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Our dear readers,

We are proud to publish the first issue of 2023 with valuable new articles. Now, our journal has completed 5 years since its establishment and we are taking firm steps towards the goals we have set. As we have mentioned before, we are increasingly contributing to the international literature day by day.

We are constantly working to raise our scientific bar and to increase the success of our journal by entering valuable international indexes such as SCIExp and Pubmed. We would like to thank all the authors who contributed to the strengthening of our journal by sending articles from both domestic and abroad.

Sincerely yours

Assoc. Prof. Alpaslan TANOGLU, MD, PhD
Editor-in-Chief

CONTENTS

Original Article

CONTENTS

Original Article

CONTENTS

Original Article

Review

Case Report

Is there any effect of long term alpha-adrenergic blocker and a single dose antibiotic usage in reducing febrile urinary tract infections after prostate biopsy?

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ABSTRACT

Aim: To investigate whether the using long term alpha-adrenergic blockers before biopsy has an effect on preventing febrile urinary tract infections (FUI) secondary to biopsy in patients undergoing prostate biopsy due to elevated prostate specific antigen (PSA) .

Material and Method: The data of 2558 patients who underwent transrectal ultrasonography-guided prostate biopsy (TRUS+BX) due to elevated PSA in our clinic between January 2008 and July 2021 were analyzed retrospectively. The patients were divided into two groups as those who used alpha-blockers for a minimum of three months or longer before biopsy (Group 1) and those who did not use alpha-blockers before applying to the outpatient clinic (Group 2) . Demographic data of the groups and post-biopsy FUI development rates were compared.

Results: It was observed that 1340 (52.4%) of the patients were using alpha-blockers (Group 1) and 1218 (47.6%) did not (Group 2) . The median age of the patients in the pre-biopsy groups was similar [Group 1=68 (IQR=9) years and Group 2=68 (IQR=9) years, p=0.887]. There was no significant difference between the groups in terms of median prostate volume [Group1=57 (31) ml and Group 2=58 (34) ml, p=0.199]. The median PSA value was found to be significantly higher in Group 1 than in Group 2 [10.50 (5.40) ng/dl vs 10.35 (6.80) ng/dl, p=0.026]. Postvoid residual urine volume (PVR) was found to be significantly higher in Group 2 [Group 1=40 (30) ml and Group2=60 (90) ml, p<0.001]. Similarly, the frequency of FUI development after biopsy was found to be significantly higher in Group 2 [Group 1=17 (1.3%) and Group 2=65 (5.3%) , p<0.001].

Conclusion: The use of alpha-adrenergic blockers for a minimum of three months or longer before prostate biopsy significantly reduces the incidence of FUI that may develop secondary to biopsy.

Keywords: Urinary tract infection, prostate, core needle biopsy

INTRODUCTION

Prostate cancer is the most common type of cancer in elderly men and ranks sixth among the causes of death from cancer (1). Today, although the development of radiological imaging methods helps to diagnose prostate cancer, the definitive diagnosis is made with transrectal ultrasound-guided prostate biopsy (TRUS+BX) (2). Although prostate biopsy is also taken transperineally in some centers, the transrectal route is still routinely used (3). Prostate biopsy is an invasive procedure and causes mini-perforation and injury in the rectal mucosa and surrounding tissues. Therefore, minor complications such as hematuria, dysuria, rectal bleeding and hematospermia are frequently observed after a biopsy (4). However, FUI that can develop after TRUS+BX is a severe complication

and may result in sepsis (5). For this reason, antibiotic prophylaxis is routinely applied before biopsy in many centers (6). It has been shown in many studies that antibiotic prophylaxis performed before TRUS+BX significantly reduces FUIs that may develop after biopsy (7). While a significant portion of the patients diagnosed with prostate cancer consist of patients using alpha-blocker medication due to lower urinary tract symptoms (LUTS), some of them are patients who have not started medical treatment yet and who have had a biopsy due to primarily elevated PSA levels. Numerous studies have shown that alpha-blocker drugs relax prostatic smooth muscle fibers, increase voiding rate (Q-max), decrease postvoid residual urine volume (PVR), and

Sarıkaya et al. Effect of alpha-blockers on post-biopsy infection

J Health Sci Med 2023; 6(1): 1-6

provide significant improvement in LUTS (8). It is also a known fact that alpha-blocker drugs make a significant contribution to the prevention of chronic prostatitis and lower urinary tract infections (9). There is not enough literature yet on whether alpha-blocker drugs have any effect on FUI developing after prostate biopsy. Therefore, in this study, we aimed to indicate whether the use of alpha-blockers for a minimum of three months or longer before TRUS+BX has an effect on preventing FUIs that may develop secondary to biopsy.

MATERIAL AND METHOD

The study was carried out with the permission of Health Sciences University Keçiören Training and Research Hospital Ethics Committee (Date: 14.09.2021, Decision No: 2012-KAEK-15/2370). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Data Collection

The data of 2558 patients who underwent TRUS+BX due to PSA elevation in our clinic between January 2008 and July 2021 were analyzed retrospectively. The patient record information in the biopsy registry of our clinic and the electronic data system of our hospital and the information in the hospitalization file were used to collect the data. The patients were divided into two groups as those who used alpha-blockers for a minimum of three months or longer (long-term) before the biopsy (Group 1) and those who did not use alpha-blockers before applying to the outpatient clinic (Group 2). Patients who could not obtain sufficient data on whether they were using alpha-blockers or who had irregular drug use were excluded from the study. The urinalysis and urine cultures of the patients who applied to the outpatient clinic and were found to have elevated PSA were examined for possible urinary tract infection. Antibiotic treatment+alpha blocker was initiated in accordance with the antibiogram sensitivity test in patients with bacterial growth in urine culture. Alpha-blocker was initiated in patients who did not have symptoms of urinary tract infection and did not have bacterial growth in urine culture. Patients with bacterial growth in their urine culture and who did not show a significant decrease in PSA level after antibiotic treatment were prepared for biopsy. On the other hand, a single dose of prophylactic antibiotic treatment was administered before biopsy in patients with elevated PSA levels and only alpha-blocker therapy. Except for PSA elevation, MRI was requested beforehand, and as a result, patients who underwent biopsy with suspicion of cancer were excluded from the study. To exclude confounding factors in our research, we also excluded patients with prostate volumes greater than 100 ml, which may cause elevated PSA. Additionally, the study did not include patients with upper urinary tract or bladder stones, urethral stenosis, previous transurethral prostate surgery, upper urinary tract or bladder tumors, and a neurogenic bladder history. Age, PSA level, prostate volume, Q-max and PVR value, pathological findings of TRUS-BX, presence of hypertension (HT) and diabetes mellitus (DM), presence of urethral catheterization and previous biopsy history of the patients in both groups were determined and recorded.

Biopsy Procedure

Two days before the biopsy, routine urine analyzes and urine cultures of all patients were performed and active urinary tract infection was ruled out. Anticoagulant drugs of the patients were discontinued one week before the biopsy if they were using. Before the biopsy procedure, the patients were informed in detail about the procedure, and patient consent forms were obtained and retained. Prophylactic single dose oral 500 mg ciprofloxacin or a single dose oral 1 gr 3rd generation cephalosporin prophylaxis was administered to all patients about 12 hours before the biopsy. Rectal swab was achieved by performing a rectal enema (fleet-enema) in all patients just before the biopsy. The patients were placed in the lateral decubitus position and all biopsies were performed using a 22 gauge biopsy needle under the guidance of transrectal ultrasound (Philips, Logiq C-2). The biopsy procedure was performed under periprostatic local anesthesia (lidocaine, prilocaine) in accordance with the recommendations of the European Association of Urology (EAU) guideline, and 12 core biopsies were taken (10).

Follow-up and Complication Management

After the biopsy, the patients were especially informed about AUE and followed closely. The patients were also informed about possible complications such as hematuria, dysuria, rectal bleeding, and hamatospermia, and these complications were followed up and treated as an outpatient without the need for hospitalization. Patients presenting with a high fever after biopsy were hospitalized and followed closely because of possible sepsis. Intravenouz catheterization was established for these patients, appropriate hydration was provided, and empirical intravenous ceftriaxone 2×1 and appropriate analgesic treatment was ordered. CBC, biochemical analyzes, urine analysis and urine cultures of patients hospitalized for FUI were studied and followed-up. The patients with bacterial growth in their urine culture were evaluated according to the antibiogram sensitivity results and their antibiotic treatments were rearranged. The groups were compared in terms of demographic data, minor complications after biopsy and incidence of FUI.

J Health Sci Med 2023; 6(1): 1-6

Sarıkaya et al. Effect of alpha-blockers on post-biopsy infection

Statistical Analysis

Data were analysed using SPSS 24.0 (IBM Corp., Armonk, NY, USA) software. The Kolmogorov-Smirnov test was used to assess the distribution of parameters. Median and interquartile range were used for describing data. Chi-Square Test was used for nominal data, while the Mann-Whitney U-test was used for nonparametric variables. Statistical significance was set at $p<0.05$.

RESULTS

It was determined that 1340 (52.4%) of the patients included in the study were using alpha-blockers (Group 1), while 1218 (47.6%) were not (Group 2). The median age of the patients was 68 (9) years, and the median prostate volume was 57 (33) ml. It was observed that 82 (3.2%) of the patients were hospitalized and treated for FUI that developed after biopsies (**Table 1**). While the median prostate volume was 57 (31) ml in Group 1, it was 58 (34) ml in Group 2 (p=0.199). While the median PSA value was 10.50 (5.40) ng/dl in Group 1, it was 10.35 (6.80) ng/dl in Group 2 (p=0.026). There was no significant difference between the groups in terms of median Q-max values [Group 1=13 (5)ml/sec and Group 2=13 (5) ml/sec ,p=0.833]. The median pre-biopsy PVR value was found to be significantly lower in Group 1 compared to Group 2 [40 (30) ml and 60 (90) ml, p<0.001] (**Table 2**).

Within the first month after biopsy, FUI was observed in 17 (1.3%) patients in Group 1, while it was developed in 65 (5.3%) patients in Group 2 (p<0.001). The number of bacterial growth in the urine cultures of patients who developed FUI was also found to be significantly higher in Group 2 than in Group 1 [43 (4%) vs 13 (1%), p<0.001]. There was no significant difference between Group 1 and Group 2 in terms of minor complications observed after biopsy [hematuria= 551 (41.1%) vs 498 (40.9%), rectal bleeding= 218 (16.3%) vs 194 (15.9%), hematospermia=260 (19.4% vs 238 (19.5), acute urinary retention (AUR)=196 (14.6) vs 175 (14.4), p=0.905, p=0.775, p=0.930 and p=0.895, respectively] (**Table 3**, **Graphic 1**). Post-biopsy PVR volume was found to be significantly lower in Group 1 than in Group 2 [35 (30) ml vs 65 (80) ml, p<0.001]. According to the pathological findings, prostate adenocarcinoma was detected in 398 (30%) patients in Group 1, while cancer findings were detected in 366 (30%) patients in Group 2 (p=0.863). In addition, chronic prostatitis was detected in 123 (8%)patients in Group 1, while it was found in 123 (10%) patients in Group 2 (p=0.036) (**Table 4**).

Table 1: Characteristics and complications of the patients	
Age, Median (IQR), years	68 (9)
PSA, Median (IQR), ng/dl	10.4 (5.8)
Prostate volume, Median (IQR), ml	57 (33)
Q-max, Median (IQR), ml/sec	13 (5)
PVR, Median (IQR), ml	40 (42.5)
Alpha- blocker, n (%)	
User	1340 (52.4)
Non-user	1218 (47.6)
Prophylaxy, n (%)	
FQ	1440 (56.3)
3rd Gen-Cephalosporine	1118 (43.7)
HT, n (%)	
(+)	1112 (43.5)
(-)	1446 (56.5)
DM, n (%)	
(+)	452 (17.7)
(-)	2106 (82.3)
Urethral catheter, n (%)	
(+)	108 (4.2)
(-)	2450 (95.8)
Secondary Biopsy, n (%)	
(+)	120 (4.6)
(-)	2438 (95.4)
FUI, n (%)	
(+)	82 (3.2)
(-)	2476 (96.8)
Urine culture growth, n (%)	
E. coli	19 (0.7)
ESBL (+) *E. coli*	14 (0.5)
Klebsiella	5 (0.2)
Enterobacter	7 (0.3)
Enterococcus	5 (0.2)
Pseudomonas	6 (0.2)
Hematuria, n (%)	
(+)	1049 (41)
(-)	1509 (59)
Rectal bleeding, n (%)	
(+)	413 (16.1)
(-)	2145 (83.9)
Hematospermia, n (%)	
(+)	498 (19.5)
(-)	2060 (80.5)
AUR, n (%)	
(+)	370 (14.5)
(-)	2188 (85.5)

IQR: Interquartile range, PSA:Prostate specific antigen, Q-max: Maximum urine flow, PVR: Post-void residual urine volume, FQ: Fluorokinolone, HT: Hypertension, DM: Diabetes mellitus, FUI: Febrile urinary tract infection, *E. coli*: Escherichia Coli, ESBL (+) *E. coli*: Extended-spectrum beta-lactamase producing Escherichia Coli, AUR: Acute urinary retention

Table 2. Characteristics and pre-biopsy findings of the groups

	Group 1 (n=1340)	Group 2 (n=1218)	p
Age, Median (IQR), years	68 (9)	68 (9)	0.887
PSA, Median (IQR), ng/dl	10.50 (5.40)	10.35 (6.80)	0.026
Prostate volume, Median (IQR), ml	57 (31)	58 (34)	0.199
Q-max, Median (IQR), ml/sec	13 (5)	13 (5)	0.833
PVR, Median (IQR), ml	40 (30)	60 (90)	<0.001
Prophylaxy, n (%)			0.895
FQ	756 (56.4)	685 (56.2)	
3 rd Gen-Cephalosporine	584 (43.6)	533 (43.8)	
HT, n (%)	582 (43.4)	530 (43.5)	0.967
DM, n (%)	239 (17.8)	214 (17.6)	0.899
Urethral catheter, n (%)	56 (4.2)	51 (4.2)	0.988
Secondary Biopsy, n (%)	60 (4.5)	58 (4.8)	0.725

IQR: Interquartile range, PSA:Prostate specific antigen, Q-max: Maximum urine flow, PVR: Post-void residual urine volume, FQ: Fluorokinolone, HT: Hypertension, DM: Diabetes mellitus

Table 3. Post-biopsy findings and complications of the groups

	Group1 (n=1340)	Group 2 (n=1218)	p
FUI, n (%)	17 (1.3)	65 (5.3)	<0.001
Urine culture growth, n (%)	13 (1)	43 (4)	<0,001
E. coli, n	4	15	
E. coli (ESBL+), n	4	10	
Klebsiella, n	1	4	
Enterobacter, n	3	4	
Enterococcus, n	0	5	
Pseudomonas, n	1	5	
Hematuria, n (%)	551 (41.1)	498 (40.9)	0.905
Rectal bleeding, n (%)	218 (16.3)	194 (15.9)	0.775
Hematospermia, n (%)	260 (19.4)	238 (19.5)	0.930
PVR, Median (IQR), ml	35 (30)	65 (80)	<0.001
AUR, n (%)	196 (14.6)	175 (14.4)	0.895

FUI: Febrile urinary tract infection, *E. coli*: Escherichia Coli, ESBL (+) *E. coli*: Extended-spectrum beta-lactamase producing Escherichia Coli, AUR: Acute urinary retention, PVR: Post-void residual urine volume.

Table 4. Pathological findings of the Groups

	Group 1 (n=1340)	Group 2 (n=1218)	p
BPH, n (%)	813 (61)	707 (58)	0.184
Chronic prostatitis, n (%)	103 (8)	123 (10)	0.036
HG-PIN, n (%)	14 (1)	12 (1)	1
ASAP, n (%)	12 (0.9)	10 (0.8)	1
Prostate adenocarcinoma, n (%)	398 (30)	366 (30)	0.863

BPH: Benign prostate hyperplasia, HG-PIN: High grade prostatic intraepithelial neoplasia, ASAP: Atypical Small Acinar proliferation
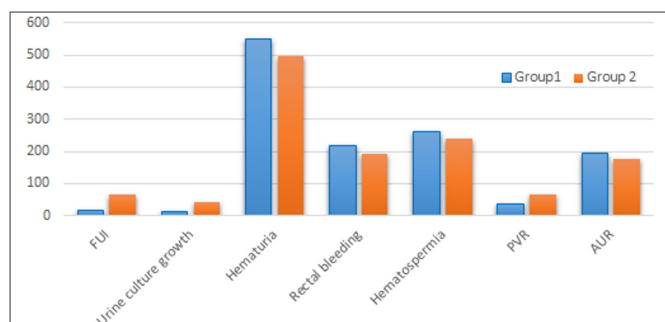
Graphic 1. Post-biopsy complications of the groups
FUI: Febrile urinary tract infection, PVR: Post-void residual urine volume, AUR: Acute urinary retention.

DISCUSSION

FUI is one of the most important complications observed after TRUS+BX procedure for the diagnosis of prostate cancer. If the infection is not intervened in a timely and correct manner, it can lead to serious life-threatening consequences that progress to septic shock (5). Mini-perforation in the rectal mucosa during biopsy and direct inoculation from there to the blood or urinary system, or inoculation of an abscess or infection focus in the prostate tissue are the main sources of infection (11). Studies have shown that up to 70% of patients develop bacteremia after biopsy (12,13). Therefore, rectal enema or rectal washing applications and antibiotic prophylaxis procedures have been established to prevent FUIs that may develop after biopsy. Hwang et al. (14) reported that FUI developed after biopsy in 16 (2%) patients in their retrospective study, in which they examined the results of 841 patients in whom they performed TRUS+BX. In this study, it was reported that rectal cleansing with povidone-iodine significantly reduced the rate of severe FUI compared to the group without rectal cleansing (0.3 vs 3.5%, p=0.001). In the study conducted by Kalkanlı et al. (15) in 400 patients, patients given a single dose of 750 mg oral ciprofloxacin before TRUS+BX and the patient groups given 500 mg oral ciprofloxacin for a total of 7 days after biopsy were compared . According to this study, it was reported that there was no significant difference between the groups in terms of infection that developed after biopsy in a total of 4 weeks of follow-up (single dose: 3% vs prolonged 3%, p>0.05). In another study conducted by Choi et al. (16) in a total of 1,995 patients, it was emphasized that FUI was seen in 39 (3.1%) patients after TRUS+BX and that quinolone resistance was the most important factor in developing infections (p=0.014), therefore the necessity of finding alternative prophylactic agents . In a retrospective study by Wu et al. (17) reported the results of 1,523 biopsy patients, fluoroquinolone (FQ) monotherapy, third-generation cephalosporin combined with FQ, and antibiotic groups started according to rectal swab culture results were compared in terms of the development of infection after biopsy. In this study, it was reported that the combined treatment provided a significant advantage in preventing post-biopsy infection from the FQ monotherapy group (1.0% vs 4.0%, p<0.001). In the same study, it was reported that there was no significant difference in the development of FUI between the antibiotic group started according to rectal swab culture and the FQ monotherapy group (p=0.349). According to the univariate analysis of this study, obesity was shown to be an important risk factor in terms of post-biopsy infection (OR=12.827, 95% CI:0.983-8.925, p=0.001). In our study, however, prophylactic combined antibiotic therapy was not used in any patient , and there was no difference in the frequency of prophylactic single

dose FQ or single dose 3rd generation cephalosporin use between the groups. It has been reported that the maximum effect of alpha-blockers occurs 4-6 weeks after the start of treatment (18). Under the guidance of this information, we included patients who used drugs for a minimum of 3 months or longer, assuming that the maximum effect of alpha-blocker drugs began in our study (Group 1). Our study observed that the use of alpha-adrenergic blockers for a minimum of three months or longer before the biopsy in addition to single-dose prophylaxis significantly decreased the frequency of FUI developing after biopsy.

Alpha-blockers constitute the most widely used first-line medical treatment option to reduce LUTS secondary to benign prostatic hypertrophy (BPH) (19). In addition to eliminating LUTS symptoms, alpha-blockers eliminate the need for surgical intervention in many patients with the significant increase in Q-max and a decrease in PVR (20). Alpha-1 adrenergic receptor blockers eliminate storage and obstructive symptoms by acting on both alpha-1a receptors in prostate tissue and alpha-1b receptors in the bladder (21). Masumori et al. (22) started tamsulosin 0.2 mg/day in a total of 112 patients with LUTS symptoms and reported 5-year follow-up results. According to their study, the mean international prostate symptom score (IPSS) of the patients decreased from 17.7±6.1 to 12.6±6.0 (p<0.001) after five years of follow-up, and the mean quality of life index (QOL) from 4.2±1.1 to 2.7±1.3, and It was reported that significant improvement was achieved (p<0.0001). Alpha-blockers have an important place in the literature in the treatment of non-bacterial or bacterial prostatitis as well as LUTS symptoms. Nickel et al. (23) conducted a study on 58 patients with chronic prostatitis/chronic pelvic pain syndrome and showed that the improvement in the group who received 0.4mg tamsulosin daily for 6 weeks was significantly higher than that of placebo (p=0.04). In another study, Barbalias et al. (24) showed the effectiveness of alpha-adrenergics in preventing recurrence in a 22-month follow-up of a patient with Category-2 chronic bacterial prostatitis, including 64 patients. According to this study, the recurrence rate was 41% in the antibiotic+alpha-blocker group, while it was 88% in the antibiotic-only group .

As stated in the studies highlighted above, many treatment and prophylaxis modalities have been described to prevent FUI after TRUS+BX. However, there is not yet sufficient literature data on alpha-blockers in this area. One of the most important factors of AUE developing after TRUS+BX is prostatic abscess and possible bacterial growth in this abscess (11). The fact that the frequency of FUI developing after biopsy was significantly lower in the group using long-term alpha-blockers in our study supports the idea that these drugs reduce the possibility

of infection by facilitating the drainage of bacterial abscess focus present in the prostate tissue. In addition, the fact that the presence of chronic prostatitis in Group 1 was significantly lower than Group 2 in the pathology findings of our study can be considered as another factor supporting this idea.

According to the EAU guideline information, a PVR value of more than 50 ml can be evaluated in favor of the presence of obstruction (25). Numerous studies have shown that alpha-blockers reduce PVR volume in patients with LUTS symptoms (26,27). Consistent with the literature data, pre-biopsy and post-biopsy PVR volumes were found to be significantly lower in the group using long-term alpha-adrenergic blockers in our study. High PVR volume is another important factor that predisposes the patient to lower urinary tract infection (28). The decrease in PVR volume by using long-term alpha-adrenergic blockers can be considered as another factor contributing to the prevention of FUI that may develop after biopsy. On the other hand, post-biopsy AUR rates were found to be similar between the groups in our study. In our study, although the post-biopsy PVR value was significantly higher in Group 2, we think that the antibiotic and alpha-blocker treatment that we started before the biopsy may be a preventive factor for AUR, and therefore, there was no significant difference between the groups in terms of the development of AUR in the post-biopsy period.

Limitations: The most important limitation of the study is its retrospective nature. Another limitation can be considered as the fact that the contribution to the post-biopsy FUI could not be evaluated due to the unknown duration of catheterization of patients with bladder catheters before the biopsy. The need for catheterization after biopsy and the effect of catheterization on the development of post-biopsy FUI could not be determined, can be considered as another limitation. However, we believe that the fact that the number of patients included in the study is quite high compared to the literature data will increase the contribution of our study to the scientific literature.

CONCLUSION

In our study, the frequency of FUI developing after TRUS+BX in patients using alpha-adrenergic blockers for a minimum of three months or longer before the biopsy was found to be significantly lower than the non-user group. Alpha adrenergic blockers recommended as first-line medical therapy to reduce LUTS symptoms. Long-term usage of these drugs could make a significant contribution to the reduction of possible FUI that may develop after TURS+BX for prostate cancer diagnosis.

Sarıkaya et al. Effect of alpha-blockers on post-biopsy infection

J Health Sci Med 2023; 6(1): 1-6

The fact that the alpha-adrenergic blockers relax the prostate smooth muscle and bladder neck and facilitate prostatic abscess drainage may explain their contribution in preventing infection. In addition, the usage of long-term alpha adrenergic blockers prevent possible urinary infection focus by reducing the PVR volume can be considered as another additional factor causing this result.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Health Sciences University Keçiören Training and Research Hospital Ethics Committee (Date: 14.09.2021, Decision No: 2012-KAEK-15/2370).

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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HEALTH SCIENCES MEDICINE

Effect of vaccine on prognosis and mortality in COVID-19

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ABSTRACT

Aim: The aim of our study is to reveal the factors affecting the clinical course of COVID-19 infection and mortality in immune and non-immune patients aged 65 and over.

