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EDITORIAL

Dear esteemed readers of JHSM,

Here we are again with a new issue. The new issue of JHSM has some very interesting studies. The development of our journal is quite well. We believe it will continue with your support. We try to work with experienced editorial members. Our referees are also in the direction of increasing the quality of the article with their positive criticism. We were very pleased with the contribution of our colleagues, who were psychologically burn out during the COVID-19 pandemic. We should be beneficial to humanity by contributing to science under all challenges. JHSM concentrates on clinical, experimental and metabolic disorders, published quarterly with a peer review system that esteems recognition. Publication is in English towards endocrinology specialists and practitioner medical doctors. JHSM is indexed by the following groups. Throughout its years within the world of international academia our journal has become more and more recognized. Presently our journal has become recognized and sourced by; Directory of open Access Journals (DOAJ), ULAKBIM TR Index. JHSM has a very rigerous review system which starts with pre evaluation for ithenticate analysis. Publications receiving an approval will be sent to editors and staff for proof reading. Articles reciving a page format and a DOI number will be published on our web site as well. The journals excellent team with its rigorous workload manages to execute the total review process in less that two months. With this professional and occupational dedication our first priority is to be indexed under PubMed framework. Receiving the necessary national and international citation our focus will be indexed by SCI-E. To achieve the proposed vision we have to work in collaboration with you devoted contributors. Knowing and appreciating your work load, we ask for more efforts towards submitting more original articles.

Wish you a very healthy and pleasant Holiday Season. Please take care of yourselves and your patients.

Best regards

Assoc. Prof. Dr. Bekir UÇAN
Assoc. Editor

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HEALTH SCIENCES
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The relationship between sarcopenia and nesfatin-1 and ghrelin levels in patients with chronic obstructive pulmonary disease

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ABSTRACT

Aim: In this study, we aimed to investigate the relationship between the levels of adipocytokines anorexigenic nesfatin-1 and orexigenic ghrelin levels and body mass index (BMI), muscle mass, muscle strength and sarcopenia in patients with chronic obstructive pulmonary disease (COPD).

Material and Method: The patients were divided into two groups according to the severity of COPD. Body mass index (BMI), body fat and muscle measurements were measured with bioelectrical impedance device. A hand-grip strength test was performed with a hand dynamometer to determine muscle strength. We used the mMRC (Modified Medical Research Council Questionnaire) questionnaire to measure the intensity of dyspnea during daily activities. A 6-minute walk test (6MWT) was used to test functional capacity and physical exercise tolerance. COPD Assessment Test (CAT) was performed to determine the health status. Serum nesfatin-1 and ghrelin levels were measured in all patients.

Results: In a total of 90 patients (16 female and 74 male) were included in the study prospectively. There was not any significant difference regarding the Nesfatin-1, ghrelin and Nesfatin-1/ghrelin ratio between mild/moderate or severe COPD patients. Muscle strength was correlated positively with 6MWT, nesfatin-1 and ghrelin levels, but negatively correlated with CAT score and mMRC score. The number of patients in the advanced stage of COPD was higher in the sarcopenia group than in the non-sarcopenia group.

Conclusion: There was a positive correlation between muscle strength and nesfatin-1 and ghrelin levels. However, muscle strength was negatively correlated with mMRC and CAT scores. Approximately 19% of COPD patients had sarcopenia. Serum nesfatin-1 and ghrelin levels were not different between sarcopenic and non-sarcopenic COPD patients.

Keywords: Chronic obstructive pulmonary disease, ghrelin, nesfatin-1, sarcopenia

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by permanent limitation of airflow and exacerbations in respiratory distress, also causing systemic effects. In addition to the inflammation process in the lungs, there is a low-grade systemic inflammation in COPD resulting in co-morbidities (1). Although not elucidated clearly yet; smoking, lung hyperinflation, tissue hypoxia and skeletal muscle dysfunction have been suggested as possible factors in the pathogenesis of systemic inflammation in COPD. Lately, adipose tissue mediated inflammation is gaining increasing interest as an important mechanism in inducing systemic inflammation in COPD (2).

Involuntary weight loss is a common complication of COPD and has been found to be associated with negative outcomes regardless of its degree. It was found that approximately 50% of hospitalized patients with COPD were below 90% of their ideal body weight. Low body weight and muscle loss are associated with accelerated

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mortality and decline in clinical status, regardless of the severity of lung disease. Sarcopenia is defined as low muscle mass together with low muscle function. Initially, sarcopenia was used to describe age-related muscle mass and loss of strength. The European Working Group on Sarcopenia in the Elderly (EWGSOP) recommends the presence of both low muscle mass and low muscle function (strength or performance) for the diagnosis of sarcopenia (3). Chronic inflammatory diseases are known to induce sarcopenia (4,5). Recently, in presarcopenic and sarcopenic individuals a higher prevalence of COPD but not other chronic diseases was reported (6).

Adipokines are protein mediators secreted by adipose tissue and are involved not only in regulating energy metabolism, but also in inflammatory responses in many chronic diseases. Nesfatin-1 is a new adipokine that was discovered in 2006 and was first associated with appetite and body weight control in rats. Nesfatin-1 is expressed in human adipose tissue and has been shown to increase secretion of tumor necrosing factor- α (TNF-α), IL-6, insulin and dexamethasone (7). On the other hand, a study examining emphysema-type COPD patients suggested that NUCB2/nesfatin-1 may be a new factor associated with systemic inflammation in COPD and may emerge as a stable new inflammatory factor (8). Ghrelin, a 28-amino acid peptide secreted from the stomach, was isolated in 1999 as an endogenous ligand for the growth hormone secretor receptor. Plasma ghrelin levels are inversely proportional to body mass index (9).

In this study, we aimed to investigate the relationship between circulating nesfatin-1 and ghrelin levels and body mass index, muscle mass, muscle strength and sarcopenia in COPD patients; this may lead to a new way of predicting the course of the disease and delaying and reducing complications.

MATERIAL AND METHOD

In this prospective study, the patients with mild, moderate, severe and very severe stable COPD who were admitted to the pulmonology outpatient clinic during the three-month period (between 30 June 2019 and 30 September 2019) were included in the study. Patients with malignancy, thyroid dysfunction, psychiatric disorders or chronic inflammatory diseases, and patients receiving corticosteroid therapy or dietary supplementation were excluded. All consecutive patients who gave consent were included in the study. The study was approved by the Health Sciences University, Okmeydanı Training and Research Hospital Clinical Research Ethics Committee (Date: 23.06.2019, Decision No: 48670771-514.10) and performed in accordance with declaration of Helsinki.

In addition to demographic characteristics, body mass index (BMI), body fat and muscle measurements were recorded with bioelectrical impedance device (Tanita BIA model TBF-300; Tanita Corporation, Tokyo, Japan). To determine the muscle strength, a handgrip strength test was performed with a hand dynamometer (Jamar dynamometer, Takei Scientific Instruments Co., LtdJapan). While the grip strength was measured, three measurements were performed with the dominant hand and averaged in kilograms (3). BMI was calculated as body weight (kg)/height2 (m2). The cut-off values for low muscle mass were ≤8.50 kg/m2 for men and ≤5.75 kg/m2 for women (3). To evaluate the muscle strength, grip strength was considered as> 20 kg in women and> 30 kg in men as normal (3). According to the European Working Group on Sarcopenia in the Elderly, patients with low muscle mass and low muscle strength were evaluated as having sarcopenia (3).

According to GOLD criteria, the post-bronchodilator FEV1/FVC <70% on spirometric measurement confirms the diagnosis of COPD. Patients were classified as ≥80% (mild), 50-79% (moderate), 30-49% (severe) and <30% (very severe) according to the expected FEV1 percentage on spirometric measurement (10). At the same time, the severity of COPD (clinical stage) was determined according to the GOLD criteria, with the post-bronchodilator FEV 1, the number of exacerbations on the last year, presence of hospitalization, and dyspnea. Dyspnea was evaluated with the mMRC and CAT scores (10). Based on these criteria, each patient was clinically classified as GOLD stage A, B, C or D (10). We divided the patients into two groups for comparison. Patients with COPD were classified as a group (1 + 2) with mild to moderate obstructive disorder according to FEV1 values, and the remaining group with severe to very severe obstructive disorder (3 + 4). COPD was also divided into two groups according to the clinical stage A + B and C + D. We used the mMRC questionnaire to measure the intensity of dyspnea during daily activities. COPD Assessment Test was performed to determine the health status. Patients with a total score of ≥10 points were evaluated as symptomatic (11). 6MWT was used to test the functional capacity and physical exercise tolerance.

Serum nesfatin-1 and ghrelin levels were measured in all patients. For blood Nesfatin-1 and ghrelin measurements, blood was transferred to yellow biochemistry tube, centrifugation was carried out, and supernatant was taken to eppendorf tube and stored at -80 degrees. Serum levels of Nesfatin-1 and ghrelin were detected by ELISA (enzyme-linked immunosorbent assay) method through commercial kits.

Statistical Analyses

Statistical analyses were performed by using SPSS program v.21 (SPSS Inc., Chicago, IL). Continuous data were expressed as mean±standard deviation or median and categorical data were expressed as percentages. Chi-square test was used for the comparison of categorical data. Pearson's correlation analysis was performed to determine the association of different test parameters. A value of $p< 0.05$ was considered statistically significant.

RESULTS

In a total of 90 patients (16 female and 74 male) were included in this prospective study. The mean age of the study participants was 59.82±9.984 years. Patients with COPD were divided into subgroups according to the spirometric FEV1 (%) measurements as mild-moderate (stage 1 and 2) and severe-very severe (stage 3 and 4). Between those two subgroups, BMI, fat mass, muscle mass and grip strength, serum nesfatin-1, ghrelin and nesfatin-1/ghrelin ratio were compared (**Table 1**). In the mild to moderate COPD group; the number of patients with normal fat mass (%) was significantly higher. There was no significant difference between the two subgroups of COPD patients in terms of nesfatin-1, ghrelin levels or nesfatin-1/ghrelin ratio.

Table 1. Comparison of serum nesfatin-1, ghrelin and nesfatin-1/ghrelin ratio according to stage of COPD

	GOLD 1+2 (n:49)	GOLD 3+4 (n:41)	P value
BMI (kg/m2)	25.25±4.58	23.47±4.31	0.09
Fat mass (%)	24.07±10.37	19.33±9.17	0.026
Muscle mass (%)	33.03±4.99	33.65±4.40	0.541
Grip strength (kg)	32.03±7.12	29.66±6.74	0.113
6MWT(m/sn)	0.92±0.08	0.77±0.14	0.001
CAT score (+)	3/49	29/41	0.001
mMRC score	1.29±0.50	2.74±0.71	0.001
Nesfatin-1(ng/ml)	19.64±15.71	22.45±14.68	0.385
Ghrelin (ng/ml)	2.74±3.83	2.92±3.02	0.803
Nesfatin-1/Ghrelin ratio	8.62±3.48	9.10±3.09	0.493

BMI:Body mass index, 6MWT:6 minutes walking test, CAT: COPD assessment test, mMRC: Modified Medical Research Council

Patients were subdivided into mild-moderate (stage A and B) and severe-very severe (stage C and D) groups according to the GOLD clinical stage. BMI, fat mass, muscle mass and grip strength, serum nesfatin-1, ghrelin and nesfatin-1/ghrelin ratio between these two subgroups were compared (**Table 2**). The number of patients with normal fat mass (%) and 6MWT was significantly higher in the mild to moderate COPD group. There was no significant difference between the two subgroups of COPD patients in terms of nesfatin-1, ghrelin and nesfatin-1/ghrelin ratio.

Table 2. Comparison of BMI, fat mass and grip strength, serum nesfatin-1, ghrelin and nesfatin-1/ghrelin ratio

	GOLD (A+B) (n:47)	GOLD (C+D) (n:43)	P value
BMI (kg/m2)	24.80±4.76	24.12±4.27	0.47
Fat mass (%)	23.02±11.00	20.92±9.01	0.032
Muscle mass (%)	33.22±5.37	33.39±3.97	0.86
Grip strength (kg)	32.00±7.63	29.93±6.20	0.16
6MWT(m/sn)	0.92±0.08	0.78±0.14	0.001
CAT score (+)	7/47	25/43	0.001
mMRC score	1.51±0.65	2.37±1.00	0.001
Nesfatin-1(ng/ml)	20.25±15.74	21.52±14.85	0.69
Ghrelin (ng/ml)	2.84±3.97	2.79±2.90	0.94
Nesfatin-1/Ghrelin ratio	8.84±3.68	8.82±2.90	0.98

BMI:Body mass index, 6MWT:6 minutes walking test, CAT: COPD assessment test, mMRC: Modified Medical Research Council

In correlation analysis, fat mass was positively correlated with BMI and negatively correlated with mMRC score; muscle mass was negatively correlated with BMI. Grip strength was positively correlated with 6MWT, nesfatin-1 and ghrelin levels, but negatively correlated with CAT and mMRC scores (**Table 3**).

Table 3. Correlation analysis between patients' clinical characteristics and fat mass, muscle mass and handgrip strength

	Fat mass (%)		Muscle mass (%)		Grip strength	
	r	p	r	p	r	p
Age (years)	0.024	0.826	-0.148	0.165	-0.123	0.248
BMI(kg/m2)	0.678	0.001	-0.469	0.001	0.077	0.468
6MWT (m/sn)	0.101	0.343	0.124	0.245	0.237	0.024
CAT score (+)	-0.172	0.105	-0.001	0.994	-0.244	0.021
mMRC score	-0.243	0.021	0.013	0.900	-0.305	0.003
Nesfatin-1 (ng/ml)	-0.158	0.137	0.170	0.108	0.221	0.036
Ghrelin (ng/ml)	-0.152	0.152	0.190	0.073	0.241	0.022
Nesfatin-1/ Ghrelin ratio	-0.003	0.978	-0.017	0.873	-0.055	0.607

BMI:Body mass index, 6MWT:6 minutes walking test, CAT: COPD assessment test, mMRC: Modified Medical Research Council

Sarcopenia was detected in 17 of the study participants. A comparison of the general characteristics and laboratory results of patients with or without sarcopenia is summarized in **Table 4**. There was no significant difference between the two groups in terms of age, gender, BMI or smoking status. However, the total cigarette pack/year (p: 0.04) and COPD duration (p: 0.02) of the patients with sarcopenia were significantly higher than those of COPD patients without sarcopenia. Similarly, the number of patients in the advanced stage of COPD was higher in the sarcopenia group than in the non-sarcopenia group. Also, FEV1 (%), FVC (%) and FEV1/FVC (%) values were worse in sarcopenia group compared to non-sarcopenia group (p: 0.001).

Table 4. Comparison of the general characteristics and laboratory results of patients with or without sarcopenia

	Sarcopenia (+) (n:17)	Sarcopenia (-) (n:73)	P value
Age (years)	62.00±6.432	58.90±9.872	0.22
Gender (F/M)	0/17	16/57	0.035
Smoking (quitted /still smoking)	7/10	24/49	0.68
Cigarette (pocket x years)	43.11±11.52	36.16±15.49	0.04
BMI (kg/m2)	23.62±4.51	24.68±4.53	0.39
GOLD stage (Predicted FEV1%)			
1 +2	3	48	0.001
3 + 4	14	25	
GOLD stage (Clinical)			
A +B	2	45	0.001
C + D	15	28	
COPD duration (years)	8.76±6.13	4.87±4.49	0.02
FEV1 (%)	44.58±19.40	66.10±19.31	0.001
FVC (%)	58.29±15.75	74.16±18.58	0.001
FEV1/FVC%	58.64±13.52	69.17±8.31	0.001
CAT score (+)/(-)	14/3	18/55	0.001
6MWT (m/sn)	0.62±0.06	0.91±0.07	0.001
mMRC score	2.76 ±1.09	1.72±0.78	0.001
CRP	5.31±1.33	4.83±1.75	0.29

BMI: Body Mass Index, GOLD: Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease, FEV1: Forced expiratory volume on the first second, FVC: Forced vital capacity, CAT: COPD assessment test, 6MWT: 6 Minute Walking test, mMRC: Modified Medical Research Council

Fat mass, muscle mass and grip strength were compared between the sarcopenia and non-sarcopenia groups, and as expected, the muscle mass and grip strength were worse in the sarcopenia group (p: 0.001) (**Table 5**).

Table 5. Comparison of fat mass, muscle mass and grip strength between sarcopenia and non-sarcopenia groups

	Sarcopenia (+) (n:17)	Sarcopenia (-) (n:73)	P value
Fat mass (%)	21.48±7.84	22.14±10.60	0.77
Muscle mass (%)	31.44±1.44	33.73±5.11	0.001
Grip strength (kg)	28.52±3.44	31.58±7.52	0.001
Nesfatin-1 (ng/ml)	25.46±16.97	19.789±14.74	0.21
Ghrelin (ng/ml)	3.15±2.43	2.74±2.52	0.66
Nesfatin-1/Ghrelin ratio	9.68±3.44	8.63±3.27	0.26

DISCUSSION

In this study, we analyzed the association of circulating nesfatin-1 and ghrelin levels with muscle loss, muscle strength, and sarcopenia in COPD patients and we determined that; 1) Fat mass (%) was significantly lower in patients with severe COPD than patients with mild to moderate COPD. Although the muscle mass did not change significantly in the severe COPD group, physical performance was significantly lower with low fat mass; 2) In correlation analysis, fat mass was positively correlated with BMI and negatively correlated with mMRC score; muscle mass correlated negatively with BMI; handgrip strength was positively correlated with 6MWT, nesfatin-1 and ghrelin levels, but negatively correlated with CAT and mMRC scores; 3) Total cigarette pack/year smoking and COPD duration were significantly higher in sarcopenia patients; FEV1 (%), FVC (%) and FEV1/FVC (%), CAT score, 6MWT and mMRC score were worse than patients without sarcopenia; 4) When patients were compared for the presence of sarcopenia or COPD, the ratio of nesfatin-1, ghrelin and nesfatin-1/ghrelin did not differ significantly.

When the mild and severe patient groups with COPD were compared, fat mass was decreased, muscle mass was preserved and BMI was not changed in severe COPD patients. Our patients are outpatients, and their mobility may explain the relative protection of muscle masses while decreasing fat masses. In a study evaluating nesfatin-1 levels in patients with cystic fibrosis (12), high nesfatin-1 levels with low fat mass were detected in patients with severe cystic fibrosis. In our study, there was no significant change in nesfatin-1 values with decreased fat mass in severe COPD patients. Circulating ghrelin levels are generally positively proportional to weight loss and fat mass reduction (9), but we did not observe a change in ghrelin levels in patients with severe COPD, with a decrease in fat mass. While the effect of adipokines nesfatin-1 and ghrelin on fat tissue was expected, no significant changes were observed in nesfatin-1 and ghrelin levels, although fat mass decreased in severe COPD patients. With the introduction of different mechanisms in COPD, the process may be altered differently in changes in body composition.

Studies on the relationship between fat mass and COPD are limited. Both being overweight or obese, have been shown to increase the severity of symptoms in patients with COPD (13). However, in a recent study, in contrast to previous findings, the mortality of overweight and obese patients with COPD was shown to be low (14). Existing studies have been performed mainly with overweight and obese COPD patients. In general, there are only a small number of studies investigating patients with COPD having normal or lower BMI. In a retrospective cohort study (15) investigating the exacerbation risk of COPD patients with a predominantly normal and low BMI, fat mass showed a significant protective effect for acute exacerbation of COPD. In our study, fat mass was decreased, muscle mass was preserved in patients with severe COPD with similar BMI, but a decrease in physical performance was observed. In spite of the possible negative effects of fat mass, especially in obese COPD patients, the protective effect of fat mass in COPD patients with normal BMI was quite significant.

In the study of Yamamoto et al (16), it was found that TNF-α (tumor necrosis factor-alpha) levels increased in stable COPD cases and there was a negative correlation between fat mass and TNF-α. Fat mass may play a

protective role in COPD. TNF-α, a good indicator of inflammation, is inversely proportional to fat mass. In our study, this finding supports the decrease in physical performance in patients with severe COPD with decreasing fat tissue and increasing the severity of inflammation.

There is significant interest in understanding the mechanisms underlying low muscle mass in patients with COPD. Systemic inflammation, oxidative stress, hypoxia, and hypercapnia are the main mechanisms suggested in sarcopenia (2). Poberezhets et al (17) examined 98 male COPD patients with a mean age of 60.4±11.2 years and reported that, increase in the frequency of COPD exacerbations was associated with the decrease in the 6-minute walk test distance and with the rise of sarcopenia. Graumam et al (18) reported that, with an increase in disease severity, there was a decrease in skeletal mass index and increase in the rate of low lean mass in COPD patients. In a cross-sectional study of 240,562 Korean adults without known lung disease, decreased skeletal muscle mass index was determined to be independently associated with a decrease in lung function in actually healthy adults (19). In this study, we also determined worse FEV1, FVC and FEV1/FVC results in patients with sarcopenia. Moreover, with an increase in COPD severity, fat mass was decreasing and the number of patients with low muscle mass was increasing.

Another important finding of this study was the correlation of handgrip strength with 6MWT, Nesfatin-1 and Ghrelin levels, and inverse correlation with CAT score positivity and mMRC score. Similar with our results Kyomoto et al (20) reported that carbon monoxide diffusing capacity of the lungs and handgrip strength were strongly correlated with 6MWT in their study on 133 patients with COPD. In previous literature, decreased ghrelin levels were associated with the nutritional status of the COPD patients and ghrelin has been suggested for the treatment of COPD patients (21-23). However, we did not determine any significant difference regarding ghrelin levels between COPD patients with or without sarcopenia or any alteration in ghrelin levels with an advance in COPD.

In previous literature, the data analyzing the association of COPD with sarcopenia is limited. The prevalence of sarcopenia in patients with COPD was reported between 15% and 25% (24). In 622 outpatients with stable COPD, Jones et al reported the Prevalence of sarcopenia as 14.5% (25). The prevalence of cachexia, which was defined as unintentional weight loss >5% and low fat-free mass index, was reported as 4.6% in 1755 outpatients with stable COPD (26). In our study the prevalence of sarcopenia was 18.8 %, which was compatible with the literature.

Another finding of the study was that in patients with sarcopenia, CAT score, 6MWT and mMRC score were all worse compared with the patients without sarcopenia. Pothirat et al (27) reported a significant correlation between fat-free mass index and mMRC, FEV_1, and CAT score in 121 patients with stable COPD. In that study, the rate of sarcopenia/ muscular atrophy was 9.9, and the rate of cachexia was as high as 35.5%.Similar with our results, Gologanu et al (28) reported that the sarcopenic COPD patients were having the lowest mean 6MWT and the higher CAT mean scores. In a cross-sectional study, Serra pratt et al (29) investigated the association of plasma ghrelin levels and sarcopenia in elderly people and reported significantly lower ghrelin levels in elderly patients with sarcopenia but this difference disappeared when stratified for gender. We did not determine any significant difference regarding ghrelin or nesfatin-1 levels between sarcopenic and non-sarcopenic COPD patients or with an advance in disease severity. In a recent meta-analysis, circulating level of ghrelin was reported to be significantly elevated in patients with COPD, especially in those underweight (30).

There are some limitations that should be mentioned. First is the low number of patients and secondly, follow-up data was not analyzed in this study.

CONCLUSION

In conclusion, while the effect of nesfatin-1 and ghrelin on fat mass was expected in COPD patients, there was no significant change in nesfatin-1 and ghrelin levels in patients with severe COPD although there was a decrease in fat mass. The decrease in fat mass in severe COPD, decreased physical performance even though muscle mass did not change. Fat mass in patients with severe COPD with normal and low BMI, may play a protective role in the course of the disease. Muscle strength was positively correlated with nesfatin-1 and ghrelin levels, but negatively correlated with mMRC and CAT scores. Approximately 19% of COPD patients had sarcopenia. When the patients were compared for the presence of sarcopenia or COPD; nesfatin-1, ghrelin and nesfatin-1/ ghrelin ratio did not differ significantly. Larger studies with longer follow-up periods are needed to determine the exact role of nesfatin-1 or ghrelin levels in COPD patients and to guide their role in treatment..

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Health Sciences University, Okmeydanı Training and Research Hospital Clinical Research Ethics Committee (Date: 23.06.2019, Decision No: 48670771-514.10).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article

HEALTH SCIENCES
MEDICINE

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The effect of gender differences on the use of valproic acid for migraine prophylaxis

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ABSTRACT

Aim: The efficacy of the drugs used for migraine prophylaxis remains unclear. At our headache polyclinic, when we observed the difference in prophylactic response especially in valproic acid (VA) treatment between genders, the response of male was found to be better than that of females so we wanted to document this difference by collecting data retrospectively

Material and Method: Forty-six chronic migraine without aura patients with VA who were enrolled in our headache outpatient clinic between 2015-2018 were included in the study (29 male, 17 female). In these patients, after VA the frequency of montly attacks between sexes, changes in visual analogue scale (VAS) scores, single VA prophylaxis and the addition of a second prophylaxis drug were evaluated.

Results: None of the men needed an additional second drug while 11 of 17 women needed the addition of a second drug. There was no gender difference in monthly attack frequency before treatment, whereas monthly attack frequency after treatment with VA was found to be significantly lower in men (before treatment; female 20.9±11.3 male 17.7±11.5, $p>0.05$) (after treatment; male 1.2±5.5 female 12.0±12.2, $p<0.05$). There was no difference between sexes in the pre-treatment VAS scores, but the VAS score was significantly lower in men after treatment with VA (before treatment; female 7.8±0.8 male 8.1±1.2, $p>0.05$)(after treatment; female 4.4±2.8 male 2.3±2.8, $p<0.05$). 75.9% of men had no post-treatment migraine attacks, while this rate was 5.9% in women ($p<0.05$). The dose range of VA treatment wasn't found to be different between genders.

Conclusion: Better response of men with migraine to valproic acid treatment is important in clinical practice, and the reason for this situation may be clarified with further studies.

Keywords: Migraine, valproic acid, visual analogue scale

INTRODUCTION

Migraine is a multifactorial neurovascular syndrome characterized by episodes of moderate to severe, unilateral, pulsating headaches with nausea and/or vomiting, worsened by light and sound, pain associated with triggering factors in genetically susceptible individuals (1). Diagnostic criteria established by the International Headache Society (IHS) are used in the diagnosis of migraine (2). The most frequent migraine episodes are encountered in the second and third decades, with the highest incidence of episodes occurring between the ages of 35 and 45 years. The course of the disease is usually with attacks , but migraine is chronic in about 4% of the patients. Migraine treatment is given acutely and/ or prophylactically. Recurring migraine that significantly interferes with the patient's daily routine despite acute treatment. For example, two or more migraine attacks/ month impairing function for ≥3 days or infrequent migraine attacks that produce profound disability during each attack/ failure of, contraindication to, or intolerable side effects to acute medications/ acute medication overuse or the risk of developing acute medication overuse prophylactic treatment should be given (3). Effective prophylactic treatment should reduce at least 50% of the monthly episode frequency within three months (3). Among beta blockers, especially propranolol, valproate and topiramate from antiepileptics, flunarizine from calcium channel blockers, and amitriptyline from tricyclic antidepressants are used for migraine prophylaxis. Topiramate and valproate are antiepileptics approved by the FDA for migraine treatment. Valproic acid can be given in a single dose 500-1800 mg/ day. Valproate has effects like providing neuronal

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hyperpolarization by enhancing potassium transport and increasing postsynaptic GABA response. Various studies using valproate have reported reductions in migraine attack frequency by 43% to 48% (4,5). The studies have used valproate at doses ranging from 500 to 1500 mg daily for migraine prophylaxis have demonstrated that valproate is significantly more effective than placebo (6,7). Prophylactic response varies among patients who take valproate at the same dosage. In our practice, we wanted to document this difference by collecting data recorded at our headache polyclinic retrospectively due to the obvious difference between genders, especially in valproate treatment.

MATERIAL AND METHOD

This study is a retrospective study and the study was approved by the Clinical Researches Ethics Committee of Balıkesir University, Faculty of Medicine (Date: 20.02.2019, Decision No: 2019/34). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Forty-six chronic migraine without aura patients who received valproic acid between 2015 and 2018 were enrolled in our headache outpatient clinic (29 men, 17 women). International Headache Institute (IHS) diagnostic criteria were used to diagnose migraine without aura in our outpatient clinic (2). The VAS pain scale, age, sex, prophylactic drugs used, presence of comorbid systemic diseases, frequency of monthly migraine headaches and time of migraine attack onset were recorded. Patients with systemic diseases, psychiatric comorbidities, >65 years of age were not included. After valproic acid, monthly attack frequency and severity, VAS score, drug dose results in the 1st, 3rd and 6th months were also recorded. We usually start valproic acid treatment with 250 mg dosage in a single dose and give information to patients about 250 mg increments weekly to reduce the adverse effects, and advise on how to decide to increase the dosage by weekly increments. Some patients have responded to a 500 mg controlled release valproic acid tablet dosage, while some others required a dose of 750 mg or 1000 mg.

Statistical Analysis

The data from this research was electronically transferred to the SPSS 20.0 statistics program, and data control and analysis were performed. Descriptive statistics (frequency, percentage distribution, mean, median) were used as statistical analysis. ManWhitney-U was used in the comparison of quantitative data as inferential statistics method test, and chi-square test was used to compare qualitative data. P<0.05 was considered to be a statistically significant value.

RESULTS

The mean age of female patients included in the study was 54.7±10.2 years, while the mean age of male migraine patients was 46.3±11.5 years. 75.9% of men had no migraine attacks post-treatment, while this rate was 5.9% in women (p=0.001). There was no gender difference in the monthly attack frequency before treatment, whereas the monthly attack frequency after treatment with valproic acid was found to be significantly lower in men (before treatment; female 20.9±11.3 male 17.7±11.5, p=0.38) (after treatment; male 1.2±5.5 female 12.0±12.2, p=0.001) (Figure 1). There was no difference between genders in the pre-treatment VAS scores, but the VAS score was significantly lower in men after treatment with valproic acid (before treatment; female 7.8±0.8 male 8.1±1.2, p=0.31) (after treatment; female 4.4±2.8 male 2.3±2.8, p=0.02) (Figure 2). The median dose of valproic acid was found to be 1000 mg in women and 750 mg in men, there was no statistically significant difference. None of the men needed the addition of a second drug while 11 of 17 women needed the addition of a second prophylaxis drug (p=0.001) (Figure 3).

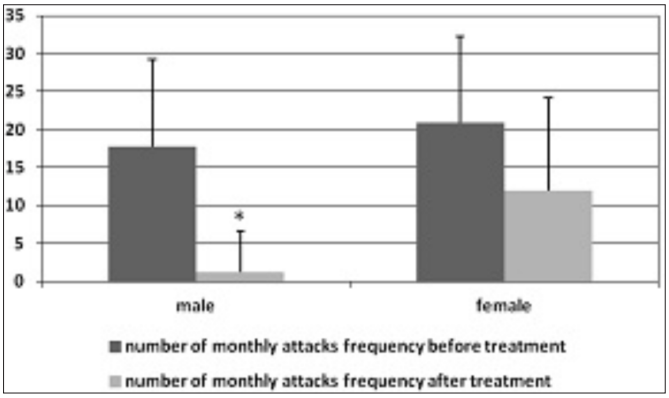

Figure 1. Monthly attack frequency (before- after valproic acid); The monthly attack frequency was found to be statistically significant in males compared to females. 75,9% of men had no migraine attack post-treatment, while this rate was 5,9% in women (p<0,05).

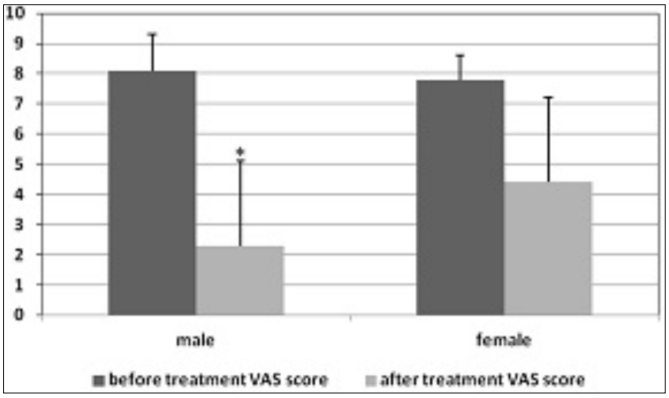

Figure 2. VAS score was found to be statistically significant in males compared to females. (before treatment; female 7,8±0,8 male 8,1±1,2) (after treatment; female 4,4±2,8 male 2,3±2,8, p<0,05) (mean±SD). VAS score (before- after valproic acid)

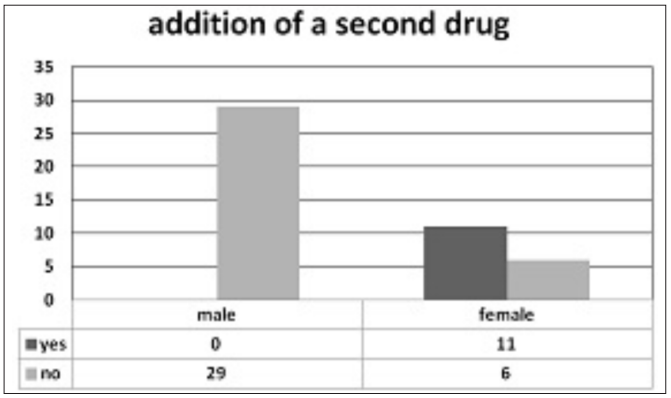

Figure 3. None of the men needed the addition of a second drug while 11 of 17 women needed the addition of a second prophylaxis drug ($p<0,05$)

DISCUSSION

The response to prophylactic therapy varies among migraine patients. Various studies have shown that polymorphic changes in drug metabolism genes, which display differences among individuals, are responsible for 20–40% of the individuals' responses to the drugs and 50% of their side effects (8). The association between antiepileptic drugs and genetic polymorphisms has been evaluated in many studies (9-11). In the current study, no statistical significance was detected between the response to other drugs used in the prophylactic treatment of migraine and the related gene polymorphisms (except for topiramate). The results suggest that migraine patients who receive topiramate prophylactic treatment should be assessed for the presence of the MDR1 C3435T polymorphism. However, there was no significant relationship between the treatment response to topiramate and either the CYP2D6 or CYP2C19 polymorphism, and there were no significant correlation between the treatment responses to amitriptyline, propranolol, and valproate and the MDR1, CYP2D6 and CYP2C19 gene polymorphisms (12). In one study, no significant or nominal association with genetic load was found for any of the prophylactic treatments, either in combination or separately (13). There is currently no solid evidence that pharmacogenomics should be used in migraine prophylaxis. Variability of the dose-response mainly depends on three factors: 1- factors associated with the disease mechanism, which are unknown for prophylactic migraine drugs; 2- pharmacodynamics, also unknown for such drugs; 3- pharmacokinetic factors. Metoprolol, propranolol, and amitriptyline have high pharmacokinetic variability, whereas valproate, topiramate, atenolol, naproxen, and candesartan have low pharmacokinetic variability; however, factors determining this variability are poorly understood (14). Another study shows that age and gender significantly influence valproate serum concentrations but several publications have reported no effects of age on total valproate serum concentrations (15,16). In our study, the frequency of attacks was independent of age in male patients who responded to valproate therapy. Ibarra et al. have shown increased reabsorbed fraction and bioavailability of valproate doses in females compared to males, hence indicating gender differences in hepatobiliary output, which emerge as higher bioavailability of valproate in females than males (17). These studies cannot help to explain why valproate is more effective in male patients.

An examination of recent studies revealed no evaluations on the response to valproate prophylaxis for migraine headache in both genders. After observing that valproate decreases the frequency and severity of migraine headache, especially in men, the results of the present retrospective study showed a decrease in attack frequency and VAS score, which was statistically significant. In addition, the effect that valproic acid alone is sufficient for prophylaxis in men and the need for adding a second drug in women are high and statistically significant, supports the gender differences in the response of valproic acid. Moreover, while valproate prophylaxis alone was sufficient in men, the rate of addition of the second drug was high and statistically significant in women and these data support the differences in valproic acid responses between genders. It still remains unclear why valproate has a different effect on migraine depending on gender. As a continuation of this study, we will search new polymorphisms for valproate with the drug blood level combination for the difference in migraine prophylaxis responses in both sexes.

The present study had several potential limitations. Firstly, this study was designed as a retrospective study. Secondly, since our study was retrospective, we could not look at the valproic acid blood levels of our patients. Thirdly our number patients were small.

CONCLUSION

We wanted to share our clinical observation with this study. Monotherapy is primarily the basis for migraine prophylaxis, and the fact that valproic acid is more effective in men than women will contribute to clinical practice.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Balıkesir University, Faculty of Medicine (Date: 20.02.2019, Decision No: 2019/34).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

HEALTH SCIENCES
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A content analysis and evaluation of outcomes of specialty theses in dentistry

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ABSTRACT

Aim: This study aimed to evaluate the characteristics of dentistry residents and their supervisors involved in the publication of dentistry specialty theses in Turkey.

Material and Method: The dentistry specialty theses uploaded to the Council of Higher Education (YÖK) National Thesis Centre website during 2015–2019 were researched, and 1381 theses were identified and selected.

Results: One thousand three hundred and forty theses were completed at state universities and 41 at foundation universities. While 477 of the theses were prepared by male residents, 904 were prepared by female residents. Four hundred eighteen of the theses were supervised by assistant professors, 413 by associate professors, and 550 by professors. Most of the theses were completed at Hacettepe University. The greatest number of theses were in orthodontics (n=341) and the fewest in oral and maxillofacial radiology (n=89). Published theses accounted for 23.4% of the total, and 15.4% were in Science Citation Index – Expanded journals.

Conclusion: This cross-sectional study revealed that dentistry specialty theses in Turkey contributed to national and international literature.

Keywords: Dentistry, graduate thesis, content analysis, article

INTRODUCTION

In Turkey, oral and maxillofacial radiology, oral and maxillofacial surgery, orthodontics, periodontology, pedodontics, restorative dentistry, endodontics and prosthodontics are postgraduate programs within departments of clinical sciences in dentistry faculties. Nowadays, to receive the title of specialist dentist in Turkey, each candidate must produce a dental specialist thesis on a chosen topic under the guidance of a faculty advisor and must successfully defend the thesis before a panel of examiners.

Students specializing in dentistry are required to prepare a thesis on a subject related to their specialty training before they can take the final examination and earn the title of specialist dentist. Thesis studies help residents gain scientific research skills by creating hypotheses in subjects they are curious about and collecting and analyzing data on their chosen topics. Although thesis studies are the building blocks of graduate education, their results should also be shared with the world of science. For this purpose, the publication of results obtained from postgraduate theses in national or international refereed journals can indicate research quality. (1-5)

This study evaluates the journal publication rates of the departments, universities, and thesis supervisors for dentistry specialty theses completed in our country between 2015 and 2019.

MATERIALS AND METHODS

Ethics committee approval for this study was given by Ordu University Clinical Research Ethics Committee (Date: 03/09/2020, Decision No: 2020/175). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. In this study, all dentistry theses uploaded to the Council of Higher Education National Thesis Center website (https://tez.yok.gov.tr/UlusalTezMerkezi/) between 2015 and 2019 were researched, and 1,381 theses were identified and selected. The registration of names and information for the relevant

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theses in the Higher Education Council Thesis Center was carried out by a single researcher (R.T.) in September 2020.

Next, for each thesis we evaluated its publication date, the university and department where the thesis was done, academic title of the thesis supervisor, co-advisor status, type of thesis subject, whether the thesis was published in a refereed scientific journal, length of the published article, journal index and database, and whether the thesis student is continuing his or her academic career. To this end, a thesis student, supervisor and two expert researchers (R.T. and S.A.) searched Google Scholar (https://scholar.google.com) and PubMed (www.ncbi.nlm.nih.gov/pubmed) databases for articles produced from the related theses. For female thesis students, the search also considered potential surname changes due to marital status. If there was no match between the title of the published article and the thesis, the relevant thesis and article abstract were carefully scanned and checked.

RESULTS

Within the scope of our study, all presentations were made between 2015 and 2019 and were successfully defended, and 1,381 dentistry specialization theses registered by YÖK Thesis Center were evaluated. While 477 theses were prepared by male students, 904 were prepared by female students. The number of dentistry specialty theses per year is shown in **Figure 1**. As is evident, the works range in length from 48 to 408 pages.

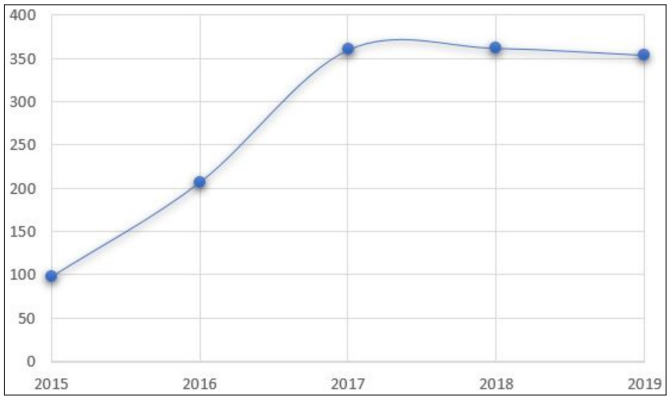

Figure 1. Distribution of theses by years.

Four hundred eighteen of the theses were supervised by faculty members, 413 by associate professors, and 550 by professors. Also, co-advisors participated in the supervision of 30 theses. These co-advisors consisted of 12 physician lecturers, four associate professors, and 14 professors.

Thirty-six Turkish state universities and three foundation universities produced dentistry specialty theses between 2015 and 2019 (**Table 1**). The greatest number were completed at Hacettepe University (99), followed by Ondokuz Mayıs (88) and Atatürk University (87).

Table 1. Distribution of theses by universities

Number	University	Number of specialty thesis in dentistry
1	Ankara Üniversitesi	47
2	Afyon Kocatepe/Afyonkarahisar Sağlık Bilimleri Üniversitesi	7
3	Aydın Adnan Menderes Üniversitesi	22
4	Akdeniz Üniversitesi	39
5	Atatürk Üniversitesi	87
6	Başkent Üniversitesi	14
7	Bolu Abant İzzet Baysal Üniversitesi	28
8	Bezmi Alem Vakıf Üniversitesi	24
9	Bülent Ecevit Üniversitesi	31
10	Cumhuriyet Üniversitesi	65
11	Çukurova Üniversitesi	40
12	Dicle Üniversitesi	43
13	Ege Üniversitesi	36
14	Erciyes Üniversitesi	31
15	Eskişehir Osmangazi Üniversitesi	25
16	Fırat Üniversitesi	1
17	GATA Üniversitesi	11
18	Gazi Üniversitesi	14
19	Gaziantep Üniversitesi	32
20	Gaziosmanpaşa Üniversitesi	26
21	Hacettepe Üniversitesi	99
22	İnönü Üniversitesi	33
23	İstanbul Üniversitesi	43
24	İzmir Katip Çelebi Üniversitesi	25
25	Karadeniz Teknik Üniversitesi	48
26	Kırıkkale Üniversitesi	54
27	Kocaeli Üniversitesi	39
28	Marmara Üniversitesi	54
29	İstanbul Medipol Üniversitesi	16
30	Necmettin Erbakan Üniversitesi	22
31	Ondokuz Mayıs Üniversitesi	88
32	Ordu Üniversitesi	33
33	Pamukkale Üniversitesi	10
34	Rize Recep Tayyip Erdoğan Üniversitesi	9
35	Sağlık Bilimleri Üniversitesi	16
36	Selçuk Üniversitesi	81
37	Süleyman Demirel Üniversitesi	71
38	Van Yüzüncüyıl Üniversitesi	16
39	Yeditepe Üniversitesi	1

The distribution of theses by department is shown in **Figure 2**. The greatest number of dentistry specialty theses were produced in orthodontics (n=341) and the fewest in oral and maxillofacial radiology (n=89). When the theses were examined according to research type, prospective-clinical and epidemiological studies were the most common (**Table 2**).

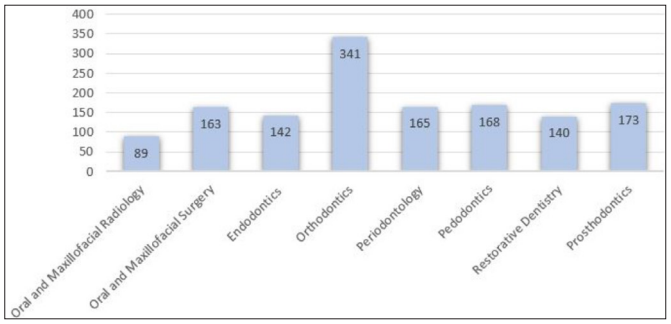

Figure 2. Distribution of the theses according to the departments.

Table 2. Types of research in theses.

Research type	Number
Survey study	72
Clinic-epidemiologic study	511
Animal study	60
In-vitro study	478
Radiographic and retrospective study	214
Finite element method study	46

An evaluation of the publication rate of theses found that 323 (23.4%) of the 1,381 evaluated theses had been turned into published scientific articles. The distribution of these articles according to the published indexes can be seen in **Figure 3**. While 15.4% of the theses were published in journals covered by Science Citation Index-Expanded (SCI-E), 4.6% were published in journals covered by TR Index. The number of published theses by department can be seen in **Figure 4**.

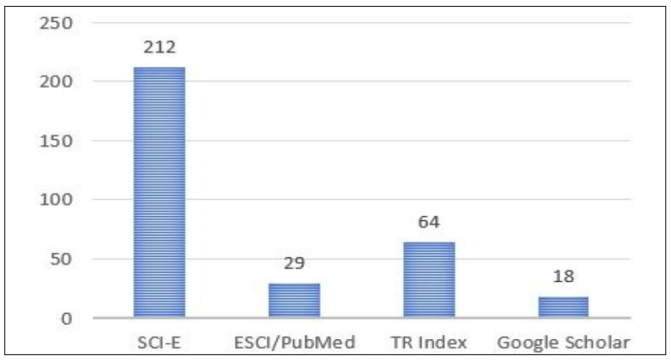

Figure 3. Distribution of the number of theses to be converted into articles in journals in national or international indexes.

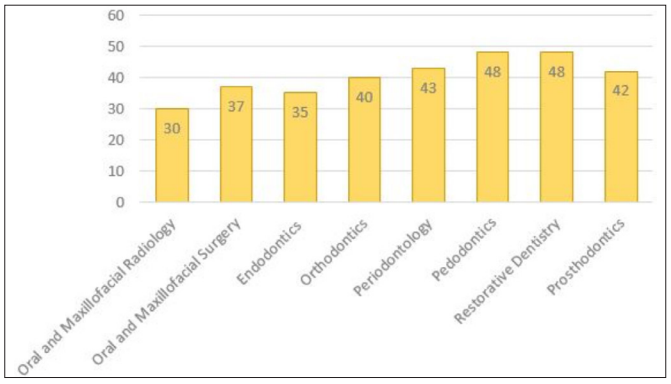

Figure 4. Distribution of the number of theses to articles according to the departments.

DISCUSSION

The first examination for the specialization training in dentistry was held in April 2012, and those who successfully submitted their theses from the relevant departments and succeeded in their final exams earned the title of specialist dentist. In this context, the dentists who won the relevant department in April 2012, which was the first exam in our country in which specialist dentists were determined, through a central examination in the Department of Clinical Sciences, were determined as 3 or 4 years. have been. For this purpose, in this study, specialty theses in dentistry between 2015 and 2019 were evaluated (6).

Specialty theses are one of the most important parts of dentistry specialty education. The specialty thesis aims to enable the trained resident to conduct scientific research. Thus, dentists who have specialized training will learn to interpret the literature, be aware of current international developments, apply reliable treatment methods to their patients as necessary, and plan the path they will follow when designing a scientific study. In addition, they will contribute to the development level of our country by laying the groundwork for scientific studies they conduct nationally and internationally.

Our study is the first and only one to evaluate specialty theses in dentistry in Turkey. In this context, using the same standards, it is possible to compare them with the studies evaluating medical specialty theses produced in Turkey. Erim and Petekkaya (7) evaluated 910 specialty theses in psychiatry in our country between 1981 and 2018. In their study, the average rate of publication of theses was 37.7%, while the rate of publication in SCI and SCI-E index journals was 28.5%. Sipahi et al. (4) examined the publication rates of medical specialty and doctoral theses in medical microbiology, clinical microbiology and infectious diseases in international journals; they determined that 94 (11.4%) of a total of 824 theses became international publications. In our study, 323 of the 1,381 specialty theses (23.4%) were published as national or international journal articles. In a study examining specialty theses in family medicine between 1981 and 2008, 2.1% were published in journals covered by SCI–E (8). In contrast, the rate of SCI–E journal publication of theses produced in the urology departments was 32.7% (9); the corresponding rates were 18% (10) for neurosurgery and 18.5% (11) for eye diseases and surgery. In our study, 212 (15.4%) of the articles obtained from specialty theses in dentistry were published in journals covered by SCI–E.

In our study, it is instructive to evaluate the number of theses uploaded to the YÖK National Thesis Center website relative to the number of dentistry specialty theses. Although such a well-established institution as

Gazi University had over 50 residents from 2012 to 2015, the fact that only 14 dentistry theses were included in the YÖK Thesis Center's database is probably because the theses were not entered into the system by the relevant administrative staff. Even though Mustafa Kemal University has produced at least one dentistry specialty student, there is no specialist thesis in dentistry at the YÖK Thesis Center from this university. In this regard, the administrators of faculties of dentistry should follow the relevant units closely and ensure that the theses completed in their faculties are entered into the system in a timely manner.

CONCLUSIONS

Although the total number of specialty theses in dentistry is not high, the field has yielded an encouraging output of articles in national and international journals. In order to improve the scientific level of our country by increasing the publication rate, institutions should support the academic education of specialty students to the same degree as their clinical education. Thesis advisors should closely follow the thesis preparation process of research assistants specializing in dentistry and play an important role in transforming the results of theses into internationally published articles. To this end, research assistants specializing in dentistry should devote more time to scientific research throughout their degree programs and complete their education with an academic perspective.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Ordu University, Clinic Research Ethic Committee (Date: 03/09/2020, Decision No: 2020/175).

Informed Consent: Not applicable.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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The impact and relationship of inflammatory markers and radiologic involvement in the COVID-19 patients

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ABSTRACT

Aim: In the study, it was aimed to investigate the relationship between inflammatory markers and radiology in COVID-19 patients.

Material and Method: The study was conducted in the quarantine wards of a tertiary hospital between March and May 2020. Patients with a definite diagnosis of COVID-19 were included in the study. The lung damage of the patients caused by COVID-19 was determined by computed tomography and the relationship between lung damage and inflammatory markers was examined.

Results: The mean age of 259 COVID-19 patients included in the study was 61.96±14.076. Except for thrombocytopenia, all variables such as ferritin, D-dimer, thoracic computerized tomography (CT) involvement rates were significantly poorer in the patients requiring the care in ICU than the patients in wards ($p<0.001$). No chronic disease was found in 193 (74.5%) of 259 patients. In multi-variate analyzes, elderly and high thoracic CT involvement rate were determined as independent variables determining the serious disease risks and are important parameters in assessing the need for ICU ($p<0.05$). Ferritin value, D-Dimer value on the third day of admission, Neutrophil lymphocyte ratio and leukocyte count were found to be correlated with thoracic CT involvement rate ($p <0.05$).

Conclusion: It was observed that there were serious changes in the infection parameters of COVID-19 cases with advanced radiological involvement in the lung.

Keywords: COVID-19, intensive care unit, prognosis, SARS-CoV-2

INTRODUCTION

In the last 10 years, infectious diseases have posed an ongoing threat, and especially acute respiratory viruses are among the most recent and re-emerging infectious diseases due to the burden led by severe disorders. Human metapneumo virus (described first in 2001), SARS-related coronavirus (described in 2003), human bocavirus (described in 2005), pandemic influenza A (H1N1) (described in 2009) and the novel Middle Eastern respiratory syndrome coronovirus (MERS-CoV) associated with severe respiratory disease in 2012 are among the examples of acute respiratory infections in the 21st century (1). The novel coronavirus 2019 pneumonia (COVID-19) led by severe acute respiratory syndrome coronavirus (SARS-CoV-2) was detected for the first time in December 2019 in the province of Wuhan, China and has become one of the fatal outbreaks of respiratory infections in the 21st century. To date, 57.8 million cases and 1.3 million deaths have been witnessed worldwide since the outbreak of the COVID-19 (2). The worries of an efficiency antiviral agent and vaccine against the virus with high infectivity and the existence of an asymptomatic population are the factors complicating the therapeutic management of COVID-19 (3). As well as the asymptomatic or mild upper respiratory symptoms, some

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cases of SARS-CoV-2 are encountered with severe course of pneumonia, characterized by fever, cough, dyspnea, bilateral pulmonary infiltration and acute respiratory disease (4). A study stated that approximately 19% of the cases progressed to severe respiratory disease, and a mortality rate of 2.3% was reported (5). Since a clinically effective drug targeting directly the virus in the cases of SARS-CoV-2 has yet to be created, it is important to identify the cases where COVID-19 is likely to progress critically or severely (6). In the study, it was aimed to examine the factors affecting the severity of the disease in COVID-19 cases, as well as to examine the relationship between infection parameters and radiology.

