences between the groups in terms of gender (p= 0,281). The mean age was 29 (18-72) years, and there were no statistically significant differences between groups in terms of mean age (p= 0.192). When the vitals of the patients who exceeded the daily dose was examined, it was found that 23 (54.8%) patients had bradycardia, and 20 (46.5%) patients had hypotension. 65.2% of the bradycardia patients and 70% of the hypotensive patients were in the propranolol overdose group (p= 0.030 p= 0.021, respectively). Mean dose of symptomatic propranolol overdose patients (n= 12) was found as 1256 (280-2000) mg, mean dose of symptomatic metoprolol overdose patients (n= 11) was found as 559 (250-1000) mg. When toxic dose (≥ 400 mg) intakes were compared, more cardiovascular effects were observed in the propranolol group (p= 0.014). As a result, it was determined that Propranolol overdose has more cardiovascular effects than metoprolol overdose and there is a linear dose-symptom relationship for Propranolol.

Keywords: Beta blocker poisoning, propranolol, metoprolol, emergency medicine

1. Introduction

Due to their blood pressure and heart rate lowering effects, beta-adrenergic receptor antagonists (B-blockers) are commonly used in adults in the treatment of hypertension, tachycardia, cardiac angina, and heart failure (1). They have a risk of causing bradycardia, hypotension, bronchospasm, myocardial infarction, heart failure, and potentially fatal toxicity when taken above the daily recommended doses (2).

The cellular toxicity of B-blockers depends on their membrane stabilizing activities (MSA), lipophilicity, and intrinsic sympathomimetic activities (ISA). Agents with high MSA (Propranolol, Carvedilol, Acebutolol, Betaxolol, and Oxprenolol) inhibit fast sodium channels and cause a wide QRS range. Agents with high lipid solubility (Propranolol, Penbutolol, Metoprolol, and Betaxolol) cross the blood-brain barrier quickly and cause neurological side effects such as seizures and delirium. Agents with ISA (Pindolol, Penbutolol, Acebutolol, and Carteolol) have partial antagonist properties. They activate or block receptors depending on the situation; however, this protective effect of ISA does not completely prevent cardiovascular toxicity at toxic doses (3).

Propranolol is one of the first generation classical non-selective B-blockers with MSA, high lipid solubility, no ISA and a half-life of 3-5 hours. On the other hand, metoprolol is a second-generation B1-selective blocker with MSA, moderate lipid solubility, no ISA and a half-life of 3-7 hours (4–6).

In National Poison Solidarity Centre (UZEM) 2018 report, among the first 50 human health agents the cases were exposed to (according to ATC name), metoprolol ranked 39th (n=865, 0.56%), while propranolol ranked 45th (n=817, 0.53%) (7). In the American Association of Poison Control Centre (AAPCC) National Poison Data System (NPDS) 2020 report, 3,328 (86.0%) drug-related death cases were reported. Of these deaths, 263 occurred due to cardiovascular drugs (82 Amlodipine, 24 Metoprolol, 17 Propranolol, 15 Digoxin, 15 Verapamil, 14 Diltiazem, 11 Carvedilol, 10 Verapamil) (8).

*Correspondence: mehmet.altuntas@erdogan.edu.tr

B-blocker exposures are generally reported as case reports or case series. There are limited numbers of studies that investigated the comparison of clinical toxicity of propranolol and metoprolol. The present study was designed to investigate the differences and potential dose-related effects of propranolol and metoprolol toxicity depending on their selectivity.

2. Materials and methods

2.1. Patients and data collections

The files of patients older than 18 years of age who were evaluated with suspicion of B-blocker toxicity in the Emergency Department (ED) of Ondokuz Mayıs University Medical Faculty were analyzed retrospectively. This study was approved by The Clinical Research Ethics Committee of Ondokuz Mayıs University Medical Faculty (Decision number. 2014/920). Of the 57 patients who were suspected of B-blocker intoxication, 43 were included in the study. In multiple drug intakes, patients who had taken cardiovascular drugs (antihypertensive-antiarrhythmic) together with B-blockers were excluded from the study. Two patients who had taken multiple drugs, including propranolol, were excluded since they had not exceeded the daily propranolol dose (120-240 mg). Other B-blockers (Carvedilol, Nebivolol, and Bisoprolol) were excluded from the analysis. Patients with a history of severe cardiac arrhythmia, renal and hepatic dysfunction, and those who left the hospital voluntarily or without permission while their follow-up was continuing, were also excluded (Fig. 1).

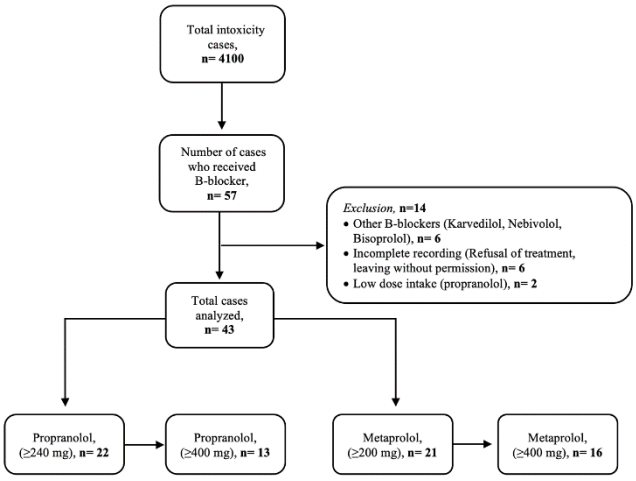

Fig.1. Flowchart

Patients were divided into groups, with a daily overdose of≥ 240 mg/day for propranolol, ≥ 200 mg/day for metoprolol, and a toxic dose of≥ 400 mg/day for both drugs (9). Heart rate (beat/min), PR interval (ms), QRS width (ms), QT distance (ms), and corrected QT (QTc) time (ms) (Bazett formula; QTC= QT/√RR) were calculated from the patients' ECGs. A PR interval of ≥ 200 ms was considered as a first-degree atrioventricular (AV) block, a QRS time of ≥100 ms was considered as interventricular conduction delay, and when patients' QTc values were calculated according to heart rate, ≥ 440 ms was considered as prolonged QT. Patients with systolic blood pressure (SBP) of ≤ 90 mmHg and a mean arterial pressure of [MAP= (SBP + 2DKB)/3] ≤ 70 mmHg at the time of admission to the hospital were considered hypotensive. A heart rate of ≤ 60 beats/minute measured at the time of admission to the hospital was considered bradycardia. The treatment of patients according to their current clinic, intoxication severity scores (o; no symptoms, 1; mild symptoms, 2; moderate symptoms, 3; severe symptoms, 4; death), the time between drug intake and hospital admission, and hospital stay were recorded.

Cardiovascular involvement was defined as:
An SBP of <90 mm Hg or a heart rate of <60 beats/min,
Symptoms suggestive of decreased end-organ perfusion (e.g., decreased consciousness, syncope, myocardial infarction) and
The need for a therapeutic intervention involving cardioactive drugs (e.g., atropine, glucagon, catecholamines) other than treatments with intravenous fluids alone
The patients who could not meet all three of the above criteria were considered as patients who did not have cardiovascular involvement.

2. 2. Statistical analysis

The statistical analyses were performed with IBM SPSS v.23. The normality of the continuous variables was analyzed with the Shapiro Wilk test. Pearson Chi-square test was used to compare the categorical variables, Mann Whitney U test was used to compare non-parametric qualitative data, and the T-test was used to compare the normally distributed data. The results were presented as mean ± standard deviation, median (min-max), frequency, and percentage. The level of significance was considered as $p < 0.05$.

3. Results

This 7-year-long retrospective cohort study was conducted among 43 adult patients who received overdose propranolol (n= 22) and metoprolol (n= 21). During the study period, 1.40% (57/4100) of adult poisoning cases were due to B-blocker intoxication. Thirty-four (79.1%) of the patients with overdose were female, and there were no statistically significant differences between the groups in terms of gender (p= 0,281). The mean age was 29 (18-72) years, and there were no statistically significant differences between groups in terms of mean age (p= 0,192). When the patients were examined according to the drugs they took, it was found that 20 (46.5%) patients had taken only B-blocker (propranolol or metoprolol), while 23 (53.5%) had taken more than one drug (multiple drugs). Antidepressants (31.0%), nonsteroid anti-inflammatory drugs (20.7%), analgesics (17.3%), and other drugs (31.0%) were the drugs most commonly taken together. When the vitals of the patients who exceeded the daily dose

was examined, it was found that 23 (54.8%) patients had bradycardia and 20 (46.5%) cases had hypotension. 65.2% of the bradycardia patients and 70% of the hypotensive patients were in the propranolol overdose group (p= 0,030 p= 0,021, respectively). No significant difference was found between groups in terms of cardiovascular involvement criteria (p= 0,151). Mean dose of asymptomatic patients (n= 20, 46.5%) was found as 734 (200-2000) mg, mean dose of asymptomatic propranolol overdose patients (n= 10) was found as 468 (240-1000) mg and mean dose of asymptomatic metoprolol overdose patients (n= 10) was found as 1000 (200-2000) mg. Mean dose of symptomatic patients (n= 23, 53.5%) was found as 923 (250-2000) mg, mean dose of symptomatic propranolol overdose patients (n= 12) was found as 1256 (280-2000) mg and mean dose of symptomatic metoprolol overdose patients (n= 11) was found as 559 (250-1000) mg. No statistically significant difference was found in terms of treatment and symptoms (Table 1).

Table 1. Clinical toxicity data in case of daily overdose

		Total, n=43	Propranolol, (≥ 240 mg), n= 22	Metoprolol (≥ 200 mg), n= 21	p
Female, n (%)		34 (79.1)	19 (55.9)	15 (44.1)	0.281[b]
Age, median (min-max)		29 (18-72)	28 (18-62)	30 (18-72)	0.192[a]
Drug dose, (mg), median (min-max)		640 (200-2000)	800 (240-2000)	500 (200-2000)	0.607[a]
Admission time, (min)		60(15-600)	60 (30-600)	60 (15-520)	0.307[a]
Drug intake, n (%)	Multiple drugs	23 (53.5)	12 (52.2)	11 (47.8)	0.887[b]
	Single drug	20 (46.5)	10 (50.0)	10 (50.0)	
Heart rate, (beats/min), median (min-max)		60 (46-92)	58 (48-80)	64 (46-92)	0.193[a]
Bradycardia, (≤ 60 beats/min), n (%)		23 (54.8)	15 (65.2)	8 (34.8)	**0.030***
Systolic BP, (mmHg), median (min-max)		100 (40-140)	90 (40-110)	100 (70-140)	**0.021[a]**
Hypotension, (≤ 90 mmHg), n (%)		20(46.5)	14 (70.0)	6 (30.0)	**0.021***
MAP, (mmHg), median (min-max)		73 (20-102)	70 (20-90)	73 (50-102)	**0.028[a]**
MAP, (≤ 70 mmHg), n (%)		21 (48.8)	15 (71.4)	6 (28.6)	**0.009***
PR distance, (ms), mean±SD		166.65 ± 32.72	173.64 ± 31.39	159.33 ± 33.22	0.154**
Long PR, (≥ 200 ms), n (%)		8 (18.6)	6 (75.0)	2 (25.0)	0.240[b]
QRS distance, (ms), median (min-max)		88 (70-172)	90 (70-106)	88 (72-172)	0.480[a]
QRS (≥ 100 ms), n (%)		6 (14.0)	3 (50.0)	3 (50.0)	0.999[b]
QTc, (ms), median (min-max)		400 (350-550)	400 (370-460)	410 (350-550)	0.642[a]
QTc, (≥ 440 ms), n (%)		8 (18.6)	3 (37.5)	5 (62.5)	0.457[b]
Cardiovascular involvement, n (%)		17 (39.5)	11 (64.7)	6 (35.3)	0.151*
Gastric lavage, n (%)		38 (88.4)	20 (52.6)	18 (47.4)	0.664[b]
Activated carbon, n (%)		38 (88.4)	20 (52.6)	18 (47.4)	0.664[b]
Therapeutic intervention, n (%)		17 (39.5)	11 (64.7)	6 (35.3)	0.151*
Atropine		15 (34.9)	9 (60.0)	6 (40.0)	0.396*
Dopamine		8 (18.6)	6 (75.0)	2 (25.0)	0.240[b]
Glucagon		3 (7.0)	2 (66.7)	1 (33.3)	0.999[b]
Symptomatic, n (%)		23 (53.5)	12 (52.2)	11 (47.8)	0.887*
Vomiting		6 (14.0)	2 (33.3)	4 (66.7)	0.412[b]
Dizziness		12 (27.9)	8 (66.7)	4 (33.3)	0.206*
Somnolence		5 (11.6)	2 (40.0)	3 (60.0)	0.664[b]
Chest pain		7 (16.3)	2 (28.6)	5 (71.4)	0.240[b]
Syncope		2 (4.7)	2 (100.0)	0	0.488[b]

*MAP; Mean Arterial Pressure, QTc; Corrected QT, a Mann Whitney U, * The Chi-square statistic, ** T test, b Fisher's Exact test*

When single-time overdose (≥ 400 mg) drug intakes were compared, more cardiovascular involvement was observed in the group that received propranolol, and it was statistically signification (p= 0,014). As expected, it was found that the group that received propranolol needed more therapeutic intervention (p= 0,014) (Table 2).

In terms of intoxication severity classification, 2 (4.7%) of the cases in the "severe" group had taken high dose propranolol. Both cases were symptomatic (syncope, chest pain), hypotensive (OAB= <40 mmHg) and had bradycardia (<40 beats/min). All of the cases were followed and treated in the ED, and they were discharged with recovery (Table 3).

Table 2. Clinical toxicity data in case of toxic overdose (≥ 400 mg)

		Total, n:29	Propranolol, (≥ 400 mg), n:13	Metoprolol (≥ 400 mg), n:16	p
Female, n (%)		22 (75.9)	11 (50.0)	11 (50.0)	0.410[b]
Age, median (min-max)		36 (18-65)	33 (19-62)	37 (18-65)	0.537[a]
Drug dose, (mg), median (min-max)		1000 (500-2000)	11000 (640-2000)	775 (500-2000)	0.036[a]
Admission time, (min)		60 (15-600)	60 (30-600)	0 (15-520)	0.288[a]
Drug intake, n (%)	Multiple drugs	15 (51.7)	6 (40.0)	9 (60.0)	0.588[b]
	Single drug	14 (48.3)	7 (50.0)	7 (50.0)	
Heart rate, (beats/min), median (min-max)		58 (48-92)	55 (48-60)	63 (48-92)	**0.031[a]**
Bradycardia, (≤ 60 beats/min), n (%)		19 (65.5)	12 (46.2)	7 (36.8)	**0.003[b]**
Systolic BP, (mmHg), median (min-max)		95 (40-120)	90 (40-110)	00 (70-120)	**0.015[a]**
Hypotension, (≤ 90 mmHg), n (%)		15 (51.7)	10 (66.7)	5 (33.3)	**0.014***
MAP, (mmHg), median (min-max)		70.86 ± 15.71	63.92 ± 16.02	76.50 ± 13.41	**0.029****
MAP, (≤ 70 mmHg), n (%)		15 (51.7)	10 (66.7)	5 (33.3)	**0.014***
PR distance, (ms), mean±SD		166 (110-242)	166 (116-242)	157 (110-240)	0.417[a]
Long PR, (≥ 200 ms), n (%)		6 (20.7)	4 (66.7)	2 (33.3)	0.364[b]
QRS distance, (ms), median (min-max)		88 (70-172)	88 (70-106)	6 (72-172)	0.481[a]
QRS (≥ 100 ms), n (%)		4 (13.8)	1 (25.0)	3 (75.0)	0.606[b]

QTc, (ms), median (min-max)	400 (350-550)	390 (370-440)	410 (350-550)	0.342[a]
QTc, (≥ 440 ms), n (%)	4 (13.8)	1 (25.0)	3 (75.0)	0.606[b]
Cardiovascular involvement, n (%)	15 (51.7)	10 (66.7)	5 (33.3)	**0.014***
Gastric lavage, n (%)	25 (86.2)	11 (44.0)	14 (56.0)	0.999[b]
Activated carbon, n (%)	25 (86.2)	11 (44.0)	14 (56.0)	0.999[b]
Therapeutic intervention, n (%)	15 (51.7)	10 (66.7)	5 (33.3)	**0.014***
Atropine	14 (48.3)	9 (64.3)	5 (35.7)	**0.042***
Dopamine	6 (20.7)	5 (83.3)	1 (16.7)	0.064[b]
Glucagon	3 (10.3)	2 (66.7)	1 (33.3)	0.573[b]
Symptomatic, n (%)	18 (62.1)	10 (55.6)	8 (44.4)	0.249[b]
Vomiting	6 (20.7)	2 (33.3)	4 (66.7)	0.663[b]
Dizziness	9 (31.0)	6 (66.7)	3 (33.3)	0.226[b]
Somnolence	3 (10.3)	2 (66.7)	1 (33.3)	0.573[b]
Chest pain	5 (17.2)	1 (20.0)	4 (80.0)	0.343[b]
Syncope	2 (6.9)	2 (100.0)	0	0.192[b]

*MAP; Mean Arterial Pressure, QTc; Corrected QT, a Mann Whitney U, * The Chi-square statistic, ** T test, b Fisher's Exact test*

Table 3. Intoxication severity and patient follow-up times in case of daily overdose

		Total, n=43	**Propranolol, (≥ 240 mg), n= 22**	**Metoprolol, (≥ 200 mg), n= 21**
Intoxication severity, n (%)	0 (no symptoms)	20 (46.5)	10 (50.0)	10 (50.0)
	1 (mild)	14 (32.6)	8 (57.1)	6 (42.9)
	2 (moderate)	7 (16.3)	2 (28.6)	5 (71.4)
	3 (severe)	2 (4.7)	2 (100.0)	0
	4 (death)	0	0	0
Follow up time, n (%)	24 hours	11 (25.6)	5 (45.5)	6 (54.5)
	24-48 hours	15 (34.9)	6 (40.0)	9 (60.0)
	48-72 hours	16 (37.2)	11 (68.8)	5 (31.3)
	72-96 hours	1 (2.3)	0	1 (100.0)

4. Discussion

The indications (angina pectoris, cardiac arrhythmias, thyrotoxicosis and migraine prophylaxis) for the use of these two B-blocker drugs, which are mainly used in the treatment of hypertension, are similar. In addition to being used in the treatment of essential tremor, hypertrophic obstructive cardiomyopathy, propranolol is also used “off-label” to treat fear of social situations, panic disorder and types of other anxiety disorders (10).

This study investigated propranolol and metoprolol toxicity in adults regardless of the intention of being exposed. Previous studies showed that propranolol is responsible for more exposure than other B-blockers and is associated with more deaths (1,3,9). In our study, it was found that propranolol and metoprolol did not differ in terms of the number of individuals exposed, gender and age.

It was found that the patients did not differ in terms of intoxication severity, frequency and distribution of symptoms following exposure. No optimal threshold at which patients became symptomatic was found for both drugs. The mean dose was found as 1256 (280-2000) mg for patients who took propranolol and as 468 (240-1000) mg for asemptomatik patients. While patients became symptomatic as the dose increased with propranolol, it was not the same with metoprolol. We could not answer with the available data whether this linear dose-response for propranolol was the class effect of B-blockers.

Neurological symptoms (seizure, delirium, somnolence, vomiting) have been reported due to high lipophilic propranolol and moderate lipophilic metoprolol, although B-blockers do not have strong sedative properties (9,11). Although there were no patients who had seizures in our study, somnolence, dizziness, and vomiting can be considered the symptoms of central nervous system depression. In terms of these symptoms, no difference was observed between groups in terms of overdose. Prominent symptoms were mainly bradycardia and hypotensive blood pressure measurements. There were more patients with bradycardia and hypotension in the propranolol group.

In a study investigating B-blocker cardiotoxicity, cardiotoxicity was found to be associated with notable ECG changes in most symptomatic patients. Negative dromotropic effects such as first-degree AV block and interventricular conduction delays were observed most frequently. Bradycardia (a negative chronotropic effect) was reported to be a less common manifestation (12). In our study, no difference was found between drug groups in terms of ECG findings. In terms of cardiovascular involvement, a statistically significant difference in favour of propranolol was found for intakes of >400 mg (p= 0,014). MSA characteristics of propranolol can be responsible for cardiovascular effects. Metoprolol is known to have high doses of MSA characteristics (5, 6).

Intravenous fluid (10-20 ml/kg saline) was started on all our patients regardless of their admission vitals. While 39.5% of the patients who exceeded the daily dose needed therapeutic intervention (atropine and/or dopamine), 51.7% of the overdose (≥ 400 mg) patients needed therapeutic intervention. A statistically significant difference in favour of the propranolol group was found in terms of atropine administration for overdose (p= 0,042). While there is weak evidence for the useful effects of glucagon, glucagon recommended in resistant shock was used only in three of our patients (13).

In our study, approximately half of the cases were symptomatic, but no deaths occurred. Groups were not found to differ significantly in terms of symptoms and intoxication severity. While there was a correlation between dose and symptoms for propranolol, there was no such correlation for metoprolol. Propranolol shows more cardiovascular effects

than metoprolol in overdose.

One of our study's limitations is that B-blocker doses were recorded according to patient reports without actual measurement of blood concentrations. For this reason, the reported doses were only predictions of potential exposure. Admission findings of the patients were obtained from records. To the best of our knowledge, there are no studies which make associations between and confirm intoxication severity and serum levels. Daily follow-up data of patients whose treatment processes started at admission were not included in the present study.

Conflict of interest

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Authors' contributions

Concept: F.Ö., M.A., C.K., Design: F.Ö., M.A., Data Collection or Processing: F.Ö., M.A., C.K., Analysis or Interpretation: F.Ö., M.A., Literature Search: F.Ö., M.A., C.K., Writing: F.Ö., M.A.

References

1. Reith DM, Dawson AH, Whyte IM, Buckley NA, Sayer GP. Relative Toxicity of Beta Blockers in Overdose. Journal of Toxicology: Clinical Toxicology. 1996; 34(3):273–8.
2. Metra M, Cas LD, Cleland JGF. Pharmacokinetic and Pharmacodynamic Characteristics of β-Blockers: When Differences May Matter. Journal of Cardiac Failure. 2006; 12(3):177–81.
3. Love JN, Howell JM, Litovitz TL, Klein-Schwartz W. Acute beta blocker overdose: factors associated with the development of cardiovascular morbidity. J Toxicol Clin Toxicol. 2000; 38(3):275–81.
4. Jaillon P. Relevance of intrinsic sympathomimetic activity for beta blockers. Am J Cardiol. 1990; 66(9):21C-23C.
5. Bylund DB, Gruetter CA. Propranolol. xPharm: The Comprehensive Pharmacology Reference. 2007, p. 1–9.
6. Gruetter CA. Metoprolol. In: xPharm: The Comprehensive Pharmacology Reference. 2007, p. 1–7.
7. Ulusal Zehir Danışma Merkezi (UZEM) Ayrıntılı Faaliyet Raporları 2014-2020 Yılları. TC Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü [Internet]. 2021; Available from: https://hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/Raporlar/Uzem/uzem_raporlari_2014-2020.pdf
8. Gummin DD, Mowry JB, Beuhler MC, Spyker DA, Bronstein AC, Rivers LJ, et al. 2020 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 38th Annual Report. Clin Toxicol (Phila). 2021; 59(12):1282–501.
9. Lauterbach M. Clinical toxicology of beta-blocker overdose in adults. Basic Clin Pharmacol Toxicol. 2019; 125(2):178–86.
10. Kriikku P, Pelkonen S, Kaukonen M, Ojanperä I. Propranolol and metoprolol: Two comparable drugs with very different post-mortem toxicological profiles. Forensic Science International. 2021; 327:110978.
11. Khalid MM, Galuska MA, Hamilton RJ. Beta-Blocker Toxicity. In Treasure Island (FL); StatPearls Publishing; July 26, 2021.
12. Love JN, Enlow B, Howell JM, Klein-Schwartz W, Litovitz TL. Electrocardiographic changes associated with β-blocker toxicity. Annals of Emergency Medicine. 2002; 40(6):603–10.
13. Rotella JA, Greene SL, Koutsogiannis Z, Graudins A, Hung Leang Y, Kuan K, et al. Treatment for beta-blocker poisoning: a systematic review. Clinical Toxicology. 2020; 58(10):943–83.

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Effects of cholecystectomy on lipid profile

Altan AYDIN[1,*], Doğan ÖZTÜRK[2]

[1]Department of General Surgery, Health Sciences University, Trabzon Kanuni Education and Research Hospital, Trabzon, Turkey
[2]Department of General Surgery, Kecioren Training and Research Hospital, Ankara, Turkey

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Abstract

Cholecystectomy is widely used to treat gallstone disease as one of the surgical procedures which have been most frequently performed worldwide. The bile acids pool has unchanged size, but it has fast circulation, which causes to expose the enterohepatic organs to increased flux of bile acids per day in cholecystectomized patients. This study aims to evaluate blood lipid levels before and after cholecystectomy. The total number of 80 consecutive patients underwent elective cholecystectomy were included in this study. Lipid parameters, biochemical analysis was obtained before surgery and after 12 weeks of cholecystectomy. Mean age was 49±13. Sixty (75%) patients were female. None of the subjects died during perioperative course. Levels of total cholesterol (191.9 ±37.1 mg/dl vs. 186.1 ±36.8 mg/dl, respectively; p=0.006), LDL-C (142.6±41.7 mg/dl vs. 133.9±35.1 mg/dl, respectively; p<0.001) were significantly reduced after eight weeks of cholecystectomy, whereas triglyceride and HDL-C levels were not significantly altered. This study demonstrated that cholecystectomy causes a significant reduction of LDL and total cholesterol levels. Prospective randomized studies should help for determining of possible clinical positive effects of cholecystectomy on atherosclerosis.

Keywords: atherosclerosis, blood cholesterol, cholecystectomy, gallstone

1. Introduction

The gallbladder supports the maintenance of lipid homoeostasis of the human body. The gallbladder plays an essential role in the digestion and absorption of lipid by concentrating and storing hepatic bile. Water is the main component of bile, the initial excretory route for organic compounds such as low water solubility drugs, lipid hormones, and cholesterol (1-2). Peripheral uptake and cholesterol synthesis are mainly located in the hepatocyte, and excess cholesterol is converted into bile salts or directly secreted into bile (3).

Cholecystectomy is widely used to cure gallstone disease as one of the surgical procedures most frequently performed worldwide. It is generally accepted that gallbladder removal is a benign condition without negatively affecting the normal health status or overall metabolic regulation. In fact, digestion and absorption of dietary fats and lipid-soluble vitamins are normal after cholecystectomy (4).

The bile acids pool has an unchanged size, but it has fast circulation, which exposes the enterohepatic organs to increased flux of bile acids per day in cholecystectomized patients (5-8).

Due to the fast circulation of the bile after cholecystectomy, the higher flux of molecules of bile acids per unit time could affect blood lipid levels. This study aimed to evaluate levels of blood lipid before and after cholecystectomy.

2. Materials and Methods

2.1. Subjects

Eighty consecutive patients undergoing elective cholecystectomy participated in this study. We conducted a detailed examination of all participants through physical examinations and taking medical histories such as lifestyle habits. We excluded the patients who received statin and fibrate therapy, had alcohol intake above 30 g per day, acute cholecystitis, liver failure, familial hypercholesterolemia, renal failure, or hypertriglyceridemia. We obtained lipid parameters and conducted biochemical analysis before surgery and after 12 weeks of cholecystectomy. We abided by the Declaration of Helsinki to have the protocol approved by the local ethics committee and received informed consent from all patients.

2.2. Cholecystectomy

We performed laparoscopic cholecystectomy using the four-trocar technique (10 mm umbilical, 10 mm subxiphoid, 5 mm subcostal and 5 mm midaxillary line).

*Correspondence: altanaydin76@hotmail.com

Extra trocars may be helpful in complex cases, but we did not use them. For patients with suspected intra-abdominal adhesions, we placed the first trocar using the open technique and the others by visual assistance.

We initiated dissection with adhesions and followed by Callot's triangle, applied double clip after the cystic artery and duct dissection and performed cholecystectomy. We routinely extracted the gall bladder from a 10 mm subxiphoid trocar and used endobag for patients with perforation.

2.3. Lipids measurement

We took fasting blood samples from participants' antecubital veins after fasting of >12 hours. We let the blood samples clot at room temperature for 20-30 minutes and then centrifuged them at 3000 xg for five minutes and froze them to below -800C until analysis. We performed enzymatic colorimetric tests to measure triglycerides (Lot No: C186, Konelab) and total cholesterol (Lot No: B540, Konelab) while performing the homogeneous enzymatic colorimetric test to measure high-density lipoprotein-cholesterol (HDL-C) (Lot No: C136, Konelab) and low-density lipoprotein-cholesterol (LDL-C) (Lot No: C435, Konelab).

2. 4. Statistical analysis

We used the Kolmogorov-Smirnov test to determine the distribution of the continuous variables. We expressed variables with skew distribution as median (minimum-maximum), continuous normally distributed variables as mean±SD, and categorical variables as percentage. We used the Wilcoxon rank-sum test for skew distributed variables and the paired sample t-test for variables with normal distribution. We identified correlations between study parameters through Spearman and Pearson analyses. A p-value<0.05 showed statistical significance in all statistics. We used SPSS 10.0 for Windows for all analyses.

3. Results

Eighty patients participated in the study. The mean age was 49±13. Sixty (75%) patients were female. None of the subjects died during the perioperative course. We performed open cholecystectomy in four patients and laparoscopic in 76 patients. The mean hospitalization duration was 2.1±0.3

Nine patients were (11%) diabetic, while 14 (17%) were hypertensive. There was no significant change in alanine aminotransferase (ALT) or gamma-glutamyltransferase (GGT) levels, plasma fasting glucose or aspartate aminotransferase (AST) after cholecystectomy.

Table 1 shows the effects of cholecystectomy on lipid and biochemical parameters. Levels of total cholesterol (191.9±37,1 mg/dl vs. 186.1±36.8 mg/dl, respectively; p=0.006) and LDL-C (142.6±41.7 mg/dl vs. 133.9±35.1 mg/dl, respectively; p<0.001) decreased significantly after eight weeks of cholecystectomy. Triglyceride (147.3±75.2 mg/dl vs 144.2±68.2 mg/dl, respectively; p=NS) and HDL-C levels (39.8±9.2 mg/dl vs. 38.7±8.3 mg/dl, respectively; p=NS) did not change significantly after eight weeks of cholecystectomy.

Table 1. Biochemical parametres before and after cholecystectomy

	Before cholecystectomy	After cholecystectomy	p-value
Fasting plasma glucose (mg/dl)	100.8±18.8	99.7±17.5	NS
Triglyceride (mg/dl)	147.3±75.2	144.2±68.2	NS
Total cholesterol	191.9±37.1	186.1±36.8	0.006
LDL-cholesterol (mg/dl)	142.6±41.7	133.9±35.1	<0.001
HDL-cholesterol (mg/dl)	39.8±9.2	38.7±8.3	NS
GGT (U/l)	29±3.7	28.5±5.1	NS
ALT (U/l)	29.2±3.7	30.1±3.4	NS
AST (U/l)	24.3±5.8	26.1±4.2	NS
Creatinine (mg/dl)	0.8±0.1	0.8±0.1	NS

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol

4. Discussion

This study evinced a significant decline in LDL-C and total cholesterol levels after eight weeks of cholecystectomy, whereas HDL-C and triglyceride levels did not change significantly.

Several controversial studies evaluated the impact of cholecystectomy on lipid levels. Juvonen et al. demonstrated that LDL cholesterol and plasma total levels significantly decreased in cholecystectomy patients at day three following the operation. The values returned to the preoperative level thereafter in 19 patients (9). Malik et al. reported a significant reduction of the plasma concentration of triglycerides, total cholesterol, and LDL cholesterol in patients on the third day of surgery and after six months in 73 patients (10). Van der Linden et al. demonstrated that removing the functioning gallbladder caused irregular but accelerated flow of activity to the duodenum using 99mTc-HIDA (11). Decreased total and LDL cholesterol levels may be associated with this higher flux of bile per unit time. Accelerated enterohepatic bile flow causes more excretion of bile acids and cholesterol from the liver. It may result in lower blood cholesterol levels.

Cholecystectomy does not negatively affect normal health status or total metabolic regulation as a benign condition. Lipid soluble vitamins and dietary fats are normally digested and absorbed after cholecystectomy. This study included a positive effect of cholecystectomy (4). Dyslipidemia is a major risk factor for cardiovascular diseases, and LDL cholesterol is highly crucial information

and progression of atherosclerosis (12-13). Decreasing total and LDL cholesterol levels may prevent this process. Clinical trials evinced cardiovascular disease risk reduction by about 2% through a 1% reduction of serum total cholesterol level. Statins trials indicate a risk reduction by about 1% through a 1% decrease of LDL cholesterol (14). We found 9 mg/dl (6%) LDL reduction after cholecystectomy. This LDL reduction may be related to an amazing effect of cholecystectomy.

Amigo et al. showed higher serum TG and hepatic concentrations in cholecystectomized mice (15). They suggested that systemic metabolic homoeostasis vitally required gallbladder function, and cholecystectomy might not be harmless. Our study did not support their findings. We found that cholecystectomy had no significant effect on triglyceride levels in humans.

This study demonstrated that cholecystectomy caused a significant reduction of LDL and total cholesterol levels. Furthermore, the results of this study showed that cholecystectomy did not affect triglyceride and HDL-C levels. Cholecystectomy is essential in preventing atherosclerosis and comorbid diseases related to atherosclerosis by reducing LDL and total cholesterol levels. Prospective randomized studies should help determine possible clinical positive effects of cholecystectomy on atherosclerosis.

Conflict of interest

Authors of this study do not have any conflict of interest.

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Authors' contributions
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References

1. Browning MG, Pessoa BM, Khoraki J, Campos GM. Changes in Bile Acid Metabolism, Transport, and Signaling as Central Drivers for Metabolic Improvements After Bariatric Surgery. Curr Obes Rep. 2019 Jun;8(2):175-184.
2. Boyer JL. Bile formation and secretion. Compr Physiol. 2013 Jul;3(3):1035-78.
3. Houten SM, Watanabe M, Auwerx J. Endocrine functions of bile acids. EMBO J 2006; 25(7):1419-25.
4. Krondl A, Vavrinkova H, Michalec C. Effect of Cholecystectomy on the Role of the Gall Bladder in Fat Absorption. Gut. 1964 Dec;5(6):607-10.
5. Almond HR, Vlahcevic ZR, Bell CC, Jr., Gregory DH, Swell L. Bile acid pools, kinetics and biliary lipid composition before and after cholecystectomy. N Engl J Med. 1973 Dec 6;289(23):1213-6.
6. Roda E, Aldini R, Mazzella G, Roda A, Sama C, Festi D, Barbara L. Enterohepatic circulation of bile acids after cholecystectomy. Gut. 1978 Jul;19(7):640-9.
7. Kullak-Ublick GA, Paumgartner G, Berr F. Long-term effects of cholecystectomy on bile acid metabolism. Hepatology. 1995 Jan;21(1):41-5.
8. Shaffer EA, Small DM. Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. J Clin Invest. 1977 May;59(5):828-40.
9. Juvonen T, Kervinen K, Kairaluoma MI, Kesaniemi YA. Effect of cholecystectomy on plasma lipid and lipoprotein levels. Hepatogastroenterology. 1995 Jul-Aug;42(4):377-82.
10. Malik AA, Wani ML, Tak SI, Irshad I, Ul-Hassan N. Association of dyslipidaemia with cholilithiasis and effect of cholecystectomy on the same. Int J Surg. 2011;9(8):641-2.
11. van der Linden J, Jr., Kempi V, van der Linden W. Bile flow before and after cholecystectomy: a study with 99mTc-HIDA. Acta Chir Scand. 1985;151(5):461-4.
12. Chen YP, Chang KC, Tseng WK, Yin WH, Chen JW, Lee YT, Wu CC. Increased Rosuvastatin Dose versus Concomitant Fenofibrate and Rosuvastatin Therapy to Achieve Lipid Goal in Patients with Diabetes or Atherosclerosis with Metabolic Syndrome. Acta Cardiol Sin. 2013 Sep;29(5):421-8.
13. Simko V, Ginter E. How far down to push the LDL cholesterol? PCSK9 where statins fail. Bratisl Lek Listy. 2016;117(4):193-4.
14. Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001 May 16;285(19):2486-97.
15. Amigo L, Husche C, Zanlungo S, et al. Cholecystectomy increases hepatic triglyceride content and very-low-density lipoproteins production in mice. Liver Int. 2011 Jan;31(1):52-64.

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Cardiovascular biomarkers in pulmonary hypertension- current applications and future directions

Asparuh G. NIKOLOV[1,*], Pavlina GLOGOVSKA[2]

[1]Department of Pre-clinical and Clinical Sciences, Faculty of Pharmacy, Medical University-Pleven, Bulgaria
[2]Department of Propedeutics of Internal Diseases, Clinic of Pneumology and Phthisiatry, Medical University-Pleven, Bulgaria

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Abstract

Biomarkers are non-invasive tools, which can be very useful in diagnosis and prognosis of a specific disease. Pulmonary hypertension (PH) is a serious health condition, characterized by increased pulmonary arterial pressure, elevated pulmonary vascular resistance often leading to right heart failure. If not treated properly, it can significantly reduce patients' quality of life and even lead to death. Nowadays, there is an increasing interest on PH markers and more specifically how they can contribute to the diagnosis, prognosis, and monitoring of the development of this disease. Recent studies on biomarkers in PH suggest several novel and promising molecules, which can be potentially useful in PH work-up and follow-up strategies. This review focuses on biomarkers for diagnosis and prognosis of PH via well-known and some novel cardiovascular disease (CVD) indicators associated with heart failure, myocardial remodelling, and injury. Current thinking holds that CVD biomarkers can detect not only heart abnormalities but also pulmonary vascular system damage.

Keywords: pulmonary hypertension, biomarkers, heart, lung, pulmonary arterial hypertension

1. Introduction

1.1. Pulmonary hypertension- overview

Pulmonary hypertension (PH) is a progressive disease caused by different etiological factors and classified into many subtypes according to the specific cause which can lead to irreversible complications and a lethal outcome if not treated properly (1). Based on the current approach towards PH pathogenic mechanisms, increased pulmonary arterial pressure (PAP) and elevated pulmonary vascular resistance (PVR) have been considered as PH's cardinal features. They often lead to right ventricular (RV) overload and right heart failure (HF) (2). According to the latest ESC and ERS guidelines, right heart catheterization has been highlighted as the definitive and confirmatory gold standard for diagnosis, prognosis, and defining the etiology of PH (3). The disease's course can be monitored by image tests such as pulmonary artery systolic pressure measurement via transthoracic echocardiography. Functional measures, such as estimation of functional class and the 6-min walk test can also be evaluated (4).