Material and Method: The study was carried out by scanning the files of a total of 1,642 COVID-19 cases aged 65 and over. The 1337 cases included in the study were divided into two groups as follows: patients who were vaccinated with the 2nd dose of CoronaVac but became infected with COVID-19 after the 14-day period in which immunization should develop (Group I) and the others who were unvaccinated, or infected with COVID-19 after a single dose of vaccination or infected after receiving a 2nd dose of vaccination in 14 days (Group II). The groups were compared with each other about mortality and the factors affecting mortality.

Results: The length of intensive care unit (ICU) stay, and the total length of hospital stay were significantly longer in Group II than Group I (p<0.05). The need for mechanical ventilation (MV) and the length of MV were also significantly higher in Group II than Group I (p<0.05). All patients enrolled in the study had lung involvement. The percentage of Computed tomography (CT) involvement over 50% was statistically significant in Group II (p<0.05). The percentage of severe and critically severe patients and mortality were significantly high in Group II vs. Group I (p<0.05).

Conclusion: When we compared the vaccinated and unvaccinated groups of 65 years of age, we found that hospitalization in the ICU and the need for MV increased mortality, and the vaccine reduced the need for intensive care and MV.

Keywords: Vaccine, COVID-19, elder age

INTRODUCTION

The COVID-19 infection is a fatal disease that has become a serious health problem for the whole world in the last 1.5 years, for which no definitive treatment has been found yet, and early diagnosis and early isolation are the most important subjects (1). Considering the fact that the persons who pulled through the disease with mild symptoms or no symptoms at all are the secret porters in the spread of the viral genome, it is obvious that the immunization plays a crucial role in protection from the disease (2).

Since the beginning of the pandemic, protein subunit vaccines, viral vector vaccines, m-RNA vaccines, and DNA vaccines have been, and continue to be, researched and developed in numerous centers to ensure immunity to the COVID-19 infection (3).

One of these vaccines, and the first one coming to our country, the CoronaVac vaccine is an inactivated vaccine, demonstrated in the research made to be inducing the neutralizing antibodies specific to SARS-COV-2 in mice, rats, non-human primates, and macaques. The Phase 1 and Phase 2 clinical studies conducted on healthy individuals between the ages of 18 and 59 and over 60 have demonstrated that the CoronaVac has been tolerated well and not caused dose-related safety concerns. The Phase 2 studies have demonstrated that the neutralizing antibodies have developed on day 14 and later following the 2nd dose of vaccination applied in divided doses. After 3 mcg doses administered on day 0 and 28, the seroconversion ratio of neutralizing antibodies detected in patients above 65 years was 94% (4).

Following the Emergency Use Authorization given in our country on January 13, 2021, the CoronaVac vaccine was administered first to the healthcare personnel designated as the high-priority group, then to the elderly patients group in which the infection could be the most severe and fatal and has been continued gradually. It has been administered in two divided doses (5).

The disease may either have an asymptomatic course or manifest itself with the mild upper respiratory tract infection symptoms like diminished taste and smell perception, back pain, joint pain, fatigue, fever, or with pneumonia, sepsis, septic shock, multiple organ dysfunction syndrome (6). It has been demonstrated that the old age, the presence of comorbid systemic diseases like chronic obstructive pulmonary disease (COPD), hypertension, cardiac disease, and the presence of lung involvement are closely related to poor prognosis and mortality (7).

The purpose of our study is to reveal the effect of the vaccine on prognosis and mortality by comparing vaccinated with CoronaVac and not vaccinated or vaccinated but deemed non-immune, ≥65 years COVID-19 patients which we have been followed in the services and ICU (intensive care units) of our hospital.

MATERIAL AND METHOD

The study was carried out with the permission of Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date: 23.06.2021, Decision No: 2021/178-23.06.2021). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The research planned as a retrospective clinical study and approved by the Ministry and the local ethics committee was conducted by screening the files of totally 1,642 65-year-old and older COVID- 19 cases monitored and treated in the relevant services and ICU of our hospital due to COVID-19 infection between the dates of 1 March 2021 and 15 May 2021. 305 patients excluded from the study for improvability by positive PCR result, treatment refusal of patient, referral to another center and hospitalization during data collection. 1,337 patients' vaccination details included in the study were recorded. The cases were examined in 2 groups: The COVID-19 patients vaccinated with the 2nd dose of CoronaVac but infected with after the 14-day period were defined as Group I (immunized), and unvaccinated, or vaccinated with a single dose, or 2 dose vaccinated but infected in 14 days after second dose vaccination as Group II (non-immunized) (**Figure 1**).
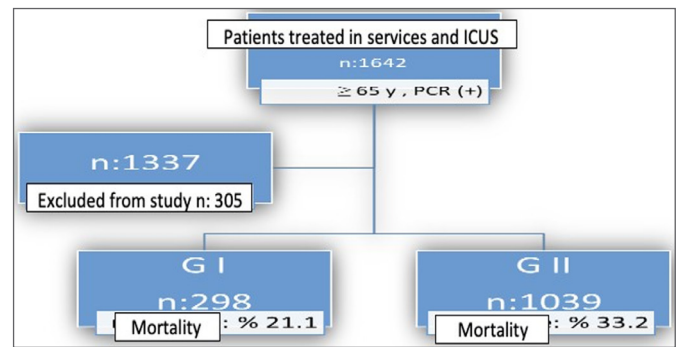
Figure 1. Study flow chart

In both groups' cases age, gender, comorbid diseases, lung involvement data according to pulmonary CT (Computed tomography) (its presence, being unilateral or bilateral, percentage) were recorded from patient's files. And also, for ICU patients, the number of transfers from the relevant service to ICU or direct ICU admissions, length of ICU stay, total length of hospital stay were noted. If ICU patients needed mechanical ventilation (MV), these patients' length of MV, and their exit methods were noted down. The mortality rate (the rate of the number of exitus cases to the number of inpatients for each group) was calculated for both groups.

All patients were categorized according to the disease severity scale against their clinical states and laboratory parameters. (Mild disease: symptomatic patients without radiographic findings; moderate disease: patients with fever, respiratory findings, and radiographically low lung involvement; severe disease: patients with dyspnea and respiratory distress, respiratory rate over 30, $PaO_2/FiO_2<300$, $SpO_2<90\%$ despite the oxygen therapy of 5 l/min; critical disease: respiratory failure, septic shock, and multiple organ dysfunction syndrome (8).

Our clinic has standard criteria for ICU admission: dyspnea and respiratory distress, respiratory rate over 30, $PaO_2/FiO_2<300$, $SpO_2<90\%$ or $PaO_2<70$ mmHg despite the oxygen therapy of 5 l/min, hypotension, development of acute organ failure, high lactate levels, arrhythmia, confusion, skin disorders such as capillary return disorder and cutis marmaratus, and immunosuppression.

Statistical Analysis

During evaluation of the results of the study, IBM SPSS Statistics 22 (IBM SPSS, Turkey) software was used for the statistical analyses. The Kolmogorov-Smirnov and Shapiro-Wilks tests were used to assess the compliance of the parameters with normal distribution; and it was found out that the distribution of the parameters was not normal. During evaluation of the study data, the Mann Whitney U test was used to compare quantitative data in addition to the descriptive statistical methods (Mean, Standard deviation, frequency). The Chi-Square test,

Fisher's Exact Chi-Square test, and the Continuity (Yates) correction were used to compare the qualitative data. The logistic analysis was used for the multivariate analysis. The significance level was p<0.05.

RESULTS

The study was conducted on 1337 cases in total; 631 males (47.2%) and 706 females (52.8%), between the ages of 65 and 99. The mean age was 75.0±7.99 years. Of the cases, 298 patients (22.3%) were immunized whereas 1,039 (77.7%) patients were non-immunized. The rate of male cases (p:0.001; p<0.05) and the mean age (p:0.001; p<0.05) were statistically significantly higher in Group I vs. Group II.

Table 1. The age- and gender-specific evaluation of groups

	Group I (n=298)	Group II (n=1039)	Total (n=1337)	p
Gender n (%)				
Male	168 (56.4%)	463 (44.6%)	631 (47.2%)	[1]0.001*
Female	130 (43.6%)	576 (55.4%)	706 (52.8%)	[2]0.001*
Age Mean±SD (median)	76.45±7.64 (76)	74.58±8.04 (73)	75.0±7.99 (74)	

[1]Chi-square test, [2]Mann Whitney U Test, *p<0.05 Group I: Immune Group II: Non-immunized

Transfer from service to ICU and direct ICU admission were significantly higher in Group II (p<0.05).

The length of ICU stays, and the total length of hospital stay were significantly longer in Group II vs. Group I (p<0.05).

The need for MV and the length of MV were also significantly higher in Group II (p<0.05) (**Table 2**). All patients enrolled in the study had lung involvement. It was lower than 50% in 61.7% of the cases in Group I, and higher than 50% in 62.4% of the cases in Group II. The percentage of CT involvement over 50% was statistically significant in Group II (p<0.05).

Table 2: Evaluations of groups

	Group I (n=298)	Group II (n=1039)	Total (n=1337)	p
Direct ICU admissionn (%)	81 (27.2%)	405 (39%)	486 (36.4%)	*[1]0.001
Transfer from Service to ICUn (%)	48 (16.1%)	298 (28.7%)	346 (25.9%)	*[1]0.001
Length of ICU stay Mean±SD (median)	2.29±4.96 (0)	3.94±8.11 (0)	3.58±7.56 (0)	*[2]0.001
Total length of hospital stay Mean±SD (median)	10.05±6.37 (9)	11.61±9.08 (9)	11.26±8.57 (9)	*[2]0.026
Need for MVn (%)	69 (23.2%)	335 (32.2%)	404 (30.2%)	*[1]0.003
Length of MV Mean±SD (median)	1.29±33.36 (0)	2.59±6.64 (0)	2.30±6.09 (0)	*[2]0.001

[1]Chi-square test, [2]Mann Whitney U Test, *p<0.05, ICU: Intensive care unit, MV: Mechanical ventilation

The rates of diabetes (DM), hypertension (HT), chronic renal failure (CRF), hyperlipidemia (HL), and cancer were higher in Group I (p<0.05). HT, DM, and coronary artery disease (CAD) were the top 3 comorbid diseases.

The rate (38.3%) of 3 and more comorbid systemic diseases in Group I was statistically significantly higher than Group II (30.9%) (p:0.017; p<0.05).

The rates of severe and critically severe patients and mortality were significantly higher in Group II vs. Group I (p<0.05) (**Table 3**).

Table 3: Evaluations of lung involvement and clinical data by groups

	Group I (n=298) n (%)	Group II (n=1039) n (%)	Total (n=1337) n (%)	p
CT finding				
Below 50%	184 (61.7%)	391 (37.6%)	575 (43%)	
Over 50%	114 (38.3%)	648 (62.4%)	762 (57%)	[1]0.001*
Comorbid diseases				
DM	141 (47.3%)	376 (36.2%)	517 (38.7%)	[1]0.001*
HT	206 (69.1%)	627 (60.3%)	833 (62.3%)	[1]0.006*
CAD	79 (26.5%)	294 (28.3%)	373 (27.9%)	[1]0.544
CRF	53 (17.8%)	96 (9.2%)	149 (11.1%)	[1]0.001*
COPD	44 (14.8%)	195 (18.8%)	239 (17.9%)	[1]0.112
HL	23 (7.7%)	40 (3.8%)	63 (4.7%)	[1]0.005*
CVD	17 (5.7%)	57 (5.5%)	74 (5.5%)	[2]0.999
Alzheimer	23 (7.7%)	56 (5.4%)	79 (5.9%)	[1]0.133
HF	33 (11.1%)	101 (9.7%)	134 (10%)	[1]0.493
Cancer	24 (8.1%)	48 (4.6%)	72 (5.4%)	[1]0.021*
Number of comorbid diseases				
Below 3	184 (61.7%)	718 (69.1%)	902 (67.5%)	
≥3	114 (38.3%)	321 (30.9%)	435 (32.5%)	[1]0.017*
Outcomes				
Discharged	235 (78.9%)	694 (66.8%)	929 (69.5%)	
Exitus	63 (21.1%)	345 (33.2%)	408 (30.5%)	[1]0.001*
Severity of disease				
Mild	96 (32.2%)	301 (29%)	397 (29.7%)	
Moderate	110 (36.9%)	244 (23.5%)	354 (26.5%)	
Severe	27 (9.1%)	152 (14.6%)	179 (13.4%)	[1]0.001*
Critical	65 (21.8%)	342 (32.9%)	407 (30.4%)	[1]0.001*

[1]Chi-square test, [2]Continuity (Yates) correction, *p<0.05, CT: Computed tomography, DM: Diabetes mellitus, HT: Hypertension, CAD: Coronary artery disease, CRF: Chronic renal failure, COPD: Chronic obstructive pulmonary disease, HL: Hyperlipidemia, CVD: Cerebrovascular disease, HF: Hearth failure

In consequence of an examination of the prognostic factors (need for MV, CT involvement, ICU admission, Pulmonary Embolism (PE)) we considered to have potential impacts on mortality; in Group I, the mortality rate was significantly high among the patients who needed MV, had over 50% CT finding, were admitted to ICU, and developed PE (p<0.05) (**Figure 2**). There was no statistically significant difference between the number of comorbid diseases and the mortality rates (p>0.05).
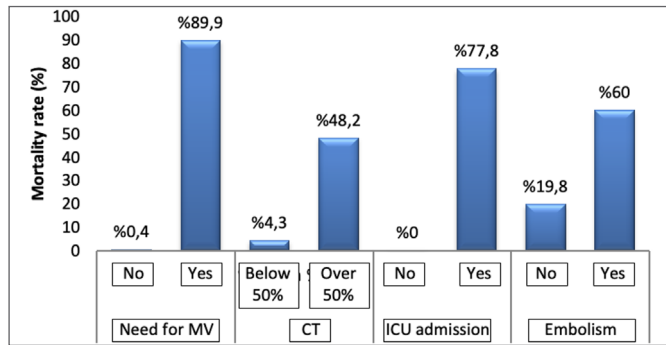
Figure 2. Evaluations of mortality in Group I

When we evaluated the effects of the parameters affecting mortality in the vaccinated cases significantly such as age, need for MV, CT finding, and ICU admission by Backward stepwise logistic regression analysis, we saw that the model was significant (p:0.001; p<0.05), the Nagelkerke R-square value was 0.897, and the exploratory factor of the model was good (97.3%). The effects of the parameters such as the need for MV and ICU admission on the model were found statistically important (p<0.05) (**Table 4**). It was seen that the need for MV increased the mortality rate by 93.25 times, and ICU admission by 30.274 times.

Table 4: Evaluation of parameters affecting mortality in vaccinated group significantly by logistic regression analysis

Vaccinated	OR	95% C.I.for OR		p
		Lower	Upper	
Step 3a				
Need for MV	93.25	14.253	610.09	0.000*
ICU admission	30.274	2.564	357.389	0.007*

aVariable(s) entered on step 1: Need for MV, CT finding, ICU admission, PE., MV: Mechanical ventilation, ICU: Intensive care unit CT: Computed tomography PE:Pulmonary emboli

In consequence of an examination of the prognostic factors (need for MV, CT involvement, ICU admission, PE) we considered to have potential impacts on mortality; in Group II, the mortality rate was high among the patients who needed MV, were admitted to ICU, and had over 50% CT involvement (p<0.05).

The mortality rate among the patients who needed MV (97%) was statistically significantly higher than the patients who did not MV (2.8%) (p:0.001; p<0.05). The mortality rate among the patients who were admitted to ICU (82%) was statistically significantly higher than the patients who were not admitted to ICU (2.1%) (p:0.001; p<0.05). There was no statistically significant difference between the mortality rates and the presence of PE (p>0.05).

The mortality rate among the patients who had minimum three comorbid diseases (38.6%) was statistically significantly higher than the patients who had less than three comorbid diseases (30.8%) (p:0.013; p<0.05).

Table 5: Evaluations of mortality in Group II

Without vaccination	Outcomes		p
	Discharged n (%)	Exitus n (%)	
Need for MV			¹0.001*
No	684 (97.2%)	20 (2.8%)	
Yes	10 (3%)	325 (97%)	
ICU admission			¹0.001*
No	621 (97.9%)	13 (2.1%)	
Yes	73 (18%)	332 (82%)	
PE			¹0.844
No	671 (66.9%)	332 (33.1%)	
Yes	23 (63.9%)	13 (36.1%)	
CT			²0.001*
Below 50%	338 (86.4%)	53 (13.6%)	
Over 50%	356 (54.9%)	292 (45.1%)	
Number of comorbid diseases			²0.013*
Below 3	497 (69.2%)	221 (30.8%)	
≥3	197 (61.4%)	124 (38.6%)	

¹Continuity (Yates) correction, ²Chi-square test, *p<0.05, MV: Mechanical ventilation, ICU: Intensive care unit CT: Computed tomography PE:Pulmonary emboli

DISCUSSION

Many studies have demonstrated that the old-age is a strong risk factor for the poor prognosis cases such as severe disease, hospitalization, ICU admission, and death in the course of COVID-19 infection (9, 10). The purpose of our study is to reveal the factors affecting the clinical course of the COVID-19 infection and mortality in the immunized and non-immunized 65-year-old and older patients. In consequence, we have found out that the need for intensive care and MV increases the mortality, that the vaccination reduces the need for intensive care and MV, and that the persons not vaccinated have more lung involvement and a more severe course of the disease.

Regardless of the factor (viral & bacterial & fungal…), bronchopneumonia is generally severe for elder patients. The changes in the lung parenchyma, decreasing compliance, chest wall deformities developing in old age increases the patient's respiratory workload during bronchopneumonia. In case of accompanying comorbidity especially, the functionality may decrease more and cause respiratory failure, and ICU monitoring may become necessary (11). A study conducted by Richardson et al. (12) had examined 5,700 inpatients monitored due to the COVID-19 infection; of these patients, 14.2% with a mean age of 68 had needed ICU treatment. The most frequently seen comorbidities in the hospitals were DM, HT, and obesity. In our study, the ICU admission rates, and ICU stay lengths of the immunized 65-year-old and older cases were statistically significantly lower than the non-immunized cases. Although HT, DM, CAD, CRF, and COPD were the most frequently observed comorbidities in all cases, the rate of presence of 3 and more systemic diseases in the immunized cases was

statistically significantly higher vs. the non-immunized cases; however, there was no significant difference between the number of comorbid diseases and the rate of mortality in the immunized group. We consider that good management of the comorbidities during infection, good evaluation of the interactions between the treatment applied and the medicines taken for a long time, and close monitoring of the clinical stability will mitigate the relevant risks.

CT is important in diagnosis, treatment, and prognosis establishment of the bronchopneumonia. According to the result of the literature review carried out by Ojha et al. (13) (with 45 studies) covering 4,410 patients, the most prevalent CT findings in the COVID-19 infection were the ground glass pattern and the mixed pattern that includes ground glass and consolidation simultaneously. The studies that have compared the CT imaging findings by age report that the rates of widespread multilobar involvement and pleural thickening are higher at the old age group. Xu et al. (14) examined the relation between the disease severity and the CT involvement in their study and found out that the rates of widespread multilobar involvement, widespread consolidation, atelectasis, and effusion were higher in the critically severe patients. When we compared the immunized and non-immunized groups in our study, we found out that the rate of over 50% involvement according to the CT examination was statistically significantly high in the non-immune group vs. the immune group even though the lung involvement was present in both groups. Consequently, we consider that the vaccine is effective in reducing the lung involvement.

In the patients with advanced pulmonary involvement caused by bronchopneumonia, the increasing need for oxygen can be met supplied by nasal cannula, high flow nasal oxygen (HFNO), non-invasive mechanical ventilation (NIMV), or mechanical ventilation. The studies conducted reveal that, the patients receiving MV are under higher risk in terms of mortality (15, 16). The study conducted by Grasselli et al. (15) on 1,591 intensive care patients (mean age: 63) has informed that 99% of the patients needed respiration support, 88% of whom needed MV support, and that the rate of mortality among the elder patients (age >64) monitored in the ICU was higher than the young patients. In a meta-analysis study conducted by Zheng Jie Lim et al. (16), the COVID-19 patients receiving invasive MV treatment were categorized in terms of case mortality rates by age group, and it was seen that the mortality increased in patients' group > 80 years old. In our study too, the need for and length of MV was statistically significantly higher among the non-immunized cases than the immunized cases; and the need for MV was increasing the mortality by 93%.

Mine Durusu et al. (17) conducted CoronaVac vaccine Phase III study on healthy adults ranging between 18 and 59 years of age, and it was shown at the end of that study that the rate of effectiveness of the vaccine was 83.5%, and that the vaccine prevented hospitalization by 100%. The study conducted by Jara et al. (18) on the other hand has reported the effectiveness of the vaccine as 65.9%. The subgroups were also examined in this study, and it was reported that the vaccine prevented the COVID-19 infection by 66%, hospitalization by 85.3%, ICU admission by 89.2%, and death by COVID-19 by 86.5% in patients' group > 60 years old. In our study, we found 21% mortality rate among the >65-year-old patients immunized with 2 doses of vaccination. This rate was significantly lower than the non-immunized Group II.

Emire Seyahi et al. (19) evaluated the Anti-spike IgG antibody levels in > 65-year-old healthy subjects and immunodeficient patients (who had negative COVID-19 anamnesis) 21 days after the 2nd dose of vaccination and found out that the antibody titers were lower in the > 60-year-old patients and immunodeficient patients' group. The Anti-spike IgG antibody study conducted on the hospital personnel immunized with CoronaVac by Aysen Bayram et al. (20) has demonstrated that the antibody levels were low in the subjects over 60 years of age and suffering from chronic diseases as well. Another vaccination study that compared the elder and young individuals for immunization is the study conducted about the Biontech vaccine, and this study found out too, that the number of neutralizing antibodies was lower in the elder individuals (21).

The immune system aging, which occurs with aging, the antibody deficiency arising from the decrease in the response, may explain the higher mortality despite vaccination among the elder people. In our study, we evaluated the factors affecting the mortality in the immune group by using the logistic regression analysis; we found out that need for MV and ICU admission increased the mortality. Therefore, we consider that the vaccination can not prevent mortality at the old age group by one hundred percent, and thus this patient group must be supported with additional measures and applications.

Limitations

During our study, only the coronavac vaccine was approved for use over the age of 65. Therefore, we could not evaluate the clinical efficacy of other vaccines in this age group. We think that comparative clinical studies showing the efficacy of different vaccines for 65 years and older will be useful.

CONCLUSION

In our study in which we examined retrospectively 1,337 patients (298 of which were immunized) above 65 years Covid-19 PCR (+) patients, we found out that the ICU admission and the need for MV raised the mortality rate. In addition, the vaccination reduced the need for intensive care and MV. Besides, the disease was more severe, and the mortality rate was higher among the patients who were not vaccinated, or who have not developed immunity despite vaccination.

Accordingly, we can say that the vaccination has positive effects on the course of the disease and reduces mortality among the 65-year-old and older Covid-19 patients. However, mortality can be seen despite vaccination due to cellular aging and immune system aging as well as organ dysfunctions caused by comorbidities. We consider that this age group requires more precautions and more studies on vaccination and medication.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date: 23.06.2021, Decision No: 2021/178-23.06.2021).

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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Effect of single serve sachet powder drinks on color stability of a nano-hybrid composite resin

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ABSTRACT

Aim: Nano-filled composite resin materials used for aesthetic purposes have better mechanical, polish ability, and color stability properties compared to other composite resin types. The aim of this in vitro study was to evaluate the effect of 5 different single serve sachet powder drinks popular among adolescents in recent years on the color change of Nano hybrid composite resin material.

Material and Method: In this study, Nano hybrid composite resin (Filtek Z550) and five different types of sachet drinks (cherry flavored powdered drink [Cherry-dp], instant sachet Turkish coffee [IS-Turkish-c], instant sachet filter coffee [IS-Filter-c], instant tea [I-tea], 3-in-1 granulated coffee [3-in-1 Granulated-c] and distilled water [DW]) were used. A total of 42 2×10 mm disc-shaped samples were prepared. The prepared samples were kept in DW in an oven at 37°C for 24 hours and the initial color measurements were made with a spectrophotometer (Vita Easy Shade Advance 4.0., Germany). The samples were divided into 6 groups (n=7) to be kept in 5 colorant solutions and DW (control group). Composite samples were kept in an oven at 37°C and the solutions were changed once a week. Color change was measured before and after 1, 7, and 28 days of immersion. Shapiro Wilk test was used to check whether the data conformed to normal distribution. The variables were analyzed with one-way analysis of variance ANOVA and post-hoc Tukey test ($p<0.05$).