MATERIAL AND METHOD

An approval was obtained from the Ethical Board of Van Training and Education Hospital (Date: 18/06/2020, Decision No: 2020/11). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients and Design

Of 259 patients who were diagnosed with definitive COVID-19 and followed-up in the quarantine hospitals in the province of Van between 1st March and 15th May 2020, were included into the study. The definitive diagnosis of COVID-19 infection was made by detecting the positivity of COVID Real-time polymerase chain reaction (RT-PCR) in the samples upper respiratory tract (nasopharyngeal and oropharyngeal swabs) and, if possible, lower respiratory tract (sputum, tracheal aspirate, or broncho alveolar lavage) of patients in quarantine hospitals. The patients were evaluated clinically, radiologically and in terms of laboratory tests, and those with definitive diagnosis of COVID-19 showing RT-PCR positivity in recurrent tests were included into the study (at least one of them was positive). The patients who are under 16 years of age and considered to be with COVID-19 based on the clinical, laboratory and radiologic investigations, and those without RT-PCR positivity in the repetitive samples from upper or lower respiratory tracts (If no COVID RT-PCR positivity is detected in at least one of the two samples) were excluded out of the study. Patients who were found to be positive in at least two COVID RT-PCR tests were being diagnosed with COVID-19 and included in the study. The parameters of the patients diagnosed with COVID-19 such as age, gender, history of additional diseases, initial time of complaints, length of hospital stay, length of staying in ICU, fever, saturation, respiratory rate, and patients' laboratory and radiological findings were acquired retrospectively from the patients' discharge and hospital medical records. Radiological imaging was not performed in cases with pregnancy and in cases where radiological imaging was not required by the clinician. According to the severity of the disease, the need for intensive care was decided with the following criteria:

- Those with dispnea and respiratory distress,
- Respiratory rate ≥30/minute,
- Arterial oxygen partial pressure (PaO_2)/fractional inspired oxygen (FiO_2) <300,
- Those requiring oxygen during the follow-up,
- Those with serum pressure oxygen-2 (SpO_2) < 90% or PaO_2 <70 mmHg despite oxygen treatment of 5 L/min,
- Hypotension [systolic blood pressure (SBP) <90 mmHg, a decrease more than 40 mmHg of normal SBP, mean arterial pressure <65 mmHg and tachycardia >100/min]
- Those with the development of immunosuppression and acute organ dysfunction, such as acute kidney injury, impairments in acute liver function tests, confusion and acute hemorrhagic diathesis
- High levels of troponin and arrhythmia
- Lactate >2 mmol (7)

Only patients hospitalized in a quarantine hospital were included in the study. Antiviral and antimicrobial treatments were applied to all patients here. In addition, supportive treatments (such as anticoagulants, immunomodulators or corticosteroids) were applied according to the laboratory and clinical conditions of the patients. Treatment modalities were personalized for each patient according to the presence of contraindications, probable development of drug-drug interactions and toxicity.

In COVID-19 cases with low saturation and increased respiratory rate accompanied by high D-Dimer, the differential diagnosis of "Pulmonary Thromboembolism" (PTE), was made by pulmonologists and cardiology physicians (the differential diagnosis of "Pulmonary Thromboembolism" (PTE). In addition to these, pulmonary angiography and echocardiography were performed by pulmonologists and cardiology physicians.

Laboratuary Assessment

Complete blood count, biochemical analysis, coagulation tests, renal function tests, C-reactive protein (CRP), ferritin and D-dimer parameters of all patients on admission and on their third day of antiviral therapy were evaluated. Lymphopenia was defined as lymphocyte count <800 cells/mm^3. Thrombocytopenia was defined platelet count <150,000/mm^3 respectively.

Radiologic Analysis

Each scanning was obtained in the supine position, and no contrast and sedation were used for the procedure. The images were acquired with a 16-slice CT (MSCT; Siemens X-Ray Vacuum Technology, Jiangsu, China) and using the following parameters: Tube voltage, 130 kV; effective mAs, 25; slice thickness, 5 mm; scanning field of the view, 350 mm; and image matrix, 512×512. Also,

the images were taken at the axial plane. Thoracic CT images were evaluated retrospectively on a PACS work station with multiplanar reconstruction by one radiologist (VB) having 10-year experience in interpreting thoracic CT images. A semi-quantitative scoring system was used to quantitatively estimate the pulmonary involvement of all these abnormalities on the basis of the area involved. In order to quantify the extent of lesions, CT score was assigned on the basis of all abnormal areas involved. Each lobe was assigned a score based on the following criteria: Score 0, 0% involvement; score 1, less than 5% involvement; score 2, 5 to 25% involvement; score 3, 26 to 49% involvement; score 4, 50 to 75% involvement; and score 5, greater than 75% involvement. The total CT score was the sum of the individual lobar scores and ranged from 0 (no involvement) to 25 (maximum involvement) (8,9). After each patient was scored on CT, a six-graded staging criterion was constituted according to total chest CT involvements as follows:

Grade 0: 0 (no involvement)

Grade 1: 0-5 (mild)

Grade 2: 6-10 (mild-to-moderate)

Grade 3: 11-15 (moderate)

Grade 4: 16-20 (severe)

Grade 5: >20 (advanced severe)

Statistical Analysis

The statistical analyzes of the data obtained in the study were carried out using the Standard Package for Social Sciences for Windows, version 18.0 (SPSS, Chicago, IL, USA). For the comparison between the groups, the student t-test was used, and a p value less than 0.05 was considered to be significant. Relationships with categorical variables such as gender of patients in ICU and in ward, were examined. While the "enter" method was used for the variables seen to be significant in the univariate analyzes, the multivariate analyzes were performed using the "forward conditional" method.

RESULTS

General characteristics of the patients: It was determined that 24 of 259 patients followed up within the scope of the study needed to be followed in the intensive care unit. The average age level of 24 patients with severe COVID-19 in ICU was determined to be 61.96±14.076 years. It was observed that the patients followed up in ICU were older (p <0.05), (**Table 1**). In multivariate analyzes, advanced age was determined as an independent variable in severe COVID-19 cases (p <0.05) (**Table 2**). In terms of gender differences, It was observed that the patients with severe COVID-19 were 8.4% male patients. When the relationship between gender and ICU need was examined, it was observed that there was no significant relationship (p> 0.005). Thirty-four (13.1%) patients were determined to have at least one chronic disease while 19 (7.33%) had two chronic diseases. The distribution of chronic diseases was determined as hypertension 15.4% (n=40), diabetes mellitus (DM) 9.2% (n=24) and chronic lung disease 7.72% (n=20). No significant relationship was found between hypertension and the requirements of ICU (p>0.005). While 95.7% (248/259) of the patients in the study were discharged from the hospital, it was observed that 4.2% (11/259) of them died. All of the cases that resulted in death were being followed up in the ICU.

Table 1. Univariate risk analysis of severe COVID-19 cases and mild-to-moderate COVID-19

Variables		Inpatients in wards (n=235)	ICU patients (n=24)	p
Age mean±SD (years)		38.37±16.593	61.96±14.076	<0.001
Gender	Male	131 (%91.6)	12 (%8.4)	0.590
	Female	104 (%89.7)	12 (%10.3)	
Clinical parameters	Fever (°C)	36.5±0.74	37.2±0.98	P=0.005
	Respiratory rate/minute	19.2±2.68	31.8±7.08	<0.001
	Oxygen saturation (%)	95.2±4.68	77.08±15.4	<0.001
	qSOFA values			
	0	217±99.1	2±0.9	<0.001
	1	17±50.0	17±50.0	
	2	1±16.7	5±83.3	
Laboratory parameters	Leukocyte (/L)	6153.48±2507.48	8774.58±4610.43	<0.001
	Lymphocyte <800 cells per mm³	1613±615.44	1070±408.28	<0.001
	Platelets <150.000 per mm³	216697.87±57445.758	195916.67±49400.684	0.089
	NLR	2.98±2.32	7.66±6.1	<0.001
	CRP (mg/L)	13.68±29.85	101.68±96.02	<0.001
	Ferritin value on admission	169.55±201.104	493.2±434.51	<0.001
	Ferritin value on 3rd day of antiviral treatment	182.44±248.42	615.12±544.97	<0.001
	D-dimer value on admission (ng/mL)	261.11±632.66	1192.59±3271.75	<0.001
	D-dimer value on 3rd day of antiviral treatment (ng/mL)	273.15±757.08	965.3±1016.45	<0.001
	Creatine (mg/dL)	0.93±0.49	1.26±0.7	<0.001
Radiologic parameters (according to involvement rates) (%)		7.56±13.09	50.48±29.2	<0.001

CRP: C-reactive protein, ICU: Intensive care unit, NLR: Neutrophil/lymphocyte ratio, SD: Standard deviation, qSOFA: Quick sequential organ failure assessment

Table 2. Analysis of independent variables through the Binary Logistic Regression in the development of severe COVID-19

Independent variables	OR	95% CI	p
Advanced age	1.07	1.0-1.13	p=0.036
Thorax CT involvement rate	1.06	1.02-1.09	p<0.001

CI: Confidence interval, CT: Computerized tomography, OR: Odds ratio

Laboratory characteristics of the patients: The mean leukocyte count in the patient group in need of ICU was 8774.58±4610.43 and the leukocyte value was statistically significantly higher in this group (p <0.05). It was observed that there was a significant increase in lymphopenia, CRP, NLR, D-Dimer, and Creatine values except thrombocytopenia in patients in the ICU group (p <0.05). All the variables of the patients requiring to be cared in ICU, except for thrombocytopenia, were significantly different from those of the inpatients followed-up in wards (p<0.001), and COVID-19 patients with the requirements of ICU were found to have poorer values (**Table 1**). The mean ferritin values of all patients were detected to be 199.47±249.48 on admission and 223.93±315.15 on 3rd day of antiviral therapy. D-dimer values of all patients were also seen as 345.79±1169.81 on admission and 343.9±812.37 on 3rd day of antiviral therapy. In addition, the average rates of neutrophil/lymphocyte ratio (NLR) and leukocyte were observed to be 3.45±3.24 and 6396.37±2859.6 on admission, respectively. In 11.7% (30/257 * 100) of the 257 patients included in the study, their first RT-PCR results were negative. RT-PCR tests performed with repeated in the repetitive samples from nasopharyngeal and oropharyngeal swabs were found positive on the 2-3rd day of the follow-up in the quarantine hospital of the patients whose first RT-PCR test was negative.

The relations of inflammatuary markers and radiological involvement: No radiological imaging was performed for 38 of 259 participants since five were pregnant, and 33 were assessed not to require the imaging by the clinician. The rate of total thoracic CT involvements in 221 patients undergoing thoracic CT was found as 11.6±19.8. However, when the rate of lung involvements was examined, no involvement was observed in the lung parenchyma in 57% (n=126) of the cases. It was seen that 7% (15/221) of all patients with thoracic CT scan had parenchymal involvement over 50% (**Table 3**). As the rate of thoracic CT involvements increased, a positive increase was also observed in almost all parameters. The parameter demonstrating the best correlation with the rate of lung involvements was found in ferritin (52% positively significant). While there was no correlation between D-Dimer value and the rate of thoracic CT involvements on admission, a weak correlation of 27% was observed between them on the 3rd day of treatment and this correlation was found to be significant (**Table 4**). It could be considered that as the lung involvements increase, D-Dimer value will increase, or vice versa. Moreover in multivariate analyzes, involvement rate was determined as an independent variable in severe COVID-19 cases (p <0.05) (**Table 2**).

Table 3. Distribution of the involvement rates in Thorax CT of the cases

The involvement rates of cases in thorax CT	Number of patients (n:)	Percent (%)
0	126	57.0
4	5	2.3
8	20	9.0
12	4	1.8
16	17	7.7
20	10	4.5
24	5	2.3
28	4	1.8
32	3	1.4
36	5	2.3
40	4	1.8
44	1	0.5
48	2	0.9
56	3	1.4
64	3	1.4
68	1	0.5
72	3	1.4
76	1	0.5
80	2	0.9
92	2	0.9
Total	**221**	**100.0**

Table 4. Correlation of radiological tutulum with each other

	Correlation coefficient r_s	P
Ferritin value on admission- Total involvement	0.521	0.00*
Ferritin value on 3rd day of antiviral treatment- Total involvement	0.422	0.00*
D-dimer value on admission- Total involvement	0.042	0.546*
D-dimer value on 3rd day of antiviral treatment- Total involvement	0.270	0.000*
NLR- Total involvement	0.406	0.000*
Leukocyte count- Total involvement	0.295	0.000*

NLR: Neutrophil/lymphocyte ratio

DISCUSSION

The patients diagnosed with COVID-19 are encountered in a wide spectrum from asymptomatic cases to severe pneumonia and even death. COVID-19 cases were reported in the WHO-China joint report in February 2020; Eighty percent of those with a definite diagnosis thanks to the laboratory tests have mild or moderate disease (with or without pneumonia), 13.8% experience the disease severely (dyspnea, breath count ≥3 / min, blood oxygen saturation ≤93%, PaO_2 / FiO_2 ratio <300,

and / or lung infiltration of> 50% between 24-48 hours) and 6.1% also have critical disease (respiratory failure, septic shock, and / or multiple organ dysfunction / failure) (10). The requirements of ICU are seen in 5% of all COVID-19 patients (11). The presence of comorbid factors, such as male gender, over 60 years of age, hypertension, DM, cerebrovascular, cardiovascular and chronic respiratory diseases, and cancers are among the risk factors leading to severe COVID-19 pneumonia (12-14). In the study, it was seen that 9.2% (24/259) of the patients needed intensive care. Similar to the data in other studies, COVID-19 was found to have a more severe prognosis in advanced aged patients in our study ($p < 0.05$). It was determined that the most common accompanying chronic disease was hypertension (15.4%, (n = 40)). However, there was no significant relationship between the severity of the disease ($p > 0.05$).

Metabolic diseases cause the immunal functions to be reduced by leading to lymphocyte and macrophage dysfunction, as well as the patient to be more sensitive to the complications of COVID-19 disease (15). During COVID-19 infection, both the innate and acquired immune system participates synergistically in the antiviral response, and a significant increase is seen in leukocytes, neutrophils and NLR, along with significant lymphopenia in severe cases (16). However, in the study in which 225 patients were investigated by Li et al., it was stated that 37 (16.44%) patients were diagnosed with severe COVID-19 disease, and patients' leukocyte (86.67%) and lymphocyte (99.11%) counts were within normal levels or decreased on admission, and the levels of CRP (mean, 60.4±57.5; normal range, 0–10 mg/L), procalcitonin (0.87±0.56; normal range, 0–0.5 mg/L) and sedimentation (mean, 55.8±25.3; normal range, 0–15 mm/h) were increased in 86.22, 10.67 and 90.22% of the patients, respectively (17). In another study by Guan et al., however, lymphocytopenia, thrombocytopenia and leukopenia were detected to be present in 83.2, 36.2 and 33.7% of the patients on admission respectively, and the levels of CRP, alanine amino transferase (ALT), aspartate amino transferase (AST), creatine kinase (CK) and D-dimer were found to be higher in general (18). In the same study, it is reported that 61.1% of the patients with severe COVID-19 presented with leukopenia, 96.1% with lymphopenia (below 1500 per mm^3) and 57.7% with thrombocytopenia, while the levels of CRP, procalcitonin and D-dimer were found higher as 81.5, 13.7 and 59.6%, respectively (18). As can be seen in the mentioned studies; In COVID-19 cases, it is known that comorbid factors as well as laboratory changes are associated with a more severe clinical course. When the laboratory parameters of the cases in our study are examined; Patients with increased levels of leukocytes, NLR, CRP, ferritin, D-dimer and creatinine and those with lymphopenia were found to be significantly more severe patients ($p < 0.001$). These data obtained in the study have added strength to the literature in this respect.

Patients with severe prognosis were found to be worse in the laboratory when evaluated both clinically and laboratory. The laboratory and clinical data of the patients in our study were found to support other studies in this area, and in this respect, it was necessary to examine the relationship between the laboratory and the radiological evaluations of patients. As known; the investigations through thorax CT imaging as an important modality in the diagnosis of lung diseases are of vital sensitivity in the diagnosis and evaluation of COVID-19 disease (19). Under The radiological imaging guide released by the World Health Organization (WHO), it is recommended that the need for imaging be supported by the patient's clinic, and the laboratory and RT-PCR results (20). As thorax CT findings can be detected as normal in half of the patients within the first 1-2 days after the symptoms begin, radiological findings can also be detected even before the symptom starts (19,21). Bilateral lung involvement develops in over 75% of thorax CT images in COVID-19 cases, and multilobar involvement is frequently observed (71%) (22). Among the most common finding in thorax CT images are ground glass opacities, and ground glass opacities were observed in varying rates between 86-77% in thorax CT series (21-23). In the study where Shi et al. examined the rate of radiological involvement in terms of weeks, leukocytosis (32%) and lymphocytosis (67%) were found to be high, as well as CRP concentration and serum amyloid A protein levels, in most of the patients (24). Ayrıca In the study by Zhang et al., as consistent with the findings reported in other studies, the increase in D- dimer was found to be statistically significant in predicting mortality (25). As it can be seen, most COVID-19 cases come with the presence of diffuse lung involvement. It is also known that there is a significant relationship between lung involvement and the laboratory. In our study, we found that the average of thorax CT involvements was found to be 50.48±29.2 and also, as the lung involvement rate increased, the severity of the disease increased.

Laboratory findings are of great importance in severe COVID-19 cases. In addition to the clinic, radiological progress should be evaluated with the laboratory. It is now well known and supported by many studies that many inflammation indicators such as ferritin, CRP, D-Dimer, NLR are associated with the severity of the disease. In our study, the relationship between ferritin, D-Dimer, Leukoid count and NLR and radiological involvement rates were examined. As a result the value of ferritin on admission was found to be correlated positively and significantly at the rate of 52%. 27% correlation was found between

D-Dimer values on 3rd day of antiviral therapy and rate of thoracic CT involvements. In the light of the data obtained in the study; It would be appropriate to say that serial measurements of ferritin and D-Dimer values in COVID-19 cases will significantly contribute to clinical findings in evaluating the progression of the disease.

CONCLUSION

COVID-19 cases present with different clinical pictures from asymptomatic to serious. In this respect, clinical and laboratory are of great importance in evaluating the severity of the disease. However, when examining laboratory parameters, the evaluation of patients' inflammatory markers becomes more important than other parameters. In particular the ferritin and D-dimer parameters should be meticulously evaluated on admission and 3rd day of the antiviral treatment, and predicting the rates of lung involvements will be effective in understanding the extent of disease severity.

ETHICAL DECLARATIONS

Ethics Committee Approval: An approval was obtained from the Ethical Board of Van Training and Education Hospital (Date: 18/06/2020, Decision No: 2020/11).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: All authors also declare no conflict of interest.

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The high co-existence rate of *Blastocystis* and *Dientamoeba fragilis* in human faecal samples and the analysis of demographic and clinical findings

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ABSTRACT

Aim: *Blastocystis* and *Dientamoeba fragilis (D. fragilis)* are among the most common protozoon species in human faecal samples. The cross-sectional studies have reported the frequencies in a variety of populations. However, we have very limited information about the co-existence rate of those protozoans. The study aimed to compare *D. fragilis* frequency in *Blastocystis* positive and negative faecal samples in order to determine the co-existence rate. The secondary objective was to analyse demographic characteristics and gastrointestinal (GI) symptoms in relation to both infections.

Material and Method: In the present study, we defined a study group that included 100 *Blastocystis* positive faecal samples and a control group that included 100 *Blastocystis* negative samples. The frequency of *D. fragilis* in samples was determined with a PCR assay specific to the small-subunit ribosomal RNA (SS rRNA) gene. A positive control of *D. fragilis* was used and the samples with amplification of the expected size (863 bp) were considered as positive. In addition to the statistical comparison of frequencies, the descriptive and clinical findings of cases were analysed retrospectively with Pearson chi-square or ANOVA tests.

Results: The frequency of *D. fragilis* was 21% in *Blastocystis* positive group and it was 10% in *Blastocystis* negative group. There was statistically significant difference in terms of *D. fragilis* positivity between the groups ($p < 0.05$). Age, gender and GI symptoms did not reveal a significant difference between the following groups: only *Blastocystis* infected (n=77), only *D. fragilis* infected (n=11), infected with both protozoans (n=34) and non-infected individuals (n=89) ($p > 0.05$).

Conclusion: Our study highlighted the high co-existence of *D. fragilis* and *Blastocystis* in human faecal samples. A possible explanation for this finding may be the faecal-oral transmission of these protozoans. In addition, analysis of clinical findings was supported common asymptomatic colonisation of *Blastocystis* and *D. fragilis*.

Keywords: *Blastocystis*, *Dientamoeba fragilis*, co-existence, clinical findings

INTRODUCTION

Dientamoeba fragilis (D. fragilis) is a common, globally distributed, enteric protozoon in humans. It was initially classified in amoebas, but the exact taxonomic position was found after phylogenetic and ultra-structural studies. It was finally defined as a member of trichomonads (1). Presence of non-motile pre-cyst and cyst forms in human faecal samples was recently confirmed (2). It colonizes large intestines of humans and some other non-human hosts such as livestock and pet animals. Frequency of *D. fragilis* in many countries has been studied using a variety of diagnostic methods. In common, higher frequencies were reported in developed countries as compared to undeveloped countries unlike other intestinal protozoans (3). Prevalence of *D. fragilis* greatly varied (between 0.4% and 82.9%) in those studies and influenced by the diagnostic methods, study groups, sample size, and geographical location (2). Despite continuous reports emerging over last 100 years, it is still often ignored as a pathogen "neglected parasite", and routine testing often not conducted by diagnostic laboratories. A study from the Netherlands reported that after implementation of faecal PCR, the number of reported *D. fragilis* cases increased 20 folds. In addition, the symptoms in *D. fragilis* infected group lasted longer when compared to *Giardia intestinalis* infected group and complete resolution of symptoms was noted after eradication of the parasite in faecal samples (4). The previous studies mostly performed in industrialized countries such as the Netherlands and Denmark using molecular techniques and they reported frequencies reaching up to 71% in particular age groups (5). In Brazil, *D. fragilis* was detected in 10.3% of children; the other protozoans were *Blastocystis* (14.1%), *Endolimax nana*

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(13.5%), *Entamoeba coli* (12.2%), and *G. intestinalis* with a frequency of 10.9% (6). A study found that frequency of *D. fragilis* (5.2%) was lower than *Blastocystis* (9.6%) but higher than of *G. intestinalis* (2%) among symptomatic and asymptomatic population in Sydney (7). The most common transmission way of intestinal, parasites is faecal-oral and they are mostly defined as food-borne pathogens. World Health Organisation (WHO) reported that the foodborne parasitic diseases including *Entamoeba histolytica*, *G. intestinalis* and *Cryptosporidium spp*. are focal and cause significant morbidity and mortality in vulnerable populations, however, *D. fragilis* was not listed (8).

Previous studies on *D. fragilis* in Turkey reported that the frequencies varied from 0% to 26.9% (9, 10). However, a systematic and comprehensive understanding of *D. fragilis* prevalence is still lacking in our country. The pathogenic or opportunistic role of *D. fragilis* in human diseases has been a controversial issue for a long period of time. Most of the infected individuals do not represent clinical symptoms and higher frequencies have been reported in healthy group as compared to symptomatic group. Although initially described as a non-pathogen, *D. fragilis* has been associated with wide-ranging symptoms. Symptomatic cases represent primarily non-specific gastrointestinal (GI) symptoms mostly abdominal pain or intermittent diarrhoea (7). In addition, many other symptoms including malaise, nausea, anorexia, fatigue, poor weight gain, and unexplained eosinophilia (almost half of the positive cases) are also attributed to *D. fragilis* infection. The symptoms may persist or re-occur in some patients until application of effective treatment (2).

Blastocystis is an intestinal anaerobic protozoan of humans and many other non-human species. Following the long-term taxonomic studies, *Blastocystis* was included in the group of Stramenopiles. The colonization in humans and the absence of a flagellated differentiates *Blastocystis* from others in this group (11). *Blastocystis* has a global distribution and has been reported as the most common protozoon in human faecal samples in many studies (12). *Blastocystis* prevalence is higher in undeveloped countries, and frequencies reaching up to 100% have been reported in Senegal. The evaluation of prevalence studies revealed estimation that 1-2 billion people around the world had *Blastocystis* infection (13). A study from Sweden evaluated retrospectively the intestinal parasite frequency for 10-year period, and found that 4.2% of intestinal parasite prevalence, all of them were positive for *Blastocystis*. However, it was noted that most had an immigration history (14). *Giardia* and *Blastocystis* were the most common protozoan species in a study from Australia (15). A study identified GI pathogens in children with diarrhoea reported 2.9% *Blastocystis* carriage and none in asymptomatic group in United Arab Emirates (16). A systematic review in China estimated overall 3.3% *Blastocystis* prevalence in the country and noted great variations between cities in terms of *Blastocystis* frequency, from 0.8% to 100% (17). *Blastocystis* prevalence in Turkey was reported at rates ranging from 1,4% to 23,5% (18, 19). There is lot of controversy regarding the pathogenicity, genetic diversity, life cycle, diagnosis and treatment of *Blastocystis* (12). Similar to *D. fragilis* the role of *Blastocystis* in the aetiology of particular GI diseases such as irritable bowel syndrome (IBS) and ulcerative colitis (UC) is an important area of interest. Recently, controversial findings suggest that both infections implicate the development of IBS. A systematic review with meta-analysis examined the possible link and reported a correlation with *Blastocystis* (21). However, there is great need for future studies to reveal the actual mechanisms. Some defined *Blastocystis* as a pathogen, an opportunistic pathogen, or a non-pathogenic microorganism. Currently, it is thought that *Blastocystis* pathogenicity is multifactorial and complicated phenomenon that depends on *Blastocystis* strains, host characteristics, therefore it is hard to explain the pathogenicity over a single feature (12).

Both *Blastocystis* and *D. fragilis* are common microorganisms in human faecal samples, worldwide. However, there is limited data about their co-existence and few studies directly investigated this relationship. The aim of the present study was to determine the co-existence of *Blastocystis* and *D. fragilis* in human faecal samples with molecular methods. In addition, we aimed to analyse some demographic characteristics and GI symptoms related to *Blastocystis* and/or *D. fragilis* infections.

MATERIAL AND METHOD

The study was reviewed and applied by No-Interventional Clinical Research Ethics Committee of Aydın Adnan Menderes University Faculty of Medicine (Date: 17.02.2021, Decision No: 2021/37). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study included two groups: *Blastocystis* positive 100 and *Blastocystis* negative 100 DNA from faecal samples. All of the 200 individuals were scanned retrospectively for the presence of any other intestinal protozoans or helminths and negatives with direct microscopy were included in the study. Genomic DNAs were previously isolated from faecal samples of the individuals during routine coprological examination in parasitology laboratory in Aydın Adnan Menderes University, Training and Research Hospital. A commercial kit (QIAamp DNA Stool mini kit, Germany) was used to isolate genomic DNA directly from fresh faecal samples. *Blastocystis* positivity was

detected by amplification of 18S rRNA gene of *Blastocystis* with the primers RD5 and BhRDr as previously reported (22). *Blastocystis* isolates were confirmed by submission of partial 18S rRNA sequences to MLST database (http://pubmlst.org) and with neighbour-joining method including reference sequences.

Determination of *D. fragilis* Positivity

Positivity of *D. fragilis* was studied with amplification of the small-subunit rRNA (SSU rRNA) (23). Reaction was set in 30-ul volume: 1 µl of template DNA, Taq DNA polymerase (0.3 U), dNTPs (0.2 mM), the primers (0.4 pmol), $MgCl_2$ and 1× Taq buffer with $(NH_4)2SO_4$. The primers DF400 (TAT CGG AGG TGG TAA TGA CC) and DF1250 (CAT CTT CCT CCT GCT TAG ACG) were used in the assay. PCR cycle was as follows: initial denaturation at 94°C for 3 min, 30 cycles (1 min at 94°C, 1.5 min at 57°C, 2 min at 72°C) and final extension at 72°C for 7 min. The PCR amplicons analysed by electrophoresis on 1% agarose gel and visualized with the UV imaging system (Vilber Lourmat, France). A previous *D. fragilis* isolate (ADUDf101), confirmed with partial sequence of SSU rDNA, was used as positive control in our experiment.

Statistical Analysis

The descriptive data was presented using Statistical Package for the Social Sciences, SPSS (IBM, USA) vs. 21.0. A chi-square test of independence was performed to show the relation between *Blastocystis* and *D. fragilis* co-existence rate. The sociodemographic characteristics (gender and age) were evaluated also analysed using Pearson chi-square. The common GI findings were compared between four groups (*Blastocystis* infected, *D. fragilis* infected, non-infected and both infected) with ANOVA.

RESULTS

The positive rate of *D. fragilis* was 23% in *Blastocystis* positive samples and it was 11% in *Blastocystis* negative samples (**Figure**, **Table 1**). *D. fragilis* positivity was significantly higher in *Blastocystis* infected individuals as compared to non-infected cases χ^2 (1, N=200)=0.1028, p=.0238. The age of study population (n=200) varied from 1 to 84 with the average of 37.2±23.5. Males accounted for the %53 (n=106) of individuals and females for %47 (n=94).

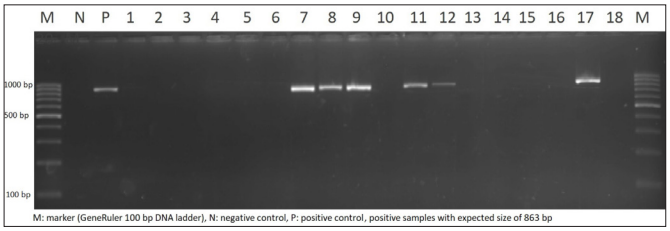

Figure. Agarose gel electrophoresis of amplicons from *D. fragilis* 18 S rRNA PCR

Table 1. The comparison of *D. fragilis* frequency in *Blastocystis* positive and negative samples

	***D. fragilis* PCR**		
	Positive n (%)	**Negative n (%)**	**Total**
Blastocystis			
Positive	23 (23)	77 (77)	100
Negative	11 (11)	89 (89)	100
Total	34 (17)	156 (78)	200

The faecal samples were sent from many different clinical departments: gastroenterology and hepatology (n=53, 26.5%), child health and diseases (n=40, 20%), dermatology (n=31, 15.5%), general internal medicine (n=20, 10%), allergy and immunology (n=17, 8.5%), oncology (n=10, 5%), infectious diseases (n=8, 4%), chest diseases (n=5, 2.5%), and the other departments (n=16, 8%) including haematology, urology, family medicine, rheumatology, otolaryngology, and nephrology.

The clinical features and diagnosis greatly varied in the study population: abdominal pain (n=39, 19.5%), diarrhoea (n=25, 12.5%), allergy (n=25, 12.5%), flatulence (n=24, 12%), constipation (n=18, 9%), pruritus (n=17, 8.5%), nausea-vomiting (n=16, 8%), general medical examination (n=14, 7%), malnutrition-developmental delay (n=13, 6.5%), urticeria (n=12, 6%), gastroesophageal reflux (n=11, 5.5%), vitamin-D deficiency (n=9, 4.5%), anaemia (n=7, 3.5%), colitis (n=4, 2%), dermatitis (n=4, 2%), dyspepsia (n=4, 2%), and the others; skin rash, dysuria, cramping, GI haemorrhage, lassitude, myalgia, and cellulitis in single patients. In addition, 12 (6%) of the studied population were cancer patients, 10 (5%) were ulcerative colitis patients, six (3%) had irritable bowel syndrome, four had Crohn's disease, four (2%) had urinary system infections, two (1%) had pneumonia, two (1%) had obesity treatment, two (1%) had renal failure, one had diabetes, and one had rheumatoid arthritis.

In the present study, we found 77 cases with single *Blastocystis* infection, 11 cases with single *D. fragilis* infection, 34 cases with both of *Blastocystis* and *D. fragilis* and 89 non-infected cases. These groups were compared for demographic characteristic in statistical analysis and no significant relation was found regarding gender and age (**Table 2**). We also analysed common GI findings including abdominal pain, diarrhoea, constipation, flatulence, and nausea-vomiting between the groups, none of these symptoms were significantly different between the groups (**Table 3**).

Table 3. The analysis of common gastrointestinal symptoms between the groups

	Abdominal pain*	Diarrhoea*	Constipation*	Flatulence*	Nausea- vomiting*
Blastocystis infected only (n=77)	15 (19.5)	10 (12.9)	5 (6.4)	8 (10.3)	5 (6.5)
D. fragilis infected only (n=11)	3 (27.3)	2 (18.1)	1 (9.1)	2 (18.1)	1 (9.1)
Blastocystis and *D. fragilis* infected (n=34)	6 (17.6)	4 (11.7)	3 (8.8)	6 (17.6)	2 (5.9)
Non-infected with both of them (n=89)	15 (16.9)	9 (10.1)	9 (10.1)	12 (13.4)	8 (9)
Chi square (p value)	0.782 (0.852)**	0.775 (0.855)**	4.873 (0.181)**	1.354 (0.716)**	0.557 (0.906)**

* number of positives and (%) in the groups ** not significant at p<0.05 level

Table 2. The comparison of some demographics of studied population between the groups

	N	Age* (mean±sd)	Gender**	
			Female (n, %)	Male (n, %)
Blastocystis infected only	77	32.2±24	37 (48.1)	40 (51.9)
D. fragilis infected only	11	42.9±28.1	5 (45.5)	6 (54.5)
Blastocystis and *D. fragilis* infected	34	39.6±22	14 (41.2)	20 (58.8)
Non-infected with both of them	89	36.4±21.4	38 (42.7)	51 (57.3)

N: number, Sd: standard deviation, *not significant, ANOVA: F (3, 207)=1.31, p=.271; ** not significant, χ^2= 0.666, p= .881

DISCUSSION

In the present study, we have tested the frequency of *D. fragilis* in *Blastocystis* positive and negative individuals with molecular methods. The findings indicate significantly high infection rate of *D. fragilis* in *Blastocystis* positive cases. In the literature, previous studies reported some data related to our findings. However, few studies directly aimed to study the co-existence rate with molecular methods. A study investigated the frequency of intestinal parasites in 580 children with diarrhoea and they reported a correlation between *Blastocystis* and *D. fragilis*. The frequency of *D. fragilis* was 2.7% among children without *Blastocystis* infection. However, the rate was 29.6% among children with *Blastocystis* infection (24). A study from Iran investigated prevalence of intestinal parasites with conventional parasitological methods and reported that 21 (17%) of 125 *Blastocystis* infected individuals were also positive for *D. fragilis* (25). In the Netherlands, *D. fragilis* was detected as the most frequent protozoon in faecal samples of 163 paediatric patients and the combination of *D. fragilis* and *Blastocystis* accounted for almost 50% of them (26). Another study found that *Blastocystis* frequency was 42% in *D. fragilis* positive cases (9 out of 21). *D. fragilis* was detected as the most common parasite species in faecal samples of studied population (27). Similarly, frequency of *D. fragilis* was studied in patients with GI symptoms and 23.7% of *D. fragilis* positives had *Blastocystis* infection (28). The most common protozoon was *D. fragilis* in *Blastocystis* infected individuals, 24% (53 of 221 *Blastocystis* infected cases). In addition, a significant correlation was found between *Blastocystis* and *D. fragilis* (29).

The proposed mode of transmission is faecal-oral for both *Blastocystis* and *D. fragilis* (2,11). Therefore, they share a common source of infection for enteric protozoans. Parallel to our findings, a study among IBS patients found that *Blastocystis* carriage was a risk factor and increased the odds for *D. fragilis* infection (30). In addition, another hypothesis about transmission of *D. fragilis* is the carriage with pinworm eggs (2), our study did not include Enterobius vermicularis positive faecal samples as well as other intestinal parasites. Therefore, we could eliminate possible effects related to this type of transmission in our study.

In our study, the overall positive rate of *D. fragilis* was 17% in Aydin. In general, the finding was in accordance with the reported frequencies from other cities of Turkey. The frequency of *D. fragilis* was studied in faecal samples collected from 121 individuals, of them 101 had GI complaints and remaining 20 cases were in control group. The overall positive rate of *D. fragilis* was 13% with iron haematoxylin staining (31). Another study from Istanbul determined 16.7% positivity of *D. fragilis* and they found a statistically significant difference between healthy individuals and patients in terms of *D. fragilis* positivity (9). In Manisa, *D. fragilis* positivity was studied with different culture methods and *D. fragilis* trophozoites were determined in 11 of 104 (10.6%) samples with Robinson's medium (32). A study from Izmir investigated *D. fragilis* positivity in 490 faecal samples with real-time PCR; they found that 59 (12%) patients were infected with *D. fragilis* (28).

A limitation of our study was the possible cross-reaction of PCR testing with other trichomonads in human faecal samples. There is currently no PCR testing protocol for laboratory detection of *D. fragilis* that is approved by U.S. Food and Drug Administration (FDA) and the validation of the tests are still in progress. It was reported that PCR method in our study was tested against various other protozoan parasites including *Blastocystis*, *Entamoeba spp*, *E. hartmanni*, *Giardia intestinalis*, *Endolimax nana*,

Iodamoeba butschlii, *Cryptosporidium spp*., *Cyclospora spp*., *Chilomastix mesnili*, *Enteromonas hominis* and no amplification was detected with having 100% specificity (23). However, recently, it was reported that conventional PCR for *D. fragilis* may result in cross reactions with other trichomonads (2). In addition, the sensitivity of PCR testing with DF400 and DF1250 primers was 93.5% in fresh faecal specimens (23).

Another limitation of the current study was the lack of permanent staining because of the retrospective nature of the study. Faecal samples were initially tested for the presence of *Blastocystis* and subjected to genomic DNA isolation. Therefore, we could present only wet-mount examination (native-Lugol's iodine) of faecal samples from hospital record. At the beginning of the study, we excluded the samples that were positive for other intestinal protozoa. It was reported that the nuclear structure of *D. fragilis* is visible when permanent staining methods are used (2). In general, molecular methods are more sensitive than examination of wet-mount preparations. A study reported frequency of *D. fragilis* with direct smear, formalin-ether concentration, culture, permanent staining and amplification of SSU rRNA and 5.8S rRNA genes. The positive rates with the methods were as follows: 0%, 0%, 1%, 5%, 6% and 13.5%, respectively (33).

In the present study, when we compared age, gender and common GI symptoms between the four groups, no statistically significant difference was noted. A number of studies reported no relation between gender and *Blastocystis* infection, as well as *D. fragilis* infection, supporting our findings (33, 34). A case control study reported that GI symptoms were more common in cases without *D. fragilis* or *Blastocystis*. In addition, they reported that both *D. fragilis* and *Blastocystis* frequency was higher in healthy controls than in cases with symptoms (35). However, some reported a correlation with age of cases and *Blastocystis* infection in particular age groups (19). Despite the relatively small size of study population, the findings on GI can be attributed to the general characteristics of both *Blastocystis* and *D. fragilis* infection. Because, there is growing body of literature that reported that these two infections are mostly asymptomatic and a small ratio of infected individuals represent GI symptoms (2,11). Diarrhoea, abdominal pain, constipation and urticarial findings were reported in symptomatic cases of *Blastocystis* infection (11-13). Similar to *Blastocystis*, non-specific GI symptoms including abdominal pain, cramps and diarrhoea were reported in symptomatic cases of *D. fragilis* (36). Nausea, vomiting, fever and eosinophilia have also been observed in cases with *D. fragilis* infection (2,37). Parallel to these findings, recent developments in the study of microbiota research revealed that the existence of *Blastocystis* and *D. fragilis* may be related to a healthy intestinal flora (38,39). It was reported that, the colonization of both *D. fragilis* and *Blastocystis*, unlike bacterial composition, diverged between healthy controls and irritable bowel syndrome (IBS) patients. Their colonization was associated with rich and diverse bacterial microbiota; but the association changed in patients with IBS (40).

CONCLUSION

The present study reported a significantly high frequency of *D. fragilis* in *Blastocystis* positive faecal samples. The current finding highlighted the importance of faecal-oral transmission of these two protozoa. The analysis of clinical findings emphasises common asymptomatic colonisation of these protozoans. However, the correlation found in our study may not directly indicate a causality and represent a direct relationship of these two pathogens. This finding provides new insights for future research that includes randomized-controlled studies with larger sample size.

ETHICAL DECLARATIONS

Ethical Committee Approval: The study was reviewed and applied by Non-Interventional Clinical Research Ethics Committee of Aydın Adnan Menderes University Faculty of Medicine (Date: 17.02.2021, Decision No: 2021/37).

Informed Consent: Because of the experimental, retrospective and non-invasive nature of the study design, no written informed consent form was obtained from patients.

Conflict of Interest Statement: The authors declare that they have no conflicts of interest.

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Author Contributions: All the authors participated in the design, in the experimental parts, and in the data analysis of the study. All authors approved the final version of the manuscript.

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Investigation of sinus vein thrombosis cases detected in the emergency department

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ABSTRACT

Objective: Sinus vein thrombosis often presents to the emergency department with headache and is a rare disease among all strokes. Epidemiological data on sinus vein thrombosis are insufficient. Our aim in this study is to examine the findings of patients with sinus vein thrombosis who applied to our emergency department.

Material and Method: 267 patients with suspected sinus vein thrombosis in patients who came to our emergency department between 2013-2018 were included in the study. Patients were divided into 2 groups as present and without sinus vein thrombosis. Women were divided into 3 subgroups as not pregnant, pregnant and postpartum period. Demographic data, comorbid diseases, hemogram parameters and radiological data of the patients were recorded in a form. The relationship of the data with sinus vein thrombosis was examined.

Results: Sinus vein thrombosis was detected in 19.48% of the patients. 76.3% of the patients were women. 65.4% of them were between the ages of 18-40. The most common complaint was headache with 51.9%. And most of the patients' neurological examinations were normal. We found that hemoglobin <8.75 g/dL increased mortality and mean platelet volume was more effective in predicting mortality (AUC=0.846).

Conclusion: Sinus vein thrombosis is more common in women under 40 years of age. While headache is the most common reason for presentation, neurological examination is mostly normal. For this, magnetic resonance imaging and venography should be taken when the disease is suspected. Anemia and mean platelet volume may indicate poor prognosis.

Keywords: Anemia, female, headache, mean platelet volume (MPV), sinus vein thrombosis

INTRODUCTION

Venous vessels of the brain are divided into superficial and deep. The cortical veins and the superior sagittal sinus form the superficial veins. Deep veins, on the other hand, consist of transverse sinus, vein of Galen, straight and occipital sinuses and drain into dural sinuses. Finally, this structure drains into the vena jugularis interna (1). Thrombus in this venous circulation in the brain causes sinus vein thrombosis (SVT) (2). It is a rare type of stroke that is less common than arterial ischemic stroke and prevalent in women and young people (2,3). Its frequency is estimated to be 1-12 per million per year (4). A study was conducted in the Netherlands demonstrating that its rate is 1.32-2.78 per hundred thousand per year in women aged 31-50 years (5). While thrombophilia is the most important risk factor, pregnancy, postpartum period, oral contraceptive drugs, trauma, infection, cancer, and inflammatory diseases increase the risk of SVT (6). Although the symptoms of the patients are in a wide range, the most common type of it which we meet are headaches. Besides, nausea, blurred vision, loss of muscle strength, seizures, altered state of consciousness, and coma may be seen in patients. Neurological findings such as papilledema, cranial nerve findings, and paralysis can be detected (7). Magnetic resonance imaging (MRI) and MRI Venography (MRV) are used for diagnosis (8).

Although SVT is easier to be diagnosed after the occurrence of neurological symptoms, it might be diagnosed late when presented only with a headache. In this study, we aimed to reveal the characteristics of SVT patients diagnosed in the emergency service.

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MATERIAL AND METHOD

Our study was conducted with Ankara Numune Training and Research Hospital Ethics Committee (Date: 28/03/2019, Decision No: E-19-2627). Our study was conducted in accordance with the declaration of Helsinki and good clinical practices. There is no conflict of interest between the authors. Our study was planned as retrospectively observational. As it was in the form of file review over the hospital automation system and as it did not contain images that would enable the identification of the patients, the patients' consents were not obtained.

The files of 657 patients, who came to our emergency department between 2013 and 2018 and underwent magnetic resonance imaging (MRI) were examined. 267 patients over the age of 18 and having MRI venography were included in the study. 390 patients, who were excluded from the study, did not have MRI venography imaging. The imaging of different anatomical regions was done in these patients due to trauma and other reasons. A form was prepared for the study. The demographic data of the patients, their accompanying diseases, their complaints of presentation to the emergency service, neurological examination findings of them, pregnancy periods of women (divided into 3 groups as non-pregnant, pregnant, and postpartum period) were included in the study. In the relationship between SVT and age; they were grouped as follows: group 1: 18-40 years old, group 2: 41-65 years old, and group 3: 66 years old. Hemogram parameters were analyzed from laboratory values. Among these, Leukocyte (WBC-reference range (RA): 4.8-10.8 10^3/μL), Neutrophil (Neu-RA: 1.8-7.7 10^3/μL), Lymphocyte (Lym-RA: 1-4.8 10^3/μL), Hemogram (Hgb-RA: 12-16 g/dL), Erythrocyte Distribution Width (RDW-RA: 11.5-14.5% CV), Platelet (PLT-RA: 130-400 10^3/μL), Mean Platelet Volume (MPV-RA: 7-12 fL), Platelet Distribution Width (PDW-RA: 9-17%) were recorded. Computerized Brain Tomography (CBT) and MRI-MRV reports were examined from the radiological images of the patients. SVT status and other diseases detected in the patients were determined according to these reports and epicrisis records. In-hospital mortality of the patients was recorded. The data was recorded by two emergency medicine specialists. The other two emergency medicine specialists checked the data.

The patients were divided into two groups as with SVT and without SVT. Besides, three subgroups were created according to the pregnancy status of women as follows: no pregnancy, pregnant and postpartum period. Relationships between admission symptoms, neurological examination findings, hemogram parameters, CBT, and MRV results were examined among the groups. The presence of SVT according to the gestational period, which constitutes our subgroup, and the effects of the anatomical variation in the sinuses on the symptoms were statistically determined.

MRV is mostly used to confirm sinus vein thrombosis. In addition, observing isointense or hyperintense filling in the sinus in T1, T2, and FLAIR sequences is an important finding to distinguish SVT from anatomical variations (1). The postpartum period includes the 6-month period after the termination of pregnancy (9).

Venous blood samples were obtained in Vacutainer tubes of Becton Dickinson (BD diagnostics, Plymouth, UK) compatible with potassium EDTA. Samples were analyzed on XT-2000i (Sysmex Corporation of America, Long Grove, Illinois, USA). Computerized tomography system was Toshiba Aquilion 64-slice CT (Toshiba Medical System Corporation, Shimoishigami, Otawara-Shi, Japan). The magnetic resonance imaging device was 1.5 tesla (General Electric (GE) Healthcare, USA).

Statistical Analysis

SPSS 22.0 for Windows software was used for statistical analysis. Shapiro Wilks test was used to determine whether the data showed normal distribution. Descriptive statistical analyses were used to evaluate demographic data and data collected from tests and scales. Independent sample T-test was used to compare the data. Data were expressed as mean±standard deviation and percentages. $P < 0.05$ was considered statistically significant. In the independent sample T-test p values showed a statistically significant or non-significant inverse interaction.

RESULTS

Sinus vein thrombosis was detected in 19.48% of the patients. When we look at the gender and age distribution of SVT, 76.3% of those, who had SVT, were women. While the mean age of patients with SVT was 37.9, it was 34.7 for patients without SVT. 65.4% of those, who had SVT, were between the ages of 18-40, and 11.5% of them were over 65 years old. No statistically significant difference was found between age groups (p=0.152). The gender distribution and comorbid diseases of the patients are summarized in **Table 1**.

No significant difference was found between the patients' probability of having comorbid diseases (p=0.581). The relationship of age and gender with SVT is summarized in **Table 2**.

When the relationship between SVT and general symptoms was examined by using an independent sample T-test, no difference was found between the two groups with p=0.435. When the relationship between SVT and neurological examination findings was examined with an independent sample T-test, there was a significant relationship between the two groups with p=0.044. It was seen that normal examination findings were higher with a rate of 75% (n=39) in those with SVT. These findings are summarized in **Table 3**.

Table 1. Demographic data of the patients

Variables			n		%	
		SVT*	Yes	No	Yes	No
Gender	Male		9	58	13.4	86.6
	Female	Not pregnant	22	100	18	82
		Pregnant	15	45	25	75
		Postpartum	6	12	33.3	66.7
		SVT	Yes		No	
Comorbid disease	None		39 (75%)		162 (75.3%)	
	Diabetes mellitus		3 (5.8%)		7 (3.3%)	
	Rheumatic disease		-		6 (2.8%)	
	Eplepsy		2 (3.8%)		5 (2.3%)	
	Preeclampsia		1 (1.9%)		-	
	Chronic renal failure		-		1 (0.5%)	
	Abortion		1 (1.9%)		2 (0.9%)	
	Thyroid disease		2 (3.8%)		-	
	Sinus vein thrombosis		1 (1.9%)		5 (2.3%)	
	Migraine		-		6 (2.8%)	
	Perianal abscess		-		4 (1.9%)	
	Hypertension		-		1 (0.5%)	
	Pseudo tumor cerebri		2 (3.8%)		7 (3.3%)	
	Psychiatric illness		-		1 (0.5%)	
	Multiple sclerosis		-		1 (0.5%)	
	Coronary artery disease		-		2 (0.9%)	
	Lymphoma		-		1 (0.5%)	
	Cerebro vascular disease		-		1 (0.5%)	
	Deep vein thrombosis		-		1 (0.5%)	
	Peripheral vertigo		-		1 (0.5%)	
	Trigeminal neuralgia		-		1 (0.5%)	
	HT and PTS **		1 (1.9%)		-	

* SVT: Sinus vein thrombosis, ** HT: Hypertension, PTS: Pseudo tumor cerebri

Table 2. Age and gender relationship with SVT

Variables			SVT* Yes (n)	SVT No (n)	p
Gender	Male		9	58	
	Female	Not pregnant	22	109	
		Pregnant	15	50	0.149
		Postpartum	6	15	
Age		18-40	34	156	
		41-65	13	49	0.152
		Above 66	5	10	

*SVT: Sinus vein thrombosis

Table 3. Relationship between symptoms and examination with MRV in patients with SVT

Variables		SVT* Yes		SVT No		p
		n	%	n	%	
Symptoms	Headache	27	51.9	96	44.6	
	Nausea, vomiting, vertigo	4	7.7	17	7.9	
	Fever	-	-	1	0.5	
	Seizure	4	7.7	23	10.7	
	Defect of vision	6	11.5	25	11.6	
	Consciousness change	3	5.8	18	8.4	0.435
	Loss of strength & hypoesthesia	7	13.5	32	14.9	
	Speech disorder	1	1.9	3	1.4	
Examination	Normal	39	75	188	87.5	
	Ptosis	1	1.9	1	0.5	
	Pleji	4	7.7	9	4.2	0.044
	Homonymous hemianopsia	-		3	1.4	
	Parazi	4	7.7	4	1.8	
	Facial asymmetry	1	1.9	5	2.3	
	Cranial nerve sign	3	5.8	4	1.8	
	Aphasia	-		1	0.5	

*SVT: Sinus vein thrombosis

197 patients underwent CBT. Sixty-five of the 70 patients did not undergo CBT as they were pregnant, and five patients did not undergo CBT as they did not accept the tomography shooting. No acute pathology was found in the results of 172 (87.4%) patients. 13 (6.6%) patients with venous infarction in the CBT were detected and 12 (6.1%) of them had SVT in MRV and 1 had sinus hypoplasia. Intracranial bleeding was seen in 4 (2%) patients. SVT was detected in one of them. Intracranial mass was seen in 4 (2%) patients, arterial ischemia in 2 (1%), arteriovenous malformation in 1 (0.5%) and cerebral edema in 1 (0.5%) patient.

When the relationship between SVT and MRV is examined with an independent sample T-test; thrombus in MRV was detected as 100% (n=52) in patients with SVT. The correlation between those with general symptoms and MRV findings was investigated, but no relationship was established (R=0.025, p=0,689). The correlation between those with neurological examination findings and MRV findings was investigated, but no relationship was established (R=-0.097, p=0.127).

The mean values of the hemogram parameters are summarized in **Table 4**. When we look at whether there is a cut-off value between mortality and Hgb or not, the 'Area under the curve' (AUC) value was found to be 0.652. However, an increase in mortality was observed if Hgb is <8.75 g/dL from the relationship between the cut-off value 1-specificity and sensitivity. Besides, the AUC value of MPV was found to be 0.846. It was found to be more valuable in predicting mortality than other hemogram parameters. The AUC values of the hemogram parameters are shown in **Figure 1**.