Biomarkers are non-invasive indicators which can be related to structural and/or functional changes in various organs and systems in human body. These parameters can be associated with specific diseases and have been proposed for assessing diagnosis, prognosis and response to therapy. Nowadays, significant advances have been made in the investigation of PH circulating biomarkers. Different blood indicators have been identified and the large majority of them have the potential to be implemented in the routine clinical practice in the future.

2. Heart structure/function related biomarkers in pulmonary hypertension

Researchers are intensively studying various molecules, which are candidates for potential PH biomarkers. Noteworthy, cardiovascular biomarkers are promising indicators for that role, because they can reveal not only heart abnormalities, but also pulmonary vascular system injury. Despite the overlap in some features of cardiovascular biomarkers, they can be classified into the following basic categories:

2.1. Markers Related to Myocardial Remodeling

Galectin-3 (Gal-3) is a protein, member of lectins family known to be secreted by macrophages as a result of mechanical and neurohumoral stimulation (5). It is presumed to have an interaction with extracellular matrix (ECM) proteins and cell surface glycoproteins. Gal-3 has abilities to interact with ECM proteins and cell surface glycoproteins in physiological and pathological conditions (6). Moreover, Gal-

*Correspondence: a_nicoloff@yahoo.com

3 can also activate other macrophages, fibroblasts and inflammatory cells. Galectin-3 has been associated with myocardial remodeling and fibrosis in patients with heart failure (7,8,9). In terms of pulmonary hypertension, Fensteret et al. (10) examined Gal-3 in patients with RV dysfunction and pulmonary arterial hypertension (PAH) and found a significant correlation between abnormal changes in RV structure and Gal-3 concentrations. Of note, Gal-3 levels were increased in all patients with PAH (11). In another investigation, Calvier et al. found increased Gal-3 concentrations in patients with PAH. The authors also described correlations between Gal-3 and functional parameters (12). Mazurek et al. conducted a research involving both PAH and PH patients due to left-sided HF. Authors reported that increased Gal-3 levels were associated with mortality in PH patients (13). In the study of Geenen et al. including 164 patients with PAH, chronic thromboembolic pulmonary hypertension (CTEPH) or PH caused by lung disease, there were no significant differences in the Gal-3 levels between subgroups (14).

Soluble ST2 (sST2) ST2 protein is part of Toll inteurleukin-1 receptor superfamily and has two isoforms: transmembrane ST2 ligand (ST2L) and soluble ST2 (sST2), which circulates in the blood (15). It has been postulated that the transmembrane form is expressed predominantly on inflammatory cells and plays an important role in the acceleration of the immune response of Th2-lymphocytes. An intriguing fact is that ST2 has also been detected in cardiomyocytes and endothelium. Interleukin-33 has been recognized as a ligand for ST2. In that way, IL-3/ST2L system plays a protective role and has anti-fibrotic and anti-hypertrophic functions. Increased ST2 serum levels have been recognized to be related with cardiac remodeling (16). Studies have shown that the ST2 gene is induced during abnormal cardiomyocyte stretch or cardiac fibrosis. ST2 has been reported to be involved in left ventricular (LV) hypertrophy, fibrosis and remodeling via interaction with interleukin-33 (IL-33).

Recently, sST2 levels have been found to correlate with RV remodeling in various PAH types. The higher sST2 levels have been suggested to be linked with RV remodeling in different types of PH (17). In 2020, Banaszkiewicz et al. studied patients diagnosed with PAH or CTEPH. Researchers found correlations between sST2 and cardiac index (CI), mean right atrial pressure (mRAP), PVR, mixed venous oxygen saturation, N-terminal pro b-type natriuretic peptide concentration and 6 min walking distance (6MWD) (18). These observations are consistent with those from other studies conducted in smaller populations of patients with precapillary PH (19,20). Contrary to that, no analogous changes in N-terminal pro b-type natriuretic peptide levels were noticed, which may be suggestive of an additional noncardiac source of sST2 in CTEPH patients. Therefore, in PH, sST2 may reflect not only heart condition, but also pulmonary vascular system and lung tissue (21). These findings support the theory that sST2 could be used as a marker for risk stratification of patients with RHF.

2.2. Indicators of Neurohumoral Activation

Mid-regional proadrenomedullin (MR-proADM) is a novel cardiovascular biomarker, which has been investigated in recent years. It is known that one of the first evidences of cardiac dysfunction is the activation of the sympatic nerve system. MR-proADM has been described as a precursor of the powerful vasodilator with inotropic properties-adrenomedullin, initially isolated from pheochromocytomic cells. MR-proADM has been shown to be increased in patients with acute and chronic heart failure (22,23,24). MR-proADM has been demonstrated as a strong predictor of clinical events such as mortality and hospitalization, even in addition of natriuretic peptides.

MR-proADM has been suggested as a promising indicator to predict prognosis and a helpful tool in the evaluation of different cardiopulmonary diseases (25,26,27). As for functional parameters, it was found that MR-proADM strongly correlates with exercise capacity in PAH patients (28).

Copeptin is also known as CT-proAVP. COP is a peptide involving 39 amino acid chains, derived by C-terminal of pre-pro-hormone of arginine vasopressin, neurophisin II and copeptin. Arginine vasopressin (AVP), known also as antidiuretic hormone (ADH) plays a key role in many cardiovascular and renal conditions. Its abnormal levels have been associated with different pathologies. Unfortunately, AVP measurement has not been incorporated in the routine clinical practice, because of its short half-life. Contrary to that, copeptin can be easily detected by immunoassays and is also used as a vasopressin secretion surrogate indicator. Copeptin has been reported as a valuable tool in the monitoring of cardiovascular pathologies such as myocardial infarction, left ventricular hypertrophy, cardiogenic shock and heart failure. Its expression correlated with survival, severity and disease prognosis (29). Copeptin also significantly correlates with 6MWD and New York Heart Association (NYHA) class as well as with kidney function in PAH patients (30,31,32). Moreover, in a study performed by Nickel et al., elevated copeptin concentration was related to a higher risk of death and it was an independent predictive factor of adverse outcomes in PAH patients (33).

2.3. Biomarkers Related to Myocardial stress/injury

Natriuretic peptides- brain natriuretic peptide (BNP) and N-terminal pro b-type natriuretic peptide (NT-pro-BNP). BNP is initially secreted as a pro-hormone (pro-BNP) by the heart ventricles in response to increased ventricular wall stress and myocardial hypoxia or ischemia. BNP and NT-proBNP are produced from pro-BNP via several enzyme-controlled reactions. BNP and NT-pro-BNP have predictive value for cardiovascular death and congestive heart failure (34).

Measurement of NT-pro-BNP increases prognostic assessment for cardiovascular death and exacerbation of HF. BNP is a powerful predictor for cardiovascular death and strongly correlates with LV-systolic stress and LV-dysfunction. Therefore, BNP and NT-pro-BNP are independent predictors for CVD morbidity and mortality in patients with HF. The American College of Cardiology (ACC) and American Heart Association (AHA) recommend BNP и NT-pro-BNP assessment in diagnosis and prognosis of HF. The European Society of Cardiology (ESC) also recommends BNP and NT-pro-BNP for evaluation of HF severity and evaluation of therapeutic response (35).

As mentioned above, BNP is produced as an inactive precursor (pro-BNP), then converted into the active form NT-pro-BNP and released from cardiomyocytes. Due to the longer half-life of NT-pro-BNP compared to BNP, NT-pro-BNP is preferred in clinical practice as a marker of heart overload and myocardial dysfunction. NT-pro-BNP remains a well-established and widely used biomarker in numerous cardiovascular diseases. NT-pro-BNP is mostly used in the diagnostic process of patients with acute or chronic HF as well as in prognostic strategies. ESC/ERS guidelines classify the risk of 1-year mortality according to NT-pro-BNP concentrations as low (<5%), intermediate (5–10%), or high (>10%), by using specific thresholds of 300 and 1400 ng/L (36). Similarly, data from the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) registry show that a baseline NT-pro-BNP level of 340 ng/L is a strong predictor of improved survival in PAH patients for up to 5 years (37). Studies by Galie et al. in 2017 and Chin et al. in 2019 demonstrated that a significant decrease in NT-pro-BNP levels among patients with PAH is associated with the response to targeted medical therapy (38,39). There is evidence that in PH patients, NT-pro-BNP concentrations correlate with right heart dysfunction. Measurement of this indicator can provide prognostic information at diagnosis and during follow-up assessment (40,41,42). In 2017, Berghaus et al. described a high variability of NT-pro-BNP levels and proposed a possible inadequate correlation with hemodynamic parameters and exercise capacity. That is why, it should only be interpreted in the clinical context (40). Currently, NT-pro-BNP is an important component in the PAH risk stratification strategy proposed by ESC/ERS guidelines (39) and is addressed in both the risk score method developed from the REVEAL registry (37,43). An important conclusion has been provided from the analysis of patients' data with CTEPH: BNP seemed to be related with both: (1) the degree of RV dysfunction and hemodynamic severity of the disease, and (2) BNP may contribute to the assessment of pulmonary endarterectomy effect with estimated BNP baseline cut-off values predictive of worse postoperative survival (44,45). In another study, Kriechbaum et al. reported two intriguing findings: (1) balloon pulmonary angioplasty (BPA) as well as pharmacological treatment result in a decrease of NT-pro-BNP concentration and (2) the procedural success of BPA in patients treated with BPA could be indicated by a reduction in NT-pro-BNP concentration (46).

Cardiac Troponins

The 2020 ESC Guidelines for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation pointed-out both cardiac troponin I (cTnI) and T (cTnT) as one of the key laboratory parameters from all ACS diagnostic methods (47). Furthermore, the development of high-sensitivity assays has made it possible to detect troponin concentrations and their association with morbidity and mortality in many chronic diseases, such as heart failure, coronary artery disease, or chronic kidney disease (48–51). Literature data describes that in most cases troponin levels correlate with left heart structural and functional parameters and some other indicators related to left HF. There is increasing evidence that the mechanism of troponins release in patients with PH seems to be associated with RV pathology. It has been theorized that RV alteration is caused by misbalance of demand/perfusion processes or microcirculatory injury. Researches by Velez-Martinez et al. and Torbicki et al. have found significant correlations between troponins' concentration and some hemodynamic parameters as mean PAP, mixed venous oxygen saturation and RV ejection fraction (52,53). Moreover, both cTnT and cTnI concentrations were associated with worse outcomes in mixed cohorts of PH patients (54,55). In order to perform accurate prognostic assessment and risk stratification of patients, ESC guidelines recommend troponin levels to be measured at the diagnosis of PAH, then at least once a year or whenever the patient presents with clinical worsening (36). In 2018, Kriechbaumet al. analysed a group of CTEPH patients undergoing interventional treatment with BPA. The authors found that high-sensitivity cTnT concentration decreases stepwise under therapy. This finding indicates a reduction of ongoing myocardial damage due to decreased right ventricular afterload following BPA therapy (56). Consequently, troponins can be useful markers in monitoring the treatment results of CTEPH patients.

3. Other potential cardiovascular markers in PH

Homocysteine is a sulfhydryl-containing amino acid, known as an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine. Homocysteine is a homologue of the amino acid cysteine. There are only two studies, which have investigated this sulfhydryl-containing amino acid and they revealed increased homocysteine levels in PH. Firstly, Ozerol et al. in 2004 examined patients with congenital heart disease and reported elevated homocysteine concentrations in patients with congenital heart disease and PAH compared to patients with left to right shunt surgery and no PAH (57). Later, a study by Sanliet al. in 2012 focused on patients with congenital heart disease. Authors found an association between homocysteine concentrations and

asymmetric dimethylarginine (ADMA) levels (ADMA is known as a natural amino acid, which plays an important role in the organism via inhibition of nitricoxide production). Researchers reported elevated homocysteine levels in patients with PAH and congenital heart disease. Furthermore, homocysteine concentrations were significantly increased in cyanotic patients compared to those without cyanosis (58).

Heart-Type Fatty Acid-Binding Protein (H-FABP). Fatty Acid-Binding Protein (FABP) plays key role in the regulation of energy metabolism. FABP can be divided into two major types: liver FABP (L-FABP) and heart FABP (H-FABP). The classification is not completely accurate, because these proteins can be found not only in the above mentioned tissues. H-FABP is expressed in the cytosol of cardiomyocytes. It has been estimated that higher levels of H-FAPB are related with increased hospitalizations because of heart failure (59). In patients with chronic heart failure, elevated serum levels of H-FABP have been associated with poor prognosis (60). H-FABP has also been proposed as an additional biomarker for early diagnosis of ACS (61). In terms of PH, Lankeit et al. explored whether H-FABP could contribute in CTEPH risk stratification. They found that H-FABP is an independent marker of adverse outcomes (62). The routine application of markers of PH in clinical practice could provide improved risk stratification and better patient outcomes (63). Fig. 1 represents the basic categories of cardiovascular biomarkers in pulmonary hypertension.

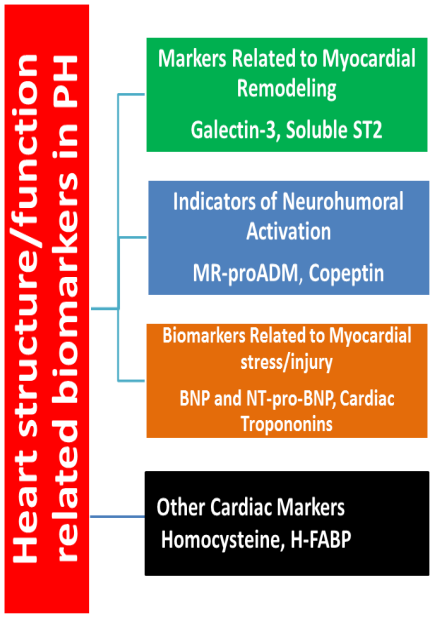

Fig. 1. Schematic presentation of heart structure/function related biomarkers in pulmonary hypertension

4. Conclusions and future directions

Nowadays, there has been an increasing interest in the research field of PH and especially on biomarkers as potential tools for diagnosis and prognosis of this disease. There are a growing number of investigations providing data for novel and promising non-invasive biomolecules, which could be useful in the evaluation of severity, prognosis and monitoring of PH. Given the complexity of PH, because of its different aetiology, hemodynamic, biochemical parameters and response to treatment, it is a great challenge for researchers to point-out only one marker that would be completely informative for clinical assessment of patients with different types of PH. That is why, a combination of different types of biomarkers would probably increase the sensitivity and specificity of the work-up and follow-up algorithms. Unfortunately, the optimal combination of blood indicators has not been discovered yet. Apparently, the use of multimarker strategies would be a more successful method. In conclusion, cardiovascular biomarkers have future potential as PH indicators and might contribute to early diagnosis, prognosis and monitoring of the development of pulmonary hypertension.

Conflicts of Interest

The authors declare no conflict of interest.

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References

1. Galie N, McLaughlin VV, Rubin LJ, et al. An overview of the 6th World Symposium on Pulmonary Hypertension. Eur. Respir. J. 2019; 53:1802148.
2. Galie N, HumbertM,Vachiery JL, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur. Respir. J. 2015; 46:903–975.
3. Simonneau G, Galie N, Rubin LJ, et al. Clinical classification of pulmonary hypertension. J Am CollCardiol 2004; 43: Suppl. 12, 5S–12S.
4. Iannuzzi GL, D'Alto M, Formisano R, Maniscalco M. Biomarkers in clinical management of pulmonary hypertension: has the emperor no clothes? A call for action. Biomark Med. 2019; 13(4):235-238. doi: 10.2217/bmm-2018-0491. Epub 2019 Mar 18. PMID: 30883210.
5. Ho JE, Liu C, Lyass A, et al. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. J Am CollCardiol. 2012;60:1249–56.
6. Brinchmann MF, Patel DM, Iversen MH. The role of galectins as modulators of metabolism and inflammation.Mediators Inflamm.2018: 9186940.
7. van Kimmenade RR, Januzzi JL, Ellinor PT, et al. Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure. Journal of the American College of Cardiology.

2006;48(6):1217–24. doi:10.1016/j.jacc.2006.03.061. PMID 16979009.

8. Lok DJ, Van Der Meer P, de la Porte PW, et al. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study. Clinical Research in Cardiology. 2010; 99(5): 323–8.

9. de Boer RA, Voors AA, Muntendam P, et al. Galectin-3: a novel mediator of heart failure development and progression. European Journal of Heart Failure. 2009; 11(9):811–7

10. Fenster BE, Lasalvia L, Schroeder JD, et al. Galectin-3 levels are associated with right ventricular functional and morphologic changes in pulmonary arterial hypertension. Heart Vessels.2016;31:939–46.

11. Li L, Li J, Gao J. Functions of galectin-3 and its role in fibrotic diseases. J PharmacolExpTher.2014;351:336–43.

12. Calvier L, Legchenko E, Grimm L, et al. Galectin-3 and aldosterone as potential tandem biomarkers in pulmonary arterial hypertension. Heart 2016; 102:390–396.

13. 13. Mazurek JA, Horne BD, Saeed W, et al. Galectin-3 Levels Are Elevated and Predictive of Mortality in Pulmonary Hypertension. Heart Lung Circ. 2017; 26:1208–1215.

14. Geenen LW, Baggen VJM, Koudstaal T, et al. The prognostic value of various biomarkers in adults with pulmonary hypertension; a multi-biomarker approach.Am. Heart J. 2019; 208:91–99.

15. Weinberg EO, Shimpo M, De KeulenaerGW,et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. Circulation 2002;106:2961–2966.

16. Sanada S, Hakuno D, Higgins LJ, et al. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. J Clin Invest 2007;117:1538-49.

17. Carlomagno G, Messalli G, Melillo RM, et al. Serum soluble ST2 and interleukin-33 levels in patients with pulmonary arterial hypertension. Int. J. Cardiol. 2013; 168:1545–1547.

18. Banaszkiewicz M, Pietrasik A, Darocha S, et al. Soluble ST2 protein as a new biomarker in patientswith precapillary pulmonary hypertension. Archives of Medical Science. 2020. doi:10.5114/aoms.2020.98635..

19. Geenen LW, Baggen VJM, Kauling RM, et al.The Prognostic Value of Soluble ST2 in Adults with Pulmonary Hypertension. J. Clin. Med. 2019; 8:1517.

20. Zheng YG, Yang T, He JG,et al. Plasma soluble ST2 levels correlatewith disease severity and predict clinical worsening in patients with pulmonary arterial hypertension. Clin.Cardiol.2014; 37:365–370.

21. Banaszkiewicz M, Pietrasik A, Florczyk M, et al. Soluble ST2 as a Biomarker for Early Complications in Patients with Chronic Thromboembolic PulmonaryHypertension Treated with Balloon Pulmonary Angioplasty. Diagnostics 2021; 11:133.

22. Kolditz M, Seyfarth H-J, Wilkens H et al. MR-proADM predicts exercise capacity andsurvival superior to other biomarkers in PH. Lung. 2015;193:901–10.

23. Kümpers P, Nickel N, Lukasz A, et al. Circulating angiopoietinsinidiopathic pulmonary arterial hypertension. Eur Heart J. 2010;31:2291–300.

24. Maisel A, Mueller C, Nowak R, et al. Mid-region pro-hormonemarkers for diagnosis and prognosis in acute dyspnea: results from the BACH(Biomarkers in Acute Heart Failure) trial. J Am CollCardiol. 2010;55:2062–76.

25. Khan SQ, O'Brien RJ, Struck J, et al. Prognostic value of midregional pro-adrenomedullinin patients with acute myocardial infarction: the LAMP (Leicester AcuteMyocardial Infarction Peptide) study. J Am CollCardiol. 2007;49:1525–32.

26. Krüger S, Ewig S, Giersdorf S, Hartmann O, Suttorp N, Welte T, and GermanCompetence Network for the Study of Community Acquired Pneumonia(CAPNETZ) Study Group. Cardiovascular and inflammatory biomarkers topredict short- and long-term survival in community-acquired pneumonia:Results from the German Competence Network, CAPNETZ. Am J RespirCritCare Med. 2010;182:1426–34.

27. Langleben D, Orfanos SE, Giovinazzo M, et al. Acute vasodilator responsiveness and microvascular recruitment in idiopathic pulmonary arterial hypertension.Ann Intern Med. 2015; 162:154–6.

28. Gaggin HK, Januzzi JL. Cardiac Biomarkers and Heart Failure. Expert Analysis. Latest in Cardiology. American Colledge of Cardiology. Feb 10 2015: https://www.acc.org/%2Flatest-in-cardiology%2Farticles%2F2015%2F02%2F09%2F13%2F00%2Fcardiac-biomarkers-and-heart-failure.

29. Acher R, Chauvet J, Rouille Y. Dynamic processing of neuropeptides: sequential conformation shaping of neurohypophysial preprohormones during intraneuronal secretory transport". Journal of Molecular Neuroscience. 2002; 18(3): 223–8.

30. D'Alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry.Ann.Intern. Med. 1991; 115: 343–349.

31. Shah SJ, Thenappan T, Rich S, et al. Association of serum creatinine with abnormalhemodynamics and mortality in pulmonary arterial hypertension. Circulation. 2008; 117: 2475–2483.

32. Benza RL, Miller DP, Gomberg-Maitland M, et al. Predicting survival in pulmonary arterial hypertension: Insights from the Registry to Evaluate Early andLong-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation. 2010; 122: 164–172.

33. Nickel NP, Lichtinghagen R, Golpon H, et al. Circulating levels of copeptin predict outcome in patients with pulmonary arterial hypertension. Respir.Res. 2013; 14, 130.

34. Kociol RD, Horton JR, Fonarow GC, et al. Admission, discharge, or change in B-type natriuretic peptide and long-term outcomes: data from Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure (OPTIMIZE-HF) linked to Medicare claims. Circ Heart Fail. 2011;4:628-36.

35. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of American College of Cardiology/American Heart Associaton Task Force on Practice Guidelines.J Am CollCardiol. 2013;62:e147-239

36. 36. GalieN,Humbert M, Vachiery JL, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur. Respir. J. 2015; 46:

903–975.

37. Benza RL, Miller DP, Barst RJ, et al. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL Registry. Chest 2012; 142:448–456.

38. Chin KM, Rubin LJ, Channick R, et al. Association of N-Terminal Pro Brain Natriuretic Peptide and Long-Term Outcome in Patients With Pulmonary Arterial Hypertension. Circulation 2019; 139: 2440–2450.

39. Galie N, Jansa P, Pulido T, et al. SERAPHIN haemodynamic substudy: The effect of the dual endothelin receptor antagonist macitentan on haemodynamic parameters and NT-proBNP levels and their association with disease progression in patients with pulmonary arterial hypertension. Eur. Heart J. 2017; 38:1147–1155.

40. Berghaus TM, Kutsch J, Faul C,et al. The association of N-terminal pro-brain-type natriuretic peptide with hemodynamics and functional capacity in therapy-naive precapillary pulmonary hypertension: Results from a cohort study. BMC Pulm. Med. 2017; 17:167.

41. Souza R, Jardim C, FernandesCJC,et al. NT-proBNP as a tool to stratify disease severity in pulmonary arterial hypertension. Respir.Med. 2007; 101: 69–75.

42. Fijalkowska A, Kurzyna M, Torbicki A, et al. Serum N-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. Chest 2006; 129:1313–1321.

43. Benza RL, Gomberg-Maitland M, Elliott CG, et al. Predicting Survival in Patients With Pulmonary Arterial Hypertension: The REVEAL Risk Score Calculator 2.0 and ComparisonWith ESC/ERS-Based Risk Assessment Strategies. Chest 2019; 156:323–337.

44. Nagaya N, Ando M, Oya H, et al. Plasma brain natriuretic peptide as a noninvasive marker for efficacy of pulmonary thromboendarterectomy. Ann. Thorac. Surg. 2002; 74:180–184.

45. Surie S, Reesink HJ, van der Plas MN, et al. Plasma brain natriuretic peptide as a biomarker for haemodynamic outcome and mortality following pulmonary endarterectomyfor chronic thromboembolic pulmonary hypertension. Interact. Cardiovasc.Thorac.Surg. 2012; 15:973–978.

46. Kriechbaum SD, Wiedenroth CB, Wolter JS, et al. N-terminal pro-B-type natriuretic peptide for monitoring after balloon pulmonary angioplasty for chronic thromboembolic pulmonary hypertension. J. Heart Lung Transplant. 2018; 37:639–646.

47. Collet JP, Thiele H, Barbato E, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Rev. Esp. Cardiol. 2021; 74,544.

48. Eggers KM, Lagerqvist B, Venge P, et al. Persistent cardiac troponin I elevation in stabilized patients after an episode of acute coronary syndrome predicts long-term mortality. Circulation 2007; 116:1907–1914.

49. de Lemos JA, Drazner MH, Omland T, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. JAMA.2010; 304:2503–2512.

50. Apple FS, Murakami MM, Pearce LA, Herzog CA. Predictive value of cardiac troponin I and T for subsequent death in end-stage renal disease. Circulation 2002; 106:2941–2945.

51. Horwich TB, Patel J, MacLellanWR,Fonarow GC. Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure. Circulation 2003; 108:833–838.

52. Banaszkiewicz M, Gasecka A, Darocha S, et al. Circulating Blood-Based Biomarkers in Pulmonary Hypertension. J. Clin.Med. 2022; 11: 383. https://doi.org/10.3390/jcm11020383.

53. Torbicki A, Kurzyna M, Kuca P, et al. Detectable serum cardiac troponin T as a marker of poor prognosis among patients with chronic precapillary pulmonary hypertension. Circulation 2003; 108:844–848.

54. Velez-Martinez M, Ayers C, Mishkin JD, et al. Association of cardiac troponin I with disease severity and outcomes in patients with pulmonary hypertension. Am. J. Cardiol. 2013; 111:1812–1817.

55. Heresi GA, Tang WH, Aytekin M, et al. Sensitive cardiac troponin I predicts poor outcomes in pulmonary arterial hypertension. Eur. Respir. J. 2012; 39:939–944.

56. Kriechbaum SD, Wiedenroth CB, Keller T, et al. Dynamics of high-sensitivity cardiac troponin T during therapy with balloon pulmonary angioplasty for chronic thromboembolic pulmonary hypertension.PLoS ONE 2018; 13: e0204683.

57. Ozerol IH, Pac FA, Ozerol E, et al. Plasma endothelin-1, homocysteine and serum nitric oxide values in patients with left-to-right shunt. Indian Heart J. 2004;56:653–7.

58. Sanli C, Oguz D, Olgunturk R, et al. Elevated homocysteine and asymmetric dimethyl arginine levels in pulmonary hypertension associated with congenital heart disease. Pediatr.Cardiol.2012; 33: 1323–1331.

59. Hoffmann U, Espeter F, Weiss C, et al. Ischemicbiomarker heart-type fatty acid bindingprotein (hFABP) in acute heart failure- diagnostic and prognostic insights comparedto NT-proBNP and troponin I. BMCCardiovascDisord 2015;15:50. doi:10.1186/s12872-015-0026-0.

60. Niizeki T, Takeishi Y, Arimoto T, et al. Persistently increasedserum concentration of heart-type fatty acid-binding protein predicts adverse clinicaloutcomes in patients with chronic heartfailure. Circ J2008;72:109–114.

61. Carroll C, Al Khalaf M, Stevens JW, et al. Heart-type fatty acid binding protein asan early marker for myocardial infarction: Systematic review and meta-analysis. Emerg.Med. J. 2013; 30:280–286.

62. Lankeit M, Dellas C, Panzenbock A, et al. Heart-type fatty acid-binding protein for risk assessment of chronic thromboembolic pulmonary hypertension. Eur.Respir. J. 2008; 31:1024–1029.

63. Kanwar MK. Biomarkers in pulmonary arterial hypertension: Moving closer toward precision medicine? J Heart Lung Transplant. 2020; 39(4):287-288. doi: 10.1016/j.healun.2020.02.020. Epub 2020 Feb 29. PMID: 32199588.

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Drug repositioning approach to target viral and host cells in terms of COVID-19 treatment: A review of in vivo experiments and clinical studies

Ali SHAHALİ [1,2,3]*, Vajihe AKBARI [1], Rasool SOLTANI [4], Shirin Sadat BADRI [4] Rashid Alijani ARDESHIR[5]**

[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
[3]Department of Pharmaceutics, College of Pharmacy, University of the Punjab, Lahore, Pakistan
[4]Department of Clinical Pharmacy and Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
[5]Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran

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Abstract

While the COVID-19 pandemic is expanding at an alarming rate, there is currently no treatment option for this disease. Therefore, it is necessary to find an effective treatment special for hospitalized COVID-19 patients at the earliest possible time. One of the promising options which should be investigated is the possible effects of old drugs or drug repositioning. This strategy has less risk with more economic advantages and can benefit the long-term control of this pandemic. Our study aimed to give an overview, update the current status of drug candidates (both virus-targeting and host-targeting drugs) for repurposing in COVID-19 infection, and assess the possible mechanism of their effect, in vivo antiviral efficacy, and clinical studies.

Keywords: COVID-19, Drug repurposing, therapeutic, pandemics.

1. Introduction

Ribonucleic acid (RNA) viruses are the most infected pathogens that can spread very fast for their capability of zoonotic potential, evolvability, and enhanced virulence (1, 2). Despite advances in technologies to study these viruses in the last two decades, it has been impossible to stop the rapid evolution of these viruses, leading to pandemics (3). The prevalent coronavirus strains that can infect humans include HCoV-OC43, HCoV-NL63, HCoV-229E, and HCoVHKU, with the capability of mild respiratory illness (4, 5). In the twenty-first century, the first outbreak of human coronavirus was the severe acute respiratory syndrome (SARS-CoV) in China, and this fatal respiratory illness killed around 750 people (6-8). The second coronavirus outbreak occurred in the Middle East countries, named Middle East respiratory syndrome coronavirus (MERS-CoV), and led to the death of 866 people (9, 10). The most extensive human coronavirus outbreak started in Wuhan in China's Hubei Province in December 2019 (11). This human coronavirus, known as coronavirus disease 2019 (COVID-19), spread quickly worldwide and was announced a pandemic in March 2020 (10-15).

Sudden and fast outbreaks of COVID-19 caused a unique challenge in choosing proper treatments within the short time available for drug analysis and development for healthcare professionals. Although there is no particular medicine against COVID-19, more than 80 clinical trials have been developed to test therapeutic approaches for COVID-19, including drug repurposing or repositioning (16). Some clinical trial steps, especially phases I and II, might not be required in the drug repositioning strategy. Existing pharmaceutical supply chains are available for formulation and distribution in drug repositioning, and new mechanisms of action for old drugs and new classes of medicines may be discovered. Therefore, this medical strategy has lower costs (17, 18). This study aimed to collect and summarize all clinical and in vivo findings related to the old drugs–COVID19 disease, and assess the mechanism of action to introduce a suitable therapy for this infectious disease.

2. Research Method

This study involved articles and relevant data investigating the antiviral activity of available drugs in clinical and preclinical phases (in vivo), published until October 3, 2021. We collected information by searching in Google Scholar, PubMed/MEDLINE, Scopus, Cochrane Library, clinicaltrials.gov website, and valid encyclopedia. We combined the keywords "Drug repositioning", OR "Drug

*Correspondence: alishahali.ir@gmail.com

repurposing", and "clinical future" with the keywords "SARS-CoV-2" OR "COVID-19" as search terms. We removed the in silico and in vitro studies. We did not restrict the search regarding the type and language of studies. We included all kinds of published research projects, reviews, comments, letters, editorials, and eBooks and used Google Translate software to review studies written in the English language.

3. Result

3.1. Classification of candidate drug targets

Potential drug targets must be selected from among several viral and host molecules to achieve a successful drug treatment of COVID-19 (Figure 1). The macro-molecule such as RNA/DNA and proteins participating in vital processes of the virus infection cycle, such as replication, cell entry, host metabolic pathways, and immune response, can be regarded in this case (19). This study comprehensively reviewed the drug repositioning used in COVID-19 in two categories: Virus targets and host drugs.

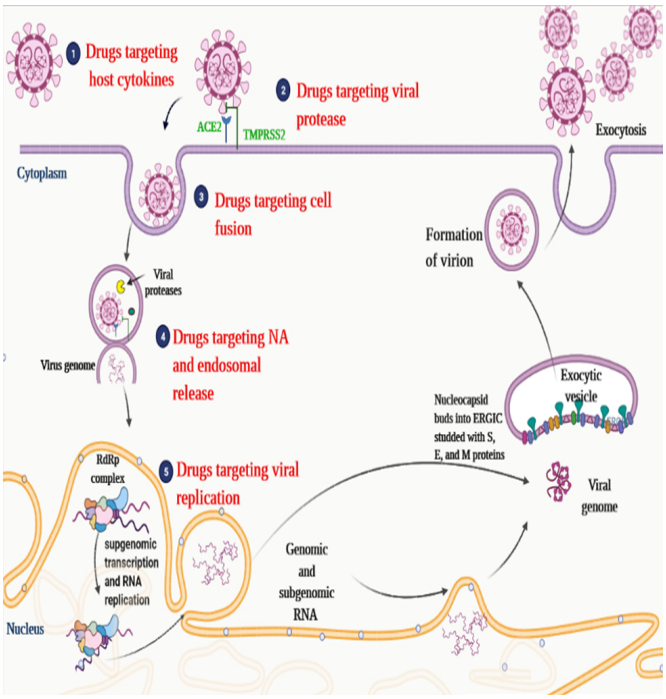

Fig. 1. Therapeutic targets for the drug repositioning to combat COVID-19 based on the virus life cycle

1: Drugs that suppress coronaviruses by enhancing host immune system activity and release interferons

2: The fusion of the viral spike proteins with the cellular ACE2 receptor causes the entrance of the virus to the host cell. Then, the ACE2 is downregulated. Therefore, the drugs targeting viral protease or enhancer ACE2 expressions, such as Angiotensin-converting enzyme inhibitors (ACEIs) and Angiotensin receptor blockers (ARBs), may have efficacy in this condition

3: After endocytosis of the virus, the drugs can disturb viral endosteal conditions, such as reducing endosomal pH and helping lysis viral structural proteins

4: The drugs that can suppress viral nucleic acid (NA) and translation of the viral proteins by host ribosomes may have antiviral activity

5: Drugs that can inhibit RNA-dependent RNA polymerase (RDRP) as the main viral protease enzyme making functional proteins to stop viral replication in the host cell

3.2. Virus based drug repositioning

3.2.1. Drug repositioning that targets viral replication

The key enzymes in the replication of the COVID-19 virus (e.g., protease and polymerase) are highly conserved, with 96% and 97% overall identity (GISAID,2020). Therefore, drugs that could bind protease or polymerase sites of the SARS virus may also be good therapeutic candidates against the COVID-19 virus (Table1).

3.2.1.1. Remdesivir

One of the novel RNA polymerase (RdRp) inhibitor drugs that have been developed against the Ebola virus for the first time is remdesivir. Remdesivir is the only FDA-approved drug for treating COVID-19 patients (20, 21). From a mechanistic point of view in the coronavirus, remdesivir in the cells is metabolized to remdesivir triphosphate (RTP), an active NTP analog (22). The RTP can be used as a substrate by the RdRp resulting in remdesivir Monophosphate (RMP) merging into the growing RNA product (23, 24). A cohort study observed clinical improvement in 36 of 53 patients (68%) with severe COVID-19 who received remdesivir (25). Similar results were also reported by other studies (26) (27) (20). On the other hand, one study reported that there was not a significant difference in clinical benefits after ten days of remdesivir prescription in hospitalized COVID-19 patients at ten hospitals in China (28). Several clinical trial studies in China and USA (NCT04252664, NCT04292899, and NCT04257656) reported that patients with COVID-19 could tolerate remdesivir prescription (28, 29). Most meta analysis studies evinced that a 5-day course remdesivir administration to severe COVID-19 patients might have more positive effects and decreased mortality with lower drug costs than a 10-day course (30-33). However, other meta analysis studies stated stronger evidence about the need to administer remdesivir to severe COVID-19 patients. (34, 35), while one meta analysis study showed that remdesivir administration should not be recommended for use especially in countries with low inclome (36).

3.2.1.2. Favipiravir

Favipiravir (approved in Japan for influenza since 2014) is a small purine analog, transformed into its active ribofuranosyl 5'-triphosphate metabolite in the cell, and can be linked to the growing RNA strand (37). Since the antiviral activity of favipiravir in clinical trials may be more potent than in vitro, this drug is being conducted in several randomized trials against COVID-19 in combination with other drugs (38-42). The prescription dose of favipiravir for COVID-19 patients is i.e. 53 mg/kg/day. Favipiravir oral bioavailability is close to 100% (43). This antiviral drug acts dose-dependent and has a short half-life of 2–5.5 h (43-45). Some studies demonstrated that favipiravir could improve clinical conditions and decrease the median time to viral clearance in mild to moderate COVID-19 patients (39). However, this was not reported for severe COVID-19 (46). Evidence from a meta-

analysis study showed that favipiravir might not decrease mortality in patients with mild to moderate COVID-19 (47). On the other hand, another meta-analysis study reported that favipiravir leads to the clearance of the virus by seven days, takes part in clinical improvement within 14 days, and can decrease the mortality rate by more than 30% (48).

Table 1. List of drug repositionings that have the potential to target the virus and may be used against COVID-19 infection.