Results: After 28 days of immersion, IS-Turkish-c, IS-Filter-c, and I-tea led to statistically significant discoloration in composite resin ($p<0.05$). In contrast, no statistically significant difference in discoloration was observed after 24 hours and 7 days of immersion ($p>0.05$).

Conclusion: The results obtained in the present study showed that the Nano hybrid composite material is sensitive to discoloration by long-term use of IS-Turkish-c, IS-Filter-c, and I-tea. It was thought that Nano hybrid composite should not be preferred especially in young people with high coffee and tea consumption or necessary warnings should be given if it is to be used.

Keywords: Nano hybrid composite resin, color change, instant sachets

INTRODUCTION

Due to the increasing demand for aesthetic dental fillings in recent years, composite resins have become the first choice for the restoration of anterior teeth in modern restorative dentistry (1). The increasing trend towards natural looks has led to the development of new restorative resins that closely mimic tooth structure, and modifications in fillers have enhanced the mechanical and aesthetic properties of conventional resins (2).

In dental restorative materials, Nano hybrid solutions have combined the strong mechanical properties of hybrid composites with the high polish ability properties of micro filled composites. Nano hybrid composites have been frequently used in anterior group direct restorations in recent years due to their advantages such as better mechanical and optical properties, better surface gloss, and high resistance to abrasion (3).

Despite the evolving characteristics of composite restorations, discoloration occurring in composite restorations, especially in the anterior region, is one of the main reasons for replacement of restorations (4,5). Discoloration in composite restorations is an aesthetic failure that leads to extra cost and time spent by the physician and the patient (6).

Akleyin et al. Color stability of a nanohybrid composite resin

J Health Sci Med 2023; 6(1): 13-17

Different internal and external factors play a role in discoloration of composite resins. Internal coloration may occur depending on the size and distribution of fillers, the composition of the resin matrix, and the photo initiators. External discoloration may occur due to insufficient polymerization, heat, UV irradiation, water absorption or absorption of colorants from food and beverages, surface roughness, and insufficient surface polish and finishes. ΔE is a standard measurement established by the Commission Internationale de l'Eclairage (International Illumination) that measures the difference in two colors visible in a product. ΔE is used to ensure that the displayed color is nearly identical to the color perceived by the human eye. The higher the ΔE value, the lower the color accuracy (7-9).

In recent years, the diversification of industrial consumption and the practicality of powdered sachet drinks have increased the use of these beverages in the daily diet. Therefore, the aim of this study was to determine the effect of instant sachets, especially those popular among adolescents in recent years, on the surface discoloration of Nano hybrid composites. The alternative hypothesis (H1) of this study was that the ΔE value of the groups other than the control group would be above the cut-off value.

MATERIAL AND METHOD

Ethics committee approval is not required as the study. All procedures were carried out in accordance with the ethical rules and the principles.

A Nano hybrid composite (Filtek Z550, A2) was used to check the color stability. The properties of this restorative material are presented in **Table 1**. Five different coloring solutions, including sour Cherry-dp, IS-Turkish-c, IS-Filter-c, I-tea, and 3-in-1 Granulated-c, were used as discolor ants. DW was used for the control group.

Table 1. Preparation of sachet drink and properties of the restorative material	
Sachet drink types and composite material	**Quantities and ingredients**
Cherry flavored powdered drink (Cherry-dp) (Nazo; Nazlı food and beverage exporter, İstanbul)	45 grams of powder/ 1 L water
Instant sachet Turkish coffee (IS-Turkish-c) (Shazel; Neon Co., Hatay)	200 mL powder/100 mL water
Instant sachet filter coffee (IS-Filter-c) (Shazel; Neon Co., Hatay)	200 mL powder/100 mL water
Instant tea (I-tea) (Lipton yellow label; Unilever, PLC, England)	200 mL powder/100 mL water
3-in-1 granulated coffee (3-in-1 Granulated-c) (Nescafe 3-in 1; Nestle Co., Sweden)	200 mL powder/100 mL water
Filtek Z550 (Nano hybrid composite resin) (Filtek, St Paul, MN, USA)	Bis-GMA, Bis-EMA, UDMA, PEGDMA, TEGDMA, zirconia silica, silica filler

Preparation of Samples

Teflon molds were used to prepare the samples. The molds were specially prepared with an inner diameter of 10 mm, an outer diameter of 12 mm, and a thickness of 2 mm. A total of 42 Nano hybrid composite materials were placed in molds and smoothed with a mouth spatula. Transparent tape and then glass coverslip were used to remove overflowing excess material. According to the manufacturer's instructions for each sample, composite resins were polymerized from both surfaces with an LED device (Valo Cordless, Ultradent, USA) for 20 seconds. The top and bottom surfaces of all samples were polished with aluminum oxide polishing discs (Sof-Lex TM, Filtek, USA).

Preparation of Solutions

All solutions were prepared according to the manufacturer's instructions. For the cherry flavored beverage solution, 45 grams of cherry powder was poured into 1 liter of water and mixed until dissolved. For teas and coffees, each sachet was poured into 200 ml of boiling water at 100°C (**Table 1**). The tea sachet was shaken gently at 0, 2 and 5 minutes and removed from the water after 5 minutes. While preparing the coffee solutions, the mixture was added to the water and mixed at 0 and 5 minutes.

Color Measurements

Composite discs prepared for color measurement were kept in DW in an incubator at 37°C for 24 hours in the absence of light. The samples were washed with DW and then dried. Initial color measurements were made with a spectrophotometer (Vita Easy Shade Advance 4.0. Germany) with the head of the instrument placed in the middle of the composite samples. Each color measurement was made on a gray background under standard conditions in the dark room with only the illumination of the Fluorescent Daylight Lamp (Master TL-D 90 Graphical 18W965SLV/10, Philips, The Netherlands). Color measurements were repeated three times for each sample and the spectrophotometer was calibrated before each measurement. After the first measurements, the samples were divided into 6 groups (n=7) (Cherry-dp, IS-Turkish-c, IS-Filter-c, I-tea, 3-in-1 Granulated-c, and DW). Prepared solutions were distributed to all groups (5 ml per group) and the samples were completely covered. Between measurements, all samples were kept in the solutions in an incubator at 37°C in the dark. The solutions were changed regularly once a week. Color measurements were repeated on days 1, 7 and 28. The samples were washed with DW for 5 minutes until all beverage residues were removed before each measurement and then dried completely with blotting paper. Each color measurement was made three times for each sample. The color change of the samples was calculated using the formula CIEDE2000 (ΔE00) (10).

J Health Sci Med 2023; 6(1): 13-17

Akleyin et al. Color stability of a nanohybrid composite resin

$$\Delta E_{00}^* = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C} \frac{\Delta H'}{k_H S_H}}$$

Equation 1: CIEDE2000

In the CIEDE2000 equation, ∆L', AC' and AH' are the difference in luminance, Chroma, and hue, respectively. RT refers to the interaction between color and hue differences in the blue region (∆R = RT (∆C'x ∆H'). SL, SC, and SH are weighting functions that adjust the total color difference in coordinates L*, a*, and b*. KL, KC, KH are correction terms for experimental conditions (11). In the present study, the CIEDE2000 parametric factors difference formula was set to 1. Ethics committee decision is not required as the study was performed on the discs we prepared. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analysis

The data were analyzed using the SPSS for Windows 25.0 program. Descriptive statistical methods (mean, standard, deviation) were used while evaluating the data. Conformity to normal distributed was evaluated with the Shapiro Wilk test. Data were analyzed with one-way analysis of variance (ANOVA) and post-hoc Tukey test. $p < 0.05$ was accepted as statistically significant in all analyses.

RESULTS

Statistical analysis of immersion results for 24 hours, 7 days, and 28 days is shown in **Table 2**. No significant difference was found between any of the composite samples evaluated at 24 hours and on day 7 in terms of color change results (p>0.05). In 28 days, IS-Turkish-c and I-tea caused significantly more coloration compared to Cherry-dp and DW, and IS-Filter-c caused significantly more coloration compared to DW (p<0.05).

There was no significant difference between the results of Cherry-dp, 3-in-1 Granulated-c, and DW at all measurement times (p>0.05). IS-Turkish-c and IS-Filter-c caused significantly more coloration in 28 days compared to 24 hours. I-tea also caused significantly more coloration in 28 days compared to 24 hours and 7 days (p<0.05).

DISCUSSION

The success of aesthetic restorations is directly related to technological developments in composites. Nanotechnology has been incorporated into dental materials to achieve a universal restorative with better mechanical and aesthetic properties (12). The present in vitro study was conducted to evaluate the coloring potential of instant sachets (IS) on Nano hybrid composite (Filtek Z550) material.

Discoloration of composite restorations may occur due to both surface adsorption and absorption of colorants (13). Chemicals in beverages can lead to surface deterioration of composite restorations, causing unaesthetic external pigmentation. The ability of resin composites to absorb water can also be affected by the chemical composition of the beverage (14). In addition, the effect of coloring agents -found in foods and beverages that modernized societies often consume- on composite restorations may be directly related to the amount and frequency of beverage intake (15). IS were preferred in the present study because have become an important part of the daily diet for both practicality and variety.

Visual or instrumental techniques can be used to assess color stability. Color differences below the visual perception threshold can be detected using spectrophotometers (16). In the present study; spectrophotometer was used to evaluate color changes in dental materials because it is more quantitative.

The color stability of the Nano composite resin was evaluated and the numerical values obtained by the spectrophotometer were analyzed with the CIEDE2000 formula. For the evaluation of color stability, CIEDE2000 reported that a modified version of the CIELab system better evaluates color acceptability and perceptibility (17). The most commonly used ∆E cut-off value, which is clinically accepted by most researchers, is 3.3 (15,18). The alternative hypothesis of this study was partially rejected as the ∆E value of sour cherry juice was below the cut-off value. In terms of ∆E values, the cut-off value of 3.3 was measured in the cherry juice and distilled water groups, while the values observed on the 7th and 28th days in the other groups were above the acceptable cut-off value.

Table 2. Mean and standard deviation values of the groups (∆E)						
	Cherry flavored	**Turkish coffee**	**Filter coffee**	**Control group**	**Instant tea**	**3-in-1 granulated coffee**
24 hours	1.86±1.11	2.33±1.64[a]	2.72±1.19[a]	1.63±0.89	1.72±0.50[a]	2.80±2.44
7 days	2.68±1.52	5.43±3.71[ab]	4.62±2.56[ab]	2.08±1.02	4.14±2.45[a]	4.48±2.32
28 days	3.29±1.36[A]	8.73±2.51[Cb]	6.96±2.01[Bb]	2.81±1.48[A]	7.75±3.77[Cb]	5.11±2.72[A]
a-b: Shows the differences within the same column. A-C: Shows the differences within the same line. * One way ANOVA						

Akleyin et al. Color stability of a nanohybrid composite resin

J Health Sci Med 2023; 6(1): 13-17

Immersion time has an effect on the color stability of composite resins. Color changes become more intense as the immersion time increases. This may be due to increased interaction between chemicals and resin, as well as better penetration of staining agents into the resin. An aqueous environment can affect the properties of composite resins and even lead to hydrolytic degradation over time (19). In the present study, the immersion process of composite samples in beverages was standardized based on previously reported immersion times in the literature. Chowdhury et al. (20) evaluated color change and reported that the lowest values were obtained on the 7th day, followed by the 14th day, and maximum color change was obtained on the 28th day. Based on this study, the samples in the present study were kept in the prepared solutions for 28 days to obtain the maximum results.

For adults, coffee is a frequently preferred beverage in daily life. According to coffee producers, a cup of coffee is consumed by a person in an average of 15 minutes. Average daily consumption of coffee is 3.2 cups per day. There are studies in the literature reporting that the in vitro immersion of samples in coffee for 24 hours is equivalent to approximately 1 month of coffee consumption in vivo (13,21,22). In the present study, 28 days of immersion simulated almost 2 years of coffee consumption.

The fillers in the structures of composite resins affect their susceptibility to coloration. Inorganic fillers on the surface cause an increase in the surface roughness of the composite by moving away from the resin matrix as a result of clinical use. Since the fillers of Nano hybrid resins are small, lower surface coloration is expected when separated from the surface (3). Color stability of Nano hybrid composites is still a controversial issue in the literature. Villalta et al. (23) and Al Kheraif et al. (24) reported that Nano hybrid composites showed higher discoloration. Özkanoğlu et al. (25). Stated that Nano hybrid composites are resistant to external discoloration.

Similar to the present study, Hasani et al. (26) and Koraç et al. (27) reported that the Filtek Z55O showed more discoloration, especially in coffee. In contrast, Gönülol et al. (28) reported that Filtek Z550 demonstrated high water absorption and the lowest ΔE values in terms of discoloration. In the present study, IS-Turkish-c, IS-Filter-c caused significantly more coloration in 28 days compared to 24 hours. I-tea also caused significantly more coloration in 28 days compared to 24 hours and 7 days (p<0.05). This result indicated that immersion time in the solutions caused an increase in discoloration of Nano hybrid composites.

Darabi et al. (29) and Domingo et al. (30) reported that coffee was the immersion medium that caused the most discoloration on the composite resin tested. There are studies reporting that the high yellow coloring agent in coffee adheres to the composite surface and interacts with the organic phase of the composite resin, resulting in discoloration of the composite (31, 32). In contrast, other studies reported that tea causes more significant discoloration than coffee (33, 34). Nasim et al. (35) reported that the most significant discoloration caused by tea occurred on the 7th and 30th days of immersion. In the present study, immersion in sachet drinks for 24 hours and 7 days did not results in significant color change results, however, IS-Turkish-c, IS-Filter-c and I-tea caused significantly more discoloration in 28 days compared to DW. This was thought to be due to the use of dense extract (tea water) in tea bags for rapid brewing and instant coffees produced from extracts obtained by brewing more concentrated coffee.

Guler et al. (13) reported that cherry juice did not cause a significant color difference in the tested resin composite with the lowest average ΔE value, and the addition of milk powder to coffee reduced the color difference compared to coffee without cream. In the present study, Cherry-dp and 3-in-1 Granulated-c did not result in significant color change compared to DW at all time periods, which is consistent with the results of this study. In our study, it was concluded that adding cream to coffee, especially in 3-in-1 Granulated-c, reduces the concentration of coffee, and milk proteins reduce discoloration by binding polyphenols.

There are certain limitations of this in vitro study. In the study, the composite resin material was not constantly exposed to different foods and beverages, did not come into contact with saliva, was not brushed as in the oral environment, and the remineralization environment of saliva was not created. All parameters were measured up to 28 days, but longer clinical studies or studies better simulating the oral environment are needed to confirm the findings.

CONCLUSION

The results obtained in the present study showed that long-term consumption of instant coffee and tea products, which are becoming increasingly popular among young people in modernizing societies, cause discoloration in Nano hybrid composites. Questioning the beverage habits while taking the patient's history may be a criterion in material selection.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval is not required as the study.

Informed Consent: Informed consent is not required as the study.

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Comparison of deep and combined serratus anterior plane block after video-assisted thoracoscopic surgery; a prospective randomized trial

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ABSTRACT

Aim: Ultrasound-guided plane blocks have been employed frequently in Video-assisted thoracoscopic surgery (VATS). The aim of this study was to evaluate the effect of deep and combined serratus anterior plane block (SAPB) after VATS.

Material and Method: The patients, in the age range of 18 to 65 years, with the American Society of Anesthesiologists (ASA) physical status of I-III, and body mass index (BMI) of 18-30 kg/m², and undergoing lung resection with VATS were included in the study. Patients were informed about the study, and their written consent was obtained. Patients were divided into Deep SAPB (DSAPB) (Group 1) and combined SAPB (CSAPB) (Group 2) groups according to the analgesia protocol.

Results: There was no statistically significant difference between the groups in terms of demographic characteristics and surgical features (p>0.05). When the groups were evaluated in terms of the block performance time, it was found to be statistically significantly longer in the CSAPB group than in the DSAPB group (p<0.001). When the groups were evaluated in terms of VAS resting scores, the 1st, 2nd, 4th, 8th, 16th, 24th, and 48th-hour VAS resting results were found to be statistically significantly higher in the DSAPB group than the CSAPB group (p<0.05). VAS cough scores were statistically significantly higher in the DSAPB group at the 1st, 2nd, 4th, 8th, 16th, 24th, and 48th-hour (p<0.05). When the groups were evaluated in terms of the side effects, additional analgesic use, and morphine consumption, they were found to be statistically significantly higher in the DSAPB group than in the CSAPB group (p: 0.026, p: 0.020, p<0.001, respectively).

Conclusion: CSAPB provided effective analgesia after VATS for 48 hours. In addition, morphine consumption and the need for additional analgesics were low in CSAPB. However, the duration of the block procedure was longer in the CSAPB application.

Keywords: Acute pain, deep serratus anterior plane block, superficial serratus anterior plane block, combined serratus anterior plane block, video-assisted thoracoscopic surgery, VATS

INTRODUCTION

Although thoracic surgery causes severe pain, the use of video ascites thoracoscopic surgery (VATS) methods has limited postoperative pain (1). However, pain is still a problem in VATS patients. Failure to control this pain is associated with postoperative complications (2). The causes of acute pain after VATS include soft tissue damage, incision line, and drain entry sites (3).

In recent years, regional anesthesia methods, multimodal analgesia techniques combined with nonsteroidal anti-inflammatory drugs (NSAIDs), and opioids have been preferred for postoperative analgesia (4). Thoracic epidural analgesia (TEA), thoracic paravertebral block (TPVB), and intercostal block are among the commonly used methods (5). However, with the widespread use of ultrasonography

(USG) in recent years, fascial plane blocks (such as erector spinae plane block and serratus anterior plane block (SAPB)) in which local anesthetic (LA) is injected into a tissue plane have become very popular (6,7). Unlike peripheral nerve blocks, no nerve or plexus block is required. LA drugs reach the desired nerve by spreading along the muscle plane (8).

SAPB, which has just been applied after VATS, targets the lateral cutaneous branches of the intercostal nerves, which originate from the intercostal nerves and pass from the deep to the surface of the serratus anterior muscle (9). SAPB is one of the plane blocks that can provide analgesia between the levels of the second thoracic vertebra (T2) and the ninth thoracic vertebra (T9) and can be applied under the guidance of USG (10). This application can be applied in two ways as deep SAPB (DSAPB) or superficial SAPB

J Health Sci Med 2023; 6(1): 18-24

Zengin et al. Deep and combined serratus anterior plane block for VATS

(SSAPB) (11,12). Although both methods are easy to apply, it has been reported that the duration of action of SSAPB is longer than that of DSAPB. In addition, it has been reported that SAPB application has similar analgesic efficacy and morphine consumption to TPVB for thoracic surgery (13).

Possible side effects and complications that may develop due to other regional anesthesia techniques are more limited in SAPB (14). In contrast to neuraxial techniques, patients with coagulopathy have very limited contraindications and no sympathetic block resulting in hypotension. Theoretically, neurological complications are also not expected (15). Although there are studies on SSAPB and DSAPB blocks, we could not find any study combining these two blocks.

In this study, we hypothesized that more nerve blocks can be created with multisite injection and more effective postoperative analgesia can be provided. Therefore, we thought that the combination of deep and superficial SAPB could provide more effective analgesia than DSAPB. We determined the postoperative first 48-hour VAS scores as the primary outcome. We determined 24-hour morphine consumption, additional analgesia needs, and side effects as the secondary outcomes.

MATERIAL AND METHOD

Study Design

The randomized and prospective trial was conducted in a high-volume tertiary thoracic surgery center after obtaining approval from the Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 20.10.2021, Decision No: E1/2066/2021). The trial was registered on clinicaltrials.gov (https://clinicaltrials.gov/) under the identifier NCT05106283. This study was conducted within the framework of the ethical rules stated in the Declaration of Helsinki and followed Good Clinical Practices. This manuscript adheres to the applicable Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Patients

The patients, in the age range of 18 to 65 years, with the American Society of Anesthesiologists (ASA) physical status of I-III, and body mass index (BMI) of 18-30 kg/m², and undergoing lung resection with VATS were included in the study. Patients were informed about the study, and their written consent was obtained.

During the preoperative evaluation, the patients were informed about pain assessment and patient-controlled analgesia (PCA). Patients with preoperative acute or chronic pain and a history of opioid therapy were excluded. Moreover, patients with bleeding disorders, infection at the injection site, allergy to local anesthetics, and patients who underwent emergency surgery were excluded from the study.

Patients were divided into DSAPB (Group 1) and CSAPB (Group 2) groups according to the analgesia protocol. Randomization was achieved with computer-generated random numbers. Group assignments were kept in a sealed envelope known only to the physician who would perform the block procedures.

Anesthesia Protocol

Patients were monitored in the operating room following the ASA standards. Patients were administered 0.03 mg/kg midazolam for premedication. Following preoxygenation, anesthesia was induced with 2 mg/kg propofol, 1 mcg/kg fentanyl, and 0.1 mg/kg vecuronium. After intubation with a left-sided double-lumen endobronchial tube, tube localization was confirmed. Anesthesia was maintained with sevoflurane in a mixture of oxygen and air. Additionally, remifentanil infusion at a dose of 0.01-0.20 mcg/kg/min was administered.

Block Procedures

Block procedures were performed under general anesthesia before the skin incision to prevent anxiety and ensure patient comfort. Following the anesthesia induction, blocks were performed under US guidance when patients were in the lateral decubitus position. After strict skin antisepsis, the needle insertion area was covered with sterile drapes. A high-frequency 6–18 MHz linear probe (MyLab six, Esaote, Genoa, Italy) in a sterile cover and a US-compatible 22-gauge and 8-mm nerve block needle (Pajunk, SonoPlexSTIM, Germany) were used in all groups. Block performance times were noted based on the entry of the block needle into the skin and the exit of the needle after the block. The following procedures were performed in the study groups:

DSAPB group (n: 30): Following the visualization of the anatomical structures, the nerve block needle was advanced via the in-plane technique until reaching the fourth rib. Hydrodissection with 2 ml of normal saline was performed beneath the serratus anterior muscle, and a volume of 20 ml of 0.25% bupivacaine was injected into the area (**Figure 1**).
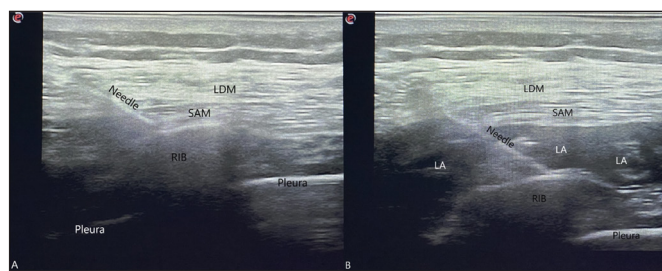
Figure 1. Deep serratus anterior plan block procedure. A: Anatomical scene before block B: Deep serratus anterior plan block; The local anesthetic spread beneath the serratus anterior muscle. SAM: serratus anterior muscle, LDM: Latissimus dorsi muscle, LA: local anesthetic.

Zengin et al. Deep and combined serratus anterior plane block for VATS

J Health Sci Med 2023; 6(1): 18-24

CSAPB group (n: 30): After visualizing the anatomical structures, the needle was first advanced via the in-plane technique until reaching the fourth rib. Hydro dissection with 2 ml of normal saline was performed beneath the serratus anterior muscle, and a volume of 10 ml of 0.25% bupivacaine was injected into the area. Then, the needle was retracted 1–2 cm above the serratus anterior muscle; hydro dissection with 2 ml of normal saline was performed in the interfascial space, and a volume of 10 ml of 0.25% bupivacaine was injected into the area (**Figure 2**).
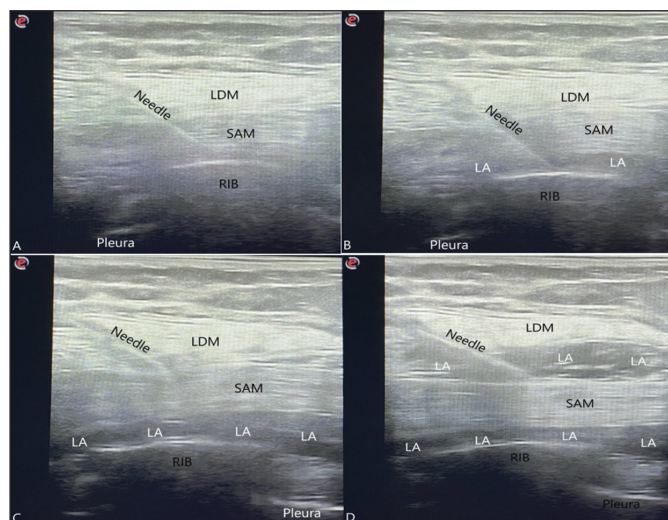
Figure 2. Combined serratus anterior plane block. A: Anatomical scene before the block. B: Deep serratus anterior plane block; The local anesthetic spread beneath the serratus anterior muscle. C: Anatomical scene before the superficial serratus anterior plane block after the deep serratus anterior plane block. D: Superficial serratus anterior plan block; The local anesthetic spread above the serratus anterior muscle. SAM: serratus anterior muscle, LDM: Latissimus dorsi muscle, LA: local anesthetic.