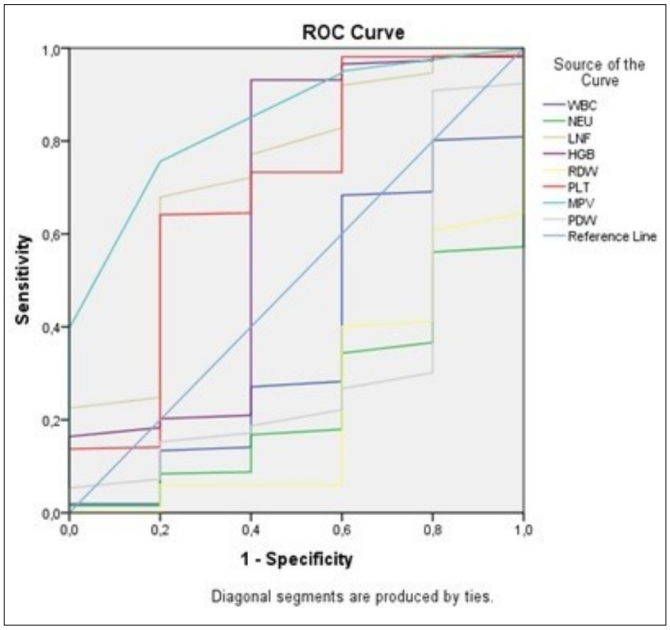

Figure 1. Relationship of hemogram parameters with mortality

Table 4. Relationship between hemogram parameters and mortality

Variables	SVT* Yes		SVT No	
	Alive (n)	Dead (n)	Alive (n)	Dead (n)
Total	48	4	214	1
Leukocyte	10.4417	10.2500	10.0637	20.4000
Neutrophil	7.4125	8.6500	6.8566	16.8000
Lymphocyte	2.1625	1.1500	2.3896	2.9000
Hemoglobin	13.2917	10.9000	13.2121	14.9000
Red blood cell distribution width (RDW)	14.2479	15.5500	14.1346	15.4
Thrombocyte	265.7917	180.2500	275.4121	346
Mean Platelet Volume (MPV)	9.2167	8.0000	9.2912	7.0
Platelet Distribution Width (PDW)	13.5271	15.1000	13.7703	16.9

*SVT: Sinus vein thrombosis

DISCUSSION

In our study, we found that SVT is more common in women under 40 years of age in the emergency service and that the headache is the most common symptom. In these patients, we mostly could not find any findings on neurological examination. MRV was of high importance for diagnosis. We found that MPV, one of the hemogram values, was more significant than other parameters in predicting mortality.

Although the intercontinental rate of SVT varies, it is more common in women than in men (10). It is seen more than twice in women, with a rate of 74.5% (11). In our study, it was seen as 76.3% for women as in line with the literature. We believe that its higher prevalence in women is due to the fact that some of the disease's identified risk factors are only found in women. It is known that pregnancy, postpartum period, and use of oral contraceptives are important risk factors for SVT formation. This situation also causes the disease to be seen more frequently in young women (12). Our study contains results in this direction. 58.3% of women diagnosed with SVT were in pregnancy or postpartum period. When we look at the age distribution in all patients, it was concentrated between the ages of 18-40 (65.4%). While the mean age was 39.1 according to the International Study on Cerebral Vein and Dural Sinus Thrombosis (ISSVT) (11), it was found to be 41.24 according to the study by Sidhom (13) et al. In our study, we found it as 37.9. However, there was no statistical difference between age groups in the age distribution (p=0.152). When we look at the ratio of SVT between genders in our study, the incidence of SVT was roughly 5-times higher in women than in men. However, there was no statistical significance of SVT between genders (p>0.149). This situation shows us that there are other risk factors besides gender and hormonal factors. Other risk factors include infection, coagulation disorder, malignancy, anemia, thrombocytosis, drugs, and trauma (14). As a limitation of our study, these data were missing from the records. However, 25% (n=13) of our patients with SVT had a comorbid disease. The most common accompanying disease was Diabetes mellitus (DM) 5.8% (n=3). It was found that DM was accompanying at the rate of 17% and 7% in SVT patients in some studies (14,15).

Headaches are the most common cause of SVT. Besides, other symptoms are nausea, vomiting, visual impairment, focal neurological deficit, change in consciousness, epileptic seizure, speech disorder, and hypoesthesia (2,10). While the headache was the most common reason (87.2%) for admission in the VENOST study conducted by Duman et al. (2), Lee et al. (16) found this rate as 63.4%. In our study, we found that headache (51.9%) was the most common presenting symptom. In addition, hypoesthesia 13.5% and visual impairment 11.5% were other common symptoms. Despite the fact that the rate of headache is the most common symptom in research, the prevalence varies from each other. The reason for this may be due to the fact that SVT of all strokes is <1% (10) and that the studies conducted did not include a great number of patients. Maali et al. (10) showed in their literature study that this rate varies between 28-36% according to continents. Besides, the headache does not have a typical feature for SVT (7). In our study, we found that headache was statistically insignificant in determining SVT (p=0.435). This showed us that headache was an important symptom for SVT, but that it was insufficient for differential diagnosis. While focal neurological findings are 15% if the presentation is in good time, it can increase up to 50% when it is delayed (17). This rate was found to be 15.4% in our study. We think this is due to the early admission of patients to our emergency service and to the easy accessibility of our MRV facility. Kamışlı et al. (18) found the rate of neurological deficit to be 47%. In our study, we found the presence of neurological findings to be statistically more significant in terms of SVT than patients without SVT (p=0.044).

In emergency services, CBT is used for patients with neurological complaints. Its sensitivity is low in the diagnosis of SVT (19). In our study, 197 patients underwent CBT. Venous infarction was seen in 13 patients (6.6%). The results of 12 patients (6.1%) were consistent with MRV and SVT. Sinus hypoplasia was detected in 1 patient. MRI and MRV have sensitivity and specificity for the diagnosis of 95% SVT (19). The European Stroke Organization (ESO) shows MRV as a good alternative to digital subtraction angiography (DSA) for the diagnosis of SVT (20). We showed thrombus along with MRV in all our patients whom we diagnosed with SVT. We detected SVT in 19.5% (n=52) of 267 patients suspected of SVT, and anatomical variation in the sinuses in 25.8% (n=69). The anatomical variation in the sinuses in MRV is between 25-36% and mostly it has no clinical result (21). When we looked at the correlation between the symptoms and findings of our patients and sinus variations in MRV, we could not find a correlation (respectively, R=0.025, p=0.689/ R=-0.097, p=0.127).

It has been shown that low Hgb may be a risk factor for SVT. Studies have reported that the association with anemia is between 7-27% in SVT patients (22). This situation has been attributed to the fact that anemia reduces the oxygen-carrying capacity and that it causes changes in blood viscosity (23). When we look at the mortality relationship between SVT and Hgb, we found AUC as 0.652. The fact that insufficient results in predicting mortality may be due to the low number of patients who died. Nevertheless, we analyzed a cut-off value between mortality and Hgb. The fact that Hgb decreases below 8.75 g/dL showed that mortality increased. One study showed that SVT is associated with anemia at a rate of 30.67%. As the anemia deepened, it was found that there was a poor outcome (endpoint) (23). Kamışlı et al. found a significant relationship between increased MPV value and SVT related to MPV, another one of the hemogram parameters. They concluded that this situation could be a predictor for poor prognostic outcomes (24). In our study, the AUC value of MPV was higher than the others (AUC=0.846). There are two different opinions about the relationship of MPV with thrombosis. It is thought MPV highness poses a risk for thrombus development. Increased volume of platelets may facilitate thrombus development. Another view is that the formed thrombus triggers the formation of a new platelet. Young and large platelets participate in the circulation. In addition, these young platelets respond poorly to antithrombotic therapies. These two theories are still debated. However, it is a common opinion that the increase of MPV is associated with poor outcome (25).

Limitations

The fact that it is a study conducted in a single-center hospital causes the number of patients to be low in terms of rare diseases. However, our easy access to MRV provided ease of obtaining the diagnosis in every patient we suspected. This enabled us to diagnose more than 19% of SVT among patients who undergone MRV. The retrospective nature of our study led to some data deficiencies. In particular, data on risk factors were insufficient. For us, the most important deficiencies in our study were 'the use of oral contraceptives, the absence of genetic research on coagulation disorder, and the lack of information on trauma and malignancy'. Our third limitation is that, due to our low mortality numbers, our analysis that we did with hemogram parameters caused insufficient results.

CONCLUSION

Especially newly developing headache that does not respond to analgesia in women under the age of 40 should suggest SVT. MRV shooting for diagnosis has a high confirmation rate. Hemogram is an analysis that is frequently checked in the emergency service. MPV is more significant in predicting mortality than other hemogram values. The retrospective nature of our study has some limitations. Therefore, multi-center prospective controlled studies will yield more specific epidemiological results for SVT. Besides, more studies are needed to reveal the relationship between Hgb and thrombocyte measurements and the prognosis of the disease. If anemia and increased MPV are indicators of poor prognosis, this situation may be a guide for early treatment of anemia and effective antithrombotic therapies for young thrombocytes.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was conducted with Ankara Numune Training and Research Hospital Ethics Committee (Date: 28/03/2019, Decision No: E-19-2627).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The effect of restorative dentistry practices on the vital signs of healthy individuals

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ABSTRACT

Aim: Clinical applications of dentistry, the tools and drugs used increase the effects of anxiety and stress factors that we encounter before the treatment, but also cause changes in the life symptoms of the patients. In this study, it is aimed to evaluate the effects of Restorative Dentistry applications on vital signs parameters such as blood pressure, heart rate, body temperature and oxygen saturation of healthy individuals before and after the interventional procedure.

Material and Method: In the study, blood pressure, pulse rate, body temperature and oxygen saturation values of 100 patients without any systemic disease were measured before clinical applications. Following the treatment intervention, the same parameters were measured again and evaluated by comparing the previous and next values.

Result: In the patients who were not applied local anesthesia, the post-treatment measurement values of the body temperature parameter were significantly higher than the initial measurement values ($p<0.05$). There was no statistically significant difference between the first and last measurement values of other parameters ($p>0.05$). In patients who were applied local anesthesia, the last measurement values of body temperature and systolic blood pressure were significantly lower than the initial measurement values ($p<0.05$). There was no statistically significant difference between the first and last measurement values of diastolic blood pressure and other parameters ($p>0.05$).

Conclusion: We think that measures such as a relationship based on mutual trust, approaching expectations with understanding, informing about interventional procedures and operating consent processes will prevent patients from anxiety during the session and keep vital parameter values at a normal level.

Keywords: Restorative dentistry, blood pressure, body temperature, oxygen saturation, pulse

INTRODUCTION

Vital findings are the parameters that determine the basic functions and physiological state of the body, in short, whether a person is healthy or not. Any deviation from the basic functions of the body is considered a sign of illness. The most important basic vital signs are body temperature, blood pressure, pulse rate and respiratory rate. Today, while oxygen saturation and pain parameters are evaluated as vital signs, the idea that blood glucose level is the vital parameter is not adopted (1,2).

It is possible for the body to carry out its normal functions if the body temperature remains constant within a certain limit. The hypothalamus region of the brain plays an important role in regulating body temperature. The most important factors affecting body temperature are age, gender, physical activity, daily heat cycle, emotional state, environment, hormonal factors and some drugs used (3,4). The average body temperature of a healthy young adult is higher than that of the elderly, and ranges from about 36-38°C. Normal body temperature in women varies compared to men, depending on the hormonal effect. Body temperature, which is low in the morning, rises during the day. In addition, with the stimulation of the sympathetic nervous system due to the increase in stress, the amount of epinephrine and norepinephrine secretion increases, metabolism accelerates and body temperature rises (4). Body temperature can be measured by conventional methods (oral, underarm, ear, rectal) or non-contact infrared thermometers. It is easy to use and it is possible to read the body temperature from the indicator in these battery operated thermometers (3).

Blood pressure refers to the pressure that blood exerts on the artery wall. Blood pressure is expressed in terms of

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mercury pressure per mm^2 (mm/Hg). During heartbeat, two different pressure values called systolic and diastolic are formed in the vessel. The normal value of systolic blood pressure in an adult individual, which is defined as the highest pressure exerted by the blood on the vascular wall during the contraction of the heart, is 120 mmHg. It is accepted that the normal value of diastolic blood pressure in an adult individual is 80 mmHg, which is defined as the lowest blood pressure measured at the vascular wall during heart relaxation (3).

Blood pressure measurement can be performed on both arms of an individual sitting and at rest. Repeated measurements should generally be based on the measurement in the arm where the higher value is obtained. Constant blood pressure out of normal values is considered a sign of illness. There may be only systolic or diastolic pressure increase, or both can be seen (3).

The main factors affecting blood pressure are age, gender, race, diet, smoking and alcohol use, physical exercise and some diseases. Blood pressure increases due to the increase in heart rate due to the stimulation of the sympathetic nervous system by some psychological factors such as pain or anxiety. In addition, some narcotic analgesics can cause low blood pressure (4-6). Blood pressure is measured with different blood pressure devices (mercury, mechanical and electronic) called sphygmomanometers. The most widely used technique is the auscultation technique, in which a stethoscope is also used in addition to the sphygmomanometer (2).

In each contraction of the heart, some blood is thrown into the arteries and vessel dilation occurs. This enlargement can be easily felt where the veins are close to the skin surface. This condition, which is repeated with every contraction of the heart, is defined as a pulse. Pulse rate, defined as the heart's rate of beats per minute, is an indicator of heart rate and rhythm (2). The resting heart rate in a healthy adult is between 60 and 100 per minute (3,4).

The main factors affecting the pulse rate are age, weight, physical exercise, high fever, bleeding, pain, systemic diseases, emotional stress and some medications used. While the pulse rate is higher at a young age, it decreases over time. In case of acute pain and increased anxiety, the pulse increases with the stimulation of the sympathetic system. In addition, chronic and long-term pains slow the pulse by stimulating the parasympathetic system. With the increase in blood volume, the pulse feels fuller. The radial artery is the area where the peripheral pulse is taken most easily in adults. The radial artery is easier to localize (3,4).

The easiest symptom to evaluate among vital signs is respiration. Respiratory rate is the number of breaths given by an individual per minute, and the resting respiratory rate in a healthy adult varies between 16-20 per minute. For approximately every 4 heartbeats, 1 breathing occurs. As the amount of CO_2 in the blood increases, the number and depth of breathing increases (1). The main factors affecting respiration are age, body temperature, physical exercise, some diseases and some medications used. In children and individuals over the age of 65, the respiratory rate tends to be higher. Pain and some psychological problems stimulate the sympathetic nervous system, thus increasing the speed and depth of breathing. Narcotic analgesics (morphine, diazem, etc.) negatively affect the rate and depth of respiration (3,4).

Oxygen saturation refers to the oxygen level in the blood circulation of the individual. Under normal conditions, the blood oxygen saturation level of a healthy person should be between 90 and 100. Devices that can measure the oxygen level in the blood in the easiest and fastest way are called pulse oximeters. With these devices, the percentage of hemoglobin saturated with oxygen is measured and recorded as peripheral oxygen saturation (SpO_2) (1,2,7).

The emotional stress that the patient is exposed to before or during dental treatment is called dental anxiety. These types of stress and anxiety cause changes in vital signs, especially cardiovascular and respiratory systems (6,7). The patient's age, gender, personality, expectations, previous treatment experiences and fear of local anesthesia are factors affecting the anxiety level. It is inevitable that such anxieties affect many parameters such as body temperature, blood pressure, pulse and respiratory rate, as well as mental state and behavior (2,5,6).

The aim of this study is to examine the effect of restorative treatment procedures on vital parameters (body temperature, blood pressure, pulse rate and oxygen saturation) measured before and after treatment and to investigate whether these changes remain within physiological limits.

MATERIAL AND METHOD

Participants

In our study, a total of 100 people (50 females, 50 males), between the ages of 16-50, who applied to Dicle University University Faculty of Dentistry, Department of Restorative Dentistry and did not have any systemic disease were included in the study. Before and after the interventional procedure, blood pressure, pulse rate, body temperature and oxygen saturation values were measured twice in total, and the informed consent form was read and signed by the patients to be compared.

Ethics Statement

Participants were informed about the purpose of the study and their consent was obtained by paying attention to the principle of volunteering. It was also ensured that the information about the participants would be kept confidential. Approval for the study was given by the Ethics Committee of Dicle University University Faculty of Dentistry (Date: 29.05.2019, Decision No: 2019/6). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Evaluation of Vital Signs

Clinical and radiological examinations of the patients whose anamnesis were taken were made and their treatments were started. Before the procedure, the vital signs of the patients who underwent routine restorative dental procedures such as cavity opening, placement of the base material, application of the adhesive system, composite resin restoration were measured and recorded. After routine treatment procedures, the effects of the procedures performed by measuring the vital signs for a second time on the vital signs parameters and whether they are within the physiological limits were investigated.

A manual sphygmomanometer with a stethoscope was used to measure systolic and diastolic blood pressure values and recorded in mmHg. Pulse rate values as well as oxygen saturation were determined with the pulse oximeter device attached to the fingertip. Body temperature was measured and recorded in degrees Celsius with a non-contact digital thermometer.

RESULTS

The data recorded before and after the treatment were analyzed with IBM SPSS Statistics package program. The Shapiro-Wilk test was used to investigate the status of the variables coming from the normal distribution, due to the unit numbers. While examining the intragroup differences, Paired Samples T-Test was used as the variables came from normal distribution. While interpreting the results, the level of significance was determined as 0.05, it was stated that there was a significant difference when $p<0.05$, and that the difference was not significant when $p>0.05$.

In patients who did not undergo local anesthesia, a statistically significant difference was observed between the first and last measurement values in terms of only the body temperature parameter ($p<0.05$). The last measurement values of body temperature were significantly higher than the first measurements (**Figure 1**). There was no significant difference between the first and last measurement values of other parameters ($p>0.05$).

A statistically significant difference was found between the first and last measurement values in terms of fever and systolic blood pressure parameters in patients undergoing local anesthesia ($p<0.05$). While the last measurement values of body temperature were significantly higher than the first measurements, the last measurement values of systolic blood pressure were found to be significantly lower than the first measurements (**Figure 2**). There was no significant difference between the first and last measurement values of other parameters ($p>0.05$). Regardless of the anesthesia application, a positive and moderately significant correlation was found between the first and last measurement values of all parameters ($p<0.05$).

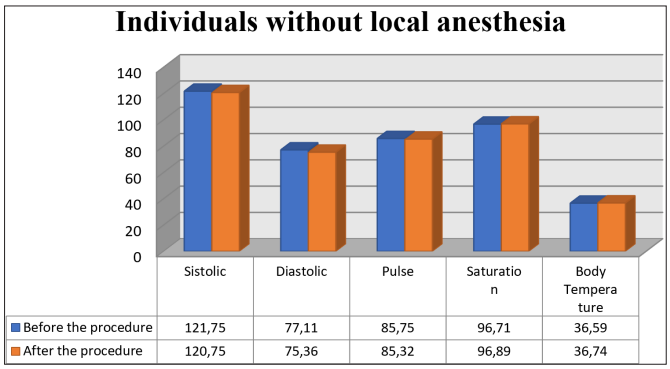

Figure 1. The distribution of vital signs measurement values before and after the procedure in patients who were not applied local anesthesia.

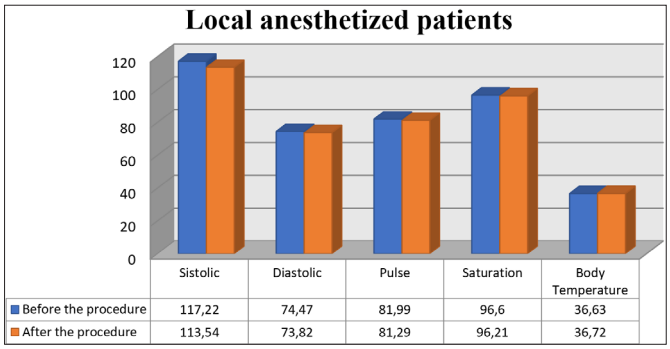

Figure 2. Distribution of vital signs measurement values before and after the procedure in locally anesthetized patients.

In all male and female patients, a statistically significant difference was observed between the first and last measurement values in terms of only the body temperature parameter ($p<0.05$). The last measurement values of body temperature were significantly higher than the first measurements (**Figure 3,4**). There was no significant difference between the first and last measurement values of other parameters ($p>0.05$). When the first and last values of the saturation parameter were excluded in female patients, a positive and moderately significant correlation was found between the first and last measurement values of all other parameters, regardless of gender ($p<0.05$).

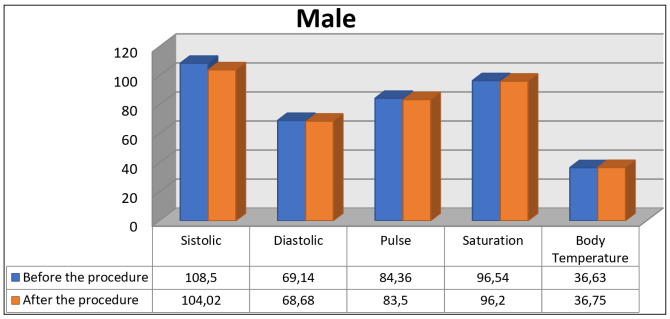

Figure 3. Distribution of vital signs measurement values before and after the procedure in male patients

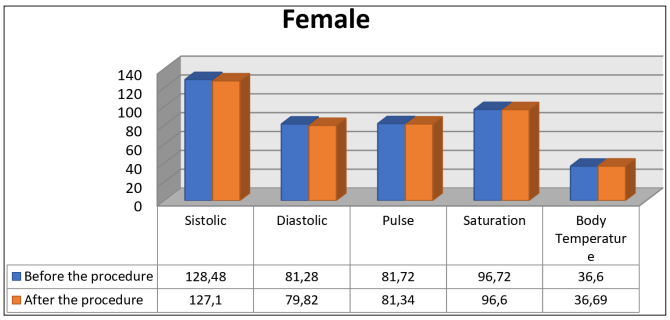

Figure 4. Distribution of vital signs measurement values before and after the procedure in female patients

DISCUSSION

Clinical practices in dentistry cause changes in the life symptoms of patients by increasing the effects of anxiety and stress factors. Anxiety has been defined as an emotional response accompanied by physical symptoms. It is a pathological uneasiness condition that is generally seen at different times in every person and develops due to fear. After a certain stage, it causes mental problems and negatively affects the vital activities and relationships of the individual. For example, blood pressure and pulse increase in the person, and physiological symptoms similar to sweating occur (10,11).

Dental anxiety is expressed as the feeling that bad things will happen during the dental treatment of the patient and the anxiety of feeling pain. Today, despite the widespread use of local anesthetics and analgesics, the absence of a decrease in the frequency and severity of dental anxiety refutes the idea that the main etiological factor is pain. In addition to the negative experiences of the person in the past, rotary devices, light devices, amalgam and compomer guns, hand tools such as probe and presses, acid injectors, injection process and waiting time also play a role in the increase of such concerns (12).

Patients generally feel more fear during waiting than when they are treated. At the root of this fear lies the anxiety of losing the teeth or physical integrity, especially the pain. The most important clinical findings are irregularity in breathing, tightening of the teeth and jaw, muscle tension, sudden silence of a talkative person or excessive conversation of a calm person, the desire to spit frequently and mouthwash, holding the physician's arm, discomfort and restlessness, respectively (12).

Age, gender, education level or socio-economic status are the main factors affecting dental anxiety level. In a study by Gedik et al. (13), they claimed that patient age, gender, education, local anesthetic volume, length of treatment and difficulty of procedure changed body parameters. Especially in young patients, it has been claimed that the level of dental anxiety is higher, however, Ay et al. (14) and Öcek et al. (15) stated that there is no relationship between age and anxiety level. In our study, it was not possible to comment on whether there was a relationship between age and anxiety level, since the distribution of 100 patients between the ages of 16-50 was not homogeneous across age groups.

Studies have shown that the higher anxiety level of women compared to men is not because they fear more, but because they express their feelings more easily (16-18). In their study, Muğlalı and Kömerik (19) attributed the lower dental anxiety level of men to the belief that men in our society should be more durable and brave, and they did not reveal their fears. Özdemir et al. (20) explained that the anxiety level in men is higher than in women. In our study, unlike these studies, no significant difference was found between the anxiety levels of men and women. The last measurement values of body temperature in all male and female patients were significantly higher than the first measurements ($p<0.05$). There was no significant difference between the first and last measurement values of other parameters ($p>0.05$).

It has been reported that patients with high socio-economic and educational levels have lower dental anxiety levels. It has been claimed that the anxiety levels of the patients decrease with the increase in the level of education and the awareness of the patients (21). This result is explained by the fact that patients with high education level cope more easily with stress. In addition, there are studies that argue that there is no relationship between education level and dental anxiety (22). In our study, the effect of patients' socio-economic status and education level on dental anxiety was excluded.

Informing patients in advance about the procedures to be performed with informed consent will provide some reduction in anxiety (12). However, sharing more than necessary medical details and possible complication information in the consent form may increase the anxiety level of the patients. In a study in which Casap et al. (23) investigated the effect of the informed consent form on the anxiety level of patients, it was stated that the heart rate increased, but blood pressure and saturation did not change statistically. In our study, an enlightened consent form was read before the treatment, and the anxiety of the

Table 1. Analysis of patients' first and last vital signs according to local anesthesia application status

Anesthesia	Parameters Before the procedure (b.p) After the procedure (a.p)	Mean	N	Std. Deviation	Std. Error mean	P	Correlation	P
Patients without local anesthesia	Sistolic (b.p)	121.75	28	13.72	2.59	0.698	0.67	**0.001**
	Sistolic (a.p)	120.75	28	18.04	3.41			
	Diastolic (b.p)	77.11	28	11.40	2.15	0.355	0.624	**0.001**
	Diastolic (a.p)	75.36	28	11.27	2.13			
	Pulse (b.p)	85.75	28	15.44	2.91	0.837	0.707	**0.001**
	Pulse (a.p)	85.32	28	10.99	2.07			
	Saturation (b.p)	96.71	28	2.43	0.46	0.624	0.637	**0.001**
	Saturation (a.p)	96.89	28	1.89	0.35			
	Body temperature (b.p)	36.59	28	0.32	0.06	**0.037**	0.403	**0.033**
	Body temperature (a.p)	36.73	28	0.30	0.05			
Patients undergoing local anesthesia	Sistolic (b.p)	117.22	72	13.98	1.64	**0.039**	0.649	**0.001**
	Sistolic (a.p)	113.54	72	19.43	2.29			
	Diastolic (b.p)	74.47	72	12.40	1.46	0.464	0.82	**0.001**
	Diastolic (a.p)	73.82	72	12.64	1.49			
	Pulse (b.p)	81.99	72	10.94	1.29	0.411	0.771	**0.001**
	Pulse (a.p)	81.29	72	9.92	1.16			
	Saturation (b.p)	96.60	72	2.10	0.24	0.186	0.316	**0.007**
	Saturation (a.p)	96.21	72	2.11	0.24			
	Body temperature (b.p)	36.62	72	0.30	0.03	**0.011**	0.477	**0.001**
	Body temperature (a.p)	36.71	72	0.27	0.03			

Table 2. Analysis of patients' first and last vital signs by gender

Gender	Parameters Before the procedure (b.p) After the procedure (a.p)	Mean	N	Std. Deviation	Std. Error mean	P	Correlation	P
Male	Sistolic (b.p)	108.5	50	10.35	1.46	0.064	0.368	**0.009**
	Sistolic (a.p)	104.02	50	17.44	2.46			
	Diastolic (b.p)	69.14	50	10.45	1.47	0.712	0.678	**0.001**
	Diastolic (a.p)	68.68	50	11.26	1.59			
	Pulse (b.p)	84.36	50	12.62	1.78	0.465	0.762	**0.001**
	Pulse (a.p)	83.5	50	10.76	1.52			
	Saturation (b.p)	96.54	50	2.565	0.36	0.34	0.49	**0.001**
	Saturation (a.p)	96.2	50	2.365	0.33			
	Body temperature (b.p)	36.634	50	0.3402	0.048	**0.016**	0.467	**0.001**
	Body temperature (a.p)	36.754	50	0.3208	0.04			
Female	Sistolic (b.p)	128.48	50	9.20	1.30	0.413	0.485	**0.001**
	Sistolic (a.p)	127.1	50	13.11	1.85			
	Diastolic (b.p)	81.28	50	10.62	1.50	0.185	0.739	**0.001**
	Diastolic (a.p)	79.82	50	10.62	1.50			
	Pulse (b.p)	81.72	50	12.16	1.72	0.751	0.726	**0.001**
	Pulse (a.p)	81.34	50	9.88	1.39			
	Saturation (b.p)	96.72	50	1.76	0.24	0.698	0.224	**0.118**
	Saturation (a.p)	96.6	50	1.72	0.24			
	Body temperature (b.p)	36.598	50	0.2818	0.0398	**0.024**	0.421	**0.002**
	Body temperature (a.p)	36.69	50	0.2288	0.0324			

patients was tried to be reduced by making explanations about the procedure to be performed.

The level at which various dental practices affect anxiety levels is also different. Wong and Lyte (24) in a study where they examined the effects of 8 different dental procedures on anxiety levels, concluded that there was a higher anxiety towards root canal treatment and surgical procedures. While a moderate level of anxiety developed against restorative treatment and prosthetic procedures, a low anxiety response occurred to tartar cleaning and examination procedures.

Dental anxiety decreases the quality of life of the person and can cause changes in body temperature, blood pressure, pulse and respiratory rate as well as psychosomatic symptoms (25). In a study by Salma et al. (6), it was determined that even a simple treatment

intervention has an impact on vital values. They reported that tooth extraction, tartar cleaning and restorative treatment procedures cause an increase in body temperature and oxygen saturation values. Gedik et al. (9) reported that blood pressure, heart rate and body temperature decreased significantly after gingivectomy, and that blood pressure and pulse did not change after frenectomy and curettage procedures, but body temperature increased significantly. In our study, the last measurement value of body temperature was found to be significantly higher than the first measurement in patients who were not applied local anesthesia and who were applied local anesthesia with vasoconstrictor ($p<0.05$). Excluding systolic blood pressure, no significant difference was found between the first and last measurement values of the other parameters ($p>0.05$).

It is claimed that local anesthesia and preparation procedures cause higher anxiety in terms of image and sound. Even if the anesthesia procedure will make the treatment application painless, it increases the anxiety level of the patient before the treatment. Nakamura et al. (26) stated that local anesthesia applied to the patient before dental surgery caused a significant increase in heart rate and systolic blood pressure. Elad et al. (27) reported that while restorative treatments performed under dental anesthesia in patients with a history of ischemic heart disease, insignificant increases in systolic blood pressure were recorded, there was no change in the number of heartbeats. In our study, contrary to these findings, the last measurement values of systolic blood pressure were found to be significantly lower than the first measurements in patients who underwent local anesthesia with vasoconstrictor ($p<0.05$). Excluding body temperature, there was no significant difference between the first and last measurement values of the other parameters ($p>0.05$).

Tomeva et al. (28), in a study in which they evaluated vital signs and hemodynamic changes after local anesthesia, did not find an increase in systolic and diastolic blood pressure, they explained that the pulse rate changed slightly, but this change was not statistically significant.

Salma et al. emphasized that the oxygen saturation value during local anesthesia injection exceeded normal physiological limits in some patients compared to the treatment procedure (6). Amoian et al. (29) stated that there is no statistically significant difference in the oxygen saturation level measured by pulse oximetry at different stages of periodontal surgery. Alemany-Martinez et al. (30) also observed the pulse, blood pressure and oxygen saturation values of healthy patients during the extraction of mandibular 3rd molar teeth under local anesthesia. It was determined that the increase in pulse rate observed during the surgical incision in patients with high anxiety returned to normal after a while. It has been reported that systolic and diastolic blood pressure increased slightly and there was no significant change in oxygen saturation value during osteotomy and dissection of the tooth.

The vasoconstrictor nature of local anesthesia can cause cardiovascular complications in addition to catecholamine secretion in patients with anxiety (30). In a different study by Mohammad Ketabi et al. (13), it was stated that the application of local anesthesia with vasoconstrictor increased blood pressure and pulse rate, but this increase was within medically and clinically normal limits. Laragnoit et al. (25) reported that there was no increase in heart rate and blood pressure during dental treatments where they applied vasoconstrictor local anesthesia on people with heart disease. Güngörmiş et al. (31) could not detect a significant effect on blood pressure and pulse values in patients who used vasoconstrictor local anesthesia. Faraco et al. (32) examined the effects of anesthetics containing epinephrine and lidocaine on the cardiovascular system during dental implant surgery and emphasized that there was no significant change in blood pressure and pulse values . In our study, it was concluded that the vital signs parameters were not significantly affected by the restorative treatment procedures in the evaluations made considering the gender difference and whether local anesthesia was performed or not.

Most of the dentists worry about dental anxiety patients and do not know what to do and do not try to solve the problem. We think this is due to lack of information. In dental treatment of such patients, pharmacological methods, behavioral approaches, additional time, patience and energy are needed. Patients with high levels of anxiety can usually cope with this, with some encouragement. In addition, it is witnessed that the level of dental anxiety spontaneously decreases over time without any physician or patient effort.

CONCLUSIONS

To establish a relationship based on mutual trust and empathy, to approach their concerns and special requests with understanding, to continue communication during the examination and treatment process, to make them think that no action will be taken that they do not allow, and to inform patients about interventional procedures in advance (tell-show-apply strategy) during the session. It will help him relax by preventing his anxiety.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for the study was given by the Ethics Committee of Dicle University University Faculty of Dentistry (Date: 29.05.2019, Decision No: 2019/6).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Relationship between the ABO blood group and mortality among the COVID-19 patients

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ABSTRACT

Objective: Differences in blood group antigen expression may increase or decrease the sensitivity of the host to many infections. Our aim in this study is to examine the relationship between ABO and Rh blood groups of COVID-19 patients and their mortality.

Material and Method: This retrospective observational study was conducted with patients who were diagnosed with COVID-19 in the emergency department of a tertiary hospital between May 1, 2020, and November 1, 2020. Patients who had a positive COVID-19 RT-PCR test and had blood group information in the HIMS database were included in the study. Blood groups, age, and gender information of the patients who included in the study were recorded on a form.

Results: The population of this study consists of 356 patients, of which 171 were women and 185 were men. There were 94 people in the O blood group, 185 people in the A blood group, 48 people in the B blood group, 29 people in the AB blood group, 37 people in the Rh-negative blood group, 319 people in the Rh-positive blood group. When the mortality status between blood groups is examined, it was observed that COVID-19 was less mortal in men with O blood group (p= 0.002).

Conclusion: COVID-19 infection is more common in those with the A blood group compared to the other groups. Additionally, we concluded that being in the O blood group is a factor that reduces mortality in men. More studies with a larger sample size are needed to confirm the results of our study.

Keywords: Blood groups, COVID-19, mortality

INTRODUCTION

COVID-19, the disease caused by the SARS-COV 2 virus, has led to a global epidemic (1). The SARS-COV2 virus has had various effects on the global population. It has been proven that older people and those with comorbidities such as cardiovascular disease, diabetes, and lung diseases are more vulnerable to severe disease of COVID-19 (2-5). To understand the underlying causes of morbidity and mortality associated with COVID-19, there has been a scientific interest to uncover characteristics that may make individuals more susceptible to COVID-19 infection and to identify risk factors associated with disease severity (6-9).

Data from Wuhan, China, the first epicenter of the COVID-19 epidemic, have shown the link between the ABO blood groups and COVID-19 infections. In a multicenter study conducted in the Wuhan region, Zhao et al. compared the general population with 2173 COVID-19 patients in terms of ABO blood groups. They reported that blood group A was associated with higher COVID-19 positivity compared to non-A groups and blood group O was associated with a significantly lower risk of infection compared to non-O groups (10).

Our aim in this study is to investigate the relationship between blood groups of COVID-19 patients and their mortality.

MATERIAL AND METHOD

After the approval of the Research Ethics Committee of Kartal Dr. Lütfi Kırdar City Hospital (Date: 29.03.2021, Decision No: 2021/514/198/29). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. This retrospective observational study was carried out with the information of patients admitted to the ED of a tertiary hospital between May 1, 2020, and November 1, 2020.

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Data of all patients over the 18-year-old and admitted to ED with suspected COVID-19 between September 1, 2020, and December 1, 2020, were scanned in the Hospital Information Management System (HIMS). The patients who had positive results in the real-time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test of nasal and pharyngeal swab samples included the study (11). The diagnosis of COVID-19 was determined based on the World Health Organization (WHO) guidelines. Patients whose RT-PCR results were negative or blood group information could not be reached were excluded from the study. Blood group information of patients diagnosed with COVID-19 was obtained from the hospital blood bank. Chronic disease information of the study population including chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), chronic renal failure (CRF), chronic neurological disease (CND), diabetes mellitus (DM), hypertension (HT), coronary artery disease (CAD) and atrial fibrillation (AF) were obtained from HIMS digital recordings.

Statistical Analysis

Statistical analyzes were performed using IBM SPSS Statistics 26 software. Mann-Whitney U test was used for the analysis of continuous data, Chi-square and Fisher's exact tests were used for the analysis of categorical data. Continuous data are reported as medians and interquartile ranges (IQR) and categorical data were reported as frequency and percentage (**Table 1, 2** and **3**). A p-value of less than 0.05 was considered statistically significant.

RESULTS

The population of this study consisted of 356 patients, of which 171 were women and 185 were men. There were 251 patients in the survivor group and 105 in the non-survivor group (**Table 1**). The median age of the study population was 64 (49-76), while the minimum age was 19, and the maximum age was 97. The median age of the survivor group was 58 (45-70), while the median age was 75 (65-82) for the non-survivor group (**Table 1**).

When the relation of chronic diseases with mortality was examined in the study population, there was a significant difference between survivor and non-survivor groups for COPD, CHF, CRF, and CND (**Table1**). Also, there was no significant difference between survivor and non-survivor groups for DM, HT, CAD, and AF (**Table 1**).

The study population consists of 94 people in blood group O, 185 in blood group A, 48 in blood group B, 29 in blood group AB, 37 in Rh-negative blood group, 319 in Rh-positive blood group (**Table 2**).

When patients grouped according to ABO blood groups were compared in terms of mortality, there was no significant difference between the groups (**Table 2**). In addition, no significant difference was found between the patients in the Rh-negative and Rh-positive groups in terms of mortality (**Table 2**).

When the relationship between mortality and ABO blood groups was assessed in COVID-19 patients classified by gender, there was a statistically significant difference between the O blood group with non-O groups for men (**Figure 1**, **Table 3**). This significant difference is important

Table 1. Demographic and comorbidity data of the study population

Variables	Category	Survivor		Non-survivor		Total	Sig.
		n	%	n	%	n	p
Gender	Female	124	72.5	47	27.5	171	0.424
	Male	127	68.6	58	31.4	185	
COPD	No	239	72	93	28	332	0.023
	Yes	12	50	12	50	24	
DM	No	188	71.2	76	28.8	264	0.620
	Yes	63	68.5	29	31.5	92	
HT	No	162	72	63	28	225	0.418
	Yes	89	67.9	42	32.1	131	
CHF	No	238	72.8	89	27.2	327	0.002
	Yes	13	44.8	16	55.2	29	
CAD	No	226	71.5	90	28.5	316	0.239
	Yes	25	62.5	15	37.5	40	
AF	No	242	71	99	29	341	0.362
	Yes	9	60	6	40	15	
CRF	No	236	72.6	89	27.4	325	0.005
	Yes	15	48.4	16	51.6	31	
CND	No	240	73.6	86	26.4	326	<0.001
	Yes	11	36.7	19	63.3	30	
		Survivor		Non-survivor		Total	
		Median	IQR	Median	IQR	Median IQR	
Age		58	45-70	75	65-82	64 49-76	<0.001

COPD: Chronic Obstructive Pulmonary Disease, DM: Diabetes Mellitus, HT: Hypertension, CHF: Congestive Heart Failure, CAD: Coronary Artery Disease, AF: Atrial Fibrillation, CRF: Chronic Renal Failure, CND: Chronic Neurological Disease Sig: Significance

as it shows that COVID-19 disease has low mortality in men with blood group O. When the relationship was investigated between mortality and ABO or Rh blood groups in COVID-19 patients classified by gender, no statistically significant relationship was found for the groups other than group O (**Table 3**).

Table 2. Relationship between mortality and blood groups in COVID-19 patients

Variables	Category	Survivor		Non-survivor		Total n	Significance p
		n	%	n	%		
O blood group	O	70	74.5	24	25.5	94	0.326
	Non-O	181	69.1	81	30.9	262	
A blood group	A	128	69.2	57	30.8	185	0.571
	Non-A	123	71.9	48	28.1	171	
B blood group	B	32	66.7	16	33.3	48	0.531
	Non-B	219	71.1	89	28.9	308	
AB blood group	AB	21	72.4	8	27.6	29	0.814
	Non-AB	230	70.3	97	29.7	327	
Rh blood group	Rh (+)	226	70.8	93	29.2	319	0.679
	Rh (-)	25	67.6	12	32.4	37	

Tablo 3. Relationship between mortality and blood groups in COVID-19 patients when grouped by gender

Gender	Category	Survivor		Non-survivor		Total n	Sig. p
		n	%	n	%		
Female (n=171)	O	30	62.5	18	37.5	48	0.067
	Non-O	94	76.4	29	23.6	123	
	A	67	76.1	21	23.9	88	0.275
	Non-A	57	68.7	26	31.3	83	
	B	17	70.8	7	29.2	24	0.842
	Non-B	107	72.8	40	27.2	147	
	AB	10	90.9	1	9.1	11	0.293*
	Non-AB	114	71.3	46	28.7	160	
	Rh (+)	112	71.8	44	28.2	156	0.763*
	Rh (-)	12	80	3	20	15	
Male (n=185)	O	40	87.0	6	13.0	46	0.002
	Non-O	87	62.6	52	37.4	139	
	A	61	62.9	36	37.1	97	0.076
	Non-A	66	75.0	22	25.0	88	
	B	15	62.5	9	37.5	24	0.486
	Non-B	112	69.6	49	30.4	161	
	AB	11	61.1	7	38.9	18	0.468
	Non-AB	116	69.5	51	30.5	167	
	Rh (+)	114	69.9	49	30.1	163	0.303
	Rh (-)	13	59.1	9	40.9	22	

* p value of Fisher's Exact Test, Sig.: Significance

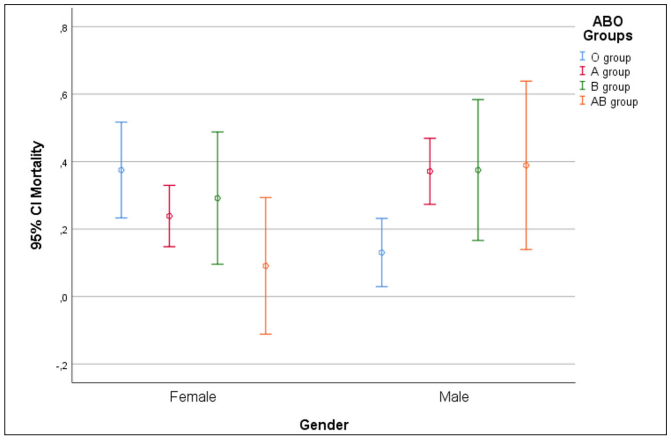

Figure 1. Bar graph in terms of mortality and ABO blood groups relationship of COVID-19 patients grouped by genders

DISCUSSION

In this study, the relationship between blood groups and mortality status of COVID-19 patients was examined. It was concluded that the disease was less mortal in the O blood group with male gender.

Landsteiner's ABO blood groups are carbohydrate epitopes found on the surface of human cells. Antigenic determinants trisaccharide parts of A and B blood groups are GalNAcα1-3 (Fuca1, 2) -Galβ- and Gala1-3- (Fuca1, 2) -Galβ-, O blood group antigen Fuca1, 2-Galβ-. While blood groups are genetically inherited, environmental factors can potentially affect which blood groups in a population will be passed on more frequently to the next generation. Viral infection sensitivity has previously been found to be associated with the blood ABO group. For example, blood group sensitivity of Norwalk virus and Hepatitis B is clear (12, 13).

Differences in blood group antigen expression can increase or decrease the sensitivity of the host to many infections. Therefore, since the beginning of the COVID-19 epidemic, many studies have been conducted on this subject. Zhao et al. studied the ABO blood group distribution in 2.173 COVID-19 patients. In this study, it was shown that the frequency of blood group A in COVID-19 patients was higher than that of non-A blood groups, and it was found that blood group O was associated with a lower risk of infection compared to non-O blood groups (10). Wu et al. (study included 187 COVID-19 patients) and H. Göker et al. (study included 186 COVID-19 patients) found similar results (14,15).

It is known that thrombotic risks are significantly reduced in the blood group O compared to non-O blood groups. Studies have shown that micro thrombosis developing in COVID-19 infection in the pulmonary vascular bed contributes significantly to acute respiratory syndrome, therefore the use of prophylactic anticoagulants is also included in the guidelines (16). There are opinions suggesting that the protective effect shown in O blood group is based on this phenomenon (17). In our study, the lower mortality rate in men with O blood group is consistent with the studies in the literature.

Blood groups are determined by sugars, and coronaviruses in cattle have surface proteins that bind to sugars. The extra sugar N-acetylgalactosamine on the surface of blood group A cells is likely to result in more pathogen exposure. This sugar is lacking in blood group O cells (18). This information is coherented with our results, such as that the majority of the population in the study consisted of individuals with blood group A and that blood group O is relatively protective.

As with any retrospective study, there are some limitations in this study. In this single-center study, the blood groups of the patients were found by querying from the electronic database of the hospital, and the patients whose blood groups could not be reached were not included in the study, resulting in a limited sample size. Another limitation of this study was the absence of a control group consisting of people who were not infected with COVID-19. However, the control group was not included in the study because COVID-19 infection can also be seen asymptomatically.

CONCLUSION

In this study, we concluded that those who with blood group A had more COVID-19 infections and that blood group O with male gender was more protective against the disease. More studies with a larger sample size are needed to confirm the results of our study.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Research Ethics Committee of Kartal Dr. Lütfi Kırdar City Hospital (Date: 29.03.2021, Decision No: 2021/514/198/29).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: All authors also declare no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Effects of essential oils obtained from wild and cultured forms of thyme (*Origanum acutidens*) on lung cancer cells membrane

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ABSTRACT

Aim: Nowadays, studies on the use of medicinal plants and their essential oils in cancer treatment are increasing rapidly and these studies are very important both scientifically and economically. However, the collection of plants from nature causes extinction. Therefore, it is of great importance to cultivate plants, especially endemic species, to reproduce and consume them. In our study, cytotoxic and membrane damaging effects of essential oils obtained from wild and cultured forms of *Origanum acutidens* (Hand.-Mazz.) Ietswaart (*Lamiaceae*) (*O. acutidens*), which is endemic to Turkey, on non-small-cell lung cancer (NSCLC) cells H1299 and A549 were compared.

Material and Method: The level of malondialdehyde, an oxidative stress biomarker, was determined in cell lysates. Assessment of cell viability was made by CellTiter-Blue® Cell Viability Assay and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay after 10- 250 µg/mL concentrations of wild and cultured forms of *O. acutidens* essential oil treated to H1299 and A549 cells for 24, 48 and 72 h. Malondialdehyde levels were assayed for determining the membrane damaging effects.

Results: Cell viability of H1299 and A549 cells incubated with essential oils obtained from wild and cultured forms of *O. acutidens* was found to decrease depending on concentration and time. 179 µg/mL, 157 µg/mL, and 132 µg/mL were calculated as IC50 values of wild form of *O. acutidens* essential oil on H1299 cells for 24, 48, and 72 h, respectively by MTT assay. 150 µg/mL, 131 µg/mL, and 110 µg/mL were calculated as IC50 values of wild form of *O. acutidens* essential oil on H1299 cells for 24, 48, and 72 h, respectively by resazurin-based assay. 118 µg/mL, 99 µg/mL, and 69 µg/mL were calculated as IC50 values of wild form of *O. acutidens* essential oil on A549 cells for 24, 48, and 72 h, respectively by MTT assay. 98 µg/mL, 83 µg/mL, and 57 µg/mL were calculated as IC50 values of wild form of *O. acutidens* essential oil on A549 cells for 24, 48, and 72 h, respectively by resazurin-based assay. Essential oils obtained from wild and cultured forms of *O. acutidens* increased malondialdehyde level on both H1299 and A549 cells. The greatest membrane damage was observed in A549 cells treated with wild form of *O. acutidens* essential oil.

Conclusion: Essential oils obtained from wild and cultured forms of *O. acutidens* had cytotoxic effect on lung cancer cells and it has been demonstrated that they showed this effect by causing membrane damage in cells.

Keywords: *O. acutidens*, essential oil, wild and cultured form, membrane damaging, anticancer

INTRODUCTION

Many of medicinal and aromatic plants are available thanks to the large differences in Turkey ecology. Species endemism is high in Anatolia provides this plant diversity. 30% of the species of flora aromatic are plants in Turkey. Aromatic plants are the main sources of essential oils (1). Many drugs with known antineoplastic properties are originated from plants. It is a current issue to investigate the treatment methods against cancer types, which are the most important diseases of our age, and the treatment possibilities with herbal origin chemicals among these methods. The fact that a sufficient treatment method has not been developed for this serious disease causes many speculations about the treatment. There is a huge gap in our country in terms of researching the antineoplastic properties of plant extracts. The most researched aromatic plant is thyme. Plant species belonging to different genera in the same family are called thyme. The genera including the thyme species that are traded and widely used in our country are Origanum, Thymbra, Coridothymus, Satureja and Thymus. Studies on the use of medicinal plants and their essential oils in cancer treatment have gained speed and these studies are so important both scientifically and economically.

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According to the report of the World Health Organization (WHO), lung cancer is the first cancer type causing death in men and the third cancer type in women (2). Among all cancer types, the incidence in women is 9% while it is 17% for men. The response to chemotherapy is as low as 30-50% in patients with non-small cell lung cancer, which constitute 80-85% of lung cancer cases (3). The failure of anticancer drugs in the treatment of lung cancer, especially NSCLC, reveals the need for the development of new chemotherapeutics.

Approximately 50% of drugs in clinical trials for anticancer activity have been isolated from natural sources or those associated with them (4,5). Many plant species that are endemic to Turkey disappear over time. However, culturing plants while preserving their biological activity characteristics can prevent the extinction of these plant species. Our aim should be to culture and propagate plants without consuming them and to use cultured plants in drug development. Origanum species, which are aromatic medicinal plants, are unconsciously collected from nature and used as spice, thyme tea and thyme oil. The use of plants in culture can prevent the extinction of endemic species and can enable us to have easy and large amount of plants. Therefore, if we compare the wild and the cultured form in our studies and reveal their effects, we can continue our studies with the cultured form. Thus, we protect the endemic species in nature.

The aim of this study was to demonstrate and compare the cytotoxic and membrane damaging effects of essential oils obtained from wild and cultured forms of *O. acutidens* in H1299 and A549 cells.

MATERIAL AND METHOD

Collection of Plant Material

O. acutidens was collected from Refahiye, Erzincan (1950-2000 m), in Turkey, in July 2017. The taxonomic identification of plant materials were confirmed by a plant taxonomist, Dr. Canan Dulgeroglu from Department of Biology, Akdeniz University, Antalya, Turkey (Voucher no: TR 1019).. The cultured form of *O. acutidens* was obtained from the Erzincan Directorate of Horticulture and was harvested in July 2017. Our study does not require any ethics committee approval. Our study was performed with cancer cell lines obtained from ATCC.

Isolation of the Essential Oil

The dried aerial parts of plants (100 g) collected were submitted to water distillation for 2 h using a Clevenger-type apparatus (Ildam Ltd., Ankara, Turkey) at Molecular Biology Department in Biology in Akdeniz University. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at +4°C until used in experiments.

Cell Lines and Culture

The human non-small-cell lung cancer (NSCLC) cell line H1299 and A549 were purchased from the American Type Culture Collection (ATCC). All cells were maintained in Roswell Park Memorial Institute 1640 medium (RPMI 1640) contained 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (penicillin, streptomycin and amphotericin) in a humidified atmosphere containing 5% CO2 at 37°C. For subculturing, cells were harvested after trypsin/ethylenediaminetetraacetic acid (EDTA) treatment at 37°C. Cells were used when monolayer confluence had reached 75%.

Cytotoxicity Assays

The cancer cells (10,000 cells/well, monolayer) were plated in a 96-well plate. The next day the cells were treated with different concentrations of wild and cultured forms of *O. acutidens* essential oils (10-250 µg/mL) for 24, 48, and 72 h. At the end of the incubation period, the cytotoxicity of this solution on cancer cells was determined by the CellTiter-Blue cell viability assay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The CellTiter-Blue cell viability assay is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Nonviable cells rapidly lose metabolic capacity and thus do not generate a fluorescent signal (6). Following cellular reduction, fluorescence was recorded at 560 nm (excitation) and 590 nm (emission) spectrofluorometrically (PerkinElmer LS 55). In the MTT assay, tetrazolium salts such as MTT are metabolized by mitochondrial dehydrogenases to form a blue formazan dye, useful for the measurement of cytotoxicity. Test reagents were added to the culture medium. Briefly, 15% volumes of dye solutions were added to each well after the appropriate incubation time. After 2 h of incubation at 37 °C, an equal volume of solubilization/stop solutions (dimethyl sulfoxide) was added to each well for an additional 1 h of incubation. The absorbance of the reaction solution at 490 nm was recorded (7). The data were expressed as average values obtained from eight wells for each concentration. IC50, and IC70 concentrations were calculated. For the calculation of these values, Microsoft Excel software was used. Essential oil was dissolved in 0.5% dimethyl sulphoxide (DMSO). So we treated 0.5% DMSO alone to H1299 and A549 cells. The reading taken from the wells with cells cultured with only the medium (untreated cells) was used as a 100% viability value.