Drug name	Drug Indication	Target	Effect	Invivo test	Clinical trials	References
emdesivir	Antiviral (Ebola and Marburg)	RdRp	viral replication inhibitor	Rhesus macaques, mouse	NCT04292730- Phase 3 NCT04292899- Phase 3 NCT04257656 Phase 3 NCT04401579- Phase 3	(20, 28, 29, 185, 186)
avipiravir	AntiRNA viral (influenza, Rhino, and Respiratory Syncytial Virus)	RdRp	viral replication inhibitor	Hamster	NCT04358549- Phase 2 NCT04346628- Phase 2 NCT04303299- Phase 3	(38-42, 187, 188)
ibavirin	Antiviral	RdRP, Inosine monophosphate dehydrogenase	viral replication inhibitor	N/A	NCT04276688- Phase 2 CTOROTSADTOC IRCT20200324046850N2- Phase 2 NCT04392427- Phase 3	(55-58)
ofosbuvir	AntiRNA viral (flaviviridae, HCV)	RdRp	viral replication inhibitor	N/A	IRCT20200624047908N1- Phase 3 IRCT20200128046294N2- Phase 3 IRCT20130812014333N145- Phase 3 IRCT20100228003449N29- Phase 2–3	(63-65)
Tenofovir/ Emtricitabine	Antiviral (HIV-1)	NRT	viral replication inhibitor	N/A	NCT04685512- Phase 2 NCT04519125- Phase2 NCT04334928-Phase 3 NCT04405271- Phase 3	(68, 69, 71, 72)
Azvudine	Antiviral (HIV-1, HBV, HCV)	NRT	viral replication inhibitor	N/A	ChiCTR2000029853 NCT04668235- Phase 3 NCT04425772- Phase 3	(74-76)
vermectin	Anti-parasit	Nuclear transport activity	viral replication inhibitor	Hamster, Mous	NCT04646109- Phase 3 NCT04381884- Phase 2 NCT04739410- Phase 4	(99, 102-105, 189)
olnupiravir	AntiRNA viral (influenza)	RNA replication	viral replication inhibitor	Hamster	NCT04405570- Phase 2	(107, 108)
arunavir	Anti-retroviral (HIV-1)	Protease	viral entery inhibitor	N/A	NCT04425382 NCT04252274- Phase 3 NCT04303299- Phase 3	(27, 80, 190), (, https :// www.sd.china news. com/2/2020/0205/ 70145.html)
Lopinavir-ritonavir	Anti-retroviral (HIV-1)	Protease	viral entry inhibitor	Ferrets	NCT04330690- Phase 2 NCT04372628- Phase 2 NCT04276688- Phase 2	(57, 86-88, 90, 91, 142, 191)
afamostat	Anticoagulant	Protease	viral entery inhibitor	Mous	NCT04418128- Phase 2,3 NCT04352400- Phase 2,3	(94-96)
Proxalutamide And 5-alpha-reductase inhibitors	Antiandrogen	Protease	viral entery inhibitor	N/A	NCT04728802-Phase 3 NCT04870606- Phase 3 NCT05009732- Phase 3 NCT04853927- Phase 3	(109, 115, 116, 118)

3.2.1.3. Ribavirin

Ribavirin is a guanosine analog causing a disturbance in virus genome replication. This antivirus drug interferes with polymerases and can disturb RNA capping, depending on natural guanosine to stop RNA degradation. In addition, ribavirin can inhibit natural guanosine generation in the virus by directly inhibiting inosine monophosphate dehydrogenase in a critical pathway of transforming guanine precursor to guanosine (49, 50). Although there is no in vivo study on the effect of ribavirin on COVID-19, a previous in vivo study on the effect of ribavirin on SARS and MERS-CoV infected mice reported that this drug could not increase the survival rate (51, 52). Moreover, monotherapy of ribavirin is insufficient to inhibit coronavirus, and combinatorial therapies with LPV/r and IFN-a for MERS-CoV (53) and with LPV/r viral protease inhibitors for SARS-CoV are recommended (54). The clinical trials' efficacy of ribavirin in treating COVID-19 patients is being tested. The oral prescription of this drug (400 mg per day) combined with another drug such as interferon-alpha or lopinavir/ritonavir could improve symptoms in mild to moderate COVID-19 (55-58).

3.2.1.4. Sofosbuvir

Sofosbuvir is a uridine nucleotide analog prodrug that causes RNA chain termination in RNA virus HCV and competitively blocks HCV NS5B polymerase (59). Since COVID-19 and HCV are both positive-sense RNA viruses, sofosbuvir as an RNA polymerase inhibitor drug is expected to be effective for COVID-19 (60, 61). Although sofosbuvir alone could not inhibit COVID-19 in Vero cells, it can do so in hepatoma Huh-7 cells (EC50= 6.2 mM, SI= 61) and human lung adenocarcinoma Calu-3 cells (EC50= 9.5 mM, SI= 54) (62). Meanwhile, a drug combination of daclatasvir and sofosbuvir could inhibit COVID-19 in all three cell lines (EC50= 0.6~1.1 mM, SI= 34~47) (60). Several clinical trial studies were carried out to assess the use of sofosbuvir/daclatasvir in COVID-19 patients. Oral prescription of sofosbuvir/daclatasvir (60-400 mg per day) or sofosbuvir/velpatasvir in moderate to severe hospitalized COVID-19 patients had improved clinical outcomes (63-65).

3.2.1.5. Tenofovir/emtricitabine

Tenofovir and emtricitabine are the analogs of adenosine 5′-monophosphate and cytidine that can inhibit nucleoside reverse transcriptase in HIV-1 (66, 67). The coadministration of these drugs is used as a backbone of antiretroviral therapies. The clinical finding showed that the use of tenofovir/emtricitabine in non-severe COVID-19 patients (68) is suitable, but the significant effect of prophylactic efficacy of tenofovir/emtricitabine against COVID-19 was not observed (69, 70). Two ongoing clinical trial studies on this subject are about the prophylactic efficacy of tenofovir/emtricitabine against COVID-19 in Healthcare Workers from Argentina and Colombia (71, 72).

3.2.1.6. Azvudine

Azvudine is a cytidine analog that can act as the chain terminator in proviral genome biosynthesis in various types of viruses (73). Several clinical studies have been carried out to assess the positive effect of this drug in COVID-19 patients (74-76). A randomized, open-label, controlled clinical trial showed that oral administration of Azvudine in mild COVID-19 patients could shorten the nucleic acid negative conversion (NANC) time compared to the control group (74).

3.2.2. Drug repositioning that targets viral protease

The RNA of the virus is converted into polypeptide through translation and then packed into virions after the cleavage by the main viral proteases (11). Since protease in COVID-19 has a 96% overall similarity with SARS-CoV (77), the use of some protease inhibitors prescribed in HIV and SARS-CoV therapy may be effective against COVID-19 (77, 78).

3.2.2.1. Darunavir

Darunavir is a non-peptide protease inhibitor against HIV-1, has more inhibitor potential than the other protease, enhances binding affinity, and reduces dissociation rate (79). Although there is no in vivo and preclinical evidence, several clinical news reports evinced that oral prescription of darunavir (600 mg tablet every 12 h) with other antiviral drugs and supportive therapy could be effective in COVID-19 patients (27). However, a randomized open-label controlled trial in China (NCT04252274) reported no improved symptoms in mild COVID-19 patients after 5-day Darunavir/Cobicistat treatment (80).

3.2.2.2. Paxlovid

Paxlovid is traditionally used to fight HIV (81). According to the last reports, this drug could bind to the COVID-19 3CL-like protease and disturb the virus's function, host cell entry, and reproduction (81, 82). The interim analysis of the clinical phases in 1219 COVID-19 adults patients that received paxlovid after three days of covid-19 symptoms showed the risk of hospital admission or death in COVID-19 patients treated with paxlovid was 89% lower than that in the placebo group (82).

3.2.2.3.Lopinavir/ritonavir

Currently, the combination of these two drugs is used to treat and prevent HIV infection. Lopinavir can make uncleavable peptidomimetic of the linkage peptide in HIV gag-pol polyprotein that inhibits HIV protease activity by binding to it (83, 84). Regarding the host proteases' rapid degradation of lopinavir in the body, a lower dose of ritonavir (a protease inhibitor) must be used to inhibit CYP3A4 and help lopinavir remain active for a longer time (85). Despite the differences in the main proteases of HIV and coronavirus, the use of nonspecific protease inhibitors in HIV therapy (Lopinavir/ritonavir) has more clinically positive effects than the control group in clinical studies of SARS-CoV patients (54). Some clinical studies reported that lopinavir and ritonavir therapy could reduce the viral load (86-88). WHO uses the prescription of lopinavir/ritonavir alone or in combination with interferon-beta (INF-β) as an option for a "solidarity" clinical trial for COVID-19 patients (89, 90). Moreover, the combination of ritonavir-lopinavir and umifenovir in COVID19-patients could substantially halt the progression of lung damage (91).

3.2.2.4. Nafamostat

This drug is a synthetic serine protease inhibitor and an anticoagulant in nature. This drug could inhibit COVID-19 in the Vero E6 cells at a 50% effective concentration of 22.50 μM (92). Nafamostat has high efficiency in inhibiting the entry of the COVID-19 virus into host cells (93). The in vivo observation showed that the mice treated with nafamostat before COVID-19 infection had less virus-induced weight loss, viral replication, and mortality than the untreated control mice (94). Several clinical trials are studying these drugs (95-97).

3.2.2.5. Ivermectin

Ivermectin is commonly used as an anti-parasitic drug that has recently exhibited efficacy against some viral infections. Although the exact antiviral mechanism of ivermectin is unknown, a recent study suggested that this drug may inhibit

the importin (IMP) α/β1-mediated nuclear import of viral proteins (98). This drug may prevent clinical deterioration due to its immunomodulatory activities through the cholinergic anti-inflammatory pathway (99). The combination of ivermectin and hydroxychloroquine may inhibit both entry and replication of COVID-19 and thus has a synergistic effect. Therefore, combination therapy for the prophylaxis or treatment of COVID-19 was suggested (100). The main problem with administering ivermectin is the need for a high dosage to achieve antiviral activity (101). The ivermectin therapy (150 mcg/Kg) for COVID-19 patients in a case-controlled study decreased the mortality rate and the duration of hospital stay (102). Some clinical studies reported an improvement in the symptoms of severe COVID-19 after the treatment (103-105).

3.2.2.6. Molnupiravir

Molnupiravir is the newest experimental antiviral drug for treating influenza and disrupts viral RNA replication (106). The in vivo study on the combined effect of molnupiravir and favipiravir on infected hamsters with COVID-19 showed strong antiviral activity and reduced transmission of the virus to uninfected contact sentinels (107). Although molnupiravir administration for COVID-19 is only just beginning to go through the first clinical trials, this drug is known as the first oral and direct-acting antiviral that has been highly effective against COVID-19. This drug has a favorable safety and tolerability profile and can reduce the nasopharyngeal COVID-19 infectious virus (108).

3.2.2.7. Proxalutamide and 5-alpha-reductase inhibitors

5-alpha-reductase inhibitors are a group of anti-androgen drugs used to treat prostate gland hyperplasia and male pattern hair loss. Proxalutamide has androgen antagonism action and downregulates androgen expression, which was developed to treat prostate and breast cancer (109). In COVID-19 infectivity, androgen signaling has a critical role. The serine 2 (TMPRSS2), androgen-promoted enzyme, and transmembrane protease in the host cell help virus entry mediated by viral spike proteins (110). The TMPRSS2 could modify viral spike proteins and, therefore, increase the virus's binding to angiotensin-converting enzyme 2 (ACE2) and viral entry into host cells (111). Some epidemiologic and clinical evidence demonstrated that the severity of COVID-19 disease is related to the androgen-mediated phenotype androgenetic alopecia (AGA) (112, 113). Therefore, COVID-19 in male patients with more advanced AGA is more likely to need more care or die (114). The newest clinical reports showed significant clinical symptoms of COVID-19 infection and a lower mortality rate in men patients undergoing androgen deprivation therapy (ADT) (109, 115-117). A recent finding showed that Proxalutamide could help nonhospitalized COVID-19 patients with mild to moderate symptoms to clear the virus much faster than those given a placebo (110, 118). Therefore, proxalutamide could be a good candidate in the global fight against COVID-19. Nevertheless, more detailed studies are needed to make more accurate comments.

3.2.2.8. Casirivimab and imdevimab

Casirivimab/imdevimab is a combinational medicine consisting of two human monoclonal antibodies that can be bound to different sites on the receptor-binding domain of the spike protein of COVID-19 and can block its attachment to the human ACE2 receptor (119). Primary clinical studies reported that administration of casirivimab and imdevimab can prevent symptomatic infection, reduce overall infection, and decrease viral load and duration of viral RNA detection in COVID-19 (120). These antibodies in high-risk patients with mild to moderate COVID-19 infection could significantly reduce hospitalization rates (121, 122). In contrast, another study reported that using bamlanivimab/etesevimab in patients infected by the Gamma variant of COVID-19 could enhance the risk of hospitalization or death (123). Therefore, knowing which COVID-19 variant infection is in question may allow more appropriate use of these drugs.

3.3. Drug repositioning targeting host cell

3.3.1. Drug repositioning targeting cell fusion

The drugs inhibiting the fusion can prevent the fusion process during viral entry into the host cells (Table 2). Therefore, they prevent the virus entry into the host cell (124, 125).

3.3.1.1. Arbidol (umifenovir)

Arbidol, also called umifenovir, is a small indole-derivative molecule, showing activity against a wide range of enveloped and nonenveloped viruses (126). This drug has an approved therapeutic effect on prophylaxis and influenza virus infection (127). Arbidol can hinder the hemagglutinin fusion machinery by inhibiting viral membrane fusion and blocking virus entry into the cell (127). According to clinical studies, arbidol treatment coupled with lopinavir/ritonavir could reduce the development of pulmonary lesions and viral load, lowering the transmission of mild to severe COVID-19 patients (90, 91, 128, 129). However, some clinical trial studies reported that the administration of arbidol and lopinavir/ritonavir monotherapies in mild/moderate COVID-19 patients had no significant effect on the viral negative conversion rate and symptom improvement (130, 131).

3.3.1.2. Baricitinib and ruxolitinib

Like other viruses, receptor-mediated endocytosis has a vital role in the COVID-19 virus's entrance into the host cells. Regulation of the process of endocytosis is undertaken by AP2-associated protein kinase 1 (AAK1). Thus, the drugs targeting AAK1 block the viral entry and the intracellular viral assembly (132). Baricitinib and ruxolitinib are Janus kinase (JAK) inhibitors with a high potential to bind to and inhibit AAK1 (133, 134). Suppression of inflammation through inhibiting the production of cytokines and chemokines by macrophage and neutrophil recruitment was observed in baricitinib-treated infected animals (135). Clinical trials on baricitinib and ruxolitinib considered the effect of these drugs on the control of cytokine storms in

severe COVID-19 patients (136, 137).

Table 2. List of drug repositionings that have the potential to target the host cell and may be used against COVID-19 infection

Drug name	Drug Indication	Target	Effect	Invivo test	Clinical trials	References
Arbidol (Umifenovir)	Antiviral (influenza A and B)	Spike glycoprotein	viral entry and post-entry inhibitor	Hamsters, Mouse, Rat	ChiCTR2000029592 NCT04286503- Phase IV NCT04260594- Phase IV NCT04255017- Phase IV	(90, 91, 128-131, 192-194)
Baricitinib	kinase inhibitors.	Janus-kinase 1/2	Cytokine storm inhibitor	Rhesus macaques	NCT04640168- Phase III NCT04693026- Phase III NCT04358614- Phase II, III	(135, 195-197)
Ruxolitinib	kinase inhibitors.	Janus-kinase 1/2	Cytokine storm inhibitor	N/A	NCT04362137-Phase III NCT04334044- Phase I, II NCT04338958- Phase I, II	(136, 137, 198, 199)
Chloroquine and hydroxychloroquine	Anti-malarial	Glycosylation of the host receptor for the virus, Change in endosomal pH, angiotensin converting enzyme 2	Viral entry inhibitor and Post-entry Inhibitor	Mous, Ferrets, Hamsters, Rhesus macaques	NCT04353336- Phase II, III NCT04331600- Phase IV NCT04334148-Phase III NCT04355026- Phase IV	(27, 143-146, 200)
Angiotensin Receptor Blockers and renin-angiotensin-aldosterone system (RAAS) inhibitors	Using treat high blood pressure and heart failure	Angiotensin converting enzyme	Viral entry inhibitor	N/A	NCT04335123- Phase I NCT04312009- Phase II NCT04428268- Phase II	(153-155)
Azithromycin	Antibiotic	Change in cytokines and endosomal pH (Not conclusive)	Inhibits IL-6 production	N/A	NCT04622891 NCT04349592 NCT04381962- Phase III NCT04332107- Phase III NCT04334382- Phase III	(158-163, 201)
Tocilizumab	Using treat rheumatoid arthritis	IL-6 receptor	Inhibits IL-6 release	N/A	NCT04445272- Phase II NCT04730323- Phase IV NCT04479358- Phase II NCT04331795- Phase II	(166, 167, 202-205)
Dexamethasone and Methylprednisolone	Corticosteroid	Inflammatory cells	Inhibits release of cytokines	Mous	NCT04325061- Phase IV NCT04395105- Phase III NCT04347980- Phase III	(168, 170, 171, 206)
Interferons (pegylated IFNα-2a and pegylated IFNα-2b)	Antiviral	B cells through a host interferon receptor, IFNAR1 signalling	Enhanced immune response against viral infections	N/A	NCT04349410- Phase III NCT04273581- Phase II NCT04379518 -- Phase I, II	(42, 176, 207)
Statins	lipid-lower	Angiotensin converting enzyme 2	Improve endothelial dysfunction	N/A	NCT04486508-Phase III NCT04380402- Phase II IRCT20190727044343N2-Phase II–III NCT04390074	(183, 184)

3.3.1.3. Chloroquine and hydroxychloroquine

Chloroquine and its derivative hydroxychloroquine, known for its antimalarial actions, can also interfere with the endosome-mediated viral entry in the host cell and the late stages of viral replication in an acidic environment (138). Therefore, these drugs have exhibited broad-spectrum antiviral activities (138). The in vivo antiviral effect of these drugs against COVID-19 was highly controversial. Hydroxychloroquine did not have antiviral activity in hamsters and macaques (139-141). In addition, it decreased the clinical scores in ferrets but did not affect the viral titers (142). Although large-scale clinical trials have not yet confirmed the efficacy of chloroquine and hydroxychloroquine against COVID-19, these drugs have been considered in several recent clinical studies to combat COVID-19. Nowadays, the answer to the question of "whether the administration of these antimalarial drugs can be repurposed for the treatment of COVID-19" has sparked much interest globally. Some clinical trial studies reported prophylactic and therapeutic efficacy of chloroquine and hydroxychloroquine against COVID-19 (27, 143-146). However, the use of chloroquine against COVID-19 needs a high dose, leading to an overdose of chloroquine and,

consequently, poisoning and death (147, 148). Although hydroxychloroquine is less toxic in animal models (149), this drug has shown side effects such as prolonged heart failure and QT interval. WHO recently stopped the hydroxychloroquine arm of the Solidarity Trial conducted to find an effective COVID-19 treatment.

3.3.2. Drug repositioning targeting host cytokines

3.3.2.1. Angiotensin receptor blockers

COVID-19, like other coronaviruses, binds to the ACE2 receptors (150). There is a significant relationship between COVID-19 infection and the process of chronological aging due to the presence of two host receptors, CD26 and ACE2, associated with senescence (151). The Angiotensin receptor blockers (ARBs) drugs inhibit the action of ACE, an isoform of ACE2 that can enhance the expression of ACE2 (152). Some clinical studies showed that ACEI/ARB drugs did not decrease the mortality rate of COVID-19 patients with cardiovascular diseases (153), while others reported that ACEIs/ARB drugs decreased the mortality of COVID-19 patients more than the control group (154). This drug can decrease the peak of viral load and level of IL-6 in peripheral blood through the increment of CD3 and CD8 T cell counts in peripheral blood. This process may positively affect the clinical condition of COVID-19 patients (154). Nevertheless, clinical studies on the effect of ACEIs/ARB drugs on COVID-19 patients are ongoing (155).

3.3.2.2. Azithromycin

Azithromycin has anti-inflammatory and antiviral properties with potential activity against COVID-19 (156). The immunomodulatory activity of azithromycin through the regulation of cellular processes involved inhibits a variety of pro-inflammatory pathways and also exhibits immunomodulatory properties (157). Some clinical evidence showed that a single oral dose of azithromycin has a positive effect on preventing COVID-19 (158, 159). However, no supportive evidence could be found for using azithromycin in COVID-19 treatment in hospitalized COVID-19 patients (160). To date, more than 100 clinical trials have been performed on the effect of azithromycin alone or combined with other drugs (such as clarithromycin and hidroxicloroquin) to control COVID-19 symptoms in mild to severe patients (161-163). However, more comprehensive studies are needed for better conclusions.

3.3.2.3. Tocilizumab

Tocilizumab is an antihuman monoclonal antibody belonging to the immunoglobulin G1k subclass responsible for binding to the human IL-6 receptor and inhibiting its signal transduction pathway (164). Tocilizumab is used against rheumatoid arthritis and cytokine release syndrome/systemic inflammatory response syndrome (165). After COVID-19 infection, the use of this drug may reduce the cytokine response of the host. The clinical study with successful treatment confirmed this hypothesis. However, the recovery of the normal T cells and the COVID-19 patient may be due to a rebound phenomenon of IL-6 level (166). Clinical studies suggest that tocilizumab is a good candidate for treating COVID-19 in patients with the risk of cytokine storms, especially in immunocompromised patients. (166, 167).

3.3.2.4. Dexamethasone and methylprednisolone

Dexamethasone and methylprednisolone are corticosteroids with anti-inflammatory, vasoconstrictive, and antifibrotic activities. This drug has been one of the conflicting treatment choices since the emergence of COVID-19. The preliminary results of the RECOVERY trial showed that the administration of dexamethasone (6 mg once daily for up to 10 days) could improve the recovery and decrease the rate of mortality in COVID-19 patients (168). However, choosing the correct steroid and its dose in the initial phase of the disease to the severe phase of COVID-19 patients and during treatment is still under study and controversy (169-171).

3.3.2.5. Interferons

The interferons (IFNs) are antiviral molecules divided into two classes: type I (IFNα, IFNβ, IFNω, and IFNτ) and type II (IFNγ). Previous research demonstrated that IFNα contributes to innate immunity against the virus, leading to its use against viral infections. The interferon alfacon-1 is recombinant of IFNα.The pegylated IFNα-2a and pegylated IFNα-2b are pegylated types (172). The Pegylated interferon alfa-2b can fight against viral infections by activating interferon receptors in host B cells and enhancing immune response (173). The in vitro study showed that the recombinant human IFNα-2b has antiviral activity, low toxicity, and a high therapeutic index against COVID-19 infection (174). Although studies in China and Iran reported that intranasal IFNα-2b combined with ribavirin showed antiviral activity in COVID-19 patients (42, 175, 176), more clinical trials are needed before using these drugs.

3.3.2.6. Statins

Statins have anti-inflammatory and immunomodulatory properties and can be used against the COVID-19 infection (177). They are commonly used to fight coronary artery diseases. They can increase the expression of the ACE2 (178). In a viral infection, stimulation of ACE2 by statins can help restore viral infection-induced endothelial dysfunction and maintain the homeostasis of the patients (179). In addition, statins showed antiviral activity by inhibiting viral protease (180). Therefore, the pleiotropic properties of this drug may be related to the therapeutic impact on COVID-19 patients through interfering with endothelial function and anti-thrombotic and anti-inflammatory effects (181, 182). Recent clinical studies reported that statin administration was associated with reduced mortality and reduced the risk of ICU admission in COVID-19 patients via modulation of cytokine overexpression (183, 184). Therefore, this drug can be an essential adjunctive substance in COVID-19 management. Further studies are required to confirm the higher observed

benefits.

4. Discussion

The COVID-19 infection as a global pandemic is a significant challenge to public health and national economies. Given that, no specific therapy has been approved to treat this infection, and repositioning existing drugs that target specific steps within the life cycle of the COVID-19 virus or host immune system could be a fast and alternative therapeutic strategy for dealing with this viral outbreak. To identify potential candidate drugs with promising antiviral activities, they should not only prove effective on animal models but be confirmed by previous human experience. In other words, although animal studies may help predict the drugs' effect in a more realistic scenario, the outcomes between animals and humans can be variable. It should be borne in mind that specific antivirals for the long-term benefit should be developed along with efficient use of repurposed drugs for COVID-19 therapy. Although the introduction and advancement of specific antivirals therapy with different targets in the life cycle of COVID-19 and the use of combination therapy may have more potential to prevent severe disease progression by reducing viral load, results indicate that symptomatic control of this infection (by targeting the host cells) has more positive outcomes compared to current antivirals (which effect the virus directly).

Drug repurposing is a promising strategy that can elevate the effectiveness of the therapy via a simplified pharmacokinetic and pharmacodynamic profile. In addition, these strategies can reduce drawbacks associated with conventional new drugs, such as drug-drug interaction and drug resistance. Optimizing drug repurposing can be done by data available on many public platforms, such as CheEMBL, PubChem, DrugBank, DrugCentral, STITCH, PHAROS, SEA, SuperTarget, TTD, along with others. that can help organize a correlation between chemical and physical properties of multiple drugs, through integrating in silico prediction and in vitro validation. The use of available drugs that can target specific steps within the life cycle of SARS-CoV-2 may be a strong alternative therapeutic strategy for dealing with this pandemic and similar future virus pandemics. To improve the success rate of drug repurposing in the clinical trial, it is recommended to personalize drug repurposing, taking into account the gene expression profile as an effective approach. Using new in silico methods and AI integrated with big data, it is possible to progress in the drug repurposing field and provide support for taking decisions for the therapeutic benefits of drugs against COVID-19. The preliminary screening of old drugs in the face of new virus diseases such as COVID-19 using the newest graph convolutional network models can help medical staff screen out potential treatments for COVID-19 in the shortest time.

Moreover, some items such as the inclusion criteria, administration route, treatment assignment, co-existing treatments, and endpoints should be carefully considered in the design and interpretation of clinical data of COVID-19 drug reposting studies. Among all of the drugs reviewed in this article, some antivirals (such as molnupiravir, lopinavir/ritonavir, paxlovid) and a few other medicines (such as corticosteroids, casirivimab/imdevimab) have more scientific evidence along with more reliable clinical trials showing anti-covid efficacy. In summary, In sum, twe want to underscore here the critical point that there are no promised pharmacotherapy guidelines to be a definite treatment for this pandemic yet. Subsequently, prescribing unnecessary medicines for infected patients, especially antibiotics (such as azithromycin -antibiotic resistance development-) and corticosteroids (if prescribed extraordinary, especially before the inflammatory phase -mucormycosis-) can expose the globe to more health-related challenges. In light of all the above, from our standpoint, complete vaccination of the majority of the worldwide population within a limited duration (to avoid further mutation of SARS-CoV-19) is the golden key to controlling and overcoming the pandemic.

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References

1. Carrasco-Hernandez R, Jácome R, López Vidal Y, Ponce de León S. Are RNA viruses candidate agents for the next global pandemic? A review. ILAR journal. 2017;58(3):343-58.
2. Duffy S. Why are RNA virus mutation rates so damn high? PLoS biology. 2018;16(8):e3000003.
3. Meganck RM, Baric RS. Developing therapeutic approaches for twenty-first-century emerging infectious viral diseases. Nature Medicine. 2021;27(3):401-10.
4. Kin N, Miszczak F, Lin W, Gouilh MA, Vabret A. Genomic analysis of 15 human coronaviruses OC43 (HCoV-OC43s) circulating in France from 2001 to 2013 reveals a high intra-specific diversity with new recombinant genotypes. Viruses. 2015;7(5):2358-77.
5. Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology. 2018;23(2):130-7.
6. Drosten C, Günther S, Preiser W, Van Der Werf S, Brodt H-R, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. New England journal of medicine. 2003;348(20):1967-76.
7. Zhong N, Zheng B, Li Y, Poon L, Xie Z, Chan K, et al. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. The Lancet. 2003;362(9393):1353-8.
8. Organization WH. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. http://www who int/csr/sars/country/table2004_04_21/en/index html. 2003.
9. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD,

Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. New England Journal of Medicine. 2012;367(19):1814-20.

10. Situation WM. January 2020. World Health Organization Regional Office for the Eastern Mediterranean. 2020.

11. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-9.

12. McIntosh K, Hirsch MS, Bloom A. Coronavirus disease 2019 (COVID-19). UpToDate Hirsch MS Bloom. 2020;5(1).

13. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. New England journal of medicine. 2020.

14. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical characteristics of coronavirus disease 2019 in China. New England journal of medicine. 2020;382(18):1708-20.

15. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA internal medicine. 2020;180(7):934-43.

16. Maxmen A. More than 80 clinical trials launch to test coronavirus treatments. Nature. 2020;578(7795):347-9.

17. Mercorelli B, Palù G, Loregian A. Drug repurposing for viral infectious diseases: how far are we? Trends in microbiology. 2018;26(10):865-76.

18. Pillaiyar T, Meenakshisundaram S, Manickam M, Sankaranarayanan M. A medicinal chemistry perspective of drug repositioning: Recent advances and challenges in drug discovery. European journal of medicinal chemistry. 2020;195:112275.

19. Senger MR, Evangelista TCS, Dantas RF, Santana MVdS, Gonçalves LCS, de Souza Neto LR, et al. COVID-19: molecular targets, drug repurposing and new avenues for drug discovery. Memórias do Instituto Oswaldo Cruz. 2020;115.

20. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19—preliminary report. New England Journal of Medicine. 2020.

21. Consortium WST. Repurposed antiviral drugs for COVID-19—interim WHO SOLIDARITY trial results. New England journal of medicine. 2021;384(6):497-511.

22. Siegel D, Hui HC, Doerffler E, Clarke MO, Chun K, Zhang L, et al. Discovery and synthesis of a phosphoramidate prodrug of a pyrrolo [2, 1-f][triazin-4-amino] adenine C-nucleoside (GS-5734) for the treatment of Ebola and emerging viruses. ACS Publications; 2017.

23. Gordon CJ, Tchesnokov EP, Feng JY, Porter DP, Götte M. The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. Journal of Biological Chemistry. 2020;295(15):4773-9.

24. Gordon CJ, Tchesnokov EP, Woolner E, Perry JK, Feng JY, Porter DP, et al. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. Journal of Biological Chemistry. 2020;295(20):6785-97.

25. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate use of remdesivir for patients with severe Covid-19. New England Journal of Medicine. 2020;382(24):2327-36.

26. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, et al. First case of 2019 novel coronavirus in the United States. New England Journal of Medicine. 2020.

27. Nicastri E, Petrosillo N, Ascoli Bartoli T, Lepore L, Mondi A, Palmieri F, et al. National institute for the infectious diseases "L. Spallanzani" IRCCS. Recommendations for COVID-19 clinical management. Infectious disease reports. 2020;12(1):3-9.

28. Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. The lancet. 2020;395(10236):1569-78.

29. Goldman JD, Lye DC, Hui DS, Marks KM, Bruno R, Montejano R, et al. Remdesivir for 5 or 10 days in patients with severe Covid-19. New England Journal of Medicine. 2020;383(19):1827-37.

30. Kaka AS, MacDonald R, Greer N, Vela K, Duan-Porter W, Obley A, et al. Major update: remdesivir for adults with COVID-19: a living systematic review and meta-analysis for the American College of Physicians Practice Points. Annals of internal medicine. 2021;174(5):663-72.

31. Angamo MT, Mohammed MA, Peterson GM. Efficacy and safety of remdesivir in hospitalised COVID-19 patients: a systematic review and meta-analysis. Infection. 2021:1-15.

32. Lai C-C, Chen C-H, Wang C-Y, Chen K-H, Wang Y-H, Hsueh P-R. Clinical efficacy and safety of remdesivir in patients with COVID-19: a systematic review and network meta-analysis of randomized controlled trials. Journal of Antimicrobial Chemotherapy. 2021.

33. Bansal V, Mahapure KS, Bhurwal A, Gupta I, Hassanain S, Makadia J, et al. Mortality benefit of remdesivir in COVID-19: a systematic review and meta-analysis. Frontiers in medicine. 2020;7.

34. Okoli GN, Rabbani R, Copstein L, Al-Juboori A, Askin N, Abou-Setta AM. Remdesivir for coronavirus disease 2019 (COVID-19): a systematic review with meta-analysis and trial sequential analysis of randomized controlled trials. Infectious Diseases. 2021:1-9.

35. Piscoya A, Ng-Sueng LF, Parra del Riego A, Cerna-Viacava R, Pasupuleti V, Roman YM, et al. Efficacy and harms of remdesivir for the treatment of COVID-19: a systematic review and meta-analysis. PloS one. 2020;15(12):e0243705.

36. Singh S, Khera D, Chugh A, Khera PS, Chugh VK. Efficacy and safety of remdesivir in COVID-19 caused by SARS-CoV-2: a systematic review and meta-analysis. BMJ open. 2021;11(6):e048416.

37. Abdelnabi R, Morais ATSd, Leyssen P, Imbert I, Beaucourt S, Blanc H, et al. Understanding the mechanism of the broad-spectrum antiviral activity of favipiravir (T-705): key role of the F1 motif of the viral polymerase. Journal of virology. 2017;91(12):e00487-17.

38. Lou Y, Liu L, Yao H, Hu X, Su J, Xu K, et al. Clinical outcomes and plasma concentrations of baloxavir marboxil and favipiravir in COVID-19 patients: an exploratory randomized, controlled trial. European Journal of Pharmaceutical Sciences. 2021;157:105631.

39. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental treatment with favipiravir for COVID-19: an open-label control study. Engineering. 2020;6(10):1192-8.

40. Udwadia ZF, Singh P, Barkate H, Patil S, Rangwala S, Pendse A, et al. Efficacy and safety of favipiravir, an oral RNA-dependent RNA polymerase inhibitor, in mild-to-moderate COVID-19: A randomized, comparative, open-label,

multicenter, phase 3 clinical trial. International Journal of Infectious Diseases. 2021;103:62-71.

41. Chen C, Huang J, Cheng Z, Wu J, Chen S, Zhang Y, et al. Favipiravir versus arbidol for COVID-19: a randomized clinical trial. MedRxiv. 2020.

42. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19), Drug Discov. Ther. 14 (2020) 58–60. 2020.

43. Du YX, Chen XP. Favipiravir: pharmacokinetics and concerns about clinical trials for 2019-nCoV infection. Clinical Pharmacology & Therapeutics. 2020;108(2):242-7.

44. Pires de Mello CP, Tao X, Kim TH, Vicchiarelli M, Bulitta JB, Kaushik A, et al. Clinical regimens of favipiravir inhibit Zika virus replication in the hollow-fiber infection model. Antimicrobial agents and chemotherapy. 2018;62(9):e00967-18.

45. Nguyen THT, Guedj J, Anglaret X, Laouénan C, Madelain V, Taburet A-M, et al. Favipiravir pharmacokinetics in Ebola-Infected patients of the JIKI trial reveals concentrations lower than targeted. PLoS neglected tropical diseases. 2017;11(2):e0005389.

46. Khamis F, Al Naabi H, Al Lawati A, Ambusaidi Z, Al Sharji M, Al Barwani U, et al. Randomized controlled open label trial on the use of favipiravir combined with inhaled interferon beta-1b in hospitalized patients with moderate to severe COVID-19 pneumonia. International Journal of Infectious Diseases. 2021;102:538-43.

47. Hassanipour S, Arab-Zozani M, Amani B, Heidarzad F, Fathalipour M, Martinez-de-Hoyo R. The efficacy and safety of Favipiravir in treatment of COVID-19: A systematic review and meta-analysis of clinical trials. Scientific reports. 2021;11(1):1-11.

48. Manabe T, Kambayashi D, Akatsu H, Kudo K. Favipiravir for the treatment of patients with COVID-19: a systematic review and meta-analysis. BMC infectious diseases. 2021;21(1):1-13.

49. Graci JD, Cameron CE. Mechanisms of action of ribavirin against distinct viruses. Reviews in medical virology. 2006;16(1):37-48.

50. Khalili JS, Zhu H, Mak NSA, Yan Y, Zhu Y. Novel coronavirus treatment with ribavirin: Groundwork for an evaluation concerning COVID-19. Journal of medical virology. 2020;92(7):740-6.

51. Day CW, Baric R, Cai SX, Frieman M, Kumaki Y, Morrey JD, et al. A new mouse-adapted strain of SARS-CoV as a lethal model for evaluating antiviral agents in vitro and in vivo. Virology. 2009;395(2):210-22.

52. Balzarini J, Keyaerts E, Vijgen L, Egberink H, De Clercq E, Van Ranst M, et al. Inhibition of feline (FIPV) and human (SARS) coronavirus by semisynthetic derivatives of glycopeptide antibiotics. Antiviral research. 2006;72(1):20-33.

53. Kim UJ, Won E-J, Kee S-J, Jung S-I, Jang H-C. Case report Combination therapy with lopinavir/ritonavir, ribavirin and interferon-α for Middle East respiratory syndrome. Antiviral therapy. 2016;21:455-9.

54. Chan K, Lai S, Chu C, Tsui E, Tam C, Wong M, et al. Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study. Hong Kong medical journal. 2003.

55. Hung I. Lopinavir/Ritonavir, Ribavirin and IFN-beta Combination for nCoV Treatment (NCT04276688)[Internet]. 2020. 2020.

56. trial C. Clinical trial on regularity of TCM syndrome and differentiation treatment of COVID-19. (CTOROTSADTOC). 2020. https://clinicaltrials.gov/ct2/show/NCT04306497. Accessed March 13, 2020.. 2020.

57. Hung IF-N, Lung K-C, Tso EY-K, Liu R, Chung TW-H, Chu M-Y, et al. Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. The Lancet. 2020;395(10238):1695-704.

58. Tong S, Su Y, Yu Y, Wu C, Chen J, Wang S, et al. Ribavirin therapy for severe COVID-19: a retrospective cohort study. International journal of antimicrobial agents. 2020;56(3):106114.

59. Bhatia HK, Singh H, Grewal N, Natt NK. Sofosbuvir: a novel treatment option for chronic hepatitis C infection. Journal of pharmacology & pharmacotherapeutics. 2014;5(4):278.

60. Ferreira AC, Reis PA, de Freitas CS, Sacramento CQ, Villas Bôas Hoelz L, Bastos MM, et al. Beyond members of the Flaviviridae family, sofosbuvir also inhibits chikungunya virus replication. Antimicrobial agents and chemotherapy. 2019;63(2):e01389-18.

61. Elfiky AA. Anti-HCV, nucleotide inhibitors, repurposing against COVID-19. Life sciences. 2020;248:117477.

62. Sacramento CQ, Fintelman-Rodrigues N, Temerozo JR, Da Silva AdPD, Dias SdSG, da Silva CdS, et al. The in vitro antiviral activity of the anti-hepatitis C virus (HCV) drugs daclatasvir and sofosbuvir against SARS-CoV-2. BioRxiv. 2020.

63. Eslami G, Mousaviasl S, Radmanesh E, Jelvay S, Bitaraf S, Simmons B, et al. The impact of sofosbuvir/daclatasvir or ribavirin in patients with severe COVID-19. Journal of Antimicrobial Chemotherapy. 2020;75(11):3366-72.

64. Sadeghi A, Ali Asgari A, Norouzi A, Kheiri Z, Anushirvani A, Montazeri M, et al. Sofosbuvir and daclatasvir compared with standard of care in the treatment of patients admitted to hospital with moderate or severe coronavirus infection (COVID-19): a randomized controlled trial. Journal of Antimicrobial Chemotherapy. 2020;75(11):3379-85.

65. Sayad B, Sobhani M, Khodarahmi R. Sofosbuvir as repurposed antiviral drug against COVID-19: why were we convinced to evaluate the drug in a registered/approved clinical trial? Archives of medical research. 2020;51(6):577-81.