Analgesia Protocol

During the skin closure, patients received dexketoprofen and tramadol intravenously. Metoclopramide was administered intravenously to avoid nausea and vomiting. In the postoperative surgical intensive care unit, intravenous morphine was administered via PCA pump for 24 hours. Pain intensity was evaluated using a 10- point (0: No pain and 10: Unbearable pain) visual analog scale (VAS). The PCA pump's dose delivery was limited to administering a bolus dose of 1 mg morphine and delivering a maximum dose of 12 mg morphine in total within 4 hours with lockout intervals of 15 minutes. Paracetamol 1 g every 8 hours and dexketoprofen 50 mg twice daily were administered intravenously for multimodal analgesia. As a rescue analgesic agent, 0.5 mg/kg tramadol was given to patients intravenously when a score of VAS at rest was $\geq$ 4. The patients were transferred to the ward in the postoperative 24th hour. Paracetamol 500 mg tablets and tramadol 50 mg capsules every 8 hours and dexketoprofen 25 mg tablets every 12 hours were given after the postoperative second day. VAS scores at rest and while coughing were recorded in the postoperative 1st hour, 2nd hour, 4th hour, 8th hour, 16th hour, 24th hour, and 48th hour. The need for additional

analgesics and side effects including allergic reactions, respiratory depression, sedation, hypotension, urinary retention, nausea-vomiting, and itching were recorded. In two groups, patients' hemodynamic data, age, BMI, gender, diagnosis, the type of surgery, intraoperative and postoperative complications, postoperative VAS scores, and postoperative additional analgesic use were recorded. The block was applied to all patients by the same attending anesthesiologist. The VAS score was followed up by the pain management nurse who was blinded to the type of block applied to the patient. All VATS procedures were applied by the same surgical team with sufficient experience in this regard in the third-level thoracic surgery center. A single polyvinylchloride chest tube was introduced, made of two openings, a camera port, and a utility.

Sample Size

The sample size was calculated using G*Power© software version 3.1.9.2 (Institute of Experimental Psychology, Heinrich Heine University, Dusseldorf, Germany). The sample size was calculated for the Mann-Whitney U-test, which was used for testing the main hypothesis of (VAS rest first hour) the present study. Depending on previous research results with two-sided (two tails) type I error 0.05 and power of 80% (1-β=0.8), effect size (d) factor 0.79, should involve $\geq$ 56 subjects.

Power Analyses

The post hoc power was calculated using G*Power© software version 3.1.9.2 (Institute of Experimental Psychology, Heinrich Heine University, Dusseldorf, Germany). The power was calculated for the Mann-Whitney U test, which was used for testing the main hypothesis of the present study (VAS rest first hour). Depending on previous research results with two-sided (two tails) type I error 0.05 and effect size (d) factor 1.07, post hoc power calculated as %97.96.

Statistical Analyses

Data analyses were performed by using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variables was normal or not was determined by the Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean±SD for normal distributions, and median (Q1: first quartile – Q3: third quartile) for skewed distributions. Categorical data were described as a number of cases (%). Statistical analysis differences in normally distributed variables between two independent groups were compared by Student's t-test, Mann Whitney U test was applied for comparisons of the not normally distributed data. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test was accepted p-value <0.05 as a significant level on all statistical analysis.

J Health Sci Med 2023; 6(1): 18-24

Zengin et al. Deep and combined serratus anterior plane block for VATS

RESULTS

66 patients were eligible for this study, and the data of 60 patients were analyzed (**Figure 3**). There was no statistically significant difference between the groups in terms of demographic characteristics and surgical features (p>0.05) (**Table 1**).
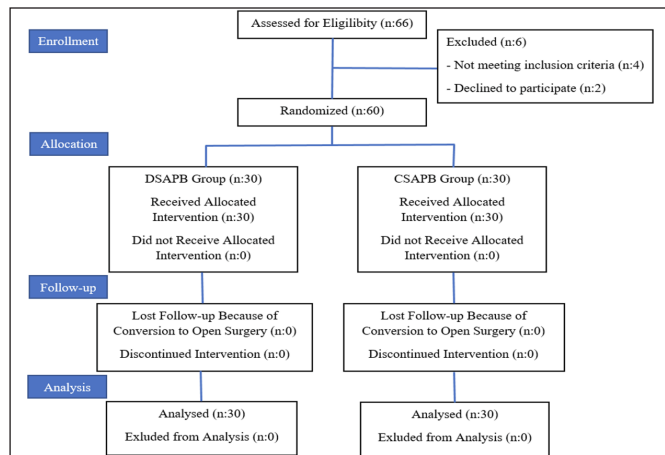
Figure 3. Flowchart of the patients. DSAPB: Deep serratus anterior plane block, CSAPB: Combined serratus anterior plane block

When the groups were evaluated in terms of the block performance time, it was found to be statistically significantly longer in the CSAPB group than in the DSAPB group (p<0.001) (**Table 1**).

Table 1. Demographic characteristics and surgical features of patients.

	DSAPB	CSAPB	p
Age, year β	51.5 (33-60)	52 (30-56)	0.569
Gender δ			
Female	4 (13.3%)	9 (30.0%)	0.117
Male	26 (86.7%)	21 (70.0%)	
BMI*	25.36±3.03	24.54±2.98	0.293
Diagnosis δ			
Pneumothorax	6 (20.0%)	7 (23.3%)	0.754
Lung Mass	24 (80.0%)	23 (76.7%)	
Operation δ			
Wedge	23 (76.7%)	25 (83.3%)	0.519
Lobectomy	7 (23.3%)	5 (16.7%)	
Block performance time (second)*	117.30±15.67	142.10±19.55	<0.001
Duration of anesthesia (minute) β	150 (120-180)	150 (120-180)	0.994
ASA δ			
ASA I	4 (13.3%)	3 (10.0%)	0.999
ASA I I	13 (43.3%)	13 (43.3%)	
ASA I I I	13 (43.3%)	14 (46.7%)	
Intraoperative remifentanil consumption (mcg) β	350 (300-450)	350 (275-450)	0.700

Continuous variables are expressed as either * the mean±standard deviation (SD) or β the median (Q1: first quartile – Q3: third quartile), and categorical variables are expressed as either δ frequency or percentage. Continuous variables were compared with a Student t-test or the Mann-Whitney U test, and categorical variables were compared using Pearson's chi-square test or Fisher's exact test. Statistically significant p-values are in bold. BMI: Body mass index. ASA: American Society of Anesthesiologists. DSAPB: Deep Serratus Anterior Plane Block, CSAPB: Combined Serratus Anterior Plane Block

When the groups were evaluated in terms of VAS resting scores, the 1st, 2nd, 4th, 8th, 16th, 24th, and 48th-hour VAS resting results were found to be statistically significantly higher in the DSAPB group than the CSAPB group (p<0.05) (**Table 2**). VAS cough scores were statistically significantly higher in the DSAPB group at the 1st, 2nd, 4th, 8th, 16th, 24th, and 48th-hour (p<0.05) (**Table 2**).

Table 2. Resting and coughing VAS scores of the patients during the postoperative 48 hours.

	DSAPB Med (Q1-Q3)	CSAPB Med (Q1-Q3)	p
VAS resting			
1st hour	3 (3-4)	3 (2-3)	<0.001
2nd hour	3 (3-3)	2 (1-3)	0.003
4th hour	2.5 (2-3)	2 (1-3)	0.016
8th hour	2 (2-3)	2 (1-2)	0.003
16th hour	2 (2-2)	1 (1-2)	0.002
24th hour	2 (1-2)	1 (0-2)	0.002
48th hour	2 (2-3)	1 (1-2)	<0.001
VAS coughing			
1st hour	5 (4-5)	4 (3-5)	0.006
2nd hour	4 (4-5)	3 (2-4)	0.001
4th hour	4 (3-4)	3 (2-3)	0.001
8th hour	3 (3-4)	3 (2-3)	0.035
16th hour	3 (3-3)	3 (2-3)	0.008
24th hour	3 (3-3)	2 (1-2)	<0.001
48th hour	3 (3-4)	3 (2-3)	0.001

Continuous variables are expressed as the median (Q1: first quartile – Q3: third quartile). Continuous variables were compared with the Mann-Whitney U test. Statistically significant p-values are in bold. DSAPB: Deep serratus anterior plane block, CSAPB: Combined serratus anterior plane block

When the groups were evaluated in terms of the side effects, additional analgesic use, and morphine consumption, they were found to be statistically significantly higher in the DSAPB group than in the CSAPB group (p: 0.026, p: 0.02, p<0.001, respectively) (**Table 3**).

Table 3. Morphine consumption during the postoperative 24-hours need for additional analgesics, and complication rates

	DSAPB (n:30)	CSAPB (n:30)	p
Morphine consumption (mg) *	30.30±9.52	18,27±4.53	<0.001
Additional analgesic use n (%) δ	14 (46.7%)	6 (23.3%)	0.028
Complication (Nausea) n (%) δ	8 (26.7%)	1 (3.3%)	0.026

Continuous variables are expressed as either * the mean±standard deviation (SD) and categorical variables are expressed as either δ frequency or percentage. DSAPB: Deep Serratus Anterior Plane Block, CSAPB: Combined Serratus Anterior Plane Block

Zengin et al. Deep and combined serratus anterior plane block for VATS

J Health Sci Med 2023; 6(1): 18-24
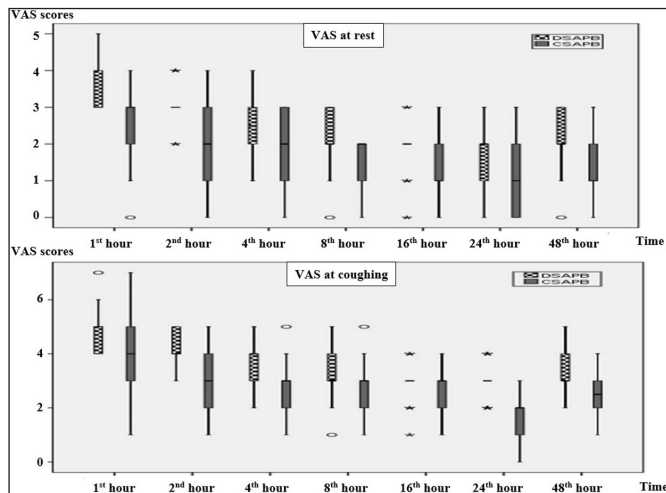
Figure 4. VAS scores at rest and VAS scores at coughing. Data are expressed as median (horizontal bars), interquartile range (boxes), and maximum and minimum values (whiskers) for the VAS scores in the 1st, 2nd, 4th, 8th, 16th, 24th hours, and 48th hours. DSAPB: Deep Serratus Anterior Plane Block, CSAPB: Combined Serratus Anterior Plane Block, VAS: Visual analog scale.

DISCUSSION

The results of this study comparing DSAPB and CSAPB applications showed that CSAPB provided effective analgesia for 48 hours postoperatively. However, morphine consumption and the need for additional analgesics remained very limited. However, the duration of the block procedure was longer in the CSAPB application. This study is special in terms of evaluating the combined block effectiveness in SAPB applications.

VATS, which is one of the developments in minimally invasive surgical techniques in recent years, provides more acceptable postoperative analgesia in patients with smaller incisions. This situation also ensures that complications are less common and therefore shortens the discharge time (16). However, severe pain in the acute postoperative period is still a problem that needs to be resolved in these patients. For this purpose, multimodal analgesia techniques are widely used and effective postoperative analgesia is provided in patients. One of the most important components of multimodal analgesia is regional and peripheral nerve blocks (17,18). For this purpose, plane blockers applied to the thoracic wall have been widely accepted in recent years. In addition, these blocks have become an indispensable part of the concept of acceleration of recovery after thoracic surgery (ERATS) due to the potential for thoracic epidural analgesia and less side effects compared to thoracic paravertebral blocks (19-22).

The analgesic effect of local anesthetics in thoracic plane blocks is still a controversial issue. The targeted area in SAPB application is the lateral cutaneous branches of the thoracic intercostal nerves, which originate from the anterior branches of the thoracic spinal nerves and extend as a neurovascular bundle just below each rib. In the midaxillary line, the lateral cutaneous branches of the thoracic intercostal nerve pass through the inner intercostal, outer intercostal, and serratus anterior muscles, which innervate the muscles of the lateral thorax (23). Local anesthetic spreading into these planes will spread along the lateral chest wall, resulting in paresthesia in the T2 to T9 dermatomes of the anterolateral thorax (23). SAPB, first described by Blanco et al. (9), has been evaluated by anatomical and radiological examination in fresh cadavers. SAPB containing the superficial plane has been reported to result in better drug diffusion and a longer duration of paresthesia (750-840 minutes) compared to SAPB containing the deep plane. It has been reported that the area of sensory loss in the pin-prick test is the same for superficial and deep SAPB injections. Moon et al. (11) found that the intraoperative analgesic efficacy is similar for deep and superficial SAPB in VATS lobectomy. Park et al. (4) evaluated the comparison of DSAPB and placebo, found that DSAPB provided effective analgesia after VATS. In a study comparing epidural analgesia and SAPB applications, Khalil et al. (15) found similar analgesic efficacy after thoracotomy and they also found that side effects were limited in the SAPB group. Baytar et al. (24) compared the DSAPB and TPVB applications reported that DSAPB and TPVB provided similar analgesic effects after VATS, and stated that DSAPB was safer. In present study is special in terms of comparing DSAPB and CSAPB applications, and according to the results of the study, more effective analgesia was provided in patients who underwent CSAPB with comparable side effects in both groups. Providing more effective analgesia in CSAPB can be explained by the more effective spread of local anesthetic applied to both deep and superficial areas by multisite injection and the compensation of a possible block failure with local anesthetic applied to the other area. Since this application is a new application and local anesthetic spread is still controversial in these blocks, new studies with different volumes may shed light on this issue.

Block implementation time is important both in terms of ease of application and easy learning. In this study, the duration of block application was longer in SSAPB than in DSAPB. This can be explained by the fact that rib imaging is performed faster in the DSAPB application since it is considered a landmark. In addition, in multisite injection, the application of injection to two points, even if it is a single needle entry, prolonged the time. However, providing more effective analgesia in CSAPB shows that this time difference is negligible.

Epidural, paravertebral, and intercostal blocks have been used in thoracic surgery for years. However,

each of these blocks has some disadvantages. It includes unnecessary bilateral blockade, including the sympathetic nervous system, and various complications such as hypotension, epidural hematoma, abscess, and dural puncture (25,26). Although it has been recommended as a paravertebral block in recent years, it also has some disadvantages such as difficult technique, despite the use of pneumothorax and ultrasound (27). Intercostal nerve block also has some deficiencies such as pneumothorax, short duration of action, high plasma absorption of local anesthetics, and the need to block multiple nerve levels (25,27). Therefore, thoracic plane blocks are preferred because of their low incidence of side effects. In present study, only PONV was seen in the patients and the incidence of PONV in CSAPB group was quite low. This situation might be related to higher consumption of morphine and rescue analgesic requirements in DSAPB group.

There are some limitations in this study. First of all, since it is a single-center study, it may not be appropriate to generalize these results to the general population. Second, the long-term analgesic effects of these two blocks and their results on chronic pain could not be evaluated.

CONCLUSION

CSAPB provided effective analgesia after VATS for 48 hours. In addition, morphine consumption and the need for additional analgesics were low in CSAPB. However, the duration of the block procedure was longer in the CSAPB application. Large-scale prospective studies with different local anesthetics volumes and concentrations will be useful in evaluating the effectiveness of these two methods.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 20.10.2021, Decision No: E1/2066/2021).

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

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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Comparison of ultrasonography and conventional radiography in the diagnosis of extremity fractures in the emergency department

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ABSTRACT

Aim: The purpose of the study is to compare the diagnostic accuracy (sensitivity and specificity) of ultrasonography (USG) with that of conventional radiography (CR), the standard imaging modality used to diagnose acute extremities fractures.

Material and Method: The prospective investigation examined 245 patients with clinical symptoms of an extremity fracture. Radiography (anteroposterior and lateral radiographs for each patient, oblique if necessary) and USG were performed on all participants and compared with all the results.

Results: CR verified 98.5% of 132 patients who were determined to have extremities fractures with USG. CR, on the other hand, confirmed 99.1% of 112 patients who were reported to have no extremities fractures by USG. The sensitivity (detection of fractures based on USG of patients with fractures detected based on the CR imaging) was 99.2% (95%CI=95.8-99.9); selectivity (no fracture was detected based on USG of patients with no fracture detected based on the CR imaging) was 98.2% (95%CI=93.8-99.7); the positive predictability was 98.48% (95%CI=94.2-99.6), whereas the negative predictability value was 99.1%(95%CI=94-99.8).

Conclusion: USG and CR showed similar diagnostic performances in the diagnosis of extremity fractures. USG can be considered an alternative to CR in the examination of extremity fractures with comparable diagnostic performance.

Keywords: Ultrasonography, accuracy, sensitivity and specificity, extremity fracture

INTRODUCTION

Suspected extremity fracturesas a result of trauma are a common reason for admissions to the emergency department (ED) (1). "We hypothesized that using the USG to exclude extremities fractures in all age groups would detect the same proportion of fractures as CR, by providing similar diagnostic efficiency (2). Alternatively, Ultrasonography (USG) is increasingly being used in practically all areas of modern medicine for procedural, screening, and diagnostic purposes, with new applications (3).

Although conventional radiographic imaging is accepted as the gold standard diagnostic tool in the diagnosis of fractures, ionizing radiation caused by its use may cause adverse effects especially in the pediatric group with sensitive tissues and in pregnant (4). The accuracy of ultrasonic imaging in identifying fractures following acute trauma was summarized in comprehensive reviews (5-7). USG of bone surfaces can be an effective approach for

assessing acute injuries (8). Using USG to detect changes in bone surfaces (discontinuities, displacement, and subperiosteal hematomas) is a reliable and manageable approach to fracture diagnosis (9). The avoidance of radiation exposure is one of the primary benefits of ultrasonic testing; this is especially true in pregnant and children, who are more vulnerable to radiation than adults (10). Ultrasonography as a diagnostic tool in situations of suspected fractures may offer economic, prognostic, and therapeutic benefits, particularly in terms of reducing unnecessary radiation exposure to human body (11).

The purpose of the present study is to compare the diagnostic accuracy of USG with that of CR, the standard imaging modality used to diagnose acute extremities fractures. We hypothesized that using the USG to exclude extremities fractures in all age groups would detect the same proportion of fractures as CR, by providing similar diagnostic efficiency.

Yıldırım et al. Ultrasonography and radiography in extremity fractures

J Health Sci Med 2023; 6(1): 25-29

MATERIAL AND METHOD

This prospective study was initiated with the approval of the Health Sciences University Afyonkarahisar Medical Faculty, Clinical Researches Ethics Committee (Date: 03.05.2019, Decision No: 166). Participants were informed in writing about the use and publishing of the anonymized data and consented willingly by returning a questionnaire. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Study Population

Before the study, the emergency physicians (EP) and triage team working in our department were informed about the study. Patients who applied with the suspicion of extremity fracture were referred to the emergency room physician who would perform USG. It was performed by an EP trained phcysian in basic ultrasonography following a therical and practical training periods.. Before the study, the physician performing USG performed ultrasonographic imaging on a sufficient number of patients with extremity fractures on radiographs. The patient was informed by the practicing doctor about the study and the subjects he wanted to learn. Exclusion criteria were as follows: presence of a local or systemic infection, missing follow-up data, and history of allergy to contrast, local anesthetic drugs, and coagulopathy.

Data Collection and Imaging

The patients were recorded with demographic characteristics (age, gender, mechanism of trauma) and evaluated in longitudinal and transverse planes with a linear probe with a valence of 4-15 MHz using the ultrasonography device (USMART-3200T model England, United Kingdom) according to the symptom locations. The presence of cortical irregularity or interruption in the bone on USG was considered a fracture. The extremity (right upper extremity, left upper extremity, right lower extremity, left lower extremity), bone and part of the bones, ultrasonographic findings (cortical irregularity, edema, periosteal thickening, fracture), and intra-articular extension were assessed in the study. USG applications were performed following the Musculoskeletal Ultrasound Technical Guide of the European Society of Musculoskeletal Radiology (12).

After the ultrasonographic examination, anteroposterior and lateral, and if necessary, oblique extremity radiography requests were routinely performed by different EP. Radiographic examinations were performed with the LISTEM brand Rex-525R model X-ray. Radiographs were interpreted by the EP who evaluated the patient. Whether a fracture was detected on the radiograph, the name and region of the examined bone, whether it was displaced if there was a fracture, the presence or absence of intra-articular extension with the fracture, and how long the patient's radiographs lasted (in minutes) and whether he felt pain when the X-ray was taken were recorded by the EP in charge. EP who interpreted the radiographs did not have any information about the USG findings.

Statistical Analysis

SPSS v23.0 software was used to analyze the data (IBM Inc,Chicago,USA).The sample's sociodemographic variables were provided descriptively (means, standard deviation, relative and absolute frequencies). The results of the two diagnostic tools (radiography and ultrasonography) and between the 2 readers were compared using Chi-Square ($\chi2$) testing and Cohen's kappa coefficient(κ)testing for significance (with Fisher-Exact correction). In the data analysis, we employed the McNemar test to evaluate our initial hypothesis. To test for systematic differences, odds ratios (ORs) with corresponding %95 confidence intervals (CIs) were calculated, along with an accurate p-value. The sensitivity and specificity, as well as the positive and negative predictive values, were calculated to test our second hypothesis. The significance criterion has been set at a probability of error of $p<0.05$.

RESULTS

Descriptives

This clinical research involved 245 patients clinical symptoms of an extremity fracture (102 females to 143 males). Thirty-five patients with multiple fractures, who did not sign the consent form, suspected of pregnancy, younger than 4 years, and diagnosed in an external center were excluded from the study.

The mechanism of extremity trauma were as follows: fall (52.7%), blunt impact (17.6%), sprain (13%), crush (8.2%), in-vehicle traffic accident (4%), non-vehicle traffic accident (3.7%), and other (0.8%). According to the fracture region detection, 27.3% of the fractures were in the right upper extremities, 26.1% in the left upper extremity, 25.7% in the right lower extremity, and 20.8% in the left lower extremity. While the USG was used to diagnose 53.9% of extremities fractures, RC was used to diagnose 53.5%. The lower extremity accounted for 39.7% of the 131 fractures diagnosed with RC, while the upper extremities accounted for 60.3%.

Comparison of USG and CR Results

The results were not significant when we compared the CR and USG methods for diagnosing extremities fractures in patients. CR verified 98.5% of 132 patients who were determined to have extremities fractures with USG. CR, on the other hand, confirmed 99.1% of 112 patients who

were reported to have no extremities fractures by USG. Although CR determined fracture, just one incidence of extremities fracture could not be recognized by USG. In addition, CR was unable to identify two incidences of extremity fractures that were determined to be fractured by USG but could not be detected by CR. The following were the characteristics of a single case that could not be detected by USG despite being fractured by CR: A 35-year-old man was determined to have a radius shaft fracture localized to the right upper extremity with a blunt trauma etiology.