Determination of Malondialdehyde Levels

Malondialdehyde (MDA) levels were determined after H1299 and A549 cells were exposed to different concentrations of wild and cultured forms of *O. acutidens* essential oils (IC50) for 24 h. They were dissolved in 0.5% DMSO. So we treated 0.5% DMSO alone to H1299 and A549 cells. H1299 and A549 cells were plated at a

density 15×104 cell/100 mm dishes. Cells were scraped off culture plates with culture medium and were centrifuged 600×g for 10 min. The cell pellets were washed with phosphate buffered saline and then sonicated (3×15 sec) in 50 mM potassium phosphate, pH 7.2, containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 µg/mL of leupeptin and centrifuged at 150,000×g for 45 min. The supernatant was used for the determination of malondialdehyde level. Malondialdehyde levels in H1299 and A549 were assayed as described in a previous method (8). This fluorometric method for measuring thiobarbituric acid-reactive substances (TBARS) in supernatant is based on the reaction between malondialdehyde and thiobarbituric acid. The product of this reaction was extracted into butanol and measured at 525 nm (excitation) and 547 nm (emission) spectrofluorometrically. Protein was determined by the Bradford method (9) with bovine serum as a standard. The experiment was performed in triplicate and mean values were recorded.

Statistical Analysis

The results of the replicates were pooled and expressed as mean ± standard error. Analysis of variance (ANOVA) was carried out. The ANOVA was used to determine whether there are any significant differences between the means of three or more independent (unrelated) groups on some variable. Tukey multiple comparisons tests were used. Significance was accepted at $p \leq 0.05$ (10). Statistical analyses were performed using the Minitab program Release 13.0.

RESULTS

Effect of Essential Oils Obtained from Wild and Cultured Forms of *Origanum acutidens* on the Viability of H1299 and A549 Cells

In this study, cytotoxic effects of wild and cultured forms of *O. acutidens* essential oils on H1299 and A549 lung cancer were investigated by CellTiter-Blue® Cell Viability and MTT tests. H1299 and A549 cells were submitted to increasing concentrations of wild and cultured forms of *O. acutidens* essential oils for 24, 48 and 72 h. The concentrations of essential oils needed to reduce growth by 50% and 70%, respectively (IC50 and IC70) were calculated using the Linear functions (The equation of a straight line). The essential oil from wild and cultured forms of *O. acutidens* were found cytotoxic in concentration and time dependent manners in H1299 and A549 cells according to both cytotoxicity assays (**Figure 1A,B**; **Figure 2A,B**). The essential oil from wild forms of *O. acutidens* was found more effective on A549 cells than essential oil from cultured forms of *O. acutidens* (**Figure 1A,B**). While the essential oil from cultured forms of *O. acutidens* was found more effective on H1299 cells than essential oil from wild forms of *O. acutidens* (**Figure 2A,B**). After 24, 48 and 72 hours incubations IC50 values were calculated respectively from CellTiter-Blue® Cell Viability test results, for essential oil from wild form of *O. acutidens* on H1299 cells, 150, 131 and 110 µg/mL, for essential oil from cultured form of *O. acutidens* on H1299 cells 80, 69 and 60 µg/mL (**Table 1**). After 24, 48 and 72 hours incubations IC50 values were calculated respectively from MTT test results, for essential oil from wild form of *O. acutidens* on H1299 cells, 179, 157 and 132 µg/mL, for essential oil from cultured form of *O. acutidens* on H1299 cells 100, 94 and 83 µg/mL (**Table 1**). Also, after 24, 48 and 72 hours incubations IC50 values were calculated respectively from CellTiter-Blue® Cell Viability test results, for essential oil from wild form of *O. acutidens* on A549 cells, 98, 83 and 57 µg/mL, for essential oil from cultured form of *O. acutidens* on A549 cells 136, 115 and 102 µg/mL. After 24, 48 and 72 hours incubations IC50 values were calculated respectively from MTT test results, for essential oil from wild form of *O. acutidens* on A549 cells, 118, 99 and 69 µg/mL, for essential oil from cultured form of *O. acutidens* on A549 cells 163, 138 and 122 µg/mL. The CellTiter-Blue cell viability assay was found to be more sensitive than the MTT assay, so we studied other parameters according to CellTiter-Blue assay results.

Table 1. Summary of the cytotoxic effects of wild and cultured forms of *O. acutidens* essential oils on H1299 and A549 cells

Cells treatments	Es. oil wild (µg/mL) (CellTiter.) X ± S.E	Es. oil wild (µg/mL) (MTT) X ± S.E.	Es. oil cultured (µg/mL) (CellTiter.) X ± S.E.	Es. oil cultured (µg/mL) (MTT) X ± S.E.
H1299, 24 h, IC_{50}	150±2.11d	179±3.71f	80±1.65a	100±1.99b
H1299, 24 h, IC_{70}	199±2.38f	239±2.98h	120±3.81c	287±3.44j
H1299, 48 h, IC_{50}	131±2.01c	157±2.03e	69±1.04a	94±1.23b
H1299, 48 h, IC_{70}	179±3.71f	215±3.11g	116±1.99c	151±1.88e
H1299, 72 h, IC_{50}	110±2.99b	132±2.01c	60±1.71a	83±1.75a
H1299, 72 h, IC_{70}	159±2.43e	191±2.38f	111±2.41c	143±2.11d
A549, 24 h, IC_{50}	98±1.22b	118±1.99c	136±2.66d	163±2.03e
A549, 24 h, IC_{70}	149±2.35d	179±2.77f	185±2.01f	224±2.78g
A549, 48 h, IC_{50}	83±1.65a	99±1.22b	115±3.81c	138±2.71d
A549, 48 h, IC_{70}	134±2.66c	160±2.88e	162±1.99e	194±2.76f
A549, 72 h, IC_{50}	57±1.00a	69±1.71a	102±1.78b	122±3.43c
A549, 72 h, IC_{70}	108±1.99b	130±1.89c	149±2.11d	178±2.77f

Values followed by different letters within a column are significantly different ($p \leq 0.05$); SE, standard error. Es. Oil, Essential oil; CellTiter., CellTiter-Blue cell viability assay. X is an average of five repetitions.

The Membrane Damaging Effect of Essential Oils from Wild and Cultured form of *O. acutidens* on H1299 and A549 Cells Membrane

The induction of cytotoxic cell death can be accompanied by membrane and DNA damage. Essential oil induced membrane damage at IC50 concentrations (**Figure 3**) than those that mediate its anticancer activities. The results of membrane damaging effects of the essential oils (IC50) on H1299 and A549 after 24 h exposure is shown in **Figure 3**. Essential oils from wild and cultured form of *O. acutidens* showed membrane damaging effects on both H1299 and A549 cells. The amounts of malondialdehyde (MDA), an end product of lipid peroxidation of membrane, increased almost 2.9 and 2.1 fold in the IC50 concentrations of the essential oil from wild form of *O. acutidens* and essential oil from cultured form of *O. acutidens* treated A549 cells compared to control cells, respectively (**Figure 3**). While the amounts of MDA increased almost 1.6 and 1.4 fold in the IC50 concentrations of the essential oil from wild form of *O. acutidens* and essential oil from cultured form of *O. acutidens* treated H1299 cells compared to control cells, respectively (**Figure 3**). The essential oil from wild form of *O. acutidens* caused the most membrane damage in both A549 and H1299 cells. Both the essential oil from wild form of *O. acutidens* and essential oil from cultured form of *O. acutidens* have been shown to cause more membrane damage in A549 than H1299 cells.

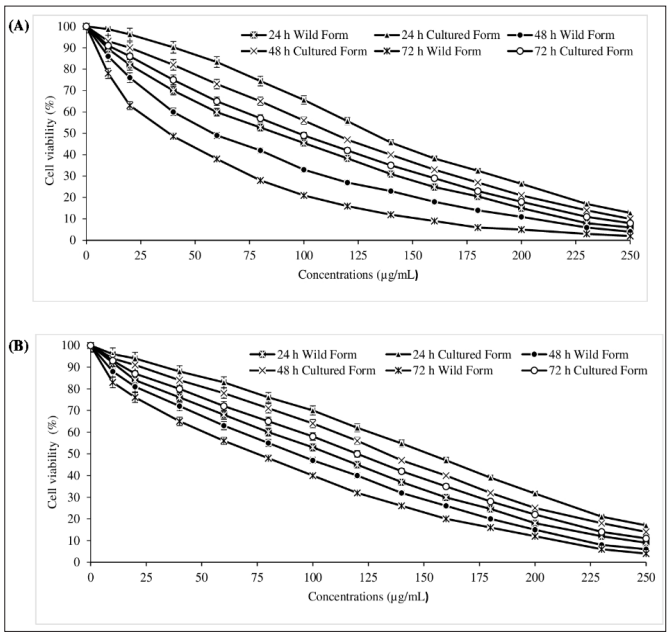

Figure 1. The cytotoxic effects of essential oil from wild and cultured forms of *O. acutidens* on A549 cells after 24, 48 and 72 h measured by (A) The CellTiter-Blue-Cell Viability Assay; (B) MTT Assay. Results are presented as viability ratio compared with the control group (treated with only the medium-untreated cells). Values are expressed as the mean of three separate experiments ± S.E. Error bars represent standard error of the mean from three replications.

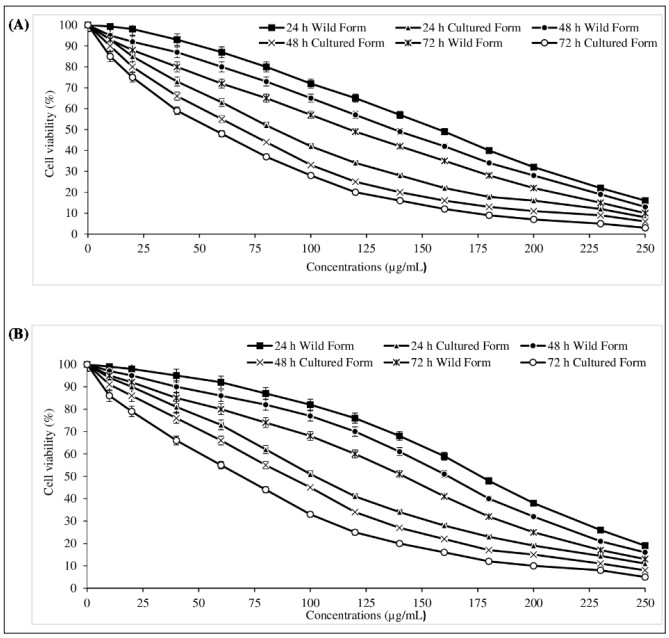

Figure 2. The cytotoxic effects of essential oil from wild and cultured forms of *O. acutidens* on H1299 cells after 24, 48 and 72 h measured by (A) The CellTiter-Blue-Cell Viability Assay; (B) MTT Assay. Results are presented as viability ratio compared with the control group (treated with only the medium-untreated cells). Values are expressed as the mean of three separate experiments ± S.E. Error bars represent standard error of the mean from three replications.

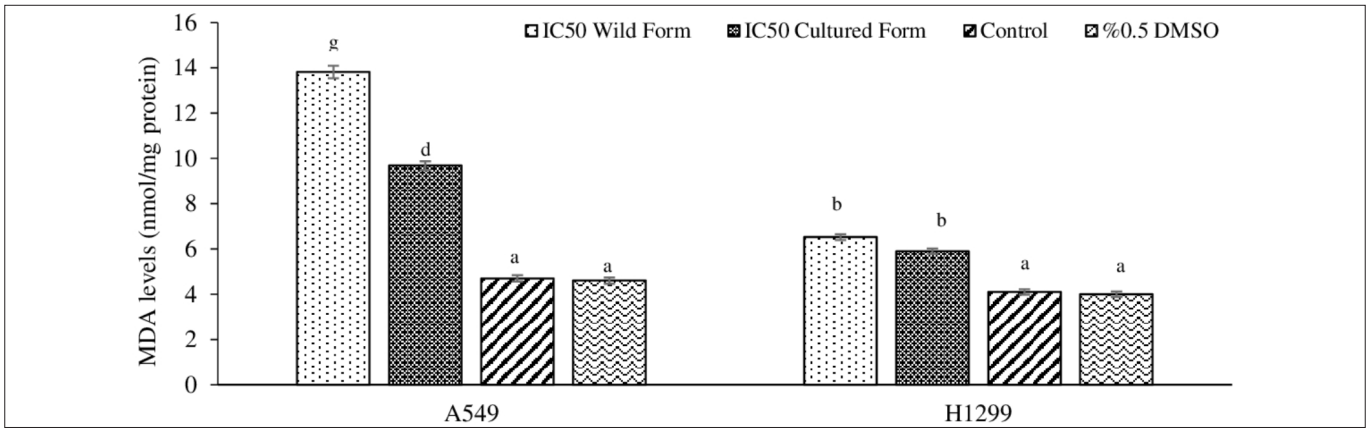

Figure 3. Membrane damaging effects of essential oil from wild and cultured forms of *O. acutidens* on H1299 and A549 cells. Values are expressed as the mean of three separate experiments ± S.E. Error bars represent standard error of the mean from three replications, and bars with the same letter indicate no significant difference (ANOVA with Tukey's test, $p \leq 0.05$). Different letters represent significant differences among treatments (ANOVA, $p \leq 0.05$) in H1299 and A549 cells.

In our study, essential oils from wild and cultured form of *O. acutidens* induced membrane damage and cytotoxicity in both A549 and H1299 cells and this can mediate its anticancer activity. The induction of cytotoxic cell death can be accompanied by membrane damage.

DISCUSSION

It is very important both scientifically and economically to obtain and evaluate the pure medicinal plants and especially the essential oils of these plants. Many drugs used today with anticancer effect such as vinblastine, irinotecan, topotecan, vincristine were obtained from plants. However, the continuous collection of these plants from nature causes their extinction. Therefore, plants to be used for drug production or other purposes can be cultivated and reproduced. We look for answers to these questions, what kind of differences in the biological action mechanisms of endemic plant species can be caused by the culture and reproduction processes we apply to prevent extinction.

One of the most important mechanisms of used in cancer treatment is apoptosis. Since various chemotherapeutics that act by using apoptotic mechanisms in cancer treatment also damage healthy cells, interest in natural herbal medicines has increased. The plant-derived products are expected to induce lesser side effects compared to synthetic drugs New cancer treatment strategies using endemic plants are increasing day by day.

According to the essential oil content analysis we conducted in another project, carvacrol and p-cymene were determined as the main components of both wild and cultured form of *O. acutidens*. However, while the rate of carvacrol was higher in wild form (Wild: 72.65%; Cultured: 67.98%), it was observed that the rate of p-cymene was higher in the cultured form (Wild: 15.19%; Cultured: 16.01%). Essential oils, which are lipophilic in nature, show their effects in the cell by crossing the plasma membrane (11). Carvacrol and p-cymene, due to their hydrophobic nature, can react with lipids of the cell membrane and mitochondria, make the membranes permeable and disrupt the structure of the cell (12). Many studies have shown that many essential oils, the main components of which are carvacrol and p-cymene, have similar anticancer effects (13-17). It has been reported that water extract of *O. acutidens* caused cytotoxic and apoptotic effects in breast cancer cells such as MCF-7, MDA-MB-468 and MDA-MB-231. At the end of the incubation, it was shown that caspase-7 protein expression and the number of TUNEL-positive cells increased, indicating an apoptotic effect (18). The antineoplastic effect of various extracts of *O. acutidens* obtained from Sivas on breast cancer cells (MDA-MB-231, MDA-MB-468) was demonstrated by Trypan blue method (19). The essential oils from wild and cultured form of Salvia pisidica showed cytotoxicity on H1299 cells24. In other studies, it has been reported that *O. acutidens* essential oil showed antimicrobial activity against different bacterial species (20, 21).

By culturing endemic species in different physical conditions, we can make them show similarities with the wild form. It has been reported in many studies that essential oils and their components obtained from plants had anticancer effects by creating cytotoxic effects on cancer cells. In our study, H1299 and A549 cells, which were exposed to essential oils obtained from wild and cultured form of *O. acutidens* for 24, 48 and 72 hours, decreased cell viability due to the increase in concentration.

Free radicals cause cytotoxicity and lipid peroxidation associated with chronic diseases such as cell senescence and cancer. Reactive oxygen species (ROS) are generated inside the cells in response to external stimuli or stress under normal conditions. ROS interacts with the double bonds of the polyunsaturated fatty acids to form lipid hydroperoxide. One of the major secondary oxidation products of peroxidized polyunsaturated fatty acids is malondialdehyde (MDA), which has a mutagenic and cytotoxic effect. The lipid peroxidation caused by free radicals causes changes in the structure, permeability and fluidity of the membrane, impairment of lysosomal balance and induction of apoptosis

Essential oils obtained from wild and cultured form of *O. acutidens* increased MDA levels according to controls in H1299 and A549 cells. Origanum onites (*Lamiaceae*) essential oil and its two phenolic components, thymol and carvacrol, induced membrane damage on Hep G2 cells (22). Carvacrol, thymol eugenol, eucalyptol, terpinen-4-ol, and camphor at higher concentrations increased MDA levels, causing membrane damage, in both parental and epirubicin-resistant H1299 cells (22, 23). Also in another study T. revolutus C. essential oil caused to increase MDA levels in Hep G2 cells according to control cells (25).

With our study, it can be suggested as a natural herbal source in the production of new anticancer drugs, since it was revealed that essential oils obtained wild and cultured form of *O. acutidens* had cytotoxic and membrane damaging effects on H1299 and A549 cells. The results of our study will also contribute significantly to the medical literature and the national economy. In addition, taking our naturally grown plants into culture will prevent their extinction. The production of cultured form of *O. acutidens* will contribute to the protection of this endemic plant by preventing its decline or extinction in nature. With the cultivation process, it will be easier to reach this endemic species and it will be available in more abundance. This will significantly reduce the cost of using this plant for therapeutic purposes.

CONCLUSION

O. acutidens essential oils may be a hope in the future for lung cancer patients who were tried to be treated with conventional chemotherapy drugs but could not achieve the desired success, in line with the results we obtained from our study. Finally, the new experiences/gains we have gained as a result of our study will also lead to the evaluation of plant-derived compounds in lung and other cancer research.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study does not require any ethics committee approval. This study was performed with cancer cell lines obtained from ATCC.

Informed Consent: For this type of study, formal consent is not required.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The evaluation of the cases with extrapulmonary tuberculosis

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ABSTRACT

Objective: Tuberculosis (TB) involving all organs is a disease progressing with a wide range of clinical presentations. It is especially difficult to diagnose cases of extrapulmonary tuberculosis (EPTB) due to the a typical course. The cases of EPTB constitute approximately 35% of all TB cases in our country. In our study, it was aimed to examine the distribution rates, diagnostic methods, and treatment processes of EPTB in terms of the involved systems.

Material and Method: Of 308 patients diagnosed with TB, 119 cases having EPTB were retrospectively analyzed in The Konya Training and Research Hospital of Health Sciences University between 1st January 2015 and 30th June 2019.

Results: Of 308 cases diagnosed with TB, 119 (38.6%) EPTB cases were included in the study. Sixty-three (52.9%) and 56 (47%) patients were women and men, respectively. The average age was found as 44.42±18.8 years (min: 18, max: 91 years). The distribution of involvement sites of EPTB was as follows: lymphadenitis, 45.4%; pleural tuberculosis, 24.4%; peritonitis, 14.3%; bone-joint tuberculosis, 7.5%; meningitis, 2.5%; miliary tuberculosis, 1.7%; others, 4.2%. In terms of EPTB, 83 (69.7%) cases were diagnosed histopathologically, and 28 (23.5%) and nine (7.5%) had culture and acido-resistant bacilli (ARB) positivity, respectively. A total of 29 (24.3%) cases were diagnosed microbiologically, and the purified protein derivative (PPD) positivity was detected in 106 (89%) cases. While the success rate of the treatment was found to be 93.2%, the mortality rate was measured as 3.3%.

Conclusion: It should be kept in mind that TB is a common multisystem disease in our country and may present itself with a wide range of symptoms. In the presence of clinical suspicion, supportive tests should be performed; biopsy and culture samples should be obtained from the appropriate tissues; anti-TB treatment should be initiated at once if clinicians have sufficient evidence.

Keywords: Extrapulmonary tuberculosis, diagnosis, histopathological, microbiological

INTRODUCTION

Presenting very wide clinical findings, tuberculosis (TB) is a disease that can involve almost any organ in humans. Extrapulmonary tuberculosis (EPTB), however, is witnessed in approximately 35% of allcases in Turkey (1). Although TB can involve all tissues and organs, the types and main involvement sites of EPTB are lymphadenitis, miliary TB, central nervous system (CNS), bone-joint TB, pleural and pericardial TB, and TB of genitourinary (GUS) and gastrointestinal (GIS) systems. The type of EPTB most commonly encountered in our country is pleural TB. It is difficult to diagnose the cases of EPTB, and so the diagnosis can often be ignored (2). When EPTB is suspected, and the diagnosis process is supported by clinical and laboratory findings, anti-tuberculosis treatment should be initiated at once. The fact that the treatment modalities are initiated at an earlier phase is so important to reduce morbidity and mortality (1).

The present study aims to draw attention to the cases with EPTB, which is still an important public health problem in our country, since its diagnosis can be generally ignored, delayed, or not considered due to the delays in the treatment by evaluating the cases in terms of patients' demographic characteristics, an anatomical sites of the involvements, diagnostic procedures and the outcomes of the treatment modalities.

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MATERIAL AND METHOD

An approval was obtained from the Medical Specialty Education Board of Konya Training and Research Hospital (Date: 01/08/2019, Desicion No: 28-01). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Among a total of 308 patients diagnosed with TB in The Konya Training and Research Hospital of Health Sciences University between 1st January 2015 and 30th June 2019, 119 cases with EPTB were included and analyzed retrospectively in our study. The patients under 18 years of age were excluded from the criteria. For the study, approval was obtained from The Medical Specialty Education Board (TUEK) of the hospital. Since the study was of a retrospective design, approval from the ethical board of an institution was considered not to be necessary. The information related to the patients was obtained by scanning the hospital automation system and analyzed retrospectively. EPTB cases were diagnosed by determining one or more of the following criteria:

1. The determination of claseified granulomatous or granulomatous necrosis in the histopathological examination on the biopsy material obtained from the current focus.
2. The demonstration of ARB in the microscopic examination or culture of material obtained from the current focus.
3. The presence of the response to the anti-TB treatment administered, as well as the positivity of the PPD test, in case of clinical/radiological compatibility with EPTB.

The demographic characteristics, accompanying comorbid diseases, PPD results, histories of previous treatment regimes (case definition), foci of involvements, diagnostic methods and treatment protocols of the patients diagnosed with EPTB were recorded. The definitions of the cases were created under The 2019 National Tuberculosis Diagnosis and Treatment Guidelines (1). The diagnostic and treatment protocols were determined under the recommendations by The World Health Organization (WHO). The treatment outcomes were also defined as the cure, completion of the treatment performed, withdrawal from the treatment, therapeutic failure, and death under the criteria by WHO (3).

Statistical Analysis

The statistical analyzes were performed using the statistical software of the Statistical Package for Social Sciences (SPSS) for Windows, version 18.0 program (SPSS Inc, Chicago, IL, USA).The descriptive variables and the findings of mean±standard deviation (SD) were also analyzed.

RESULTS

Among 308 cases reported to have TB, 119 (38.6%) were detected to be EPTB patients and included in the study. Of 119 patients, 63 (52.9%) and 56 (47%) were female and male, respectively, and the patients' average age was measured as 44.42±18.8 years (min: 18, max: 91 years). While 99 (82.2%) of the patients were Turkish citizens, 20 (16.8%) were citizens of other countries, such as Syria, Afghanistan, and Azerbaijan.

Considering the existence of predisposing comorbid diseases, the distribution of the disorders was as follows: diabetes mellitus (DM) in eight (6.7%) patients, malignancy in six (5%) patients, chronic renal failure (CRF) in eight (6.7%) patients, and chronic obstructive pulmonary disease (COPD) in five (4.2%) patients. Of 119 EPTB patients in the study, while 103 (86,6%) were examined in the out-patient clinics, 16 (13.4%) were treated in the in-patient units. Even so, while 111 (92.2%) were newly diagnosed TB patients, seven (5.8%) were detected to have recurrent TB, and one (0.8%) case was also seen to abandon the treatment. The distribution of EPTB is shown in **Table 1**, and the distribution of lymphadenitis under the involvement sites is demonstrated in **Table 2**.

Table 1. The distribution of extrapulmonary tuberculosis cases under the systems

EPTB	n	%
Lymphadenitis	54	45.4
Pleural tuberculosis	29	24.4
Peritonitis	17	14.3
Bone-joint tuberculosis	9	7.5
Meningitis	3	2.5
Miliary tuberculosis	2	1.7
Laryngeal tuberculosis	2	1.7
Tuberculosis of GIS	2	1.7
Tuberculosis of GUS	1	0.8
Total	119	100

EPTB: Extrapulmonary tuberculosis, GIS: Gastro-intestinal system, GUS: Genito-urinary system

Table 2. Distribution of the involvement sites of lymphadenopathies

Localizations of LAPs	n	%
Cervical LAP	28	51.9
Axillary LAP	23	42.6
Mediastinal LAP	3	5.5
Total	54	100

LAP: Lymphadenopathy

In terms of EPTB, 83 (69.7%) cases were diagnosed histopathologically, 28 (23.5%) and nine (7.5%) were found to have culture and ARB positivity, respectively. On the other hand, a total of 29 (24.3%) cases were diagnosed microbiologically, and the PPD positivity was detected in 106 (89%) cases. The diagnostic methods, and the PPD positivity and mortality rates concerning the involvement sites of EPTB are presented in **Table 3**.

Table 3. Diagnostic methods, and PPD positivity and mortality rates according to EPTB involvement regions

Involvements sites of EPTB	Number of cases n (%)	Clinical-radiological n (%)	Microbiological (culture-ARB)		Histopathological n (%)	PPD positivity n (%)	Mortality rates n (%)
			n (%) Culture	n (%) ARB			
Lymphadenitis	54 (45.4)	-	6 (11.1)	2 (3.7)	54 (100)	49 (90.7)	
Pleural tuberculosis	29 (24.4)	7 (24.1)	10 (34.4)	2 (6.8)	13 (44.8)	25 (86.2)	
Peritonitis	17 (14.3)	4 (23.5)	6 (35.2)	3 (17.6)	10 (58.8)	17 (100)	1 (5.8)
Bone-Joint tuberculosis	9 (7.5)	5 (55.5)	2 (22.2)		4 (44.4)	8 (88.8)	
Meningitis	3 (2.5)	2 (66.6)	2 (66.6)	1 (33.3)	-	3 (100)	1 (33.3)
Miliary tuberculosis	2 (1.7)	1 (50)	1 (50)		-	-	1 (50)
Laryngeal tuberculosis	2 (1.7)	-		1 (50)	-	2 (100)	1 (50)
Tuberculosis of GIS	2 (1.7)		-	-	2 (100)	2 (100)	
Tuberculosis of GUS	1 (0.8)	-	1 (100)	-	-	-	
Total	119 (100)	22 (18.4)	29 (24.3)		83 (69.7)	106 (89)	4 (3.3)

ARB: Acido-resistant bacilli, EPTB: Extrapulmonary tuberculosis, GIS: Gastro-intestinal system, GUS: Genito-urinary system, PPD: purified protein derivative

The histopathological findings were seen to be compatible with TB in 13 of 29 patients with tuberculous pleurisy. In seven cases where tuberculous pleurisy could not be diagnosed histopathologically or microbiologically, and the clinical picture was compatible with TB, the diagnosis was confirmed through the positive response to anti-TB treatment as a result of the presence of exudate pleural fluid and/or the positive tuberculin test under the dominance of lymphocytes.

A quadruple anti-TB treatment was initiated in 110 of the patients with isoniazid (H) + rifampicin (R) + pyrazinamide (P) + ethambutol (E), and the treatment was maintained with the combination of isoniazid + rifampicin (HR). Since the resistance to H was detected in one pleural TB patient under the treatment, the combination of isoniazid + rifampicin + ethambutol (HRE) was administered as the maintenance therapy, and the treatment period was extended to nine months. In eight patients (seven with recurrent TB, and one case had also abandoned the treatment), with the addition of streptomycin (S) to the classical quadruple treatment, the modality was completed as the combination of isoniazid + rifampicin + ethambutol + pyrazinamide + streptomycin (HERZS) for the first 2 months, as the combination of HRZE for the following one month, and then as HRE for the following 5 months (1). The treatment period of the patients diagnosed with lymphadenitis, pleural TB, and tuberculous peritonitis was completed in six months, while the period of those diagnosed with meningitis and bone-joint TB was completed within 12 months. During the treatment and follow-up period, a total of four cases died, one due to miliary TB, one due to larynx TB, one due to tuberculous meningitis, and the last due to tuberculous peritonitis. While no comorbidity was seen in the cases having miliary TB and TB meningitis with mortal outcomes, malignancy was witnessed in the case with laryngeal TB, and CRF and DM were also seen in the case with tuberculous peritonitis. While the recurrence was observed in one case with peritonitis, two cases (one with pleural TB and the other with bone-joint TB) abandoned the follow-up, and one case with cervical lymphadenitis decided to quit the treatment. While the success rate of the treatment was found to be 93.2%, the mortality rate was measured as 3.3%. A total of 111 cases were cured and/or completed the treatment period.

DISCUSSION

Keeps on being an important health challenge in developing countries, TB is a disease that can frequently involve other systems, especially the lungs in humans. The clinical signs and symptoms of TB vary according to the involvement site, the load of bacilli, and the host response to TB (4). The patients with TB are admitted to the clinics and out-patient clinics from many different branches depending on the involved organs and systems, and the severity of the disease. For this reason, the awareness of physicians including all branches should be increased about EPTB.

Considering the data related to TB around our country, the rates of EPTB were found to be 55% in the study performed by Inonu et al. (5), 15.8% in the study by Oztop et al. (6), 28.1% in the study of Özdemir (7), and 21.7% in the study carried out by Demiralay et al (8). Under the data released in 2017 by The Tuberculosis Control Department of The Turkish Ministry of Health, the number of those with extrapulmonary organ involvement was announced as 33.9%.

In the province of Konya, while the total diagnosis of TB was reported as 236, the number of EPTB diagnoses was stated to be 104 (44.1%) in 2017 (9). The rate of EPTB (38.8%) detected in our study is consistent with the numbers reported for both the province of Konya and the national data. Among the leading predisposing factors in

the development of infection are immunosuppressive diseases (6,10). It is observed today that the prevalence of EPTB cases is increasing (9). When the presence of comorbid diseases was taken into account in our study, the following comorbid diseases were seen to accompany the patients' tables as DM in eight (6.7%) patients, malignancy in six (5%), CRF in eight (6.7%), and COPD in 5 (4.2%) patients. As a gender difference, EPTB is stated to be seen more commonly in the female gender in the literature (6,9,11,12). As consistent with the findings in the literatüre, 52.9% of the cases were female, and 47% were male in our study.

Pleural involvement and lymphadenitis are the most common forms encountered in EPTB. TB bacilli are settled in the nearest lymph node with a hematogenous spread following the primary infection, and so lymphadenitis occurs with the reactivation of these bacilli. In the cases led by TB bacilli, the cervical lymph nodes are the most commonly involved sites (13). Tuberculous lymphadenitis was the most common type of EPTB in our study. As the most common involvement site, cervical lymph nodes (n=28, 51.8%) were detected in our study, and the cervical lymph nodes were followed by the axillary (n=23, 42.5%) and mediastinal lymph node (n=3, 5.5%) involvements, respectively. Based on previous studies, the rates of lymphadenitis were reported as 31.8% in the study performed by Inonu et al. (5), 53.3% in the study by Tavusbay et al. (11), and 26% in the study of Demiralay et al. (8). However, in the study conducted by Aslan et al. (14), lymphadenitis ranked first with a rate of 34.7% and involved most commonly the cervical region with a rate of 25.7%. In the same study, pleural TB was detected as the second most common type (18.8%). The findings in our study are compatible with those reported in the study by Aslan et al. (14) While the rates of lymphadenitis stated by Tavusbay et al. (11) are well-coincided with our findings, the rates detected in Inonu et al. and Demiralay et al.'s studies are lower than our findings. The differences in the findings reported by various studies may have also been affected by regional factors, as well as the influences stemming from the physicians' approaches to the follow-up of LAPs. For the cases of pleural TB, the diagnosis is often confirmed with the treatment in case of radiological and clinical compatibility. While the rates of pleural TB were reported to be 47.7% in the study by Inonu et al. (5), 50.2% in the study by Kolsuz et al. (15), and 52.8% in the study by Demiralay et al. (8) we found the rate of pleural TB as 24.3% in our study. Compared with the findings in those studies, the rate of pleural TB is seen to be lower in our study; the reason for such a difference between the previous studies and our study may be that some pleural TB cases are accompanied by pulmonary TB, and the diagnostic intervention is avoided for pleural TB both because of the predominance of pulmonary TB symptoms, and because pulmonary TB is diagnosed easily. Another reason is that a group of patients give no consent to diagnostic invasive interventions for pleural TB. Because the treatment protocols for the involvements of pleural and pulmonary TB are the same, and the treatment should be initiated without delay in both conditions, it is suggested that the unrecorded cases of pleural TB are treated along with the current cases of pulmonary TB. Considering our findings related to pleural TB, it is seen that 76% of the cases were diagnosed through the microbiological and/or histopathological examination of the material (biopsy, pleural fluid) obtained via the invasive intervention, and only 24% were diagnosed clinically and radiologically. Such a situation suggests that a part of our pleural TB cases accompanying pulmonary TB could not be recorded due to the lack of histopathological and microbiological evidence, whereas these pleural TB cases in our study could have been diagnosed accurately in the light of clinical and radiological findings. Seen between 1 to 5% of the cases, tuberculous peritonitis is a rare form of EPTB (8). In previous studies, the rates of tuberculous peritonitis were stated as10% in the study by Tavusbay et al. (11), as 5.78% in the study by Sirin et al. (16), and as 3.8% in the study by Rieder et al. (10). In our study, the rate of cases with tuberculous peritonitis was detected to be higher with a rate of 14.2%. The cases of tuberculous peritonitis detected in our study are generally those examined in the hospitalized patients, with no growth in the cultures and undergoing further investigation due to their unresponsiveness to the combination therapy with various antibiotics. Fourteen of 17 cases with tuberculous peritonitis were diagnosed microbiologically and histopathologically. We consider that such a higher rate of accurate diagnosis was related to the fact that the necessary diagnostic procedures can be performed in our hospital because it is a tertiary research hospital, and the patients referred from the secondary centers were eventually diagnosed in our hospital.

It is difficult to diagnose in EPTB, and so and the first step in the diagnosis process is to always keep in mind the likelihood of EPTB in suspected cases since the clinical symptoms and findings in the patient vary according to the affected organ in EPTB. The diagnosis of pulmonary TB is performed through the direct smear or demonstration of TB bacilli in the culture. Even so, this is not the case in the cases of EPTB, and the situation is slightly different. In diagnosing EPTB, histopathological methods have come to the fore frequently, and invasive interventions are usually required for diagnostic procedures. Therefore, the diagnosis of EPTB is more difficult (12). Of the patients with EPTB, 69.7% were diagnosed pathologically in our hospital. Such a rate is quite high and shows that the physicians in our hospital have reached a high level

in diagnostic biopsy procedures. On the other hand, it can also be asserted that the awareness level of the physicians performing biopsies is lower in terms of TB because the material obtained by the surgical branches is usually sent to the laboratory for histopathological examination in 10 percent formaldehyde saline and microbiological examination is neglected. Although the material is sometimes sent to the laboratory for bacterial culture, mycobacterial culture and ARB examination are often overlooked. Considering the increasing anti-TB resistance today, it is obvious that mycobacterial culture plays a role in the regulation of optimal anti-TB therapy. Even in some involvements, mycobacterial culture is of crucial importance.

In the study performed by Inonu et al. (5), the rate of pathological diagnosis was 61%. while the pathological diagnosis was observed to rank first in the diagnosis of EPTB as 69.7% in our study, the rates of culture positivity, ARB positivity, microbiological diagnosis, and PPD positivity were found to be 23.5, 7.5, 24.3, and 89%, respectively. As similar to our findings, while the pathological diagnosis ranked first with the rate of 60.9% in the study conducted by Kolsuz et al. (15), the rate of culture positivity was found to be quite lower (2.5%), compared to that in our study. Even so, the rate of PPD positivity was found at a higher rate (95.7%), similar to our study finding. In the study by Demir et al. (17), ARB positivity was found to be 9.2%. With the samples obtained repeatedly, the demonstrability of the bacilli increases, and so a higher rate of ARB positivity was achieved as 30% in the study by Kurt et al. (18). The rates of histopathological diagnoses were found much higher than those of microbiological diagnoses both in our study and in other studies. The alteration in the rates of microbiological diagnosis encountered in various studies is related to the awareness level of physicians about EPTB. Such a situation suggests that the diagnostic biopsy is carried out in terms of the differentiation between malignant and benign samples; infectious factors are sometimes considered by referring bacterial culture to the laboratories, but TB is not taken into consideration. Therefore we consider that the awareness of EPTB should be increased among all physicians, especially in surgical branches.

While the success rate of the treatment was found as 93.2%, the mortality rate was detected to be 3.3% in our study. The forms of our EPTB patients resulting in mortality were as follows: one case with miliary TB, one with TB meningitis, one with TB peritonitis, and the last one with larynx TB. The last two cases also had additional comorbidities. The mortality rates were determined as 33.3 and 50% in TB meningitis and miliary TB cases, respectively. In the study conducted by Arıbas et al., the mortality rate among TB meningitis cases was found to be 13.1% (22). In various previous studies, the mortality rates of TB meningitis cases have been reported between 15-50% (23). The mortality rate is seen to be between 25-30% in the cases with miliary TB (24). However, we consider that higher mortality rates of both TB meningitis and miliary TB were associated with the low number of cases in our study since the presence of one case in each form led to higher mortality rates. While the treatment success and mortality rates were found to be 91 and 4.1% in the study conducted by Gonlugur et al. (19) respectively, the rates of the treatment success and mortality were detected as 91.3 and 2.2% in the study by Kolsuz et al. (15) respectively. However, in the study by Sengul et al. (20), the treatment success and mortality rates were reported as 94 and 2.4%, and the treatment success was found to be 80% in the study by Yılmaz et al. (21). The treatment success and mortality rates determined in our study are largely compatible with those found in various studies.

Since our study is of a retrospective design, the fact that no data were available from the patients' files can be seen as a limitation of our study.

CONCLUSION

Lymphadenitis and pleural TB were determined as the most common involvement form of EPTB in the province of Konya. It should be known that TB is a multisystemic disease and can manifest itself with a wide range of symptoms. In our country where the incidence of TB is encountered at a higher rate, it should also be kept in mind by the physicians in the differential diagnosis that EPTB is difficult to diagnose. If the clinical picture is compatible with EPTB, supportive tests should be performed, the biopsy samples should be obtained from appropriate tissues, and microbiological examinations should be implemented for TB. Finally, if there is sufficient evidence, anti-TB treatment should be commenced immediately.

ETHICAL DECLARATIONS

Ethics Committee Approval: An approval was obtained from the Medical Specialty Education Board of Konya Training and Research Hospital (Date: 01/08/2019, Desicion No: 28-01).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: All authors also declare no conflict of interest.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Investigation of factors associated with nausea and vomiting in pregnant women

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ABSTRACT

Aim: In the study, it was aimed to determine the factors that may be associated with nausea and vomiting in pregnant women.

Material and Method: The research was conducted between November 2020-February 2021. The sample consisted of 434 pregnant women. Included were primiparous and multiparous pregnant women aged 18 and older, at 6-16 weeks of gestation, able to read, write and understand the Turkish language. "Personal Information Form" and "Pregnancy-Unique Quantification of Emesis and Nausea-PUQE" were used as data collection tools. The data were collected by online questionnaire created in Google Forms. The data obtained from the study were analyzed using a statistical package program. A value of $p<.05$ was accepted as the level of significance.

Results: 77.6% of the pregnant women had nausea and vomiting during pregnancy. The results of this study showed that pregnant women who were old age, unemployed, had nausea and vomiting in their previous pregnancies, did not use any vitamin supplements during their pregnancy, and whose fetüs was a girl were at high risk for nausea and vomiting during pregnancy.

Conclusion: Although nausea and vomiting during pregnancy are a natural physiological process, the findings obtained from this study provide more information about the potential factors associated with nausea and vomiting during pregnancy. Understanding potential risk factors associated with pregnancy can provide better guidance of pregnant women about nausea and vomiting and treatment options by healthcare professionals. Further studies are needed to examine lifestyle changes and psychological conditions during pregnancy, which are factors that can cause and increase nausea and vomiting.

Keywords: Pregnancy, nausea, vomiting, associated factors

INTRODUCTION

Nausea and vomiting are common experiences in pregnancy and occurring in 56–80% of all pregnancies during the first trimester. There is a wide range of severity of nausea and vomiting, from mild, occasional nausea to severe, persistent vomiting requiring hospitalization (1). Nausea and vomiting of pregnancy (NVP) is exacerbated and can cause maternal weight loss, electrolyte imbalance, and dehydration. This condition, defined as hyperemesis gravidarum (HG), is one of the most common causes of hospitalization in pregnant women (2).

NVP symptoms usually begins between 4-6 weeks of pregnancy, and peaks between 7-12 weeks (3,4) and usually resolves before the 16th week of pregnancy (5). NVP can also be defined as "morning sickness" due to the fact that it usually occurs in the morning (6).

Although the etiology of NVP remains unclear, it appears to be positively associated with serum levels of estrogen and human chorionic gonadotropin (hCG). In addition, different studies have reported that factors such as age, ethnicity, number of pregnancies (7), gastrointestinal problems (8), personality traits, depression (3) are associated with NVP. However, the data in the studies are not consistent (1-7). Treatment -of NVP depends on the severity of symptoms and accompanying complications. Although non-pharmacological medications, diet and lifestyle changes can be effective in mild nausea and vomiting, they are ineffective in more severe cases. Pharmacotherapy is required when non-pharmacological interventions are not effective (1,9).

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NVP is a condition that has negative effects on the individual, family, social and work life of the woman. NVP prevents women from practicing self-care, decreases their strength and makes them highly sensitive (6). Recognizing and treating NVP in a timely manner can prevent NVP from progressing to HG. All women with symptoms related to NVP should be counseled about safe and effective treatments early in their pregnancy. The pathogenesis of NVP is poorly understood, and the etiology is likely to be multifactorial. Identifying NVP and associated factors prevents progression of NVP and can assist with monitor treatment (10). The aim of this study was to investigate the factors affecting the nausea and vomiting of pregnancy.

MATERIAL AND METHOD

Permission was obtained from the Non-Interventional Ethics Committee of Eskisehir Osmangazi University (Date: 16.10.2020, Decision No: 08). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Sample

A cross-sectional study was conducted from November 2020-February 2021. The sample consisted of 434 pregnant women. Included were primiparous and multiparous pregnant women aged 18 and older, at 6-16 weeks of gestation, able to read, write and understand the Turkish language. Diabetic disease, hyperthyroidism, heart disease, multiple pregnancy, molar pregnancy, kidney disease and gastrointestinal problems that could cause NVP were excluded from the study. These diseases were questioned according to medical diagnosis.

Data Collection

The data were collected with "Personal Information Form" and "Pregnancy-Unique Quantification of Emesis and Nausea-PUQE".

Personal Information Form

There were 24 questions in the Personal Information Form. The form was developed by the researcher to determine the socio-demographic characteristics of women, obstetric properties and symptoms related to NVP.

Pregnancy-Unique Quantification of Emesis and Nausea-PUQE

The Pregnancy-Unique Quantification of Emesis and Nausea-PUQE modified from the Rhodes scoring system (11) and based on the 3 items that included the number of daily vomiting episodes (for how long have you felt nauseated or sick to your stomach?), the length of nausea per day in hours (how many times do you vomit or throw up?), and the number of retching episodes (how many times have you had retching or dry heaves without bringing anything up?). Responses were then grouped into 5 different categories that were scored from 1 to 5 according to the severity of the symptom. Symptoms include last 24 hours (12). The scale was adapted to Turkish by Sucu et al (2009). In the PUQE test score between 3–6 points was defined as mild NVP, 7–12 points as moderate NVP and 13-15 points as severe NVP (13).

Data Collection

Data were collected via an online questionnaire reated in Google Forms. The form was shared in groups on Instagram and Facebook in which pregnant women participated extensively. The page administrators who created the groups were contacted and it was provided that the questionnaire was shared in groups at regular intervals. Before the web-based questionnaire, pregnant women approved the consent form that they agreed to participate in the study. The aim of the study and they can withdraw from the study at any time were explained in the consent form The pregnant women were asked whether they were diagnosed with diabetes, hyperthyroidism, heart disease, multiple pregnancy, molar pregnancy, kidney disease and gastrointestinal problems that may cause NVP and those with one of these diseases were excluded from the study.

Statistical Analysis

Descriptive statistics results of the sample are given as mean and standard deviation. Whether there is a difference in terms of socio-demographic, obstetric and other complaints in pregnant women with and without nausea-vomiting was evaluated with chi-square analysis for non-continuous variables.

The relationship between PUQE test scores of pregnant women with and without nausea-vomiting and some discontinuous control variables were analyzed with Kruskal-wallis on the number of groups (>2). The significance level was considered $p<.05$.

RESULTS

The socio-demographic and obstetric characteristics of the pregnant women are given in **Table 1**. The mean age of the participants 27.77±4.21, 79.3% are university graduates. A total of 41.5% are between 6-12 weeks and 58.5% are between 13-16 weeks of pregnancy.

77.6% of the pregnant women had NVP. 39.2% of them used drug due to NVP. 21% of multiparous pregnant women experienced NVP in their previous pregnancies (**Table 2**).

The distribution of the average PUQE score of pregnant women classified as mild, moderate and severe NVP is given in **Table 3**. Half of pregnant women have moderate NVP (59.1%).

Table 1. Socio-demographic and obstetric characteristics of pregnant women

Socio-demographic and obstetric characteristics	Mean	SD
Age	27.77	4.21
	n	%
Education status		
Primary education	6	1.4
High school	84	19.4
University	384	79.3
Working status		
Working	176	40.6
Not working	258	59.4
Number of pregnancies		
Primiparous	299	68.9
Multiparous	135	31.1
Pregnancy week		
6-12	180	41.5
13-16	254	58.5
Gender of fetus		
Female	112	25.8
Male	102	23.5
Unknown	220	50.7

Table 2. Distribution of the characteristics of nausea and vomiting during pregnancy of women

The characteristics of nausea and vomiting during pregnancy	n	%
Experiencing nausea and vomiting during pregnancy		
Yes	337	77.6
No	97	22.4
Experiencing nausea and vomiting in previous pregnancies*		
Yes	91	21.0
No	44	10.1
Having sensitivity to smell during pregnancy		
Yes	396	91.2
No	38	8.8
Drug use due to nausea and vomiting*		
Yes	132	39.2
No	205	60.8

* Only multiparous pregnant women answered.
* Those who had nausea and vomiting during pregnancy answered.

Table 3. Distribution of the mean PUQE score of pregnant women

PUQE Classification	n	%
Mild NVP	120	35.6
Moderate NVP	199	59.1
Severe NVP	18	5.3
PUQE Total score (Mean.± SD)	7.36±2.66	

The relationship between the PUQE mean scores of the pregnant women and some socio-demographic, obstetric, and NVP related characteristics were examined (**Table 4**). As the age decreased, the severity of the NVP increased (KW=9.524, p=.023). 24.4% of working mothers did not have NVP, while this rate was 20.9% for non-working mothers. NVP rate in non-working mothers were higher than in working mothers (X2=702.140, p=<.001). Participants who smoke during pregnancy had less NVP than non-smokers (X2=13.920, p=.034). Women who experienced NVP in their previous pregnancy had severe NVP in this pregnancy (X2=47.630, p=<.001). The presence and severity of NVP decreased in those who used vitamins during pregnancy compared to those who did not use vitamins (X2=10.346, p=.016). Since the fetal gender is determined in the following weeks and the severity of NVP usually decreases in the following weeks, the effect of the fetal gender on the NVP had been evaluated according to whether the pregnant woman had NVP or not. Pregnant women with a female fetus (82.1%) had a higher rate of NVP compared to pregnant women with a male fetus (67.6%) (X2=6.020, p=.014).

Table 4. The effects of some socio-demographic, obstetric and nausea-vomiting characteristics on the mean PUQE score of pregnant women

Some characteristic	Nausea-Vomiting				Statistical analysis
	None	Mild	Moderate	Severe	
Age (Mean±SD)	28.91±4.26	28.55±4.63	27.47±4.39	27.29±3.67	KW=9.524 p=.023*
Education status, n (%)					
Primary education	0 (0.0)	4 (66.7)	2 (33.3)	0 (0.0)	X^2=6.892 p=. 311**
High school	15 (17.9)	26 (31.0)	39 (46.4)	4 (4.8)	
University	82 (23.8)	90 (26.2)	158 (45.9)	14 (4.1)	
Working status, n (%)					
Working	43 (24.4)	69 (39.2)	58 (33.0)	6 (6.4)	X^2=702.140 p=<.001***
Not working	54 (20.9)	51 (19.8)	141 (54.7)	12 (4.7)	
Number of pregnancies, n (%)					
Primipar	69 (23.1)	84 (28.1)	138 (46.2)	8 (2.7)	X^2=5.336 p=.149***
Multipar	28 (20.7)	36 (26.7)	61 (45.2)	10 (7.4)	
Smoking during pregnancy, n (%)					
Smoking	9 (42.9)	2 (9.5)	10 (47.6)	0 (0.0)	X^2=13.920 p=.034**
Not smoking	84 (22.2)	112 (29.6)	167 (44.1)	16 (4.2)	
Used before pregnancy, not during pregnancy	4 (11.8)	6 (17.6)	22 (64.7)	2 (5.9)	
Presence of nausea and vomiting in previous pregnancies, n (%)					
Yes	4 (4.4)	27 (29.7)	50 (54.9)	10 (11.0)	X^2=47.630 p=<.001***
No	24 (54.5)	9 (20.5)	11 (25.0)	0 (0.0)	
Using a multivitamin during pregnancy					
Yes	85 (25.4)	94 (28.1)	143 (42.8)	12 (3.6)	X^2=10.346 p=.016***
No	12 (12.0)	26 (26.0)	56 (56.0)	6 (6.0)	
	Yes		No		
Fetus gender, n (%)					
Female	92 (82.1)		20 (17.9)		X^2=6.020 p=.014****
Male	69 (67.6)		33 (32.4)		

* Kruskal-wallis, ** Exact chi-square, *** Pearson chi-square, **** Fisher's exact test

DISCUSSION

In our study, 77.6% of the pregnant women stated that they had NVP complaints. While this rate is similar to the results of some studies indicating NVP rates in the literatüre (2, 14, 15), it was found higher than from some (16, 17). In our study and some studies showing a higher incidence of NVP, the NVP complaints of women were questioned in the early weeks, this provided better detection of early pregnancy conditions.

In this study older women were associated with mild NVP; this result is consistent with previous knowledge that increased age affects NVP. (18, 19).

The literature on the relationship between NVP and parity is uncertain: both primiparity (20, 21) and multiparity (17, 18, 22) have been associated with NVP. Similar to our study, in studies that found a relationship between NVP and multiparity have shown that women who had NVP in their previous pregnancy have an increased risk of NVP in their subsequent pregnancies (17, 18, 22). NVP tends to recur in subsequent pregnancies. Therefore, the absence of a history of NVP in previous pregnancies makes the diagnosis less likely (23).

Compared with working women, non-working women were at increased risk of NVP. The finding of our study are consistent with some studies which were identified non-working as a risk factor of NVP (16, 17). However, it remains unclear whether employment status is a true risk factor for NVP. Because women may stop working due to symptoms and may choose not to work pregnancy complications and the need to care for other children (16).

Due to the negative effect of smoking on the placenta during pregnancy, some data have been revealed that the placental volume remains small and therefore NVP complaint may decrease due to the decrease in hCG and estrogen hormone levels secreted (24). Our study and some study results in the literature showed that NVP decreased in pregnant women who smoke compared to non-smokers (9, 24).

In our study, vitamins were associated with a decreased risk for NVP. In addition, NVP severity decreases in pregnant women who have NVP and use vitamins. An association between vitamin supplementation and prevention of NVP has previously been described. However, no information was given about the pathogenesis of this relationship (9). One of the possible etiology of NVP is shown as vitamin B6 deficiency. The effect of vitamin B6 on nausea and vomiting is generally explained by the fact that vitamin B6 acts as a coenzyme in the reactivity of the amino acid lysine. It is explained by the fact that lysine reactivity reduces nausea and vomiting caused by increased estrogen levels in pregnant women (25). It is thought that NVP may decrease in these pregnant women due to vitamin B6, which may be in the content of the vitamins used by the participants.