66. Orkin C, Llibre J, Gallien S, Antinori A, Behrens G, Carr A. Nucleoside reverse transcriptase inhibitor-reducing strategies in HIV treatment: assessing the evidence. HIV medicine. 2018;19(1):18-32.

67. Kearney BP, Flaherty JF, Shah J. Tenofovir disoproxil fumarate. Clinical pharmacokinetics. 2004;43(9):595-612.

68. Parienti J-J, Prazuck T, Peyro-Saint-Paul L, Fournier A, Valentin C, Brucato S, et al. Effect of Tenofovir Disoproxil Fumarate and Emtricitabine on nasopharyngeal SARS-CoV-2 viral load burden amongst outpatients with COVID-19: A pilot, randomized, open-label phase 2 trial. EClinicalMedicine. 2021;38:100993.

69. Ayerdi O, Puerta T, Clavo P, Vera M, Ballesteros J, Fuentes ME, et al., editors. Preventive efficacy of tenofovir/emtricitabine against severe acute respiratory syndrome coronavirus 2 among pre-exposure prophylaxis users. Open Forum Infectious Diseases; 2020: Oxford University Press US.

70. Polo R, Hernán M. Randomized clinical trial for the prevention of SARS-CoV-2 infection (COVID-19) in healthcare personnel

(EPICOS). ClinicalTrials. gov; 2020. 2020.

71. Ignacio HUS. Daily Regimen of Tenofovir/Emtricitabine as Prevention for COVID-19 in Health Care Personnel in Colombia. ClinicalTrials gov. 2020.

72. TAF/FTC. TAF/FTC for Pre-exposure Prophylaxis of COVID-19 in Healthcare Workers (CoviPrep Study). https://ClinicalTrials.gov/show/NCT04405271. Accessed October 12, 2020. 2020.

73. Wang R-R, Yang Q-H, Luo R-H, Peng Y-M, Dai S-X, Zhang X-J, et al. Azvudine, a novel nucleoside reverse transcriptase inhibitor showed good drug combination features and better inhibition on drug-resistant strains than lamivudine in vitro. PloS one. 2014;9(8):e105617.

74. Ren Z, Luo H, Yu Z, Song J, Liang L, Wang L, et al. A Randomized, Open-Label, Controlled Clinical Trial of Azvudine Tablets in the Treatment of Mild and Common COVID-19, a Pilot Study. Advanced Science. 2020;7(19):2001435.

75. A. A Clinical Trial for Azvudine in the Treatment of Novel Coronavirus Pneumonia (COVID-19） https://ClinicalTrials.gov/show/NCT04425772. 2020.

76. s. Study on Safety and Clinical Efficacy of AZVUDINE in COVID-19 Patients (SARS-CoV-2 Infected)https://ClinicalTrials.gov/show/NCT04668235. 2020.

77. Chen Y, Yiu C. B, Wong KY. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CL) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates [version 1. 2020.

78. Savarino A. Expanding the frontiers of existing antiviral drugs: possible effects of HIV-1 protease inhibitors against SARS and avian influenza. Journal of clinical virology. 2005;34(3):170-8.

79. Liaño JP, Tenorio CH. Chemical characteristics, mechanism of action and antiviral activity of darunavir. Enfermedades infecciosas y microbiologia clinica. 2008;26:3-9.

80. Chen J, Xia L, Liu L, Xu Q, Ling Y, Huang D, et al. Antiviral activity and safety of darunavir/cobicistat for the treatment of COVID-19. Open Forum. Infectious Diseases. 2020.

81. Ledford H. Covid antiviral pills: what scientists still want to know. Nature. 2021;599(7885):358-9.

82. Mahase E. Covid-19: Pfizer's paxlovid is 89% effective in patients at risk of serious illness, company reports. British Medical Journal Publishing Group; 2021.

83. Adedeji AO, Singh K, Kassim A, Coleman CM, Elliott R, Weiss SR, et al. Evaluation of SSYA10-001 as a replication inhibitor of severe acute respiratory syndrome, mouse hepatitis, and Middle East respiratory syndrome coronaviruses. Antimicrobial agents and chemotherapy. 2014;58(8):4894-8.

84. Cvetkovic RS, Goa KL. Lopinavir/ritonavir. Drugs. 2003;63(8):769-802.

85. Chandwani A, Shuter J. Lopinavir/ritonavir in the treatment of HIV-1 infection: a review. Therapeutics and clinical risk management. 2008;4(5):1023.

86. Lim J, Jeon S, Shin HY, Kim MJ, Seong YM, Lee WJ, et al. Case of the index patient who caused tertiary transmission of COVID-19 infection in Korea: The application of lopinavir/ritonavir for the treatment of COVID-19 infected pneumonia monitored by quantitative RT-PCR. Journal of Korean medical science. 2020;35(6):e79-e.

87. Xu K, Cai H, Shen Y, Ni Q, Chen Y, Hu S, et al. Management of COVID-19: the Zhejiang experience. Journal of Zhejiang University (medical science). 2020;49(2):147-57.

88. Han W, Quan B, Guo Y, Zhang J, Lu Y, Feng G, et al. The course of clinical diagnosis and treatment of a case infected with coronavirus disease 2019. Journal of medical virology. 2020;92(5):461.

89. WHO. "Solidarity" Clinical Trial for COVID-19 Treatments. 2020. 2020.

90. Zhu Z, Lu Z, Xu T, Chen C, Yang G, Zha T, et al. Arbidol monotherapy is superior to lopinavir/ritonavir in treating COVID-19. Journal of Infection. 2020;81(1):e21-e3.

91. Deng L, Li C, Zeng Q, Liu X, Li X, Zhang H, et al. Arbidol combined with LPV/r versus LPV/r alone against Corona Virus Disease 2019: A retrospective cohort study. Journal of Infection. 2020;81(1):e1-e5.

92. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell research. 2020;30(3):269-71.

93. Hoffmann M, Schroeder S, Kleine-Weber H, Müller MA, Drosten C, Pöhlmann S. Nafamostat mesylate blocks activation of SARS-CoV-2: new treatment option for COVID-19. Antimicrobial agents and chemotherapy. 2020;64(6):e00754-20.

94. Li K, Meyerholz DK, Bartlett JA, McCray Jr PB. The TMPRSS2 Inhibitor Nafamostat Reduces SARS-CoV-2 Pulmonary Infection in Mouse Models of COVID-19. Mbio. 2021;12(4):e00970-21.

95. Hospital GNU. Clinical Efficacy of Nafamostat Mesylate for COVID-19 Pneumonia,ClinicalTrials.gov Identifier: NCT04418128,June 5, 2020. 2020.

96. Padova UH. Efficacy of Nafamostat in Covid-19 Patients (RACONA Study) (RACONA),ClinicalTrials.gov Identifier: NCT04352400,April 20, 2020. 2020.

97. Takahashi W, Yoneda T, Koba H, Ueda T, Tsuji N, Ogawa H, et al. Potential mechanisms of nafamostat therapy for severe COVID-19 pneumonia with disseminated intravascular coagulation. International Journal of Infectious Diseases. 2021;102:529-31.

98. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral research. 2020;178:104787.

99. de Melo GD, Lazarini F, Larrous F, Feige L, Kornobis E, Levallois S, et al. Attenuation of clinical and immunological outcomes during SARS-CoV-2 infection by ivermectin. EMBO molecular medicine. 2021;13(8):e14122.

100. Patrı A, Fabbrocini G. Hydroxychloroquine and ivermectin: a synergistic combination for COVID-19 chemoprophylaxis and/or treatment. J Am Acad Dermatol. 2020:30557-0.

101. Momekov G, Momekova D. Ivermectin as a potential COVID-19 treatment from the pharmacokinetic point of view: antiviral levels are not likely attainable with known dosing regimens. Biotechnology & biotechnological equipment. 2020;34(1):469-74.

102. A. P. Usefulness of ivermectin in COVID-19 illness. ; 2020 Available at: SSRN 3580524. 2020.

103. University AHS. Ivermectin for Severe COVID-19 Management,ClinicalTrials.gov Identifier: NCT04646109,November 27, 2020. 2020.

104. S.A. LEP. Ivermectin Effect on SARS-CoV-2 Replication in Patients With COVID-19,ClinicalTrials.gov Identifier:

NCT04381884,May 11, 2020. 2020.

105. Dentistry FCoMa. ffectiveness of Ivermectin in SARS-CoV-2/COVID-19 Patients,ClinicalTrials.gov Identifier: NCT04739410,February 4, 2021. 2021.

106. Toots M, Yoon J-J, Hart M, Natchus MG, Painter GR, Plemper RK. Quantitative efficacy paradigms of the influenza clinical drug candidate EIDD-2801 in the ferret model. Translational Research. 2020;218:16-28.

107. Abdelnabi R, Foo CS, Kaptein SJ, Zhang X, Do TND, Langendries L, et al. The combined treatment of Molnupiravir and Favipiravir results in a potentiation of antiviral efficacy in a SARS-CoV-2 hamster infection model. EBioMedicine. 2021;72:103595.

108. Fischer WA, Eron JJ, Holman W, Cohen MS, Fang L, Szewczyk LJ, et al. Molnupiravir, an Oral Antiviral Treatment for COVID-19. medRxiv. 2021.

109. McCoy J, Cadegiani FA, Wambier CG, Herrera S, Vaño-Galván S, Mesinkovska NA, et al. 5-alpha-reductase inhibitors are associated with reduced frequency of COVID-19 symptoms in males with androgenetic alopecia. Journal of the European Academy of Dermatology and Venereology: JEADV. 2020.

110. McCoy J, Goren A, Cadegiani FA, Vaño-Galván S, Kovacevic M, Situm M, et al. Proxalutamide Reduces the Rate of Hospitalization for COVID-19 Male Outpatients: A Randomized Double-Blinded Placebo-Controlled Trial. Frontiers in Medicine. 2021:1043.

111. Qiao Y, Wang X-M, Mannan R, Pitchiaya S, Zhang Y, Wotring JW, et al. Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and TMPRSS2. Proceedings of the National Academy of Sciences. 2021;118(1).

112. Wambier CG, Vaño-Galván S, McCoy J, Pai S, Dhurat R, Goren A. Androgenetic alopecia in COVID-19: compared to age-matched epidemiologic studies and hospital outcomes with or without the Gabrin sign. Journal of the American Academy of Dermatology. 2020;83(6):e453-e4.

113. Ramos PM, Ianhez M, Miot HA. Alopecia and Gray Hair Are Associated with COVID-19 Severity. Experimental dermatology. 2020.

114. Salazar Arenas MÁ, Muñoz Del Carpio-Toia A, Aybar Galdos J, Rodriguez-Morales A. Alopecia and severity of COVID-19: a cross-sectional study in Peru. Le infezioni in medicina. 2021:37-45.

115. Goren A, Wambier CG, Herrera S, McCoy J, Vaño-Galván S, Gioia F, et al. Anti-androgens may protect against severe COVID-19 outcomes: results from a prospective cohort study of 77 hospitalized men. Journal of the European Academy of Dermatology and Venereology. 2020.

116. Cadegiani FA, McCoy J, Wambier CG, Goren A. Early antiandrogen therapy with dutasteride reduces viral shedding, inflammatory responses, and Time-to-Remission in males with COVID-19: a randomized, double-blind, placebo-controlled interventional trial (EAT-DUTA AndroCoV trial–biochemical). Cureus. 2021;13(2).

117. Zarehoseinzade E, Allami A, Ahmadi M, Bijani B, Mohammadi N. Finasteride in hospitalized adult males with COVID-19: A risk factor for severity of the disease or an adjunct treatment: A randomized controlled clinical trial. Medical Journal of the Islamic Republic of Iran. 2021;35:30.

118. Cadegiani FA, McCoy J, Wambier CG, Vaño-Galván S, Shapiro J, Tosti A, et al. Proxalutamide significantly accelerates viral clearance and reduces time to clinical remission in patients with mild to moderate COVID-19: Results from a randomized, double-blinded, placebo-controlled trial. Cureus. 2021;13(2).

119. Horby PW, Mafham M, Peto L, Campbell M, Pessoa-Amorim G, Spata E, et al. Casirivimab and imdevimab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. medRxiv. 2021.

120. O'Brien M, Neto E, Chen K, Isa F, Heirman I, Sarkar N, et al. Casirivimab with imdevimab antibody cocktail for COVID-19 prevention: Interim results. Topics in Antiviral Medicine. 2021:33-4.

121. Ganesh R, Philpot LM, Bierle DM, Anderson RJ, Arndt LL, Arndt RF, et al. Real-World Clinical Outcomes of Bamlanivimab and Casirivimab-Imdevimab among High-Risk Patients with Mild to Moderate Coronavirus Disease 2019. The Journal of Infectious Diseases. 2021;224(8):1278-86.

122. Razonable RR. Real-World Clinical Outcomes of Bamlanivimab and Casirivimab-Imdevimab among High-Risk Patients with Mild to Moderate Coronavirus Disease 2019. 2021.

123. Falcone M, Tiseo G, Valoriani B, Barbieri C, Occhineri S, Mazzetti P, et al. Efficacy of bamlanivimab/etesevimab and casirivimab/imdevimab in preventing progression to severe covid-19 and role of variants of concern. Infectious diseases and therapy. 2021;10(4):2479-88.

124. Frediansyah A, Tiwari R, Sharun K, Dhama K, Harapan H. Antivirals for COVID-19: A critical review. Clinical Epidemiology and global health. 2021;9:90-8.

125. Ghosh RK, Ghosh SM, Chawla S. Recent advances in antiretroviral drugs. Expert opinion on pharmacotherapy. 2011;12(1):31-46.

126. Wang X, Cao R, Zhang H, Liu J, Xu M, Hu H, et al. The anti-influenza virus drug, arbidol is an efficient inhibitor of SARS-CoV-2 in vitro. Cell discovery. 2020;6(1):1-5.

127. Blaising J, Polyak SJ, Pécheur E-I. Arbidol as a broad-spectrum antiviral: an update. Antiviral research. 2014;107:84-94.

128. Wang Z, Chen X, Lu Y, Chen F, Zhang W. Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment. Bioscience trends. 2020.

129. Rosa SGV, Santos WC. Clinical trials on drug repositioning for COVID-19 treatment. Revista Panamericana de Salud Pública. 2020;44:e40.

130. Li Y, Xie Z, Lin W, Cai W, Wen C, Guan Y, et al. An exploratory randomized, controlled study on the efficacy and safety of lopinavir/ritonavir or arbidol treating adult patients hospitalized with mild/moderate COVID-19 (ELACOI). MedRxiv. 2020.

131. Union Hospital TMC, Huazhong University of Science and Technology. Clinical Study of Arbidol Hydrochloride Using for Post-exposure Prophylaxis of 2019-nCoV in High-risk Population Including Medical Staff.ChiCTR; 2020-02-05; TrialID: ChiCTR2000029592 2020.

132. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The lancet. 2020;395(10224):565-74.

133. Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, et al. Baricitinib as potential treatment for 2019-nCoV acute

respiratory disease. Lancet (London, England). 2020;395(10223):e30.

134. Bagca BG, Avci CB. The potential of JAK/STAT pathway inhibition by ruxolitinib in the treatment of COVID-19. Cytokine & growth factor reviews. 2020;54:51-61.

135. Hoang TN, Pino M, Boddapati AK, Viox EG, Starke CE, Upadhyay AA, et al. Baricitinib treatment resolves lower-airway macrophage inflammation and neutrophil recruitment in SARS-CoV-2-infected rhesus macaques. Cell. 2021;184(2):460-75. e21.

136. Giudice V, Pagliano P, Vatrella A, Masullo A, Poto S, Polverino BM, et al. Combination of ruxolitinib and eculizumab for treatment of severe SARS-CoV-2-related acute respiratory distress syndrome: a controlled study. Frontiers in pharmacology. 2020;11:857.

137. Pharmaceuticals N. tudy to Assess the Efficacy and Safety of Ruxolitinib in Patients With COVID-19 Associated Cytokine Storm (RUXCOVID),ClinicalTrials.gov Identifier: NCT04362137,April 24, 2020. 2020.

138. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases. The Lancet infectious diseases. 2003;3(11):722-7.

139. Rosenke K, Jarvis MA, Feldmann F, Schwarz B, Okumura A, Lovaglio J, et al. Hydroxychloroquine proves ineffective in hamsters and macaques infected with SARS-CoV-2. BioRxiv. 2020.

140. Maisonnasse P, Guedj J, Contreras V, Behillil S, Solas C, Marlin R, et al. Hydroxychloroquine use against SARS-CoV-2 infection in non-human primates. Nature. 2020;585(7826):584-7.

141. Lou B, Li T-D, Zheng S-F, Su Y-Y, Li Z-Y, Liu W, et al. Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. European Respiratory Journal. 2020;56(2).

142. Park S-J, Yu K-M, Kim Y-I, Kim S-M, Kim E-H, Kim S-G, et al. Antiviral efficacies of FDA-approved drugs against SARS-CoV-2 infection in ferrets. MBio. 2020;11(3):e01114-20.

143. Mitjà O, Clotet B. Use of antiviral drugs to reduce COVID-19 transmission. The Lancet Global Health. 2020;8(5):e639-e40.

144. University T. Efficacay of Chloroquine or Hydroxychloroquine in COVID-19 Treatment,ClinicalTrials.gov Identifier: NCT04353336,ClinicalTrials.gov Identifier: NCT04353336. 2020.

145. Hernandez A. Healthcare Worker Exposure Response and Outcomes of Hydroxychloroquine (HERO-HCQ), ClinicalTrials.gov Identifier: NCT04334148,April 6, 2020. 2020.

146. Celje GaTH. Use of Bromhexine and Hydroxychloroquine for Treatment of COVID-19 Pneumonia,ClinicalTrials.gov Identifier: NCT04355026,April 21, 2020. 2020.

147. Doyno C, Sobieraj DM, Baker WL. Toxicity of chloroquine and hydroxychloroquine following therapeutic use or overdose. Clinical Toxicology. 2021;59(1):12-23.

148. Watson JA, Tarning J, Hoglund RM, Baud FJ, Megarbane B, Clemessy J-L, et al. Concentration-dependent mortality of chloroquine in overdose. Elife. 2020;9:e58631.

149. McChesney EW. Animal toxicity and pharmacokinetics of hydroxychloroquine sulfate. The American journal of medicine. 1983;75(1):11-8.

150. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. nature. 2020;579(7798):270-3.

151. Singh TU, Parida S, Lingaraju MC, Kesavan M, Kumar D, Singh RK. Drug repurposing approach to fight COVID-19. Pharmacological Reports. 2020:1-30.

152. Aghdashi M, Aghdashi M, Rabiepoor M. Osteopoikilosis: pain as a presenting symptom in three family members. Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders. 2011;4:CMAMD. S7035.

153. Xie Y, You Q, Wu C, Cao S, Qu G, Yan X, et al. Impact of cardiovascular disease on clinical characteristics and outcomes of coronavirus disease 2019 (COVID-19). Circulation Journal. 2020:CJ-20-0348.

154. Meng J, Xiao G, Zhang J, He X, Ou M, Bi J, et al. Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. Emerging microbes & infections. 2020;9(1):757-60.

155. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. Journal of virology. 2020;94(7):e00127-20.

156. Oliver ME, Hinks TS. Azithromycin in viral infections. Reviews in medical virology. 2021;31(2):e2163.

157. Venditto VJ, Haydar D, Abdel-Latif A, Gensel J, Anstead MI, Pitts MG, et al. Immunomodulatory effects of azithromycin revisited: potential applications to COVID-19. Frontiers in Immunology. 2021;12:285.

158. Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, et al. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. New England Journal of Medicine. 2020;383(6):517-25.

159. Abaleke E, Abbas M, Abbasi S, Abbott A, Abdelaziz A, Abdelbadiee S, et al. Azithromycin in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. The Lancet. 2021;397(10274):605-12.

160. Butler CC, Dorward J, Yu L-M, Gbinigie O, Hayward G, Saville BR, et al. Azithromycin for community treatment of suspected COVID-19 in people at increased risk of an adverse clinical course in the UK (PRINCIPLE): a randomised, controlled, open-label, adaptive platform trial. The Lancet. 2021;397(10279):1063-74.

161. University SV. Clarithromycin Versus Azithromycin in Treatment of Mild COVID-19 Infection,ClinicalTrials.gov Identifier: NCT04622891,November 10, 2020. 2020.

162. Corporation HM. Hydroxychloroquine With or Without Azithromycin for Virologic Cure of COVID-19,ClinicalTrials.gov Identifier: NCT04349592,April 16, 2020. 2020.

163. Gautret P, Lagier J-C, Parola P, Meddeb L, Mailhe M, Doudier B, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. International journal of antimicrobial agents. 2020;56(1):105949.

164. Bersanelli M. Controversies about COVID-19 and anticancer treatment with immune checkpoint inhibitors. Future Medicine; 2020.

165. Sheppard M, Laskou F, Stapleton PP, Hadavi S, Dasgupta B. Tocilizumab (actemra). Human vaccines & immunotherapeutics. 2017;13(9):1972-88.

166. Zhang X, Song K, Tong F, Fei M, Guo H, Lu Z, et al. First case of COVID-19 in a patient with multiple myeloma successfully treated with tocilizumab. Blood advances. 2020;4(7):1307.

167. Khiali S, Khani E, Entezari-Maleki T. A comprehensive review of tocilizumab in covid-19 acute respiratory distress syndrome. The Journal of Clinical Pharmacology. 2020;60(9):1131-46.

168. Horby P, Mafham M, Linsell L, Bell JL, Staplin N, Emberson JR, et al. Effect of hydroxychloroquine in hospitalized patients with COVID-19: preliminary results from a multi-centre, randomized, controlled trial. MedRxiv. 2020.

169. Theoharides T, Conti P. Dexamethasone for COVID-19? Not so fast. J Biol Regul Homeost Agents. 2020;34(3):1241-3.

170. Group TRC. Dexamethasone in hospitalized patients with Covid-19—preliminary report. The New England journal of medicine. 2020.

171. Ranjbar K, Moghadami M, Mirahmadizadeh A, Fallahi MJ, Khaloo V, Shahriarirad R, et al. Methylprednisolone or dexamethasone, which one is superior corticosteroid in the treatment of hospitalized COVID-19 patients: a triple-blinded randomized controlled trial. BMC infectious diseases. 2021;21(1):1-8.

172. Li J, Lehmann C, Chen X, Romerio F, Lu W. Total chemical synthesis of human interferon alpha-2b via native chemical ligation. Journal of peptide science. 2015;21(7):554-60.

173. Thomas H, Foster G, Platis D. Mechanisms of action of interferon and nucleoside analogues. Journal of hepatology. 2003;39:93-8.

174. Wang H-Q, Ma L-L, Jiang J-D, Pang R, Chen Y-J, Li Y-H. Recombinant human interferon alpha 2b broad-spectrum anti-respiratory viruses pharmacodynamics study in vitro. Yao xue xue bao= Acta pharmaceutica Sinica. 2014;49(11):1547-53.

175. Lu H. Drug treatment options for the 2019-new coronavirus (2019-nCoV). Bioscience trends. 2020;14(1):69-71.

176. Darazam IA, Shokouhi S, Pourhoseingholi MA, Irvani SSN, Mokhtari M, Shabani M, et al. Role of interferon therapy in severe COVID-19: the COVIFERON randomized controlled trial. Scientific reports. 2021;11(1):1-11.

177. Phadke M, Saunik S. COVID-19 treatment by repurposing drugs until the vaccine is in sight. Drug development research. 2020;81(5):541-3.

178. Tikoo K, Patel G, Kumar S, Karpe PA, Sanghavi M, Malek V, et al. Tissue specific up regulation of ACE2 in rabbit model of atherosclerosis by atorvastatin: role of epigenetic histone modifications. Biochemical pharmacology. 2015;93(3):343-51.

179. Fedson DS. Treating the host response to emerging virus diseases: lessons learned from sepsis, pneumonia, influenza and Ebola. Annals of translational medicine. 2016;4(21).

180. Castiglione V, Chiriacò M, Emdin M, Taddei S, Vergaro G. Statin therapy in COVID-19 infection. European Heart Journal-Cardiovascular Pharmacotherapy. 2020;6(4):258-9.

181. Lee KCH, Sewa DW, Phua GC. Potential role of statins in COVID-19. International Journal of Infectious Diseases. 2020;96:615-7.

182. Subir R. Pros and cons for use of statins in people with coronavirus disease-19 (COVID-19). Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2020;14(5):1225-9.

183. Zhang X-J, Qin J-J, Cheng X, Shen L, Zhao Y-C, Yuan Y, et al. In-hospital use of statins is associated with a reduced risk of mortality among individuals with COVID-19. Cell metabolism. 2020;32(2):176-87. e4.

184. Vahedian-Azimi A, Mohammadi SM, Beni FH, Banach M, Guest PC, Jamialahmadi T, et al. Improved COVID-19 ICU admission and mortality outcomes following treatment with statins: a systematic review and meta-analysis. Archives of Medical Science: AMS. 2021;17(3):579.

185. de Wit E, Feldmann F, Cronin J, Jordan R, Okumura A, Thomas T, et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. Proceedings of the National Academy of Sciences. 2020;117(12):6771-6.

186. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Science translational medicine. 2017;9(396).

187. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proceedings of the Japan Academy, Series B. 2017;93(7):449-63.

188. Driouich J-S, Cochin M, Lingas G, Moureau G, Touret F, Petit PR, et al. Favipiravir and severe acute respiratory syndrome coronavirus 2 in hamster model. bioRxiv. 2020.

189. Arévalo A, Pagotto R, Pórfido J, Daghero H, Segovia M, Yamasaki K, et al. Ivermectin reduces in vivo coronavirus infection in a mouse experimental model. Scientific Reports. 2021;11(1):1-12.

190. De Meyer S, Bojkova D, Cinatl J, Van Damme E, Buyck C, Van Loock M, et al. Lack of antiviral activity of darunavir against SARS-CoV-2. International Journal of Infectious Diseases. 2020;97:7-10.

191. Choy K-T, Wong AY-L, Kaewpreedee P, Sia SF, Chen D, Hui KPY, et al. Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. Antiviral research. 2020;178:104786.

192. Zhao H, To KK, Lam H, Zhou X, Chan JF-W, Peng Z, et al. Cross-linking peptide and repurposed drugs inhibit both entry pathways of SARS-CoV-2. Nature communications. 2021;12(1):1-9.

193. Leneva I, Pshenichnaya N, Bulgakova V. Umifenovir and coronavirus infections: a review of research results and clinical practice. Terapevticheskii arkhiv. 2020;92(11):91-7.

194. Li H, Liu R, Zhang R, Zhang S, Wei Y, Zhou H, et al. Protective effect of arbidol against pulmonary fibrosis and sepsis in mice. Frontiers in Pharmacology. 2020;11:2504.

195. NIAID. Adaptive COVID-19 Treatment Trial 4 (ACTT-4),ClinicalTrials.gov Identifier: NCT04640168,November 23, 2020. 2020.

196. Hospital MARMCa. Efficacy of Ramdicivir and Baricitinib for the Treatment of Severe COVID 19 Patients,ClinicalTrials.gov Identifier: NCT04693026,January 5, 2021. 2021.

197. Cantini F. Baricitinib Therapy in COVID-19,ClinicalTrials.gov Identifier: NCT04358614,April 24, 2020. 2020.

198. Malignas GCdH. Treatment of SARS Caused by COVID-19 With Ruxolitinib, ClinicalTrials.gov Identifier: NCT04334044,April 3, 2020. 2020.

199. Jena Uo. Ruxolitinib in Covid-19 Patients With Defined Hyperinflammation (RuxCoFlam),ClinicalTrials.gov Identifier: NCT04338958, April 8, 2020. 2020.

200. University WM. Chloroquine as Antiviral Treatment in Coronavirus Infection 2020, ClinicalTrials.gov Identifier:

NCT04331600,April 2, 2020. 2020.

201. Poschet JF, Perkett EA, Timmins GS, Deretic V. Azithromycin and ciprofloxacin have a chloroquine-like effect on respiratory epithelial cells. BioRxiv. 2020.

202. SEIMC-GESIDA F. Clinical Trial to Evaluate the Effectiveness and Safety of Tocilizumab for Treating Patients With COVID-19 Pneumonia,ClinicalTrials.gov Identifier: NCT04445272,June 24, 2020. 2020.

203. Dentistry FCoMa. TOCILIZUMAB - An Option for Patients With COVID-19 Associated Cytokine Release Syndrome; A Single Center Experience,ClinicalTrials.gov Identifier: NCT04730323,January 29, 2021. 2021.

204. Chicago Uo. Low-dose Tocilizumab Versus Standard of Care in Hospitalized Patients With COVID-19 (COVIDOSE-2,ClinicalTrials.gov Identifier: NCT04479358,July 21, 2020. 2020.

205. Chicago Uo. Tocilizumab to Prevent Clinical Decompensation in Hospitalized, Non-critically Ill Patients With COVID-19 Pneumonitis (COVIDOSE,ClinicalTrials.gov Identifier: NCT04331795,April 2, 2020. 2020.

206. Hosseinzadeh MH, Shamshirian A, Ebrahimzadeh MA. Dexamethasone vs COVID-19: An experimental study in line with the preliminary findings of a large trial. International Journal of Clinical Practice. 2021;75(6):e13943.

207. 207. Institute RPC. Rintatolimod and IFN Alpha-2b for the Treatment of COVID-19 in Cancer Patients, ClinicalTrials.gov Identifier: NCT04379518,May 7, 2020. 2020.

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Hematopoetic stem cell transplantation in children

Canan ALBAYRAK

Department of Pediatric Hematology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

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Abstract

Bone marrow transplantation is now called hematopoietic stem cell transplantation (HSCT) because, apart from bone marrow, peripheral blood and umbilical cord blood can also be used as stem cell sources. HSCT can be performed in three ways. One is autologous stem cell transplantation. The other is allogeneic stem cell transplantation. Another type of transplantation applied in recent years is haploidentic transplantation. More than 20% of all allogeneic HSCTs are performed in patients under 20 years of age. HSCT has become a well-established lifesaving treatment procedure for many patients with hematological malignancies, hemoglobinopathies, inborn errors, or bone marrow failure syndromes. HSCT is now integrated as an essential part in many treatment concepts and protocols. HSCT is a treatment that provides an increase on success compared to other treatments when it is performed in suitable patients and in accordance with the standards. Selection of the appropriate transplant type, appropriate donor, appropriate preparation regimen, and close monitoring of complications are essential.

Keywords: hematopoietic stem cell transplantation, child, indication, graft versus host disease

1. Introduction

Bone marrow transplantation is now called hematopoietic stem cell transplantation (HSCT) because, apart from bone marrow, peripheral blood and umbilical cord blood can also be used as stem cell sources (1, 2).

HSCT can be performed in three ways. One is autologous stem cell transplantation. It is the freezing of stem cells taken from the patient and giving them to him after high-dose chemotherapy. In this way, in many malignant diseases, intense chemotherapy can be given to cause severe myelotoxicity and cure can be provided. The myelotoxicity problem is solved by the proliferation of the stem cells given to the patient in the bone marrow. The stem cell source may be bone marrow or peripheral blood. It is frequently used for multiple myeloma in adults and solid tumors in children (1, 2).

The other is allogeneic stem cell transplantation. Stem cells that are fully suitable or 9/10 suitable for human leukocyte antigens (HLA) tissue antigens are collected from another person and given to the patient. First, the relatives of the patients are screened for HLA antigen compatibility. If there is no suitable relative, the bone marrow bank is applied to the unrelated donors for screening. The source of stem cells can be bone marrow, peripheral blood, or cord blood. Chemotherapy or radiotherapy should be given before the patient is given stem cells. With this application, which is called the preparation regime, the cells in the bone marrow are cleaned, space is opened for new stem cells to be given, cancer cells, if any, are also cleaned, and immune suppression is applied to prevent the harm that the given stem cells can cause to the patient. It is frequently used in hematological malignant diseases, hemoglobinopathies, and metabolic diseases (1, 2).

Another type of transplantation applied in recent years is haploidentic transplantation. If stem cell transplantation is required in patients who do not have a complete or nearly complete HLA match, stem cells are collected from two or more antigen-incompatible individuals. The probability of finding a donor in these transplants is high. However, the probability of severe graft versus host disease (GVHD) is very high due to inappropriate antigens. Haploidentical stem cell transplantation is indicated in patients whose only treatment is stem cell transplantation, but who do not have a suitable donor (1, 2).

The HSCT stages are; preparative regimen (starting from day -10 or -7), stem cell infusion (day 0), engraftment; often +10 to +20 days (for neutrophils; the first day when the neutrophil count is 0.5x 106/L for 3 consecutive days, for platelets; the first day without platelet suspension for a week), chimerism monitoring, GVHD monitoring, monitoring for possible side effects and infections.

Chimerism should be followed up after engraftment in allogeneic or haploidentic HSCT. It shows sensitively whether the leukocytes produced are of patient or donor origin. The most widely used and accepted method today is the determination of short repeat sequences by polymerase chain reaction, and its sensitivity is 1-3%. Sensitivity can be increased (<1%) with chimerism testing in specific cell lines.

*Correspondence: can68ucar@yahoo.com.tr

Peripheral blood cells are sufficient for chimerism measurement. Bone marrow samples can be used to evaluate recurrences early in patients with acute leukemia. Patients at high risk of graft rejection, recurrence in malignant diseases, and development of graft versus host disease can be identified using serial chimerism determinations, and this information can be used to guide the initiation and timing of necessary applications (increasing, decreasing, terminating immunosuppressive, donor lymphocyte infusion, and so on) (1, 2).

More than 20% of all allogeneic HSCTs are performed in patients under 20 years of age. HSCT has become a well-established lifesaving treatment procedure for many patients with hematological malignancies, hemoglobinopathies, inborn errors, or bone marrow failure syndromes. HSCT is now integrated as an essential part in many treatment concepts and protocols (1, 2).

The first successful stem cell transplantation application in history started in 1952 with a case diagnosed with acute leukemia, for this reason E. Donnall Thomas was awarded the Nobel Prize in 1990 (3). HSCT indications and applications have changed significantly in the last 20 years. Developing sources of hematopoietic stem cells, less toxic conditioning regimens, and successes in graft versus host disease prophylaxis and treatment have led to the application of hematopoietic stem cell transplantation in increasing numbers from malignant to nonmalignant.

Indications for HSCT in children

The indications for HSCT in children recommended by the European Blood and Bone Marrow Transplantation Group (EBMT) are based on current clinical practices in Europe and America (1, 2).

It is essential to perform HSCT on the right patient at the right time, with the right steps. It is necessary to start by evaluating patient-related factors and disease-related factors separately when making a decision for transplantation in a patient.

It has been published as a guide by many transplant associations (EBMT, IBMTR) on which disease and when to transplant. There is an indication guide published by the Ministry of Health in Turkey, which is updated from time to time. The last update date is July 2017 (4). Guidelines show routine indications; standard treatment is left to clinical preference or not recommended. These guidelines are very important, but patient-related factors are at least as important as the indication when making the transplant decision. The length of the process, responsibilities in the process, continuous support, and organization should be explained to the patient and his family, and it should be stated that family support in this process will increase success.

In Turkey, 87.5% of the 6620 stem cell transplants performed in childhood are allogeneic HSCTs, and 53.5% of these transplants were performed on non-malignant diseases, mostly on thalassemia major patients. In the malignant disease group, ALL is the most common followed by AML and other diseases (5).

Acute Lymphoid Leukemia (ALL)

HSCT is indicated in the high-risk group in ALL patients in first complete remission. Event-free survival is less than 50% in this group in most studies. A high-risk ALL group was defined in each chemotherapy protocol. The presence of some molecular and chromosomal anomalies and minimal residual disease positivity observed during certain periods of treatment are defined as high risk and HSCT is indicated. In ALL patients with early and very early bone marrow relapse, transplantation from an HLA-matched relative or unrelated donor is indicated.

Acute Myeloid Leukemia (AML)

In childhood AML cases, the cure rate is around 60% with intensive chemotherapy and intensive supportive care. Better outcomes have been reported for patients with good prognostic markers. HSCT is not recommended for these patients. HSCT is recommended in high-risk and very high-risk patients and in patients with relapse.

Myelodysplastic syndrome, Juvenile myelomonocytic leukemia and Secondary leukemia

Allogeneic HSCT from a suitable sibling or unrelated donor is recommended.

Chronic Myeloid Leukemia (CML)

Although allogeneic HSCT is the only curative therapy for CML patients, the introduction of specific tyrosine kinase inhibitors (TKI) has affected treatment strategies. If children in the chronic phase are in remission with TKIs, treatment can be continued for many years, and close molecular monitoring is required after discontinuation of treatment. There is insufficient evidence for treatment discontinuation. It may be necessary to start again. HSCT is recommended for patients in the chronic phase who cannot use different TKIs due to side effects or intolerance, or who cannot achieve remission despite using them. In the accelerated and blastic phase, HSCT is required.

Hemoglobinopathies

β-Thalassemia and sickle cell anemia are the most common single gene diseases in the world. Although regular transfusion and chelation and supportive treatments such as hydroxyurea for sickle cell anemia significantly improve clinical findings and quality of life, it is not possible to exclude the disease and prevent treatment-related complications with these approaches. It is accepted today that HSCT is the only curative treatment in this patient group. We retrospectively enrolled 1469 patients with thalassemia major who underwent their first HSCT between 1988 and 2020 in

25 pediatric centers in Turkey. The median follow-up duration and transplant age were 62 months and 7 years, respectively; 113 patients had chronic graft versus host disease (GVHD). The 5-year overall survival, thalassemia-free survival, and thalassemia-GVHD-free survival rates were 92.3%, 82.1%, and 80.8%, respectively (6). The prognosis is much better, especially in cases of young age and in the low-risk group. For this reason, HSCT is recommended to be performed in early childhood before iron load and disease-related complications develop. In recent years, positive results have been reported in thalassemia with incompletely matched relative or compatible unrelated donor transplants. Recently, it has been reported that the rates of GVHD are low in cord blood transplants in β thalassemia and/or sickle cell diseases. However, graft failure and recurrence of the disease are still important problems for cord blood transplantation (7).

Currently, HSCT indications in sickle cell anemia in Turkey are limited, and it is indicated only in children with stroke, central nervous system complications, magnetic resonance imaging findings, or organ damage (4).

Primary immunodeficiencies

Allogeneic HSCT is currently the only accepted curative treatment for most immune deficiencies. Severe combined immune deficiency, various T cell deficiencies, Wiskott-Aldrich syndrome, leukocyte adhesion defect, chronic granulomatous disease, Chediak-Higashi syndrome, Griscelli syndrome, familial lymphohistiocytosis, X-linked lymphoproliferative disease can be counted in this group. If there is no HLA-matched family donor in this patient group, there is also an indication for transplantation from an unrelated donor (1, 2).