As a result of the logistic regression analysis, the CR could be anticipated by examining the results of the USG method (p<0.001), and the Nagelkerke pseudo R2 value was 0.953. The Nagelkerke pseudo R2 score indicated that the USG result accounts for roughly 95% of the variance in the CR. Furthermore, the rate of fracture identification in the USG was determined to be 98.8% in patients with fractures diagnosed in CR. As seen in **Figure**, Receiver Operating Characteristic (ROC) was performed to acquire more specific information about this classification [Area Under Curve (AUC)= 0.987 (95%CI=0.971-0,998)]. The next step was to calculate the sensitivity, specificity, and positive and negative predictability values (Positive and Negative Predictive Values). According to the findings, the sensitivity (detection of fractures based on USG results of patients with fractures detected based on the CR imaging result) was 99.24 % (95%CI=95.8-99.9); selectivity (no fracture was detected based on USG results of patients with no fracture detected based on the CR imaging result) was 98.25 % (95%CI=93.8-99.7); the positive predictive value was 98.48% (95%CI=94.2-99.6), whereas the negative predictability value was 99.1%(95%CI=94-99.8).
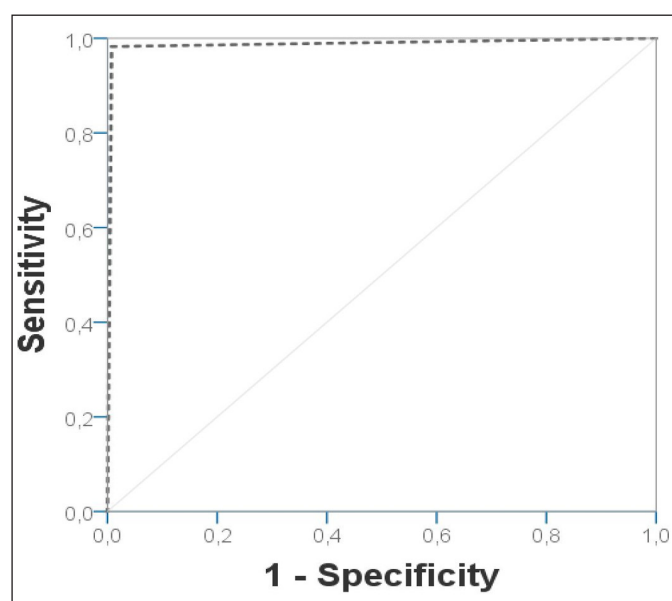
Figure. The ROC for comparison of ultrasonography and conventional radiography

Comparison of Fracture Characteristics

Ultrasonographic examination revealed that it reached the joint in 7.3% of extremities fractures, but not in 46.5%. Cortical irregularity was present in 53.9% of the patients but was absent in 46.1%. The radiographic examination revealed that it reached the joint in 13.1% of extremities fractures, but not in 40.4%, and 30.2% of the extremities fractures were displaced, while 23.3% were not. There were significant discrepancies between the results of CR and USG imaging when assessing the extent of joint fractures(p<0.001). 12.5% of the fractures diagnosed by CR imaging results extended to the joint, but they did not extend to the joint according to the USG result. 83.3% of the 12 patients who complained of pain during the USG procedure also complained of pain during the CR procedure. 98.7% of the 233 people who indicated they didn't feel pain during USG indicated the same thing with CR.

The average time to administer the USG was 3.6 minutes, with a standard deviation of 0.7 minutes. The USG had the quickest application time of 2 minutes and the longest application time of 5 minutes. The mode and median were discovered to be three. The average time to administer the CR was 13.9 minutes, with a standard deviation of 6.5 minutes. The CR had the quickest application time of 5 minutes and the longest application time of 60 minutes.

DISCUSSION

In the present study, we compared ultrasonography with radiography, which is the gold standard in the diagnosis of extremity fractures, and assessed the sensitivity and specificity of USG in recognizing fractures. We have reached important conclusions that USG is a useful instrument for more use in the diagnosis of extremity traumas in emergency departments, due to its duration and cost, and its results being similar to radiography.

Conventional radiographic imaging is the first-line diagnostic tool accepted as the gold standard in the diagnosis of patients admitted to the emergency department with a suspected fracture (13). Serious complications such as bleeding and neurovascular injuries can be seen in bone fractures caused by extremity trauma. While taking critically ill patients to the radiography unit poses many risks, there may not be a radiography unit, especially in regions where geographical conditions are not suitable. For this reason, studies on finding alternative imaging techniques that are safer, economical, and easily accessible have intensified (14). Extremity injuries can occur due to many reasons. Wang et al. (15) reported that the trauma mechanism was caused by falls in the first place and traffic accident in the second place. In a large study by Khorgami et al. (16),

Yıldırım et al. Ultrasonography and radiography in extremity fractures

J Health Sci Med 2023; 6(1): 25-29

falls, traffic accident, motorcycle accidents, and gunshot wounds cause extremity injuries, in order of frequency. In the present study, falls were determined as the most common cause of trauma.

When the fractures detected on X-rays of our patients included in our study were defined according to the region and bone localization, 39.7% were in the lower extremities and 60.3% were in the upper extremities. While distal radius fractures were most common in the upper extremity, phalangeal fractures were most common in the lower extremities. Bozorgi et al. (17) tested the reliability of USG in detecting extremity fractures and observed that the most fractured bone in the upper extremity was the radius, and the femur in the lower extremity. Wang et al. (15) emphasized that upper extremity fractures were detected more than lower extremity fractures. Although conventional radiographic imaging is accepted as the gold standard diagnostic tool in the diagnosis of fractures, ionizing radiation caused by its use may cause adverse effects especially in the pediatric patients with sensitive tissues and in pregnant (18).

On ultrasonography, bone fractures are seen as cortical irregularity and interruption in the continuity of the cortex on longitudinal scanning (19). In our study, 130 of the 132 fractures detected by USG were also detected by radiographic imaging. Of the 113 fractures that could not be detected by USG, 112 could not be detected in radiographic imaging. With the results obtained, the sensitivity of USG in detecting extremity fractures was 99.24%, the selectivity was 98.25%, the positive predictive value was 98.48% and the negative predictability value was 99.1%. Patel et al. (20) found the sensitivity of ultrasonography as 97%, selectivity at 93%, positive predictability value of 95%, and a negative predictability value of 96% in the identification of fractures. In the study conducted by Dallaudiere et al. (21), the sensitivity, selectivity, positive predictability, and negative predictability of USG in the diagnosis of extremity fractures were respectively; 98.3%, 96%, 98.3%, and 96%. In the Netherlands, Epema et al. (22) found the sensitivity of USG to be 95%, its selectivity to 86%, positive predictability to 92%, and negative predictability to 91%. In our study and the literature, ultrasonography indicates high sensitivity and selectivity in recognizing bone fractures.

In our study, there were significant differences between X-Ray imaging and USG results in determining the extent of fractures to the joint. Of 32 fractures that showed extension to the joint on plain radiography, only 18 of them could be detected by USG. Bozorgi et al. (17) observed only 24 of 50 fractures with intra-articular extension, which they observed in X-ray imaging,

by ultrasonography. Tsung et al. (23) found that the sensitivity of ultrasonography was lower in children with active epiphyseal enlargement, especially in the detection of fractures close to the joints. These results show that ultrasonography is insufficient to determine the extension of the fracture into the joint space. In the studies of Avcı et al. (24), and Beltrame et al. (25) on the contrary, fractures that could not be diagnosed by X-Ray were detected by ultrasonography. In a study conducted in Erzincan, CT, USG, and plain radiograms were compared in patients who applied to the emergency department due to elbow injury, and it was reported that 8 fractures that could not be detected by X-Ray were detected by USG (24). In one case in our study, the distal tibia fracture, which was visualized by USG, could not be observed on X-ray. In this case, who was brought to our emergency department due to traffic accident, CT imaging was performed after the orthopedic consultation and a fracture was found in the distal tibia.

The main limitation of the present study was the low number of patients with fractures extending to the joint. In addition, all patients who applied to the emergency department with the suspicion of extremity fractures during the study dates were not included in the study, since the ultrasonographic examination was performed by a single physician. Randomization was achieved because the day and night study times were approximately equal. Advanced imaging techniques such as CT or MRI were not applied to patients with inconsistency between the results of ultrasonography and direct radiography. Hence, fractures in which USG and direct X-ray gave false positive and false negative results could have been detected. USG is a dynamic and user-specific examination, and both its sensitivity and specificity are tightly connected with user experience and training that credentials of the person performing the US may result in bias.

CONCLUSION

USG and radiography showed similar diagnostic performances in our study results. Ultrasound, which can be carried easily in triage areas and pre-hospital environments where X-Ray devices cannot be carried, does not contain radiation, and is easy to learn with short and simple training, can be used in the diagnosis of tissue and organ injuries. Radiographs may be insufficient in the diagnosis of some fractures because thin fractures may be hidden by overlapping structures or missed by X-rays that are not perpendicular to the fracture line. The USG can be used at the bedside and gives results in a short time, saving time in intensive emergency room conditions, and allowing early diagnosis, especially in the evaluation of critical patients.

J Health Sci Med 2023; 6(1): 25-29

Yıldırım et al. Ultrasonography and radiography in extremity fractures

ETHICAL DECLARATIONS

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The effect of polycystic ovary syndrome history on neonatal anogenital distance: a prospective study in Turkish population

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ABSTRACT

Aim: Embryonic anogenital length depends on fetal sex and testosterone. Children of women with a history of polycystic ovary syndrome (PCOS) who became pregnant may have different anogenital lengths due to hyperandrogenemia. Therefore, the aim of this study was to compare the anogenital lengths of male and female newborns of women with and without a history of PCOS.

Material and Method: The study was designed prospectively. Pregnant women with PCOS and control group who gave birth at term (≥37-42 weeks) between March 2019 and March 2020 in University of Health Sciences, Tepecik Training and Research Hospital, Department of Obstetrics and Gynecology were included in this study.

Results: A total of 119 patients, including 21 mothers with PCOS and female newborns, 35 mothers with female newborns in the control group, 21 mothers with PCOS and male newborns and 42 mothers with male newborns in the control group, were included in this prospective study. Anogenital distance-anus fourchette (AGD-AF) measurement was significantly higher in the female newborns from mother with PCOS than in the female newborns from control group (18.1±2.5 vs. 14.1±1.6, p=0.046). Also, AGD-AF was 20.3±3.5 in the PCOS with hirsutism group and 15.2±1.5 in the PCOS without hirsutism group, and the difference between them was statistically significant (p=0.041).

Conclusion: The anogenital distance may change in female newborns in the presence of maternal PCOS. Considering that AGD reflects fetal testosterone exposure, the findings may reflect increased testosterone exposure in female fetuses of pregnant women with PCOS. The results pave the way for new studies.

Keywords: Anogenital distance, polycystic ovary syndrome, fetus, newborn, testosterone

INTRODUCTION

The first 6-week period in human embryos is the "undifferentiated stage" in which the bipotential organ plans are formed. During this period, primitive sex cells (germ cells) have not yet differentiated (1). Sexual development proceeds as a hormone-dependent and dynamic process after the sixth week of gestation. From this week onwards, the genital tubercle lengthens and forms the phallus in the male and the clitoris in the female (2). Anogenital distance (AGD) is defined as the distance between the fetal caudal extremity and the base of the genital tubercle, and this distance is fetal sex and testosterone dependent (3). Testosterone is required for sexual differentiation in males. AGD and phallic length increase with the effect of dihydrotestosterone (DHT) (4).

Polycystic ovary syndrome (PCOS) is a metabolic disease that is common in women of reproductive age and affects approximately 4% to 20%, associated with oligo-ovulation or anovulation, androgen excess symptoms and multiple ovarian cysts (5). This disease is a syndrome also known as ovarian hyperandrogenemia, and excess testosterone is the hallmark of the disorder (6).

In previous studies, it has been shown that maternal exposure to androgenic or anti-androgenic environmental agents affects AGD in the fetus (7). Given this situation, newborn of women with a history of PCOS may have different androgen exposure, and hence different AGD, from women with a normal reproductive cycle (8,9).

Studies on this subject are very limited due to the difficulty in finding the sample size and the difficulty of conceiving patients with PCOS (9,10). In this prospective study conducted in term pregnant women, it was planned to investigate the effects of pre-pregnancy PCOS history and environmental factors on the AGD of the newborn. In addition, AGD values were examined according to pre-pregnancy PCOS symptoms.

MATERIAL AND METHOD

The study was carried out with the permission of İzmir Tepecik SUAM Non-interventional Clinical Researches Ethics Committee (Date: 13.02.2019, Decision No: 2019/2-2). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All participants were informed and informed voluntary consent was obtained.

Study Design

The study was designed prospectively. Pregnant women with PCOS and control group who gave birth at term (≥37-42 weeks) between March 2019 and March 2020 in University of Health Sciences, Tepecik Training and Research Hospital, Department of Obstetrics and Gynecology were included in this study. Participants were selected from pregnant women who were admitted to the delivery room and gave birth. Information about hirsutism, oligomenorrhea and polycystic ovary appearance in ultrasonography of pregnant women with PCOS was questioned before delivery.

Data of newborns were collected in the delivery room after the first postnatal examination. The body length, head circumference and birth weight of the newborns were measured. In male newborns, anogenital distance-anus scrotum (AGD-AS): The distance between the center of the anus and back of the scrotum and anogenital distance-anus penis and (AGD-AP): The distance between the center of the anus and the posterior base of the penis were measured. (**Figure 1**).
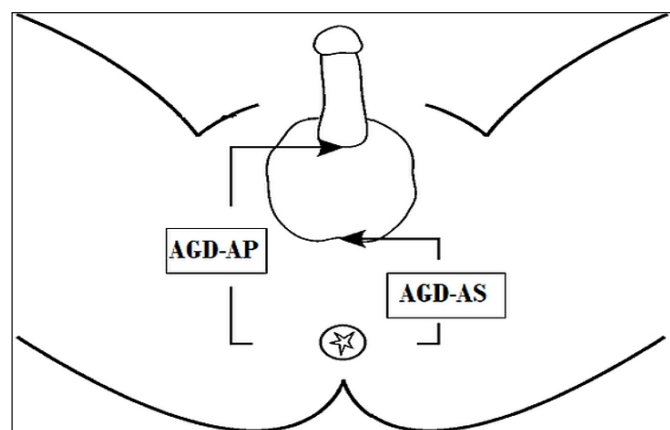
Figure 1. Neonatal anogenital distance (AGD) measurement in males

In female newborns, anogenital distance-anus fourchette (AGD-AF): The distance between the center of the anus and posterior fourchette and anogenital distance-anus clitoris (AGD-AC): The distance between the center of the anus and the clitoris floor were measured. (**Figure 2**). Each measurement was made by a single person (B.B.), repeated three times, and the average of these three values was used in the analysis.
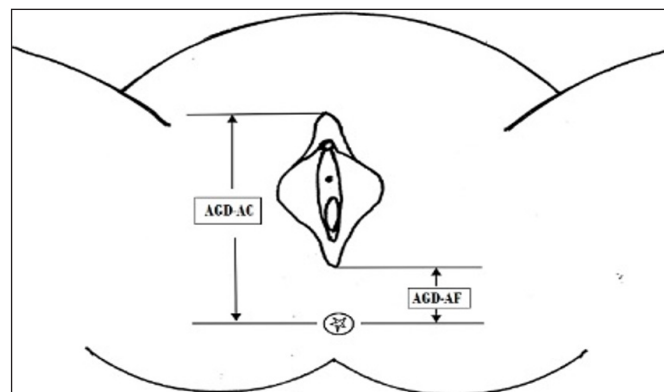
Figure 2. Neonatal anogenital distance (AGD) measurement in females

Study Participants

As inclusion criteria; singleton and term (≥37 weeks, <42 weeks) pregnant women with PCOS history and control group who gave birth in our hospital were included. Multiple pregnancies, in vitro fertilization (IVF) pregnancies, fetuses with major or minor fetal anomalies, pregnant women with any disease (such as: thyroid disease, diabetes, hypertension/preeclampsia etc.) pregnant women using medication, pregnant women with fetal growth restriction (FGR) were excluded from the study. For the number of samples, power analysis was performed with G-power 3.1.9.7 version using similar studies in the literature. Accordingly, the minimum number of patients for each group was calculated as 11.

Statistical Analysis

Statistical Package for the Social Sciences version 26.0 (IBM Corporation, Armonk, New York, USA) was used for data analysis. Significance level was taken as $p < .05$ in all analyzes. Shapiro-Wilk test was used to determine the distribution of the data. Student's t-test was used for normally distributed data in comparisons and data were presented as mean ± (SD). Mann-Whitney U test was used to compare the data that could not show normal distribution and the data were shown as median ± (min, max). Chi-square test was used to compare categorical variables and Odds ratio (95% Confidence interval) calculations were made.

RESULTS

A total of 119 patients, including 21 mothers with PCOS and female newborns, 35 mothers with female newborns in the control group, 21 mothers with PCOS and male newborns

and 42 mothers with male newborns in the control group, were included in this prospective study. Maternal, obstetric and neonatal characteristics of study participants who gave birth to female fetuses were analyzed in **Table 1**. Accordingly, maternal ages and body mass indexes (BMIs) of both groups were statistically similar. In the group of mothers with PCOS, 4 (19%) mothers were smokers, and 7 (20%) mothers from the control group were smokers (p=0.930). The mean gestational age at delivery of both groups was statistically similar (39±2.2 vs. 39.2±1.4, p=0.736). The birth weight (3443.3±665.9 vs 3231±3594), head circumference (33.6±1.3 vs 33.6±1.77) and body length (49.3±1.7 vs 49.8±1.2) of newborns was similar between the groups (p=0.189, p=0.881, and p=0.286, respectively). AGD-AF measurement was significantly higher in the female newborns from mother with PCOS than in the female newborns from control group (18.1±2.5 vs. 14.1±1.6, p=0.046). However, AGD-AC measurement was similar between the groups (36.7±2.7 vs. 36.3±2, p=0.512). (**Table 1**).

Table 1. Maternal, obstetric, and neonatal characteristics of study participants who gave birth to female fetuses

	Female newborns from mother with PCOS n=21	Female newborns from control group n=35	P
Maternal age (year) median (min,max)	28 (18-35)	29 (18-38)	0.529
Parity (n,%)			0.264
Nulliparous	7 (33.3%)	7 (20%)	
Multiparous	14 (66.7%)	28 (80%)	
BMI (kg/m2)	31.1±3.7	30.1±2.8	0.225
Smoking (n,%)	4 (19%)	7 (20%)	0.930
Gestational age at delivery (week) (mean±SD)	39±2.2	39.2±1.4	0.736
Delivery type (n,%)			0.833
Vaginal delivery	12 (57.2%)	21 (60%)	
Cesarean section	9 (42.8%)	14 (40%)	
Birth weight (g) (mean±SD)	3443.3±665.9	3231±3594	0.189
Head circumference of newborns (cm) (mean±SD)	33.6±1.3	33.6±1.77	0.881
Body length of newborns (cm) (mean±SD)	49.3±1.7	49.8±1.2	0.286
AGD-AF (mm) (mean±SD)	18.1±2.5	14.1±1.6	0.046
AGD-AC (mm) (mean±SD)	36.7±2.7	36.3±2	0.512
Abbreviations: BMI: Body mass index, AGD-AF: The distance between the center of the anus and posterior fourchette, AGD-AC: The distance between the center of the anus and the clitoris floor			

Maternal, obstetric and neonatal characteristics of study participants who gave birth to male fetuses were analyzed in **Table 2**. Accordingly, maternal ages and BMIs of both groups were statistically similar. In the group of mothers with PCOS, 2 (9.5%) mothers were smokers, and 5 (11.9%) mothers from the control group were smokers (p=0.776). The mean gestational age at delivery of both groups was statistically similar (39.5±0.3 vs. 39.2±1, p=0.756). The birth weight (3554.8±174.6 vs 3514.2±332.4), head circumference (34.9±0.5 vs 34.8±1.2) and body length (50.7±1.1 vs 51.2±1.3) of newborns was similar between

the groups (p=0.802, p=0.718, and p=0.656, respectively). AGD-AS measurement (24.8±1.2 vs. 23.5±3.5, p=0.195) and AGD-AP (55.4±1.5 vs. 55±4.1, p=0.406) measurement was similar between the groups. (**Table 2**).

Table 2. Maternal, obstetric, and neonatal characteristics of study participants who gave birth to male fetuses

	Male newborns from mother with PCOS n=21	Male newborns from mother with PCOS n=42	P
Maternal age (year) median (min,max)	29 (18-39)	28 (18-37)	0.718
Parity (n,%)			0.236
Nulliparous	4 (19%)	14 (33.3%)	
Multiparous	17 (81%)	28 (66.7%)	
BMI (kg/m2)	31.1±0.9	31.8±3.2	0.336
Smoking (n,%)	2 (9.5%)	5 (11.9%)	0.776
Gestational age at delivery (week) (mean±SD)	39.5±0.3	39.2±1	0.756
Delivery type (n,%)			0.444
Vaginal delivery	13 (61.9%)	30 (71.4%)	
Cesarean section	8 (38.1%)	12 (18.6%)	
Birth weight (g) (mean±SD)	3554.8±174.6	3514.2±332.4	0.802
Head circumference of newborns (cm) (mean±SD)	34.9±0.5	34.8±1.2	0.718
Body length of newborns (cm) (mean±SD)	50.7±1.1	51.2±1.3	0.656
AGD-AS (mm) (mean±SD)	24.8±1.2	23.5±3.5	0.195
AGD-AP (mm) (mean±SD)	55.4±1.5	55±4.1	0.406
Abbreviations: BMI: Body mass index, AGD-AS: The distance between the center of the anus and back of the scrotum, AGD-AP: The distance between the center of the anus and the posterior base of the penis			

Anogenital distance measurements were compared by dividing the PCOS group into two groups according to the presence or absence of hirsutism. AGD-AF was 20.3±3.5 in the PCOS with hirsutism group and 15.2±1.5 in the PCOS without hirsutism group, and the difference between them was statistically significant (p=0.041). AGD-AC measurement was similar in PCOS with and without hirsutism groups (37.7±2.9 vs. 35.3±2.1, p=0.306). In addition, AGD-AS (26.2±1.7 vs. 23±1.1, p=0.156) and AGD-AP (58.4±2.6 vs. 54.4±0.5, p=0.332) measurements were similar in both groups. (**Table 3**).

Table 3. Anogenital distance of pregnant women with PCOS with and without hirsutism

Female	PCOS with hirsutism n=14	PCOS without hirsutism n=7	P
AGD-AF (mm) mean±SD)	20.3±3.5	15.2±1.5	0.041
AGD-AC (mm) (mean±SD)	37.7±2.9	35.3±2.1	0.306
Male	**PCOS with hirsutism n=12**	**PCOS without hirsutism n=9**	
AGD-AS (mm) (mean±SD)	26.2±1.7	23±1.1	0.156
AGD-AP (mm) (mean±SD)	58.4±2.6	54.4±0.5	0.332
Abbreviations: AGD-AF: The distance between the center of the anus and posterior fourchette, AGD-AC: The distance between the center of the anus and the clitoris floor, AGD-AP: The distance between the center of the anus and the posterior base of the penis, AGD-AS: The distance between the center of the anus and back of the scrotum			

DISCUSSION

In this study, it was shown that female newborns born to women with PCOS history had higher AGD-AF measurements indicating testosterone exposure. In addition, when the PCOS group is examined within itself, AGD-AF measurement increases significantly in the presence of hirsutism. The findings may indicate the effect of testosterone in female fetuses.

Anogenital distance is accepted as a sensitive indicator of masculization of the external genitalia (11). Maternal androgens pass to the fetus via the placenta, and fetal androgens pass into the maternal circulation in the same way. In previous animal and human studies, it has been shown that maternal administration of androgenic and anti-androgenic agents affects AGD in the fetus (7). In various studies, it has been shown that AGD is shortened in the use of Bisphenol A, Triclosan, and Phthalate (7,12). In one study, females born to high-stress couples had a significantly longer AGD than females born to low-stress couples. In the same study, no significant difference was observed between males (13). All AGDs increase in female or male infants of women exposed to testosterone during pregnancy (14). This has also been supported in animal experiments (15). Based on this situation, Barret et al. (9) in 2018, AGDs of newborns of pregnant women with PCOS were examined. In this study, longer AGD-AF measurements were observed in the daughters of mothers with PCOS compared to the control group. However, AGD-AC measurements of both groups were similar. This result is similar to our study. Male fetuses were not evaluated in this study. Unlike this study, male fetuses were examined in our study and it was found that having a mother with PCOS did not affect AGD. Glintborg et al. (10) analyzed the AGDs of both male and female infants at 3 months born to mothers with PCOS in 2019. There was no effect of being a mother with PCOS on AGD in both female and male infants. In their study, maternal testosterone levels were also examined and maternal testosterone levels (total and free testosterone) were positively associated with AGD-AS and AGD-AP in boys, while AGD measurements in girls were not found to be associated with maternal testosterone levels. We think that the different result in this study (similarity of AGD-AF measurements between groups in female fetuses) may be due to the measurement performed at different times (at the 3rd month), racial differences, and patient selection criteria.

We compared the anogenital distance measurements in the PCOS group by dividing them into two groups according to the presence or absence of hirsutism. To the best of our knowledge, such a comparison has been made for the first time in the literature. The closest to our comparison is Barret et al. (9) in 2018, they compared the AGDs of the isolated hirsutism group (without PCOS), PCOS with hirsutism group and control group. They did not observe a significant AGD difference in the unadjusted analyzes between all three groups. In our study, we observed a significantly higher AGD-AF value in the PCOS with hirsutism group compared to the PCOS without hirsutism group. This difference may be due to testosterone exposure, which is the cause of hirsutism, but this needs to be supported by testosterone measurements.