As a social belief around the world, it is thought that the presence and intensity of NVP can be predictive of fetal gender. According to this belief, if a pregnant woman does not experience frequent NVP, she will probably give birth to a boy, and if she experiences intense NVP, she will give birth to a girl (21). In our study, it was determined that pregnant women with female fetus had a higher rate of NVP compared to pregnant women with male fetus. In a retrospective study conducted with 9,980 women giving birth in the United Kingdom, it was found that the incidence of hyperemesis gravidarum (HG) was higher in pregnant women whose babies were female than those whose babies were male (26). The pathogenesis of the relationship between NVP and fetüs gender is not fully known. However, the effect of hCG, an endocrine factor that can play an important role in NVP and mediate the effect of fetal gender on NVP, has been shown in some studies. hCG production may differ in terms of gender due to different placental gene expression (27), and chromosome-based sex differences in placenta can activate the pathways that lead to NVP (21).

Limitations

In the study, effect of nutritional habits and psychological factors on the NVP has not been evaluated.

PUQE test evaluates nausea and vomiting in the last 24 hours. Nausea and vomiting are evaluated together in the scale, but these symptoms may respond differently to pharmacologic and nonpharmacologic treatment. NVP symptoms usually begins between 4-6 weeks of pregnancy and resolves before the 16th week of pregnancy. Therefore, pregnant women at different weeks of gestation were included, but this wide range of gestational weeks may lead to different results.

CONCLUSION

In our study, it was found that compared to some previous studies, a higher prevalence of NVP and pregnant women were at higher risk for NVP due to some characteristics. The results of this study showed that pregnant women who were old age, unemployed, had NVP in their previous pregnancies, did not use any vitamin supplements during their pregnancy, and whose fetüs was a girl were at high risk for NVP. Although NVP is a usual physiological process, the findings obtained from this study provide more detailed

information about the potential factors associated with NVP. Understanding potential risk factors associated with pregnancy can provide better guidance of pregnant women about NVP and treatment options by healthcare professionals. Further studies are needed to examine lifestyle changes and psychological conditions during pregnancy, which are factors that can cause and increase NVP.

ETHICAL DECLARATIONS

Ethics Committee Approval: Permission was obtained from the Non-Interventional Ethics Committee of Eskisehir Osmangazi University (Date: 16.10.2020, Decision No: 08).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Conflict of Interest Statement: The author have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they all participated in the design and the conduct of the study, and in the analysis of the study data. All authors have approved the final version.

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Laboratory parameters can be used to differentiate renal infarction and urolithiasis in patients who are admitted to the emergency department with flank pain

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ABSTRACT

Aim: We aimed to determine the predictive value of laboratory parameters for the distinction between urolithiasis (UL) and renal infarction (RI) in patients presenting to the emergency department with flank pain complaint.

Material and Method: In our retrospective study, the files of 73 patients who presented to the emergency department (ED) with flank pain and whose costovertebral angle tenderness was positive were reviewed. Routine blood tests and the results of contrast-enhanced abdominal computed tomography were obtained. The patients were divided into two groups as RI and UL according to the results of computed tomography. Accordingly, 8 patients were found to have RI, and 65 had UL.

Results: Lactate dehydrogenase (LDH) and platelet lymphocyte ratio (PLR) values were significantly higher in the RI group compared to the UL group ($p<0.001$-$p=0.045$). In ROC curve analysis, the AUC values of LDH and PLR were determined as LDH (AUC=0.983, $p<0.001$) and PLR (AUC=0.719, $p=0.015$) for their diagnostic performance in distinguishing between RI and UL.

Conclusion: We think that the use of LDH level and PLR value may be guiding in making a distinction between UL, one of the frequent reasons for presenting to ED, and RI, in which rare but early diagnosis of plank pain is critical in terms of renal parenchymal damage.

Keywords: Emergency department, flank pain, urolithiasis, renal infarction

INTRODUCTION

Flank pain is one of the important reasons for presenting to the emergency department (ED). Although many diseases, such as pyelonephritis, urinary system diseases such as ureteropelvic junction obstruction, or intestinal, gynecological, and retroperitoneal diseases can present with similar clinics, most of them turn out to be urolithiasis (UL) cases (71.4%) (1). Even though it is rare (0.004-0.007%), one of the most important etiology is renal infarction (RI). RI is permanent renal parenchymal damage characterized by impaired blood flow to the kidney as a result of renal artery occlusion. It has an increased prevalence in the population over the age of 40 (2,3). Apart from flank pain, patients present to ED due to nonspecific reasons, such as fever, nausea, vomiting, uncontrolled hypertension, hematuria, or acute renal failure. This may delay the diagnosis of the disease and increase the risk of possible complications and renal failure (4). Clinical presentation similar to acute pyelonephritis, especially UL, and other diseases that constitute acute abdominal clinics, may cause delays in the diagnosis of infarction (5). The most important step in diagnosis is to consider RI disease because there are no specific laboratory parameters. Contrast-enhanced abdominal computed tomography (CT) is the preferred imaging method to evaluate a variety of acute abdominal conditions. The gold standard method for the diagnosis of RI is contrast-enhanced abdominal CT because it is easy to apply and non-invasive (6,7). Demonstration of a wedge-shaped hypodense lesion in the peripheral area is diagnostic. In addition, elevated white blood cell (WBC), C-reactive protein (CRP), lactate dehydrogenase (LDH), decreased glomerular filtration rate (GFR), and increased creatine (Cr) in patients may help us. LDH is an indicator of cell necrosis, and its high levels have been shown as a promising marker for predicting the renal prognosis (3,4,6-8).

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This study aimed to investigate whether laboratory parameters could be used to make a distinction between patients with RI, which is a rare clinic in ED, and patients with UL, which is one of the main reasons for flank pain presentations.

MATERIAL AND METHOD

The study, which was designed retrospectively, was approved by the The study was approved by the Balıkesir University Faculty of Medicine Clinical Research Ethics Committee (Date: 23.12.2020, Decision No: 2020/238). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. This study was conducted with the documentation of patients who were aged 18 or older and who presented to University Faculty of Medicine Emergency Department with flank pain between February 01, 2019 and November 01, 2020. Patients younger than 18 years of age were not included in the study. According to the documentation, it was determined that 181 patients presented to the ED with flank pain complaints. However, some of them were excluded from the study because 18 had trauma, 58 had an infection in their complete urinalysis, and 32 had incomplete examinations. A total of 73 patients were included in the study. Of the laboratory values of the patients, WBC, neutrophil, lymphocyte, platelet (Plt), mean platelet volume (MPV), Urea, creatinine (Cr) and LDH were recorded. Neutrophil lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) were calculated. The final diagnosis of all patients was confirmed by contrast-enhanced abdominal computed tomography results. Our data were collected by trained researchers.

Statistical Analysis

Shapiro-Wilk test was used to test the normality of variables. Continuous variables were presented as mean±standard deviation values for normally distributed variables and with median (1st quartile-3rd quartile) values for non-normal variables. Mann-Whitney U-test was used for comparing two independent groups. Categorical variables were expressed by counts and percentages. Comparisons between the groups were performed with Fisher's exact chi-square test for categorical variables. Risk factors were also evaluated with binary logistic regression analysis. Receiver operating characteristics (ROC) curve analysis was performed to evaluate and compare the performances of diagnostic markers. Youden J index was used to obtain optimal cutoff value, and related sensitivity, specificity, positive predictive, and negative predictive values were given. The significance level was taken as α=0.05. The statistical analyses were performed with IBM SPSS Statistics version 22.0 (IBM Corp., USA) and MedCalc version 12.3.0.0 software packages.

RESULTS

The study included 73 patients, 8 (10.96%) of whom had renal infarct (RI) and 65 (89.04%) of whom had urolithiasis (UL). The mean age of the study sample was 45.45±14.37 years. Of the 73 patients, 44 (60.27%) were male, and 29 (39.73%) were female. Among the RI group, complete resolution of the thrombus was observed in 3 (37.50%) patients, and partial resolution of the thrombus was observed in 5 (62.50%) patients.

There was a significant difference between RI and UL groups in terms of LDH (p<0.001) and PLR (p=0.045). The values were significantly higher in the RI group compared to the UL group. There was no significant difference between the two groups in terms of the other variables (**Table 1**).

Table 1. Comparison of patient characteristics between RI and RC groups

Variable		RI	UL	p-value
Age (years)		51.00 (47.00-56.00)	44.00 (36.00-53.50)	0.152
Gender	Female	3 (37.50)	26.00 (40.00)	1.000
	Male	5 (62.50)	39 (60.00)	
WBC ($\times10^3$)		10.40 (7.25-12.85)	10.30 (8.80-11.90)	0.818
Neutrophil ($\times10^3$)		7.80 (5.35-10.95)	7.40 (5.63-9.00)	0.578
Lymphocyte ($\times10^3$)		1.30 (0.90-1.95)	1.80 (1.40-2.35)	0.097
PLT ($\times10^3$)		246.50 (211.75-302.25)	244.50 (208.50-306.50)	0.923
MPV (fL)		8.50 (7.93-9.18)	8.20 (7.45-9.15)	0.372
Urea (mg/dL)		33.50 (25.00-49.75)	35.00 (26.00-44.50)	0.916
Creatinine (mg/dL)		1.03 (0.75-1.47)	0.97 (0.83-1.27)	0.951
LDH (IU/L)		964.00 (344.75-1963.25)	198.00 (173.00-221.50)	<0.001
NLR		6.71 (3.92-11.11)	4.21 (2.23-6.19)	0.154
PLR		200.14 (137.28-256.67)	136.53 (102.71-184.34)	0.045

Data given as median (1st quartile-3rd quartile) or n (%)
WBC: White Blood Cell, PLT: Platelet MPV: Mean Platelet Volume, LDH: Lactate Dehydrogenase, NLR: Neutrophil Lymphocyte Ratio, PLR: Platelet Lymphocyte Ratio

We performed ROC curve analyses to evaluate the diagnostic performances of WBC, neutrophil, lymphocyte, Plt, MPV, urea, Cr, LDH, NLR, and PLR in discriminating patients who had RI and UL. Significant diagnostic performances were obtained for LDH (AUC=0.983, p<0.001) and PLR (AUC=0.719, p=0.015). Optimal cutoff values were obtained according to the Youden J index, and corresponding sensitivity and specificity values were given. The WBC (p=0.848), neutrophil (p=0.632), lymphocyte (p=0.083), Plt (p=0.912), MPV (p=0.271), urea (p=0.933), Cr (p=0.960), and NLR (p=0.175) values did not yield significant diagnostic performance in distinguishing between RI and UL groups (**Table 2, Figure 1**).

Table 2. Diagnostic accuracy measures for LDH and PLR

Diagnostic accuracy measure	LDH	PLR
AUC	0.983	0.719
p-value	<0.001	0.015
Cutoff value	>259	>225
Youden-J index	0.892	0.391
Sensitivity (95% CI)	100.00 (63.10-100.00)	50.00 (15.70 – 84.30)
Specificity (95% CI)	89.23 (79.10 – 95.60)	89.06 (78.80 – 95.50)
PPV (95% CI)	53.3 (25.70-79.50)	36.4 (10.90-69.20)
NPV (95% CI)	100.0 (93.80-100.00)	93.4 (84.10-98.20)

AUC: Area under the curve, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, LDH: Lactate dehydrogenase, PLR: Platelet lymphocyte ratio

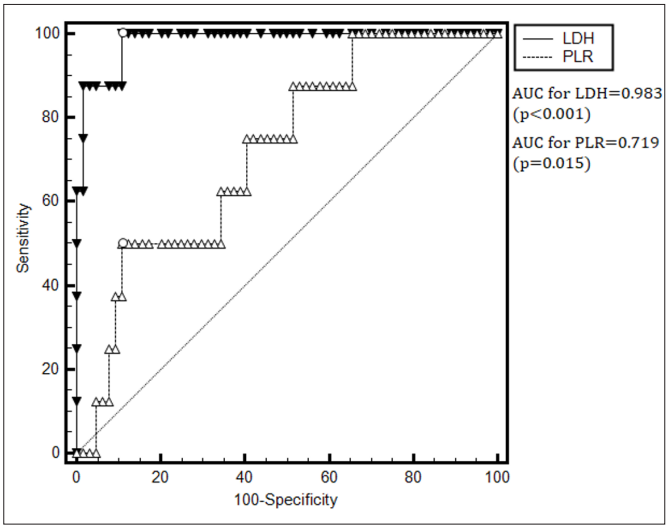

Figure 1. ROC curve for LDH and PLR

We grouped patients according to their LDH and PLR values for the cutoff point obtained from the ROC curve analysis. In the RI group, 8 of the 8 patients (100%) had LDH values of >259 IU/L, and 58 of the 65 patients (89.23%) in the UL group had LDH values of ≤259 IU/L (**Figure 2**). Also, in the RI group, 4 of the 8 patients (50%) had PLR values of >225, and 57 of the 64 patients (89.06%) in the UL group had PLR values of ≤225.

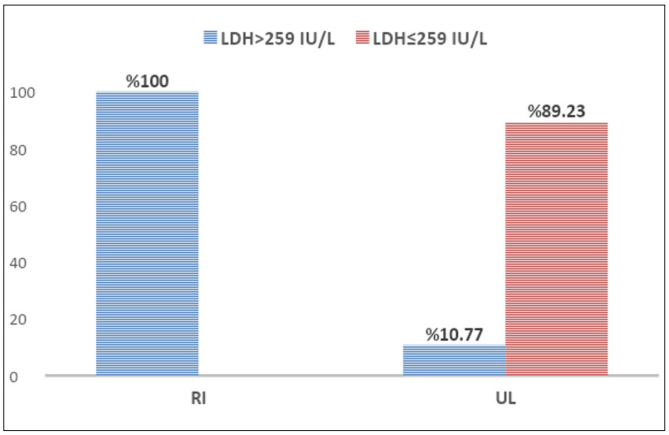

Figure 2. Percentages of RI and UL groups for LDH>259 and LDH≤259 patients

DISCUSSION

The mean age of ED presentations with flank pain concentrates in the 4th decade of life. According to the results of the study involving 400 patients which was conducted by Taşlıdere (9) (2018), the mean age of the patients was 38.22±14.5 years (1). Similarly, RI is more common in patients over the age of 40 (2). In our study, the mean age of all patients presenting with flank pain was 45.45±14.37, and the median age of patients with RI was 51 years, which is consistent with the literature.

A significant increase in WBC and LDH levels has been found as the most common laboratory parameter in RI patients (10). According to the results of a review study consisting of 102 patients, which was conducted by Antopolsky et al. (10), LDH levels were found to be significantly higher in 95% of patients with RI, and WBC was shown to increase, as well (11). According to the results of a study that involved 121 patients with RI and was conducted by Eren et al. (2), LDH was found high (696±93 IU/l). In the literature, the RI dimension and LDH level were determined in direct proportion, and it was specified as high (6-11). Similarly, LDH was found high in our study, too. WBC and CRP are directly proportional to decreased acute stage renal function (6). According to the study of Silverberg et al. (5) (2016), WBC was found to be high in 67% of the patients with RI, and CRP was high in 72% of them. In our study, we found that the WBC count was not increased in both the RI group and the UL group. We think that this was because patients with flank pain due to infection were not included in the study. Besides, the Cr values of our patients were within the normal range, which may explain the absence of an increase in WBC and CRP levels.

NLR and PLR increase secondary to inflammation, and it is stated in the literature that it can be employed as an alternative to WBC (12). As a result of a 30-day mortality survey on 688 patients who presented to ED with abdominal pain, it was reported that the increase in NLR and PLR was directly proportional to mortality (13). When the rate of NLR and PLR of patients with UL and acute appendicitis (AA) was compared, it was found to be significantly higher in the AA group (14). However, there are no studies in the literature regarding the correlation between PLR and NLR between RI and UL groups, and the results of our study are the first in this regard. While we did not find a significant change in the NLR rate, we found the PLR rate high, and we think it can be used for diagnosis.

In studies conducted on patients with UL, Cr values (0.7±0.56 mg/dL-0.96±0.19 mg/dL) were found to be normal (9-15). According to the study of Eren et al. (2) (2018), the mean Cr value of 121 patients was

reported to be minimally high as 1.5±0.1 mg/dl, and ABY was determined in 35% of the patients. According to the study of Antopolsky et al. (10) (2012), a moderate increase was found in Cr levels of 43.96% of the patients. According to our results, no significant increase was observed in the Cr value, and no difference was found between the groups, either. We think this may have been because the diagnoses of the patients had been made in the emergency department before the kidney functions of the patients were impaired and the patients may have increased their oral hydration for urolithiasis.

CONCLUSION

Urolithiasis-focused radiological scanning alone in patients who present to ED with flank pain may cause the diagnosis of RI to be overlooked. Therefore, complications, morbidity, and mortality will increase in these patients. We argue that the LDH value should definitely be studied in patients who present to ED with flank pain, additionally, PLR rate should be calculated, and that in case of detection at high levels, RI diagnosis should be ruled out.

Limitations: Our study was carried out in a single center. The majority of the articles on RI are case reports, and similarly, we have some limitations in terms of the number of patients. The reason why our study group included a small number of patients is that RI is a very rare disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Balıkesir University Faculty of Medicine Clinical Research Ethics Committee (Date: 23.12.2020, Decision No: 2020/238).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Adaptation of COVID-19 risk perception and COVID-19 prevention guidelines compliance scales to Turkish: a validity and reliability study

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ABSTRACT

Aim: The aim of this study is to make Turkish adaptation and psychometric analysis of the COVID-19 Risk Perception and COVID-19 Prevention Guideline Compliance scales.

Material and Method: The COVID-19 Risk Perception and COVID-19 Prevention Guideline Compliance scales administered to a total of 385 healthcare workers (Emergency medical technician, paramedic, ambulance driver etc.) and 50 healthcare workers were retested two weeks later. The opinions of 10 experts were taken for the content validity of the scale, the confirmatory factor analysis was performed for the construct validity, the Cronbach's alpha reliability coefficient was calculated to determine the internal consistency, and the test-retest reliability was performed and the results were evaluated with Pearson correlation analysis.

Results: It can be concluded that an agreement among experts according to the results of the content validity index of the Turkish version of COVID-19 Risk Perception scale was found to be 0.91, the COVID-19 prevention guideline compliance scale was found to be 1. The test-retest reliability correlation of the scales was 0.85.

Conclusion: COVID-19 Risk Perception and the COVID-19 prevention guideline compliance scales were suitable for Turkish culture, and they are valid and reliable.

Keywords: COVID-19, risk perception, prevention guidelines, validity and reliability

INTRODUCTION

According to World Health Organization (WHO) (1) currently the world has been witnessing a global epidemi of 2019 novel coronavirus (SARS-CoV-2) which causes COVID-19 disease. Available data highlighting the actual prevalence of the disease support this view (2,3). It is a well-known fact that the spread of the disease is affected by the willingness of the public to adopt preventive public health behaviors which are often associated with the public's risk perception.

Risk perception is significantly associated with preventive health behaviors reported in all ten countries (4). In order to slow down the spread of the virus some countries have been implementing drastic measures such as severe travel restrictions, border closures, lockdowns, curfews, limiting personal contact with people except family members or permitting only one person who is not a family member. Most people around the world have felt the need to follow the rules of hand sanitation and social distancing in order to prevent coronavirus and its spread. Nevertheless, while some people are strictly obedient to the restrictions, others either ignore or delay restrictions of governments. The fact that individuals act very differently from each other during this period is a sign that risk perception of this disease strongly differs from different places and individuals. Moreover, since this situation can influence the number of new positive cases, it is an indicator that risk perception is a potentially powerful modifier of epidemic evolution (5).

As the number of people dying of the disease increase all around the world, it becomes more significant to understand the risk perception of the public (6). The success of policies that slowed down the rapid spread of the disease during previous pandemic cases is partly attributed to the existence of people who perceived individual and social risk factors in the correct way. In fact, collectively human behavior can fundamentally influence and change the spread of a pandemic (7-9). Threat assessment and

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risk perception can be expressed as fundamental features of protection-motivation theory (10,11). In this context, it is known that collaboration of the community and their willingness to adapt preventive behaviors (frequent hand washing, social distancing, avoiding public places and wearing face masks, etc.) in time to protect their health are significant determinants during pandemics (12). In other words, correct public risk perceptions are critical to effectively managing public health risks (4).

MATERIAL AND METHOD

Even though there is important medical and epidemiological information on severity of the disease and contagiousness of the virus in the corona virus literature, psychosocial responses of the population still is not fully known (13). It is thought that there is limited research particularly on responses of individuals to COVID-19 prevention guidelines. It is considered potentially significant to discover the premises of compliance to these guidelines since it might help us identify the high risk groups and take necessary steps to improve the level of compliance. Permission was obtained from Nejc Plohl via e-mail, in order to use C-RP and C-PGC scales. Plohl sent information on the scale and how to apply the scale via e-mail.

The study protocol was approved by the Ethics Committee of İstanbul Okan University (Date: 23.12.2020, Decision No: 130). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Type of Research

This study has been carried out in a methodological way.

Study Population and Sample

The population of the study is composed of healthcare workers employed within geographical borders of Istanbul province. However, while determining the sample for the objective of the study it was planned to conduct the research on EMTs, paramedics and ambulance drivers, who are considered as special risk groups of priority. Convenience sampling was preferred as sampling method and 385 healthcare workers employed at pre-hospital medical institutions who have approved of the study participated in the scope of the study. In scale adaptation studies, for reliability and validity analysis it is recommended to reach a number of participants that is 10 times the number of items in the scale (14, 15). This rule was taken into account in order to determine the sample size.

Data Collection Tool

Participant Information Form, C-RA and C-PGC scales developed by Plohl and Musil (16) were used. The data which is used for the research had been collected between 1- 20 January 2021 using online questionnaire method. The questionnaires were prepared and uploaded on Google forms database and were sent to the employees' e-mail addresses.

Participant Information Form

This form, created by the researchers, was used in order to collect demographic information about the participants. The form includes questions about participants' gender, age, education level, marital status, occupation, employment state, whether they have a chronic disease, whether they smoke, their responds to health problems and questions about COVID pandemic.

COVID-19 Risk Perception Scale (C-RP)

The scale was developed to evaluate risk perceptions of individuals related to COVID-19 pandemic. The scale was rated with a 7-point likert ranging between strongly agree (1 point) and strongly disagree (7 points). Cronbach's alfa reliability coefficient of the original scale was found to be 0.72 (16). The scale consists of six questions in total and as the score increases a negative situation occurs.

COVID-19 Prevention Guidelines Compliance Scale (C-PGC)

The scale was developed in order to evaluate individuals' compliance levels to measurements taken against COVID-19 pandemic. It consists of 11 actions determined by WHO and CDC that has to be followed to protect against COVID-19. The scale was rated with a 4 Likert chart ranging from Never (1 point) to Always (4 points). The Cronbach's alpha reliability coefficient of the original scale was found to be 0.76 (16). There are a total of 11 questions in the scale and as the score increases, the situation arises.

Data Analysis

The data was analyzed using SPSS 26 and AMOS 20 programs. To determine the adequacy of sample size and evaluate suitability of the items to factor analysis KMO, Barlett sphericity tests were used and to analyze the data about descriptive characteristics frequency tables and central-prevalence criteria were used. In order to analyze validity of the scales, content and construct validity analyses were used and to analyze reliability an internal consistency analysis method Cronbach's alpha coefficient and test r-test analysis were used.

Validity Study of C-RP and C-PGC Scales

Language equivalence-cultural adaptation and content validity: The scales were originally written in English. The original scale was translated into Turkish by two independent linguists competent in their field to create the form in Turkish. The Turkish form created was translated back into English by two different linguists. Finally a third independent linguist revised the items of the scale and determined the most suitable options to create the English

form. The items both on the Turkish form and the original one were examined by group of ten experts consisting of academics for the appropriateness of the translation and content validity. For content validity, the consulted experts were asked to rate suitability and intelligibility of each item of the scale between 1-4 points. The experts were asked to choose one of the options "unsuitable" (1), "the item must be made more suitable" (2), "suitable but small changes are necessary" (3), or "very suitable" (4) for each item. Necessary arranges were made in line with the recommendations of the experts (17). The English form created was submitted for the approval of the author who developed the scale. For content validity, content validity index was calculated.

Construct Validity

Initially CFA assumptions were reviewed and the extent to which the theoretical model explained the relationships in data set was tested. At the last stage analyses were made on alternative models. Chi Square ($\chi 2$), degree of freedom (sd), mean squared error of the predictions (RMSEA), standardized root mean square residual (SRMR) and comparative fit index (CFI), match mismatch coefficients were evaluated. As a result of the exploratory factor analysis performed for the C-RP scale, it was seen that the scale explained two-way factor, 78.97% of the total variance, and the C-PGC scale explains with one-way factor, 64.26% of the total variance.

Reliability

Reliability of the scales was evaluated through internal consistency and test-retest. In order to evaluate internal consistency "Cronbach's alpha reliability coefficient" was used. In the evaluation of the Cronbach's's alpha coefficient, it is stated that 0.50 and below shows low reliability, 0.50-0.70 medium reliability, 0.70-0.90, high reliability, 0.90 and above show excellent reliability (18). In order to assess time invariance, 50 people having the same characteristics of the sample group were retested two weeks following the first data collection time. The relationship between test-retest scores was evaluated using Pearson correlation analysis.

RESULTS

Average age of the participants is 26.39±3.51 and 41.8% are female 58.2% are male 52.5% are single, 67.8% have a university degree. 17.9% of the participants have a chronic disease, 46.8% of them smoke. 90.6% of the participants are employed, 47.0% of them are EMT, and when they have a health condition 53.0% go to a hospital (**Table 1**).

85.5% of the participants access news about COVID-19, on the media, 93.0% of them know methods to protect against COVID-19, 92.7% of them follow "Stay Home Turkey" movement 28.6% have difficulty to supply masks (**Table 2**).

Table 1. Introductory results of students

Variables	Min.-Max.	X ± Sd
Age	18-38	26.39 ± 3.51
	n	%
Gender		
Female	161	41.8
Male	224	58.2
Marital status		
Single	202	52.5
Engaged	56	14.5
Married	127	33.0
Education		
High school	121	31.4
Bachelor	261	67.8
Post graduate	3	0.8
Chronic disease		
Yes	69	17.9
No	316	82.1
Smoking		
Yes	180	46.8
No	205	53.2
Employment		
Yes	349	90.6
No	36	9.4
Profession		
EMT	181	47.0
Paramedic	117	30.4
Ambulance driver	87	22.6
The means they choose for a health problem		
Counseling family or friends	11	2.9
Searching the Internet	19	4.9
Self-treatment	76	19.7
Chemist's	5	1.3
Family doctor	44	11.4
Hospital	204	53.0
Waiting for it to pass	26	6.8
Total	385	100.0

Table 2. Results regarding COVID-19

Variables	n	%
Follow up with media for COVID-19		
Yes	329	85.5
No	56	14.5
Knowing the ways to prevent COVID-19		
Yes	358	93.0
No	27	7.0
Following "Stay home Turkey" movement		
Yes	357	92.7
No	28	7.3
Mask supply		
I have difficulty	110	28.6
I don't have difficulty	275	71.4
Total	385	100.0

Item Analysis and Reliability Results

According to data obtained from 385 participants Cronbach's alpha reliability coefficient of C-RP was found to be 0.79, Cronbach's alpha reliability coefficient of C-PGC was found to be 0,94. Test-retest reliability correlation was found to be 0.85. C-RP score averages of the participants is 28.81±7.84; C-PGC score averages of them is 37.85±7.13 (**Table 3**).

Table 3. COVID-19 reliability analyses and descriptive statistical values of risk perception and compliance with prevention guidelines scales (n=385)

Factor (min- max)	Mean	Sd	Test-Re Test	Cronbach's alfa
COVID-19 Risk Perception Scale (8-42)	28.81	7.84	.85	.79
COVID-19 Compliance with Prevention Guidelines (11-44)	37.85	7.13	.85	.94

Results Regarding Validity

Sample sizes of the scales were evaluated using KMO and Barlett Tests. Sample size of C-RP (KMO=0.725, X^2=1402.478, p=0.00) and C-PGC (KMO=0.958, X^2=3037.842, p=0.00) was found to be medium for C-RP, excellent for C-PGC. Content validity indices of the scales were found to be 0.91 for C-RP and 1 for C-PGC.

Results Regarding Structure Validity

In the Confirmatory Factor Analysis, the factor loads of the 6 two-way factor items of C-RP were found to be between 4.17 and 0.49. As a result of the examination of the fit indices values, it was found that the factor structure showed a high fit (X^2=8.828; χ^2/ df=1.26; GFI=0.99; AGFI=0.97; CFI=0.99; RMSEA=0.026) (**Figure**).

It is found that 11 items of C-PGC scale are one-way factor, and factor loads of items are 0.59 and 0.86. As a result of the examination of the fit indices it was found that the factor structure showed a high fit (X^2=154.971; χ^2/ sd=3.52; GFI=0.92; AGFI=0.88; CFI=1.0; RMSEA=0.081) (**Figure**).

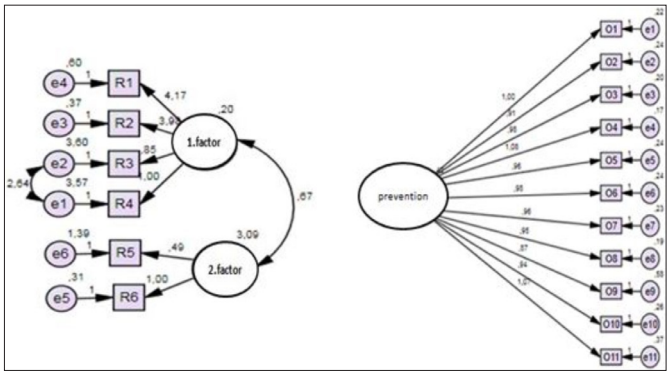

Figure. C-RP and C-CPG item- factor loads

DISCUSSION

In order to define risk perceptions of individuals of COVID-19 and to determine to what extent individuals comply with prevention guidelines, it is necessary to have measurements that are valid and reliable in the field index. In this study, it was determined that the Turkish version of the COVID-19 Risk Perception scale had two factors with 6 items (1st factor; contagion and 2nd factor; fear), and the Turkish version of the Compliance with Prevention Guidelines Scale had 11 items and one factor. It was found that being valid and reliable instruments Risk Perception and COVID-19 Prevention Guideline Compliance scales are valid and reliable scales as well.

In order to evaluate the scope validity it is recommended to consult experts who are acquainted with the subject being researched and the methods of creating scale items. It is stated in the literature that opinions of at least 3 and at most 20 experts should be obtained and percentage of compliance and Content Validity Index (CVI) should be calculated (17). For the CVI, Grant and Davis (19) state that 80 %of the scale items must score 3 points or more. The mean opinion scores given by ten experts consulted for this study is between 3.13-3.43 for C-RP and between 3.83-4 for C-PGC. The CVI was 0.91 for C-RP and 1 for C-PGC showing that scale items are appropriate for our culture and the structure aimed to be measured can be measured.

Confirmatory factor analysis (CFA) which is one of validity methods is used to develop scales or adapting a developed scale to another culture (20). According to the (CFA) results of this study, general coefficient concordance of COVID-19 Risk Perception Scale are found to be X^2=8.828; χ^2/sd=1.26; GFI=0.99; AGFI=0.97; CFI=0.99; RMSEA=0.026) and general coefficient concordance of COVID-19 Prevention Guideline Compliance scales were found to be X^2=154.971; χ^2/sd=3.52; GFI=0.92; AGFI=0.88; CFI=1.0; RMSEA=0.081. Hair et al. (14) state that if the number of items is between 12 and 30 and the number of people is > 250 the value of X^2 is expected to be statistically significant, besides they sate that when CFI is greater than .90 and RMSEA is less than .08 general coefficient concordance of the model can be accepted as adequate. As a result of this, it is possible to claim that theoretical model adequately explains the correlations between the items.

The fact that all items on a measurement instrument measure the desired property and when measured again it gives consistent and stable results (21-24). Internal consistency is the determination of the reliability of the scale that shows concordance of scale items with each other. One of the most frequently used methods to determine internal consistency reliability is Cronbach's alpha coefficient (25). For alpha coefficient of the scales

0.50 or below low, 0.50-0.70 medium, 0.70-90 high and 90 is indicate excellent reliability levels (18). Cronbach's alpha coefficient of the C-RP scale of the original study was found to be 0.72. Cronbach's alpha coefficient of this study was found to be 0.72 which indicates high reliability level. Cronbach's alpha coefficient of the C-PGC scale of the original study was found to be 0.76. Whereas in this study the alpha coefficient of the scale was found to be 0.94 which is a better level than the original scale's. Cronbach's alpha coefficient of sub dimension of internal benefit was found lower than the original study and on a medium level.

Test retest reliability is measurements that are carried out in order to assure that the measurement tool gives consistent results and it is invariable against time. These measurements need to be repeated at least with a 2 week gap or at most a 4 week gap (26) with at least 100 participants (27) and should have a correlation coefficient of ≥ 0.40 (29). On the original study a test-retest correlation was not applied. In this study the test-retest reliability coefficient that was carried out two weeks after following the first data collection work on 50 participants was found to be 0.85 as high level.

Due to the fact that the research was carried out via online questionnaire it is limited to prehospital employees who are able to use the Internet and accepted to participate in the study. Therefore, it will be beneficial to conduct the research on different sample and occupational groups and analyze them.

CONCLUSION

In conclusion, validity and reliability of Turkish form of C-RP and C-PGC scales developed by Plohl and Musil (16) and originally written in English was found to be on a good level. Confirmatory factor analysis carried out to evaluate construct validity verified the original factor structure that is present in the literature. In accordance with these results Turkish form of C-RP and C-PGC can be used.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of İstanbul Okan University (Date: 23.12.2020, Decision No: 130).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article

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Evaluation of non-traumatic intracranial hemorrhages as cause of cardiac arrest in emergency department

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ABSTRACT

Aim: Cardiac arrests can be brought to emergency departments with an intervention from out-of hospital, and they can also occur for different reasons in the emergency department. Due to the high rates of mortality in this important clinical situation, regulations are made through guidelines and algorithms. Most of these regulations cover acute coronary syndromes and special conditions. The aim of our study is to evaluate intracranial hemorrhages in non-traumatic cardiac arrest cases in our emergency department for a period of 10 years.

Material and Method: The data of patients 18 years of age and over who were found to have cardiac arrest in the emergency department between January 2011 and January 2021, who did not have trauma, were retrospectively scanned from the hospital information management system. Demographic information of all patients, computed tomography examinations for intracranial hemorrhage and emergency department outcomes were evaluated.

Results: Of the 173 patients included in the study, 81 (46.8%) were women. The median age of the patients was determined as 72.00 (IQR 64.00-80-00). In the whole patient group, 20 (11.6%) patients had intracranial bleeding and 10 of these patients were women. More intrcranial hemorrhage was detected in the group that underwent CT before cardiopulmonary resuscitation (CPR) and it was statistically significant ($p<0.001$). In 4% of the patients who underwent CT after CPR, intracranial bleeding was detected. No difference was found in terms of the presence of bleeding or the timing of tomography in terms of discharge from the hospital.

Conclusion: With this study, we found the rate of intracranial hemorrhage (ICH) to be 11.6% in patients with in-hospital cardiac arrest. As changes occur in the treatment and management of patients in the presence of ICH, we think that brain CT should be performed in the early period in cardiac arrest cases of unknown cause, as stated in the guidelines. Especially in the presence of change in consciousness and high blood pressure, it is important to organize algorithms in order to detect ICH.

Keywords: Cardiac arrest, intracranial hemorrhage, emergency department

INTRODUCTION

Guidelines on Advanced Cardiac Life Support (ACLS) have suggested acute coronary syndromes as the cause of sudden cardiac arrest (1,2). In addition to fatal dysrhythmias and acute coronary syndromes, intracranial hemorrhages (ICHs) may also cause arrests. However, the studies on their incidence are limited in number. This condition occurs in both pre-hospital and in-hospital settings, and it continues to be an important clinical condition in cardiac arrest(3). In the studies conducted, the incidence of ICH in out-of-hospital cardiac arrests (OHCA) was found to be approximately 4%, while this rate was found to be 1% in in-hospital cardiac arrests (IHCAs) (4,5). IHCAs often develop due to cardiac and pulmonary causes, and ICHs are also among the important causes of mortality and morbidity (6,7). However, the necessity of computed tomography (CT) scan of the brain in patients with cardiac arrest is still unclear (8). The use of CT scan of the brain and thorax is recommended for the management of unexplained cardiac arrests in the guidelines (2). Intracranial pathologies detected in CT require many interventions in patients, including avoidance of antifibrinolytic, anticoagulant, and antiplatelet therapies and referral to neurology and critical care (5). Studies have shown that brain CT pathologies are considerably common in patients who are evaluated with cardiac arrest in the emergency department; therefore, further studies are needed to raise awareness on this issue (9).

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In this study, we aimed to investigate the incidence and the general characteristics of ICH detected by brain CT in patients who presented to our emergency department and developed IHCA, the role of resuscitation in these cases, and the outcomes.

MATERIAL AND METHOD

The study was initiated after obtaining approval from the Non-Interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University (Date: 09.02.2021, Decision No: 2021-68-10). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The research was conducted as a single-center, retrospective study using data from patients who presented to the emergency department of a tertiary university hospital between January 1, 2011 and January 1, 2021. Our emergency department is a tertiary center, which was nationally accredited as a department. The list of patients who underwent CPR in the emergency department was accessed through the transaction codes and evaluated. An average of 110,000 patients are admitted annually, and adult ACLS trainings are regularly held (10). Care was provided in accordance with the Utstein protocol in all cardiac arrest patients (11). Patients who developed cardiac arrest during their follow-up in the emergency department were included in the study.. Brain CT scans of these patients were examined as before and after cardiopulmonary resuscitation (CPR). Those who underwent CT before CPR were primarily believed to have an intracranial pathology based on their clinical condition. However, CT scan and achieving return of spontaneous circulation (ROSC) after CPR require additional examination, as in OHCA patients.

Among the patients who developed IHCA and underwent CPR, those who had undergone brain CT scan were included in the study. Patients <18 years of age, those with traumatic arrests and OHCA, those who could not undergo CT scan, and pregnant patients were excluded from the study. Patients who were given fibrinolytic therapy without imaging during CPR in the emergency department were also excluded from the study.

Demographic data, vital parameters at presentation, Glasgow Coma Scale at presentation, presence of hemorrhage on brain CT scan, emergency department outcomes, hospitalization periods, and hospitalization outcomes as well as modified Rankin scale (mRS) scores were recorded. The hemorrhage has been investigated in the medical records and the presence of the hemorrhage has been evaluated by emergency physicians.

Statistical Analysis

Continuous data were presented as mean±standard deviation. Categorical data were given as percentage (%). Shapiro–Wilk test was used to investigate the normality of the distributed data. For comparison of groups that do not conform to normal distribution, Mann–Whitney U test was utilized in cases where the number of groups was two. Pearson's Chi-Square and Pearson's Exact Chi-Square analyses were employed for the analysis of the cross tables created. IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) software was used to perform the analyses. The criterion for statistical significance was accepted as $p<0.05$.

RESULTS

Among the patients who were brought to the emergency department owing to non-traumatic cardiac arrest or who developed cardiac arrest in the emergency department, 294 underwent CT scan of the brain. Of these patients, 173 experienced IHCA in the emergency department, and the evaluation was made using them. Intracranial bleeding was detected in 20 (11.6%) of the 173 patients comprising the study group. Of these 20 patients, 10 were women. The demographic characteristics, presenting complaints, and vital signs of the patients are listed in **Table 1**. Altered mental status and syncope are the major neurological symptoms and %60 of the ICH group has these initial complaints. Regarding the assessment of the vital parameters, since eight patients were found to have an arrest when they were taken on a stretcher to the emergency department, their vital parameters were not included in the statistical evaluation. Systolic blood pressure, diastolic blood pressure, and oxygen saturation were statistically significantly higher in the ICH group than in the others ($p=0.003$, $p=0.007$, and $p=0.035$, respectively). Of the patients with intracranial bleeding, seven (35.0%) were consistent with subdural hemorrhage, seven (35.0%) with subarachnoid hemorrhage, and six (30.0%) with intraparenchymal hemorrhage. There was no significant difference between the groups with and without ICH in terms of hospital outcomes. Of the 20 patients with ICH, three were discharged from the hospital. Furthermore, it was observed that two of these three patients were discharged with an mRS of 5 and one with an mRS of 4.

In the evaluation of the timing of CT scan and CPR, it was found that 16 (80.0%) of the patients with hemorrhage underwent CT scan prior to CPR, and this was found to be statistically significant ($p < 0.001$). Emergency department outcomes, hospital outcomes, and lengths of hospital stay are presented in **Table 2**.

Table 1. Comparison of all patients whose spontaneous circulation returned after CPR regarding the presence of ICH

	ICH + n: 20	ICH - n: 153	p-value
Age, years [IQR]	70.50 [64.25-78.00]	73.40 [64.00-81.00]	0.610
Female n (%)	10 (50.0%)	71 (46.4%)	0.762
Complaint			**0.033**
Dyspnea, n (%)	1 (5.0%)	56 (36.6%)	
Angina pectoris, n (%)	0	2 (1.3%)	
Altered mental status, n (%)	10 (50.0%)	30 (19.6%)	
Loss of appetite, n (%)	6 (30.0%)	49 (32.0%)	
Syncope, n (%)	2 (10.0%)	8 (5.2%)	
Abdominal and back pain, n (%)	1 (5.0%)	8 (5.2%)	
Vital Signs			
SBP, mmHg [IQR]	150.00 [92.50-177.50]	100.00 [80.00-130.00]	**0.001**
DBP, mmHg [IQR]	75.00 [60.00-100.00]	60.00 [50.00-80.00]	**0.003**
Pulse rate[IQR]	102.00 [80.00-123.50]	104.00 [78.75-127.25]	0.929
SpO_2, % [IQR]	91.50 [83.50-94.75]	82.50 [72.00-92.00]	**0.016**
Respiratory rate, [IQR]	21.00 [20.00-24.00]	24.00 [20.00-30.00]	0.088
GCS, [IQR]	7.00 [3.25-13.75]	12.00 [7.00-14.00]	0.107

ICH: Intracranial hemorrhage, IQR: Interquartile range, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, SpO_2: Oxygen saturation, GCS: Glasgow coma scale

Table 2. Relationship of CT timing, emergency department outcome, and hospital outcomes with the presence of ICH

	ICH + n: 20	ICH - n: 153	p-value
CT timing			**<0.001**
Before CPR, n (%)	16 (80.0%)	57 (37.3%)	
After CPR, n (%)	4 (20.0%)	96 (62.7%)	
Emergency outcome			0.478
Admission, n (%)	16 (80.0%)	111 (72.5%)	
Exitus, n (%)	4 (20.0%)	42 (27.5%)	
Hospital outcome			0.715
Discharge, n (%)	3 (15.0%)	18 (11.8%)	
Exitus, n (%)	17 (85.0%)	135 (88.2%)	
Length of stay, hours [IQR]	23.00 [4.50-66.00]	9.50 [3.00-25.00]	0.072

CT: Computerized tomography, ICH: Intracranial hemorrhage, CPR: Cardiopulmonary resuscitation, IQR: Interquartile range

Computed tomography scan status of the patients before and after the development of cardiac arrest was examined. The number of patients who developed cardiac arrest and subsequently achieved ROSC and underwent CT scan of the brain was 100. It was determined that four patients in this group had intracranial hemorrhage. Of these patients, two had subdural hemorrhage, one had intraparenchymal hemorrhage, and one had subarachnoid hemorrhage. While the patient with intraparenchymal hemorrhage was using warfarin, the patient with subarachnoid hemorrhage (SAH) was using acetylsalicylic acid. The other two patients were not using any antiaggregants or anticoagulants. Demographic data comparing the characteristics of these four patients with the other 96 patients, vital parameters, and hospital outcome information are presented in **Table 3**. The mRS scores of the groups with and without intracranial hemorrhage were found to have a median value of six in both groups.

Table 3. General evaluation of brain CT in patients with return of spontaneous circulation after CPR.

	After CPR CT ICH+ n: 4	After CPR CT ICH– n: 96	p-value
Age, years [IQR]	77.50 [72.50-81.00]	72.00 [62.50-80.00]	0.268
Female, n (%)	1 (25.0%)	41 (42.7%)	0.637
Complaint			0.283
Dyspnea, n (%)	0	48 (50%)	
Angina pectoris, n (%)	0	2 (2.1%)	
Altered mental status, n (%)	1 (25.0%)	9 (9.4%)	
Loss of appetite, n (%)	2 (50.0%)	27 (28.1%)	
Syncope, n (%)	1 (25.0%)	6 (6.3%)	
Abdominal and back pain, n (%)	0	4 (4.2%)	
Vital Signs			
SBP, mmHg [IQR]	120.00 [77.75-190.00]	100.00 [70.00-130.00]	0.404
DBP, mmHg [IQR]	75.00 [50.00-115.00]	60.00 [40.00-80.00]	0.255
Pulse rate, [IQR]	96.00 [68.50-140.00]	108.00 [80.00-130.00]	0.777
SpO_2, % [IQR]	84.00 [53.75-94.00]	75.00 [70.00-88.00]	0.552
Respiratory rate, [IQR]	17.00 [11.00-29.00]	28.00 [21.00-32.00]	0.107
GCS, [IQR]	5.00 [3.00-13.00]	12.00 [6.00-14.00]	0.317
Emergency outcome			0.999
Admission, n (%)	4 (100%)	79 (82.3%)	
Exitus, n (%)	0	17 (17.7%)	
Hospital outcome			0.999
Discharge, n (%)	0	10 (10.4%)	
Exitus, n (%)	4 (100%)	86 (89.6%)	
Length of stay, hours [IQR]	17.50 [5.25-29.75]	10.00 [4.00-22.00]	0.524

ICH: Intracranial hemorrhage, IQR: Interquartile range, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, SpO_2: Oxygen saturation, GCS: Glasgow coma scale, CT: Computerized tomography, CPR: Cardiopulmonary resuscitation,

DISCUSSION

In our study, the median age was 72.00 [64.00–80.00] years. In a study by Wallmuller et al. in which IHCA was examined, the mean age was found to be 64 (4). In a study by Naples et al. examining the brain CT scans of the patients who underwent CPR, the mean age was 58.5 and 60% of the study participants were men (9). In our study, the proportion of male participants was found to be 53.1%. The difference between our demographic data and the literature could be due to the fact that the study involved different sociodemographic groups.

In a study conducted by Kürkciyan et al., SAH was shown to be the cause of cardiac arrest in 4% of the patients (12). Shin et al. investigated ICH in patients with OHCA and found that ICH occurred at a rate of 11.4% in these patients (13). Naples et al. determined the rate of ICH to be 18% in their study (9). In a work by Cocchi et al. in which the role of brain CT scan after cardiac arrest was examined, this rate was found to be 11.7% (5). Similar to the literature, the incidence of ICH in patients who underwent CT scan of the brain was found to be 11.6% in our study. Wallmuller et al. estimated the rate of ICH to be 1% in their study on IHCA (4). However, this difference may be due to the fact that the study by Wallmuller et al. included only patients in whom ROSC was successfully achieved.

Inamasu et al. found pre-cardiac arrest neurological symptoms at a rate of 5% in their study (14). In our study, 60% of the patients with ICH had neurological symptoms prior to cardiac arrest, and this was statistically significant. This variation may be due to the difference in the location and the amount of bleeding.

In the present study, when the vital parameters were examined, higher blood pressure was observed in those with ICH than in those without ICH, which was statistically significant. It is considered that this may be a result of increased susceptibility to ICH in patients with high blood pressure and the neuroendocrine mechanisms occurring after ICH (15). In a study by Shin et al., the sPO_2 value was found to be lower in patients with ICH than in those without ICH. Conversely, in our study, the sPO_2 value was found to be higher in patients with ICH. This difference may be due to the fact that Shin et al. examined OHCA cases, but the present study included only IHCA cases; thus, the vital parameters at presentation could be measured.

In our study, ICH was more common in patients who underwent CT scan prior to CPR. The reason for this observation may be that the symptoms of the patients could be evaluated before CPR and imaging requested for preliminary diagnosis.

In a study performed by Shin et al. in patients with OHCA, it was found that no patients with ICH had favorable neurological outcomes (13). Similarly, Gelber et al. found that the survival rate of patients with ICH was 33% in OHCA and that no patients had good neurological survival (16). In the present study, no significant difference was observed between those with and without ICH in terms of discharge and survival, which may be due to the fact that our study consisted of patients with IHCA and that they were resuscitated early.

When the presenting complaints of the patients were examined, it was found that ICH was more common in the brain CT scan of the patients with an altered state of consciousness and impaired general condition. Thus, early evaluation of the patients for ICH when they present to the emergency department with the complaints would be useful.

In our study, we found ICH at a rate of 4% in patients in whom ROSC was achieved after CPR and who underwent brain CT scan due to cardiac arrest of unknown cause. Similarly, this rate was found to be 3.5% in a study by Gelber et al., which examined the incidence of ICH in cases with OHCA (16). We believe that this rate is similar in cases with OHCA and IHCA. Taking into account that some treatments applied during CPR in cases with IHCA would increase the likelihood of ICH, we believe that this rate of 4% is at a considerable level. Therefore, it would be appropriate to evaluate the patients using CT scan of the brain as soon as ROSC is achieved in those with cardiac arrest of unknown cause.

Limitations

The most important limitation of our study is that it was single-centered and retrospective. In addition, we are not able to provide accurate information about the correct rates since patients with ROSC were mainly evaluated. We believe that further studies are needed on these rates since we did not have the chance to perform autopsy or postmortem CT. In our study, we did not find any patient who received fibrinolytic therapy and had bleeding, but we were unsure about whether they had intracranial hemorrhage at the center in which they were hospitalized.

CONCLUSION

In this study, we found the rate of ICH in patients who developed IHCA to be 11%. We believe that this rate is considerably high. Since ICH warrants changes in the treatment and management of patients, its presence should be excluded with brain CT scan in cases with cardiac arrest of unknown cause, as stated

in the guidelines. It would be a logical choice to use CT scan of the brain, which can be easily accessed in the emergency departments, in patients with ROSC. This should be the standard approach whenever the procedure would not cause any delay in treatment and/or while simultaneously initiating treatment even if the cause is determined by 5H-5T. When all of these factors are taken into consideration, we believe that the algorithms for ICH should be revised. More detailed studies are needed for cases with IHCA of unknown cause, especially if altered state of consciousness and high blood pressure are detected.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated after obtaining approval from the Non-Interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University (Date: 09.02.2021, Decision No: 2021-68-10).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Original Article

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The predictors of complicated acute appendicitis: large unstained cells, gamma-glutamyl transferase, monocyte to platelet ratio, age and gender

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ABSTRACT

Aim: In this study, we sought to investigate possible biomarkers markers that can preoperatively distinguish complicated and non-complicated acute appendicitis.

Material and Method: Patients who underwent appendectomy between February and December 2019 were screened retrospectively. Patients with pathology findings other than appendicitis were excluded. Patients with a confirmed diagnosis of acute appendicitis were categorized as complicated and non-complicated appendicitis for analysis of sociodemographic characteristics, comorbidities and preoperative laboratory parameters.

Results: A total of 575 patients were included in the study. Among these, 432 (75.1%) were diagnosed with non-complicated appendicitis and 143 (24.9%) were diagnosed with complicated appendicitis. The mean (SD) age was 34.2±14.2 years. Hypertension, diabetes mellitus and hypothyroidism were the most frequent comorbidities. Age (OR, 1.026; p=0.010), male gender (OR, 1.837; p=0.044), LUC (OR: 19.868; p=0.034) and GGT (OR: 1.013; p=0.013) were associated with a higher risk of complicated appendicitis. An increase in monocyte to platelet ratio (MPR) (OR: 0.920; p=0.047) was associated with a lower risk of complicated appendicitis.

Conclusion: In patients with acute appendicitis, parameters including age, gender, as well as LUC, GGT and MPR, which are easily available and relatively cheap biomarkers, can be useful to distinguish non-complicated and complicated cases preoperatively.

Keywords: Acute appendicitis, appendectomy, large unstained cells

INTRODUCTION

Acute appendicitis is one of the most common abdominal emergencies worldwide. It occurs more often in males than females, with a lifetime incidence of 8.6% and 6.7%, respectively (1). Appendicitis is provoked by direct luminal obstruction. Although the certain etiology remains unknown, genetic, environmental and infectious factors could be the triggers (2). Appendectomy is one of the most commonly performed operations in emergency settings and is the gold standard treatment for acute appendicitis. Even the mortality rate is low (0.09-0.24%), postoperative adverse event rates of 8.2-31.4% have been reported (2,3). Since Bailey published the non-operative treatment algorithm in 1930, conservative treatment with antibiotics has been proposed, but this is not without controversies (4). A meta-analysis of five randomized trials including 980 patients with uncomplicated appendicitis showed that in patients treated conservatively, the relative odds of complications by 46%. Furthermore, the analgesic consumption decreased, and the duration of sick leave was shorter in the patients treated with antibiotics (5). Even if there are scoring systems such as the Alvarado score to determine the likelihood of acute appendicitis, there is no classifier to distinguish between complicated and non-complicated appendicitis (6).

In this study, we investigated the factors including demographic data, comorbidities, type of surgery and laboratory findings at admission, which could be effective to distinguish complicated and non-complicated appendicitis in patients with acute appendicitis.