Acquired severe aplastic anemia

Transplantation is the first treatment option for severe aplastic anemia with compatible sibling donors. If there is no familial donor, immunosuppressive therapy including ATG and cyclosporine should be tried first. In cases unresponsive to this treatment, transplantation from an unrelated donor or cord blood is indicated (1, 2).

Hereditary bone marrow failure syndromes

Fanconi anemia is a rare genetic disease characterized by progressive bone marrow failure and a predisposition to malignancy, especially AML, accompanied by various bodily abnormalities. In Fanconi anemia cases, the only way to correct the hematological disorder is HSCT, and transplantation can be done from an HLA-matched sibling, relative or unrelated donor.

Diamond Blackfan Anemia is an inherited type of anemia characterized by the reduction or absence of erythroid precursors in the bone marrow. Allogeneic HSCT is indicated in cases with compatible sibling donors and unresponsive to steroids.

Amegakaryocytic thrombocytopenia is an autosomal recessive genetic disease that presents within days or weeks after birth. Allogeneic HSCT is the only curative treatment.

Congenital neutropenia is a disease characterized by severe neutropenia and severe bacterial infections from early childhood. In cases where patients do not respond to granulocyte colony-stimulating factor or develop MDS/AML, HSCT is indicated even if there is no matched family donor.

Familial Hemophagocytic lymphohistiocytosis (HLH)

The only curative treatment modality in familial HLH cases is allogeneic HSCT.

Metabolic Diseases

Metabolic diseases with an indication for transplantation are generally in the group of lysosomal storage diseases. It is based on the transfer of the missing enzyme from donor cells to the reticuloendothelial system and solid organs. Diseases with the most experience are adrenoleukodystrophy, type 1 mucosaccharidosis (Hurler's syndrome) and osteopetrosis.

Solid tumors

Data from the European Blood and Bone Marrow Transplant Group showed that transplantation prolongs the course in children with neuroblastoma and Ewing tumor. In other solid tumors, patients may benefit from autologous transplantation in the presence of some of the following special conditions (1,2):

Germ cell tumors: In the presence of relapse or progressive disease,

Soft tissue sarcoma: Stage 4 or after relapse with no chance of resection,

Wilm's tumor: In the presence of high-risk histology or relapse,

Brain tumors: Chemotherapy-responsive medulloblastoma or high-grade gliomas.

Donor Selection for Pediatrics

The outcome of HSCT depends in part on the matching between the donor and the recipient for HLA, encoded by a group of genes on chromosome 6; genes and products are labelled as the major histocompatibility complex. The HLA system is the most polymorphic genetic region known in the human genome. A set of HLA gene alleles, called haplotype, is inherited from each parent; therefore, the probability that a child inherits and shares both parental haplotypes with a full sibling is 25%. Such an HLA-identical sibling is still considered an optimal donor (8).

Stem Cell Sources

1.Bone Marrow

The classically accepted stem cell source for HCST is bone

marrow. Multiple punctures of the iliac crest are performed in general anesthesia by experienced physicians and practitioners. The bone marrow is harvested by aspirations through adequately dimensioned needles. In very small children and if the iliac crest is anatomically not suitable for punctures, the aspirations could also be performed by punctures of the proximal tibia. The recommended number of nucleated cells for a successful "engraftment" is 2-4X108 per recipient body weight. Although the application of granulocyte colony stimulating factor to increase the amount of stem cells in the bone marrow has been reported in adult donors, data on pediatric donors are very limited (8).

2. Peripheral Blood Stem Cell (PBSC)

The stem cell source preferred by many centers for autologous transplantation is peripheral stem cells. In recent years, PBSC has been used with increasing frequency in relative and even unrelated transplants from adult donors. Although there are reservations about donors, especially in the pediatric age group, there are publications in the literature reporting that the method is safe and the desired cell count can be easily reached. The greatest advantage of the use of peripheral blood stem cells is the shorter expected neutrophil and platelet engraftment times, resulting in fewer infectious problems, hospital stays and transfusion requirements. All of these factors directly affect the cost of transplantation. However, the collection process itself, particularly the difficulties in providing an appropriate venous route, the potential short- and long-term side effects of the drugs used in mobilization, and the increased risk of GVHD are the main considerations in the decision to use.

3. Cord Blood

The greatest advantage of cord blood is the low risk of viral transmission and GVHD. In addition, it can be used immediately without needing time for donor preparation, and it can be used with 1-2 HLA incompatibility, especially for cases with rare tissue group. The most restrictive factor regarding its use is the limited number of cells. The lowest acceptable cell counts are 2.5x107/kg for nucleated cells and 1.7x105/kg for CD34+ cells (8).

Preparative (Conditioning) Regimens

The aim of the preparative regimen in HSCT is to prepare the patient for transplantation and has three separate components: “bone marrow clearance”, “immunosuppression” and “disease eradication”. Making room in the bone marrow is necessary for donor stem cells to reach the "niche" and "engraft". Rejection of the graft by recipient immune cells can be prevented by immunosuppression. Since the long-term course is related to disease control in malignancies, the main goal of the preparative regimen in this group is the eradication of the disease. The side effects of the preparative regimen are generally better tolerated in children than in adults, allowing for higher doses. On the other hand, total body irradiation (TBI) regimens may cause late complications such as growth retardation, pubertal failure or delay, which is especially important for the pediatric age group. The most frequently used regimens in children are the regimens in which cyclophosphamide and busulfan are used together. Especially in congenital genetic diseases, different chemotherapeutic agents are added according to the underlying disease.

In order to reduce the side effects of the preparative regimens, reduced-intensity regimens have come to the fore. Fludarabine is the most commonly used basic agent in reduced-intensity protocols, and different agents are added according to the protocols (8).

Stem Cell Cryopreservation

Stem cell cryopreservation should be processed and stored in accordance with the respective Medical Council, responsible local and overarching authorities as well as scientific society’s guidelines (8). If necessary, collected cells can be stored for a maximum of up to 72 hours at 2–6 °C before cryopreservation. However, cryopreservation within 48 hours or less is recommended to maintain the optimal viability of the cells. In the case of storage for >24 h prior to cryopreservation, the maximum NC concentration should not exceed 2 × 108/mL. For cryopreservation, a number of different protocols are used worldwide. Usually, the maximum

acceptable NC concentration is ≤4 × 108/mL. If necessary, PBSC products can be diluted with autologous plasma or commercial resuspension medium. Increasing the cell concentration by volume depletion minimizes the number of cry stored bags needed, but the upper limit of the NC concentration needs to be considered. The final product includes 5–10% dimethyl sulfoxide (DMSO) as a cryoprotectant and 0.05–0.25 mL of ACD-A stabilizer solution per ml of transplant. It is recommended to freeze at a controlled rate of 1–2 °C per minute. Cells need to be stored in vapor phase nitrogen at a temperature of ≤−140 °C. Cross-contamination while preparing and storing the cells must be prevented by taking appropriate measures.

At the time of autolog HSCT, cryopreserved bags must be thawed at the site of transplantation, and PBSCs should be reinfused within a maximum time span of 10–20 min of thawing using standard transfusion filters in order to minimize the detrimental effect of DMSO upon hematopoietic stem cells (HSCs). Previous washing for purposes of DMSO depletion is not routinely performed, as the loss and damage of HSCs are regarded as too high (8).

Collection of HSCs in Children

Collecting or harvesting HSCs from children is a challenge, not only because children have different physiological and therefore anatomical situations but also because psychological, legal and ethical concerns in minors are sometimes more difficult compared to adult donors. In

addition, parents and/or legal guardians have to be addressed on all issues. The main difference to the adult setting is the small bodyweight; the difficulties in accessing venous access, especially in the leukapheresis setting; and the need for blood cell substitution in case of BM harvest. In children, the indications for autologous HSC harvesting are well-established. Using children in the allogeneic setting as donors is a completely different issue. Children should not donate HSCs if a comparable adult volunteer HSC donor is available, if the indication for the stem cell therapy is not first line, or if the therapy is experimental.

The main resources to harvest HSCs are BM and PBSCs. The basic techniques are quite similar to the techniques used in adults. For BM collection punctures of the iliac crests or in very small children, the tibia is used. For harvesting HSCs from the PB, leukapheresis is used with the same apheresis systems as in adults. To perform these procedures in children, physicians and nursing practitioners must have working knowledge about the normal age-dependent physiological parameters, like vital signs, growth, and psychological and motor development, and should be trained in the communication with children, parents, and/or their legal guardians (8).

Transplant-Related Complications

High-dose radiotherapy and/or chemotherapy used in preparative regimens may affect all organs of the recipient and cause secondary effects of varying severity, either early or late. It is known that the development of complications may be associated with individual predisposition, immunosuppressive treatments, toxicities associated with pre-transplant treatments, and the presence of other concomitant factors during transplantation.

Graft Versus Host Disease (GVHD)

GVHD is one of the most important complications of allogeneic transplantation. Although the risk is lower in pediatric cases than in adults, its frequency has increased, especially with the use of alternative donors.

In its simplest form, graft versus host disease occurs as a result of foreign recognition of recipient antigens by donor T cells. It is possible to classify them into two different groups: acute (aGVHD) and chronic (cGVHD). The time of onset is generally used to distinguish between acute and chronic, and those that develop before the first 100 days after transplantation are called acute, and those that begin later are called chronic. Occasional overlaps between the two groups suggest that this definition is not sufficiently deterministic. The clinical results of both are different and require different treatment methods on the basis of immunological differences. The most important risk factor is HLA incompatibility between donor and recipient, and the risk of GVHD increases as the mismatch rate increases. It has been reported that the risk of GVHD is increased in cases where peripheral blood is used as the stem cell source, and the risk is lower in the use of cord blood. It has been reported that the frequency of Stage 3/4 aGVHD may be as high as 30-50% in non-relative transplants.

A significant improvement in GVHD rates was achieved by identifying 10 HLA loci at the allele level with the high-resolution method and selecting a more suitable donor. Other factors thought to be at risk for GVHD; high age of the recipient and donor, gender incompatibility, especially the recipient being male, the donor being a multiparous woman, the presence of malignant disease and the use of intensive preparative regimens.

The pathophysiology of acute GVHD is explained with three phase examples: tissue damage caused by the priming regimen, activation and proliferation of donor T cells, and damage to the recipient. Tissue damage caused by the priming regimen leads to the uncontrolled release of cytokines such as interferon-γ (IFNγ), interleukin-1 (IL-1), and tumor necrosis factor-α (TNFα). The release of these cytokines increases major histocompatibility complex expression in various tissues of the recipient and exacerbates the graft versus host activity of donor T cells. The small intestine and liver are particularly susceptible to organ damage caused by the myeloablative regimen. For this reason, it has been suggested that the risk of acute GVHD is higher in cases with intense diarrhea due to the preparative regimen. In the second step, the antigen presenting cells of the donor and recipient, together with inflammatory cytokines, stimulate the donor-derived T cells, causing them to proliferate and transform into effector cells. Stimulation of T cell proliferation initiates the third phase, and inflammatory cytokines such as IL-2, IFNγ and TNFα released from T cells directly or indirectly cause tissue damage in the recipient. In addition to cytotoxic soluble vehicles, cellular cytotoxicity such as perforin-granzyme-B-mediated cytolysis and Fas-Fas ligand-mediated apoptosis also play an important role in the pathogenesis. This three-phase event results in specific clinical manifestations in which the skin, intestine, and liver are affected at different rates and can be graded according to their involvement rates (8).

Since graft versus host disease usually has a poor prognosis, preventive approaches to prevent its development are more important than treatment. Cyclosporin A, tacrolimus, mycophenolate mofetil, methotrexate and methyl prednisolone are used as preservatives.

The mortality rate is significantly lower in cases that show an early response to low-dose steroid therapy. However, cases without an early response should be evaluated in terms of other immunosuppressants without delay. New drugs, new monoclonal antibodies, complementary therapies, and immunomodulatory procedures such as intensive immunosuppression and extracorporeal photopheresis can provide remission, but the side effects of these treatments and especially infections are important problems that need to be

overcome. It may be possible to achieve immunotolerance with cellular therapies (mesenchymal stem cells) and it looks promising.

The pathophysiology of chronic (cGVHD) is not as well understood as the acute form. It is thought that both donor-derived alloreactive T cells, similar to aGVHD, and autoreactive T cell clones that cannot be deleted as a result of thymic damage. The main feature of many clinical findings in chronic (cGVHD) is extensive collagen deposition, and the clinical course often resembles autoimmune diseases.

Sinosoidal Obstruction Syndrome (SOS)

SOS, formerly called veno-occlusive disease of the liver (VOD), is the term used to designate the symptoms and signs that appear early after HSCT because of conditioning regimen-related hepatic toxicity.

The new EBMT Diagnostic Criterias for SOS in children are: (9)

No limitation for time of onset of SOSa

The presence of two or more of the followingb

• Unexplained consumptive and transfusion-refractory thrombocytopeniac

• Otherwise, unexplained weight gain on 3 consecutive days despite the use of diuretics or a weight gain >5% above baseline value

• Hepatomegaly (best if confirmed by imaging) above baseline valued

• Ascites (ideally confirmed by imaging) above baseline valued

• Rising bilirubin from a baseline value on 3 consecutive days or ≥2 mg/dL within 72 h

aUp to 20% of children present late SOS

bWith the exclusion of other potential differential diagnoses

cWeight-adjusted platelet substitution/day to maintain institutional transfusion guidelines

dSuggested: imaging (US, CT, or MRI) immediately before HSCT to determine baseline value for both hepatomegaly and ascites

Defibrotide (1B): Despite the absence of randomized studies, it is the only agent approved by

FDA and EMA to treat severe SOS (>80% mortality).

Other Complications

Bacterial, fungal and viral systemic infections (cytomegalovirus, herpes virus, BK virus)

Mucositis

Lung complications

Pulmonary edema,

Bacterial, fungal and viral infections,

Idiopathic pneumonia syndrome

Diffuse alveolar hemorrhage

Kidney complications

Nephrotoxicity,

Hemolytic uremic syndrome-Thrombotic microangiopathy

Hemorrhagic cystitis

Heart complications

Cardiotoxicity,

Conduction disorders

Thrombosis within the catheter-connected heart

Endocrinological late complications

Hypothyroidism,

Adrenal insufficiency (due to steroid use),

Testicular or ovarian insufficiency,

Developmental delay

Secondary cancers

As a result, HSCT is a treatment that provides an increase in success compared to other treatments when it is performed on suitable patients and in accordance with the standards. Selection of the appropriate transplant type, appropriate donor, appropriate preparation regimen, and close monitoring of complications are essential.

References

1. Sureda A, Bader P, Cesaro S, Dreger P, Duarte RF, Dufour C, et al. Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2015. Bone Marrow Transplant. 2015; 50(8): 1037-56.

2. Majhail NS, Farnia SH, Carpenter PA, Champlin RE, Crawford S, Marks DI, et al. Indications for Autologous and Allogeneic Hematopoietic Cell Transplantation: Guidelines from the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2015; 21(11): 1863-1869.

3. Thomas ED, Lochte HL, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N Engl J Med. 1957; 257: 491-6. 4.https://www.saglikaktuel.com/d/file/11249,pediatrik-endikasyon-listesipdf.pdf

4. Kansoy S. TPHD Ulusal Pediatrik KİT Online Veritabanı, 2018

5. Yesilipek MA, Uygun V, Kupesiz A, G Karasu, G Ozturk, M Ertem, et al. Thalassemia-free and graft-versus-host-free survival: outcomes of hematopoietic stem cell transplantation for thalassemia major, Turkish experience. Bone Marrow Transplant. 2022; 57: 760–767.

6. Locatelli F, Rocha V, Reed W, Bernaudin F, Ertem M, Grafakos S, et al. Eurocord Transplant Group. Re¬lated umbilical cord blood transplant in patients with thalassemia and sickle cell disease. Blood 2003; 101: 2137-43.

7. Carreras E, Dufour C, Mohty M, Kröger N, editors. The EBMT Handbook Hematopoietic Stem Cell Transplantation and Cellular Therapies, Springer open; 2019.

8. Corbacioglu S, Carreras E, Ansari M, Balduzzi A, Cesaro S, Dalle JH, et al. Diagnosis and severity criteria for sinusoidal obstruction syndrome/venoocclusive disease in pediatric patients: a new classification from the European society for blood and marrow transplantation. Bone Marrow Transplant. 2018; 53: 138–45.

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Stem cell and biomaterials

Rukiye DEMİR

Department of Biomedical Engineering, Faculty of Engineering, Samsun University, Samsun, Turkey

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Abstract

Stem cells are cells that are not yet differentiated, can divide asymmetrically, differentiate into different cell types, and perform functional tissue repair. They are recognized as major cellular candidates for the regeneration of damaged tissues. Biomaterials and biomaterial scaffolds are essential in tissue engineering applications using stem cells. Recently, studies examining stem cell biomaterial interactions in different aspects have attracted attention. This review presents current information about the general properties of stem cells and biomaterials, stem cell-biomaterial interactions, three-dimensional (3D) tissue scaffolds used in stem cell studies, and 3D bioprinting.

Keywords: stem cells, biomaterials, biomaterial stem cell interaction, 3D tissue scaffold

1. Introduction

Stem cells, found in the niches of various healthy tissues, have the potential to self-renew and differentiate after trauma, disease or ageing (1). Stem cells (SC) make an indispensable contribution to tissue morphogenesis, repair and the homeostatic cell cycle in the body. Due to these properties, they are recognized as major cellular candidates for the regeneration of injured, aged or diseased tissues (2). The capacity for self-renewal and the potential to generate many different types of cells form the basis for stem cells to benefit the field of regenerative medicine (3). SC therapies are gradually being recognized as a critical building block in tissue regeneration, offering treatments for various diseases. However, many challenges, such as low cell retention and engraftment and poor long-term maintenance of SC function, limit the successful use of SC translation in clinical practice (4).

The biomaterial was once described as a nonviable material used in a medical device intended to interact with biological systems. It was later revised as a material designed to interface with biological systems to evaluate, treat, augment or modify any tissue, organ or function of the body (5). Biomaterials have benefited patients with increased longevity and improved quality of life (6). They are synthetic or natural materials used to replace, improve or interact with a biological system (7). Extensive efforts have been made to mimic in vivo microenvironments to direct and control stem cells into specific cell types required for regenerative medicine (8).

2. Stem Cells

Stem cells are defined as cells that can divide asymmetrically, renew themselves and differentiate into mature cells (9). Many types of stem cells can be isolated from embryonic or adult tissues with potencies ranging from pluripotent to unipotent, depending on the type of SC (10). Totipotent stem cells can divide and differentiate into cells of the whole organism. Totipotency has the highest differentiation potential and allows cells to form both embryonic and extra-embryonic structures. The zygote formed after a sperm fertilizes an egg is totipotent (11). SC can be multipotent, as in the blood sample, or unipotent, as in the testicles (12).

In general, stem cells can be characterized as embryonic and adult stem cells.

Embryonic stem cells (ESC) are pluripotent stem cells isolated from the inner cell mass within the blastocyst. They can differentiate into all three germ layers. They express pluripotent markers such as Sox2, Oct4 and Nanog. Despite the differentiation potential of these cells, they have some disadvantages, such as ethical problems arising from the destruction of the human embryo and the formation of teratomas when transplanted directly as undifferentiated cells (13). Adult stem cells have been identified in a wide range of adult tissues, including the brain, heart, lungs, kidney, and spleen. SC in adult tissues produces differentiated cells suitable for that tissue. Somatic stem cells in adult tissues can be reprogrammed into a pluripotent state in vitro. These resulting cells are called induced pluripotent stem (iPS) cells (12). The most famous adult stem cell subgroup is mesenchymal stem cells (MSC), which can efficiently differentiate into all cell types derived from the mesoderm. These cells can be relatively easily isolated from bone

*Correspondence: rukiye.demir@samsun.edu.tr

marrow, adipose tissue, and umbilical cord blood (14). MSC are a source of precursor cells that can be replicated in vitro and used for tissue regeneration for different clinical applications (15).

Adult stem/progenitor stem cells reside in a specialized microenvironment called the stem cell niche, which houses a large number of cells such as fibroblasts, endothelial cells, and/or stromal components. The stem cell niche, a highly dynamic microenvironment housing stem cells, is usually composed of different cells (e.g., stem cells, immune regulatory macrophages and T cells), soluble secreted factors (e.g. growth factors, chemokines, hormones and androgens) (16). Niche and its components tightly regulate the behavior and function of stem cells through direct interactions and/or signaling cues from soluble factors (17). Traditionally, stem cell differentiation has been regulated through soluble signals such as growth factors. While these signals are important, factors from niche or extracellular matrix (ECM) molecules also contribute to stem cell activity and fate.

Understanding the microenvironment factors that influence stem cell fate, such as mechanical properties, topography, and specific ECM ligands, is essential for designing advanced biomaterials (18).

The development and regeneration of tissues is largely the result of stem cell function. In order to achieve this goal, stem cells can self-renew by symmetrical cell division (19). Self-renewal is the result of cell division, which takes place in the niche where stem cells are located. Stem cell division can occur as asymmetric division or symmetric division. Asymmetric division forms a progenitor cell and a daughter cell, which remain stem cells. In a symmetrical division, two new stem cells are formed (20). SC can increase in number by dividing symmetrically and renewing themselves asymmetrically to form a differentiated generation (9).

Cancer stem cells (CSCs) are defined by their potential for self-renewal, differentiation and tumorigenicity. They are considered responsible for drug resistance and relapse (21). CSCs are associated with the metastatic nature of cancer and the recurrence of cancer after treatment (22). New biomaterials have been widely evaluated as in vitro platforms for their ability to mimic the cancer microenvironment. Biomanufacturing methods and models designed with biomaterials offer opportunities to investigate signaling pathways and related phenomena that control cancer progression and drug response (21).

3. Biomaterials

Biomaterials are synthetic or natural materials that are used to replace, improve, or interact with a biological system. Various types of biomaterials are used for medical purposes, such as drug delivery, stents, and implants (7). Biomaterials are compounds that interact with biological systems, thereby affecting the growth and health of cells around them. Biomaterials have been used for targeted differentiation to generate a variety of cell types (3). Biomaterials with tunable biophysical and biochemical properties to maintain and enhance stem cell function, conjugated growth factors, and tissue-derived ECM are vehicles for the survival and differentiation of transplanted cells. Studies have shown that, with the increased effects of engraftment and differentiation, engineered materials transplanted with stem cells can facilitate functional recovery and structural integrity, such as angiogenesis and electromechanical enhancement, providing a suitable niche for tissue regeneration (4).

The biocompatibility of biomaterials is important. Biocompatibility is defined as the ability of a material to perform desired functions relative to a medical therapy without any risk of injury, toxicity, or rejection, to induce an appropriate host response in a given application, and to interact with living systems (6). Biomaterials are often designed to have favorable biochemical and biophysical properties, including molecular compatibility, high porosity, and favorable mechanical strength that mimic the microenvironment of the natural ECM (23). Biomaterials preserve the properties of stem cells, such as self-renewal, proliferation and differentiation. In some cases, they can induce the microenvironment of stem cells by mimicking the natural ECM (13).

Compared with the traditional cell-type-specific biomaterial, new stem cell-interacting biomaterials are designed to meet the needs of various cell types due to the presence of bioactive cues. Such studies aim to identify materials that can regulate cell function and find the appropriate biomaterial-stem cell combination for the human body (23). In an in vitro cell and tissue culture, biomaterials are designed to provide chemical, mechanical, and physical cues that activate several molecular signaling pathways, thereby determining the fate of the cell (7).

Next-generation biomaterials are intended to be used as scaffolds to mimic native ECM and provide a 3D environment to sustain body adhesion, migration, proliferation and differentiation (24). 3D bioprinting is a digital model-based technology for printing all kinds of materials layer by layer to create objects with complex structures. Biomimetic scaffolds, biomaterials, cells and other bioactive molecules can be created accurately and efficiently by using them as units. By creating a personalized bionic 3D scaffold to simulate the diverse tissue microenvironment, proliferation and differentiation of stem cells can be induced (25). The biomaterial will transmit specific signals to the cells, especially depending on their composition and structure. Therefore, biomaterials' topography, chemistry, and physical properties are critical parameters for guiding cell fate (20). Numerous studies have shown that the simple addition of biophysical factors to biocompatible biomaterials without any chemical factors can significantly affect stem cell

behavior and differentiation into desired cell lines (16).

3.1. Natural and Synthetic Biomaterials

Biomaterials must provide informative microenvironments that allow stem cells to interpret biomaterial instructions and change their fate accordingly. Biomaterials for modulating stem cell differentiation can be broadly categorized as natural and synthetic polymers (5). Natural biomaterials have been used for a long time because of their superior biocompatibility, biodegradability, low toxicity and low allergenicity. It turns into degradation products that are less cytotoxic and more easily metabolized by host tissues (26). Natural biomaterials contain a structure similar to biological tissue, where they can serve as reparative materials for tissue regeneration (24). Natural biomaterials are materials that can support stem cell proliferation and act as a natural base for regulating the behavior of implanted cells or even the differentiation of stem cells into the target tissue (13). Protein-based biomaterials such as collagen, fibrin, elastin, and silk-based material scaffolds are known to be suitable for tissue engineering applications such as stem cell differentiation, transplantation and wound repair. Hyaluronan, also known as hyaluronic acid, has been used as natural polysaccharide-based biomaterials for stem cell cultures in the field of tissue engineering and regeneration (24). They have similar mechanical and adhesive properties as natural ECM. It has some disadvantages such as short degradation time, difficult purification and quality control. Natural biomaterials can regulate the proliferation and differentiation of implanted stem cells into the target tissue (13). Despite their superior biocompatibility, natural biomaterials face poor mechanical strength due to rapid degradation once implanted. Time is needed for the newly formed tissue to become fully functional. Therefore, rapid degradation should be avoided. To improve their mechanical integrity, natural biomaterials are often combined with synthetic ones to produce hybrid or composite biomaterials that achieve the advantages of both categories (17).

Synthetic materials are attractive due to their more adjustable mechanical properties and ease of manufacture on a large scale (26). Synthetic materials have the advantages of controllable degradation, mechanical properties, and controllable composition of materials. However, synthetic materials often lack cell adhesion sites and cell recognition signals (24). Synthetic biomaterials can be obtained from Food and Drug Administration (FDA)-approved polymers with excellent biodegradable and biocompatible properties, such as poly (lactic acid) (PLA), polylactide caprolactone (PCL), polyglycolide (PGA) (17). Adapting synthetic materials is accomplished by adding biochemical modifications, modulating the material's mechanical properties, and/or determining the microscale structure and topography. The presentation of growth factors and morphogens is a complementary approach to giving additional biochemical functionality to synthetic material (27).

4. Biomaterial Stem Cell Interaction

Research has shown how biomaterial/structure cues in the form of biomaterial chemistry, material hardness, surface topography, porosity, and degradation properties play an important role in controlling cellular events in vitro and in vivo (28). Biomaterial selection alone can affect the behavior of stem cells (27). Poly-lactic-co-glycolic acid (PLGA) and self-gelling alginate are used to generate neuronal stem cells, astrocytes, adipocytes, osteoblasts, cardiomyocytes and chondrocytes (3). Chemical and biological modifications can directly affect SC behavior by changing substrate properties, surface interactions, scaffold degradation rate, microenvironment architecture, and ultimately manipulating signal transduction pathways in SC (29). It has been stated that microenvironment factors such as ECM proteins, growth factors (GF), stiffness and topography play a critical role in guiding stem cell behavior and fate (1).

Naturally derived ECM components such as fibrillar proteins or glycosaminoglycans (GAGs) offer an attractive starting point for biomaterials to guide the differentiation of stem cells. Most of these components are found in the natural stem cell niche and contain bioactive motifs and cell binding domains of stem cells that can promote cell survival and proliferation (27).

The mechanical properties of a scaffold or culture surface can also have a significant impact on the differentiation of the seeded stem cell. By exerting traction forces on a substrate, many mature cell types such as epithelial cells, fibroblasts, muscle cells and neurons sense the stiffness of the substrate and show different morphology and adhesive properties (30). Surface hardness regulates fate. The stiffness of most tissues is several times lower than that of tissue culture plastic or glass and can vary within a given tissue as a function of age or disease. Changes in bulk stiffness of ECM-coated hydrogels elicit differential responses in stem cell populations. Bone differentiation of mesenchymal stem cells is favored by hard substrates, while soft substrates promote adipocyte differentiation. Substrate stiffness also prompts skeletal muscle stem cells to self-renew or differentiate with moderately rigid substrates that mimic normal muscle stiffness that most effectively supports stem cell status (12). The biomaterial stiffness, which restricts stem cell differentiation in various lineages, matches the stiffness of the native tissue microenvironment in which these cells reside. It seems that the differentiation of stem cells from different tissues or species demands quite different stiffness ranges. SC from different sources may respond differently to mechanical stiffness. Several studies have indicated that hard matrix can lead to osteogenic origin by stem cell differentiation, medium stiffness to the myogenic origin, and soft matrix directs stem cells to neuronal cells (8).

Studies have revealed that the size of the topographic

features and the conformations of cavities, folds, pits, pores, symmetries, etc., are also important (30). Biophysical properties of biomaterials, such as porosity, micro/nano-scale surface patterns, architecture, and stiffness/resilience, can influence endogenous stem cells' behavior by changing the local microenvironment through cell-biomaterial interactions after implantation (17). Parameters such as surface topography, surface wettability, and physicochemical properties, including surface charge, strongly influence cell-material interactions (31). The cells were found to perceive micro- and nano-meter topography with uniform chemical properties and align and orient themselves along the grooves. In particular, it has been observed that the groove pattern exerts a dynamic effect in relation to stem cell alignment and elongation (20).

Within vivo regenerative medicine, cell-free biomaterials can be introduced into the body to stimulate and instruct the activity of endogenous adult stem and progenitor cells by changing the niche to increase the body's natural reparative capacities (32). Damaged tissues often also lose deeper layers that contain stem cell niches. In such cases, biomaterials can be useful tools to re-establish the functionality of niches. Artificial niches must contain appropriate 'homing' signals that can attract and localize endogenous stem cells through known cell-cell or cell-matrix adhesive interactions (20).

5. 3D Tissue Scaffold

Tissue engineering aims to regenerate damaged tissues by combining cells from the body with highly porous scaffold biomaterials that act as templates for tissue regeneration (33). Stem cells are a good source of cells for tissue engineering with the potential to turn into a large number of desired cell types (8). Growth factors, stem cells, and scaffolds are collectively known as the tissue engineering triad (34). Biomaterials and stem cells coupled with growth factors are imperative to increase the survival rate of stem cells and further facilitate tissue regeneration in vivo (4). The current tissue engineering strategy uses living cells, biomaterials and appropriate biochemical, physical factors and combinations to create tissue-like structures. The ultimate goal is to incorporate these tissue-like structures into the body to repair damage or replace dysfunctional organs (35). Combining stem cells with biomaterial scaffolds offers a promising strategy for the future of biomedicine and regenerative medicine. (36). An ideal scaffold should provide chemical stability or degradability to support cytocompatibility, adhesion, proliferation, stability and mechanical strength, and physical properties suitable for the surrounding tissue (31).

The incomplete differentiation of stem cell populations to the desired target remains an unresolved challenge. Various biomaterials have been designed to mimic the natural ECM action in vitro. Applying biomaterials in a 3D environment can also help create a human-based model that can reduce animal use in research (14). Considering that it once served only as a physical structure, it is now clear that the chemical composition of biomaterial scaffolds can guide, improve and redefine cell behavior (32). Bone marrow-derived mesenchymal stem cells (BMSCs) are considered to be the most commonly studied stem cells in tissue engineering. In recent years, the application of biomaterial scaffolds based on Adipose-derived mesenchymal stem cells (ADMSCs) has become an increasingly hot topic (35).

The scaffolds act as a synthetic ECM to organize cells in a three-dimensional architecture and deliver stimuli that drive the growth and formation of the desired tissue. Depending on the tissue of interest and the specific application, the required scaffold material and properties differ (37). Regardless of tissue type, biocompatibility, biodegradability, mechanical properties, scaffold architecture, production technology, and biomaterials are important in a scaffold (33).

Various synthetic and naturally derived materials can be used to form hydrogels for their scaffolds. Synthetic materials include poly (ethylene oxide) (PEO), poly (vinyl alcohol) (PVA), poly (acrylic acid) (PAA), poly (propylene fumarate-co-ethylene glycol) (P(PF-co-EG)) and polypeptides. Naturally derived polymers include agarose, alginate, chitosan, collagen, fibrin, gelatin and hyaluronic acid (37).

The tissue structure environment of cells must resemble its native counterpart for cells to maintain their phenotype, establish appropriate cell-cell interactions, and express tissue-specific proteins in conjunction with the ECM (38).

3D cell cluster configurations provide a more natural anatomical environment than single-layer cell cultures (38). The use of 3D culture techniques has become more common in research (10). Among the various techniques available for scaffolding, 3D printing technology is considered a superior technique. 3D printing is a technology that involves the sequential production of the same or different materials through an automated process layer by layer, resulting in the creation of a complete 3D structural object (39). 3D cultures more effectively summarize cell-cell and cell-matrix interactions in vivo, and cells in 3D cultures exhibit many unique and desirable properties. 3D stem cell culture can use a variety of matrices or scaffolds in addition to cells to support complex structures (10).

Organoids are a class of engineered 3D tissues that exhibit similar biological properties to their in vivo counterparts. 3D models, particularly organoids, offer opportunities to design culture systems that bridge the gap between the limitations of two-dimensional (2D) tissue culture and the in vivo organisms of the whole animal or human subjects. The production of tissue-specific organoids is based on the biological principles of organ development, where stem cells are regulated to differentiate and develop into functional tissues and organs (40).

6. Stem Cell and 3D Bioprinting

3D bioprinting is a tissue engineering production method that uses the spatial model of combining living cells and other biological materials in a layer-by-layer deposition approach to construct living tissues and organ analogues.

Bio-ink is a combination of inert printing medium seeded with living cells and forms the raw material deposited on the collection substrate. Ideal bio-ink has high mechanical integrity, high stability, insoluble in cell culture medium, non-toxic and non-immunogenic; and should be able to promote cell adhesion (38). 3D bioprinting technology is based on biomaterials, cells and other bioactive molecules as units that can accurately and efficiently form complex bionic functional scaffolds (25). 3D stem cell culture can use a variety of matrices or scaffolds in addition to cells to support complex structures (10)

7. Results

An excellent feature is that stem cells are not yet differentiated cells and have the ability to turn into different types of cells. With these features, stem cells have been and will continue to be the subject of scientific research from different aspects. Biomaterials are used in many fields today. Biomaterials play an important role in creating micro-environments to support cells for tissue regeneration. Properties such as biocompatibility, biodegradability, mechanical properties, production technology, the material used, surface topology, roughness, and hardness are important for the functionality of biomaterials. For tissue regeneration, the characteristics of the natural microenvironment of the cells forming the target tissue should also be considered. Tissue scaffolds formed from various biomaterials must be compatible with the targeted tissue properties. Research on stem cell-biomaterial interactions in the stem cell field has shown how important the properties of biomaterials to be used in tissue scaffolds are. Developing technological opportunities have carried stem cell research to different dimensions. Research results with tissue scaffolds to be obtained by 3D bioprinting to mimic the targeted tissue will provide new information about stem cell and tissue regeneration in the future.

Conflict of interest

None to declare.

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References

1. Park MH, Subbiah R, Kwon MJ, Kim WJ, Kim SH, Park K, Lee K. The three dimensional cues-integrated-biomaterial potentiates differentiation of human mesenchymal stem cells. Carbohydr Polym. 2018 Dec 15;202:488-496. doi: 10.1016/j.carbpol.2018.09.010
2. Gjorevski N, Lutolf M. Biomaterials approaches in stem cell mechanobiology. Prog Mol Biol Transl Sci. 2014;126:257-78 doi: 10.1016/B978-0-12-394624-9.00011
3. Sharifi E, Khazaei N, Kieran NW, Esfahani SJ, Mohammadnia A, Yaqubi M. Unraveling molecular mechanism underlying biomaterial and stem cells interaction during cell fate commitment using high throughput data analysis. Gene. 2022 Feb 20;812:146111 doi: 10.1016/j.gene.2021.146111.
4. Zhao, X., Li, Q., Guo, Z. Li, Z. Constructing a cell microenvironment with biomaterial scaffolds for stem cell therapy. Stem Cell Res Ther 12, 583 (2021). https://doi.org/10.1186/s13287-021-02650-w
5. Zhao X, Zhu Y, Laslett AL, Chan HF. Hepatic Differentiation of Stem Cells in 2D and 3D Biomaterial Systems. Bioengineering (Basel). 2020 May 25;7(2):47. doi: 10.3390/bioengineering7020047.
6. Ghasemi-Mobarakeh, L, Kolahreez, D, Ramakrishna, S, Williams, D. Key terminology in biomaterials and biocompatibility. Current Opinion in Biomedical Engineering 10 (2019): 45-50.
7. Bello AB, Park H, Lee SH. Current approaches in biomaterial-based hematopoietic stem cell niches. Acta Biomater. 2018 May;72:1-15. doi: 10.1016/j.actbio.2018.03.028
8. Lv H, Wang H, Zhang Z, Yang W, Liu W, Li Y, Li L. Biomaterial stiffness determines stem cell fate. Life Sci. 2017 Jun 1;178:42-48. doi: 10.1016/j.lfs.2017.04.014.
9. Eridani, S. Stem Cell Applications: An Overview. In: Shiffman, M., Di Giuseppe, A., Bassetto, F. (eds) Stem Cells in Aesthetic Procedures. Springer, Berlin, Heidelberg; 2014, 3-5 https://doi.org/10.1007/978-3-642-45207-9_1
10. Ylostalo, JH. 3D stem cell culture. Cells 2020: 9.10 -2178.
11. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019 Feb 26;10(1):68. doi: 10.1186/s13287-019-1165-5.
12. 12. Watt FM, Huck WT. Role of the extracellular matrix in regulating stem cell fate. Nat Rev Mol Cell Biol. 2013 Aug;14(8):467-73. doi: 10.1038/nrm3620.
13. 13. Hashemzadeh MR, Taghavizadeh Yazdi ME, Amiri MS, Mousavi SH. Stem cell therapy in the heart: Biomaterials as a key route. Tissue Cell. 2021 Aug;71:101504. doi: 10.1016/j.tice.2021.101504
14. Nugud A, Alghfeli L, Elmasry M, El-Serafi I, El-Serafi AT. Biomaterials as a Vital Frontier for Stem Cell-Based Tissue Regeneration. Front Cell Dev Biol. 2022 Mar 24;10:713934. doi: 10.3389/fcell.2022.713934.
15. Menicanin D, Bartold PM, Zannettino AC, Gronthos S. Genomic profiling of mesenchymal stem cells. Stem Cell Rev Rep. 2009 Mar;5(1):36-50. doi: 10.1007/s12015-009-9056-2.
16. Wan, X, Liu, Z, Li L. Manipulation of Stem Cells Fates: The Master and Multifaceted Roles of Biophysical Cues of Biomaterials. Advanced Functıonal Materıals. 2021, 31.23: 2010626.
17. Safina I, Embree MC. Biomaterials for recruiting and activating endogenous stem cells in situ tissue regeneration. Acta Biomater. 2022 Apr 15;143:26-38. doi: 10.1016/j.actbio.2022.03.014.
18. Wilems T, Vardhan S, Wu S, Sakiyama-Elbert S. The influence of microenvironment and extracellular matrix molecules in driving neural stem cell fate within biomaterials. Brain Res Bull. 2019 May;148:25-33. doi: 10.1016/j.brainresbull.2019.03.004.
19. Lensch, MW., Daheron, L. & Schlaeger, TM. Pluripotent stem cells and their niches. Stem Cell Rev 2006;2, 185–201. https://doi.org/10.1007/s12015-006-0047-2
20. Martino S, D'Angelo F, Armentano I, Kenny JM, Orlacchio A.