The AGD is longer in males than females and is routinely used to determine sex (11,16). These lengths are also an indirect indicator of fetal androgen activity. In the study of Fowler et al. (17), AGD was found to be 1.4 times longer in males than females at 11-13 weeks, 1.8 times longer at 14-16 weeks, and twice times longer at 17-20 weeks. However, in the first trimester, when external genital organ differentiation has just begun, the role of these lengths in sex determination is also limited. Salazar-Martinez et al. (3) reported the male/female distance ratio at birth as 1.9, while Sathyanarayana et al. (2015) (18) reported as 1.5. In our study, AGDs of male fetuses were higher in both the PCOS group and the control group, which is consistent with the literature. Also, AGD is associated with varying degrees of birth weight, depending on the population studied (3,19,20). In fact, this correlates with fetal sex as well, because male fetuses are generally heavier than female fetuses.

This study had some limitations. First, we could not measure blood testosterone levels of pregnant women. Other limitation was that the diagnosis of PCOS was based on the patient's questioning in most cases. Our strengths are our prospective projection of single center data on a subject. To the best of our knowledge, this is the first study on this subject in the Turkish population. We also tried to examine a transparent population by excluding IVF pregnancies and pregnancies with drug use. We think that the results of the study will contribute to the literature.

CONCLUSION

The anogenital distance may change in female newborns in the presence of maternal PCOS. Considering that AGD reflects fetal testosterone exposure, the findings may reflect increased testosterone exposure in female fetuses of pregnant women with PCOS. The results pave the way for new studies; as a result of long-term AGD follow-up of newborns born to women with PCOS and lifestyle changes and treatments of PCOS patients, AGD values can be investigated further.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İzmir Tepecik SUAM Non-interventional Clinical Researches Ethics Committee (Date: 13.02.2019, Decision No: 2019/2-2).

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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HEALTH SCIENCES
MEDICINE

Analysis of clinical findings and serum micronutrients in pediatric patients with nonalcoholic fatty liver disease

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ABSTRACT

Aim: Nonalcoholic fatty liver disease (NAFLD) is the hepatic finding of systemic lipid and energy metabolism disorder. NAFLD is frequently observed in people with higher body mass index. Serum micronutrient levels play an important role in hepatic metabolism.

Material and Method: This study included 60 NAFLD and 66 control patients. . NAFLD and control groups were compared in terms of ultrasonography (USG) and shear wave elastography (SWE) results. The two groups were compared in serum lipid profile, aminotransferase, insulin, glucose, and HOMA-IR. Both groups were then analyzed in terms of serum ferritin, B12, and vitamin D levels.

Results: . 35% (n=21) of the patients in the NAFLD group had grade 1, 55% (n=33) had grade 2 and 10% (n=6) had grade 3 adiposity. HOMA-IR and insulin levels were higher in the NAFLD group (p=0.02; p=0.001). While the serum ferritin level of the patients in the NAFLD group was higher than the control group (p=0.001); the B12 level was lower (p=0.006). In terms of vitamin D, there was no difference (p=0.368).

Conclusion: It is essential to identify risk factors in children on follow-up due to NAFLD. USG and liver function tests remain the first option in the diagnosis and screening of NAFLD in children. Serum ferritin, B12, and vitamin D levels of children on follow-up due to NAFLD should be analyzed in consideration of liver fattening.

Keywords: Fatty liver, vitamin, ferritin, elastography

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the hepatic finding of systemic lipid and energy metabolism disorder. Impairment in carbohydrate and lipid metabolism leads to lipid accumulation in hepatocytes. NAFLD is more frequently observed in people with higher body mass index (Body mass index: BMI >30) and certain ethnic groups (1). The term NAFLD is a general terminology covering a wide diagnostic spectrum ranging from simple fatty liver to steatohepatitis. There are two subtypes of NAFLD: nonalcoholic fatty liver and nonalcoholic steatohepatitis (NASH). Unlike nonalcoholic fatty liver, NASH is considered a progressive form of the disease (2). NAFLD has been associated with obesity, Diabetes Mellitus (DM), hypertension, and dyslipidemia. Insulin resistance (IR) is also considered an independent risk factor for NAFLD severity. It is also thought to be an initiating factor (3).

The fact that NAFLD is recently observed highly regularly and at increasing frequency suggests that urgent counter-measures are necessary. Following the definition of the disease, it was understood that approximately 40% of patients previously diagnosed with cryptogenic cirrhosis were NAFLD (4). The prevalence of NAFLD is around 75% in obese patients (5). The incidence of NAFLD and NASH is increasing in children and adolescents due to obesity, IR, and metabolic syndrome resulting from a sedentary life (6).

The liver exerts a significant effect in micronutrient metabolism. Disruption of energy and nutrient metabolism causes NAFLD. Lipid accumulation in cells leads to lipotoxicity. Fibrosis may develop with the liver inflammatory and parenchymal cell reaction. It is thought that micronutrients play an important role in this cycle (7). There are studies on antioxidant vitamins A, B, and D in particular (8,9). Iron also has an association with NAFLD. It has been determined that liver lipid peroxidation and damage increase with the rise of iron and ferritin levels in the blood (10).

Vitamin D, another micronutrient, was found to be low in obese and NAFLD patients. It was noted that NAFLD is increased in obese rats with vitamin D deficiency. (11).

Micronutrients act as cofactors and play a key role in the liver synthesis, and inflammatory processes. Studies on micronutrients in children with NAFLD are limited. The objective this study; is a comprehensive evaluation of micronutrients, which are as important as carbohydrate and lipid metabolism, together with clinical, laboratory and imaging findings in children with NAFLD. It is thought that the results obtained in this study will guide the follow-up of children with NAFLD in averting progress and assisting treatment.

MATERIAL AND METHOD

This study is a single-center and cross-sectional study which was conducted with the permission of Gaziosmanpaşa Training and Research Hospital Ethics Committee (Date: 19.08.2020, Decision No: 144). All procedures involving human participants were performed in accordance with 1964 Declaration of Helsinki and subsequent amendments.

Children between the ages of 8 and 18 who applied to the pediatric gastroenterology clinic of Gaziosmanpasa Training and Research Hospital between 2018 and 2020 were admitted to the study. NAFLD patients were diagnosed with ultrasonography (USG). A liver biopsy was not performed since it is an invasive method. In the control group, children with similar age and gender characteristics and followed in the Pediatric Gastroenterology outpatient clinic were included in the study. In both NAFLD and control groups, children with BMI > 25 kg/m2 were admitted. According to clinical and laboratory findings, USG and shear wave elastography (SWE) USG results, children were assigned to NAFLD and the control groups. ALT value > 40 IU/L is considered high (12). Serum samples for liver function tests, lipid profile and ferritin, B12, and vitamin D levels were obtained after 12 hours of fasting. BMI values of NAFLD patients and control group were calculated. BMI was calculated using the standard body weight (kg)/height2 (m2) formula.

Abdominal USG was performed after 6 hours of fasting. All patients were analyzed with special age-appropriate probes along with SWE. It was accepted as a reliable measurement if at least 5 valid measurements were taken by SWE.

The exclusion criteria of the control and NAFLD groups are underlying chronic liver disease, drug-induced liver damage, hematological diseases, hypothyroidism, hyperthyroidism, type 1 Diabetes Mellitus, adrenal insufficiency, renal failure, and thrombosis history. Furthermore, patients who took vitamin and iron preparations in the last year were also excluded from the study.

Statistical Analysis
IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for statistical analysis. While evaluating the study data, the normality assumption was checked with the Kolmogorov-Smirnov (K-S) test. In addition to descriptive statistical methods (mean, standard deviation, frequency), Student's t-test was applied for intergroup comparisons of normally distributed parameters between two groups, and the Mann-Whitney U test was deployed for comparisons of abnormal distributed variables. The data followed an abnormal distribution. Fisher-Freeman-Halton test and Continuity (Yates) Correction were made to compare qualitative data. Pearson correlation analysis was used to analyze the relationships between normally distributed variables, and Spearman's rho correlation analysis was utilized to examine the relationships between the abnormal parameters. A p value of less than 0.05 was considered significant.

RESULTS

There were 60 children in the NAFLD group and 66 children in the control group, respectively.. NAFLD and control groups were similar in age and gender (p=0.07; p=0.216). There was no difference in terms of BMI and waist circumference (p=0.06). The ALT (alanine aminotransferase) and GGT (gamma-glutamyl transferase) values of the patients in the NAFLD group were higher (p=0.01; p=0.03). There was no difference between the two groups in terms of AST (aspartate aminotransferase) and lipid profile. The intergroup comparison of total cholesterol, triglyceride, LDL and HDL values revealed that only HDL value was lower in the NAFLD group (p=0.02). In terms of HOMA-IR, an indicator of insulin resistance, NAFLD group had a higher score (p=0.020). Similarly, insulin levels were also higher in the NAFLD group while no difference was detected in blood sugar levels (p=0.001; p=0.31) (**Table 1**).

NAFLD and control groups were analyzed in terms of USG and SWE results. Grade 1 adiposity was observed in 35% (n=21) of the patients in the NAFLD group, grade 2 adiposity was observed in 55% (n=33) and grade 3 adiposity was observed in 10% (n=6). Liver sizes are larger in the NAFLD group due to adiposity. SWE assessment was higher in the NAFLD group (p=0.002) (**Table 2**).

Table 1. Comparison of general clinical findings between NAFLD and control groups

	NAFLD group mean±SD	Control group mean±SD	p
Female	34 (56.60%)	48 (73%)	[2]0.216
Male	26 (43.40%)	18 (27%)	
Age	13.10±2.70	12.20±3.80	[1]0.070
BMI (kg/m2)	28.60±2.80	27.40±2.50	[1]0.060
Waist circumference (cm)	94.50±13.00	92.40±10.90	[1]0.060
ALT (IU/L)	63.50±26.00 (54.50)	27.60±8.30 (16)	[3]0.010*
AST (IU/L)	45.90±37.70 (30)	28.30±11.00 (26.50)	[3]0.138
GGT (IU/L)	32.00±20.30 (24)	17.60±9.00 (12)	[3]0.030*
Total cholesterol (mg/dl)	178.50±38.00	162.50±36.50	[1]0.376
Trigliserid (mg/dl)	158.60±76.10 (120.50)	139.00±48.10 (147)	[3]0.247
HDL (mg/dl)	43.50±8.60	49.80±9.70	[1]0.020*
LDL (mg/dl)	104±39.60 (104.50)	104.10±27.60 (102)	[2]0.850
Homa-IR	4±2,50 (3.40)	2.30±0.60 (2.40)	[1]0.020*
Insulin (median)	20±11.30 (14.40)	12±3.70 (11)	[2]0.001*
Blood sugar (mg/dl)	92.80±8.00	90.10±10.50	[1]0.310

ALT, alanine aminotransferease; AST, serum aspartate aminotransferease; BMI, body mass index; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease ; HOMA-IR, homeostatic model assessment insulin resistance ; 1Student t test ; 2Continuity (yates) correction ; 3Mann Whitney U test; *p<0,05

Table 2. Comparison of shear wave elastography (SWE) and USG results between NAFLD and control groups

	NAFLD group Mean±SD	Control group Mean±SD	p
Liver long axis (mm)	151.13±12.85	123.70±16.10	[1]0.001*
Liver SWE mean (m/s)	1.82±0.40 (1.74)	1.65±0.16 (1.60)	[2]0.002*
Hepatosteatosis n(%)			[3]0.001*
None	0 (0%)	66 (100%)	
Grade 1	21 (35%)	0 (0%)	
Grade 2	33 (55%)	0 (0%)	
Grade 3	6 (10%)	0 (0%)	

2Mann Whitney U test; 3Fisher Freeman Halton test; *p<0,05

Patients in the NAFLD group were compared with the control group in terms of micronutrients. While the ferritin level of the patients in the NAFLD group was higher (p=0.001); the B12 level was lower than the control group (p=0.006). In terms of vitamin D, there was no difference between the two groups (p=0.368) (**Table 3**).

Table 3. Evaluation of ferritin, B12 and vitamin D parameters between groups

	NAFLD group Mean±SD (median)	Control group Mean±SD (median)	p
Ferritin (mg / dl)	43.14±50.73 (26.80)	19.66±16.85 (16.80)	0.001*
B12 (mg / dl)	226.05±84.24 (215)	296.91±121.86 (269)	0.006*
Vitamin D (IU)	21.26±12.35 (18.80)	22.27±10.39 (22.50)	0.368

Mann Whitney U test; *p<0,05

DISCUSSION

NAFLD and related diseases, including insulin resistance and Diabetes Mellitus, may remain asymptomatic until they develop. NAFLD is the cause of nearly 7-11% of elevated liver function tests in obese patients. Moreover, in liver biopsies performed in obese patients, 74% of patients are diagnosed with NAFLD (13). Various clinical and biochemical indicators have been investigated for recognizing the presence of NAFLD in the early stage and distinguishing it from simple steatosis. These include clinical, biochemical, and metabolic laboratory results (14). In this study, NAFLD and control groups were evaluated in terms of age, gender, BMI, and waist circumference. The biggest risk factors for pediatric NAFLD are overweight and obesity. NAFLD is observed in 2-7% of normal-weight children as opposed to a rate of 50-80% in overweight and obese children (15). Manco et al. (16) reported that 92% of pediatric NAFLD patients had a Body Mass Index (BMI) higher than the 85th percentile and 84% of patients had a waist circumference greater than the 90th percentile. Furthermore, in a cross-sectional study, a significant correlation has been reported between waist circumference, adiposity, and the incidence of NAFLD with intra-abdominal fat tissue (17). Therefore, waist circumference may be a simple, useful, and beneficial screening tool in pediatric NAFLD.

AST and ALT, which are among the aminotransferases, are elevated in many liver diseases including NAFLD. In a multicenter study, AST and GGT were predictive for both NAFLD and NASH. However, it is not sufficient on its own to distinguish cases of NASH from simple steatosis thoroughly (18). It is also known that elevated aminotransferase levels are not specific for demonstrating liver damage and inflammation. In another pediatric study on NAFLD, approximately 65% of children with NASH had normal serum ALT and AST levels despite the progressed illness (19). Normal AST and ALT levels may not exclude liver injury and fibrosis in pediatric NAFLD. However, along with the high results in these tests, necessary screening tests for NAFLD should be performed in children who are overweight or obese. In the study, ALT values of in the NAFLD group were higher. In terms of AST, there was no difference between the two groups.

Serum lipid analysis results may show abnormal lipid metabolism (20). However, there is insufficient research on pediatric liver disease. In an adult study, analysis of molecular lipid concentrations in blood samples from 679 patients indicated that low-carbon number and double-bond triglycerols rose as lysophosphatidylcholine decreased in NAFLD patients (21). In present study, however, there was no difference in the lipid profile of NAFLD and control groups. It is expected that the HDL level is lower in the NAFLD group than in the control group.

Insulin Resistance (IR) is a significant metabolic abnormality related with NAFLD. It is also an important sign of disease seriousness in children. The intensity of IR was linked with hepatic fat deposition independent of general body adiposity. The prevalence of NAFLD is, therefore, higher in patients with hyperglycemia and Diabetes Mellitus. This mechanism has also been attributed by researchers to the fact that insulin resistance and hyperglycemia directly or indirectly increase advanced glycation products and proinflammatory cytokines and stimulate fibrosis (22). Similarly, insulin levels were higher in the NAFLD group in this study. Furthermore, there was no difference in bloodglucose levels.

USG is accepted as an effective method in diagnosing fatty liver in children and it has led to a great increase in NAFLD diagnosis lately. In NAFLD, the liver is usually expanded and it appears echogenic. This indicates the accumulation of fat in the parenchyma. However, it cannot determine the true extent of steatosis. The sensitivity of USG is significantly reduced in severely obese (BMI >40) and severely NASH individuals when hepatic fat deposition remains below 30% (23). USG can not reliably distinguish between simple steatosis and steatohepatitis and cannot exclude fibrosis. SWE can identify hepatic fibrosis in pediatric NAFLD using a technique comparable to ultrasound to noninvasively measure hepatic "stiffness" (24). In this study, NAFLD and the control group were analyzed for USG and SWE results. 35% of the patients in the NAFLD group had grade 1 adiposity, 55% had grade 2 adiposity, and 10% had grade 3 adiposity. It was also determined that liver sizes increased in the NAFLD group due to adiposity. The SWE assessment was higher in the NAFLD group, which was associated with fatty liver and fibrosis.

NAFLD is also related with aberrations in iron metabolism, and in the absence of genetic hemochromatosis, it causes elevations of intrahepatic free iron with slightly elevated serum ferritin and transferrin (25). This change is due to pro-inflammatory cytokines and adipokines.. In present study, ferritin levels in the NAFLD group were higher.

Vitamin B12 deficiency in the maternal diet has been associated with fatty liver in animal experiments (26). Defects in pathways related to fatty acid metabolism, amino acid metabolism, and glycolysis have also been observed in children born to mothers with B12 deficiency (27). Similarly, in this study, vitamin B12 levels in NAFLD group were lower than in the control.

Vitamin D deficiency is more common in obese patients than in normal-weight patients (28). Recent studies also indicate that low serum vitamin D is linked with insulin resistance and Type II diabetes, and that vitamin D supplementation can reduce insulin resistance (29). It has been noted that vitamin D deficiency in adults is related with liver steatosis, necroinflammation, and fibrosis in NAFLD patients (30). Contrary to these findings, there was no difference in vitamin D levels between NAFLD and control groups in this study. There is insufficient evidence regarding both the vitamin D level and the prevalence and severity of NAFLD as well as the effect of vitamin D supplementation in patients with NAFLD (31) Nevertheless, screening for vitamin D deficiency may still be beneficial for patients that are considered to be at high NAFLD risk.

Limitations of the Study

This study is subject to certain limitations. Patients in the NAFLD group were divided into three main groups based on the level of fatty liver. Due to the small number of patients in this group, the relationship between the level of fatty liver and ferritin, B12, and vitamin D levels could not be compared. Moreover, since liver biopsy could not be performed (since it is an invasive method and not an adequate indication), the patients were divided into two groups as NASH and simple steatosis. Therefore, an evaluation in terms of clinical findings and serum micronutrients was not possible.

CONCLUSION

Abdominal ultrasound and liver function tests are the most preferred tools for the diagnosis and screening of NAFLD in children. SWE can assist in the evaluation of hepatic fibrosis in a noninvasive manner compared to biopsy. All obese patients and children with a BMI >25 kg/m² are at risk and should be investigated for NAFLD. Serum ferritin, B12, and vitamin D levels of children on follow-up for NAFLD should be analyzed with consideration to their relationship with fatty liver.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gaziosmanpaşa Training and Research Hospital Ethics Committee (Date: 19.08.2020, Decision No: 144).

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

Referee Evaluation Process: Externally peer-reviewed.

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HEALTH SCIENCES MEDICINE

Investigation of changes in young cardiac pathology cases before and during the pandemic process

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ABSTRACT

Aim: It was aimed to evaluate the effect of pandemic in cardiac pathologies, especially acute coronary syndrome in young cases.

Material and Method: Between January 2019-May 2021, 510 young patients aged between 18-50 years with acute coronary syndrome, arrhythmia or pericarditis were evaluated. The patients were divided into two groups as pre-pandemic and pandemic period, and the pandemic period was divided into two groups as Coronavirus Disesase 2019 (COVID) (-)/(+). In addition, patients were divided into groups according to their diagnosis. Demographic data, diagnostic classifications, COVID-PCR results, white blood cell, mean corpuscular volume, neutrophil, lymphocyte, neutrophil lymphocyte ratio, platelet, platelet lymphocyte ratio, C reactive protein, glucose, troponin values and survival data of the patients were recorded.

Results: The median age of 510 patients included in the study was 44(39-48) years, 395(77.5%) were male. When the diagnoses were put into groups by time, unstable angina was the most common diagnosis in each group. In the COVID(+) group, 39(23.8%) non-ST-elevation myocardial infarction (NSTEMI), 17(10.4%) inferior MI, and 14(8.5%) anterior MI were found. Twenty three (4.5%) of all cases resulted in mortality. According to the diagnoses, the most common mortality was in the inferior MI group with 10(28.6%) cases (p<0.001). During the pandemic period, 13(7.9%) of the COVID(+) patients resulted in mortality (p=0.016).

Conclusion: Acute coronary syndrome cases and cardiac pathologies other than unstable angina increased in young cases during the pandemic process. Mortality rates in all groups increased significantly during the pandemic and especially in COVID(+) cases compared to pre-pandemic.

Keywords: Emergency department, COVID-19, acute coronary syndrome, mortality, pandemic

INTRODUCTION

Acute coronary syndrome (ACS) is defined as all clinical symptoms of acute myocardial ischemia. Clinical diseases such as unstable angina pectoris, non-ST-segment elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI) are included in the phrase ACS (1). Although myocardial infarction (MI) is a condition that is usually seen in people over the age of 45 and frequently affects the elderly population, some younger patients may also present with MI clinic. ACS was found to affect 0.5% of men and 0.18% of women between the ages of 35 and 44. It affected 20.5% of men over 60 and 17.1% of women (2).

The 2019 Coronavirus Disease (COVID-19) is an infection requiring a comprehensive approach. In addition to kidney and liver damage, the nervous system and cardiovascular system can be affected by this infection (3). Individuals with cardiovascular disease are predisposed to COVID-19 infection. In patients with cardiovascular dysfunction who are infected with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), there is an elevated risk of COVID-19-related adverse outcomes. There is also an increase in cardiovascular complications in these individuals (4). Most significantly, acute myocardial injury and troponin increase are severe prognosis and death indicators in COVID-19 cases. Therefore, these cases should be followed carefully and closely (5). Labile heart rate and aberrant blood pressure response to activity are common in COVID-19 patients, as are myocarditis and pericarditis, decreased myocardial flow reserve due to microvascular injury, myocardial infarction, heart failure, life-threatening arrhythmias, and sudden cardiac death. Venous and arterial thromboembolic disorders, such as coronary artery aneurysm, aortic aneurysm, accelerated atherosclerosis, and life-threatening pulmonary embolism, can also exist (6).

We aimed to compare and evaluate acute coronary syndrome and other cardiac pathologies in terms of epidemiological, demographic, laboratory and survival before and during the pandemic period with especially young cases. In addition to the fact that there is no such data in previous studies, we were encouraged to evaluate it in a specific age range, such as young cases, which made study more meaningful.

MATERIAL AND METHOD

The study was carried out with the permission of Medipol University Training and Research Hospital, Noninvasive Clinical Researches Ethics Committee (Date: 26.10.2021, Decision No:1064). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Population

This study involves 510 patients aged 18-50 years (115 females, 395 males; median age 44(39-48) years, range 19-50 years) who presented to the emergency department between January 1, 2019 and May 11, 2021 and were diagnosed with acute coronary syndrome, pericarditis, and atrial fibrillation/flutter.

According to the hospitalization period of the patients, two groups were formed: pre-pandemic and pandemic. The pre-pandemic phase between January 1, 2019 and March 10, 2020, and the pandemic period between March 11, 2020 and May 11, 2021, each consist of 14 months. Since the 11th of March, 2020 has been designated as the beginning of the COVID-19 pandemic in Turkey, this date has been used as a point of reference. Patients who applied during the pandemic period, those who had COVID-19 disease or those with a positive Polymerase Chain Reaction (PCR) test were divided into two groups as COVID (+) and the others as COVID (-). Patients were classified into six groups based on their diagnosis: unstable angina (UA), NSTEMI, inferior MI (IMI), anterior MI (AMI), pericarditis, and arrhythmia. Patients with atrial fibrillation and atrial flutter rhythm were included in the arrhythmia group. Individuals experiencing other heart rhythms were excluded from the study. In addition, patients were analyzed based on their survival and mortality at the conclusion of the treatment process.

Cases without defined additional disease were selected in the pre-pandemic group and the COVID(-) group of the pandemic group. In the pandemic period COVID (+) group, only the cases with COVID-19 disease or positive PCR test were included. These patients were admitted to the emergency department and laboratory tests were studied. In addition, all patients had an electrocardiogram (ECG). Patients' age, gender, ECG reports, laboratory results, and clinical course were recorded in the hospital data system.

Patients with no additional disease, aged between 18-50 years, with complete demographic, laboratory and ECG reports and definitive diagnosis records were included in the study. The study excluded patients with a history of cerebrovascular disease, cardiac pathology, arrhythmia, chronic and congenital heart disease, hormone-based disease, psychiatric drug history, chronic liver disease, renal failure and dialysis, infectious disease, chronic inflammatory disease, malignancy, severe anemia and anemia treatment, hematological disease, collagen tissue disease, and pregnancy. In addition, patients who did not have hemogram and biochemistry tests, as well as ECG or missing data records, were excluded from the study. Patients under the age of 18 and over the age of 50 were also excluded from the study.