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MATERIAL AND METHOD

Patient Selection

Patients who underwent surgery for acute appendicitis between February and December 2019 at Ankara City Hospital were screened for this study. Exclusion criteria were pathology findings other than acute appendicitis (e.g., normal appendix, malignancy) and missing data. Approval for the study was granted by the Ankara City Hospital No 1 Clinical Researches Ethics Committee (Date: 18.08.2020, Decision No: E1-20-977). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Comorbidities

Sex, age, type of surgery (open or laparoscopic), comorbidities including hypertension, diabetes mellitus, chronic obstructive pulmonary disease, coronary artery disease, congestive heart failure, peripheral arterial disease, hyperlipidemia, cerebrovascular disease, rheumatological diseases, hypothyroidism, hyperthyroidism, osteoporosis, cancer, hyperprolactinemia, polycystic ovary syndrome, benign prostate hyperplasia and history of organ transplantation were recorded using electronic health records, physician and nursery reports.

Laboratory Findings

Admission laboratory parameters [white blood cell (WBC), neutrophil, lymphocyte, monocyte, eosinophil, basophil, red blood cell (RBC), hemoglobin hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count, mean platelet volume (MPV), procalcitonin (PCT), platelet distribution width (PDW), large unstained cells (LUC), urea, creatinine, C-reactive protein (CRP), lactate dehydrogenase (LDH), sodium, potassium, direct/indirect/total bilirubin, total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin] were recorded for each patient. Neutrophil-lymphocyte-platelet ratio (NLPR), mean platelet volume/platelet count ratio (MPR), lymphocyte-to-monocyte ratio (LMR), systemic immune inflammation index (SII), prognostic nutritional index (PNI) and monocyte to platelet ratio were calculated for each patient. The SII was calculated by the formula: neutrophil×platelet/lymphocyte and the PNI was calculated as 10 × serum albumin (g/dl) + 0.005 × total lymphocyte count (per mm^3).

Outcomes

Patients were classified into two groups as complicated and non-complicated appendicitis. Those with perforation, peritonitis and/or gangrenous/necrotizing changes on pathology were classified as complicated appendicitis or simple appendicitis without complication were classified as non-complicated appendicitis as described previously in details (2,7).

Statistical Method

All statistical evaluations were done using Stata MP 16. The normal distribution of the data was evaluated by the Kolmogorov-Smirnov test. Mean values (with standard deviation) were used for numerical variables with normal distribution. Categorical variables were specified as numbers and percentages. Student T test was used to compare numerical variables with normal distribution between the two groups, and Mann-Whitney U test was used to compare numerical variables without normal distribution. A stepwise backward regression model was used to identify independent predictors of acute complicated appendicitis. The diagnostic performance of the regression model was evaluated by receiver operating characteristic (ROC) analysis. Cases where type-1 error level was below 5% ($p < 0.05$) were considered as statistically significant.

RESULTS

Patient Characteristics

Totally 615 patients underwent appendectomy, of which 32 (5.2%) who had normal appendix, and 8 (1.3%) who had malignant tumors in appendix were excluded. Finally, 575 patients [432 (75.1%) with non-complicated appendicitis and 143 (24.9%) with complicated appendicitis] were included in the study.

Laparoscopic surgery was performed in 49 patients. (13 complicated and 36 non complicated cases) (**Figure 1**).

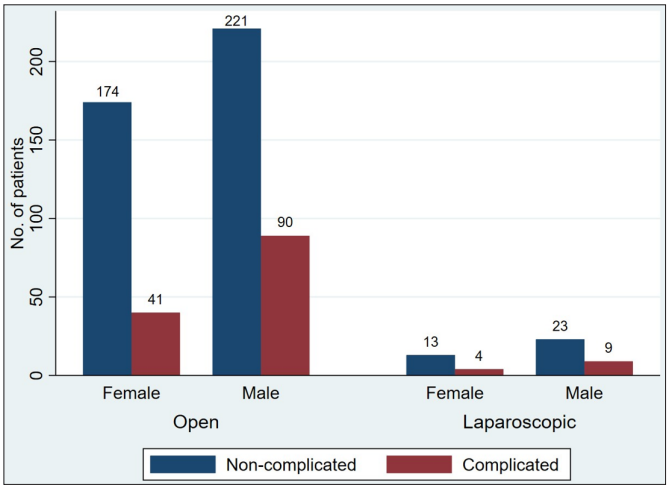

Figure 1. Number of patients according to sex and type of surgery in complicated vs. non-complicated groups

The mean (SD) age of the enrolled 575 patients was 34.2±14.2. Of these patients, 343 (59.7%) were male and 232 (40.3%) were female. Hypertension (7.5%, n=43), diabetes mellitus (4.7%, n=27) and hypothyroidism (3.8%, n=22) were the most frequent comorbidities in the study population. Male gender was significantly higher in complicated group [n=99 (68.5%) in complicated vs. n=244 (56.4%) in non-complicated group (p=0.011)]. Among the comorbidities, cerebrovascular disease was significantly higher in complicated group [n=2 (1.4%) in complicated vs. n=0 (0%) in non-complicated group (p=0.014)]. Demographic characteristics of the enrolled patients are summarized in **Table 1**.

Laboratory Findings

The mean (SD) levels of monocyte count [0.7 (0.3) in complicated vs. 0.6 (0.3) in non-complicated group (p=0.031)], CRP [105.8 (86.6) in complicated vs. 61.6 (76.5) in non-complicated group (p<0.001)], ALT [27.4 (19.6) in complicated vs. 23.1 (17.5) in non-complicated group (p=0.016)], GGT [30.3 (28.4) in complicated vs. 22.5 (19.0) in non-complicated groups (p<0.001)] were significantly higher in the complicated appendicitis compared to non-complicated appendicitis group. While the mean of RDW [13.4 (1.0) in complicated vs. 13.7 (1.3) in non-complicated group (p=0.021)] was significantly lower in complicated appendicitis group. The detailed laboratory findings of enrolled patients are presented in **Table 2**.

The Predictors of Complicated Appendicitis

In backward stepwise regression, age (OR, 1.026; 95% CI, 1.006-1.046; p=0.010), male gender (OR, 1.837; 95% CI, 1.017-3.318; p=0.044), the mean levels of LUC (OR, 19.868; 95% CI, 1.260-313.243; p=0.034) and GGT (OR, 1.013; 95% CI, 1.003-1.023; p=0.013) were associated with a higher risk of complicated appendicitis. In addition, the monocyte to platelet ratio (OR, 0.920; 95% CI, 0.847-0.999; p=0.047) was associated with a lower risk of complicated appendicitis (**Table 3**). In the ROC curve analysis of stepwise backward model AUC was 0.69 (95% CI, 0.64-0.72; p<0.001) (**Figure 2**).

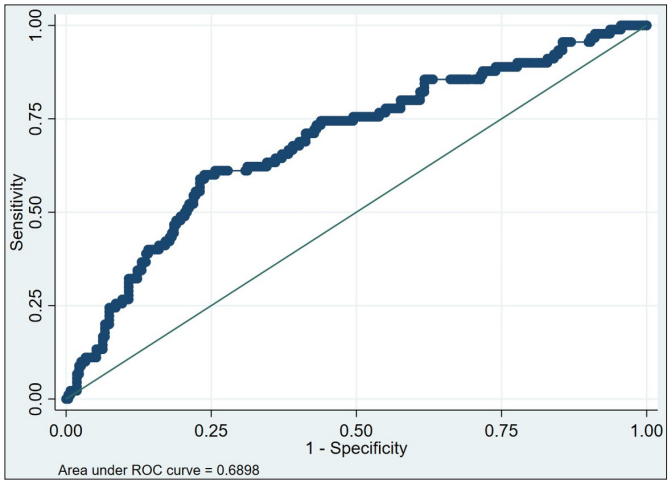

Figure 2. ROC curve of regression model for predicting complicated acute appendicitis

Table 1. Baseline characteristics of patients in complicated vs. non-complicated groups

	Total n=575	Non-complicated n=432	Complicated n=143	p
Age, mean (SD)	34.2 (14.2)	33.6 (14.1)	36.0 (14.3)	0.078
Male, n (%)	343 (59.7%)	244 (56.4%)	99 (68.5%)	0.011
Hypertension, n (%)	43 (7.5%)	32 (7.4%)	11 (7.7%)	0.91
Diabetes mellitus, n (%)	27 (4.7%)	22 (5.1%)	5 (3.5%)	0.43
Chronic obstructive pulmonary disease, n (%)	5 (0.9%)	4 (0.9%)	1 (0.7%)	0.81
Coronary artery disease, n (%)	15 (2.6%)	11 (2.5%)	4 (2.8%)	0.87
Congestive heart failure, n (%)	4 (0.7%)	3 (0.7%)	1 (0.7%)	0.99
Peripheral arterial disease, n (%)	1 (0.2%)	0 (0.0%)	1 (0.7%)	0.082
Hyperlipidemia, n (%)	3 (0.5%)	1 (0.2%)	2 (1.4%)	0.093
Cerebrovascular disease, n (%)	2 (0.3%)	0 (0.0%)	2 (1.4%)	0.014
Rheumatoid disease, n (%)	11 (1.9%)	9 (2.1%)	2 (1.4%)	0.60
Hypothyroidism, n (%)	22 (3.8%)	16 (3.7%)	6 (4.2%)	0.79
Hyperthyroidism, n (%)	2 (0.3%)	2 (0.5%)	0 (0.0%)	0.42
Osteoporosis, n (%)	1 (0.2%)	1 (0.2%)	0 (0.0%)	0.57
Cancer, n (%)	2 (0.3%)	1 (0.2%)	1 (0.7%)	0.82
Hyperprolactinemia, n (%)	1 (0.2%)	1 (0.2%)	0 (0.0%)	0.57
Polycystic ovarian syndrome, n (%)	1 (0.2%)	1 (0.2%)	0 (0.0%)	0.57
Benign prostate hyperplasia, n (%)	3 (0.5%)	2 (0.5%)	1 (0.7%)	0.73
History of organ transplantation, n (%)	1 (0.2%)	0 (0.0%)	1 (0.7%)	0.082

Table 2. Admission laboratory findings of the study population in complicated vs. non-complicated groups

	Total n=575	Non-Complicated n=432	Complicated n=143	p
White blood cell, (x109/L)	13.6 (4.2)	13.4 (4.3)	13.9 (3.8)	0.28
Neutrophil, (x109/L)	11.0 (4.2)	10.9 (4.3)	11.3 (3.9)	0.32
Lymphocyte, (x109/L)	1.7 (0.8)	1.7 (0.8)	1.7 (0.8)	0.90
Monocyte, (x109/L)	0.6 (0.3)	0.6 (0.3)	0.7 (0.3)	0.031
Eosinophil, (x109/L)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.060
Basophil, (x109/L)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.49
Red blood cell, (x1012/L)	4.9 (0.5)	4.9 (0.5)	4.9 (0.5)	0.13
HGB, (g/dL)	14.2 (1.8)	14.2 (1.9)	14.4 (1.6)	0.25
HCT, (%)	42.4 (5.1)	42.3 (5.3)	42.8 (4.4)	0.30
MCV, (fL)	87.0 (6.4)	87.1 (6.7)	86.8 (5.2)	0.57
MCH, (pg/cell)	29.2 (2.5)	29.2 (2.5)	29.2 (2.2)	0.90
MCHC, (g/dL)	33.5 (1.3)	33.5 (1.3)	33.6 (1.4)	0.43
RDW, (%)	13.6 (1.3)	13.7 (1.3)	13.4 (1.0)	0.021
PLT, (x109/L)	265.9 (66.4)	266.0 (68.7)	265.9 (59.1)	0.99
MPV, (fL)	7.7 (0.9)	7.7 (0.9)	7.8 (0.9)	0.49
PCT, (%)	0.2 (0.1)	0.2 (0.1)	0.2 (0.0)	0.69
PDW, (%)	55.2 (9.8)	55.3 (10.2)	55.0 (8.4)	0.74
LUC, (x109/L)	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	0.27
Urea, (mg/dL)	27.6 (8.8)	27.6 (9.2)	27.7 (7.5)	0.95
Creatinine, (mg/dL)	0.8 (0.8)	0.9 (0.9)	0.8 (0.2)	0.60
C-reactive protein, (g/L)	72.1 (81.1)	61.6 (76.5)	105.8 (86.6)	<0.001
LDH, (U/L)	208.5 (56.6)	208.2 (56.4)	209.4 (57.4)	0.84
Sodium, (mEq/L)	138.9 (2.4)	139.0 (2.4)	138.9 (2.6)	0.59
Potassium, (mEq/L)	4.1 (0.3)	4.1 (0.3)	4.1 (0.3)	0.63
Direct Bilirubin, (mg/dL)	0.4 (4.2)	0.5 (4.8)	0.3 (0.2)	0.61
Indirect Bilirubin, (mg/dL)	0.8 (0.7)	0.8 (0.7)	0.7 (0.6)	0.59
Total Bilirubin, (mg/dL)	1.1 (0.8)	1.1 (0.9)	1.1 (0.7)	0.87
AST, (U/L)	23.3 (10.8)	23.1 (10.7)	24.0 (11.0)	0.41
ALT, (U/L)	24.2 (18.1)	23.1 (17.5)	27.4 (19.6)	0.016
GGT, (U/L)	24.4 (22.0)	22.5 (19.0)	30.3 (28.4)	<0.001
ALP, (U/L)	78.7 (26.2)	77.8 (26.9)	81.2 (23.7)	0.18
Albumin, (g/L)	45.7 (3.3)	45.7 (3.3)	45.7 (3.3)	0.99
Total Protein, (g/L)	70.2 (4.9)	70.2 (4.7)	70.2 (5.5)	0.96
NLPR Index	3.4 (2.9)	3.3 (2.6)	3.6 (3.8)	0.19
MPR Index	40.2 (12.7)	40.3 (13.1)	39.9 (11.3)	0.73
LMR Index	3.2 (2.1)	3.3 (1.9)	3.1 (2.6)	0.31
SII Index	2154.2 (1595.1)	2132.5 (1632.5)	2219.7 (1479.7)	0.57
PNI Index	450.9 (62.9)	449.9 (66.6)	454.1 (50.3)	0.49

Table 3. Significant predictors of complicated acute appendicitis according to stepwise backward model

	Odds Ratio (95% Confidence Interval)	p
Age	1.026 (1.006-1.046)	0.010
Male	1.837 (1.017-3.318)	0.044
LUC	19.868 (1.260-313.243)	0.034
GGT	1.013 (1.003-1.023)	0.013
Monocyte to platelet ratio (MPR)	0.920 (0.847-0.999)	0.047

DISCUSSION

In addition to risk of surgical complications and absence from work, appendectomy, including the admission and 1-year follow-up, is known to be 1.6 times more expensive than conservative treatment (8). To avoid these disadvantages of appendectomy, non-operative treatment with antibiotics should be considered in non-complicated patients in certain circumstances.

The present retrospective cohort study was performed to evaluate the predictive factors of complicated appendicitis. Our results demonstrate that age is one of the predictors of complicated appendicitis. Similarly, in a study comparing complicated and non-complicated appendicitis based on patient characteristics and imaging features, Atema et al recently showed that the age over 45 years is associated with a higher risk of complicated appendicitis (9). In their study including 895 patients who underwent appendicectomy, Eddama et al. also reported that increased age was associated with increased risk of complicated appendicitis (10).

Even if it is known that acute appendicitis incidence is higher in male gender (11), the previous studies did not find an association between male gender and complicated appendicitis (12). In the present study after adjusting for confounding variables, we observed that male gender was associated with a higher risk of complicated appendicitis.

Several studies reported that CRP and bilirubin levels were significant predictors of complicated appendicitis (13,14). As well, in this study CRP and GGT levels were significant factors in predicting complicated appendicitis.

Although increased levels of LUC have previously been associated with leukemia, viral and fungal infections, to the best of our knowledge, this is the first study demonstrating that the increase in large unstained cells (LUC) is a statistically significantly risk factor in the prediction model of complicated appendicitis (15). The reason for this increase remains uncertain (16).

In addition to biomarkers, we compared comorbidities in patients with complicated and non-complicated appendicities and found that the rates of comorbidities did not differ significantly between the two groups. Although the rate of cerebrovascular disease seemed to be significantly higher in the complicated group, this appeared to be due to its low frequency in the study population and absence in the non-complicated group.

This study had several limitations. First, the study was a retrospective study and not randomized. Secondly, imaging methods such as CT scan and USG has not been included to analysis. Furthermore, the study does not contain the medical examination and surgical observation reports of patients.

In conclusion, in this study, it was demonstrated that, age, male gender, the mean levels of LUC, GGT and monocyte to platelet ratio were predictors of complicated appendicitis. Using these parameters, non-complicated appendicitis could be distinguished from complicated cases to be treated conservatively with antibiotics..

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for the study was granted by the Ankara City Hospital No 1 Clinical Researches Ethics Committee (Date: 18.08.2020, Decision No: E1-20-977).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The effects of anogenital condylomas on female sexuality and psychology: a case-control study

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ABSTRACT

Aim: Human Papillomavirus is a sexually transmitted virus with over two hundred subtypes. It can cause anogenital condyloma, cervical dysplasia, and cervical cancer. Human Papillomavirus types 6 and 11 are responsible for anogenital condylomas. The effect of condylomas on female sexual life and psychological state is investigated in our study.

Material and Method: Seventy-six vulvar condyloma patients and 80 participants as the control group were included in the study after excluding the subjects not meeting the inclusion criteria. After an initial evaluation, condylomas excised using carbon dioxide laser followed by a medical assessment in the 3rd post-excisional month. Female sexual function index and Beck depression inventory scales were used on a group of healthy patients with anogenital condyloma at 0-3 months.

Results: There was a statistically significant difference in the FSFI scores at 0 and 3 months between the case and control groups ($p<0,001$). Although no statistically significant difference was found, a trend of improvement in the Female sexual function index scores of cases from 0 to 3 months was observed ($p=0.194$). A statistically significant difference was found in the Beck depression inventory scores of cases from 0 to 3 months ($p=0.002$).

Conclusion: Anogenital condylomas cause negative effects on female psychology and sexuality, and excisional intervention seems to have a beneficial effect on psychology and sexual functions.

Keywords: HPV, condyloma, sexual functioning, depression

INTRODUCTION

Human Papillomavirus (HPV) infection, which is the most common sexually transmitted disease worldwide, affects the skin and mucosal surfaces and can cause anogenital condylomas, cervical intraepithelial neoplasia, and cervical cancer (1). More than 200 HPV subtypes that show tropism to different body parts have been identified (2). Cutaneous warts on hands and feet are mostly caused by HPV 1, 2, 4, 27, or 57 subtypes (3). HPV 6 and 11 subtypes that are called low-risk HPV are responsible for anogenital condylomas (4). The prevalence of anogenital warts in women aged 30-65 was found to be 154/100,000 in Turkey (5). Anogenital localization of condylomas has a negative effect on female sexuality. It may challenge the loyalty of the couple to each other and affect their sexual functions (6). The condylomas have a recurrence rate of 15-37% even after excision (5). The presence of condylomas, frequent recurrences, and consequent repetitive treatments can lead to vulvodynia and dyspareunia (7). The recent screening programs for HPV in some countries led to a significant increase in the diagnostic rate of HPV. Although studies have been conducted to determine whether there is a relationship between HPV infection, precancerous lesions, and sexual life, there is a limited number of studies on the effects of anogenital condylomas on sexual and psychological life.

In this study, we aimed to evaluate the sexual functions and psychological status of women diagnosed with anogenital condyloma and infection with low-risk HPV types.

MATERIAL AND METHOD

This cross-sectional study was approved by the İstanbul Gelişim University Ethics Committee (Date: 11.09.2020, Decision No: 2020-24-03). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Before the study, all participants were informed, and written consent was obtained.

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Sexually active patients aged 18-45, who were diagnosed with anogenital condyloma in a private health facility in the district of Silivri in Istanbul between February 2020 and January 2021, and sexually active volunteers of reproductive age without any health problems were included in the study. Patients with known chronic conditions, such as psychiatric, neurological, endocrinological, cardiological, and renal diseases, and patients with at least one incident of recurrent condyloma were excluded from the study.

Ninety-two patients diagnosed with vulvar condyloma were included in the study. Initially, the lesions were examined by inspection. A biopsy was taken from the condyloma. HPV PCR test and histopathological examination were performed on the sample taken for differential diagnosis. Finally, the condylomas were destroyed using fractional carbon dioxide laser. DNA of the samples was isolated with Magnesia Viral Nucleic Acid Extraction kit and HPV DNA PCR method was performed with Montania 4896 Real-Time PCR device. 100 HPV types are detected by genotyping with the Ampliquality HPV-Type Express v3.0 kit. The most important 40 types, including low, probable high and high risk groups, are determined. HPV Types in the low risk group are Type 6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 64, 71, 72, 81, 83, 84, 87, 89, 90. HPV Types in the possible high-risk group are Type 26, 53, 66, 67, 68, 69, 70, 73, 82. HPV Types in the high risk group are Type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59. Eleven patients whose molecular test results indicated high-risk HPV types, possible high risk HPV types and multiple HPV types including at least one high risk hpv were excluded from the study. Five patients with recurrent condylomas during the 3-month follow-up period were treated with laser and excluded from the study. The control group subjects consisted of 86 sexually active and healthy women who were also screened for HPV using molecular testing of cervical smear samples, and six women who tested positive for HPV DNA were excluded from the study. All of the patients were unvaccinated.

As a result, 76 vulvar condyloma patients and 80 controls were included in the study. The study flow is shown in **Figure 1**.

All patients and controls were evaluated with Female Sexual Function Index (FSFI) and Beck Depression Inventory (BDI) at 0 and 3 months of the study.

Female Sexual Function Index (FSFI)

The structure of FSFI is made up of 19 questions consisted of six domains: desire, arousal, lubrication, orgasm, satisfaction, and pain. Items are scored on a five-point Likert scale. The raw scores from domains are multiplied by 0.6 for desire, by 0.3 for arousal and lubrication, and by 0.3 for orgasm, satisfaction, and pain. The total score is obtained by adding the raw scores from each domain. The highest total score that can be obtained is 36.0, while the lowest is 2.0. Higher scores mean healthier sexual functions. In our study, we used the test validated for the Turkish language by Aygin and Aslan (8).

Beck Depression Inventory (BDI)

BDI is a scale that consists of 21 multiple-choice questions. The items are scored on a 0-3 point Likert scale and are designed to measure the severity of depression. Higher scores mean more severe depression. The validation and translation of the scale into Turkish was done by Hisli (9).

Statistical Analysis

The strength of the study was calculated using the FSFI scores and BDI scores according to the data collected from Russ Lenth's power and sample size analysis module and another reference study (10). The calculated sample size was 52 for each group. After a calculation of 76 + 80, 156 participants were recruited, and the strength of the study was 95% for a 5% type-1 error. The clinical characteristics of both groups were compared with Statistical Package for Social Sciences (SPSS) for Windows, version 22 (SPSS Inc. IL, USA). The normality of data distribution was tested with the Kolmogorov-Smirnov test. The reliability statistics of the FSFI and BDI were tested with the Cronbach Alpha test. Data were presented as mean±SD for continuous variables. The independent samples t-test was used to evaluate the variable differences between groups. The dependent t-test was used to evaluate the variation of variables within the group. Mann-Whitney U test and Bonferroni correction were used for non-normally distributed results. $P < 0.05$ values were considered statistically significant. The Cronbach Alpha value of the FSFI and BDI scoring was calculated as 0.941 and 0.882, respectively.

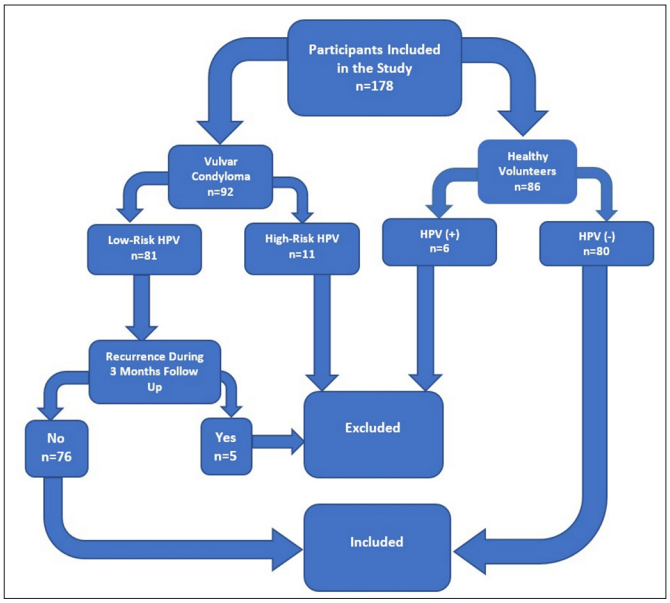

Figure 1. Study diagram

RESULTS

There was no significant difference between the groups in terms of age, age of first coitus, preferred contraception methods and education levels (p>0.05). However, a significant difference was observed between the two groups in terms of parity and smoking (p=0.001 and p=0.01 respectively). Demographic data of the participants are presented in **Table 1**. HPV Genotype distribution of condylomas are HPV Type 6 (n=40 ; 52.6%) , HPV Type 11 (n=20 ; 26.3%), HPV Type 6 and Type 11 (n=10; 13.1%), Other Low-Risk HPV Types (n=6 ; 7.8 %).

The relationship between the patients and controls regarding the evaluation with FSFI and BDI scales at the initial visit (0-month) and in the 3rd-month follow-up (3-month) were presented in **Table 2**. As seen in the table, a significant difference was observed between the two groups in terms of all FSFI subgroups and total FSFI score, except for the pain subdomain at both 0 and 3rd months. There was no significant difference between the groups in terms of BDI scores at either 0 or 3rd months.

When the groups were evaluated in terms of 3-month change within themselves, there was no significant difference between the 0 and 3-month FSFI scores of both the patients in the condyloma group and the patients in the control group (p>0.05). When the BDI scores of the patients in the condyloma group were compared, a significant decrease was observed at 0 and 3 months, and this difference was statistically significant (p=0.002). However, there was no significant difference between the BDI scores of the patients in the control group at 0 and 3 months (p>0.05). The relationships within the groups regarding the evaluation with FSFI and BDI scales at the initial visit (0-month) and in the 3rd-month follow-up (3-month) were presented in **Table 3**.

Table 1. Demographic data

Demographic data	Condyloma (n=76)	Control (n=80)	P
Age	31.61±6.42	31.74±5.62	>0.05[a]
Age at first coitus	21.34±2.87	21.95±3.33	>0.05[a]
Parity			**0.001[b]**
0	44	23	
1	11	26	
2	13	29	
≥3	8	2	
Contraception			>0.05[b]
Condom	27	24	
Combined oral contraceptive	9	11	
Intrauterine device	13	10	
Other	4	8	
None	23	27	
Smoking			**0.01[b]**
Yes	48	34	
No	28	46	
Education			>0.05[b]
Primary school	6	2	
Secondary school	14	9	
High school	15	27	
University	32	37	
Masters degree	7	4	
Doctorate	2	1	
HPV Genotype			
HPV 6	40		
HPV 11	20		
HPV 6&11	10		
Other Low-Risk HPV	6		

[a]: Independent Samples t-Test, [b]: Mann Whitney U Test

Table 2. The relationships between condyloma patients and controls

FSFI[a] Domains	0-Month			3- Month		
	The Condyloma Group (n=76)	The Control Group (n=80)	P[c]	The Condyloma Group (n=76)	The Control Group (n=80)	P[c]
Desire	3.02±1.27	3.86±1.13	**0.001**	3.14±0,96	3.92±1.01	**0.001**
Arousal	3.34±1.50	4.01±1.60	**0.008**	3.41±1.44	4.03±1.51	**0.010**
Lubrication	3.52±1.55	4.09±1.38	**0.017**	3.57±1.44	4.05±1.29	**0.031**
Orgasm	3.52±1.83	4.41±1.68	**0.002**	3.56±1.72	4.38±1.60	**0.003**
Satisfaction	3.59±1.83	4.53±1.62	**0.001**	3.64±1.73	4.51±1.54	**0.001**
Pain	2.59±1.61	2.64±1.53	0.842	2.62±1.51	2.65±1.42	0.908
FSFI Total	19.76±7.92	23.56±5.80	**0.001**	19.97±7.06	23.55±5.49	**0.001**
BDI[b] Total	17.41±9.97	16.85±10.27	0.731	16.16±9.51	16.96±9.88	0.605

[a]: Female sexual function index, [b]: Beck depression inventory, [c]: Independent Samples t-test

Table 3. Changes within the condyloma and control groups

FSFI[a] Domains	Condyloma			Control		
	0-Month (n=76)	3-Month (n=76)	P[c]	0-Month (n=76)	3-Month (n=80)	P[c]
Desire	3.02±1.27	3.14±0.96	0.104	3.86±1.13	3.92±1.01	0.270
Arousal	3.34±1.50	3.41±1.44	0.135	4.01±1.60	4.03±1.51	0.320
Lubrication	3.52±1.55	3.57±1.44	0.070	4.09±1.38	4.05±1.29	0.090
Orgasm	3.52±1.83	3.56±1.72	0.117	4,41±1,68	4,38±1,60	0,176
Satisfaction	3,59±1,83	3,64±1,73	0,145	4.53±1.62	4.51±1.54	0.339
Pain	2.59±1,61	2.62±1.51	0.283	2.64±1.53	2.65±1.42	0.691
FSFI Total	19.76±7.92	19.97±7.06	0.194	23.56±5.80	23.55±5.49	0.903
BDI[b] Total	17.41±9.97	16.16±9.51	**0.002**	16.85±10.27	16.96±9.88	0.690

[a]: Female sexual function index, [b]: Beck depression inventory, [c]: Dependent Samples t-test

DISCUSSION

HPV infection, which is the most common sexually transmitted disease in the world, has a prevalence of approximately 11-12% globally (1,11). Mostly low, rarely high-risk HPV types are responsible for anogenital condylomas. HPV infection can cause psychogenic effects by increasing fear and anxiety due to its oncogenic potential. Because condylomas can recur and cause pain during sexual intercourse, they can distract women from sexuality by being psychologically depressing (12).

Although there have been many previous studies on HPV positivity, HPV-related cervical precancerous lesions, sexual dysfunction, depression, marital relationship status, and anxiety, there number of studies on anogenital warts due to low-risk HPV types is insufficient (6,12–20).

Anogenital warts negatively affect the libido and cause sexual dysfunction because of their apparent recurrence tendency and challenges it poses on the intimacy, such as shaking self-confidence and questioning the partner loyalty (10,21–23).

Embarrassment due to the fact that HPV infection is a sexually transmitted disease, fear of stigma, relationship problems with the partner, anxiety due to the oncogenic potential of the virus, and the fear of infecting the partner, may cause a decrease in sexual desire, depression, and anxiety (23,24).

The high number of nullipars in the condyloma group in our study compared to the control group can be explained by the variations in HPV infection prevalences in different ages. It is known that HPV infection is most common in women under the age of 25; the prevalence decreases with age before peaks again after the age of 45 (25). The presence of many nullipars among the condyloma patients in the current study can thus be explained by the age distribution in the groups as the rates of patients and controls under 25 years old were 34.21% and 18.75%, respectively.

In a review by Kaderli et al. (26), a relationship between smoking and the occurrence of anogenital HPV infection and the anogenital wart was determined. In our study, consistent with previous studies, the rate of smoking in the condyloma group was significantly higher than in the control group.

A review by Lam et al. (27) indicated that people who used condoms regularly had a lower risk of being infected with HPV. In our study, the preference for condom use was similar in both groups. After the diagnosis of condyloma, those who did not use condoms before might have started to use condoms. Another reason might be the insufficient sample size. Studies with larger samples might demonstrate that condyloma patients are people who prefer condoms less.

Dinh et al. (28) reported that HPV Types 6 and 11 were responsible for more than 90% of the condyloma cases. Wiley et al. (29) reported that external condyloma usually occurred with low-risk HPV types, especially Type 6 and Type 11. In our study, the distribution of the HPV type was consistent with the results of previous studies.

Elesawy et al. (21), who examined the relationship between the anogenital wart and sexual dysfunction, found sexual dysfunction in 98% of the participants. The FSFI scores of all subgroup parameters in the condyloma group in our study were lower than those of the control group at both the 0- and 3-month. The differences in the subgroups except the pain were statistically significant.

Ma et al. (30) used the FSFI scale in patients with benign cervical pathologies and healthy controls. The prevalence of sexual pain in patients with benign cervical pathologies was lower than that of the controls. The researchers could not interpret their results and suggested that other studies with larger sample sizes were needed.

In an Egyptian study by Elesawy et al. (21), subjects with a history of condyloma for at least three months were included and compared with a healthy control group by using the FSFI test. A significant difference was observed in the control group regarding the FSFI total score. The average FSFI score in our study was two points higher than the average score observed in the study by Elesawy et al. The difference might be due to the fact that the patients in our patient group were started treatment as soon as they were diagnosed, and there were no active and visible condylomas during the study period.

In a Turkish study, Ilgen et al. (31) used FSFI in HPV positive and negative patients. Although the scores of the HPV positive group were lower than those of the control group, the difference was not statistically significant. In contrast, Mercan et al. (32) used the Arizona Sexual Experience Scale to compare the HPV positive patients and the controls, and significantly lower scores in all tested domains (desire, arousal, genital lubrication, orgasm experience, and satisfaction) were observed in the HPV group compared to the control group.

Many studies have addressed the psychological effects of anogenital warts, cervical dysplasia, and HPV positivity on male and female patients. In a study conducted in Iran, Nahidi et al. (22) compared condyloma patients with a control group. The marital relationships of condyloma patients, especially those with a history of previous condyloma treatment, were observed. With the prolongation of the disease, deterioration in marital relations and an increase in conflicts between couples were found.

McCaferry et al. (15) compared women who had cervical screening and tested positive and negative for HPV and found that HPV-positive women showed more severe depressive symptoms compared to the controls. Mercan et al. (32), who compared HPV-positive patients with a control group from a region with a high socio-cultural background in Istanbul, found a significant difference in BDI results between the groups. In another study conducted by Uzun et al. (33) in a different region of Istanbul, no significant difference in BDI scores was observed between the HPV positive and the negative groups.

The mean BDI score of the condyloma group was higher than that of the control group; however, the difference was not statistically significant. When the BDI test was used for evaluating the condyloma patients three months after the initiation of the treatment, a statistically significant decrease in the depression severity compared to the 0-month was observed. Similar to the FSFI scores, the score improvement might be due to the absence of visible condylomas and the reduction in the pressure of oncogenicity in high-risk HPV. Based on this observation, it can be hypothesized that the depression seen in HPV infection might be related to the oncogenic potential of HPV. Moreover, it could be contemplated that the likelihood and severity of depression would be less when the possibility of high-risk HPV disappears.

The discrepancy between our BDI scores and previous literature results might be due to the use of different tests or to the socio-cultural differences of the places where the studies were conducted. As the awareness about the disease increases in regions with high educational levels, it could be expected that the severity of depression in patients would be higher than the controls. Our study was conducted in a region with high socio-cultural background. Due to the high level of awareness about the disease and the lack of the oncogenic risk of the existing condyloma, no significant difference in BDI scores was observed between the groups. Moreover, even after the detection of low-risk HPV, an improvement in scores might have been observed. When the BDI scale was used again in the 3-month, the difference in the mean score was found to be statistically significant.

Having a healthy control group is one of the strengths of our study. Patients with high-risk HPV types were excluded from the study to remove the oncogenic risk of HPV in the condyloma group, which was a major concern. Besides, excluding recurrent patients from the study enabled us to measure the effect of "only condyloma diagnosis" on the individual, with no recurrences. Treatment of patients as soon as they are diagnosed in addition to not following an active condyloma case during the study has provided us with the opportunity to evaluate the effects of "exposure to HPV virus" on sexuality and psychological state rather than the physical disadvantages. The use of scales translated and validated in the Turkish language is another strength of our study.

In order to prevent bias in the study, all patients who met the criteria determined at the time of the study were included in the study group. Withdrawal bias did not occur as there was no withdrawal while the study was in progress.

One of the weaknesses of our study is that the evaluation period as short as three months. If this period were longer, we might observe a more significant improvement in sexual functions and depression severity. One of the other weaknesses of the study is that the participants consisted of patients taken care of in private health institutions and did not reflect the general public.

Anogenital condylomas have serious effects on the sexual and psychogenic life of women. In order to evaluate those effects of condylomas, different studies with larger sample sizes and other scales are needed.

CONCLUSION

HPV infection and condylomas, even with the low-risk HPV types, have a negative effect on female sexual functions and psychogenic status.

ETHICAL DECLARATIONS

Ethics Committee Approval: This cross-sectional study was approved by the İstanbul Gelişim University Ethics Committee (Date: 11.09.2020, Decision No: 2020-24-03).

Informed Consent: Before the study, all participants were informed, and written consent was obtained.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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GLIM criteria for the evaluation of nutrition in palliative care patients, a comparison of MNA-SF and NRS-2002

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ABSTRACT

Aim: A new malnutrition diagnostic criterion called the Global Leadership Initiative on Malnutrition (GLIM) was created by an initiative of the same name as these criteria in 2018. The present study aims to evaluate the differences and superiority of MNA, NRS-2002 screening tests, and GLIM criteria in patients hospitalized in palliative care with a diagnosis of malnutrition.

Material and Method: 148 patients who were hospitalized in palliative care due to clinical malnutrition were included in the study. MNA, NRS-2002, and GLIM screening tests were filled out by dieticians for each patient within the first 48 hours of hospitalization. Within the framework of GLIM criteria, patients were recorded for weight loss from phenotypic criteria based on information obtained from their relatives (more than five percent in the last six months or ten percent or more over the last six months). Hand dynamometer and calf circumference measurements were made to show muscle loss. Low Body Mass Index (BMI) was accepted as 20 kg/m² for individuals under the age of 70 years, and <22 for individuals above the age of 70 years. Decreased food intake which is among the etiologic criteria was detected by calculating the mean daily consumed calories of a patient. Individuals with CRP>5 mg/L were accepted as inflammation positive.

Results: Mean age in the total series was 72.98 with 70.4 in males and 75.5 in females including a total of 148 patients. Among the patients 50.67% (n=75) were males and 49.32% (n=73) were females. Malnutrition was found to be present in 141/148 (94.6%) patients according to the GLIM screening test. Malnutrition risk was present in 131/148 (87.9%) and 139/148 (93.2%) according to MNA-SF and NRS-2002, respectively. The results of the GLIM criteria and the other two screening tests were compared. While the results of the GLIM criteria and NRS-2002 test were similar, a significant difference was found between the GLIM test results and the results of MNA-SF.

Conclusion: The GLIM screening test is an easy-to-use and sensitive test for the diagnosis of patients hospitalized in palliative care centers. GLIM test and NRS- 2002 were found to be similar for diagnosing malnutrition. Although the results of MNA and GLIM tests were close, a significant difference was found between them in the diagnosis of malnutrition.

Keywords: Palliative care, malnutrition, nutrition assessment, elderly

INTRODUCTION

Malnutrition is a condition that is accompanied by weight loss as a result of the inability to consume sufficient nutrients and calories and is a serious health problem. The European Society for Clinical Nutrition and Metabolism (ESPEN) has defined malnutrition as inflammatory and disease-related or unrelated malnutrition as a basic concept of clinical nutrition. Excess weight and obesity are evaluated as separate concepts, not malnutrition (1). Malnutrition is a major cause of mortality and morbidity. It increases the duration of hospital stay and costs (2). Therefore, early diagnosis and the detection of patients at risk are important. There are many nutrition tests available for the diagnosis of these patients. Some of them are easily applied and can be used for all patients. The validity and reliability of the commonly used tests, Nutritional Risk Screening (NRS)-2002 and Mini Nutritional Assessment (MNA) has been proven in the diagnosis and screening of malnutrition in hospitalized patients (3,4).

A new malnutrition diagnostic criteria, called the Global Leadership Initiative on Malnutrition (GLIM), was created by an initiative of the same name to create a common test for the easy identification of the nutritional status of the medical community (5). These criteria were

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published in the ESPEN guidelines in September 2018 (5). Few studies have been published on the validity, reliability, and superiority of these criteria to other tests in palliative care centers. The present study aimed to evaluate the differences and superiority of one to another of the screening tests, MNA and NRS-2002 and GLIM criteria in a palliative care center with a high incidence of malnutrition. Our objective was to detect the degree of reliability and differences of these newly developed tests with other screening tests in diagnosing malnutrition in patients in palliative care centers.

MATERIAL AND METHOD

Population and Setting:

The study commenced following the decision of the Ethics Board of the University of Health Sciences, Erzurum Regional Training and Research Hospital (Date: 03.02.2020, Decision No: 37732058-514.10). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Between February 2020 and December 2020, there were a total of 232 palliative hospitalizations for various reasons. Informed consent forms were obtained from all participating individuals. Nutritional tests with malnutrition (low daily calorie intake) over 18 years of age followed in palliative care were retrospectively analyzed and 148 of 232 (63.7%) patients were included in the study. The study examined MNA, NRS-2002 and GLIM screening tests for each patient, which were filled by dietitians within the first 48 hours of the patient's admission to palliative care. Patients with a hospital stay of fewer than 48 hours and with missing data were excluded from the study. The reason for selecting the MNA-SF and NRS-2002 tests in the comparison of nutritional tests was based on the suggestion of evidence based guidelines (3).All three screening tests were applied in all patients.

Measurements

GLIM criteria consist of 5 criteria, including phenotypic and etiological criteria.

Phenotypic criteria: Weight loss=Within the framework of GLIM criteria, patients were recorded for weight loss from phenotypic criteria based on information obtained by self-reporting or from their relatives (more than five percent in the last six months or ten percent or more over the last six months).

Reduced muscle mass=Hand dynamometer and calf circumference measurements were taken to show muscle loss. Handgrip power was measured three times in both hands using a Jamar Digital Hand Dynamometer and the highest value was recorded. Handgrip power cut-off value was accepted as <22 and <32 for women and men, respectively (6). Calf circumference was measured at the widest level between the knee and ankle while the patient was lying on his/her back. Individuals with a calf circumference of <31 cm (the cut-off value) were considered to have muscle loss.

Low body mass index (BMI) was accepted as 20 kg/m² for individuals under the age of 70 years, and <22 for individuals above the age of 70 years.

Etiological criteria: Reduced food intake=Calculations for the daily calorie intake of the patients were based on the food consumption cards that patients and relatives filled in. A decreased intake of food, as one of the etiologic criteria, was determined by calculating mean calories during the patients' hospital stay.

Inflammation= C-reactive protein (CRP), procalcitonin and prealbumin values were used for the detection of inflammation. Individuals with CRP>5 mg/L were accepted as inflammation positive. Also, any inflammatory diseases among the present diseases of the patients were determined.

Patients were diagnosed as having malnutrition when at least one point was given for phenotypical and etiologic criteria (5).

The MNA-SF screening test was applied. Patients with seven points or less (the total score is 14) were diagnosed as having malnutrition.

Patients with a ≥3 (total points is six) in the NRS- 2002 test were diagnosed as having malnutrition. One point was added for patients over 70 years (7).

Statistical Analysis

All data entered into the database were verified by a second independent person. Descriptive statistics, as the mean and standard deviation for normally distributed continuous variables and relative frequencies for categorical (qualitative) variables, were generated for all variables. SPSS 22 Windows software (SPSS Inc, Chicago, Ill, USA) was used in the statistical analysis. If the p value is <0.05, it indicates that there is a significant difference between groups

RESULTS

The study included 148 of 232 (63.7%) malnutrition patients followed up at the palliative care center. Mean age was 72.98 years in the total series and 70.4 and 75.5 years in males and females, respectively. Among the patients 50.67% (n=75) were males and 49.32% (n=73) were females. **Table 1** shows the demographic characteristics of the patients in detail. The mean duration of hospitalization in the palliative care center was found to be 46.49 days. The first three most

common diseases among the diagnoses of the patients were terminal stage cancer, cerebrovascular diseases, and dementia in 36% (n=54), 24% (n=36) and 19% (n=29), respectively. **Table 2** presents the Laboratory values of the patients in detail. The mean calorie intake of the patients was calculated as 1273.07 kcal/day and the men were found to have more daily calorie intake compared with the women. The mean CRP was calculated as 59.88. CRP was significantly higher in the group diagnosed as having malnutrition according to the GLIM criteria. The prealbumin level was found to be significantly lower in the group with malnutrition (**Table 3**) ($p<0.05$). Mean hand grip power was 8.2 kg and 4.2 kg in men and women, respectively. Calf circumference was measured as 27 cm and 29.94 cm in men and women, respectively. (**Table 1**). Malnutrition was found to be present in 141/148 (94.6%) patients according to the GLIM screening test. Malnutrition risk was present in 131/148 (87.9%) and 139/148 (93.2%) according to MNA-SF and NRS-2002, respectively. The results of the GLIM criteria and the other two screening tests were compared. While the results of the GLIM criteria and NRS-2002 test were similar, a significant difference was found between the GLIM test results and the results of MNA-SF. The reason for the difference was considered to be the fact that more patients were diagnosed as having malnutrition when GLIM and NRS- 2002 tests were used, and the number was found to be smaller when MNA-SF was used (**Table 4**). Mean daily food consumption and the anthropometric measurements of the patients diagnosed to be normal according to MNA-SF were compatible.

DISCUSSION

In an extensive study carried out in Turkey, being at risk of malnutrition was found to be 28% (1). The rate of malnutrition among hospitalized patients was found to be 22% (8). However, in palliative care this rate escalated to 58.6% (9) and in our study 63.7% which is very high and was attributed to the high number of elderly cases with post-long-term intensive care hospitalization and with terminal stage cancer. The great majority of those patients were the ones who were hospitalized for a nutritional treatment in this clinic. These rates could increase up to 80-85% in patients with cancer and elderly cases in intensive care (10,11). Patients with malnutrition or at a high risk of malnutrition, in particular, are hospitalized in palliative care clinics.

GLIM criteria is a newly developed nutrition screening test. This present study is among the first to be conducted in a palliative care center. Practical and easily applied nutrition tests are very important in palliative care centers where patients with malnutrition

Table 1. Patient characteristics

	Male	Female	mean
Age, year	70.4	75.51	72.99
Sex (male)	50.67% (75)	49.32% (73)	100% (148)
Height (cm)	171.4	154.6	163
Weight (kg)	48.7	57.4	53.05
BMI, kg/m^2	16.51	22.47	19.39
Handgrip (kg)	8.2	4.2	6.2
Calf circumference	27	29.94	28.47
Mean calories (daily, kcal)	1345.47	1200.68	1273.07
Percutaneous endoscopic gastrostomy(PEG)	17	12	29
Total Parenteral Nutrition	19	21	41

Table 2. Laboratory of patients

Parameters	Mean	Min	max
Glucose (mg/dl)	128.32	51.00	482.00
Albumin (mg/dl)	2.98	1.70	4.80
BUN (mg/dl)	25.15	6.00	125.00
Creatinine (mg/dl)	0.87	0.32	4.66
Calcium (mg/dl)	8.56	5.40	11.90
ALT (IU/L)	23.19	6.00	268.00
AST (IU/L)	22.40	7.00	132.00
Protein (g/dl)	5.90	3.64	8.77
Prealbumin (mg/dl)	0.14	0.02	0.52
Procalcitonin (ng/mL)	2.44	0.00	80.88
CRP (mg/L)	59.89	3.10	456.00
WBC (cell/μL)	9.44	2.57	30.85
Uric acid (mg/dl)	4.56	1.30	20.40
FT4 (ng/dl)	1.04	0.40	1.85

BUN: Blood urea nitrogen, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRP: C-reactive protein, WBC: White blood cells,FT4: Free T4

Table 3. Malnutrition for GLIM criteria and laboratory difference

Parameters	Glim negative (n=8)			Glim positive (n=140)		
	Mean	Min	Max	Mean	Min	Max
*Prealbumin (mg/dl)	0.22	0.07	0.32	0.14	0.02	0.52
*CRP (mg/L)	27.95	3.1	103	61.71	3.1	456
Procalcitonin (ng/mL)	0.15	0.01	0.48	2.57	0	80.88
WBC (cell/μL)	9.13	5.51	14.57	9.46	2.57	30.85
Glucose (mg/dl)	126.75	72	184	128.4	51	482
Albumin (mg/dl)	3.29	2.6	4.1	2.95	1.7	4.8
Protein (g/dl)	6.24	5.38	7.8	5.88	3.64	8.77
TSH	2	0.16	6.02	2.59	0	69.08

Parameters mean, median, min and max. * $p<0.05$
Laboratory differences between patients with and without malnutrition according to GLİM criteria

Table 4. Malnutrition test GLIM vs MNA and NRS 2002

GLIM	MNA-SF		NRS 2002	
	Negative	Positive	Negative	Positive
Negative	6	2	4	4
Positive	12	128	6	134
	p<0.05		p>0.05	

are most found. MNA-SF and NRS-2002 tests have a high sensitivity and specificity (12). MNA-SF effectively reflects social functions, presentation to the hospital, and mortality and morbidity, while NRS 2002 reflects the nutritional status of the inpatients depending on the severity of the disease (7,13). Their validity and reliability have been proven; however, the development of a test is required that can be used universally, as one of the tests may be superior or another, according to the area of application (14). One or some of the tests may better detect the risk of mortality or rehospitalization (15). In a study by Matsumoto et al, similar to the present study, malnutrition was detected in 97.7% using the MNA-SF test and GLIM test (16). The GLIM criteria were developed for an easy-to-apply and universally used nutrition test. This present study aimed to compare the applicability and reliability of this test in palliative care patients.

Limitation

The limitations of this study were similar to those of other studies in which it is challenging to measure height, weight and loss of muscle mass in intensive care patients and patients with a poor general condition (17,18) even though these measurements are among the parameters of total nutrition tests. Measuring height and weight was difficult in most of the patients as they were bedridden. The measurement of fat-free mass was performed using anthropometric measurements (19) as in this group of patients bia or DEXA would yield false results. Daily calorie consumption was based on the records of those accompanying the patients and this might have resulted in the record showing as deficient or excess consumption. Also, many of the patients were hospitalized before being admitted to palliative care and they also had weight loss in that period. Notably, many patients were hospitalized in the intensive care unit. Errors in the patients' measurements may have occurred due to edema or the patients being confined to bed.

CONCLUSION

The GLIM screening test is an easy-to-use and sensitive test in the diagnosis of patients hospitalized in palliative care centers. Both the GLIM test and NRS- 2002 were found to be similar in diagnosing malnutrition. Although the results of the MNA and GLIM tests were close, a significant difference was found between them in the diagnosis of malnutrition. We suggest that the NRS- 2002 or GLIM test is more practical and easier to use when screening for malnutrition in patients in palliative care centers. Future studies on the applicability and ease of these tests in different populations are required.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study commenced following the decision of the Ethics Board of the University of Health Sciences, Erzurum Regional Training and Research Hospital (Date: 03.02.2020, Decision No: 37732058-514.10).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Prognostic value of blood gas lactate levels among COVID-19 patients who visited to emergency department

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ABSTRACT

Introduction: Our aim in this study, to determine the relationship between the mortality status and the İntensive Care Unit (ICU) requirement of COVID-19 patients, with their blood gas lactate levels at the time of admission to the Emergency Medicine (ED).

Material and method: This study was planned as retrospectively, all COVID-19 patients over the age of 18 who were admitted to the ED, had oropharyngeal /nasopharyngeal swabs and hospitalized between April 1, 2020 and June 31, 2020 were included. The primary outcome was in-hospital mortality. The secondary outcome is to determine the relationship between lactate levels and the ICU requirement

Results: The study was completed with 265 patients after the inclusion and exclusion criteria were applied. The median (IQR) age of the patients was 43.0 (31.0-59.0), and 132 (49.8%) were female. The comorbid diseases are; hypertension (HT), cardiovascular disease and diabetes mellitus (DM) were in the first three places with 23.0%, 17.0%, 9.1%, respectively. The cut-off value of lactate to determined for ICU admissions was 2.92, and 4.25 for mortality. The AUC for ICU requirement was 0.718±0.038 (95% CI, 0.644 - 0.792), and AUC for mortality was 0.752±0.058 (95% CI, 0.637 - 0.867), ($p < 0.001$ for both).

Conclusion: The COVID-19 pandemic has caused a serious mortality and morbidity problem worldwide. Our study found that hyperlactatemic COVID-19 patients had higher ICU requirement and mortality rates.

Keywords: COVID-19, lactate, mortality, emergency department

INTRODUCTION

In December 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in Wuhan, China (1). The World Health Organization (WHO) named this infection as COVID-19. As a result of the rapid succession of cases all over the world, WHO announced a pandemic (2). The epidemic caused a serious mortality and morbidity problem worldwide, causing approximately 153 million cases and 3.2 million deaths as of May 1, 2021 (3).

Although most patients with COVID-19 are only mildly symptomatic, a proportion of patients can deteriorate significantly, causing multiple organ failure and resulting in death (4). The 32% of all positive patients require admission to the intensive care unit (ICU), and death may occur (5). Therefore, early diagnosis is important for the patients who have risk of serious illness and the potentially life-threatening conditions (6).

Lactate is a reversible product of anaerobic respiration in glucose metabolism and increases during ischemia. The presence of high serum lactate levels is strongly associated with morbidity and mortality in various critically ill populations (7).