Stem cell-biomaterial interactions for regenerative medicine. Biotechnol Adv. 2012 Jan-Feb;30(1):338-51. doi: 10.1016/j.biotechadv.2011.06.015.

21. Hassan G, Afiffy SM., Kitano, S, Ishii, H, Shang, Y, Matsusaki, M, Seno, M. Cancer stem cell microenvironment models with biomaterial scaffolds in vitro. Processes 9.1 2020: 45.

22. Ravi M, Ramesh A, Pattabhi A. Contributions of 3D Cell Cultures for Cancer Research. J Cell Physiol. 2017 Oct;232(10):2679-2697. doi: 10.1002/jcp.25664.

23. Xu Y, Chen C, Hellwarth PB, Bao X. Biomaterials for stem cell engineering and biomanufacturing. Bioact Mater. 2019 Dec 2;4:366-379. doi: 10.1016/j.bioactmat.2019.11.002.

24. Hiew VV, Simat SFB, Teoh PL. The Advancement of Biomaterials in Regulating Stem Cell Fate. Stem Cell Rev Rep. 2018 Feb;14(1):43-57. doi: 10.1007/s12015-017-9764-y.

25. Hou Y, Liu X, Guo Y, Liu D, Guo P, Liu J. Strategies for Effective Neural Circuit Reconstruction After Spinal Cord Injury: Use of Stem Cells and Biomaterials. World Neurosurg. 2022 May;161:82-89. doi: 10.1016/j.wneu.2022.02.012

26. Han X, Alu A, Liu H, Shi Y, Wei X, Cai L, Wei Y. Biomaterial-assisted biotherapy: A brief review of biomaterials used in drug delivery, vaccine development, gene therapy, and stem cell therapy. Bioact Mater. 2022 Jan 19;17:29-48. doi: 10.1016/j.bioactmat.2022.01.011.

27. Zimmermann JA, Schaffer DV. Engineering biomaterials to control the neural differentiation of stem cells. Brain Res Bull. 2019 Aug;150:50-60. doi: 10.1016/j.brainresbull.2019.05.007.

28. Kim H, Kumbar SG, Nukavarapu SP. Biomaterial-directed cell behavior for tissue engineering. Curr Opin Biomed Eng. 2021 Mar;17:100260. doi: 10.1016/j.cobme.2020.100260.

29. 29. Dawson E, Mapili G, Erickson K, Taqvi S, Roy K. Biomaterials for stem cell differentiation. Volume 60, Issue 2, 14 January 2008, Pages 215-228.

30. Chai C, Leong KW. Biomaterials approach to expand and direct differentiation of stem cells. Mol Ther. 2007 Mar;15(3):467-80. doi: 10.1038/sj.mt.6300084.

31. Neuss S, Apel C, Buttler P, Denecke B, Dhanasingh A, Ding X, Grafahrend D, Groger A, Hemmrich K, Herr A, Jahnen-Dechent W, Mastitskaya S, Perez-Bouza A, Rosewick S, Salber J, Wöltje M, Zenke M. Assessment of stem cell/biomaterial combinations for stem cell-based tissue engineering. Biomaterials. 2008 Jan;29(3):302-13. doi: 10.1016/j.biomaterials.2007.09.022

32. Nissar AA, Martowirogo A, Gilbert PM. Targeting the stem cell niche with regenerative biomaterials. Current Opinion in Solid State and Materials Science, Volume 20, Issue 4, August 2016, Pages 180-192

33. O'brien FJ. Biomaterials & scaffolds for tissue engineering. Materials today 14.3 2011: 88-95.

34. Riha SM, Maarof M, Fauzi MB. Synergistic Effect of Biomaterial and Stem Cell for Skin Tissue Engineering in Cutaneous Wound Healing: A Concise Review. Polymers (Basel). 2021 May 12;13(10):1546. doi: 10.3390/polym13101546.

35. Liu T, Xu J, Pan X, Ding Z, Xie H, Wang X, Xie H. Advances of adipose-derived mesenchymal stem cells-based biomaterial scaffolds for oral and maxillofacial tissue engineering. Bioact Mater. 2021 Jan 30;6(8):2467-2478. doi: 10.1016/j.bioactmat.2021.01.015.

36. Golchin A, Farzaneh S, Porjabbar B, Sadegian F, Estaji M, Ranjbarvan P, Kanafimahbob M, Ranjbari J, Salehi-Nik N, Hosseinzadeh S. Regenerative Medicine Under the Control of 3D Scaffolds: Current State and Progress of Tissue Scaffolds. Curr Stem Cell Res Ther. 2021;16(2):209-229. doi: 10.2174/1574888X15666200720115519.

37. Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials. 2003 Nov;24(24):4337-51. doi: 10.1016/s0142-9612(03)00340-5.

38. Dhawan A, Kennedy PM, Rizk EB, Ozbolat IT. Three-dimensional Bioprinting for Bone and Cartilage Restoration in Orthopaedic Surgery. J Am Acad Orthop Surg. 2019 Mar 1;27(5):e215-e226. doi: 10.5435/JAAOS-D-17-00632.

39. Yadav LR, Chandran SV, Lavanya K, Selvamurugan N. Chitosan-based 3D-printed scaffolds for bone tissue engineering. Int J Biol Macromol. 2021 Jul 31;183:1925-1938. doi: 10.1016/j.ijbiomac.2021.05.215.

40. Hoang P, Ma Z. Biomaterial-guided stem cell organoid engineering for modeling development and diseases. Acta Biomater. 2021 Sep 15;132:23-36. doi: 10.1016/j.actbio.2021.01.026.

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Cerebral palsy and oral health

Manolya İLHANLI[1,*], İlker ILHANLI[2], Peruze ÇELENK[1]

[1]Department of Dentomaxillofacial Radiology, Faculty of Dentistry, Ondokuz Mayıs University, Samsun, Türkiye
[2]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

Cerebral Palsy (CP) is a disease characterized by posture, motor and movement disorders, which are caused by non -progressive but permanent damage of the fetal or infant brain in the early stages of life. In addition to these disorders abnormal motion patterns, spasticity and weakness, vision and hearing problems, epilepsy, cognitive function disorders may be accompanied. Neuromuscular problems due to CP adversely affect oral health. In addition to orofacial problems, the influence of the extremities prevents the independence of the individual affected in many areas from oral hygiene to nutritional failures. Sialorrhea, dental caries, periodontal disease, bruxism, malocclusion, dental erosion, traumatic dental injuries, enamel defects, temporomandibular joint disorders and delay in dental eruption are frequently experienced in patients with CP. The aim of this study was to examine the publications related to oral health in CP patients in the literature and to compile oral health problems and methods of managing these problems in patients with CP. 'Cerebral Palsy, Oral Health, Oral, Dental, Health, Care, Hygiene, Caries, Dysphagia, Malocclusion, Bruxism, Tooth Brushing 'and combinations of these words are scanned in Turkish and English in health databases (PubMed, Cochrane, Embase, Medline and others). Studies containing at least one subject for oral health in CP patients have been included in the examination. Existing approaches and standards were investigated. The populations examined in the studies are considered to be any subtypes of the CP, to be included in our study. The references of all scanned articles were also sought and examined. As a result, CP, one of the diseases with special requirements, brings many oral health problems. These problems adversely affect the quality of life as well as the general health of the affected individual. It is important that health professionals especially dentists, patients, patients' parents and caregivers should be aware of the oral health problems that may be encountered, should have information about protective and therapeutic methods and determine the risks and be encouraged to configure the correct treatment protocols.

Keywords: caries, cerebral palsy, dental hygiene, oral health

1. Introduction

Cerebral Palsy (CP) is a disease characterized by posture, motor and movement disorders, which are caused by non - progressive but permanent damage of the fetal or infant brain in the early stages of life (1-3). In addition to posture disorders, abnormal motion patterns, spasticity and weakness, vision and hearing problems, epilepsy, cognitive function disorders may be accompanied (2, 4, 5).

While CP is seen in an average of 2-2.5/1000 live births around the world (2,6) it has been reported to be 4.4 in Turkey (7). The high prevalence in Turkey is related with excess prevalence of consanguineous marriage, excessive infectious and febrile illnesses, inadequacy of nutrition in infants, negativity in birth conditions, inadequate baby care and diseases during pregnancy (8).

While the underlying etiological factors can be demonstrated in approximately half of the cases (9), the risk factor cannot be determined in approximately one third (10-12). While prenatal causes are held responsible in almost 70-80 %of patients (multiple pregnancies, intrauterine infections, cervical insufficiency, placenta anomalies, bleeding, intravenous clotting, pregnancy toxication, hyperthyroidism, drug use, iodine deficiency, genetic, hypertension, mental retardation, Metabolic and hormonal diseases such as epilepsy and diabetes mellitus), perinatal causes in 10-20 % (placenta infarction, vaginal bleeding, asphyxia, prematurity, placenta previa, low birth weight, corioamniotis, cord wandering, abnormal presentation, early membrane rupture, low score by APGAR scoring system (scoring by evaluating the appearance of the newborn, heart rate, reflex response, tonus and respiration) and postnatal causes (encephalopathies, polycythemia, hypoglycemia, CNS infection, intracranial bleeding, coagulopathy, convulsions, hyperbilirubinemia) in 10% of patients are held responsible (12, 13).

Tonus disorder in CP is at the forefront. According to the type of tonus disorder, CP can be classified as spastic, ataxic, dyskinetic and mixed types (14). Almost three quarters of cases are spastic types. It is characterized by increased muscle tone in the rapid angular movement of the joint in the extremity affected by the first motor neuron damage. This may cause problems such as impaired posture, limitation of

*Correspondence: manolya_dmrcn@hotmail.com

movement, difficulty in coordination, joint contracture and deformity (6,15,16). Balance disorder and tremor are at the forefront in the ataxic type. It occurs as a result of damage to cerebellum. It has an unbalanced walking pattern with a wide gait surface and swinging. Explosive speech can be seen. Ataxia cannot be diagnosed until the patient starts to walk. The only symptom is hypotonia before starting to walk. It contains approximately 5 % to 10 % of patients with CP and usually affects all extremities and trunk. Dyskinetic type CP is the form of athetoid, choreic and choreoathetoid movements. Dystonia can also be seen (17). While these cases are usually hypotonic at birth, findings of extrapyramidal system are characterized by the hypotonia and hypertonia in which they are seen in fluctuations. (18). There are swings, curls and snake movements in the extremities. Dysarthric type speech disorder may occur. Extrapyramidal movements are also reflected in the tongue of wormy, and in these patients, the difficulty of swallowing, salivation problem, speech disorder, oral-motor dyskinesia are seen as severe (19). The mixed type contains the spastic, dyskinetic and ataxic form of CP. Approximately 10 %of the cases are this type. It takes place with the involvement of both pyramidal and extrapyramidal system (18). CP can also be classified according to the affected extremity. When one half of the body is affected, it can be mentioned as hemiplegic, monoplegic when a single extremity is affected, diplegic when the lower extremities are affected, tetraplegic/quadriplegic CP when both the upper and lower extremities are affected together (18, 20).

Additional problems can be seen in CP (21). Mental retardation is one of them. As the loss of motor function increases, mental retardation increases. Dyskinetic and hemiplegic types are at least affected by mental retardation, while 70 % of the quadriplegic type CP have mental retardation (22). Mental retardation with epilepsy is thought to be related. The frequency of epilepsy in all children is 2-12/1000 (23), while in spastic tetraplegic individuals, this frequency is 42-94 % and seizures are the generalized tonic clonus type. In hemiplegic individuals, this frequency is 30-65 %and seizures are of focal types. Dyskinetic type CP is at least risky in terms of epilepsy. Epilepsy treatment in children with CP is very difficult and combined drug use is required (24,25). In 42-81 % of CP cases, speech disorders such as difficulty in speech and sound producing, such as dysarthria or aphasia are seen. There is no conversation in the cases of tetraplegic cases with mental retardation (26). Oral problems are more in this group (27,28). Difficulties in carrying out related actions due to insufficient muscles that provide suction, swallowing and chewing can be encountered. Vision problems are 28-90 %in children with CP. While vision defects and strabismus are most common; blindness, glaucoma, nystagmus can also be seen (29). Hearing loss can be seen in 12 %. Hearing loss can be prevented by routine controls (29). Speech disorder is seen in 38 %of children with CP. Inclusion of intercostal muscles, tongue muscles and larynx muscles cause this situation. (6). Apnea, bronchitis, asthma, atelectasis, pneumonia may occur due to aspiration. It is very important because aspiration is the cause of mortality and morbidity (30).

Children with CP cannot get enough nutrients for reasons such as suction, chewing and swallowing disorders, loss of appetite, rejection of food and prolonged food time. Nutritional failure is associated with mortality and morbidity (31). GIS problems are seen in 80-90 %of children with CP (32). One of the most common GIS problems is gastroesophageal reflux (GER). It can cause discomfort, restlessness, irritability, vomiting, esophagitis and aspiration pneumonia in children (32). Aspiration, GER and constipation may cause the child to reduce the interest of food and reject food. (33). Children with ataxic and spastic dyskinetic type CP can be fed to themselves, while children with spastic tetraplegic and dyskinetic type CP cannot feed on themselves due to the severity of motor functional disorder (34). Due to the difficulty of chewing and swallowing, the eating time causes malnutrition. This leads to important problems such as retardation in growth development, deterioration of the immune system, delay of wound healing and weakness (34). While the parents of normal children spend the average of 0.8 hours a day to feed their children, the parents of CPs spend 3.5-7.5 hours. Natural gagging and cough reflexes may have never developed in individuals with CP. The absence of these reflexes may cause irritation and inflammation in the respiratory tract. Sometimes these reflexes may become severe enough to reject food intake during consumption of foods (33). In children with CP, the distress in making the food into a bolus (soft, chewed food mass) is due to chewing problems of insufficient tongue and side jaw movements. In particular, the limitation of the temporomandibular joint (TMJ) movement in spastic tetraplegic CP causes the chewing problem to become even more severe (35).

Neuromuscular problems due to CP affect oral health negatively (36-39). In addition to orofacial problems, the influence of the extremities prevents the independence of the individual affected in many areas from the provision of oral hygiene to nutritional failure (9, 28, 36, 40-41). As the intensity of the disease increases, oral problems also increase (6,42). Sialorrhea, dental caries, periodontal disease, bruxism, malocclusion, dental erosion, traumatic dental injuries, enamel defects, temporomandibular joint disorders and delay in dental eruption are among the most frequent oral health problems in CP patients (43-46). Some studies show that oral-dental health is not different from healthy children as a result of appropriate oral care in children with CP, while in some studies, if there is no oral hygiene motivation, it has been shown that oral-dental health is significantly affected (38-49)

The aim of this study was to examine the publications

related to oral health in CP patients in the literature and to compile oral health problems and methods of managing these problems in patients with CP.

2. Materials and Methods

Cerebral Palsy, Oral Health, Oral, Dental, Health, Care, Hygiene, Caries, Dysphagia, Malocclusion, Bruxism, Tooth Brushing ’and combinations of these words are scanned in Turkish and English in health databases (PubMed, Cochrane, Embase, Medline and others). Studies containing at least one subject for oral health in CP patients have been included in the examination. Existing approaches and standards were investigated. The populations examined in the studies are considered to be any subtypes of the CP, to be included in our study. The references of all scanned articles were also sought and examined.

3. Results and Discussion

3.1. Sialorrhea

In fact, sialorrhea can be seen as normal in infants and children under the age of 4 where coordination is not fully developed. More saliva occurs in the anterior part of the oral cavity. Deterioration of swallowing and coordination defect of orofacial muscles can cause sialorrhea in patients with CP (50-53). Sialorrhea is not socially accepted and can produce significant negative effects on the psychosocial health and quality of life (54). The incidence of sialorrhea in CP was reported as 10-58 % (52, 55-57). Sometimes sialorrhea is related to an irritating lesion, such as dental caries or throat infection, resulting in increased production of saliva. Severe sialorrhea may get worse with some antiepileptic drugs, such as clonazepam (55). Sialorrhea has been shown to be the most serious in tetraplegic spastic CP (52,55). Cosmetic problems, perioral redness, infection and even dehydration may occur as a result of sialorrhea (58-60).

Sialorrhea must be treated if it is at the level to wet the child's clothes and toys (61). While oral-motor treatments (speech therapy, physiotherapy, biofeedback treatment) are appropriate in mild to medium intensity cases, pharmacotherapy and more radical methods can be applied in severe cases and it is recommended to lie on the side to protect the patient from aspiration (59,61,62). Conservative treatments are preferred to maintain normal mouth, teeth and jaw development. In cases where these are not effective, radiotherapy, pharmacotherapy and surgical procedures may be preferred. In children with cooperation, behavioral therapies should be applied for 6 months before considering advanced treatment options (63). Rehabilitation applications for the mouth and face region are the most frequently applied treatment option. These methods are methods aimed at healing muscle tone with lip, jaw and tongue movements (64). Often lip shrinkage, blowing, cheek inflation, tongue suppressing, rounding, tongue to the nose and chin, kiss and laughing exercises are performed (1).

Biophunctional apparatuses (ISMAR (Innsbruck sensory motor activator and regulator) (65), Castillo Morales (66) and Dr. Hinz (67) apparatuses) can be used because they increase oral awareness, help to start swallowing and increase tongue movements. ISMAR apparatus is a combination of monoblocs and Frankel apparatus that improves eating and drinking functions rather than improvement of Sialorrhea control. It is used for 1 year or longer (65). Castillo Morales apparatus stimulates the tongue with the help of a stimulator in the palatal area and ensures its position in the mouth, while stimulating the upper lip with the acrylic part on the surface of the vestibule. It is stated that it can be used in preventing sialorrhea and fighting with nutrition and speech problems (66,68). In patients with CP, the vestibule plaque developed by Dr. Hinz can be used to control sialorrhea. This apparatus encourages breathing through the nose and prevents the external forces formed from the lips and cheeks to the teeth and activates the muscle tone of the lip. With the correct positioning of the tongue, the mouth remains open (67). These apparatuses are used before moving to advanced treatment methods in cases where physiotherapy and behavioral methods are insufficient. It is recommended to apply to children older than 6 years old (69).

The use of anticholinergic drugs such as benztropine, glycopyrrolate and benzhexol hydrochloride can reduce sialorrhea (70) however, it should be remembered that they have side effects such as dry mouth, increase in salivary viscosity, urinary retention, irritability, sedation, blurred vision, constipation and flushing (51,59,62,71).

Botulinum toxin A injection in the salivary glands is another treatment option, especially parotid (72,73). Side effects include a decrease in saliva, difficulty chewing, dry mouth and difficulty in swallowing (74). Side effects such as oral asymmetry, ecchymosis and ptosis may also be seen. In a study conducted for the frequency of application of Botulinum toxin, it was emphasized that the efficacy was 3 months and that it was necessary to repetition at intervals (75,76). Drug treatment with rehabilitation is the first choice of treatment, but botulinum toxin injection is more effective and it has been reported to have less side effects than pharmacological treatment (74).

Surgical interventions can be performed in order to prevent sialorrhea, but as a result of ripening oral muscles by increasing age sialorrhea may decrease and no surgical treatment is recommended until oral muscles complete the maturation (54,62). Submandibular gland excision, changing the location of the parotid/submandibular canal, ligation of parotid ductus, tympanic neurotomy or combinations of these methods can be applied for saliva control (62, 77).

3.2. Dental caries

Dental caries is a multifactorial disease caused by microorganisms fermenting sugar -containing foods that provides acids destructing the harsh tissues of the tooth (78). Differences in food form, prolongation of the time of food in

the mouth, difficulty in cooperation, structural disorders in the teeth may cause dmft-dmfs values to increase in children with CP (39,79). In dmft index, d: caries, m: missing teeth f: the number of filled teeth, while the dmfs index express the number of affected surfaces. In addition to the higher dmft-dmfs values, it has been found that children with CP have more drawn tooths and untreated tooths than healthy children (79-82). According to a study conducted among children with CP during the primary dentition, children with CP have higher percentage of dental caries than normal children and this result was reported to be independent of oral hygiene habits and diet (83).

Oral hygiene was found worse in CP (84,85). Other risk factors are the effect of the drugs used, oral respiration and enamel hypoplasia (86). In addition, saliva flow rate, acidity and buffering capacity is lower (87). In patients with CP, a strong relationship was found between increased saliva osmolarity and caries formation (50). More severe disease is related to more caries (88, 89). Severe motor incoordination affects the ability to perform adequate oral hygiene and cognitive deficits makes cooperation for effective oral care more difficult (90).

Patients and caregivers should be informed that drugs have sugar content and reduce saliva. Dietary habits should be arranged, frequent consumption of fruit juices and sugar -containing drinks, consumption of adhesive foods that have a karyogenic affect should be limited. Instead, snacks that can be easily cleaned from teeth should be preferred. In addition, foods such as daily milk, yogurt and cheese, vegetables, fruits, cereals and protein -containing eggs, meat, chicken and fish can be recommended (91). Sealants and fluorine applications can be applied as preventive treatment (92). Caregivers should inspect the mouth after each meal or dose of medicine and remove food or medicine from the mouth by rinsing with water, sweeping the mouth with a finger wrapped in gauze, or using a disposable foam applicator swab.

3.3. Periodontal disease

Inadequate in the removal of the dental plaque and the fact that the parent cannot comprehend the importance of oral hygiene, more gingivitis and other periodontal diseases are seen in children with CP compared to normal children (92). Several studies have shown that gingival hyperplasia and associated bleeding occurs with higher frequency in children with CP (3). This high frequency may be due to the same factors predisposing to dental caries and leading to biofilm buildup (3). Difficulties in conducting daily oral hygiene, intraoral sensitivity, malocclusion, and oro-facial motor dysfunction are the main contributing factors (84, 85, 93, 94). Another important factor is the use of antiepileptic drugs, particularly phenytoin (95). Gingival hyperplasia is predictive for periodontal diseases. Eighty percent gingivitis was detected in children with CP aged 6-8 years. This frequency is 50 %under 6 years of age, 90 %over the age of 9 (96). Higher gingival index scores are seen in the tetraplegic CP than hemiplegic CP (83). Saliva has an important place in the protection of oral hygiene with its mechanical and chemical cleaning ability. The osmolarity of saliva in patients with CP increases with the rise in proinflammatory cytokines and immunoglobulin A levels, thus increasing the incidence of gingivitis, tooth stone and plaque (97).

The mechanism and treatment of these diseases in CP is the same as in periodontal diseases seen in normal children (98, 99). CP patients should be provided with oral care daily in the prevention and treatment of periodontal disease. For this purpose, it can be ensured to achieve self -care with personal modifications in appropriate cases. The use of special toothbrush techniques and dental floss to the patient should be taught to the patient or those who provide care (95). It has been reported that the plaque removal increases by the personalized modifications such as connecting the toothbrush into the hand of the child and thickening of the handle of the brush. (100). Three -dimensional toothbrush was found to be more effective than traditional toothbrushes in reducing plaque and periodontal problems, as well as the use of three -dimensional brush in patients with CP, the time for brushing, vomiting reflex and gingival bleeding decrease (101). In addition, it may be recommended to use power sonic toothbrushes. There are studies that indicate that power toothbrushes can be an alternative to three -dimensional toothbrushes in plaque removal (102). necessary, the use of mouthwash should be encouraged. Antimicrobials such as chlorhexidine have been reported to be effective (95).

3.4. Bruxism

Bruxism, the habitual grinding of teeth, is a common problem in children with CP, particularly those with severe motor and cognitive deficits (56,103). Bruxism may lead to teeth abrasion, flattening of biting surfaces, damage to the periodontal tissues, alveolar bone resorption and TMJ dysfunction. The incidence of bruxism in individuals with CP is between 25-51 % (56,103-106). In healthy children, this frequency has been reported as 15.29 % (107).

Although the cause is not certain, neurological disorders, irregularities in myophunctional complex and inadequacies to control of the lower jaw, dopamine function, sleep disorders, anxiety, stress and neuroleptic drug use have been reported to have related. In addition, GER, which is frequently monitored in CP patients, has been reported to play a role in the etiology of bruxism (1, 108).

In the treatment of bruxism, the cause should be determined first. Methods such as release of parafunctional habits, splint administration, pharmacological treatment and regulation of occlusion can be used in order to eliminate the cause of bruxism. There are publications that cold application applied to the face or cheek during tooth squeezing significantly reduces bruxism, however, that pharmacological treatment has no effect (109). In addition, botulinum toxin

application in the temporalis and masseter muscles and in advanced cases maxillary osteotomy can be considered. Medications that improve the sleep-wake cycle, such as melatonin, should be used and may also result in improved daytime behavior (110).

3.5. Malocclusion

Malocclusion has been reported with increasing frequency in children with CP, most commonly over-bite and anterior open-bite (3). Increased muscle tone caused by excessive stimulation of motor neurons in children with CP causes malocclusion (111). These abnormalities have been reported to get worse with age. Mouth breathing, lip incompetence and long face are contributing factors (112). The disturbances of the facial, masticatory and tongue musculature cause abnormal facial growth and increase the incidence of malocclusion (113). Pseudo-bulbar palsy, orofacial incoordination and hypotonia could further add to the risk of developing malocclusion (3). Poor chewing efficiency is associated with few occlusal contacts (114).

Orthodontic treatment application for children with CP is useful but, it is thought to be unnecessary (115). Unfortunately, correcting malocclusion is almost impossible in people with moderate or severe cerebral palsy. Orthodontic treatment may not be an option because of the risk of caries and enamel hypoplasia. However, a developmental disability in and of itself should not be perceived as a barrier to orthodontic treatment. The ability of the patient or the caregiver to maintain good daily oral hygiene is critical to the feasibility and success of orthodontic treatment (116).

3.6. Dental Erosion

The loss of hard tissues of the tooth with chemical factors is called dental erosion (117). There is a consensus among the researchers that dental erosion is caused by acidic foods and beverages. (118,119). It has been reported that CP is not an etiological factor for erosion, but erosion may be increased in children with CP due to vomiting, chewing and swallowing problems (120). Increased intra-abdominal pressure, neuromotor insufficiency, long-term inpatient position, delay of stomach discharge, convulsions, a wide variety of drug use are also one of the causes that increase the risk of GER and indirectly the erosion in CP (121). In a study, 73% of CP patients with dental erosions had history of GER (122). Both primary and permanent teeth can be affected, most commonly the upper molars, lower molars and upper incisors (123). The maxillary central teeth's palatine surfaces are the most affected sites by the stomach content reaching the mouth (124). Other surfaces of the teeth are less affected by erosion as they are close to salivary glands (125). This influence starts from enamel level and can reach the dentin and pulp (126).

Active factors should be determined and eliminated for the prevention and treatment of erosion. It is important to provide oral hygiene and to regulate nutrition and diet. Foods with erosive potential such as, fruit juices, carbonated and sports beverages such as non -alcoholic beverages, fresh fruits, acetic acid foods, pickles, potato chips should be reduced in the diet. The use of soft -haired toothbrushes and the use of toothpastes with low erosive features with low fluoride ratio should be encouraged (1).

3.7. Traumatic dental injuries

Although they move less than healthy children, children with CP are exposed to more trauma because of the increased overjet, coordination disorder in walking and uncontrolled head movements (127,128). Children with CP presented four times greater probability of dental trauma (129). In a study, trauma was detected in 57 % of 68 children with CP. Sixty two percent of these traumas is the fracture of the enamel and dentine. The most affected tooth by trauma is maxillary central tooth. Females are exposed to more trauma than males (128).

Tooth-saving kits can be suggested for group homes. Caregivers should be emphasized that traumas require immediate professional attention. Also, caregivers should locate any missing pieces of a fractured tooth, and radiographs of the patient's chest may be necessary to determine whether any fragments have been aspirated (116).

3.8. Enamel defects

Children with CP are at an increased risk for having developmental enamel defects (44,130). Developing germs of the primary teeth are very sensitive to systemic problems and drugs used in the prenatal and postnatal period from the 4th month of intrauterine life to 1 years old. These problems may cause enamel defects (36).

3.9. Dysphagia

Dysphagia is frequently seen in CP (131). According to the literature, the prevalence of dysphagia in children with and without CP are 19-25% vs. 45-60% (132,133). Dysphagia causes feeding difficulty and increased feeding time that impairs the quality of life (134). Aspiration is associated with dysphagia, vomiting and regurgitation (135). Causes of dysphagia in children with CP are the presence of anatomical pathologies, spastic and hypotonic posture, undeveloped swallowing reflex, inadequacy in tongue, lips, jaw and oral functions, lack of body control, deep sensation loss, inability to communicate, no lip closing, inadequate tongue movements, inadequate tongue push, inadequate jaw push, decreased tone on the cheeks, presence of tonic biting reflex, oral hypersensitivity, existence of hyperactive gag reflex, limited upper lip movement (136,137).

The first step in the treatment of dysphagia is compensatory exercises. The aim is to ensure the safety of swallowing and to prevent aspiration (138). They include replacement of posture, changes in the volume and velocity of food intake, changes in food consistency, temperature and taste, change of auxiliary tools, techniques to increase the feeling of oral sense (139). Neuromuscular electrical stimulation can be used in pharyngeal dysfunction (141). In

cases where airway safety cannot be achieved and other methods are not successful enough, the transfer of nutrients to the stomach can be achieved by orogastric, nasogastric or percutaneous endoscopic gastrotomy to ensure adequate calorie intake. Even if one of these methods is used, oral motor therapy should be applied both to switch to oral nutrition and to prevent salivary aspiration (141).

3.10. Delay of the dental eruption

Delay of the dental eruption is associated with oral dysfunction. It is more common in children who cannot be fed oral (142). Delay of the dental eruption is frequent in children with CP (45,49,143). In children with CP, the lack of chewing function and gingival hyperplasia also cause delay of the dental eruption (144).

3.11. Temporomandibular joint disorders

Children with CP are at a significantly higher risk for developing signs and symptoms of TMJ disorders (145). Male gender, the presence and severity of any malocclusion, mouth breathing, and mixed dentition were all identified as risk factors for developing signs and symptoms of TMJ disorders in CP patients (28).

3.12. Dental management

CP patients' oral health is worse than non -CP, as well as the frequency of treatment was found to be low. The reason for this is that dentists do not have sufficient information and experience about this patient group that requires special interest, or that these patients may have difficulties to apply to the dentist.

Some practical challenges are commonly encountered when handling children with CP. These include apprehension, fear from strangers, and communication difficulties (146). Special seating and positioning adjustments are needed for children with abnormal posture. Assistance from the parents and dental assistant is often needed particularly for immobilization and during radiographic procedures (28). Sedation and anesthesia are frequently needed in non-compliant children (147).

When the CP patient comes to the dentist clinic, first, he should be evaluated in detail. The presence of additional problems should be questioned and consulted to the relevant doctors. Patient's cooperation should be achieved by applying patiently the tell-show-apply method (148).

As for all individuals, there should be preventive approaches for individuals with CP. The training of the family, caregiver and child, making oral hygiene habits a part of the daily life, regular examination of children by dentists, taking professional preventative measures are among the first step approaches (149,150).

CP, one of the diseases with special requirements, brings many oral health problems. These problems adversely affect the quality of life as well as the general health of the affected individual. It is important that health professionals, especially dentists, patients, parents and caregivers should be aware of the oral health problems that may be encountered, should be encouraged to have information about preventative and therapeutic methods and to determine the risks and to configure the correct treatment protocols.

Conflict of interest

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References

1. Kırzıoğlu Z, Bayraktar C. Serebral Palsili Çocuklarda Sık Rastlanan Oral Problemler, Ağız Dışına Salya Akışı ve Tedavi Önerileri. Sdü Sağlık Bilimleri Enstitüsü Dergisi. 2018; 9(2):156-62.

2. Bax M, Goldstein M, Rosenbaum P, Leviton A, Paneth N, Dan B, et al. Executive committee for the definition of cerebral palsy. Proposed definition and classification of cerebral palsy. Dev Med Child Neurol. 2005; 47: 571-6.

3. Jan MM. Cerebral palsy: comprehensive review and update. Ann Saudi Med. 2006; 26: 123-32.

4. Rosenbaum P, Stewart D. The World Health Organization International Classification of Functioning, Disability, and Health: a model to guide clinical thinking, practice and research in the field of cerebral palsy. Semin Pediatr Neurol. 2004; 11: 5-15.

5. Gokkaya NK, Caliskan A, Karakus D, Ucan H. Relation between objectively measured growth determinants and ambulation in children with cerebral palsy. Turk J Med Sci. 2009; 39: 85-90.

6. Sankar C, Mundkur N. Cerebral palsy-definition, classification, etiology and early diagnosis. Indian J Pediatr. 2005; 72(10): 865-8.

7. Serdaroglu A, Cansu A, Ozkan S, Tezcan S. Prevalence of cerebral palsy in turkish children between the ages of 2 and 16 years. Dev Med Child Neurol. 2006; 48: 413-6.

8. Livanelioglu A, Kerem Gunel M. Serebral palsi'de fizyoterapi. Ankara Yeni Ozbek Matbaası; 2009, p. 5-12.

9. Sehrawat N, Marwaha M, Bansal K, Chopra R. Cerebral palsy: a dental update. Int J Clin Pediatr Dent. 2014; 7(2): 109- 18.

10. Berker AN, Yalcin MS. Cerebral palsy: orthopedic aspects and rehabilitation. Pediatr Clin N Am. 2008; 55: 1209-25.

11. Shevell MI, Majnemer A, Morin I. Etiologic yield of cerebral palsy: a contemporary case series. Pediatr Neurol. 2003; 28(5): 352-9.

12. Jacobsson B, Hagberg G. Antenatal risk factors for cerebral palsy. Best Pract Res Clin Obstet Gynaecol. 2004; 18(3): 425-

36.

13. Nelson KB, Ellenberg JH. Children who "outgrew" cerebral palsy. Pediatrics. 1982; 69(5): 529-36.

14. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. Surveillance of Cerebral Palsy in Europe (SCPE). Dev Med Child Neurol. 2000; 42(12): 816-24.

15. Mukherjee S, Gaebler-Spira DJ. Cerebral Palsy. In: Braddom RL (Ed.). Physical Medicine & Rehabilitation 3th. Philedelphia: Saunders Elsevier; 2007, p.1243-67.

16. Diamond M, Armento M. Children with Disabilities. In: D. J. A. (Ed.). Physical Medicine & Rehabilitation Principles and Practice. 4th. Philedelphia: Lippincott Williams & Wilkins; 2005, p. 122.

17. Molnar GE, Alexander MA. Pediatric Rehabilitation. Philedelphia, USA: Hanley Belfus inc;1999.

18. Koman LA, Smith BP, Shilt JS. Cerebral palsy. Lancet. 2004; 363(9421): 1619-31.

19. Hoyer H, Limbrock GJ. Orofacial regulation therapy in children with Down syndrome, using the methods and appliances of Castillo-Morales. ASDC J Dent Child. 1990; 57(6): 442-4.

20. Yakut, A. Serebral Palside Yeni Gelişmeler. Türkiye Klinikleri. 2008;4(4): 127-38.

21. Russman BS, Ashwal S. Evaluation of the child with cerebral palsy. Semin Pediatr Neurol. 2004; 11: 47-57.

22. Sala DA, Grand AD. Prognosis for ambulation in cerebral palsy. Dev Med Child Neurol. 1995; 37(11): 1020-6.

23. Cowan LD. The epidemiology of the epilepsies in children. Mental Retardation and Developmental Disabilities Research Reviews. 2002; 8(3): 171-81.

24. Serdaroglu A, Ozkan S, Aydin K, Gucuyener K, Tezcan S, Aycan S. Prevalence of epilepsy in Turkish children between the ages of 0 and 16 years. Journal of Child Neurology. 2004;19(4): 271-74.

25. Zelnik N, Konopnicki M, Bennett-Back O, Castel-Deutsch T, Tirosh E. Risk factors for epilepsy in children with cerebral palsy. European Journal of Paediatric Neurology. 2010; 14(1): 67-72.

26. Odding E, Roebroeck ME, Stam HJ. The epidemiology of cerebral palsy: Incidence, impairments and risk factors. Disability and Rehabilitation. 2006; 28(4): 183-91.

27. Eltumi M, Sullivan PB. Nutritional management of the disabled child: the role of percutaneous endoscopic gastrostomy. Dev Med Child Neurol. 1997; 39: 66-68.

28. Jan BM, Jan MM. Dental health of children with cerebral palsy. Neurosciences (Riyadh). 2016;21(4):314-318.

29. Duman O, Imad FM, Kızılay F, Yucel I, Balkan S, Haspolat S. Serebral palsili hastaların işlevsel kapasitelerine göre görme sorunlarının değerlendirilmesi. Çocuk Sağlığı ve Hastalıkları Dergisi. 2005; 48: 130-5.

30. Fitzgerald DA, Follett J, Asperen PPV. Assessing and managing lung disease and sleep disordered breathing in children with cerebral palsy. Paediatr Res Rev. 2009; 10(1): 18-24.

31. Vega-Sanchez R, de la Luz Gomez-Aguilar M, Haua K, Rozada G. Weight-based nutritional diagnosis of Mexican children and adolescents with neuromotor disabilities. BMC Res Notes. 2012; 5: 218.

32. Ceriati E, Peppo FD, Cıprandı G, Marchettı P, Sılverı M, Rıvosecchı M. Surgery in disabled children: General gastroenterological aspects. Acta Paediatr. 2006; 95: 34-7.

33. Erkin G, Kacar S, Ozel S. Serebral palsili hastalarda gastrointestinal sistem ve beslenme problemleri. Türkiye Fiziksel Tıp ve Rehabilitasyon Dergisi. 2005; 51(4): 150-5.