Laboratory Analysis

Patients' levels of White Blood Cell (WBC), Mean Corpuscular Volume (MCV), neutrophil, lymphocyte, neutrophil lymphocyte ratio (NLR), platelet, platelet lymphocyte ratio (PLR), C reactive protein (CRP), glucose, and troponin were measured. A Beckman Coulter Automated CBC Analyzer was used to measure hemogram (Beckman Coulter, Inc., Fullerton, CA, USA). Cobas 6000 was used to conduct biochemistry analysis (C6000-Core, Cobas c-501 series, Hitachi, Roche, USA). Analyzers STAT Elecsys and Cobas e-411 Hitachi Roche were used to evaluate cardiac Troponin T (cTn-T). At the time of emergency room admission, a 12-lead electrocardiogram was recorded at the bedside using a Cardiofax ECG-9132K (Nihon Kohden, Tokyo, Japan). Cardiac troponin T values greater than 14 pg/mL were considered as the core (Reference range: 0-14 pg/mL).

Statistical Analysis

SPSS 20 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses on the data collected for this investigation. The Kolmogorov-Smirnov test was performed to check if the variables followed normal distributions. Case counts and percentages were displayed for nominal variables. Under the assumption of normality, the one-sample Kolmogorov Smirnov test concluded that the distribution was not normal in the variables at the $p < 0.05$ level of significance. Therefore, the variables reported as median and interquartile range (IQR) were subjected to the non-parametric Mann-Whitney U-test and Kruskal-Wallis Test. Chi-square analysis was used to look at the connections between the categories of nominal variables. Results were considered statistically significant when the p value was less than 0.05.

RESULTS

The median age of 510 patients included in the study was 44 (39-48) years, 395 (77.5%) were male. 227 (44.5%) of the cases formed pre-pandemic, 119 (23.3%) pandemic COVID (-), 164 (32.2%) pandemic COVID (+) group. During the pandemic period of the patients, in COVID (+) cases; WBC (p<0.001), neutrophil (p=0.039), NLR (p=0.023), CRP (p<0.001), Troponin T (p<0.001) were significantly higher. In the classification of diagnosis according to periods, UA was the most common diagnosis in each period. UA was less in 45 (27.4%) cases in the pandemic COVID (+) group than in the other groups. In the COVID (+) group, 39 (23.8%) NSTEMI, 17 (10.4%) inferior MI, and 14 (8.5%) anterior MI were observed, which was more in the evaluation made with other groups. While the frequencies of pericarditis, arrhythmia, STEMI and NSTEMI were similar in pre-pandemic and COVID (-) patients, a significant increase in all these diseases was detected in COVID (+) patients (p<0.001, **Table 1**).

There was no significant relationship between mortality and gender. Twenty three (4.5%) of all cases resulted in mortality. According to the diagnoses, the most common mortality was in the inferior MI group with 10 (28.6%) cases (p<0.001). During the pandemic period, 13 (7.9%) of the COVID (+) patients resulted in death (p=0.016). In the mortality group, CRP 24 (12-28) mg/L (p=0.018) and Troponin T 140 (114-166) pg/mL (p<0.001) values were significant, while other laboratory results were not associated with mortality (**Table 2**).

Table 2. Evaluation of mortality in terms of gender, laboratory results, diagnose classification and pandemic groups

	Mortality		P value*
	No n(%)	Yes n(%)	
Gender			0.924
Female	110 (95.7)	5 (4.3)	
Male	377 (95.4)	18 (4.6)	
Diagnose classification			<0.001
Unstable Angina	294(98.7)	4(1.3)	
Pericarditis	52(98.1)	1(1.9)	
Inferior MI	25(71.4)	10(28.6)	
NSTEMI	58(98.3)	1(1.7)	
Arrhythmia	34(100)	0(0)	
Anterior MI	24(77.4)	7(22.6)	
Pandemic Group			0.016
Pre-pandemic	221(97.4)	6(2.6)	
COVID(-)	115(96.6)	4(3.4)	
COVID(+)	151(92.1)	13(7.9)	
Total	487(95.5)	23(4.5)	
	Median (IQR)	Median (IQR)	
Laboratuary Findings			
WBC, 103/uL	9 (8-11)	11(8-12)	0.214
NEU, 103/uL	6 (5-8)	6(5-7)	0.601
LYM, 103/uL	3(2-4)	4(2-4)	0.176
NLR, %	1.75 (1.33-3)	1.75 (1.4-2.5)	0.525
PLT, 103/uL	257 (214-313)	233 (208-325)	0.660
PLR, %	87 (64.75-114.67)	77.33 (54.25-103)	0.096
CRP, mg/L	15 (6-24)	24 (12-28)	0.018
Glucose, mg/dL	113 (98-146)	125 (104-193)	0.087
Troponin T, pg/mL	52 (13-121)	140 (114-166)	<0.001

Data are given in number (percentile) or median (IQR): (25th-75th percentile). WBC: White Blood Cell, MCV: Mean Corpuscular Volume, NEU: neutrophil LYM: lymphocyte NLR: neutrophil lymphocyte ratio PLT: platelet PLR: platelet lymphocyte ratio CRP: C reactive protein COVID: Coronavirus Disease MI: myocardial infarction NSTEMI: Non ST myocardial infarction p: Statistical Significance (<0.05) * Chi-square test was used for gender and disease classification and pandemic groups, while Man Whitney–U test was used for other variables.

Table 1. Evaluation of pre-pandemic&pandemic groups in terms of laboratory results and diagnose classification

	All Patients n: 510, Median (IQR)	Pre-pandemic n:227, Median (IQR)	Pandemic		P-value*
			COVID (-) n: 119, Median (IQR)	COVID (+) n: 164, Median (IQR)	
Baseline Chracteristics					
Age (year)	44 (39-48)	43 (39-47)	43 (35-48)	45 (40-48)	0.107
Gender (F(%) / M(%))	115 (22.5)/395 (77.5)	56 (24.7)/171 (75.3)	30 (25.2)/89 (74.8)	29 (17.7)/135 (82.3)	0.193
Laboratuary Findings					
WBC, 103/uL	9 (8-11)	9 (8-11)	9 (8-11)	10 (8.25-12)	<0.001
NEU, 103/uL	6 (5-8)	6 (4-8)	5 (5-8)	6 (5-8)	0.039
LYM, 103/uL	3 (2-4)	3 (2-4)	3 (2-4)	3 (2-4)	0.300
NLR, %	1.75 (1.33-3)	1.67 (1.33-3)	1.67 (1.25-2.67)	2 (1.4-3)	0.023
PLT, 103/uL	256 (214-313)	256 (210-311)	259 (222-325)	253 (214.25-317)	0.149
PLR, %	86.58 (64.23-114.5)	85.33 (64.75-108.33)	85.33 (64-117.5)	88.5 (63.75-117.94)	0.705
CRP, mg/L	15 (6-25)	12 (5-23)	9 (5-18)	18 (10.5-29)	<0.001
Glucose, mg/dL	114.5 (98-146.25)	111 (98-140)	115 (98-146)	117.5 (100.25-160)	0.092
Troponin T, pg/mL	60 (14-124)	21 (6-63)	40 (14-113)	125 (96-158)	<0.001
Diagnose Classification [n(%)]					<0.001
Unstable Angina	298 (58.4)	182 (80.2)	71 (59.7)	45 (27.4)	
Pericarditis	53 (10.4)	8 (3.5)	13 (10.9)	32 (19.5)	
Inferior MI	35 (6.9)	10 (4.4)	8 (6.7)	17 (10.4)	
NSTEMI	59 (11.6)	11 (4.8)	9 (7.6)	39 (23.8)	
Arrhythmia	34 (6.7)	7 (3.1)	10 (8.4)	17 (10.4)	
Anterior MI	31 (6.1)	9 (4)	8 (6.7)	14 (8.5)	

Data are given in number (percentile) or median (IQR): (25th-75th percentile). F: Female M: Male WBC: White Blood Cell, MCV: Mean Corpuscular Volume, NEU: neutrophil LYM: lymphocyte NLR: neutrophil lymphocyte ratio PLT: platelet PLR: platelet lymphocyte ratio CRP: C reactive protein COVID: Coronavirus Disease MI: myocardial infarction NSTEMI: Non ST myocardial infarction p: Statistical Significance (<0.05), * Chi-square test was used for gender and diagnose classification, while Kruskal-wallis test was used for other variables.

When the age was evaluated according to the diagnostic groups, it was 41 (29.5-47.5) years in pericarditis patients, while it was 48 (43-50) years in the inferior MI group (p=0.001). While anterior MI was present in 30 (7.6%) of male patients, it was detected in only 1 (0.9%) of female patients. The frequencies of pericarditis and NSTEMI were remarkable in both genders (p=0.016). Platelet and PLR were not associated with the diagnostic groups. Glucose 126 (112-160) mg/dL (p=0.003), WBC 12 (9-13) 103/uL (p<0.001) , neutrophil 7 (5-9) 103/uL (p=0.046) and lymphocyte 4 (2-4) 103/uL (p=0.001) values were higher in the anterior MI group. Troponin T value was highest in the NSTEMI group with 137 (110-166) pg/mL (p<0.001, **Table 3**).

DISCUSSION

Recently, both acute cardiac pathologies and COVID-19 infection are among the most important causes of mortality and morbidity in emergency services and even in all medical units. Although the conditions of cardiac pathologies, especially acute coronary syndrome, during and before the pandemic have been partially evaluated by some studies in the general population, we aimed to evaluate the relationship between the two, especially in the young patient population, and contribute to the literature.

COVID-19 disease causes respiratory system, vascular endothelial, heart, intestine, and immune system cell infections (7). This factor enhances membrane fusion by binding to the highly expressed angiotensin-converting enzyme-2 (ACE-2) receptor via the spike protein (8). Particularly, endothelial cells and pericytes display high levels of ACE-2, which renders the cells extremely vulnerable to the COVID-19 interaction. The potential mechanisms underlying acute coronary syndrome in COVID-19 infection have not been clearly elucidated. In individuals with a confirmed diagnosis of myocardial infarction, the pathophysiological mechanisms may be explained by the angiographic appearance of unoccluded coronary arteries, numerous thrombotic lesions, and stent thrombosis (9,10). Occasionally, myocardial infarction has been examined as the initial symptom of the disease, which suggests that acute coronary syndrome is a particular thrombotic consequence of COVID-19 infection (11). The most widely acknowledged mechanisms are cytokine-mediated systemic inflammation reactions, endothelial dysfunction, prothrombotic stimulation of the coagulation cascade, and hypoxia destruction due to an imbalance in oxygen supply and demand. It may develop due to atherosclerotic plaque activation due to hyperinflammation or vasoconstriction. Another possible pathophysiological process may be related to microvascular thrombosis due to hypercoagulopathy due to COVID-19 disease (12).

In addition, despite the fact that this infection causes complications in the form of coronary plaque instability and myocardial oxygen supply, numerous researchers from all over the world have reported a significant decrease in the hospitalization rate for ACS during the peak of the pandemic.   In addition to all this, increased lengths of ischemia and gate-balloon time were observed in 2020 patients (13). During this period of time, there was a correlation between a lower admission rate for STEMI and a higher incidence of cardiac arrest outside of the hospital as well as mechanical complications (14).

Showkathali et al. (15) examined the admission and clinical results of acute coronary syndrome cases in the same two-month timeframes during the pandemic process and the previous 2 years, and showed that all

Table 3. Evaluation of disease classification in terms of age, gender and laboratory results							
	Unstable Angina Median (IQR)	**Pericarditis** Median (IQR)	**Inferior MI** Median (IQR)	**NSTEMI** Median (IQR)	**Arrhythmia** Median (IQR)	**Anterior MI** Median (IQR)	**P-value***
Age (year)	43(38-47)	41(29.5-47.5)	48(43-50)	44(41-48)	46.5(41-49)	46(41-49)	0.001
Gender							0.016
F (%)	73(63.5)	16(13.9)	7(6.1)	11(9.6)	7(6.1)	1(0.9)	
M (%)	225(57)	37(9.4)	28(7.1)	48(12.2)	27(6.8)	30(7.6)	
Laboratuary Findings							
WBC, 103/uL	9(8-11)	9(8-11)	10(9-11)	10.1(8-12)	10(8.7-12.2)	12(9-13)	<0.001
NEU, 103/uL	6(4.78-7.25)	6(4-8)	6(5-7)	6(5-7)	8(5-9)	7(5-9)	0.046
LYM, 103/uL	3(2-4)	2(2-3)	3(2-3.4)	3(2-4)	3(2-4)	4(2-4)	0.001
NLR, %	1.6(1.3-2.7)	2.5(1.5-3.4)	2(1.7-2.5)	2(1.4-3)	2.5(1.5-3.5)	2(1.2-3.7)	0.003
PLT, 103/uL	259(217-313)	238(194.5-286)	268(222-321)	248(210-319)	255(231-324.5)	257(209-358)	0.129
PLR, %	83.6(64.1-110.1)	94(73.5-119.7)	101.3(74-117.5)	90(60.5-117.5)	84(63.4-117.4)	87.2(59.2-104.7)	0.330
CRP, mg/L	11(5-21)	21(13.2-39.7)	15.5(8.5-24)	15(9-25.2)	15(5.5-24.5)	15(6-34)	<0.001
Glucose, mg/dL	110.5(97-141.3)	108(98-136)	120(106-172)	118(106-165)	112(94-147.2)	126(112-160)	0.003
Troponin T, pg/mL	18(6-52)	96(69.5-131.5)	120(90-152)	137(110-166)	101(58-128.7)	118(98-138)	<0.001

Data are given in number (percentile) or median (IQR): (25th-75th percentile). F: Female M: Male WBC: White Blood Cell, MCV: Mean Corpuscular Volume, NEU: neutrophil LYM: lymphocyte NLR: neutrophil lymphocyte ratio PLT: platelet PLR: platelet lymphocyte ratio CRP: C reactive protein MI: myocardial infarction NSTEMI: Non ST myocardial infarction p: Statistical Significance (<0.05), * Chi-square test was used for gender while Kruskal-Wallis test was used for other variables.

cases of NSTEMI, STEMI and UA decreased during the pandemic process. Again, in the study conducted for the same purpose, Braiteh et al. (16) In his study with an 8-week follow-up period, he showed a 41% reduction in applications for acute coronary syndrome. Although the decrease in isolated STEMI admissions was not significant, NSTEMI cases decreased significantly. Mimoso (17), on the other hand, stated that there was a decrease in the number of cases admitted in the pandemic and pre-pandemic evaluation, but the rate of STEMI increased during the pandemic and more clinically serious patients applied. Again, in the same study, it is not overlooked that the intervention period for these cases was prolonged. Zachariah et al. (18) evaluated the cases of acute coronary syndrome with 41,832 cases in 187 centers. They observed a 35.4% decrease in applications during the pandemic period. They found the increase in the rate of STEMI in the cases significant. Considering the mortality, the mortality rate in all myocardial infarction cases, which was 4.6% before the pandemic, was 4.9% during the pandemic period. In this study, no significant change was observed in mortality of STEMI cases, while mortality in NSTEMI cases was 2% before the pandemic, and it was found to be 2.8% in the pandemic, and it was significantly higher.

In this study, anterior and inferior myocardial infarction cases, which are STEMI groups, showed an increase in the distribution of cardiac pathologies during the pandemic process. This increase was observed more especially in COVID (+) cases. We believe that this increase is due to the fact that a fatal clinical condition such as myocardial infarction does not affect emergency hospital admissions during the pandemic process, especially in young cases, and that its numerical increase is due to the above-mentioned cardiac effects of COVID-19. In addition, there was an increase in NSTEMI cases. Contrary to similar studies, the increase seen in general myocardial infarction groups may be due to the younger age group and long period of time. Unstable angina cases were significantly decreased. We attribute these reductions to the effect of social lockdowns and curfews, to fear of coming into contact with patients infected with COVID-19. Mortality rates were found to be high during the pandemic process and especially in COVID (+) cases. The fact that the mortality rate is higher in cases with COVID (-) compared to the pre-pandemic period may be due to the late admission of the cases to the hospital or the partial deterioration of health service delivery in this period. Other potential causes include asymptomatic patients, misleading negative PCR results in patients, and negative results following COVID. We attribute the higher mortality rates in COVID (+) cases to the fact that the event has a worse prognosis with infection.

It has been previously known that myocardial damage and troponin elevation are not only indicators of myocardial infarction, but can also be seen with respiratory tract disorders. Viral diseases, of which we can give an example of the Middle East respiratory syndrome coronavirus, have been found to cause myocarditis with myocardial damage and troponin elevation (19,20). SARS-CoV-2 may also cause myocardial damage and myocarditis by directly or indirectly affecting the cardiovascular system (20). In COVID-19 individuals, the exact pathophysiology of acute pericarditis and myopericarditis is not yet completely characterized. An important step in the development of SARS-CoV-2 infection is dysregulation of the immune system, which can cause certain patients to produce an excessive amount of proinflammatory cytokines. This can lead to what is known as a "cytokine storm" (21). This elevated inflammatory response could be a contributing factor in the various cardiovascular manifestations that are linked to COVID-19, such as pericarditis and myopericarditis. Pericarditis cases, which we evaluated in cardiac pathologies in study, also increased in the pandemic process and especially in COVID (+) cases. We think that this is also related to the reaction of the infection in the cardiac layers during the inflammatory process.

Arrhythmia is among the presenting findings in patients with COVID-19 infection. It is observed that 7.3% of the patients diagnosed with COVID-19 have palpitations among their complaints (22). While arrhythmia is observed in 17% of hospitalized patients, this rate reaches 44% in patients followed in the intensive care unit (23). Patients with COVID-19 may have a higher risk of short- and long-term unfavorable clinical outcomes if they have atrial fibrillation, a common arrhythmia. Inciardi et al. (24) gathered data on 53 COVID-19 patients with a history of hospitalization for heart disease and pneumonia, including their demographics, clinical manifestations, and prognosis. Forty percent had a previous diagnosis of heart failure, and 36 percent were diagnosed with atrial fibrillation (AF). A review and meta-analysis of 187,716 people indicated that 8% of COVID-19 patients had AF, but that the true prevalence of AF could be as high as 27% due to substantial discrepancies between studies (25). In this study we conducted with young cases, we found that the frequency of arrhythmias, especially AF, increased gradually during the COVID process and in positive cases. Considering this result, we can say that the clinical status of COVID infection increases the frequency of AF in young people with the inflammatory process, but this is not associated with mortality.

The single-center and retrospective nature of the study can be considered among the major limitations. In addition, false negativity and positivity in PCR results, the possibility of partial disruption of patient registration and comorbidity information during the pandemic, and possible etiological errors in mortality data can be counted among other limitations.

CONCLUSION

It can be said that there is an increase in cardiac pathologies other than unstable angina during the pandemic process. Mortality in young cardiovascular pathologies, especially in ACS, has increased significantly during the pandemic process and especially in COVID(+) cases. On the other hand, the cases of unstable angina have also decreased. More prospective multicenter studies are needed to reveal cardiovascular effects in young COVID-19 cases.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Medipol University Training and Research Hospital, Noninvasive Clinical Researches Ethics Committee (Date: 26.10.2021, Decision No:1064).

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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Does the distance of the fixation points to the fracture affect healing in tibial shaft fractures treated with openable distal claw intramedullary nail?

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ABSTRACT

Objective: A retrospective examination was made of tibia shaft fractures treated with tibia intramedullary nail (IMN), which are designed with distal retractable claws, unlike classic imtramedullar nails. It was aimed to evaluate the effect on healing of the nail diameter and the distance between the fracture line and the proximal and distal fixation points of the IMN.

Material and Method: The study included 28 patients (18 males, 10 females; mean age 43.75 (18-69) years) treated with distal retractable claw tibia IMN (Dunitech Nite Tibial IMN Oliga Med Ankara-Turkey) for a diagnosis of unilateral tibia diaphyseal fracture between January 2020 and January 2022. The midpoint of the fracture line (F), the proximal fixation point of the locking screw (S), the distal fixation point of the retractable claw (T), and the isthmus mid-point (I) were determined as reference points. The FT, ST, and IT distances, the nail diameter (ND) and isthmus diameter (ID) were measured and the FT/ST ratio was calculated. Statistical evaluations were made of the relationships between the diameter and length measurements and the visual analog scale (VAS) and radiographic union score for tibia (RUST) scores at the end of one year.

Results: No statistically significant correlation was determined between the RUST and VAS scores and the diameter measurements or the distance between the proximal and distal fixation points of the IMN.

Conclusion: There was no effect on the fracture healing scores of the nail diameter or the distance between the proximal and distal fixation points in tibia shaft fractures treated with a tibia nail with distal retractable claws. The nail design with retractable claws provides strong fixation and stable fracture healing. The operating time is shorter resulting in less radiation exposure.

Keywords: Tibia Fracture, intramedullary nail, fracture healing

INTRODUCTION

Fixation with intramedullar nailing is the most preferred method in the surgical treatment of long bone fractures. IMN's are implants with the advantages of being minimally invasive, can be applied rapidly, provide good fracture fixation, and allow early mobilisation (1,2). Soft tissue trauma, blood loss, infection rates and wound complications are less in intramedullary fixation methods (3). The factors determining the efficacy of IMN are the nail design, whether or not it is grooved, the number and diameter of the locking screws, and bone quality. The distance between the locking screws and the fracture region is also important in stability (2,4,5).

Tibial nails designed with distal retractable claws are IMN's with a different design which are now being used in the treatment of tibial shaft fractures. In this design, while proximal fixation is made with classic locking screws, distal fixation is provided by the retractable claws attaching to the inner surface of the bone cortex. Distal retractable claws eliminate the problems of distal screw application (6,7). However, there is no study in the literature related to what the distance should be of the distal claws from the fracture region, even though these measurements are known to affect fracture healing. The question to which an answer was sought in this study was whether an increase in the distance from the fracture of the fixation claws in the distal of the fracture has a negative effect on fracture stability and associated fracture healing. As the medullar canal in the tibia widens towards the distal metaphyseal region, this could reduce the attachment strength of the claw structure in the medullar region with a larger diameter and this could have a negative effect on fracture healing. In this study, a

J Health Sci Med 2023; 6(1): 46-50

Alıç et al. Does the distance of the fixation points to the fracture affect healing?

retrospective evaluation was made of the results of patients applied with tibia IMN designed with distal retractable claws in the treatment of a tibial shaft fracture. It was also aimed to reveal relationships between the radiographic union score for tibia scores (RUST) and the visual analog scale (VAS).

MATERIAL AND METHOD

The study was carried out with the permission of Hitit University Non-Invasive Ethics Committee (Date: 28.09.2022, Decision No: 2022-20). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In this study, patients who were operated for tibia fractures between January 2020 and January 2022 were analyzed. Fractures were classified according to the AO/OTA classification. The retrospective evaluations and measurements of the patients were made by two senior experienced orthopaedists and a radiologist. The patients included were those with a unilateral tibia fracture, closed or Gustilo-Anderson type 1 open fracture, AO/OTA 43 tibia diaphyseal fracture, and at least one year of recorded follow up. Patients were excluded if they had any chronic systemic disease, multiple system trauma, or more than one fracture.

When applying IMN, the patient was positioned supine with the knee in 90° flexion. Entry was made with a mid-patellar incision in the proximal tibia and the patellar tendon was separated to two sides. The medullar canal was entered with a guide K-wire. Under fluoroscopy guidance, closed reduction of the fracture was performed. The medullar canal was reamed with 8, 9, 10, 11 mm diameter reamers consecutively over the guidewire. Following the reaming procedure, a 9 mm intramedullar nail was placed. Distal fixation was provided by fully opening the distal claws with a torque screwdriver. Proximal fixation was provided by first drilling over the guide apparatus then placing the locking screws. Alignment was checked under fluoroscopy then the opeeration was terminated.