Our aim in this study, to determine the relationship between the mortality status and the ICU requirement of COVID-19 patients, with their blood gas lactate levels at the time of admission to the emergency department (ED).

MATERIAL AND METHOD

In this study, patients who were admitted to ED of İstanbul Education and Training Hospital between April 1, 2020 and June 31, 2020 and were diagnosed with COVID-19 were examined retrospectively. Permission for this study

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was obtained by Health Sciences University, İstanbul Training and Research Hospital Clinical Researches Ethics Committee (Date: 30.04.2021, Decision No: 2816). All study procedures were conducted in compliance with the principles of the Declaration of Helsinki.

All COVID-19 patients over the age of 18 who were visited to the ED, had oropharyngeal /nasopharyngeal swabs and hospitalized between April 1, 2020 and June 31, 2020 were included in this study. Patients whose reverse transcriptase polymerase chain reaction (RT-PCR) test results were negative and whose blood gas lactate value was not checked at the time of admission were excluded from the study. Additionally, patients who visited to the ED due to cardiac arrest and who were received inotropic support at the time of admission were not included in the study. Data were collected from electronic medical hospital records. The patients, all who included the study, were recorded in a form with their age, gender, blood gas lactate levels. Blood gas lactate levels were measured using the ABL800 FLEX blood gas analyser radiometer.

The primary outcome was in-hospital mortality. The secondary outcome is to determine the relationship between lactate levels and the ICU requirement. Outcomes were retrospectively assessed by reviewing of the hospital medical database.

Statistical Analysis

Categorical variables were presented as frequency and percentage. Continuous variables were tested for distribution using the Kolmogorov–Smirnov and Shapiro-Wilk tests. The asymmetrically distributed variables were expressed as the median interquartile range (25%-75%). All variables were compared for ICU admission and mortality outcomes using Pearson's chi-squared, and Mann–Whitney U tests as appropriate. Receiver operating characteristic (ROC) analyses were performed to determine the predictive power of lactate in terms of both outcomes. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of all cut points were calculated. The cut-off point that achieves the maximum Youden's index is referred to as the optimal cut-off. A 2-sided P-value of 0.05 was regarded as statistically significant. All data analyses were performed using SPSS version 23.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

The study was completed with 265 patients after the inclusion and exclusion criteria were applied. The median (IQR) age of the patients was 43.0 (31.0-59.0), and 132 (49.8%) were female. When our study sample was examined in terms of comorbid diseases; hypertension (HT), cardiovascular disease and diabetes mellitus (DM) were in the first three places with 23.0%, 17.0%, 9.1%, respectively. ICU admission was required in 68 (25.7%) of all patients and 35 (13.2%) died. The baseline characteristics of the patients were shown in **Table 1**.

Table 1. Baseline characteristics of 265 patients

Variables, median (IQR) or n(%)	Total
Age, years	43.0 (31.0-59.0)
Gender	
Female	132 (49.8)
Male	133 (50.2)
Comorbidities	
HT	61 (23.0)
DM	24 (9.1)
Cardiovascular disease	45 (17.0)
Pulmonary disease	19 (7.2)
Cerebrovascular disease	20 (7.5)
Chronic renal failure	9 (3.4)
Malignancy	15 (5.7)
Any comorbidity	105 (39.6)
Lactate, mmol/L	1.88 (1.13-3.30)
ICU	
(-)	197 (74.3)
(+)	68 (25.7)
Mortality	
(-)	230 (86.8)
(+)	35 (13.2)

HT: Hypertension, DM: Diabetes mellitus, ICU: Intensive care unit

We compare age, gender and lactate variables in terms of ICU admission requirement and mortality. The median age in the group with ICU need was 57.0, and it was 37.0 in the other group. On the other hand, the median age of the patients who died was 61.0 years and those who recovered were 40.5. These differences were statistically significant ($p<0.001$ for both). No significant difference was found in the gender variable in ICU need and mortality ($p=0.379$, $p=0.837$; respectively). When we compare the lactate values, the lactate median was 3.12 in the group with ICU need and 1.58 in the group without ICU need. In terms of mortality, the difference was more dramatic. The lactate median of the patients who died was 4.90, and that of those who recovered was 1.75. The differences between both outcome groups were statistically significant ($p<0.001$). The comparison of age, gender and lactate variables according to ICU admission and mortality outcomes were shown in **Table 2**.

ROC analysis was performed to determine the potency of lactate in predicting ICU admissions and mortality. The AUC for ICU requirement was 0.718 ± 0.038 (95% CI, 0.644 - 0.792), (**Figure 1**) and AUC for mortality was 0.752 ± 0.058 (95% CI, 0.637 - 0.867), (**Figure 2**); ($p<0.001$ for both).

Table 2. The comparison of age, gender and lactate variables according to ICU admission and mortality outcomes

Variables	ICU (-)	ICU (+)	p	Mortality (-)	Mortality (+)	p
Age	37.0 (29.0-51.0)	57.0 (47.0-74.3)	<0.001*	40.5 (31.0-55.0)	61.0 (48.5-73.5)	<0.001*
Gender			0.379**			0.837**
Female	95 (48.2)	37 (54.4)		114 (49.6)	18 (51.4)	
Male	102 (51.8)	31 (45.6)		116 (50.4)	17 (48.6)	
Lactate	1.58 (1.07-2.46)	3.12 (2.00-3.89)	<0.001*	1.75 (1.11-2.74)	4.90 (2.89-7.22)	<0.001*

ICU: Intensive care unit, *Mann-Whitney U test, ** Pearson's chi-squared test

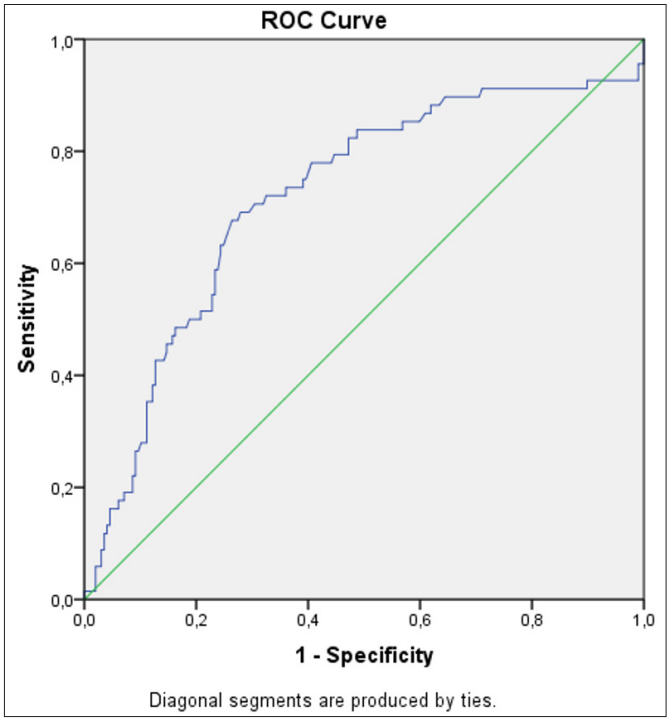

Figure 1. The ROC curve of lactate for ICU admissions.
AUC: 0.718±0.038 (95% CI, 0.644 – 0.792), (p<0.001).

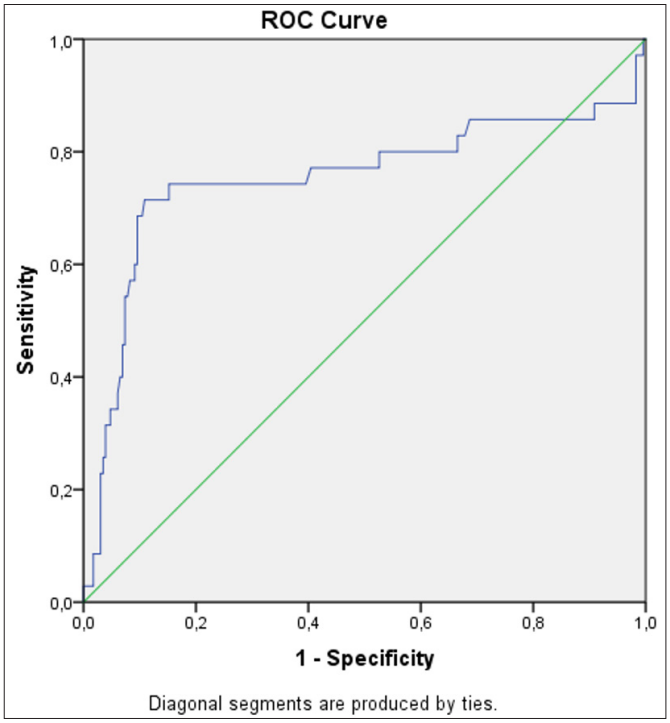

Figure 2. The ROC curve of lactate for mortality.
AUC: 0.752±0.058 (95% CI, 0.637 – 0.867), (p<0.001)

Then, the optimum cut-off points for the relevant outcomes were determined using the Youden's Index. The cut-off value determined for ICU admissions was 2.92, and 4.25 for mortality. The sensitivity, specificity, PPV, NPV, AUC and Youden's Index values of these cut-off points were shown in **Table 3**.

DISCUSSION

In this study, we aimed to determine the power of blood gas lactate levels at the time of ED admission in predicting in-hospital mortality and ICU requirement, in patients diagnosed with COVID-19 and hospitalized. In this cohort, we concluded that increased lactate levels could be useful in predicting in-hospital mortality and ICU requirement.

In a study evaluating the clinical course and risk factors on hospitalized COVID-19 patients, ICU requirement was 26% and the mortality prevalence among the patients admitted to the ICU was 22%. (8). Although our study is consistent with the literature, the relatively low mortality rates can be linked to the low average age of our study population.

In this study, it was found that the average age of the patients with a mortal course and ICU requirement was high. There are similar studies in the literature examining the effect of age on poor outcome. In a retrospective study of 710 patients, the ICU requirement and 28-day mortality were examined, and it was emphasized that the outcome was poor in the elderly population (9).

Table 3. Optimum cut-off points* of Lactate in predicting ICU admissions and in-hospital mortality

	Cut-off point	Sens (%)	Spec (%)	PPV (%)	NPV (%)	AUC	Youden's Index
ICU admissions	2.92	67.65	74.62	47.92	86.98	0.718	0.423
Mortality	4.25	71.43	89.13	50.00	95.35	0.752	0.606

* Cut-off points with the highest Youden's index value were shown, ICU: Intensive care unit, Sens: Sensitivity, Spec: Specificity, PPV: Positive predictive value, NPV: Negative predictive value AUC: Area under the curve

The ICU requirement and the mortality are more common in patients with COVID-19 in the presence of some chronic diseases (10). For this purpose, studies examining the comorbidity of patients are available in the literature. In a study conducted in China on COVID-19 patients, it was reported that the most common comorbidity was hypertension (16.9%), followed by diabetes (8.2%), and 8.2% of patients with two or more comorbidities (11). In a retrospective study conducted in Wuhan, it was reported that hypertension was the most common comorbidity, followed by diabetes and coronary heart disease (12). The most common comorbidity in our study is hypertension, followed by cardiovascular diseases and diabetes mellitus. These results were found to be suitable with the literature.

Lactate, which is produced in almost all considerable tissues (skeletal muscle, brain, erythrocytes, and kidneys) even under conditions where oxygen is sufficient, is kept below 1 mmol/L in both arterial and venous blood, especially by liver metabolism and recycling into the pyruvate. The lactate levels in the blood begin to increase, if insufficient oxygen is provided to the tissues or the oxygen demand of the tissues increases (13). Lactate is the reversible product of anaerobic respiration in glucose metabolism and it increases during ischemia. In the case of hypoxia, the formation of NAD (Nicotinamide Adenine Dinucleotide) decreases, thus the NADH/NAD ratio increases and the lactate level increases (14).

While the hyperlactatemia is defined as a persistent, mild to moderate (2-4 mmol/L) increase in blood lactate concentration without metabolic acidosis, the lactic acidosis is characterized with the blood lactate levels (usually> 5 mmol/L), which consistently increased by metabolic acidosis (15).

In this study, we found that high lactate levels observed in COVID-19 patients were significant in predicting ICU requirement and in-hospital mortality. The prognostic ability of lactate has been proven as independent of organ failure and shock, in patients with severe sepsis (7). Previous studies have shown that lactate can be used as an early identifier of organ failure and latent shock before any detectable changes in a patient's vital signs ocur (16). In a study conducted in patients admitted to the emergency department with a diagnosis of pneumonia, it is stated that lactate is more valuable than CURB-65 in predicting mortality, hospitalization, and ICU requirement (17). In another prospective study conducted on sepsis patients, it is stated that the high lactate level is effective in determining the severity of sepsis (18).

As with any retrospective study, there are some limitations in this study. The sample size of this single centre study was relatively small; therefore, more studies with a larger sample size are needed to confirm these results.

CONCLUSION

The COVID-19 pandemic has caused a serious mortality and morbidity problem worldwide. Our study found that hyperlactatemic COVID-19 patients had higher ICU requirement and mortality rates. Prospective studies with larger sample sizes are necessary to adequately assess the relationship between lactate and mortality in COVID-19 patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: Permission for this study was obtained by Health Sciences University, İstanbul Training and Research Hospital Clinical Researches Ethics Committee (Date: 30.04.2021, Decision No: 2816).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: 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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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The relationship between nutritional status, anthropometric measurements and hemogram parameters in preobese and obese women before and after menopause

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ABSTRACT

Objective: The nutritional status of pre-obese and obese women in the premenopausal and postmenopausal period is thought to be associated with anemia. In this study, we aimed to reveal the extent to which they meet their daily energy and nutrient needs and the relationship with the development of anemia by examining the food consumption records of women in the premenopausal and postmenopausal periods.

Material and Method: Women who applied to the Ataşehir District Health Directorate Healthy Nutrition and Active Life Unit for "Nutrition and Diet Consultancy" between May-July 2019 were included in the study. Women were divided into two groups as premenopause (36-45 years old) and postmenopause (46-73 years old) and their anthropometric measurements and nutritional status were evaluated. Serum glucose, blood urea nitrogen (BUN), creatinine, total-cholesterol, triglyceride, HDL, LDL, AST, ALT, iron, iron binding capacity, ferritin, vitamin B12, TSH, free T4, vitamin D and hemogram parameters of all participants were recorded.

Results: The waist circumference of 67.5% of the women in the premenopause group and 75% of the women in the postmenopausal group were above 88 cm. The blood BUN and HDL levels of premenopausal women were found to be lower than those in the postmenopausal period (BUN: 10.6±3.51 versus 15.06±4.96 and HDL: 54.1±9.1 versus 59, respectively. 3±13.5; $p < 0.05$). Premenopausal women had lower blood ferritin levels and higher iron binding capacity (WBC) compared to postmenopausal women (Ferritin: 15.8±11.5 versus 33.5±25.4 and DBT: 311.12±61.7 vs 287.50±41.93; $p < 0.05$). One of the important results of the study was the higher levels for vitamin D, AST and ALT in women in the post-menopausal period ($p < 0.05$).

Conclusion: It was determined that women in the premenopausal period did not receive enough iron and vitamin D to meet their needs. For this reason, daily food consumption should be adjusted accordingly, and lifestyle changes should be made to acquire healthy eating habits.

Keywords: Anemia, anthropometric measurements, nutrition, hemogram, menopause

INTRODUCTION

While deprivation and malnutrition are the main causes of disease and death in developing countries, eating disorders and obesity are more dominant problems in developed countries (1). Becoming overweight or pathological weight loss is inevitable when adequate and balanced nutrition cannot be achieved. Excessive weight gain is described as obesity, while excessive weight loss is defined as cachexia.

Body mass index (BMI), recommended by the World Health Organization, is still used in calculating obesity today. Different methods are also used for obesity classification, including waist circumference (WC) and central-peripheral fat mass. Recent data show that the incidence of obesity is rapidly increasing. This situation has made obesity a worldwide public health problem because excessive weight gain poses a high risk for many

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diseases, especially cardiovascular diseases, diabetes and cancers (2). Weight gain in the menopausal period is particularly striking. It was determined that women in the menopausal period gain approximately 0.7 kilograms per year, regardless of their racial-ethnic origin (3-5).

Menopause is characterized by low serum estradiol levels and high follicle stimulating hormone (FSH) levels due to complete or partial depletion of the follicles in the ovaries (6). Generally, between the ages of 45 and 55, there is a transition period to menopause (pre-menopausal period lasting 3 to 8 years). Since there is no monthly blood loss after menopause, the need for iron is less than in the pre-menopausal period (7,8).

In this study, the aim was to conduct a retrospective study to reveal iron deficiency anemia, which is thought to be related to nutritional status, by comparing the anthropometric measurements and blood parameters of pre-obese and obese women in the premenopausal and postmenopausal periods.

MATERIAL AND METHOD

The study is an analytical study and retrospective observational (case-control) study. For the research, permission was obtained from the Ethics Committee of the Health Sciences University Hamidiye Non-Interventional Ethics Committee (Date: 29.03.2019, Session No: 2019/3, Decision No: 19/33). This study was conducted with the approval (Date: 26/06/2019 6028, Decision No: 16867222-604-01-01) of the Republic of Turkey Ministry of Health Research Platform. Permission was obtained from the Istanbul Provincial Health Directorate for the participants to be included in the study. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

A questionnaire was applied, and anthropometric measurements were performed for to voluntary preobese and obese women who applied to the Ataşehir District Health Directorate Healthy Nutrition and Active Life Unit for "Nutrition and Diet Consultancy". Women were grouped according to their age as premenopause and postmenopause. A total of 80 preobese or obese participants, with 40 people between the ages of 35-44 and a postmenopausal group of 40 people between the ages of 45-72 were included in the study. Those who had any acute or chronic disease that may cause iron deficiency anemia (hyperthyroidism, hypothyroidism, diabetes mellitus, infection, bleeding, hemorrhoids, cancer and hematological diseases, etc.), regular medication users (excluding vitamins and minerals), those who received iron replacement therapy in the last 6 months, those who had gynecological surgery, who had any metabolic disease, and any hormone replacement therapy (HRT) were excluded from the study.

Research Plan

First of all, the "Informed Consent Form" was read to the women participating in the study voluntarily and their consent was obtained. Survey questions were answered during face-to-face interviews. In the first part, there were questions determining the demographic characteristics of the participants. The second part included the "24-Hour Nutrition Form" including nutritional status evaluations of the individuals. Anthropometric measurements were height, body weight, waist circumference and body fat ratio measurements.

Anthropometric Measurements

Body weight and height were measured by using Tanita brand SC240MA model bioelectric impedance device with a sensitivity of 100 grams. In order to secure accurate measurements, all measurements were made in bare and dry feet after ensuring the of conditions such as three hours after getting out of bed, after going to the toilet, three hours after exercising, and approximately three hours after meals and excessive fluid intake. The same scale was used for all measurements.

Height was measured with feet side by side, head on the Frankfurt plane (eye triangle and auricle parallel to the ground) with a 0.01 cm sensitive height measuring device. Waist circumference was measured with a non-stretch tape measure. Again, the body fat ratio was automatically measured by the device during body weight measurements.

Determination of Food Consumption Status

Food consumption was determined by asking the participants face-to-face using the 24-hour reminder method. While recording food consumption, the volunteer participant was asked to indicate the amount of food consumed by hand and finger measurements and/or kitchen measurements.

Food consumption quantities were calculated according to references included in the located in Turkish Nutrition Guide "standard portion sizes and quantities of foods according to food group in Turkey" (9). In these definitions, food consumption was recorded by questioning the volunteer participants in detail using kitchen measurements (bowl, tablespoon, ladle, water glass, cup, dinner plate) and hand and finger measurements (fist and palm). Following the determination of the nutrient consumption amounts, volunteers' daily energy, water, fiber, polyunsaturated fat, cholesterol, macro (protein, fat, carbohydrate) and micro (A, E, B1, B2, B6 and C vitamins, carotene, folate, sodium, potassium, calcium, magnesium, phosphorus, zinc) nutrient consumption and the amount of iron they received from foods were calculated using the

Nutrition Information Systems Package Program (BEBIS) version 7.2. Consumption amounts were compared with the recommended adequate intake in the Turkish Nutrition Guide (9). Energy and nutrient supply ratios of 66% were classified as under consumption and ≥133% as excess consumption (10).

Biochemical Analysis

After the blood samples were centrifuged, they were sent to the Public Health Laboratory by couriers and measurements were made under the supervision of laboratory experts with appropriate kits. Glucose, blood urea nitrogen (BUN), creatinine, total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate transaminase (AST), alanine transaminase (ALT), iron, total iron-binding capacity (TBIC), ferritin, vitamin B12, thyroid stimulating hormone (TSH), free T4, 25-hydroxy vitamin D and hemogram parameters were tested. Moreover, all participants in the study were asked to bring any biochemical analyses which were performed by the Family Medicine Unit in the last six months with them.

Statistical Analysis

Microsoft excel was used for all graphical representations and IBM Statistical Package for Social Sciences version 25 (SPSS) Chicago, IL, USA software was used for statistical analysis. Unpaired t test was used for parametric data in comparison of premenopause and postmenopausal groups and the Mann-Whitney U test was used for the analysis of nonparametric data. Pearson correlation analyses were performed for the analysis of non-parametric data of all variables of the study, and Spearman correlation was used for the analysis of parametric data.

RESULTS

When **Table 1** is examined, the difference between the ages in the premenopause and postmenopausal groups was statistically significant as expected (p<0.001). While the difference in favor of women in the premenopausal group in terms of mean height was statistically significant (p<0.05), there was no significant difference between mean weight (p> 0.05). The mean BMI of both groups were found to be statistically close to each other (p> 0.05).

Looking at **Table 2**, 12.5% of the participants in the premenopause group and 37.5% of the participants in the postmenopausal group had at least one chronic disease and the difference was statistically significant (p<0.05). There was also a difference in terms of anemia status against women in the premenopausal group (p<0.05). Of those in the premenopause group with chronic diseases, 40% stated that they had asthma and 40% had coronary heart disease. In the postmenopausal group, 73.3% of those with chronic diseases stated that they had hypertension and 20% had asthma.

Table 1. Some demographic and anthropometric data of the participants in the premenopausal and postmenopausal groups

	Premenopausal group	Postmenopausal group	P value
N	40	40	-
Age, year	41.4±3.0	54.7±6.6	a<0.0001
Height, cm	160.0±5.2	156.2±5.5	a<0.0022
Weight, kg	81.28±17.13	77,16±11.20	a0.2068
BMI, kg/m2	31.74±6.47	31,74±5.19	a0.9970
Waist circumference, cm	95.20±13.23	96.65±11.20	a0.5983
Body fat percentage, %	38.76±6.34	39.36±4.88	a0.6394

a Unpaired t test, p<0.05 is statistically significant.
N: Number of the patients BMI: Body Mass Index

Table 2. Statement of anemia and chronic disease status of the participants in the premenopausal and postmenopausal groups

	Premenopausal group	Postmenopausal group	P value
N	40	40	-
Anemia condition			
Yes, n (%) No, n (%)	12 (%30) 28 (%70)	3 (%7.5) 37 (%92.5)	b0.0442
Chronic disease status			
Yes, n (%) No, n (%)	5 (%12.5) 35 (%87.5)	15 (%37.5) 25 (%62.5)	b0.0491

b Mann-Whitney Test.
N: Number of the patients

BUN, HDL, AST and ALT levels of women in the premenopausal period were found to be lower than the postmenopausal group (p<0.05) (**Table 3**). Compared to the postmenopausal group, women in the premenopausal group had lower blood ferritin levels, whereas iron binding capacity values were higher (p<0.05). In addition, 25-OH Vitamin D levels of women in the premenopausal group were lower than postmenopausal group (p<0.05). Hgb levels were lower in premenopausal women than in the postmenopausal group (p<0.05). However, it was found that HCT, MCV and RDW values did not accompany this decrease (p>0.05).

Macro and micronutrient consumption rates in the diets of women in the premenopause and postmenopausal groups are shown in **Tables 4** and **Table 5**. There was no statistically significant difference between the women in the premenopausal and postmenopausal groups in terms of daily energy, water, protein, fat, carbohydrate, fiber, and polyunsaturated fat intake in their diets (p>0.05). This means that both groups are similar in terms of energy, water, protein, fat, carbohydrate, fiber, and polyunsaturated fat intake. However, daily cholesterol intake was found to be statistically higher in the premenopausal group compared to the postmenopausal group (p<0.05).

Table 3. Data on blood biochemistry analysis results of the premenopausal and postmenopausal women

	Premenopausal group	Postmenopausal group	P value
N	40	40	
Glucose, mg/dL	91.2±8.4	97.1±14.2	b0.0697
BUN, mg/dL	10.6±3.51	15.06±4.96	a<0.0001
Creatinine, mg/dL	0.65±0.1	0.68±0.09	a0.2080
Cholesterol, mg/dL	212.7±33.8	227.58±41.22	a0.0826
Triglyceride, mg/dL	123.8±61.1	137.4±68.5	a0.3498
HDL, mg/dL	54.1±9.1	59.3±13.5	a0.0492
LDL, mg/dL	133.3±29.4	141.6±34.9	a0.2540
AST, IU/L	18.3±4.8	22.1±7.2	a0.0069
ALT, U/L	16.9±7.3	23.7±12.3	a0.0034
Iron, µg/dL	66.7±23.2	70.43±21.30	a0.4567
TIBC, µg/dL	311.12±61.7	287.50±41.93	a0.0482
Ferritin, ng/mL	15.8±11.5	33.5±25.4	a0.0001
Vitamin B_{12}, pg/mL	240.7±211.5	261.4±143.3	a0.6106
25-hydroxy vitamin D, ng/mL	16.91±6.14	24.94±19.54	a0.0152
Hgb, g/dL	12.41±1.03	12.87±0.92	a0.0391
WBC, 10^3/µL	7.06±1.38	6.28±1.09	a0.006
RBC, 10^6/µL	4.52±0.29	4.60±0.35	a0.2146
HCT, %	37.89±2.85	39.01±2.52	a0.0654
MCV-fL	82.15±12.74	84.96±4.78	a0.1950
RDW-%	13.97±1.05	13.88±1.13	a0.7120
PLT, 10^3/µL	273.18±67.45	246.03±51.57	a0.0466

a Unpaired t test, b Mann-Whitney Test,
N: Number of patients in the group. BUN: Blood urea nitrogen, HDL: High-density lipoprotein (HDL), LDL: Low-density lipoprotein, AST: Aspartate transaminase, ALT: Alanine transaminase, TIBC: Total iron-binding capacity, Hgb: Hemoglobin, WBC: White blood cell, RBC: Red blood cell, HCT: Hematocrit

Table 4. Macro nutrient and water consumption amounts of the participants in the premenopausal and postmenopausal groups

	Premenopausal group	Postmenopausal group	P value
N	40	40	
Dietary energy, kcal/day	1650±392	1500±309	a0.055
Water, mL/day	2839±792	2625±1059	a0.311
Dietary protein, gr/day	61.4±19.7	56.8±14.2	b0.444
Dietary fat, gr/day	70.5±26.2	62.8±19.1	b0.201
Dietary carbohydrate, gr/day	188.8±46.0	173.3±58.6	b0.064
Dietary fiber, gr/day	23.6±7.9	22.9±7.6	b0.422
Dietary cholesterol, mg/day	362.1±254.1	242.3±147.6	b0.014

a Unpaired t test, b Mann-Whitney Test,
N: Number of the patients

DISCUSSION

Menopause is one of the important periods of a woman's life and is a natural process of normal aging (11,12). Changes occur in blood biochemistry with hormonal changes due to menopause. As a reflection of this, eating habits can change, the tendency to obesity increases, and metabolic and physical changes can also be involved. Therefore, knowing what kind of changes might occur in women in this period will enable them to take precautions in advance and acquire nutritional habits according to the needs.

Table 5. Micronutrient consumption amounts of the participants in the premenopausal and postmenopausal groups

	Premenopausal group	Postmenopausal group	P value
N	40	40	
Dietary vitamin A, µg/day	1153± 741	1380±1859	b0.3552
Dietary carotene, mg/day	4.42±3.98	4.67±4.75	b0.5037
Dietary vitamin E, mg/day	15.6±8.16	14.2±8.17	b0.4945
Dietary vitamin B1, mg/day	0.93±0.29	0.88±0.25	b0,3865
Dietary vitamin B2, mg/day	1.45±1.06	1.16±0.43	b0.1503
Dietary vitamin B6, mg/day	1.25±0.51	1.17±0.38	b0.6580
Dietary folate, µg/day	339.7±172.1	308.6±130.1	b0,2877
Dietary vitamin C, mg/day	142.9±99.5	149.5±106.3	b0.7111
Dietary sodium, mg/day	3497±1296	3395±1075	b0.9962
Dietary potassium, mg/day	2651±821.6	2519±839	b0.3288
Dietary calcium, mg/day	798.9±248.0	775.2±233.7	b0.5605
Dietary magnesium, mg/day	302.5±100.8	284.3±90.7	b0.2834
Dietary phosphorus, mg/day	1051±310.9	983.9±253.5	b0.4162
Dietary iron, mg/day	11.26±4.75	9.66±3.08	b0.1410
Dietary zinc, mg/day	9.96±3.54	8.49±2.33	b0.0767

a Unpaired t test, b Mann-Whitney Test, N: Number of the patients

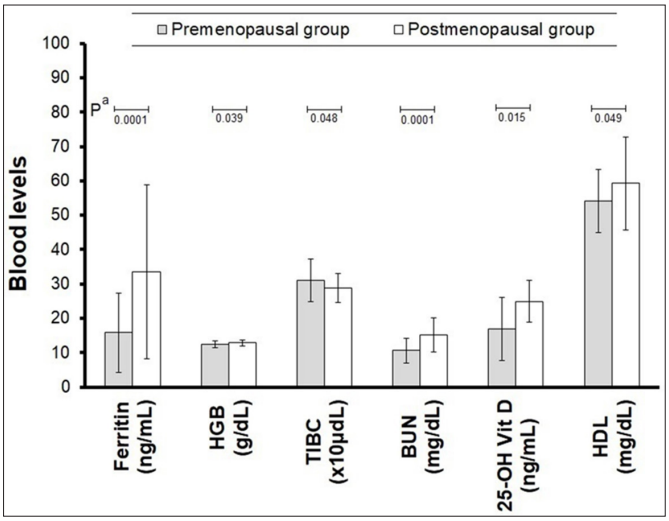

Figure. Ferritin, HGB, TIBC, BUN, 25-OH Vit D and HDL levels of premenopausal and postmenopausal women. The differences between the two groups in terms of parameters are seen clearly in the figure.

The premenopausal women who participated in our study were taller and this finding was parallel to the national data. Furthermore, these findings are consistent with data indicating that Turkey has increased the average height as an indicator of better nutrition in the younger population (13).

When the groups are examined in terms of anemia status in our study, this situation was higher in premenopausal women. The most likely reason for this difference is iron deficiency anemia due to menstrual bleeding in the premenopausal period (14). Nutritional anemia may be associated with prolonged inadequate intake of folate, vitamin B12, iron, protein and vitamin C. Although it was reported that older women after menopause may be at high risk in terms of insufficient micronutrient intake (15), this study shows that losses due to the menstrual cycle are more prominent in women. In addition, the lower levels of ferritin, TIBC and Hgb in premenopausal women also supports our view.

In a study conducted by Schwarz et al. (16) the proportion of premenopausal women with one or more chronic diseases was 29.2% while this rate for postmenopausal women was 13.1%. These rates were found to be 16.6% and 24.2% in premenopausal and postmenopausal women with three or more chronic diseases, respectively. These findings are similar to the results of our study in terms of the presence of more than one chronic disease. The probable reason for this finding is that postmenopausal women have a higher risk of developing chronic diseases due to the adverse metabolic consequences of both aging and menopause (17).

Although BUN is often used as an indicator of renal function and/or hydration status, it is also known to be an independent predictor of mortality in a large number of clinical settings and patient populations (18). In our study, unlike creatinine levels, postmenopausal women had higher BUN levels compared to the premenopausal group. This finding is thought to arise due to aging. That is, the total number of nephrons in a normal person gradually decreases with aging and diseases. Due to these losses, blood creatinine and BUN levels can be higher in the postmenopausal period (19). In our study, since creatinine levels were similar in both groups, the BUN levels of women in the postmenopausal group were attributed to the use of antihypertensive diuretic drugs rather than nephron loss. The presence of a history of hypertension in a significant portion of women in the postmenopausal group supports this conclusion because like creatinine, urea freely filters through the kidney glomeruli. However, compared to creatinine, urea can undergo substantial tubular reabsorption. This tubular reabsorption of urea may increase due to neurohormonal activation through direct effects on the distal nephron or indirect effects of water reabsorption due to reduced renal blood flow (20,21). Neurohormonal activation induced by some anti-diuretics, such as loop diuretics, will also reduce BUN clearance. This explains why serum BUN levels may be higher in people using diuretics.

Today, HDL is known to increase due to physical activity. Therefore, HDL levels are expected to be low in postmenopausal women, where we expect less physical activity. On the contrary, the fact that HDL levels of postmenopausal women were significantly higher in our study was attributed to the antihyperlipidemic drugs used by postmenopausal women to relieve cardiovascular complications. Moreover, the finding that postmenopausal women are highly hypertensive confirms our claim. Studies reporting that physical activity and HDL levels decrease with aging (22) and the lack of difference in lipid profile in both groups other than HDL also support our claim. In addition, it is reported that estrogen is very effective on blood lipids and decreases LDL while increasing HDL. After menopause, the protective effect of estrogen disappears and causes an increase in LDL and triglyceride levels and this situation negatively affects HDL levels. Moreover, the deterioration of HDL/LDL ratio is an important risk factor for cardiovascular diseases. All this information is evidence that postmenopausal women in our study may have used antihyperlipidemic drugs to reduce their cardiovascular risk. Factors in the development of post-menopausal cardiovascular diseases include not only the estrogen hormone, but also dietary habits of pre-menopausal women, obesity, and smoking (23,24).

The correlation between the development of menopause and impaired lipid metabolism leads to the emergence of obesity-related disorders, including metabolic syndrome. Therefore, in order to protect cellular structures from oxidative stress caused by estrogen deficiency, it is important for women who have entered menopause to eat adequate and balanced nutrition from antioxidative foods (25). In a recent cross-sectional study, postmenopausal women diagnosed with metabolic syndrome had higher plasma HDL cholesterol levels. On the other hand, the proportion of women with low HDL levels was higher in the premenopausal group. Therefore, it is thought that low HDL level may be the main characteristic finding of metabolic syndrome in postmenopausal women (26). In addition, in a study examining the lipid profile of healthy premenopausal and postmenopausal women (27), postmenopausal women had higher levels of total cholesterol, triglyceride, LDL and HDL.

According to data from the Turkish Nutrition and Health Survey (TNHS), mean AST level in women aged 31-50 years was 18.3 IU/L and mean ALT level was 17.5 IU/L. Mean AST level in the 51-64 age group was 21.5 IU/L and mean ALT level was 22,2 IU/L (28). In our study, AST values of the participants in the premenopausal group were lower than those in the postmenopausal group, but were similar to the results of the above study. We attributed the probable reason for the lack of difference

between the groups as being due to the liver function tests of women changing significantly during the transition to menopause. This idea is confirmed by a recent study in which an increase in AST and ALT levels associated with triglyceride was found in postmenopausal women (29). As it is known, sedentary lifestyle and decreased physical activity with aging is characterized by fattening. This fattening is also observed in internal organs such as the liver. Fatty liver usually manifests itself with ALT elevation, and in some cases, elevated AST accompanies this (30).

According to TNHS data 28 in women between the ages of 31-50, mean serum iron level was 71.7 µg/dL, serum TIBC was 332.19 µg/dL, mean serum ferritin level was 26.6 ng/mL, blood mean hemoglobin level was 12.7 g/dL and hematocrit value was 38.9%. These values were 76.0 µg/dL, 315.7 µg/dL, 55.9 ng/mL, 13.2 g/dL and 40.3%, respectively, in women between the ages of 51-64. Compared to these results, our ferritin levels were found to be lower in premenopausal women. We think that this finding occurs due to menstrual bleeding in premenopausal women. Moreover, serum iron levels were heavily affected by daily dietary changes. Studies reported that people with iron deficiency develop anemia only in advanced stages and that it can be reflected in hemoglobin and hematocrit levels due to the depletion of ferritin stores (31). In addition, TIBC was found to accompany this tableau in inverse proportion to serum ferritin levels (31). On the other hand, as iron loss will disappear with the end of menstrual bleeding in the postmenopausal period, body iron stores will be filled, and ferritin level will be higher (32,33).

According to the TNSH data, the vitamin D levels of women between the ages of 28, 31-50 and 51-64 years are 15.9 ng/mL and 17.3 ng/mL, respectively. These values were similar to the findings in our study. However, it was higher in postmenopausal women who could benefit less from daylight because they were not in an active life period. However, some researchers (34), contrary to our results, found higher serum vitamin D levels in women in the premenopausal period. They found that vitamin D levels were lower especially in those who had dark skin, were overweight and elderly (35). Therefore, they recommended special diets and vitamin supplements to increase low serum vitamin D levels. The main reason why we found a different result from these researchers may be dietary. In addition, vitamin D level is determined by measuring 25-OH vitamin D, which is a prohormone. Because 25-OH vitamin D is the most stable and abundant form of vitamin D in serum due to its half-life of 3 weeks, using this metabolite is the most reliable method in measurements. For the diagnosis of vitamin D insufficiency, 30 ng /ml value is used as the cut-off value of serum 25-OH vitamin D level (36). In another study, it was revealed that the serum 25-OH vitamin D level should be below 20 ng/mL in order to meet vitamin D deficiency (37). Vitamin D deficiency is a common finding accompanying obesity, increasing age, and unhealthy lifestyle. There are studies that show that adequate vitamin D level can be beneficial for bone, cardiovascular and general health, especially in women in the postmenopausal group (38). In light of the above information and our results, premenopausal women should take supplements for vitamin D.

It was reported that leukocytes decrease due to aging of bone marrow and decreases in its activity (39). Similarly, in our study, the WBC value of postmenopausal women was found to be lower compared to women in the premenopausal group. On the other hand, no difference was found between the daily zinc intake of women in the premenopause and postmenopausal groups. However, all women should be supported in dietary terms for iron and zinc (40).

CONCLUSION

Obese women in premenopause and postmenopausal periods should obtain adequate and balanced nutrition in accordance with their ages and conditions and their physical activity levels should be increased in order to increase their quality of life and protect their health. It should also be considered that the premenopausal group of obese women should take supplements in terms of vitamin D and iron.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for the study was granted by the Ethics Committee of the University of Health Sciences Turkey, Hamidiye Scientific Research (Date: 29.03.2019, Session No: 2019/3, Decision No: 19/33). This study was conducted with the approval (Date: 26/06/2019 6028, Decision No: 16867222-604-01-01) of the Republic of Turkey Ministry of Health Research Platform.

Informed Consent: "Informed Volunteer Consent" was obtained from the women participating in the study.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Predictive value of C-reactive protein/albumin ratio in predicting poor outcome of hospitalized patients with COVID-19

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ABSTRACT

Introduction: For more than a year, COVID-19 has caused a high number of mortality and morbidity, and negatively affects life all over the world. Early detection tools that can be used to predict prognosis are particularly important in patients who need critical care. Among the acute phase reactants, CRP can be higher without any other findings. Otherwise, the cytokine storm that occurs in hospitalized COVID-19 cases can cause critical hypoalbuminemia, and low albumin levels can predict the course of the disease independently of other indicators. Our aim in this study is to determine the relationship between CRP / albumin ratio (CAR) and prognosis of COVID-19 patients.

Material and Method: In this study, from February 1, 2021 to April 30, 2021, patients who visited to the emergency department, diagnosed with COVID-19 and hospitalized, were selected to examine retrospectively.

Results: The study was completed with total of 273 patients. We divided the patients into two groups as those who require ICU and those who do not. The CAR was found to be more than 2 times higher in the ICU required group than the non-ICU need group (1.43 - 0.61, respectively). The area under the curve (AUC) of CRP, albumin and CAR were 0.708, 0.321 and 0.729 for the prediction of ICU admissions, respectively. In terms of mortality, AUC values were calculated as 0.660, 0.304 and 0.725, in the same order, and the predictive power of CAR was higher than CRP and albumin alone in both outcomes.

Conclusion: We found that the patients with high CAR values had further ICU requirements and further mortality rates. CAR is a simple, convenient and inexpensive prognostic marker that can be used in predicting the severity of COVID-19.

Keywords C-reactive protein, albümin, COVID-19, intensive care

INTRODUCTION

In late 2019, many pneumonia cases of unknown origin were detected in Wuhan, China (1). The pathogen that caused the cases was later identified as a novel enveloped RNA betacoronavirus and named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The World Health Organization (WHO) has declared coronavirus disease (COVID-19) as an internationally considerable public health emergency (2). For more than a year, COVID-19 has caused a high number of mortality and morbidity, and negatively affects life all over the world.

Early diagnosis tools that can be used to predict prognosis are crucial for the functioning of health systems, especially in patients who require critical care.

Inflammation is triggered the liver to synthesize a large number of the acute phase reactants. One of these such reactant is C-reactive protein (CRP), which can be used as a biomarker in the presence of rheumatoid arthritis, cardiovascular disease, and infection (3). It has been reported that in severe COVID-19 cases, CRP levels can be high without any findings observed on computed tomography, therefore, CRP can be used to determine the severe cases at an early stage (4).

Otherwise, cytokine storm induced in hospitalized COVID-19 cases, may cause critical hypoalbuminemia, increase the risk of death, and low albumin levels at the time of admission stage can predict the course of the disease independently than other indicators (5).

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Recently, the CRP/albumin ratio (CAR), that a combination of markers for systemic inflammation and nutritional status, has been extensively studied as an independent prognostic marker in patients with infection, malignancy, and other diseases (6). Our aim in this study is to determine the relationship between the CAR and the prognosis of COVID-19 patients.

MATERIAL AND METHOD

The study was approved by the ethics committee of Istanbul Education and Research Hospital (Date: 31.04.2021 Decision No: 2817), and conducted in accordance with the Declaration of Helsinki, the ethical principles.

Study Design

In this study, from February 1, 2021 to April 30, 2021, patients visited to the ED who diagnosed with COVID-19 and hospitalized, were selected to examine retrospectively.

All COVID-19 patients over the age of 18 who were visited to the ED, had oropharyngeal/nasopharyngeal swabs and hospitalized between February 1, 2021 and April 31, 2021 were included the study. Patients whose reverse transcriptase polymerase chain reaction (RT-PCR) test results were negative and whose CRP and/or albumin levels were not measured at the time of admission were not included in the study. Additionally, patients who visited to the ED due to cardiac arrest and who were received inotropic support at the time of admission were not included in the study.

Data were collected from electronic medical hospital records. All of the patients were recorded in a form, with their age, gender, CRP, albumin levels. After recording the CRP and albumin levels, the ratio of CRP/albumin was measured.

The primary outcome was to determine the relationship between CAR and ICU requirement. The secondary outcome was to determine the in-hospital mortality. Outcomes were retrospectively assessed by reviewing of the hospital medical database.

Statistical Analysis

Categorical variables were presented as frequency and percentage. Continuous variables were tested for distribution using the Kolmogorov–Smirnov and Shapiro-Wilk tests. The asymmetrically distributed variables were expressed as the median interquartile range (25%-75%). All variables were compared for ICU admission and mortality outcomes using Pearson's chi-squared, and Mann–Whitney U tests as appropriate. Receiver operating characteristic (ROC) analyses were performed to determine the predictive power of the variables in terms of both outcomes. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of all cutpoints were calculated. The cut-off point that achieves the maximum Youden's index is referred to as the optimal cut-off. A 2-sided P-value of 0.05 was regarded as statistically significant. All data analyses were performed using SPSS version 23.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

The study was completed with total of 273 patients after the inclusion and exclusion criteria were performed. The median (IQR) age of the patients was 52.0 (39.0–66.0), which 126 (46.2) were female. When our study sample was examined in terms of comorbid diseases, first three places were hypertension (HT), cardiovascular disease and pulmonary disease with 38.8%, 36.2%, 33.7%, respectively. ICU admission was required in 53 (19.4%) of all patients and 28 (10.3%) of them died. The baseline characteristics of the patients were shown in **Table 1**.

We divided the patients into two groups as those who require ICU and those who do not. Then we compared the variables in these two groups of age, gender, vital signs, comorbid diseases, laboratory findings, and mortality (**Table 1**). The median (IQR) age in the group with ICU requirement was 76.0 (67.0–79.0), and it was 47.0 (36.5–58.0) in the other group (non-ICU) and the difference was statistically significant ($p<0.001$). No significant difference was found in gender variable in the groups according to ICU requirements ($p=0.171$).

When we examined the vital signs, while the body temperature, pulse and respiratory rate increased significantly in the ICU requirement group, the saturation O2 was decreased ($p<0.001$ for all). But there was no significant difference between the groups in systolic-diastolic blood pressure and mean arterial pressure ($p>0.05$ for all).

In comorbidities, pulmonary disease (83.0%), cardiovascular disease (77.4%) and hypertension (60.4%) were the most common in ICU required patients, and there was a significant difference compared to the group that non-ICU patient ($p <0.001$). All ICU required patients had at least one comorbidity. While the comorbidity median (IQR) of the ICU requirement group was 1.0 (0.0–2.0), the comorbidity median of the non-ICU patients was 3.0 (2.0–4.0) and this difference was statistically significant ($p <0.001$).

In the laboratory results, the median value of CRP was significantly higher (49.0 - 23.5, respectively) and albumin was lower (36.5 - 40.0, respectively) in the ICU requirement group. The CAR was found to be more

than 2 times higher in the ICU requirement group than the non-ICU group (1.43 - 0.61, respectively), and all these differences were statistically significant (p <0.001 for all).

The ROC analysis was performed to determine the predictive power of CRP, albumin and CAR in ICU admissions and mortality outcomes. The area under the curve (AUC) of CRP, albumin and CRP/albumin were 0.708, 0.321 and 0.729 for the prediction of ICU admissions, respectively. In terms of mortality, AUC values were calculated as 0.660, 0.304 and 0.725, in the same order, and the predictive power of CAR was higher than CRP and albumin alone in both outcomes (**Table 2**).

The optimum cut-off points for the relevant outcomes were determined using the Youden's Index. The cut-off value of CAR determined for ICU requirement was 0.74. At this cut-off value, sensitivity was calculated as 86.79%, specificity 56.82%, PPV 32.62% and NPV 94.70%. The cut-off value of CAR for mortality was calculated as 0.91 and for this cut-off value, sensitivity was 78.57%, specificity 61.22%, PPV 18.80% and NPV 96.15%. The cut-off points of CRP, albumin and CAR with sensitivity, specificity, PPV, NPV, AUC and Youden's Index values were shown in **Table 2**.

Table 2. Optimum cut-off points* of CRP, albümin and CRP/albumin ratio in predicting ICU admissions and mortality

	Cut-off point	Sens (%)	Spec (%)	PPV (%)	NPV (%)	AUC	Youden's Index
ICU admissions							
CRP	27	84.91	55.91	31.69	93.89	0.708	0.408
Albumin	53	1.89	99.09	33.33	80.74	0.321	-0.143
CRP/albumin	0.74	86.79	56.82	32.62	94.70	0.729	0.436
Mortality							
CRP	27	82.14	51.43	16.20	96.18	0.660	0.336
Albumin	53	0	98.78	0	89.63	0.304	-0.122
CRP/albumin	0.91	78.57	61.22	18.80	96.15	0.725	0.398

*Cut-off points with the highest Youden's index value were shown
CRP: C-reactive protein, ICU: Intensive care unit, Sens: Sensitivity, Spec: Specificity, PPV: Positive predictive value, NPV: Negative predictive value AUC: Area under the curve

Table 1. Baseline characteristics of 273 patients

Variables Median (IQR), n(%)	All n=273	ICU (-) n=220	ICU (+) n=53	p
Age. years	52.0 (39.0–66.0)	47.0 (36.5–58.0)	76.0 (67.0–79.0)	<0.001*
Gender. n (%)				**0.171****
Female	126 (46.2)	106 (48.2)	20 (37.7)	
Male	147 (53.8)	114 (51.8)	33 (62.3)	
Vital Signs				
Body temperature	37.8 (37.2–38.2)	37.7 (37.2–38.0)	39.0 (38.0–39.2)	<0.001*
Saturation O_2	96.0 (93.0–98.0)	97.0 (95.0–98.0)	79.0 (74.0–90.0)	<0.001*
Pulse	83.0 (73.0–97.0)	80.5 (71.8–91.0)	108.0 (90.0–126.0)	<0.001*
Systolic blood pressure	124.0 (116.0–159.0)	124.0 (116.0–150.0)	136.0 (108.0–176.0)	0.337*
Diastolic blood pressure	82.0 (71.0–100.0)	81.0 (71.0–100.0)	83.0 (69.0–105.0)	0.780*
Mean arteriel pressure	95.7 (85.3–119.0)	95.0 (85.6–116.0)	101.0 (80.3–131.0)	0.529*
Respiratory rate	16.0 (14.0–18.0)	15.0 (13.0–16.0)	24.0 (21.0–27.0)	<0.001*
Comorbidities				
Diabetes	36 (13.2)	27 (12.3)	9 (17.0)	0.363**
Hypertension	106 (38.8)	74 (33.6)	32 (60.4)	<0.001**
Cardiovascular disease	99 (36.2)	58 (26.4)	41 (77.4)	<0.001**
Pulmonary disease	92 (33.7)	48 (21.8)	44 (83.0)	<0.001**
Hepatitis B	6 (2.2)	3 (1.4)	3 (5.7)	0.055**
Malignancy	36 (13.2)	16 (7.3)	20 (37.7)	<0.001**
Cerebrovascular disease	9 (3.3)	2 (0.9)	7 (13.2)	<0.001**
Chronic renal failure	18 (6.6)	8 (3.6)	10 (18.9)	<0.001**
Immunodeficiency	3 (1.1)	2 (0.9)	1 (1.9)	0.540**
Any comorbidities	203 (74.4)	150 (68.2)	53 (100.0)	<0.001**
Number of comorbidities	1.0 (0.0–2.0)	1.0 (0.0–2.0)	3.0 (2.0–4.0)	<0.001*
Laboratory				
CRP. mg/L	28.0 (14.0–56.0)	23.5 (12.0–48.5)	49.0 (34.0–67.0)	<0.001*
Albumin. g/L	39.1 (36.0–43.0)	40.0 (37.0–43.7)	36.5 (30.8–40.6)	<0.001*
CRP/albumin ratio	0.76 (0.35–1.56)	0.61 (0.31–1.25)	1.43 (0.91–1.84)	<0.001*
Mortality	28 (10.3)	1 (0.5)	27 (50.9)	<0.001**

*Mann-Whitney U test, **Pearson's chi-squared test
ICU: Intensive care unit, CRP: C-reactive protein

The AUC of CAR for ICU requirement was 0.729±0.034 (95% CI, 0.662–0.797), (**Figure 1**) and mortality was 0.725±0.046 (95% CI, 0.635–0.815), (**Figure 2**); (p <0.001 for both).

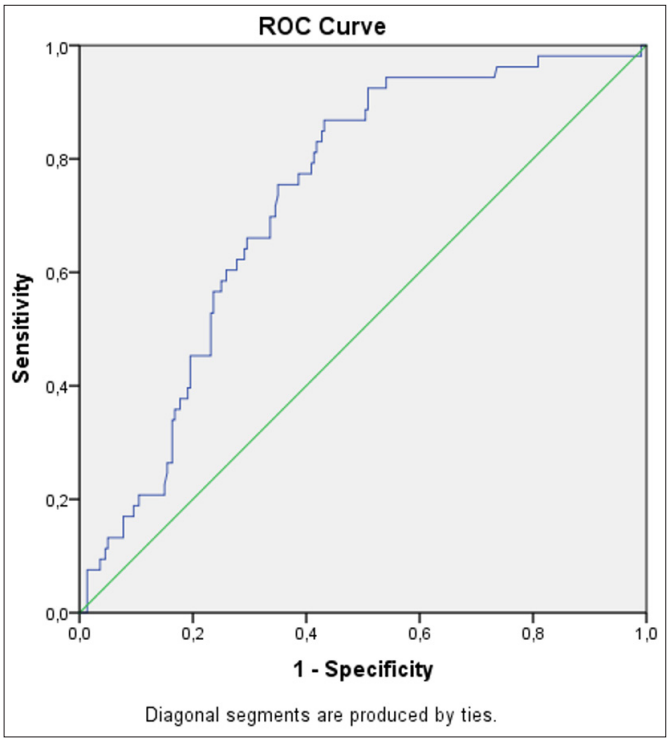

Figure 1. The ROC curve of CRP/albumin ratio for ICU admissions.
AUC: 0.729±0.034 (95% CI, 0.662 – 0.797), (p<0.001).