34. Dahlseng MO, Finbraten AK, Juliusson PB, Skranes J, Andersen G, Vik T. Feeding problems, growth and nutritional status in children with cerebral palsy. Acta Paediatr. 2012; 101(1): 92-8.

35. Pelegano JP, Nowysz S, Goepferd S. Temporomandibular joint contracture in spastic quadriplegia: effect on oral-motor skills. Dev Med Child Neurol. 1994; 36(6): 487-94.

36. Bhat M, Nelson KB. Developmental enamel defects in primary teeth in children with cerebral palsy, mental retardation, or hearing defects: a review. Advances in Dental Research. 1989; 3:132-42.

37. Cohen MD, Diner H. The significance of developmental dental enamel defects in neurological diagnosis. Pediatr. 1970; 46:737-47.

38. Murray GS, Johnsen DC, Weissman BW. Hearing and neurologic impairment: insult timing indicated by primary tooth enamel defects. Ear and Hearing. 1987; 8:68-73.

39. Grzić R, Bakarcić D, Prpić I, Jokić NI, Sasso A, Kovac Z, et al. Dental health and dental care in children with cerebral palsy. Coll Antropol. 2011; 35: 761-4.

40. Bhowate R, Dubey A. Dentofacial changes and oral health status in mentally challenged children. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2005; 23(2):71-3.

41. Altun C, Guven G, Basak F, Akbulut E. Altı-onbir yaş grubu çocukların ağız-diş sağlığı yönünden değerlendirilmesi. Gülhane Tıp Dergisi. 2005; 47:114-8.

42. Jones MW, Morgan E, Shelton JE. Primary care of the child with cerebral palsy: a review of system (Part II). J Pediatr Health Care. 2007; 21: 226-37.

43. Faulconbridge RVL, Tranter RM, Moffat V, Green E. Review of management of drooling problems in neurologically impaired children: a review of methods and results over 6 years at Chailey Heritage Clinical Services. Clinical Otolaryngology. 2001; 26(2): 76-81.

44. Lin X, Wu W, Zhang C, Lo EC, Chu CH, Dissanayaka WL. Prevalence and distribution of developmental enamel defects in children with cerebral palsy in Beijing, China. Int J Paediatr Dent. 2011; 21(1): 1-6.

45. Moslemi M, Vejdani J, Sadrabad ZK, Shadkar MM. A study on the eruption timing of permanent dentition in patients with cerebral palsy. Spec Care Dentist. 2013; 33(6): 275-9.

46. Inga CJ, Reddy AK, Richardson SA, Sanders B. Appliance for chronic drooling in cerebral palsy patients. Pediatric Dentistry. 2001; 23 (3):241-2.

47. de Carvalho RB, Mendes RF, Prado RR, Jr. Moita Neto JM. Oral health and oral motor function in children with cerebral palsy. Spec Care Dentist. 2011; 31(2): 58-62.

48. Katz CR. Integrated approach to outpatient dental treatment of a patient with cerebral palsy: a case report. Spec Care Dentist. 2012; 32(5): 210-7.

49. Rodrigues dos Santos MT, Masiero D, Novo NF, Simionato MR. Oral conditions in children with cerebral palsy. J Dent Child (Chic). 2003; 70(1): 40-6.

50. Santos MT, Ferreira MC, Mendes FM, de Oliveira Guaré R. Assessing salivary osmolality as a caries risk indicator in cerebral palsy children. Int J Paediatr Dent. 2014; 24(2): 84-9.

51. Fairhurst CB, Cockerill H. Management of drooling in children. Arch Dis Child Educ Pract Ed. 2011; 96(1): 25-30.

52. Hegde AM, Pani SC. Drooling of saliva in children with cerebral palsy-etiology, prevalence, and relationship to salivary flow rate in an Indian population. Spec Care Dentist. 2009; 29(4): 163-8.

53. Tahmassebi JF, Curzon ME. The cause of drooling in children with cerebral palsy - hypersalivation or swallowing defect? Int J Paediatr Dent. 2003; 13(2): 106-11.

54. Meningaud JP, Pitak-Arnnop P, Chikhani L, Bertrand JC. Drooling of saliva: a review of the etiology and management options. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101: 48-57.

55. Tahmassebi JF, Curzon ME. Prevalence of drooling in children with cerebral palsy attending special schools. Dev Med Child Neurol. 2003; 45(9): 613-7.

56. Ortega AO, Guimarães AS, Ciamponi AL, Marie SK. Frequency of parafunctional oral habits in patients with cerebral palsy. J Oral Rehabil. 2007; 34(5): 323-8.

57. Roberto LL, Machado MG, Resende VL, Castilho LS, Abreu MH. Factors associated with dental caries in the primary dentition of children with cerebral palsy. Braz Oral Res. 2012; 26(5): 471-7.

58. Dougherty NJ. A review of cerebral palsy for the oral health professional. Dent Clin N Am. 2009; 53: 329-38.

59. Hussein I, Kershaw AE, Tahmassebi JF, Fayle SA. The Management of Drooling in Children and Patients with Mental and Physical Disabilities a Literature Review. International Journal of Pediatric Dentistry. 1998; 8: 3-11.

60. Siegel L, Klingbeil M. Control of drooling with transdermal scopolamine in a child with cerebral palsy. Dev Med Child Neurol. 1991; 33: 1013-4.

61. Nunn JH. Drooling: review of the literature and proposals for management. J Oral Rehabil. 2000; 27(9): 735-43.

62. Lal D, Hotaling AJ. Drooling. Curr Opin Otolaryngol Head Neck Surg. 2006; 14(6): 381-6.

63. Van der Burg JJ, Didden R, Jongerius PH, Rotteveel JJ. Behavioral treatment of drooling: a methodological critique of the literature with clinical guidelines and suggestions for future research. Behav Modif. 2007; 31(5): 573-94.

64. Ruscello DM. Nonspeech oral motor treatment issues related to children with developmental speech sound disorders. Lang Speech Hear Serv Sch. 2008; 39(3): 380-91.

65. Johnson HM, Reid SM, Hazard CJ, Lucas JO, Desai M, Reddihough DS. Effectiveness of the Innsbruck Sensorimotor Activator and Regulator in improving saliva control in children with cerebral palsy. Developmental Medicine & Child Neurology. 2004; 46: 39-45.

66. Castillo-Morales R, Brondo J, Hoyer H, Limbrock GJ. Treatment of chewing, swallowing and speech defects in handicapped children with Castillo-Morales orofacial regulator therapy: advice for pediatricians and dentists. Zahnarztl Mitt. 1985; 75(9): 935-42, 947-51.

67. Hinz R. Habits and Their Prevention. Düsseldorf: HPSS-Gmbh; 1995.

68. Limbrock GJ, Hoyer H, Scheying H. Drooling, chewing and swallowing dysfunctions in children with cerebral palsy: treatment according to Castillo-Morales. ASDC J Dent Child. 1990; 57(6): 445-51.

69. Little SA, Kubba H, Hussain SS. An evidence-based approach to the child who drools saliva. Clin Otolaryngol. 2009; 34(3): 236-9.

70. Jongerius PH, van Tiel P, van Limbeek J, Gabreëls FJ, Rotteveel JJ. Ansystematic review for evidence of efficacy of anticholinergic drugs to treatndrooling. Arch Dis Child. 2003; 88(10): 911-4.

71. Bachrach SJ, Walter RS, Trzcinski K. Use of glycopyrrolate and other anticholinergic medications for sialorrhea in children with cerebral palsy. Clin Pediatr (Phila). 1998; 37(8): 485-90.

72. Hanagası HA. Hipersekretuvar Bozukluklar ve Botulinum Toksini. Nöropsikiyatri Arşivi. 2010; 47: 48-51.

73. Porte M, Chaléat-Valayer E, Patte K, D'Anjou MC, Boulay C, Laffont I. Relevance of intraglandular injections of Botulinum toxin for the treatment of sialorrhea in children with cerebral palsy: a review. Eur J Paediatr Neurol. 2014; 18(6): 649-57.

74. Chaléat-Valayer E, Porte M, Buchet-Poyau K, Roumenoff-Turcant F, D'Anjou MC, Boulay C, et al. Management of drooling in children with cerebral palsy: A French survey. Eur J Paediatr Neurol. 2016; 20(4): 524-31.

75. Koyuncuoglu HR, Demirci S. Application of botulinum toxin at a neurology clinic: an eleven-year experience. Turk J Neurol. 2016; 22: 8-12.

76. Gonzalez-L MD, Martinez C, Bori Y, Fortuny I, Suso-Vergara S. Factors in the efficacy, safety, and ımpact on quality of life for treatment of drooling with botulinum toxin type a in patients with cerebral palsy. Am J Phys Med Rehabil. 2017; 96(2): 68-76.

77. Reed J, Mans CK, Brietzke SE. Surgical management of drooling: a meta-analysis. Arch Otolaryngol Head Neck Surg. 2009; 135(9): 924-31.

78. Nguyen DH, Martin JT. Common dental infections in the primary care setting. Am Fam Physician. 2008; 77(6): 797-802.

79. Albert DA, Park K, Findley S, Mitchell DA, McManus JM. Dental caries among disadvantaged 3- to 4-year-old children in northern Manhattan. Pediatr Dent. 2002; 24(3): 229-33.

80. Cardoso AM, Gomes LN, Silva CR, Soares RD, De Abreu MH, Padilha WW, et al. Dental caries and periodontal disease in Brazilian children and adolescents with cerebral palsy. Int J Environ Res Public Health. 2014; 12: 335-53.

81. Botti MT, Biancardi M, Oliveira R, Jardim JR. Caries prevalence in patients with cerebral palsy and the burden of caring for them. Spec Care Dentist. 2010; 30:206-10.

82. Dini EL, Holt RD, Bedi R. Prevalance of caries and developmental defects of enamel in 9-10 year old children living in Brazil with differing water fluoride histories. British Dental Journal. 2000; 188:146-49.

83. Guare Rde O, Ciampioni AL. Prevalence of periodontal disease in the primary dentition of children with cerebral palsy. J Dent Child (Chic). 2004; 71(1): 27-32.

84. de Oliveira R, Ciampioni AL. Prevalence of Periodontal Disease in the Primary Dentition of Children with Cerebral Palsy. J Dent Child. 2004; 71:27-32.

85. Rennan Y, Colman McG, Cynthia K, Nigel M. Oral health in pres¬chool children with cerebral palsy: a case–control community-based study. International Journal of Paediatric Dentistry. 2010; 20:330-5.

86. Mani SA, Mote N, Kathariya M, Pawar KD. Adaptation and development of dental procedure in cerebral palsy. Pravara Med Rev. 2015; 7(4): 17-22.

87. dos Santos MT, Masiero D, Simionato MR. Risk factors for dental caries in children with cerebral palsy. Spec Care Dentist. 2002; 22(3): 103-7.

88. Santos MT, Guare RO, Celiberti P, Siqueira WL. Caries experience in individuals with cerebral palsy in relation to oromotor dysfunction and dietary consistency. Spec Care Dentist. 2009; 29: 198-203.

89. Dourado Mda R, Andrade PM, Ramos-Jorge ML, Moreira RN, Oliveira-Ferreira F. Association between executive/attentional functions and caries in children with cerebral palsy. Res Dev Disabil. 2013; 34: 2493-9.

90. Subasi F, Mumcu G, Koksal L, Cimilli H, Bitlis D. Factors affecting oral health habits among children with cerebral palsy: pilot study. Pediatr Int. 2007; 49: 853-7.

91. Jones MW, Morgan E, Shelton JE, Thorogood C. Cerebral palsy: introduction and diagnosis (Part I). J Pediatr Health Care. 2007; 21(3):146-52.

92. Kosif R, Gunalp Z, Safi Oz Z. Evaluation of the dental-gingival health in children with cerebral palsy in Zonguldak. The New Journal of Medicine. 2013; 30(1):37-41.

93. Gunel MK, Mutlu A, Tarsuslu T, Livanelioglu A. Relationship among the Manual Ability Classification System (MACS), the Gross Motor Function Classification System (GMFCS), and the functional status (WeeFIM) in children with spastic cerebral palsy. Eur J Pediatr. 2009; 168: 477-85.

94. Cokpekin F, Koymen G, Basak F, Akbulut E, Altun C. Engelliler okuluna devam eden çocukların ağız diş sağlığı yönünden değerlendirilmesi. Gülhane Tıp Dergisi. 2003: 45(3):228-32.

95. Mani SA, Mote N, Kathariya M, Pawar KD. Adaptation and development of dental procedure in cerebral palsy. Pravara Med Rev. 2015; 7(4): 17-22.

96. Weisman EJ. Diagnosis and treatment of gingival and periodontal disorders in children with cerebral palsy. J Dent Child (Chic). 1956; 23: 73-80.

97. Santos MT, Ferreira MC, Guaré RO, Diniz MB, Rösing CK, Rodrigues JA, et al. Gingivitis and salivary osmolality in children with cerebral palsy. Int J Paediatr Dent. 2016; 26(6): 463-70.

98. Bozkurt FY, Fentoglu O, Yetkin Z. The Comparison of Various Oral Hygiene Strategies in Neuromuscularly Disabled Individuals. J Contemp Dent Pract. 2004; 4(5): 23-31.

99. Mitsea AG, Karidis AG, Donta-Bakoyianni C, Spyropoulos ND. Oral health status in Greek children and teenagers, with disabilities. J Clin Pediatr Dent. 2001; 26(1): 111-8.

100. Damle SG, Bhavsar JP. Plaque removing efficacy of individually modified toothbrushes in cerebral palsy children. ASDC J Dent Child. 1995; 62(4): 279-82.

101. Yitzhak M, Sarnat H, Rakocz M, Yaish Y, Ashkenazi M. The effect of toothbrush design on the ability of nurses to brush the teeth of institutionalized cerebral palsy patients. Spec Care Dentist. 2013; 33(1): 20-7.

102. Dogan MC, Alacam A, Asici N, Odabas M, Seydaoglu G. Clinical evaluation of the plaque-removing ability of three different toothbrushes in a mentally disabled group. Acta Odontol Scand. 2004; 62(6): 350-4.

103. Santos MT, Duarte Ferreira MC, de Oliveira Guaré R, Guimarães AS, Lira Ortega A. Teeth grinding, oral motor performance and maximal bite force in cerebral palsy children. Spec Care Dentist. 2015; 35(4): 170-4.

104. Zarowski M, Mojs E, Gajewska E, Steinborn B, Samborski W. Prevelance of sleep problems in children with cerebral palsy. Preliminary study. Ann. Acad. Med. Stetin. 2008; 54: 59-64.

105. Miamoto CB, Pereira LJ, Ramos-Jorge ML, Marques LS. Prevalence and predictive factors of sleep bruxism in children with and without cognitive impairment. Braz Oral Res. 2011; 25(5): 439-45.

106. Abanto J, Ortega AO, Raggio DP, Bönecker M, Mendes FM, Ciamponi AL. Impact of oral diseases and disorders on oral-health-related quality of life of children with cerebral palsy. Spec Care Dentist. 2014; 34(2): 56-63.

107. Fonseca CM, dos Santos MB, Consani RL, dos Santos JF, Marchini L. Incidence of sleep bruxism among children in Itanhandu, Brazil. Sleep Breath. 2011; 15(2): 215-20.

108. Souza VA, Abreu MH, Resende VL, Castilho LS. Factors associated with bruxism in children with developmental disabilities. Braz Oral Res. 2015; 29: 1-5.

109. Lang R, White PJ, Machalicek W, Rispoli M, Kang S, Aquilar J, et al. Treatment of bruxism in individuals with developmental disabilities: a systematic review. Res Dev Disabil. 2009; 30(5): 809-18.

110. Jan MM. Melatonin for the treatment of handicapped children with severe sleep disorders. Pediatr Neurol. 2000; 23: 229-32.

111. Yoshida M, Nakajima I, Uchida A, Yamaguchi T, Nonaka T, Yoshida H, et al. Characteristics of lower-jaw-position sensation with respect to oral-jaw functions in patients with cerebral palsy. Pediatric Dental Journal. 2004; 14(1): 23-8.

112. Miamoto CB, Ramos-Jorge ML, Pereira LJ, Paiva SM, Pordeus IA, Marques LS. Severity of malocclusion in patients with cerebral palsy: determinant factors. Am J Orthod Dentofacial Orthop. 2010; 138: 394-5.

113. Franklin DL, Luther F, Curzon ME. The prevalence of

malocclusion in children with cerebral palsy. Eur J Orthod. 1996; 18: 637-43.

114. Henrikson T, Ekberg EC, Nilner M. Masticatory efficiency and ability in relation to occlusion and mandibular dysfunction in girls. Int J Prosthodont. 1998; 11: 125-32.

115. Becker A, Chaushu S, Shapira J. Orthodontic treatment for the special needs child. Seminars in Orthodontics. 2004; 10: 281-92.

116. Practical oral care for people with cerebral palsy. U.S. Department of Health and Human Services, National institutes of Health, National institute of Dental and Craniofacial Research. 2009. Available at https://www.nidcr.nih.gov/sites/default/files/2017-09/practical-oral-care-cerebral-palsy.pdf

117. Barron RP, Carmichael RP, Marcon MA, Sandor GK. Dental erosion in gastroesophageal reflux disease. J Can Dent Assoc. 2003; 69: 84-9.

118. Jarvinen VK, Rytomaa II, Heinonen OP. Risk factors in dental erosion. J Dent Res. 1991; 70(6): 942-7.

119. Millward A, Shaw L, Smith AJ, Rippin JW, Harrington E. The distribution and severity of tooth wear and the relationship between erosion and dietary constituents in a group of children. Int J Paediatr Dent. 1994; 4(3): 151-7.

120. Shaw L, Weatherill S, Smith A. Tooth wear in children: an investigation of etiological factors in children with cerebral palsy and gastroesophageal reflux. ASDC J Dent Child. 1998; 65(6): 484-6, 439.

121. Erkin G, Kacar S, Ozel S. Serebral palsili hastalarda gastrointestinal sistem ve beslenme problemleri. Türkiye Fiziksel Tıp ve Rehabilitasyon Dergisi. 2005; 51(4): 150-5.

122. Su JM, Tsamtsouris A, Laskou M. Gastroesophageal reflux in children with cerebral palsy and its relationship to erosion of primary and permanent teeth. J Mass Dent Soc. 2003; 52: 20-4.

123. Goncalves GK, Carmagnani FG, Correa MS, Duarte DA, Santos MT. Dental erosion in cerebral palsy patients. J Dent Child. 2008; 75:117-20.

124. Lussi A. Dental Erosion from Diagnosis to Therapy. 2nd ed. Vol 20. Switzerland, Karger; 2006, 36-41.

125. Polat Z, Akgun O, Turan I, Polat GG, Altun C. Evaluation of the relationship between dental erosion and scintigraphically detected gastroesophageal reflux in patients with cerebral palsy. Turk J Med Sci. 2013; 43:283-8.

126. Dahshan A, Patel H, Delaney J, Wuerth A, Thomas R, Tolia V. Gastroesophageal reflux disease and dental erosion in children. J Pediatr. 2002; 140(4): 474-8.

127. Yasui EM, Kimura RK, Kawamura A, Akiyama S, Morisaki I. A modified oral screen appliance to prevent self-inflicted oral trauma in an infant with cerebral palsy: a case report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004; 97(4): 471-5.

128. Holan G, Peretz B, Efrat J, Shapira Y. Traumatic injuries to the teeth in young individuals with cerebral palsy. Dent Traumatol. 2005; 21(2): 65-9.

129. Batista C, Ramos-Jorge ML, Coelho M, De Oliveira M, Gonçalves R, Silva L. Dental trauma in individuals with severe cerebral palsy: prevalence and associated factors. Braz Oral Res. 2011; 25:319-23.

130. Jaffe M, Attias D, Dar H, Eli I, Judes H. Prevalence of gestational and perinatal insults in brain-damaged children. Isr J Med Sci. 1985; 21(12): 940-4.

131. Dodrill P, Gosa MM. Pediatric dysphagia: physiology, assessment, and management. Ann Nutr Metab. 2015; 66(5): 24-31.

132. **132.** Lloyd Faulconbridge RV, Tranter RM, Moffat V, Green E. Review of management of drooling problems in neurologically impaired children: a review of methods and results over 6 years at Chailey Heritage clinical services. Clin Otolaryngol Allied Sci. 2001; 26: 76-81.

133. Lefton-Greif MA. Pediatric dysphagia. Phys Med Rehabil Clin N Am. 2008; 19: 837-51.

134. Sullivan PB, Lambert B, Rose M, Ford-Adams M, Johnson A, Griffiths P. Prevalence and severity of feeding and nutritional problems in children with neurological impairment: oxford feeding study. Dev Med Child Neurol. 2000; 42: 674-80.

135. Alsaggaf AH, Jan MM, Saadah OI, Alsaggaf HM. Percutaneous endoscopic gastrostomy (PEG) tube placement in children with neurodevelopmental disabilities: parents' perspectives. Saudi Med J. 2013; 34: 695-700.

136. Yılmaz S, Basar P, Gisel EG. Assessment of feeding performance in patients with cerebral palsy. Int Journal Rehabil Res. 2004; 27(4): 325-9.

137. Mathisen B, Skuse D, Wolke D, Reilly S. Oral-Motor Dysfunction and Failure to Thrive among Inner-City Infants. Developmental Medicine and Child Neurology. 1989; 31(3): 293-302.

138. Morasch HS, Bartolome G. Swallowing disorders: Pathophysiology and rehabilitation of neurogenic dysphagia. NeuroRehabilitation. 1998; 10: 169-89.

139. Logemann JA. Evaluation and treatment of swallowing disorders (2nd);1998.

140. Park JW, Oh JC, Lee HJ, Park SJ, Yoon TS, Kwon BS. Effortful swallowing training coupled with electrical stimulation leads to an increase in hyoid elevation during swallowing. Dysphagia. 2009; 24(3): 296-301.

141. Haberfellner H, Schwartz S, Gisel EG. Feeding skills and growth after one year of intraoral appliance therapy in moderately dysphagic children with cerebral palsy. Dysphagia. 2001; 16(2): 83-96.

142. Cumella S, Ransford N, Lyons J, Burnham H. Needs for oral care among people with intellectual disability not in contact with Community Dental Services. J Intellect Disabil Res. 2000; 44 (1): 45-52.

143. Pope JE, Curzon ME. The dental status of cerebral palsied children. Pediatr Dent. 1991; 13: 156-62.

144. Suri L, Gagari E, Vastardis H. Delayed tooth eruption: pathogenesis, diagnosis, and treatment. A literature review. Am J Orthod Dentofacial Orthop. 2004; 126(4): 432-45.

145. Miamoto CB, Pereira LJ, Paiva SM, Pordeus IA, Ramos-Jorge ML, Marques LS. Prevalence and risk indicators of temporomandibular disorder signs and symptoms in a pediatric population with spastic cerebral palsy. J Clin Pediatr Dent. 2011;

35: 259-63.

146. Jan MM. Neurological examination of difficult and poorly cooperative children. J Child Neurol. 2007; 22: 1209-13.

147. Wongprasartsuk P, Stevens J. Cerebral palsy and anaesthesia. Paediatr Anaesth. 2002; 12: 296-303.

148. Komerik N, Kırzıoglu Z, Efeoglu CG. Zihinsel Engele Sahip Bireylerde Ağız Sağlığı. J Dent Atatürk Uni. 2012; 22(1): 96-104.

149. Tesini DA, Fenton SJ. Oral health needs of persons with physical or mental disabilities. Dent Clin North Am. 1994; 38(3): 483-98.

150. Ferguson FS, Cinotti D. Home oral health practice: the foundation for desensitization and dental care for special needs. Dent Clin North Am. 2009; 53: 375-87.

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Case Report

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Perioperative anesthesia management in a patient with Eagle's syndrome

Özgenur BACAKSIZ *, Elif Bengi ŞENER

Department of Anesthesia and Reanimation, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

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Abstract

Eagle syndrome, a prolonged styloid syndrome, is a clinical disorder caused by an elongated, enlarged, and angulated styloid process. Most of the cases are asymptomatic. Symptomatic cases are presented as continuous, intermittent pain in the face and anterolateral neck region, depending on position; reflected pain may be seen in the ipsilateral ear and temporomandibular joint. Patients with a bilateral prolonged styloid process may present unilateral complaints. Symptoms can manifest themselves with foreign body sensations in the throat, episodes of dysphagia, and syncope. Syncope episodes are one of the most serious clinical manifestations of the syndrome and are called 'Carotid-Styloid Syndrome'. Patient anamnesis and physical examination are the most important steps for diagnosis. Palpation of the styloid process in the tonsillar fossa is a clue for Eagle syndrome. Imaginary examinations should confirm the diagnosis. The local anesthetic injection technique into the tonsillar fossa can be used for differential diagnosis. The reduction of post-injection pain is a finding favoring Eagle's syndrome. Furthermore, surgical and medical methods can treat Eagle's syndrome. In this article, the perioperative anesthesia management of a 38-year-old woman with Eagle syndrome planning to undergo axillary lymph node biopsy in Ondokuz Mayıs University Hospital is presented as a case report.

Keywords: Eagle syndrome, elongated styloid process, pain syndromes, stylohyoid syndrome, neurological monitorization

1. Introduction

The Styloid process (SP) is part of the temporal bone, located anterolaterally, and forms the lower part of the bone. It is lateral to the tonsillar fossa between the internal and external carotid arteries (1). SP lies adjacent to the styloglossus, stylopharyngeus, and stylohyoid muscles. Embryologically, the structures found in this region originate from Reichert's cartilage (second branchial arch). The Styloid process is located in the maxilla-vertebra-pharyngeal space where the carotid artery, internal jugular vein, facial nerve, glossopharyngeal nerve, vagus nerve, and hypoglossal nerve pass. The tip of the styloid process is connected to the lesser horn of the hyoid bone via the stylohyoid ligament. The clinical disorder resulting from the elongation of the styloid process, calcification or ossification of the ligaments it is associated with (stylohyoid, stylomandibular ligament), is called Eagle's syndrome (2).

W. W. Eagle described the clinic, radiological diagnosis, and treatment of Eagle's syndrome in 1937 (3). Eagle defined the normal length of the styloid process as 25 mm (millimeters) and named protrusions longer than 25 mm as long styloid process. Despite that W. W. Eagle was the first to describe the syndrome, Italian surgeon Pietro Marchetti detected styloid process elongation and ligament calcification in 1652 but did not mention the clinical reflections of this pathology (4).

Surgical trauma, blunt trauma, chronic inflammation of the ligaments (especially the stylomandibular ligament), styloid process osteitis, tendinitis, and mucositis may be responsible for the syndrome's pathogenesis (5). However, the etiological factor may also be calcification due to aging (6). In endocrine diseases or chronic renal failure, hypercalcemia may also cause ligament calcification. Apart from these etiological reasons, prosthesis elongation can also be congenitally observed with the malformation of Reichert's cartilage in the fetal period (7).

Most people with an elongated styloid process are not diagnosed with Eagle's syndrome if they are asymptomatic. Even if they are not symptomatic, the perioperative anesthesia approach should be known, and necessary precautions should be taken in cases with an elonged SP.

2. Case Report

A 38-year-old, 50 kg (kilogram) female patient was referred to us for axillary lymph node biopsy to prepare for preoperative anesthesia. In her anamnesis, the patient who had Eagle's syndrome, asthma without attacks and untreated follow-up, and history of hypotension attacks several times a month did not describe syncope. She had undergone

*Correspondence: ozgenur15@hotmail.com

tonsillectomy and bilateral styloidectomy with intraoral approach twice in chronological order. Enquiring the visible symptoms due to Eagle's syndrome, the patient did not describe pain when the head was in the supine and neutral position; there was a pain in the neck and tongue root in the deviations of the head to the left and right, and the pain reflected in the bilateral ears. The patient did not describe syncope attacks, vertigo, or vision-related symptoms. After the preoperative evaluation, we planned the operation day and took the patient to the operating room. Following the ASA (American Society of Anesthesiologists) standard monitoring recommendations, we performed electrocardiography, pulse oximetry, and noninvasive blood pressure monitoring. We enquired the patient again about the positions of her head she did not feel pain in and repeated her neurological examination. We fixed the patient's head in a neutral supine position and took measures to prevent deviance from right to left. We placed cerebral oximetry sensors appropriately and took the basal value to monitor cerebral blood flow closely. We recorded cerebral oximetry values for the left and right hemispheres intraoperatively every ten minutes. After recording the cerebral oximetry basal values, the patient was preoxygenated. We administered 1mg midazolam intravenously (i.v.), and started 0.2 µg/kg/min remifentanil infusion. We administered 40 mg i.v. lidocaine, 2mg/kg i.v. for induction propofol, and 1mg/kg i.v. rocuronium and performed mask ventilation for one minute so that the position of the head was not distorted. We successfully performed fiberoptic intubation, avoiding anteflexion and extension of the head. We provided intraoperative anesthesia maintenance with a MAC (minimum alveolar concentration) value of one, sevoflurane, oxygen-air mixture, and 0.2-0.5µg/kg/min remifentanil infusion. The operation lasted for 75 minutes. We observed no intraoperative hemodynamic instability and recorded no pathological value in the cerebral oximetry follow-up. The patient's muscle relaxation was reversed by 2mg/kg sugammadex and taken to the recovery unit for close postoperative follow-up at the end of the operation. The patient had no problems in the postoperative follow-up, and we transferred her to the recovery room with the head in a neutral position, hemodynamically stable, conscious, with normal neurological examination, and a modified Aldrete score of nine.

3. Discussion

According to the Genetic and Rare Diseases Information Center (GARD) data, 4% of the entire population has elongated SP. 4-10.3% of cases with an elongated styloid process are symptomatic. The incidence of Eagle syndrome is estimated to be 0.16%. Yavuz et al.'s (8) study in 2008 evinced that the length of the styloid process in Turks varies between 3.5 and 8 cm, with an average of 5 cm on the left SP and 5.2 cm on the right SP.

Clinical complaints are usually seen in patients aged 50 years and older and detected three times more in women than men (7). Studies have shown that the syndrome is more symptomatic in women than men (9) and more common in older women. This suggests that it may be associated with menopause (10). The syndrome generally progresses asymptomatically. In addition to the clinical course of an asymptomatic character, only unilateral complaints can be observed in patients with bilaterally prolonged SP. Clinically, earache, foreign body sensation in the throat, a sharp and blunt pain aggravated by swallowing, and neck rotation may be seen on the side with an elongated SP. If the elongated process presses on the carotid, syncope episodes are encountered. There are two types of the syndrome: the classical type (stylohyoid syndrome) is usually seen after tonsillectomy or pharyngeal surgery, and symptoms occur when fibrotic tissues compress the cranial nerve (5, 7, 9, and 10th cranial nerve). Pain-related complaints are in the foreground in the classical type (11). The second form, called carotid syndrome (Stylocarotid syndrome), occurs due to the end part of elongated SP or pathological ligaments compressing the sympathetic fibers adjacent to the carotid artery. It progresses with syncope attacks, and radiating pain can be present in the carotid zone (11). Symptoms are related to SP's length, width, angulation, the side of deviation, and the ligaments' ossification level (12). There is no relationship between patient age and symptoms (9). The technique of pain formation or exacerbation of pain by palpating the styloid process in the tonsillar fossa can be used in the physical examination. The styloid process, whose length is within normal limits, is not palpable in the tonsillar fossa (13). After the physical examination of patients considered to have Eagle's syndrome, an otolaryngologist should visualize the process with maxilla facial computed tomography or three-dimensional computed tomography. Radiologically, computed tomography is the most valuable method in diagnosis (14).

The primary treatment for Eagle's syndrome is the surgical shortening of the prolonged SP (styloidectomy). Analgesics, anti-inflammatory drugs, antidepressants, and corticosteroid-containing drugs can be used in medical treatment. Relieving pain by injecting local anesthetic agents into the tonsillar fossa is a method that can be used in diagnosis and treatment (15).

Eagle's syndrome is mostly asymptomatic, and the clinical status of symptomatic cases can be confused with many diseases. While creating the differential diagnosis list, Eagle's syndrome must be added to the list. Even if asymptomatic, if being taken to surgery for other reasons, there are points to be considered in patients with an elongated styloid process;

- The subtype of Eagle syndrome should be determined.
- Neurological examination and neurological monitoring should be performed (especially in cases with the carotid syndrome).

• Preoperatively, the position in which the patient's pain aggravates should be determined, and the intraoperative position should be planned according to this data.

• If the treatment of Eagle's syndrome is planned medically, antidepressant and steroid-containing drugs should be questioned, and preoperative drug regulation should be performed.

• If there is ligament calcification in the syndrome's pathogenesis, it should be kept in mind that chronic renal failure and endocrinological diseases may cause this condition.

Anesthesiologists should be aware of this syndrome and should take the necessary perioperative measures for the patient's medical well-being. Although it is a rare disorder, it requires finesse in anesthesia management.

Conflict of Interest

There is no conflict of interest between the authors.

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References

1. Kurmann PT, Van Linthoudt D. Eagle syndrom: a rare cause of lateral neck pain. Praxis. 2007; 96(8): 297-300.
2. Fini G, Gasparini G, Filippini F, Becelli R, Marcotullio D. The long styloid process syndrome or Eagle's syndrome. Journal of cranio-maxillofacial surgery. 2000; 28(2): 123-127.
3. Eagle WW. Symptomatic elongated styloid process report of two cases of styloid process–carotid artery syndrome with operation. Archives of otolaryngology. 1949; 49(5): 490-503.
4. Fusco DJ, Asteraki S, Spetzler RF. Eagle's syndrome: embryology, anatomy, and clinical management. Acta neurochirurgica. 2012; 154(7): 1119-1126.
5. Eagle WW. Elongated styloid processes: report of two cases. Arch Otolaryngol. 1937; 47:584-87.
6. Beder E, Ozgursoy OB, Ozgursoy SK, Anadolu Y. Three-dimensional computed tomography and surgical treatment for Eagle's syndrome. Ear, nose & throat journal. 2006; 85(7): 443-445.
7. Prasad KC, Kamath MP, Reddy KJM, Raju K, Agarwal S. Elongated styloid process (Eagle's syndrome): a clinical study. Journal of Oral and Maxillofacial Surgery. 2002; 60(2): 171-175.
8. Yavuz H, Caylakli F, Yildirim T, Ozluoglu LN. Angulation of the styloid process in Eagle's syndrome. European archives of oto-rhino-laryngology. 2008; 265(11): 1393-1396.
9. Piagkou M, Anagnostopoulou S, Kouladouros K, Piagkos G. Eagle's syndrome: a review of the literature. Clinical Anatomy: The Official Journal of the American Association of Clinical Anatomists and the British Association of Clinical Anatomists. 2009; 22(5): 545-558.
10. Rizzatti-Barbosa CM, Ribeiro MC, Silva-Concilio LR, Di Hipolito O, Ambrosano GM. Is an elongated stylohyoid process prevalent in the elderly? A radiographic study in a Brazilian population. Gerodontology. 2005; 22(2): 112-115.
11. Roy AK, Reader ST, Das SK, Mahmud SA, Readre NS. Case Study of Asymptomatic Eagle's Syndrome, International journal of Current Research. 2018;10(1): 64186-64189.
12. İlgüy M, İlgüy D, Güler N, Bayirli G. Incidence of the type and calcification patterns in patients with elongated styloid process. Journal of international medical research. 2005; 33(1); 96-102.
13. Rechtweg JS, Wax MK. Eagle's syndrome: a review. American journal of otolaryngology. 1998; 19(5): 316-321.
14. Eagle WW. Elongated styloid process: further observations and a new syndrome. Archives of otolaryngology. 1948; 47(5): 630-640.
15. Glogoff MR, Baum SM, Cheifetz I. Diagnosis and treatment of Eagle's syndrome. Journal of Oral Surgery. 1981; 39(12): 941-944.

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Placental chorioangioma with Dysgenesis of corpus callosum: A case report

Elif DAĞDEVİREN*, Nur DOKUZEYLÜL GÜNGÖR

Department of Obstetrics and Gynecology, Faculty of Medicine, Bahçeşehir University, İstanbul, Türkiye

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Abstract

Chorioangioma is the most common benign tumor of the placenta which results from vascular malformations. Their clinical significance changes according to their associated complications such as preeclampsia, premature placenta release, polyhydramnios, intrauterine growth restriction, fetal anemia, fetal cardiomegaly and fetomaternal hemorrhage. This case is about a 32-year-old patient whose pregnancy was complicated by a large placental chorioangioma in the third trimester.

Keywords: placental disease, chorioangioma, preeclampsia, vascular malformations

1. Introduction

Chorioangioma is the most common benign tumor of the placenta, which is non-trophoblastic in origin and believed to result from differentiation and proliferation defects of vascular structures (1). The incidence of placental chorioangioma in microscopically examined placentas is approximately 1% (2). These vascular malformations, so-called hamartomas, are of clinical importance when associated with various gestational complications such as preeclampsia, premature placenta release, polyhydramnios, intrauterine growth restriction, fetal anemia, fetomaternal hemorrhage and congestive heart failure of the fetus (3, 4). We report a pregnancy complicated by preeclampsia associated with a large placental chorioangioma, emphasizing the importance of closely monitoring both fetus and mother throughout pregnancy, both at antenatal and postnatal periods, to prevent potential complications.

2. Case Report

A 32-year-old primigravida consulted our obstetrics and gynecology service at ten weeks gestation. History revealed no significance for any disease. She did not smoke, and her blood group was A Rhesus+. She had a singleton pregnancy. The Combined test was below the cut-off value. She did not have any complaints in the previous half of the pregnancy. After referral for a detailed ultrasound in the second trimester, an ultrasound scan at 20+6 weeks gestation showed corpus callosum dysgenesis without additional major anomalies. We informed the patient about the potential neurological problems and accompanying genetic diseases. Fetal Doppler parameters did not demonstrate evidence of either anemia or cardiac dysfunction. An oral glucose tolerance test excluded gestational diabetes. Maternal serology for TORCH infections revealed no abnormality. We performed amniocentesis during the 21st gestational week to exclude any genetic or metabolic diseases. The test result did not demonstrate any quantitative or gross anomalies. Furthermore, we detected no clinically significant chromosomal copy number changes. Karyotype and array-CHG analysis results were normal.

At the 27th gestational week, Doppler US revealed a mass with significant vascularization, a flow rate of 33 cm/sec, 37x28mm, located near the caudal placental pole, away from the umbilical cord insertion area. We recommended weekly MCA follow-up.

We performed a fetal cranial MR imaging (Fig. 1a, 1b) at 28+6 weeks gestation to evaluate the fetal neuronal development, and the results confirmed ‘corpus callosum dysgeneses’.

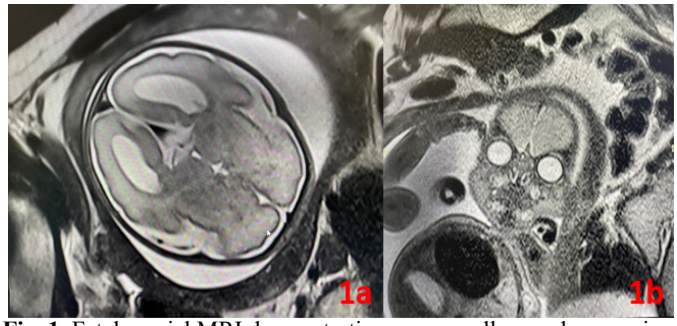

Fig. 1. Fetal cranial MRI demonstrating corpus callosum dysgenesis

Although the mass remained asymptomatic for a while, she complained of nausea, vomiting and pregnancy-induced hypertension accompanied by significant proteinuria towards the end of the pregnancy. At the 36th week of gestation, fetal heart rate was 140 bpm rhythmic and regular, with no evidence of cardiomegaly or fetal anemia. The vascular mass,

*Correspondence: elifdagdeviren1@gmail.com

considered placental chorioangioma, measured 55x49x51mm, with a 33cm/sec flow rate. MCA PSV:0.95 MoM. At the 37th week of gestation, MCA PSV:1.11MoM. Non-stress Tests were reactive. We recommended NST once in 3 days.

Caesarean delivery was performed at the 39th week without intervening complications. The newborn was male with an Apgar score of 8 for five minutes. During the postpartum period, both mother and neonatal were healthy. We obtained informed consent from the patient during the postpartum period to publish her data and images. They were discharged from the hospital on the third day.

3. Discussion

Placental chorioangiomas are vascular masses originating from placental tissue, resulting from development defects of vascular structures and associated with increased angiogenic growth factors as stated by Guschmann et al. (3) by immune-histochemical investigation of 136 cases. Placental chorioangioma is associated with severe pregnancy outcomes related to its mass size and the degree of hydrops fetalis (1). They could be associated with significant problems, including polyhydramnios, a tendency to preterm labour due to increased uterine distention, fetal anemia due to hemorrhage, fetal hydrops, fetal cardiomegaly, fetal growth restriction and fetal demise related to decompensated heart failure (2). For those complicated cases, pregnancy evaluation should be individualized to interfere in time and manage with appropriate modalities (5). In this case, we report a pregnancy complicated by a large placental chorioangioma in the third trimester. Histopathological examinations of the delivered placenta confirmed the diagnosis. (Fig. 2, 3)

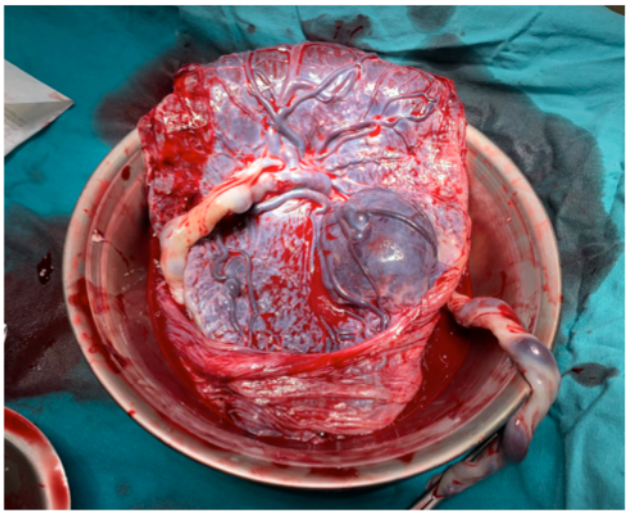

Fig. 2. Fetal surface of expelled placenta demonstrating placental chorioangioma

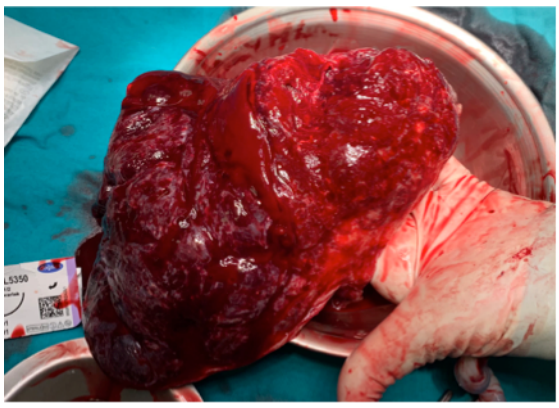

Fig. 3. Maternal surface of expelled placenta demonstrating placental chorioangioma

Placental chorioangioma can be demonstrated by various imaging techniques such as grey-scale ultrasonography, CDI, three-dimensional (3D) and four-dimensional (4D) ultrasound. Grey-scale ultrasound remains the primary diagnostic tool (2). We determined the placental mass by CDI, which is also beneficial in demonstrating the vascular nature of the mass. Besides the complications caused by placental chorioangioma, the fetal development defect causing corpus callosum dysgenesis was demonstrated by a detailed ultrasound examination in an earlier period, and genetic analysis was performed to evaluate the risks. The case demonstrates the critical role of close surveillance by imaging modalities such as Doppler ultrasound and Gray-scale ultrasound at regular intervals. Detailed ultrasound is also indispensable in evaluating fetal development and prepares us for associated risks. By the end of the second-trimester mother had signs of preeclampsia. It is shown that even preeclampsia symptoms could be a clue for us to suspect a background pathology. We managed the late pregnancy period with frequent follow-ups to protect maternal and fetal health.

In conclusion, as revealed in this case report and many other studies, placental chorioangioma is associated with vast clinical significance ranging from pregnancy-induced hypertension to fetal demise. It is crucial to remember that there could be an underlying pathology such as placental chorioangioma in patients suffering from irrelevant symptoms. Approaching suspiciously is substantial in diagnosis. Close surveillance of both fetal and maternal status with serial imaging modalities should be performed to recognize the possible complications earlier and interfere on time. Even though it is conducted with expectancy management in the reported case, close monitoring is essential in management.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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References

1. Buca D, Iacovella C, Khalil A, Rizzo G, Sirotkina M, Makatsariya A, Liberati M, Silvi C, Acharya G, D'Antonio F. Perinatal outcome of pregnancies complicated by placental chorioangioma: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2020 Apr;55(4):441-449. doi: 10.1002/uog.20304. PMID: 31034661.

2. Fan M, Skupski DW. Placental chorioangioma: literature review. J Perinat Med. 2014 May;42(3):273-9. doi: 10.1515/jpm-2013-0170. PMID: 24334427.

3. Guschmann M, Henrich W, Dudenhausen JW. Chorioangiomas--new insights into a well-known problem. II. An immunohistochemical investigation of 136 cases. J Perinat Med. 2003;31(2):170-5. doi: 10.1515/JPM.2003.023. PMID: 12747234.

4. Haak MC, Oosterhof H, Mouw RJ, Oepkes D, Vandenbussche FP. Pathophysiology and treatment of fetal anemia due to placental chorioangioma. Ultrasound Obstet Gynecol. 1999 Jul;14(1):68-70. doi: 10.1046/j.1469-0705.1999.14010068.x. PMID: 10461342.

5. Al Wattar BH, Hillman SC, Marton T, Foster K, Kilby MD. Placenta chorioangioma: a rare case and systematic review of literature. J Matern Fetal Neonatal Med. 2014 Jul;27(10):1055-63. doi: 10.3109/14767058.2013.847424. Epub 2013 Oct 17. PMID: 24460422.

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Miscarriage in a patient with Glanzmann Thrombasthenia and low ovarian reserve: A case report

Isılsu Ezgi ULUISIK [1,*], Muhammed Mert SONKAYA [1], Nur DOKUZEYLUL GUNGOR [2]

[1]Intern, School of Medicine, Bahçeşehir University, İstanbul, Türkiye
[2]Department of Gynecology and Obstetrics, Bahcesehir University Medical Park Göztepe Hospital, Istanbul, Türkiye

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Abstract

Glanzmann thrombasthenia (GT), which has an autosomal inheritance pattern, is a hemorrhagic disorder mostly complicated by mucocutaneous bleeding. The severity of this bleeding disorder varies from mild bruising to frequent severe bleeding. Prepartum, peripartum and postpartum bleeding risks are increased in these patients. In addition to the hemorrhagic complications that endanger maternal and fetal well-being, the risk of miscarriage is increased due to maternal antibodies to the platelets found on conceptus-derived trophoblast (placental) cells. In this case report, we report a patient who had a miscarriage in 7+1 week. Our patient had GT, low ovarian reserve, HPV positivity, bilateral dermoid cyst and was complicated by infertility.

Keywords: in vitro fertilization, assisted reproductive technics, glanzmann thrombasthenia, miscarriage

1. Introduction

Glanzmann thrombasthenia (GT) is a rare autosomal recessive inherited bleeding disorder, first described in 1918, characterized by qualitative and quantitative disorder of αIIbβ3 integrin, a platelet membrane transmembrane glycoprotein (1, 2). Integrin αIIbβ3 has a vital role in platelet functions, thrombus formation and hemostasis in case of injury (3). The encoding gene (*ITGA2B* and *ITGB3)* of αIIbβ3 is located on chromosome 17 with 38 known mutations in glycoprotein IIb and 25 mutations in glycoprotein IIIa (4).

As αIIbβ3 integrin mediates primary hemostasis, the bleeding sites vary in patients with GT: epistaxis (60-80%), gum bleeding (20-60%) and menorrhagia (60-90%) (5). Pregnancy in women with GT has serious risks both for maternal and fetal bleeding. Antepartum, peripartum and postpartum bleeding are pregnancy complications in GT patients. An immune response can be provoked by the passage of B3 integrin expressing fetal cells into the maternal circulation. In response to this passage, maternal antibodies can traversethe placenta and cause neonatal alloimmune thrombocytopenia (NAIT) (6). In a mouse model, antibodies against B3 integrin can promote natural killer cell activation, leading to trophoblast apoptosis in the uterus (7). It is hypothesized that maternal anti-B3 integrin IgG may cause the formation of immune complexes on trophoblast cells, and maternal immune response to fetal antigens can provoke miscarriage (7). In addition, it is reported that maternal anti-B3 integrin IgG antibodies promote intracranial hemorrhage (7).

Here, we report on the case of a 38-year-old patient with GT, complicated by infertility, bilateral dermoid cyst and HPV1 positivity, who had a pregnancy by in-vitro-fertilization resulting in miscarriage in 7+1 week.

2. Case report

A 38-year-old Turkish primigravida (in-vitro-fertilization) with GT presented to our infertility clinic for further evaluation and management. She was diagnosed with GT in her childhood with symptoms of frequent nose and gum bleedings. The hematology department regularly reviews the patient. We observed a bilateral dermoid cyst during her infertility evaluation. We conducted a colposcopy, and its result came negative. When she applied at our infertility clinics, her AMH result was 0.76. On her first visit, we observed two follicles in her right ovary. Her partner's sperm parameters were in the range of expected values with a concentration of 103.10^6 and progressive motility of 46%. We provided reproductive genetic counseling to her and her partner. We made a chromosome analysis to exclude any chromosomal associated infertility etiology and detected no chromosomal (46 XX for female and 46 XY for male). Due to the high prolactin levels (table 1), we gave cabergoline treatment before the IVF (in-vitro fertilization) procedure. We did the hematologic workup on her first admission to our infertility clinic (Table 1). These items demonstrated low hemoglobin (10.9 g/dL), hematocrit (34.6 %), MCV (78.6 fl), MCH (24.8 pg), MCHC (31.5 g/dL), PLT (105 K/uL), PCT (0.14 %) and AMH/MIS (0.76 ng/mL) levels (Table 1).

*Correspondence: isilsuezgiuluisik@gmail.com

Table 1. Hematologic workup of the patient before the IVF treatment

Parameter	Results	Unit	Reference Range
WBC	4.37	K/ul	4,.23-10.2
RBC	4.4	M/uL	4.04-5.48
HGB	10.9	g/dL	12.2-16.2
HCT	34.6	%	37.7-47.9
MCV	78.6	fl	80-97
MCH	24.8	pg	27-31.2
MCHC	31.5	g/dL	31.8-35.4
PLT	105	K/uL	142-424
RDW	17.2	%	10-20
MON%	7.4	%	4.7-12.5
MON#	0.32	K/uL	0.24-0.86
BASO%	0.4	%	0.1-1.2
EOS%	2.5	%	0.7-5.8
BASO	0.02	K/uL	0.01-0.08
EOS#	0.11	K/uL	0.04-0.36
NEUT#	2.02	K/uL	1.56-6.13
NEUT%	46.2	%	34-71.1
PCT	0.14	%	0.15-0.7
MPV	13.4	fl	9.1-11.9
PDW	16.8	fl	9-19
LYM%	43.5	%	19.3-51.7
LYM#	1.9	K/uL	1.18-3.57
Prolactin (PRL)	64.42	ng/mL	4.79-23.3
Anti-Mullerian Hormone (AMH/MIS)	0.76	ng/mL	0.14-7.

Tubal factor infertility was the reason for IVF in this patient. We did a laparoscopic tubal ligation procedure due to bilateral hydrosalpinx before IVF and relaparoscopy was made on the patient with intra-abdominal bleeding after laparoscopy. Postsurgery, two units of blood were transfused, and she was followed up for one night in the intensive care unit. The patient received NovaSeven 2 x 4770 mcg (IV) in the intensive care unit. In the first IVF trial, two oocytes were aspirated with a guide of standard ultrasound equipment and a 19-gauge single lumen needle. After that, we froze two embryos on the third day. There was no vaginal bleeding and intraovarian hemorrhage after the oocyte aspiration procedure. We then transferred embryos in the thaw cycle, but pregnancy did not occur. Afterwards, we made a second attempt. We transferred two embryos on the third day of the fresh cycle, and she conceived in her second IVF attempt. We applied the antagonist cycle was applied in both treatments. We performed ovulation induction with 225 rFSH.

The pregnancy was uneventful until six weeks. We documented anembryonic pregnancy in her 7+1 week control. After one week, she went dilatation and curettage procedure to terminate this anembryonic pregnancy.

3. Discussion

GT is one of the most commonly studied autosomal recessive inherited bleeding disorders caused by quantitative or qualitative deficiencies of the αIIbβ3 integrin coded by the ITGA2B and ITGB3 genes located at 17q21–23 (8). Failure of platelet aggregation leads to hemorrhagic complications, which vary from minimal bruising to major bleeding (4).

Pregnancy loss in a patient with Glanzmann thrombocytopenia with low ovarian reserve (AMH: 0.76 ng/mL) who became pregnant with IVF was reported for the first time in our study. Yougbaré et al. reported that maternal anti-B3 integrin IgG antibodies generated during pregnancy traverse the placenta and target paternally inherited antigens on platelets leading to NAIT(7). Furthermore, Yougbaré's study group adds that this B3 integrin antigen is also found in conceptus-derived trophoblast (placental) cells (7). These findings can be the reason for the immune-mediated pregnancy loss of our patient.

Moreover, the ovarian reserve of our patient was low, and her age was advanced (38 years, AMH: 0.76 ng/mL). These factors also can contribute to miscarriage. A meta-analysis focusing on pregnancies with assisted reproductive technology showed that the miscarriage rate is higher in women with low AMH compared to women with a medium or high serum AMH concentration (12,042 women, random-effects model, odds ratio (OR) 1.35; 95% CI, 1.10–1.66; $P = 0.004$; $I2$=50%) (9). Schumacher et al. reported that as AMH levels increase, the risk of pregnancy loss is diminished (10). They also mentioned that women with low AMH levels (AMH ≤ 0.4 ng/mL) are twice as likely to have a miscarriage than women with high AMH levels (AMH ≥ 1 ng/mL) (hazard ratio, 2.3; 95% CI, 1.3, 4.3) (10).

In conclusion, the immune-mediated mechanism due to GT, low ovarian reserve and advanced age of the mother can be the main reasons for miscarriage in this case. An obstetrician, hematologist and anesthetist should follow the pregnancy period of a patient with GT. Patients with GT have multiple risks for both mother and fetal well-being. Preconceptional counseling should be given to the parents to prevent maternal and fetal complications.

Conflict of interest

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References

1. Nurden AT. Glanzmann thrombasthenia. Orphanet J Rare Dis. 2006 ;1(1):10.
2. Poon MC, Di Minno G, d'Oiron R, Zotz R. New Insights Into the Treatment of Glanzmann Thrombasthenia. Transfusion Medicine Reviews. 2016 ;30(2):92–9.
3. Clemetson KJ. Platelets and Primary Haemostasis. Thrombosis Research. 2012 ;129(3):220–4.
4. Wijemanne A, Watt-Coote I, Austin S. Glanzmann

thrombasthenia in pregnancy: Optimising maternal and fetal outcomes. Obstet Med. 2016 ;9(4):169–70.

5. Botero JP, Lee K, Branchford BR, Bray PF, Freson K, Lambert MP, et al. Glanzmann thrombasthenia: genetic basis and clinical correlates. Haematologica. 2020 ;105(4):888–94.

6. Nurden AT. Acquired Antibodies to α IIbβ3 in Glanzmann Thrombasthenia: From Transfusion and Pregnancy to Bone Marrow Transplants and Beyond. Transfusion Medicine Reviews. 2018 ;32(3):155–64.

7. Yougbaré I, Tai WS, Zdravic D, Oswald BE, Lang S, Zhu G, et al. Activated NK cells cause placental dysfunction and miscarriages in fetal alloimmune thrombocytopenia. Nat Commun. 2017 ;8(1):224.

8. Nurden A, Pillois X, Fiore M, Heilig R, Nurden P. Glanzmann Thrombasthenia-Like Syndromes Associated with Macrothrombocytopenias and Mutations in the Genes Encoding the αIIbβ3 Integrin. Semin Thromb Hemost. 2011 ;37(06):698–706.

9. Busnelli A, Somigliana E, Cirillo F, Levi-Setti PE. Is diminished ovarian reserve a risk factor for miscarriage? Results of a systematic review and meta-analysis. Human Reproduction Update. 2021 ;27(6):973–88.

10. Lyttle Schumacher BM, Jukic AMZ, Steiner AZ. Antimüllerian hormone as a risk factor for miscarriage in naturally conceived pregnancies. Fertility and Sterility. 2018 ;109(6):1065-1071.e1.

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A case of secondary syphilis presenting with optic neuropathy

Fadime KARAMAN ATASEVER*, İnci GÜNGÖR

Department of Ophthalmology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

Syphilis is a chronic bacterial infection caused by Treponema pallidum. Syphilis is a significant individual and public health problem given the increased risk of HIV infection and lifelong morbidities in children born to infected mothers. Syphilis can be diagnosed at any stage and can affect multiple or a single organ, mimicking many diseases. It is known as the 'great imitator' as it manifests itself in various forms, often causing misdiagnosis with other conditions. Ocular findings can be seen in all stages of syphilis. In this case report, we have reported a patient with ocular syphilis diagnosed with the secondary syphilis stage. The patient has provided informed consent for publication of the case.

Keywords: syphilis, optic neuropathy, ocular, neurosyphilis

1. Introduction

The main clinical sign of primary syphilis is the presence of a painless, usually solitary, indurated, clear-based ulcerative lesion (chancre) that typically occurs approximately 2-3 weeks after direct contact with another person's infectious lesion (1). In secondary syphilis, more than 80% of patients have mucocutaneous lesions that may affect any body surface. The latent stage is characterized by positive serological tests in settings of the absence of clinical signs or symptoms and normal CSF findings (2). Tertiary syphilis can occur at any time after secondary syphilis, even years after latent syphilis (3). Transplacental spread of spirochete leads to congenital syphilis. Syphilis in pregnancy, if left untreated, has a teratogenic effect on the fetus (4).

2. Case Report

A 56-year-old male patient presented with the complaint of blurred vision in the left eye that had persisted for a week. He had a diagnosis of hypertension and a history of smoking. In the ocular examination, the best corrected visual acuity was 1.0 in the right eye and 0.7 in the left eye. Intraocular pressures were measured as 14 mmHg in the right eye and 12 mmHg in the left eye. Anterior segment examination was normal in both eyes. In the fundus examination, the right eye was normal, and the optic disc margins on the left were blurred and raised (Fig. 1). No limitation in eye movements was observed in both eyes. In the visual field examination, it was observed that there were normal right side, mild blind spot enlargement on the left and peripheral nonspecific defects (Fig 2). On admission, macular optic coherence tomography was performed, and optic disc oedema was observed in the left eye; No macular oedema was observed in both eyes (Fig. 3). Fundus fluorescein angiography was performed, and optic disc staining was observed in the left eye (Fig. 1). In his neurological examination, no motor deficit was noted. However, deep tendon reflexes were bilaterally hyperactive, cerebellar tests and vibration sense were normal, and Babinski sign was negative.

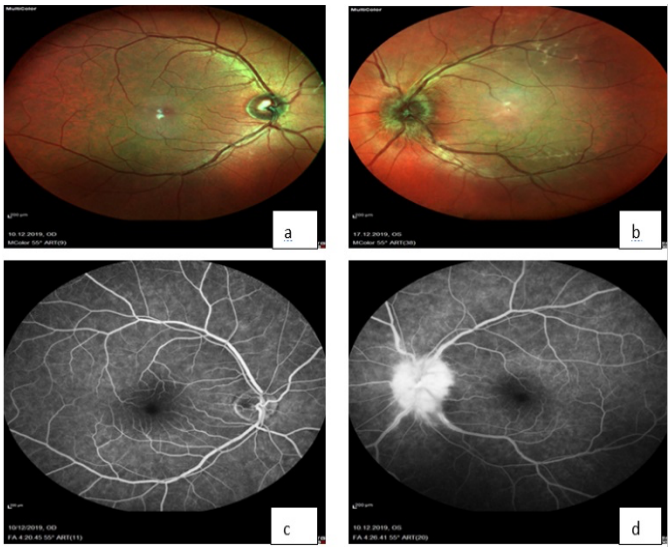

Fig. 1. Fundus photograph showing the right eye was normal (1a), in left eye blurred and elevated disc (1b); Fluorescein angiography of right and left eye: the right eye was normal (1c), optic disc staining was observed in the left eye (1d)

*Correspondence: fadimekaraman5@gmail.com

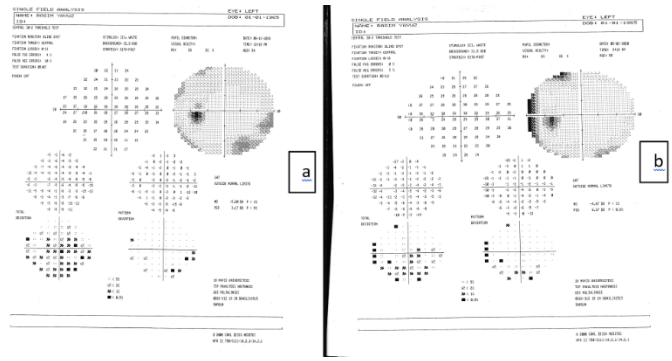

Fig. 2. Visual fields in both eyes, in the visual field examination, it was observed that there were normal right side (2a), mild blind spot enlargement on the left and peripheral nonspecific defects (2b)

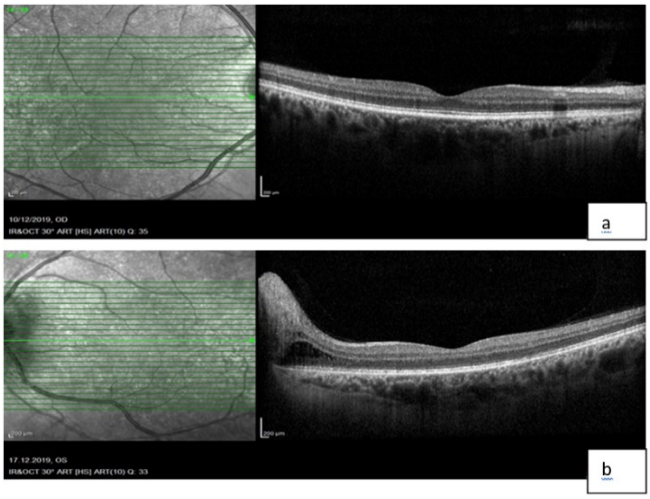

Fig. 3. Macular optic coherence tomography was performed and optic disc edema was observed in the left eye (3b); no macular edema was observed in both eyes (3a and 3b)

In the examination performed by the infectious diseases department, syphilis-specific lesions were observed on the palms of the hands, soles of the feet and inside the mouth. The patient reported that these rashes appeared on his hands and feet two weeks before the admission.

Blood biochemistry and hemogram were normal. Venereal Disease Research Laboratory (VDRL) positive and Treponema pallidum hemagglutination (TPHA) positive in serum. Tests for other infective agents were found negative.

Carotid artery Doppler examination and echocardiography were reported as normal. Cerebral magnetic resonance imaging (MRI) was normal. In orbital magnetic resonance imaging, there was a slight thickening at the optic disc level on the left, and a slight increase in contrast enhancement was observed at this level after contrast. It was reported as significant in terms of optic disc neuropathy/papillopathy.

Lumbar puncture was performed and cerebrospinal fluid (CSF) analysis showed glucose 74 mg/dL, lactate dehydrogenase (LDH) 43 U/L, sodium 147 mmol/L, potassium 2.9 mEq/L, chlorine 125.3 mEq/L, protein 24 mg/Dl. In the CSF culture examination (in a sterile tube), no microorganism could be seen as a result of direct microscopy staining, and there was no growth in its culture.

In the light of current examination findings and tests, the patient was diagnosed with secondary syphilis with central nervous system involvement and was treated with penicillin G 4x4 million units for 14 days. No complications were observed during the treatment. On the sixth day of treatment, visual acuity improved in the left eye. On the tenth day of treatment, visual acuities were at 1.0 in both eyes, and optic disc borders began to become evident in the left eye.

The patient has provided informed consent for publication of the case in December 2019.

3. Discussion

Syphilis can affect all ocular structures; the most common findings are posterior uveitis and panuveitis. Ocular manifestations may be associated with neurosyphilis, and if left untreated, ocular syphilis can lead to blindness (4).

In a recently published meta-analysis study by Furtado JM et al., previous literature on retrospective ocular syphilis was assessed and showed that the majority of patients with ocular syphilis were male (ranging from 58% to 100% of the total cases). Only one study reported a third gender category (transgender). Only four articles reported more cases in women than in men. Most studies report being diagnosed with ocular syphilis in the fifth decade of their life. Seven studies reported cases over 80 years of age, and all described cases were diagnosed within the past two decades. Published articles were in consensus that ocular syphilis is more common in men than women in HIV-positive individuals (ranging from 85.7% to 100% of cases combined). Of the 52-case series for which gender-related information was available, only 4 reported more cases in women than in men (5). Chorioretinitis, necrotizing retinitis, retinal vasculitis, neuroretinitis, retinal vascular occlusions, and exudative retinal detachment are among the posterior segment findings in ocular syphilis. Although it is a rare cause of scleritis and episcleritis, syphilis should be considered in the differential diagnosis (5). In a retrospective study by Akpek et al., assessing 134 patients with scleral inflammation, only 7.5% of the subjects had an associated infectious disease, and no treponemal infection was reported (6). The optic disc involvement may be alone or as well as together with other posterior segment manifestations. In a study by Fonollosa et al., papillitis was found to be the most common finding of ocular syphilis, followed by vitritis (7). In a meta-analysis study by Zhank et al., papillitis emerged as the most common finding of syphilitic uveitis (8).

Latent and tertiary syphilis, including neurosyphilis, is treated with aqueous crystalline penicillin G, 3 to 4 million units intravenous every 4 hours for 10 to 14 days, or benzathine penicillin G, 2.4 million units intramuscularly weekly for 3 weeks. Patients receiving treatment should be monitored for Jarisch-Herxheimer reaction (4).

In conclusion, ocular syphilis can present with different clinical pictures. For this reason, syphilis should be considered when investigating the etiology in a case with optic disc involvement. Patients with syphilis with eye involvement should also be evaluated for possible central system involvement and concomitant HIV infection.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: K.A.F., G.İ., Design: K.A.F., G.İ., Data Collection or Processing: K.A.F., G.İ., Analysis or Interpretation: K.A.F., G.İ., Literature Search: K.A.F., G.İ., Writing: K.A.F., G.İ.

References

1. Hook EW 3rd. Syphilis. Lancet. 2017 Apr 15;389(10078):1550-1557. doi: 10.1016/S0140-6736(16)32411-4.
2. Beers MH. Syphilis. In: Keryn A.G.Lane, Porter RS, editörs. The Merck Manual of Dıagnosıs and Therapy, Merck & Company, Incorporated ;2006, p. 1407-1663.
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4. Koundanya VV, Tripathy K. Syphilis Ocular Manifestations. 2022 Feb 21. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 32644383.
5. Furtado JM, Simões M, Vasconcelos-Santos D, Oliver GF, Tyagi M, Nascimento H, et al. Ocular syphilis. Surv Ophthalmol. 2022 Mar-Apr;67(2):440-462. doi: 10.1016/j.survophthal.2021.06.003.
6. Akpek EK, Thorne JE, Qazi FA, Do DV, Jabs DA. Evaluation of patients with scleritis for systemic disease. Ophthalmology. 2004 Mar;111(3):501-6. doi: 10.1016/j.ophtha.2003.06.006.
7. Fonollosa A, Martinez-Indart L, Artaraz J, Martinez-Berriotxoa A, Agirrebengoa K, Garcia M, et al. Clinical Manifestations and Outcomes of Syphilis-associated Uveitis in Northern Spain. Ocul Immunol Inflamm. 2016;24(2):147-52. doi: 10.3109/09273948.2014.943349.
8. Zhang T, Zhu Y, Xu G. Clinical Features and Treatments of Syphilitic Uveitis: A Systematic Review and Meta-Analysis. J Ophthalmol. 2017;2017:6594849. doi: 10.1155/2017/6594849

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Case Report

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Management of subcutaneous emphysema due to penetrating oropharyngeal trauma

Emre DEMİREL[1], Emel TAHİR[1,*], Hatice ALBAYRAK[2], Nazik YENER[2], Sinan ATMACA[1]

[1] Department of Otorhinolaryngology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey
[2] Division of Pediatric Critical Care, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

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Abstract

Penetrating trauma to the oropharynx is a rare problem in children. Although the majority of cases heal with no long-term consequences, it they can result in fatal complications. In this case report we discuss how a spoon-related oropharyngeal trauma case was treated. We discuss how a spoon-related oropharyngeal trauma case was treated in this case report. A 7-year-old boy fell facedown when eating ice cream, and the spoon became stuck in his throat. A vertical 3-4 cm laceration in the midline of the posterior wall of the pharynx was discovered. There was subcutaneous and retropharyngeal air values extending into the upper mediastinum in his computed tomography scan. His computed tomography scan showed subcutaneous and retropharyngeal air values extending into the upper mediastinum. The patient was intubated and monitored in the pediatric intensive care unit. To prevent infection problems, broad-spectrum antibiotics were started. He was successfully extubated as his subcutaneous emphysema regressed. In penetrating trauma of the oropharynx to avoid fatal complications, early administration of antibiotic therapy, and close monitoring of the in the intensive care unit is vital.

Keywords: oropharynx, pneumomediastinum, emphysema, trauma

1. Introduction

Foreign body cases are commonly present in otolaryngology practice, particularly in the pediatric age group. It is more common in boys, and a male/female ratio of 3:1 has been documented (1). Lethal complications such as subcutaneous emphysema, deep neck infection, pneumomediastinum, pneumopericardium, carotid artery thrombosis, and mediastinitis can occur due to these injuries (1, 2). While the soft palate and tonsils are more commonly affected in penetrating pharyngeal injuries, the hard palate, tongue, and posterior oropharynx are less frequently involved (3). This case presentation discusses a case of spoon-related posterior pharyngeal wall injury and its management.

2. Case Report

A previously healthy male patient, aged 7, fell off a chair while holding a metal spoon. The mother removed the spoon stuck in the child's mouth, which resulted in bleeding and coughing. The pediatricians and otolaryngologists evaluated the patient who presented to the emergency room. In the first examination, there was an ecchymotic look and laceration on the posterior pharyngeal wall and soft palate. Flexible fiberoptic endoscopic inspection revealed a vertical 3-4 cm lacerated area on the oropharynx's posterior wall with no evidence of respiratory distress. During transnasal fiberoptic endoscopic examination, salivary pooling in the pyriform sinuses was identified. CT scan of the neck demonstrated diffuse free air values from the retropharyngeal area to the anterior mediastinum.

In addition, although we observed air values in the left carotid sheath, no signs of vascular injury were observed in angiography. We continued the patient's oral intake and administered ceftriaxone and paracetamol. The patient was unable to swallow secretions, and his oxygen saturation declined to 85%. We planned an emergency tracheotomy in the event of upper airway edema. A previously undetected subcutaneous emphysema in the patient's submandibular region and anterior thoracic wall was found. We used a syringe to drain subcutaneous emphysema. In the presence of imminent respiratory failure, we performed elective endotracheal intubation using direct laryngoscopy without incident. The child was transported to the pediatric intensive care unit and placed on mechanical ventilation. On the first day of his hospitalization, he had a fever, and we added metronidazole to his regimen to prevent mediastinitis. Because of the persistent fever, we changed the treatment regimen to piperacillin-tazobactam, teicoplanin, and fluconazole. On the third day of hospitalization, we performed a neck ultrasound to examine for a possible deep neck infection and found heterogeneous fluid values around the thyroid gland. On the fifth day of his hospitalization, the patient was extubated and

*Correspondence: emeltahir@hotmail.com

transferred to a high-flow nasal cannula. We administered dexamethasone to alleviate airway edema. On the seventh day, we started oral feeding, and the patient no longer required oxygen. We examined the patient in service conditions the same day after a significant regression in pneumomediastinum. Observing a deep neck infection on the control tomography, we transferred the patient to the ward. He was discharged on the 17th day with no additional problems detected in the second week and second-month follow-ups following discharge.

We obtained informed consent from the family for this case presentation.

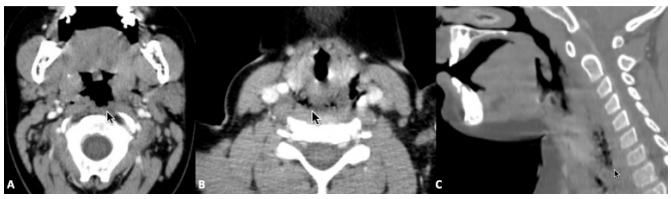

Fig. 1. The traumatic region at posterior oropharynx (A) and subcutaneous emphysema at axial (B) and sagittal (C) sections of neck CT.

3. Discussion

Penetrating pharyngeal injuries are most common in children who fall prone with a foreign body in their mouth. Toothbrushes, pencils, cylindrical toys, and straws are the most common foreign bodies that cause penetrating pharyngeal injuries (3). Although serious sequelae from penetrating pharyngeal injuries are uncommon, some occurrences might result in severe morbidity and mortality. Potentially serious complications include carotid artery injury, neurological sequelae from thrombosis, sepsis, shock, cervical emphysema, pneumothorax, and pneumomediastinum (4).

Because the posterior wall of the pharynx is susceptible, subcutaneous emphysema in the head and neck region can be noticed with a shift in pressure balance following a penetrating injury, especially in children. Air collection in the subcutaneous tissues can also be observed due to the colonization of the subcutaneous area by various gas-producing bacteria (5).

Following pharyngeal mucosal injury, air can enter the tissue between the pharyngeal constrictor muscles and then into the parapharyngeal and retropharyngeal spaces. It may extend superiorly to the submandibular and sublingual areas, advanced zygomatic arches, and retro auricular regions. Second, the air in the retropharyngeal area may move superiorly at the skull base from the prevertebral fascia and the visceral layer of the deep cervical fascia to the posterior mediastinum up to the level of the diaphragm posteroinferiorly. Third, it can spread from the deep cervical fascia's pretracheal layer to the hyoid bone, thorax, middle mediastinum, and pericardium (4).

Additionally, oropharyngeal flora infection within 24 hours, retropharyngeal abscess, and mediastinitis are among the potentially fatal consequences. After admission, we detected subcutaneous emphysema, which then generated pneumomediastinum.

In these situations, targeted radiological imaging should be conducted. A posteroanterior and lateral X-ray is recommended if pneumomediastinum is suspected, while recommending a tomography if mediastinitis and deep neck infection are suspected. When a severe vascular injury is suspected, a CT-angiography should be ordered. Although surgical intervention may be required based on the location of the damage, its extent, and the presence of bleeding, a conservative approach is sufficient for most patients (6). First and foremost, airway safety and proper oxygen support are required. Oxygen support was insufficient in our case at the 10th hour of the trauma; thus, we intubated the patient and administered mechanical ventilator support. During intubation, tracheotomy preparation should be made. In patients with a secure airway, oral intake should be halted, and prophylactic broad-spectrum antibiotics and analgesics should be given and monitored for at least 72 hours (4-6). When a patient has a high fever and elevated inflammatory markers, the antibiotic spectrum should be broadened, and the patient should be assessed for deep neck infection and mediastinitis. A high index of suspicion, early antibiotic administration, and close monitoring of the patient's vital signs in the intensive care unit are crucial for managing these potentially lethal disorders. The dangers of oropharyngeal trauma must be clearly mentioned to parents and caregivers.

Conflict of interest

The authors declare there are no conflicts of interest—financial or otherwise—related to the material presented herein.

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All procedures performed in the case report were in accordance with the ethical standards of the Helsinki Declaration. We obtained informed consent from the parents of the patients included in the study.

Authors' contributions

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References

1. Chauhan N, Guillemaud J, El-Hakim H. Two patterns of impalement injury to the oral cavity: Report of four cases and review of literature. Int J Pediatr Otorhinolarngol. 2006;70(8): 1479-1483.

2. Song N, Liu F, Liu Y. The chopstick: a rare and serious cause of penetrating oropharyngeal trauma in a 6-year-old. Br J Hosp Med. 2020; 2;81(7):1.

3. Ezerarslan H, Ataç G.K, Kaplan T, Beriat G.K. deep cervicofacial

emphysema and pneumomediastinum due to penetrant oral trauma. International Journal of Case Reports and Images. 2014;5(1):58-61.

4. Younessi OJ, Alcaino EA. Impalement injuries of the oral cavity in children: a case report and survey of the literature. Int J Paediatr Dent. 2007;17(1):66-71.

5. Nishino H, Kenmochi M, Kasugai S, Okada T, Ohashi T. Subcutaneous emphysema secondary to tonsillectomy: a case report. Auris Nasus Larynx. 2003;30(Suppl):135-136.

6. Lei W, Yu J, Huang Y, Zhao L, Sun J. Pediatric penetrating oropharyngeal trauma. Can J Anaesth. 2019;66(7):836-837.

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