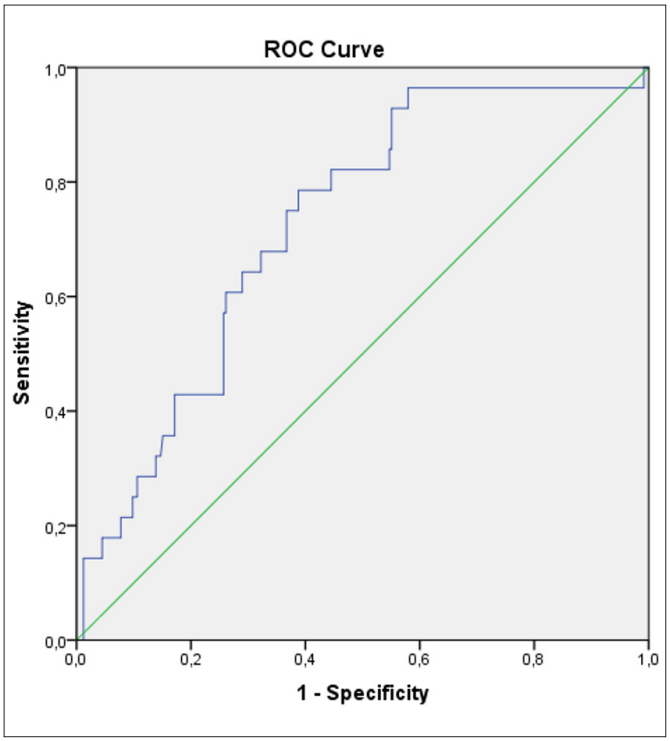

Figure 1. The ROC curve of CRP/albumin ratio for mortality
AUC: 0.725±0.046 (95% CI, 0.635 – 0.815), (p<0.001).

DISCUSSION

In this study, it is concluded that the elevation of CAR was successful in determining the ICU requirement and mortality rate of COVID-19 patients. Additionally, it was observed that the advanced age, high CRP, low albumin and the presence of comorbidity was to accompany CAR in predicting poor outcome.

It is known that systemic inflammatory response plays an important role in infections. With the release of proinflammatory cytokines and the formation of an inflammatory microenvironment, the immune system protects metabolism against existing pathogens. Recent studies have shown that many important inflammation-based prognostic scores are associated with survival outcomes in various infections (7).

It has been previously reported in the literature that high markers of inflammation at the time of admission and advanced age are significantly associated with the severity of COVID-19 (8,9). In particular, it has been reported that comorbidities such as hypertension, coronary artery disease, diabetes mellitus and the presence of malignancy are another indicator for the severity of COVID-19. Metabolic disturbances in the pathway of glycolipid can affect the severity of COVID-19 by creating an imbalance between angiotensin converting enzyme-2, causing a cytokine storm (10). The results of our study are consistent with the literature on this topic.

CRP is an acute phase protein and is produced in hepatocytes. When bound to macromolecular ligands, CRP, strongly activates the classical complement pathway and can regulate alternative pathway amplification as well as C5 transformers (11). CRP levels are known to be associated with severe sepsis, heart failure, and other inflammatory diseases (9). In a study comparing the prognosis and CRP levels of COVID-19 patients, it was observed that CRP values were significantly higher in patients with poor prognosis (12).

Albumin is a main protein synthesized in the liver, with a normal serum albumin concentration of approximately 35 to 50 g/L in healthy adults. There are many physiological functions defined for albumin. The primary ones of these defines are, the binging and transport, colloid-osmotic pressure effect, free radical scavenging, anticoagulant effect and increasing capillary membrane permeability (13). However, it can be significantly adversely affected by factors such as inflammation, which can cause a decrease in serum albumin levels by downregulating albumin synthesis through IL-6 and TNF-α or by increasing the catabolism (10). On the other hand, in the literature, there are studies include many desease which hypoalbuninemia is associated with poor outcome (14,15). Low serum albumin levels have also been reported in these studies, to be indicator of poor prognosis in COVID-19 patients (5).

This study showed that the diagnostic value of albumin and CRP in COVID-19 patients is not as good as CAR. Also, hypoalbuminemia can be caused by previous diseases or nutritional disorders, therefore, using CRP or albumin alone as a biomarker could be difficult.

In a study conducted on patients with sepsis, it was stated that CAR is an independent marker for mortality and it is provides a higher accuracy than the CRP values alone (16). In a study examining community-acquired pneumonia (CAP) patients, it was stated that CAR could be used effectively to predict the severity of pneumonia (17). In another study, in the preoperative prognosis study of ovarian cancers, it was reported that CAR may be a new independent marker of poor prognosis (18).

In the literature, there are only a few studies evaluating the efficacy of CAR in COVID-19 patients. In a retrospective study on 1077 patients, conducted by Canan D. it was stated that CAR was more successful than isolated CRP and albumin in predicting poor outcome. In another study, Xiaoyue W et al. reported that the height of CAR and age can be an early warning for severe COVID-19 disease. Another study conducted in Turkey, Faysal S. et al. reported that increased CAR could be used as an independent predictor of in-hospital mortality in hypertensive COVID-19 patients. (19–21). Studies in the current literature are consistent with our study.

Limitations

As with any retrospective study, there are some limitations in this study. Limitation of this study was the small sample size. Therefore, more studies with a larger sample size are required to confirm these results.

CONCLUSION

The COVID-19 pandemic has caused a serious death and morbidity problem worldwide. In this study, we found that the patients with high CAR values had further ICU requirements and further mortality rates. We are defending the idea of that the CAR is a simple, convenient and inexpensive prognostic marker that can be used in predicting the severity of COVID-19.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the ethics committee of Istanbul Education and Research Hospital (Date: 31.04.2021, Decision No: 2817).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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MEDICINE

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The association between PTX3 and serum manganese levels of welders in comparison with controls: An application of anti-inflammatory biomarker

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ABSTRACT

Aim: The purpose of this study was to compare serum PTX3 levels of manganese-exposed welders with non-exposed controls to evaluate the nature of the manganese-induced inflammatory response.

Material and Method: Overall, we collected 103 research samples (Mn-Exposed Welders Group:51 and Non-Exposed Controls: 52). PTX-3 levels were analyzed in the serum samples by the ELISA method, while Mn levels in whole blood specimens were quantified by the inductively coupled plasma (ICP-MS) method.

Results: The mean values of manganese and Pentraxin-3 of the control group were found to be significantly lower than those of the exposure group (Mn levels: 5.04±2.32 µg/L vs. 11.54±3.09 µg/L; PTX-3: 36.96±24.20 ng/mL vs. 48.29±27.13 ng/mL; $p<0.05$).

Conclusion: This hypothetical and observational investigation highlighted the relationship between Mn levels and PTX-3 for the first time.

Keywords: Manganese, exposure, pentraxin-3, inflammation

INTRODUCTION

Welding fumes contain Manganese (Mn), which may lead to Mn accumulation responsible for adverse effects in the central nervous system. Prolonged exposure often results in a Parkinson-like syndrome, called manganism (1). Generally, Mn exposures and ensuing toxicities occur in a variety of environmental mediums, dietary sources, contaminated food, infant formula, and natural or artificial contaminations in water, soil, and air (2). Mn is a solid and silvery metal primarily used as an industrial alloy, especially with iron in stainless steel (1). It is also an essential element found in bones predominantly. Mn is functional in bone formation, nutrient metabolism, and antioxidant defense system (3,4). Mn plays an important role as a cofactor in several enzymes and numerous vital processes, including nerve and brain development and cognitive functioning (5). Overexposure to Mn causes toxicity on the central nervous system and affects motor activities disrupting dopaminergic functions (6,7). In the brain, the target sites of Mn are the striatum, globus pallidus, and substantia nigra (6,8). People exposed to manganese may develop clinical signs and symptoms resembling Parkinson's disease (2).

One of the mechanisms that play a role in manganese toxicity is the increase in reactive oxygen species and, in turn, increased oxidation (9). N-acetylcysteine, an antioxidant, is known to protect the striatum, hippocampus, and hindbrain against Mn toxicity (10).

Pentraxin-3 (PTX3) from the pentaxin family, mainly produced by endothelial and dendritic cells, fibroblasts, and macrophages, is an essential acute-phase protein in initiating the innate immune response (11). PTX3 is known to be responsible for the pathogenesis, exacerbation, or control of many diseases, including

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psoriasis, juvenile idiopathic arthritis, and inflammatory rheumatic diseases (12–14). It was previously reported that Mn might have cardioprotective and atheroprotective properties in cardiovascular diseases and be a related marker in acute and chronic kidney injury as it correlates with the severity of the damage (14,15).

Ultimately, the objective and novelty of this paper were based on a comparison of serum PTX3 levels of Mn-exposed welders with those of non-exposed controls to assess the nature of Mn-induced inflammatory response for the first time.

MATERIAL AND METHOD

This study included 51 manganese exposed welders and 52 controls with no history of toxic metal exposure, including Mn. We extracted all sociodemographic characteristics, including alcohol consumption, smoking, and employment history. Yet, we had to exclude twelve subjects having coronary vascular disease, hypertension, diabetes mellitus, accrue upper respiratory tract infection, and cancer. All procedures followed the 1964 Helsinki Declaration and its later amendments. All subjects in the control group were healthy with no acute and/or chronic disease and selected among non-smokers and alcohol users. This study was conducted with the approval of Ankara Keçiören Training and Research Hospital Ethics Committee (Decision No:22.02.2012- B.10.4.ISM.4.06.68.49).

Collection of Blood Samples for PTX-3 Analysis

We analyzed PTX-3 levels in serum samples obtained with centrifugation of blood samples at 1500 rpm for 10 mins and transferred to 2 mL Eppendorf tubes to be saved at -20°C until analyses. The corresponding ELISA kit was used for PTX-3 assays, and samples were prepared according to the manufacturer's kit instructions. We then placed the samples on microplates and analyzed them using a CLARIOstar ELISA plate reader. (BMG LABTECH, Ortenberg, Germany). The wavelength was set at 450 nm. We found the r2 value of the calibration curve to be 0.9996.

Collection of Blood Samples for Mn Analysis

For Mn analysis, we put 1 mL of whole blood sample in Teflon tubes. Then, 5 mL of 65% nitric acid and 5 mL of ultrapure water were added to the tubes and resolved in a Milestone microwave digestion unit. The digested samples were then transferred to 50 mL polypropylene tubes with ultrapure water to obtain the total volume of 20 mL and stored at +4°C until analysis (16). We determined Mn levels with inductively coupled plasma mass spectrometry (ICP-MS). The operating parameters of ICP-MS were set as follows: RF power=1550 W, nebulizer gas=0.90 L/min, plasma gas=0.80 L/min, nebulizer pressure=3.1 bar, dwell time=0.01, and spray chamber temperature=3.3°C. We washed the sampler probe between injections by rinsing with ultrapure water for 30 s, followed by washing with 2% HNO_3 for 45 s and rinsing with ultrapure water for 45 s. Afterward, the instrument automatically ran the next sample. The r2 value of the calibration curve was 0.9999, and the interval of the calibration was set at 0.1–1000 µg/L manganese. We repeated the sample and standard of measurements three times and performed method validations using CRM Seronorm™

We ran Seronorm Whole Blood L-2 CRM 5 times on the same day and different days. Moreover, we used the average of the repeated measurements to validate the method whereby the relative standard deviation (RSD) of the values did not exceed 5%. We found the coefficient of variation (CV) and recovery to be 2.92% and 101.12%, respectively. On the other hand, the ICP-MS method for Mn analysis provided the limit of detection (LOD) and lowest limit of quantification (LOQ) equal to 0.029 and 0.146, respectively.

Statistical Analysis

We utilized the SPSS 20.0 software in statistical analyses. The Kolmogorov Smirnov test was performed to check whether the parameters showed a normal distribution. We found the data distributed normally; therefore, we used parametric tests. We presented continuous variables as mean±standard deviation. The differences between the groups were evaluated using the T-Test, while we deployed Pearson's correlation analysis to explore the relations between the variables.

RESULTS

In this study, we investigated a total of 103 employees, 51 of whom were exposed to manganese and 52 were volunteers working in the same workplace. **Table 1** presents the relationships between the main parameters and groups. Mean ages were found to be 35 and 39 years for the control and Mn-exposed workers, respectively. However, there was no difference between the exposed and control groups by age ($p>0.05$). The mean values of manganese and Pentraxin-3 of the control group were found to be significantly lower than those of the exposure group, respectively (Mn levels: 5.04±2.32 µg/L vs. 11.54±3.09 µg/L; PTX-3: 36.96±24.20 ng/mL vs. 48.29±27.13 ng/mL; $p<0.05$) (**Table 1**).

Table 2 shows the correlations between the continuous variables. Accordingly, we found a negative correlation between manganese and WBC levels ($r= -0.215$; $p<0.05$) (**Figure 2**), while manganese was positively correlated with Pentraxin-3 levels ($r=0.202$; $p<0.05$) (**Figure 1**). Finally, we reached a negative relationship between age and Pentraxin-3 levels ($r= -0.247$; $p<0.05$). (**Table 2**).

Table 1. The relationships between the main parameters and groups (n=103).

	Groups	N	Mean	SD	t	p
Mn levels (µg/L)	Control	52	5.04	2.32	12.101	<0.001**
	Mn-Exposed	51	11.54	3.09		
Age (years)	Control	52	35.42	8.86	1.988	0.06
	Mn-Exposed	51	39.18	10.24		
BMI (kg/m^2)	Control	52	27.10	3.02	0.396	0.693
	Mn-Exposed	51	27.32	2.57		
WBC (µl/ml)	Control	52	7.95	1.90	1.389	0.168
	Mn-Exposed	51	7.45	1.75		
HGB (g/dL)	Control	52	15.31	1.49	0.336	0.738
	Mn-Exposed	51	15.22	1.39		
HCT (%)	Control	52	45.47	3.78	0.381	0.704
	Mn-Exposed	51	45.73	3.29		
PLT ($10^3/\mu L$)	Control	52	242.67	59.85	1.275	0.205
	Mn-Exposed	51	228.65	51.37		
ALT (IU/L)	Control	52	25.21	14.58	0.24	0.811
	Mn-Exposed	51	25.92	15.41		
AST (IU/L)	Control	52	20.40	5.65	0.01	0.992
	Mn-Exposed	51	20.39	5.81		
Pentraxin-3 (ng/mL)	Control	52	36.96	24.20	2.237	0.027*
	Mn-Exposed	51	48.29	27.13		

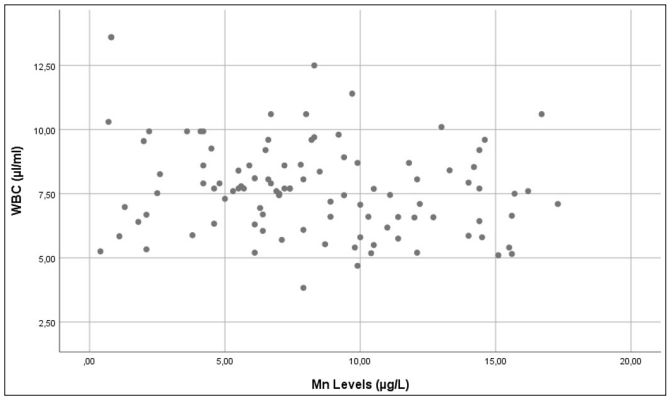

Figure 2. The relationships between Mn and WBC levels.

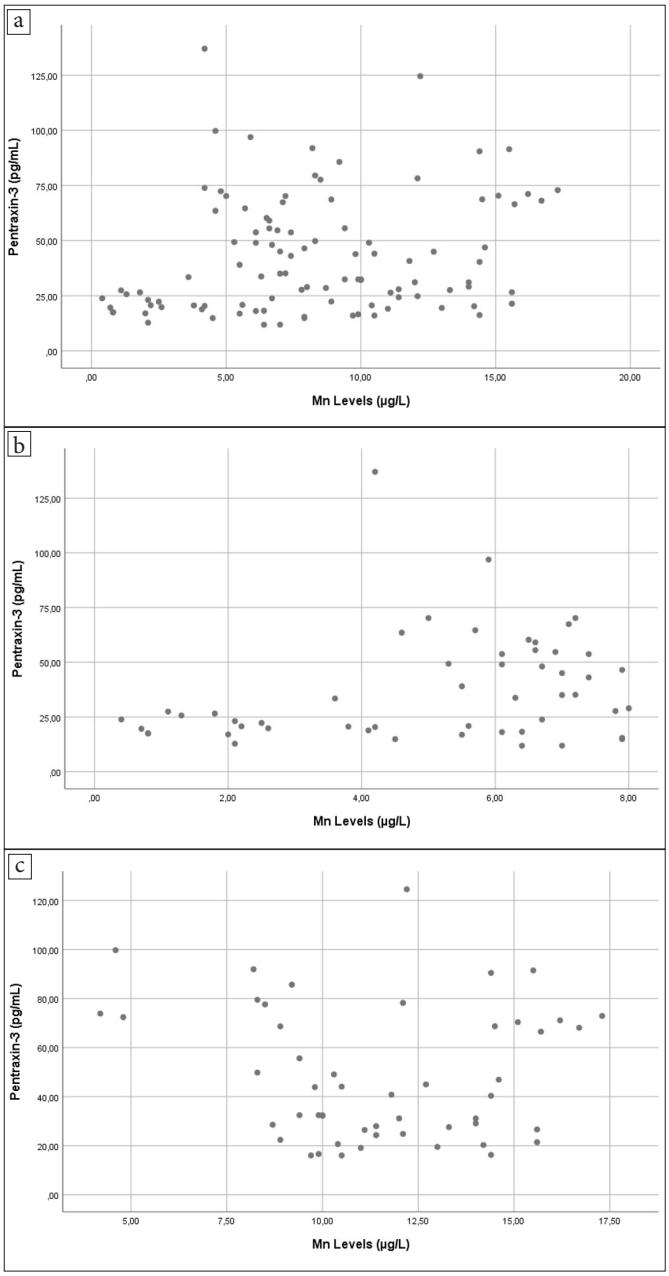

Figure 1. The relationships between Mn and PTX-3 levels (**a:** all groups; **b:** control group; **c:** Mn-Exposed group).

Table 2. Pearson correlations of continuous variables

	Mn levels	Age	BMI	WBC	HGB	HCT	PLT	ALT	AST
Age	0.165	1							
BMI	0.136	.349**	1						
WBC	-.215*	-0.055	-0.186	1					
HGB	0.096	-0.009	-0.029	0.075	1				
HCT	0.089	-0.063	-0.075	0.078	.882**	1			
PLT	-0.139	0.054	-0.104	.275**	-0.01	-0.179	1		
ALT	-0.048	-0.117	0.145	0.024	.209*	.229*	0.165	1	
AST	-0.069	-0.083	0.106	0.004	0.157	0.145	0.133	.710**	1
Pentraxin-3	.202*	-.247*	-0.165	0.029	-0.156	-0.048	0.065	-0.043	-0.139

*p<0.05; **p<0.01

DISCUSSION

In this study, we investigated the relationship between manganese exposure and the inflammatory biomarker pentraxin 3 (PTX-3). The results revealed that increased manganese level was associated with increased PTX-3 levels. Biological screening in toxicological research is important for the assessment of the risk of metal exposure for human health. Metal exposure in humans was previously found to be associated with adverse health effects such as neurological and cardiovascular harms, diabetes mellitus, skin lesions, and skin, lung, kidney, and liver cancers (6,16,17–22).

Studies showed that manganese triggers inflammatory processes through various pathways and shows neurotoxic effects. In a study examining neurotransmitter and metabolite levels after manganese exposure in the mouse brain, the researchers found increased dopamine, DOPAC (3,4-dihydroxyphenylacetic acid), and homovanillic acid (HVA) levels in the striatum (23). In another study, increases in norepinephrine and serotonin levels were observed in addition to these neurotransmitters (24). In a study on cell culture, exposure to manganese was shown to play an important role in the development of manganese-induced oxidative stress, inflammation, and apoptosis in microglia with the LRRK2 molecule. In the present study, the effect on inflammation was realized by the increase in manganese-induced TNF-α production (25).

Although the liver is the second most crucial storage organ following the brain, manganese-related hepatotoxicity in the liver has not been extensively studied so far (2). In a study with mice, hepatic accumulation was observed after manganese exposure, but no histopathological damage was observed (26). In our study, we could not find a difference in liver enzyme levels between the groups. On the other hand, like hepatotoxic effects, renal toxic effects of manganese have not been appropriately investigated. In a study conducted with predialysis patients, chronic renal failure was found to be associated with increased levels of manganese (27). However, in our study, there was no association between manganese levels and renal function tests.

We could not find a study examining the relationship between PTX-3 and manganese exposure in the literature. Yet, studies show that pentraxin 3 is related to neuroinflammatory processes in the brain (28). The correlation between manganese elevation and PTX-3 in our study suggests that a pathological process may progress through the PTX-3 molecule in the toxic effects of manganese in the brain.

Limitations

The lack of clinical endpoints is one of the most important limitations of our study. However, the observational nature of the research and the absence of a temporal relationship may have prevented the release of a causal relationship between the variables. Since blood or urine manganese levels are not clinically correlated, separating groups according to manganese levels may not coincide with clinical outcomes.

CONCLUSION

In conclusion, we suggested the relationship between manganese levels and PTX-3 for the first time in this hypothetical observational study. Accordingly, this study highlights the need for further studies to investigate the pathophysiology of both neurotoxic and other systemic toxic effects of manganese. Also, it is needed to perform animal or human studies with clinical outcomes that may provide evidence of causality.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was conducted with the approval of Ankara Keçiören Training and Research Hospital Ethics Committee (Decision No:22.02.2012- B.10.4.ISM.4.06.68.49).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Case Report

HEALTH SCIENCES
MEDICINE

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Brief review of parathyroid adenoma; problems caused by water clear cells

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ABSTRACT

Water clear cells; they can be seen in many tumors such as germ cell tumors, kidney tumors, lymphomas, sarcomas, thymic carcinomas, parathyroid carcinomas, paraganglioma. They may be primary or may occur as a result of metastatic conditions. Therefore, histopathologically differential diagnosis may be difficult or confusing. Clinical findings, radiological findings and histopathological evaluation may be required for differential diagnosis. Here, as immunohistochemically; a case with parathormone, CD10 positive and synaptophysin negative and CD10 positivity in the area where clear cells are dominant is presented.The case was reported as water clear cell (WCC) component dominant parathyroid adenoma with clinical, biochemical, radiological evaluation and histopathology and the differential diagnosis is discussed.

Keywords: Water clear cells, parathyroid, adenomas, hyperplasia, carcinomas, metastasis

INTRODUCTION

Parathyroid glands (PG) are defined as four glands in more than 80 % of normal adults. However, it can be seen in numbers ranging from 2 to 12 (1).

In light microscopy, the majority of the PG parenchyma consists of chief cells. Oxyphil cells and fat cells are also found in the stroma (2). There are publications that mention three types of epithelial cells (primary chief cells and transition phase cells between oxyphils) (1).

PG is one of the main organs involved in the regulation of calcium (Ca +2) metabolism synthesizes and releases the parathyroid hormone (PTH) (3). Therefore, when there is a parathyroid-related pathology, Ca and PTH level and parathyroid gland size may be clues.

Increase in parathyroid hormone level may occur for primary, secondary or tertiary reasons. This distinction is important for treatment. Primary hyperparathyroidism can develop hereditary (5-10%) or sporadic (90-95%). Parathyroid hyperplasia (PH) parathyroid adenoma (PA) or parathyroid carcinoma (PC) are among the causes (4).

Here, surgery was performed due to PA and the deceptive findings encountered in histopathological evaluation are presented.

CASE REPORT

A 61-year-old female patient. She has had complaints about the skeletal system since 2017. In her anamnesis, it was determined that she had been treated for peptic ulcer in 2018 and she was a patient with hypertension. The biochemical results were examined, the preoperative PTH was 136 pikogram (15-65), Ca 11.35 mg/dL (8.6-10.2) and P: 2.99 mg/dl (2.5-4.5). Parathyroidectomy was planned by the general surgery department due to swelling in the left lower pole in the vicinity of the thyroid in ultrasonography (USG) evaluation and focal radiotracer accumulation in the lower pole region of the left thyroid lobe in parathyroid scintigraphy. During the operation, the material for frozen was sent to the pathology department. It was interpreted as hypercellular parathyroid tissue in frozen evaluation. In the follow-up, a light brown nodular area of 1.2×1.5×1 cm in size, surrounded by a smooth capsule, with a diameter of 1 cm in a cross-sectional area, was observed macroscopically. Clear cells drew attention in the microscopic evaluation of this area. An immunohistochemical (IHC) study was performed in terms of metastasis. PTH, CD10 and synaptophysin were performed in the IHC study. PTH was uncertain. CD10 was positive and synaptophysin was evaluated as negative.

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The PTH (with IHC) study was repeated and weak positive expression was observed. Since the clear cells were CD10 positive, they were investigated for renal cell carcinoma. No mass lesion was observed in the patient's renal USG and there was no other malignancy in the history. Biochemically, PTH and calcium levels were compatible with hyperparathyroidism. In the postoperative period, PTH was measured as 59.4 and Ca 8.8. Since PTH and Ca were high in the initial biochemical evaluation and PTH antibody was positive in the second IHC study, this case was evaluated as water clear cell (WCC) component dominant PA.

Case reports include histopathological evaluation. The patient was informed about the presentation.

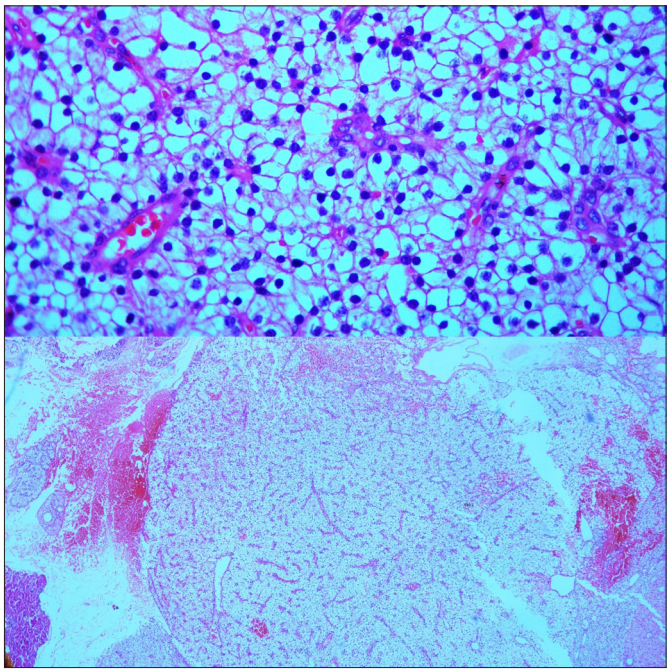

Figure 1. A nodular area with a clear cytoplasm and a prominent vascular network was observed. There is also an increase in cellularity in other cells. Normal parathyroid tissue is seen around (A:H&EX40 (down area), B:H&EX400 (upper area)).

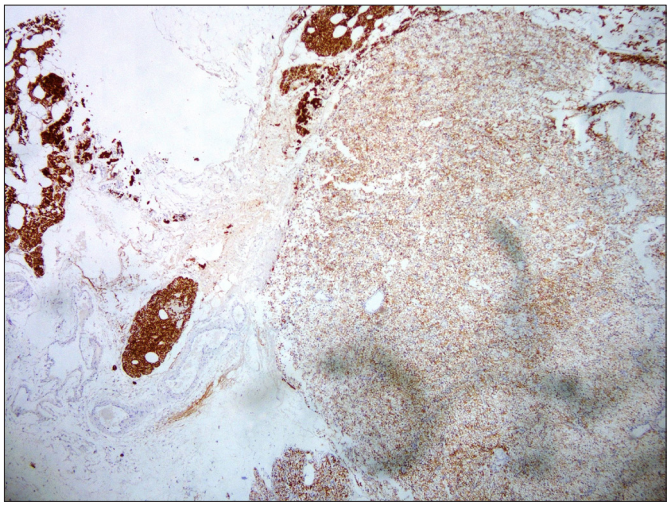

Figure 3. Weak positive staining with PTH was observed in the nodular area with these clear cells (PTH X100).

DISCUSSION

Mainly two types of cells are seen in PG. It has been reported that WCCs are not a component of the normal histology of the parathyroid gland, but transformed from the chief cells. WCCs are more likely to be seen with hyperparathyroidism, Roth reports (5).

One study suggested that all parathyroid parenchymal cells, including principal cells, oxyphilic cells and WCCs are variants of a single cell type (6). Most of the WCCA cases present with hyperparathyroidism (6).

Biochemical and clinical features were compared with the diameter of the adenoma (A) in a study (7). They suggested that the calcium level of WCCA cases did not rise until the A reached a significant size, and therefore had low endocrine activity (7).

In another study, an A with a diameter of 6×2.5×2.cm was presented, but preoperative Ca and PTH values were not available in this study (8). When WCCA was searched on

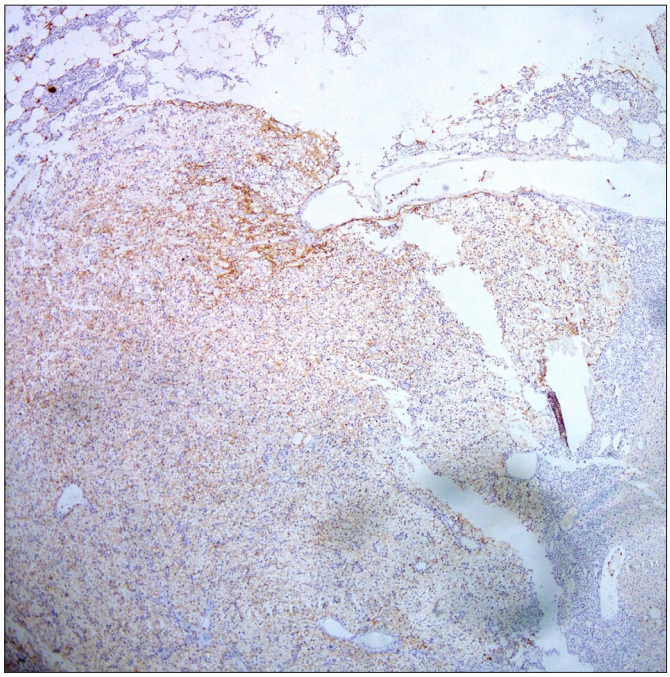

Figure 2. Clear cells were positive, other areas were negative with CD 10 (CD10 X100).

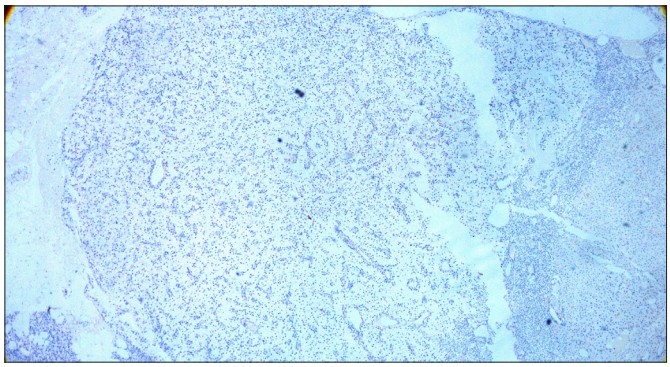

Figure 4. Synaptophysin was negative in the nodular area with clear cells (internal control staining available) (Synaptophysin X40).

the favorite search engine, 35 titles were seen. About 20 cases were reported as As.

PA should be considered in this age group due to its prevalence, especially postmenopausal (8).

The etiology of most PA is unknown. A small proportion of cases may be associated with hereditary multiple endocrine neoplasia. External radiation exposure of the head and neck region during childhood and long-term lithium use also in the etiology of PA is thought to take place (1,8).

PA consists of cells but may have variants. These; oncocytic (oxyphilic variant) A, parathyroid lipoadenoma (hamartoma), clear cell A, atypical PA. In the differential diagnosis of parathyroid adenoma, it should be considered in cases of hyperplasia, PC and metastatic events as well as variants.

PC is a malignant neoplasia that develops from parathyroid parenchymal cells (1,8). These tumors are extremely rare and account for less than 1% of primary HPT cases. And the age of diagnosis is reported to be 56, and it is common in men and women (1). Neighboring tissue invasion, vascular invasion (capsular and/or extracapsular), perineural invasion and/or proven metastasis constitute the criteria for malignancy and are diagnostic for malignancy other than at least one of these criteria (1,9).

PH is seen at a rate of ~ 10-15 %. Growth in all glands may be asymmetrical. In total of all glands are 1-3 grams. Normalization of enlarged glands the gland structure is not visible. There may be diffuse, nodular, or diffuse/nodular patterns. Major cell hyperplasia sometimes can be clear cell hyperplasia. The oil is not visible or reduced. There may be mitotic activity. They can be clonal.

As in our case, more pronounced water clear cells, more analysis and more distinct, nodular from larger network can be found in differential diagnosis of WCCA. Paraganglioma, metastatic renal cell carcinoma, clear cell sarcoma, rare clear cell type tumors, germ cell thymic carcinoma tumors (seminoma and dysgerminoma) and parathyroid carcinoma should also be considered (1,8).

With the exception of paraganglioma zellballen structures and rosettes, differential diagnosis can be made. Histological features and immunohistochemical studies are important in metastatic renal cell carcinoma (1,8).

Nuclear pleomorphism and nucleoli may be useful in differential diagnosis in clear cell sarcoma. For thymic carcinoma, the spindle of the cells and the presence of epithelioid cells are important. Lymphocytes on polygonal cells and septa are an important finding for seminoma (residual) (1,8).

In terms of parathyroid carcinoma, a decrease in expression of parafibromine, Bcl-2a, retinoblastoma (Rb) and p27 PGP9.5, as well as vascular and distant organ metastasis may help (1,8).

In this case, PTH was slightly positive and synaptophysin was negative. Cellular atypia and invasion were not present.

Histological appearance with the immune profile, widespread positivity for GATA3, focal weak positivity for parathyroid hormone and negativity for PAX8, thyroglobulin, TTF1, synaptophysin, chromogranin, and S100 were also present in the Flea and Arc study (10). GATA-3 was not studied in this study (since it is not in our laboratory).

In an article that caused a similar confusion in the literature, RCC marker positivity was detected in intrathyroidal parathyroid carcinoma, but the differential diagnosis was made with extrarenal positivity for RCC marker and other histopathological, clinical and immunohistochemical findings (11).

In case, no clinical finding suggestive of a kidney tumor was found. In terms of RCC, other markers such as vimentin, CAIX, SDH, PAX8 were not needed.

We could not find any literature on CD10 positive PA in search engines. For this reason, we considered as abnormal staining.

We did not perform any additional immunohistochemical studies since we found sufficient histopathological findings, postoperative biochemical and clinical follow-up results and immunohistochemical PTH positivity.

CONCLUSION

Parathyroid adenomas are important in terms of both clinical and differential diagnosis. In this differentiation, metastatic conditions should not be forgotten and should be excluded. The shortest way to do this is to evaluate the results of all relevant sections together.

ETHICAL DECLARATIONS

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Case Report

HEALTH SCIENCES
MEDICINE

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A neonatal case of pial arteriovenous fistulas and the review of literature

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ABSTRACT

Pial arteriovenous fistulas (PAVFs) are extremely rare vascular malformations of the brain, and associated with greater morbidity and mortality in neonates and infants. We reported a neonatal case of giant multi-hole PAVFs presented with heart failure and local brain atrophy. The challenges associated with the complexity, outcome and therapeutic options of neonatal PAVFs were discussed. In addition, a review of the published literature on cases of PAVFs diagnosed at neonatal age, over the last two decades, was performed. Comprehensive prognostic evaluation and an optimal treatment strategy offered by a multidisciplinary team at specialized centers should be assured for each case affected.

Keywords: Pial arteriovenous fistula, cerebral arteriovenous malformation, treatment, outcome, neonate

INTRODUCTION

Pial arteriovenous fistulas (PAVFs) are extremely rare vascular lesions of the brain. They are comprised of single or multiple arterial feeders draining directly into the venous channel without intervening tangle of blood vessels (1-3). It has been estimated that they make up approximately 1.6% of all intracranial vascular malformations in the general population, and up to 7.3% in the pediatric population (1-4). The morbidity and mortality associated with this anomaly seem higher in the neonatal period due to more complicated vasculature and age-related features (2-7). Herein, a neonatal case of giant multi-hole PAVFs presented with heart failure and local brain atrophy is discussed. The informed consent was obtained from the parents to publish this case.

CASE REPORT

A 10-day-old male infant was referred to the neonatal intensive care unit for cardiomegaly. He was the first child of a non-consanguineous marriage, born vaginally at 38 weeks of gestation. Despite irregular antenatal visits, the antenatal course was uncomplicated, and there was no history of skin lesions or vascular malformation in the family. On the fifth day after birth, he was admitted to a hospital where he received intravenous fluids because of poor feeding and dehydration. There the results of baseline laboratory tests were unremarkable, but chest radiograph showed increased the heart size, and therefore he was transitioned for further evaluation. On admission, he had decreased responsiveness with poor feeding, respiratory rate of 68 breaths per minute without retractions, heart rate of 184 beats per minute and caput succedaneum. On auscultation, a continuous murmur had been heard over his anterior fontanelle. The rest general examination, as well as routine laboratory analyses were unremarkable. During hospitalization, left-sided focal seizures were detected, and taken under control with anticonvulsant. Echocardiography revealed that the heart was structurally normal but grossly enlarged with moderately affected function. Transfontanelle ultrasound showed a significantly increased blood flow within the right hemisphere, indicating a brain magnetic resonance angiography and venography imagination. Brain T2-weighted magnetic resonance image (MRI) showed a giant PAVF in the right hemisphere with parenchymal destruction (**Figure**). The patient was consulted to the experts from pediatric neurosurgery and pediatric neurology. The parents were thoroughly informed about the complications of the interventions and poor prognosis, and then offered them to refer the patient to a specialized center which unfortunately only very few are available in the country. Because of socioeconomic reasons and apparently poor

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outcome, they refused further interventions at that moment. After two weeks of the discharge with medical treatment for cardiac failure and seizures, the patient suddenly deteriorated and died.

DISCUSSION

In the present case report, it was aimed to emphasize the challenges associated with the complexity, outcome and therapeutic options of PAVFs, along with literature review of neonatal cases. No more than 200 cases of PAVFs have been reported in the literature. Because of the rarity and that most authors have reported their own experiences, the exact incidence of PAVFs is not known (1). Depending on the angio-architecture of PAVFs, which varied among different ages, the presentation, treatment and outcome may be variable. A literature review of neonatal PAVF from 2005 to 2020 are summarized on **Table** (2-14).

It has been thought that most pediatric PAVFs develop antenatally, but prenatal diagnosis is rare. The cases detected antenatally are more prone to be symptomatic, either during the antenatal period or just after birth (2,3,5). Abnormal angiogenesis seems to be the underlying pathophysiology in the formation of PAVF. It has been postulated that during early stage of vascular development, fistulous connection of the dilated capillary networks randomly persisted, and progression to the structured and more mature capillary network is disrupted (1,4,15). This theory is supported by the evidence of molecular signaling pathways defects in some vascular syndromes including Klippel-Trenaunay-Weber syndrome, capillary malformation-AVM and hereditary hemorrhagic telangiectasia, which are frequently associated with PAVFs (4,15). However, clinical features may not be apparent in infants, because the degree of the phenotypic expression in these syndromes increases with age (2,4,15).

The PAVFs occur anywhere in the brain but frequently above the tentorium, showing multiplicity, particular in children (2,3). PAVF consists of a single or multiple arterial feeders in direct connection to a single venous drainage without an intervening tangle of vessels (1-4). The high pressure blood flow from arterial feeder directly into the venous drainage may result in venous varix formation, which detected more frequently in pediatric patients (1). The absence of capillary blood flow causes PAVFs to have a limited functional role in vascular feeding of the cerebral parenchyma. Therefore, these lesions may affect normal brain development, called as melting brain syndrome, and cause cognitive impairment due to low perfusion in the normal brain tissue and intracranial venous congestion (2,4). Although it usually occurs bilaterally and symmetrically, brain atrophy around the lesion, as seen in the present case, can be present as a focal expression of the same phenomenon. The PAVFs with multiple arterial feeders are observed more often in infants, having high-flow arteriovenous shunting that is more likely to lead to congestive heart failure (1-4). They may also be presented with focal neurological symptoms such as a seizure or a hemorrhage (2,3). In older children, hydrocephalus, developmental delay, focal neurologic deficits, headaches, seizures, and cerebral hemorrhage become common manifestations (1,3,4).

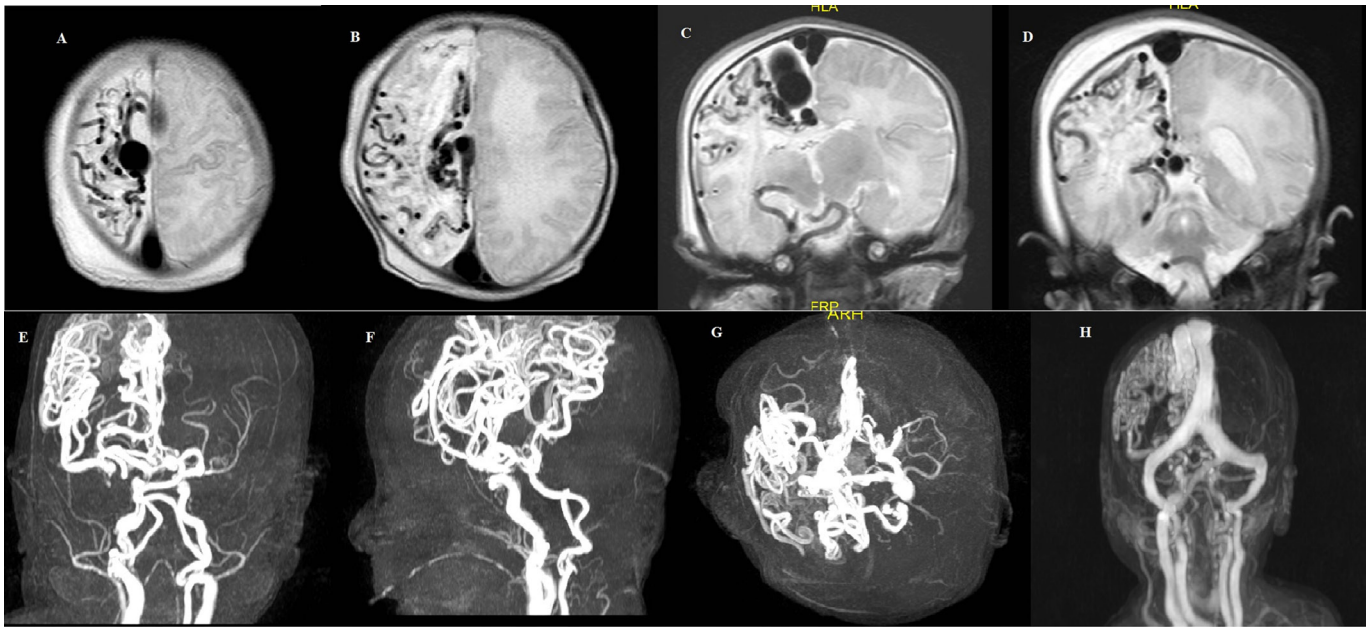

Figure. Axial **(A,B)** and coronal **(C,D)** T2-weighted magnetic resonance images show multiple tortuous signal void lesions on the right cerebral hemisphere, venous pouch and change of the signal intensity of gray matter and atrophy around the lesion. Cranial magnetic resonance angiography demonstrates a complex structure of multiple fistulas supplied by the branches of the right middle and posterior cerebral arteries communicating directly to an enlarged venous pouch beside the superior sagittal sinus **(E-H)**.

Table. Literature review of published cases of intracranial pial AVF diagnosed at neonatal age

Author, Year	No of case	Prenatal diagnosis	Clinical presentation	Treatment	Outcome†
Garel, 2005	3	Yes (2/3)	Cardiac failure (2/3) Asymptomatic (1/3)	Endo (3/3)	Death (1/3) Left hemiparesis (1/3) Normal (1/3)
Weon, 2005	21	Yes (4/21)‡	Cardiac failure (15/21) Seizures (3/21) Macrocrania (2/21) Asymptomatic (1/21)	Endo (17/21) Untreated (4/21)§ Radiotherapy (1/21)	Death (4/21) Normal(14/21) Moderate or severe NS (3/21)
Köroglu, 2006	1	Yes	Cardiac failure	Endo	Neurologically delayed
Lizuka, 2011	1	No	Tachypnea	Endo	Normal
Mascarenhas, 2012	1	No	Cardiac failure	Endo	Moderate psychomotor delay, left hemiparesis
Paramasivam, 2012	6	Yes (2/6)	Cardiac failure (4/6) Intracerebral hemorrhage (1/6) Asymptomatic (1/6)	Endo	Normal (5/6) Death (1/6)
Hetts, 2012	8	Yes (2/8)	Cardiac failure (7/8) Hydrocephalus (1/8)	Endo (5/8) Endo + surgery (3/8)	Normal (2/8) Death (2/8) Severe or moderate NS (4/8)
Kim, 2015	2	No	Intracranial hemorrhage (2/2)	Endo	Developmental delay (2/2) Left hemiparesis (1/2)
Komiyama, 2016	3	Not specified	Cardiac failure (3/3)	Endo	Normal (1/3) Vegetative state (1/3)£ Death (1/3)£
Ago, 2017	1	No	Tachypnea	Surgery	Good
Pedicelli, 2017	1	Yes	Asymptomatic	Endo + Surgery	Mild development delay
Maejima, 2018	1	Yes	Cardiac failure	Endo	Death £
Terada, 2018	12	Not specified	Hidrovenous disorder (8/12) Hemorrhage (7/12) Cardiac failure (6/12) Seizures (3/12)	Endo (10/12) Surgery (4/12) Conservative (1/12)	Favorable (5/12) Unfavorable (7/12)

† The definition of outcome may differ as per authors.
‡ Two of them were diagnosed as vein of Galen aneurysmal malformation in utero. At birth three of them had heart failure; one was asymptomatic.
§ In 3 neonates, embolization was believed to be contra-indicated because of already existing brain damage. Two of them died, and the other one had severe disability. The last one of untreated cases was lost at follow-up.
£ Despite existing brain damage at birth, they had been treated at the request of parents.
Endo, endovascular embolization; NS, neurological symptoms/signs

In the suspected cases, MRI is the preferred imaging to demonstrate the anatomical location, possible feeders, presence of venous varix, and to show evidence of regional or diffuse cerebral injury (4). Digital subtraction angiogram is the gold standard for the diagnosis, and useful in identifying the angio-architecture of the fistula and in treatment planning (1,4). Unfortunately, in the case presented here, digital subtraction angiogram could not be performed, so the anatomic and hemodynamic characteristics of the lesion were not detailed.

Indication and treatment strategy should be decided based on assessment of neurological, cardiac and other systemic manifestations and imaging studies of the lesion and the brain parenchyma. Because of the high risk of mortality and poor neurocognitive prognosis and that spontaneous obliteration of the fistula is extremely rare, curative treatment is the occlusion of the arteriovenous shunt by surgical, endovascular route or a combination of these modalities (1). Despite the presence of various factors influencing the choice of treatment strategies, endovascular approach is more preferable due to the less invasive nature of the procedure, the improvements in modern endovascular techniques and technologies, and increased endovascular experience (2-4,9). N-butyl-2-cyanoacrylate-assisted (NBCA) and Onyx (ethylene vinyl alcohol copolymer) are the most commonly used materials during endovascular embolization (1-4). Each material has advantages and disadvantages (1). Endovascular treatment may be more challenging in featured conditions such as the presence of multiple feeders draining into the fistula or high flow feeder, and in small infants having heart failure and fragilities of the cerebrovascular system including difficulty of catheter access (2,6). The presence of these conditions may necessitate a modification of the technique or perhaps a complementary surgical intervention for successful obliteration (2,4). Surgical treatment is only reserved for a few cases in whom embolization is inconclusive, complicated with intraoperative intracranial hemorrhage or assumed too dangerous (2,4). Small total blood volumes and coexistence of congestive heart failure make the surgery more risky in neonates (2).

The fact that complex fistulas with multiple arteriovenous connections are more common in small infants makes these lesion more likely to require several embolization sessions (2). Because only a limited the volume of contrast

medium can be used in infants, it has been suggested that the aim here is to temporize congestive heart failure until after the first several months of life at which definitive treatment is more manageable (2). Furthermore, in case of a neonate who diagnosed with a PAFV prenatally or postnatally, even if being asymptomatic, an intervention can be considered to reduce shunting through the fistula because of its adverse effects on a developing brain (2,3,6).

Clinical outcome seems to mainly depend on the complexity of the lesion and the age at diagnosis. The patients under 2 years of age have higher rates of mortality and procedural complications as well as poor neurologic and developmental outcomes (2). On follow-up after treatment, the patient should be monitored with MRI and if it is necessary, with cerebral angiogram, since recanalization, reactive angiogenesis with shunt, hydrocephalus and dural AVF may develop (2,4). The natural history of brain damage, especially in prenatal cases, is extremely poor because they are irreversible, despite curative treatment. Therefore, existing brain damage is considered a contraindication to aggressive treatment (3,6,8). As seen in the present case, when existing the findings of melting brain syndrome on the imagine studies, the parents should be carefully consulted regarding either high-risk procedures or poor prognosis. Nonetheless, therapeutic abstention might not be always possible if the parents requested to treat their child, despite eventually an unfavorable outcome (6,8).

CONCLUSION

PAVFs are extremely rare vascular lesions, however, the rate of neonatal cases among all published ones seems remarkable. Neonatal PAVFs are likely to be more complicated, and usually presented with cardiac failure and more severe cerebral complications. Therefore, the treatment, either endovascular or surgical, is more challenging and associated with higher morbidity and mortality in neonates. Comprehensive prognostic evaluation and an optimal treatment strategy should be provided by a multidisciplinary team at specialized centers.

ETHICAL DECLARATIONS

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Letter to the Editor

HEALTH SCIENCES
MEDICINE

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Flaxseed: a promising alternative for polycystic ovarian syndrome therapy

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Dear Editor,

Polycystic ovarian syndrome (PCOS) is a syndrome of ovarian dysfunction associated with menstrual irregularities, hirsutism, obesity, insulin resistance (IR) and hyperinsulinemia. (1) It affects 6-15% of the female population around the globe (2).

Obesity and IR play a pivotal role in the pathogenesis of PCOS. Obesity causes low-grade inflammation by the production of inflammatory mediators like cytokines, adipokines, and high C-reactive protein (CRP) levels (2). Patients with PCOS are also susceptible to oxidative stress with decreased serum antioxidant levels (2).

For PCOS, lifestyle modification is important which includes weight loss and exercise. A 5% reduction in weight results in regularity in menstruation and ovulation (2). With the increasing incidence of PCOS, the use of herbal medicines has expanded and have shown promising results in PCOS management which include herbs like Linum usittatissimum (Flaxseed) (4). Flaxseeds(linseeds) is food and fiber that are high in omega 6, 3 fatty acids (alpha linolenic acid, ALA) which have shown benefits in insulin concentration, inflammation as flaxseeds contain Secoisolariciresinol Diglucoside (SDG), which has an anti-inflammatory effect on the human body (5), and oxidative stress by decreasing lipid peroxidation through its antioxidant activity (6). Previous studies (3) show that omega 3 fatty acids supplemented from flaxseed oil when given for 12 weeks improve hyperinsulinemia by reducing the activation of the nuclear factor-kappaB (NF-kB) transcription factor (7) and suppressing pro-inflammatory mediators, very low density lipoprotein (VLDL)- cholesterol levels and have also shown a reduction in triglyceride levels by increasing lipoprotein lipase activity. All these changes led to an improvement in the manifestations of PCOS. This was also observed in a 2020 randomized controlled trial in which the results showed an improvement in metabolic syndrome and as it is associated with PCOS, flax seeds can be used to treat it (8).

Clinical trials indicate that dietary supplementation with flaxseed in PCOS patients can reduce BMI and weight but had no effect on other anthropometric indices (9). Along with that, a clinical trial demonstrated that flaxseed supplementation shows no side effects, thus, encouraging its use in the management of PCOS (7).

According to a clinical trial, the flaxseed supplementation also leads to decreased leptin levels which is important for the management of obesity (5). Several studies also reported the effect of flaxseed on ovarian morphology and showed a significant reduction in ovarian volume and number of follicles, as well as regularity in menstrual cycles (4).

It is tempting to hypothesize that flaxseeds can decrease the incidence of PCOS by alleviating hypercholesterolemia, hirsutism, insulin resistance, and obesity and the use of flaxseed oil may lead to a reduction in the low-grade inflammation, however, there is confusing literature as some of the studies encourage the use of flaxseed while some of them recommend the use of flaxseed oil to manage PCOS.

Therefore, there is a dire need to design further studies to understand the exact mechanism of action of flaxseeds and their oil in the alleviation of PCOS. Considering the cost-effectiveness, easy availability, minimal side effects, and health benefits, an evidence based purposeful management plan should be developed with flaxseeds and its oil as a part of the medicinal regime in the future..

Keywords: Polycystic ovarian syndrome, flaxseed, flaxseed oil, hyperinsulinemia, oxidative stress.

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Abbreviations: Polycystic ovarian syndrome: PCOS, Insulin resistance: IR, Alpha linolenic acid: ALA, Diabetes Mellitus: DM, Nuclear factor-kappaB: NF-kB, Very low density lipoprotein: VLDL, Secoisolariciresinol Diglucoside: SDG

ETHICAL DECLARATIONS

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PUBLICATION RULES, PUBLICATION POLICY, GENERAL PRINCIPLES AND SUBMISSION RULES

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