Measurements were taken on the radiological images of the fracture retrieved from the patient records (**Figure 1**). The medullar canal mid-point of the fracture line (F) and the mid-point of the tibial isthmus (I) were taken as reference points on the radiographs. The most distal point from the proximal locking screws (S), and the most proximal point of the retractable claws making the distal fixation (T) were determined. The distances between these points were measured as FT, ST, and IT, and the isthmus diameter (ID) was measured (**Figure 2**). In addition to these measurements, parameters such as operation time, hospital stay, union time, delayed union, development of nonunion, open fracture, and necessity for secondary surgery were examined. The development of an axial or rotational deformity on the radiographs was analyzed.
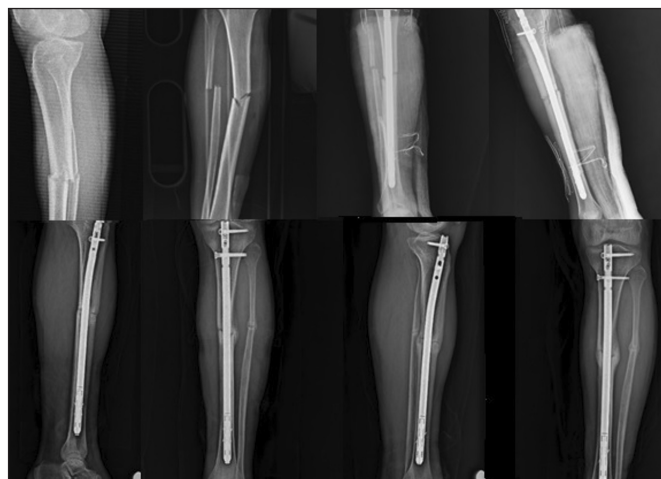
Figure 1. The appearance of the intramedullary nail in the direct radiographs of the patients
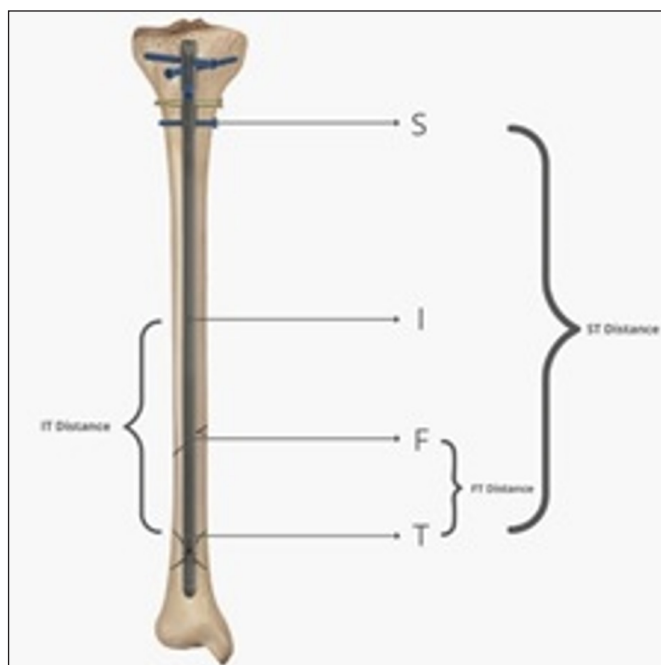
Figure 2. Length and diameter measurements drawing

RUST score for radiological healing and the VAS for functional healing of the patients were examined at the end of one year and recorded. The relationships between the distance and diameter measurements and the VAS and RUST values were statistically evaluated.

Statistical Analysis

The SPSS (Version 22,0, SPSS Inc, Chicago, IL, USA) package program was used to perform statistical analyses on the data gathered in this study. The normal distribution of the data was tested with the Shapiro-Wilk tests. Descriptive statistics of continuous variables were reported using median (min-max) and mean ± standard deviation (SD). Descriptive statistics of categorical data were reported as numbers and percentages (%). Correlations between numerical variables were investigated with Spearman correlation coefficient in accordance with the depending on data distribution. The statistical significance level was evaluated as $p < 0.05$.

Alıç et al. Does the distance of the fixation points to the fracture affect healing?

J Health Sci Med 2023; 6(1): 46-50

RESULTS

The study included 28 patients with a unilateral AO/OTA 42 tibia diaphyseal fracture treated with distal retractable claw tibia IMN in our clinic. The patients comprised 10 (35.7%) females and 18 (64.3%) males with a mean age of 43.75±17.89 years. In all the patients, fixattion was applied with a 9 mm diameter intramedullary nail. The mean operating time was 36.68±8.87 mins and the mean length of stay in hospital was 8.96±6.26 days. In five (17.9%) patients, the fracture was Gustilo-Anderson type 1 open fracture. Delayed union developed in four (14.3%) patients and union was obtained without the need for any additional surgical procedure. The mean time to union was 15.28 weeks (range, 12-26 weeks). No axial or rotational misalignment was observed on the radiographs during follow up. The descriptive statistics of the demographic and clinical characteristics of the patients are shown in **Table 1**.

There was no statistically significant correlation between radiological length measurements (FT, ST, IT) with radiological fracture healing score (RUST) and visual analog scale (VAS).

There was no statistically significant relationship between radiological length measurement ratio (FT/ST ratio) with radiological fracture healing score (RUST) and visual analog scale (VAS).

There was no statistically significant relationship between radiological diameter measurement ratio (ND/ID ratio) with radiological fracture healing score (RUST) and visual analog scale (VAS).

There was no statistically significant relationship between radiological diameter measurement difference (ID-ND diameter difference) with radiological fracture healing score (RUST) and visual analog scale (VAS) (**Table 2**).

In four patients, the nails were removed on patient request without any problems after full fracture union. Deep vein thrombosis, pulmonary embolism, infection or compartment syndrome, shortness or malunion, refracture development, intramedullary nail failure or breakage in the locking screw or claw structure were not observed in any patient.

DISCUSSION

The biomechanical characteristics of different IMN designs used in tibia shaft fractures can change the duration of application, duration of radiation exposure, fracture stability, healing and the functional effect. Nail designs which are easy to apply and provide stable fracture healing should be more preferred. Shorter operating times, less radiation exposure and stable fracture fixation have been obtained with different IMN designs used in tibia fractures. However good the stability is in locking IMN's, there are the disadvantages of difficulties in application (6-9). The ease of application of expandable nail designs or nails with retractable claws for distal fixation is a great advantage for orthopaedic surgeons (6,7,10,11). Nails designed to be completely expandable without locking screws eliminate the complications that can develop during

Table 1. Statistics on socio-demographic and clinical characteristics		
	n	**Percent (%)**
Gender		
Male	18	64.3
Female	10	35.7
Mechanism		
Fall	22	78.6
Traffic accident	5	17.9
Crush İnjury	1	3.6
Side		
Right	17	60.7
Left	11	39.3
Fracture type		
Closed Fracture	23	82.1
Open Fracture	5	17.9
AO Classification		
A2	14	50
A3	14	50
Union		
Delayed Union	4	14.3
Complete Union	24	85.7
	Mean±SD	**Median (min-max)**
Age	43.75±17.89	45.5 (17-69)
Hospitalization Days	8.96±6.26	6 (3-24)
Operation Time (minute)	36.68±8.87	37.5 (22-53)
Number of Scopy Shots	35.11±13.14	33.5 (15-56)
Union time (week)	15.28±3.88	14 (12-26)

Tablo 2. Relationships between the RUST and VAS scores and the F-T, S-T, I-T distances, the FT/ST ratio, the diameter difference in the ID-ND, and the ND/ID ratio).						
	F-T distance (mm)	**S-T distance (mm)**	**FT/ST ratio**	**I-T distance (mm)**	**ID-ND difference (mm)**	**ND/ID ratio**
RUST Score						
r	-.234	-.118	-.206	-.293	.004	-.004
p	.230	.551	.292	.130	.983	.983
VAS Score						
r	.131	-.045	.120	.247	.006	-.006
p	.505	.819	.542	.205	.977	.977
Spearman correlation coefficient						

J Health Sci Med 2023; 6(1): 46-50

Alıç et al. Does the distance of the fixation points to the fracture affect healing?

the application of locking screws and the lengthening of the operating time (12,13). The nail design we used in our study was preferred because it has these features. We think that the average operation times, the necessity of using fluoroscopy and the length of hospital stay are reduced. In fracture fixation made with classic locking intramedullar nails, the distance of the distal screws from the fracture line is important in stabilty. Pourmokhtari (4) used the definition of functional length in IMN's. This definition is basically the distance between the points fixed to the bone with screws in the proximal and distal of the nail. The placement of these screws affects the biomechanics of the fracture. The closer the screw is to the fracture region, the greater the exposure to pressure. Therefore, to increase fracture stability, there should be a distance of at least 2 cm between the screws at the end of the nail and the fracture region (4). Similarly, Eveleigh (14) named the part remaining between the fixation points in the proximal and distal of the IMN as the "working length". This definition may also be at the point which is fixed when the nail is in contact with the medullar canal wall. The torsional rigidity of an IMN and the bone structure where it is applied is in inverse proportion to the working length of the nail. It was also stated that intramedullar implants of similar diameter have similar bending rigidity (14). In another study it was reported that a distance of more than 3cm between the distal screw region and the fracture line in femoral IMN could cause complications such as non-union, malalignment, fixation failure, or progressive reduction loss (15). While research of classic locking IMN's is still ongoing there should also be examinations of IMN's of different designs. In nails designed with distal fixation applied with retractable claws, the effects on fracture healing of the claws placed close to or far from the fracture line are not known.

In our study, the ST distance, defined as the working length, was measured as 25.2 cm on average. No relationship was observed between ST distance and torsional or an axial deformity. In addition, ST distance had no effect on fracture healing. Although the lengths between the proximal and distal fixation points were reported as biomechanical fracture fixation failure in other nail designs, we found that it had no effect on the retractable claw nail design used in our study. Because although mechanical stability is very important, in our opinion, the biological aspect of fracture healing is also important. We found the fracture point and retractable claw FTs to be 8.7 cm on average. Despite this, no problems were observed in fracture stability and fracture healing. This showed us the biomechanical durability of the removable distal hook fixation.

Another important point in IMN is the nail diameter. The ratio of the diameter of the femoral intramedullary canal in the isthmus region (FI ratio) as ≥ 2 has been presented as a parameter predicting complications (15). A previous biomechanical study reported that it is possible to increase the stability of locking IMN and provide the least movement in the fracture region with the use of the thickest nail and performing the least reaming (16). However, the use of the smallest diameter nails is more often recommended because they can be applied more quickly with a shorter operating time and less radiation exposure (17). In all the cases in the current study with a large diameter (mean 13.3 mm) medullar canal, small diameter (9 mm) nails were used. However, the absence of serious rates of union problems shows the functional strength of the retractable claw design. The design of the distal claw means that it can be opened in an intramedullar canal of up to 38mm. It is thought that even if the IMN is of small diameter, when there is strong distal and proximal fixation, no problems will be experienced in fracture healing. There is a clear need for biomechanical studies showing the fixation strength, and new studies are being planned in this respect.

Another important parameter defined in tibial shaft fractures is the ratio of the nail diameter to the intramedullar canal diameter. It has been reported that this ratio should be between 0.80 and 0.99 for ideal fracture healing (18). In the current study cases, the mean nail-canal ratio was 0.68 (0.52-0.81) but this was not consistent with the fracture healing scores. With further studies of larger patient series applied with IMN and where union problems are seen, this ratio could be significant. In the current study, no cases of non-union were seen and delayed union was determined in 14.3%. The most commonly observed fracture healing problems after IMN in patients with tibia shaft fracture have been reported to be delayed union at 22.8% and non-union at 16% (19, 20). A nail diameter/reamer diameter ratio of 0.80-0.99 in the application of tibia IMN has a positive effect on fracture healing (19). In the current study, the IMN diameter was 9 mm and the reamer diameter was 10 or 11 mm in all the patients. As the nail/reamer diameter ratio in this study was 0.81-0.90, this supports the literature.

Limitations

The limitations of our study were the use of a single type of intramedullary nail and the lack of comparison with a different intramedullary nail. In addition, the number of patients remained small because it was a single-center study. Another limitation was the inability to perform biomechanical studies.

CONCLUSION

In the application of Dunitech Nite Tibial Intra Medullary Nail to AO/OTA 42 A2 and A3 fractures, the distance between the fracture line and the proximal screw and the distal retractable claw had no effect on the fracture healing scores. Even though the nail diameter was small, fracture healing and positive functional results were obtained. The complication rate was low. As the distal claw structure in the design of the nail provides extremely good attachment, there is no need for distal screw fixation. In this way, the ease-of-application results in a shorter operation with less exposure to radiation.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Non-Invasive Ethics Committee (Date: 28.09.2022, Decision No: 2022-20).

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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Nutrition knowledge levels and nutritional supplement beliefs of professional karate athletes

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ABSTRACT

Aim: The aim of this study is to evaluate the relationship between the nutritional knowledge levels, nutritional supplement belief levels, and body mass indexes of professional karate athletes.

Material and Method: Professional karate athletes (1st Dan and above) attending various karate schools in the province of Ankara, Turkey, and volunteering to participate in the study were included in the study. A total of 106 sportspeople, 52 male and 54 female, with an age average of 24.08±6.54 years, participated in the study. The participants answered the Nutrition for Sports Knowledge Questionnaire and the Sports Supplements Belief Scale questions after the questionnaire in which their demographic and anthropometric characteristics were questioned.

Results: When the sportspeople's average nutrition knowledge level was examined, it was determined that they are at a weak level of knowledge (34.18±13.84) and that they are less prone to using doping and similar banned nutritional supplements (17.79±9.01) in terms of their approach to sports nutritional supplements. No statistically significant difference was found in terms of the sports supplements belief scale and the nutrition for sports knowledge questionnaire based on the sportspeople's sex, age, karate categories, training duration, and BMI (p>0.05).

Conclusion: Adequate and balanced nutrition habits are very important for sportspeople to have good physical performance as well as health. Clubs, coaches and sportspeople seeking success in national and international fields should cooperate with a nutrition expert.

Keywords: Combat sports nutrition, karate sports nutrition, nutrition knowledge, sports supplement, body mass index

INTRODUCTION

Combat sports represent approximately 25% of all Olympic games and include a wide variety of contact sports disciplines, in which two opponents with similar physical characteristics face each other in order to knock out the opponent or get more points than the opponent (1). Karate sport has also been included in the "Olympic Combat Sports" in recent years. Apart from karate, boxing, judo, taekwondo, fencing, and wrestling are also included in this group of sports (2). Karate is an ancient martial art that is thought to have emerged on the Japanese island of Okinawa and its origins date back to China. It is a martial art in which hands and feet are used to disperse and block beats. Karate aims to develop oneself physically with certain fighting techniques and to establish a balance between mind control and body and mind (3). Karate tournaments may include Kata and/or Kumite competitions, which may consist of individual and team components. Kata involves the use of predetermined techniques without direct confrontation, while Kumite involves free fighting with an opponent using the same techniques (4).

Exercise performance describes the efforts exerted by the sportsperson within the prescribed time to achieve a certain goal. In general, the concept of sportsperson is divided into two as professional sportsperson and amateur sportsperson. While professional sportspeople are people who perform sports as their profession and continue their lives thanks to the sports they are active in, amateur sportspeople are people who struggle for the sports they are interested in, with the purpose of winning, but without expecting financial return from their sports activities (5). Factors such as aerobic and anaerobic energy capacity, technique, tactical skills, and motivation affect sports results by helping sportspeople develop their potential in the field of sports (6). There are

numerous interactions between nutrition and exercise, and nutrition has a great influence on determining long-term exercise performance results. Sportspeople's nutrition has become a subject that attracts more and more attention in recent years, on which a lot of studies has been performed. Accordingly, it is an area where sports scientists, sportspeople, their families, and coaches should have accurate and sufficient information. The most important goal in sportspeople's nutrition is to protect and improve the general health of the sportsperson, to increase his performance, and to make the sportsperson have an adequate and balanced diet depending on his age, sex, energy expenditure, physical activity, and dietary habits (7). Sportspeople consist of a population with special nutritional needs. With proper body composition and proper nutrition, it is ensured that the sportsperson can train more and longer, while at the same time, with faster recovery, the risk of fatigue, illness, and injury is reduced and his athletic performance is increased (8). Evidence-based sports nutrition principles should be applied to optimize the health and performance of sportspeople. Inadequate intake of macronutrients and micronutrients may lead to decreased training adaptation of the sportsperson, decreased performance, and increased risk of injury and disease (9). Increasing sportspeople's nutrition knowledge is of great importance in improving their nutritional behavior (10). Sports nutritionists aim to increase sportspeople's sports performance by increasing their nutrition knowledge levels in order to make positive changes in their food consumption (7). Nutrition education programs are developed to increase nutrition knowledge in order to consume adequate and balanced food, improve health status and provide higher athletic performance for sportspeople. Sportspeople's nutrition knowledge is generally similar or better than the general population (11). Nutrition knowledge is one of the determinants of dietary behaviors and affects individuals' food intake levels. As with all individuals, correct nutrition knowledge enables sportspeople to make informed decisions about their health (12). The increase in sportspeople's nutrition knowledge is associated with an increase in their performance levels by gaining healthier dietary habits. However, many factors including sex, age, socio-demographic characteristics, and educational level are defined as demographic factors affecting nutrition knowledge. In studies on sportspeople, there are some studies showing that factors that can increase focus on food intake, especially athletic ability, type of exercise, and physical capacity, are associated with nutrition knowledge (7,13,14).

Nutritional supplements are used by sportspeople for the purpose of protecting health, meeting appropriate

energy and nutrient needs, eliminating or preventing nutrient deficiencies, directly increasing performance or providing support for training, reducing the risk of injuries and diseases, and achieving an indirect performance increase as a result of accelerating the recovery process and improving the mood. Some supplements, when used properly, can help sportspeople meet their nutrition goals and provide performance enhancement (15,16).

It is known that there is a strong interaction between training and nutrition, and nutrition plays an important role in adaptation to training so that sportspeople can achieve high-level exercise performance. The aim of this study is to evaluate the relationship between the nutritional knowledge levels, nutritional supplement belief levels, and body mass indexes of professional karate athletes. Although there are studies on nutrition knowledge levels and the use of nutritional supplements in other sports branches in the literature, no research is found on karate athletes, so it is thought that this study will shed light on the literature.

MATERIAL AND METHOD

The study was carried out with the permission of Ankara Medipol University, Noninvasive Clinical Researches Ethics Committee (Date: 03.12.2021, Decision No: 56). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Participants

The study was carried out with the sportspeople aged 18 and over who volunteered to participate in the study and professionally engage in karate, and live in Ankara between December 2021 and January 2022. The sportspeople included in the study were selected from two different centers. A total of 106 karate athletes, 52 male and 54 female participated in the study. The study data were collected using the face-to-face survey technique. After informing the participants about the purpose of the study consent was obtained for each individual.

Instruments

The participants answered the Nutrition for Sports Knowledge Questionnaire (17) and the Sports Supplements Belief Scale (18) questions after the questionnaire in which their demographic and anthropometric characteristics were questioned.

The Demographic, Health, Nutrition Information and Anthropometric Measurements

In the first part of the questionnaire applied face-to-face to individuals who volunteered to participate in

the study, the individuals were asked questions about their age, sex, duration of performing karate, seniority, karate category, number of trainings per week, and training duration, educational level, nutritional education status, and the person from whom they receive training, their state of thinking whether their nutritional knowledge is sufficient or not, the amount of water consumed daily, the amount of water consumed during training, the use of nutritional supplements, their state of thinking whether their nutrition is sufficient or not, smoking status, number of main and snack meals per day, frequency of eating out. The body mass index of all individuals participating in the study was calculated by dividing the body weight (kg) reported by the participants by the square of the height (m) (BMI=kg/m²). According to the World Health Organization (WHO), 18.5 kg/m² of BMI is classified as underweight, 18.5-24.9 kg/m² as normal, and 25 kg/m² and above as overweight (19).

The Nutrition for Sports Knowledge Questionnaire (NSKQ)

The questionnaire, originally named "The Nutrition for Sports Knowledge Questionnaire" (NSKQ), which was developed by Trakman et al. in 2017 to evaluate the nutrition knowledge of adult sportspeople, composes of a total of 89 items and the reliability coefficient (Cronbach's Alpha) was found to be 0.906 (17). In the Turkish validity and reliability study of the Nutrition for Sports Knowledge Questionannaire (NSKQ) conducted by Çırak and Çakıroğlu (2019), The use of the questionnaire in the evaluation of the Turkish sportspeople's nutrition knowledge was found appropriate and recommended (20). The scoring information of the questionnaire was obtained from Çakıroğlu herself, and according to the information received, the overall performance in NSKQ (68 items were accepted as a total of 100) is evaluated using the scoring system as "poor" knowledge (0-49%), "average" knowledge (50-65%), "good" knowledge (66-75%), and "excellent" knowledge (76-100%). In this study, the Cronbach's alpha coefficient of the questionnaire was found to be 0.859.

The Sports Supplements Belief Scale

The validity, reliability, and validation study of the scale, orginally named The Sports Supplements Belief Scale (18) was conducted by Karanfil et al. (21). The scale is a 6-point Likert-type scale consisting of six questions. The scale items are in the form of -1- strongly disagree, -6- strongly agree. The lowest score that can be obtained from the scale can be calculated as 6 points by giving the answer I strongly disagree -1-, and the highest possible score can be calculated as 36 points by giving the answer I strongly agree -6- to all questions.

Statistical Analysis

SPSS for Windows, version 24.0, was used to analyze the data (SPSS Inc., Chicago, IL, United States). Frequency tables and descriptive statistics were used to interpret the findings. For the measurement values suitable for normal distribution, parametric methods were used. In accordance with the parametric methods, while the "Independent Sample-t" test (t-table value) method was used to compare the measurement values of two independent groups, the "ANOVA" test (F-table value) method was used to compare three or more independent groups. For the measurement values not suitable for normal distribution, the non-parametric methods were used. In accordance with the non-parametric methods, while the "Mann-Whitney U" test (Z-table value) method was used to compare the measurement values of two independent groups, the "Kruskal-Wallis H" test (χ2-table value) method was used to compare three or more independent groups. The "Spearman" correlation coefficient was used to examine the relationship between two quantitative data not having a normal distribution. The Binary Logistic Regression: Backward LR model was used to determine the factors affecting poor knowledge status.

RESULTS

The general characteristics and dietary habits of the participants are given in **Table 1**. It was determined that 54 individuals (50.9%) were female, 41 (39.6%) were in the 21-25 age group, 42 (39.6%) had been practicing karate for 6-10 years, and 60 (56.6%) were 1st Dan. It was determined that 61 individuals (57.6%) performed Kumite, 38 (35.7%) trained 3 days a week, 75 (70.8%) spent 2 hours in training, and 60 (56.6%) had high school/equivalent educational level. It was determined that 86 individuals (81.1%) were single, 83 (78.3%) did not receive education on nutrition, 16 (69.6%) received education on nutrition from a dietitian, and 46 (43.4%) considered their nutrition knowledge insufficient. It was determined that 100 individuals (94.3%) thought that nutrition and sports were closely related, 54 (50.9%) had 3 main meals, 53 (50.0%) had 2 snacks and 62 (58.5%) did not skip meals. It was determined that 21 individuals (47.7%) skipped lunch, 25 (56.8%) skipped meals due to lack of appetite, 63 (59.4%) ate out 1-4 times a week, and 52 (49.1%) had 2-3 liters of water daily. It was determined that 64 individuals (60.4%) consumed 1-1.5 liters of water during training, 12 (11.3%) used supplements, 80 (75.5%) had a BMI in the normal range, and 92 (86.8%) had an average/good nutritional status (**Table 1**).

Table 1. Distribution of socio-demographic findings and dietary habits of individuals					
Variable (n=106)	**n**	**%**	**Variable (n=106)**	**n**	**%**
Sex			Marital status		
Female	54	50.9	Single	86	81.1
Male	52	49.1	Married	20	18.9
Age [x-±SD→24.08±6.54 (years)]			Nutrition and sport relationship		
≤20	37	34.9	Closely related	100	94.3
21-25	41	38.7	Unrelated	6	5.7
>25	28	26.4	Daily water consumption		
Duration of performing karate [x¯±SD→9.75±6.29 (years)]			1 liter	41	38.7
≤5	29	27.4	2-3 liters	52	49.1
6-10	42	39.6	>3 liters	13	12.2
>10	35	33.0	Water consumption during training		
Seniority			0.5 liter	33	31.2
1st Dan	60	56.6	1-1.5 liters	64	60.4
2nd Dan	27	25.5	>1.5 liters	9	8.4
3rd Dan and over	19	17.9	Use of nutritional supplements		
Karate category			Yes	12	11.3
Kata	17	16.0	No	94	88.7
Kata and Kumite	28	26.4	BMI [x-±SD→22.4±3.31 (years)]		
Kumite	61	57.6	Underweight	8	4.7
Number of training per week			Normal	80	75.5
1 day	2	1.9	Overweight	17	16.0
2 days	9	8.5	Obese	4	3.8
3 days	38	35.8	Nutritional status assessment (self-assessment)		
4 days	29	27.4	Bad	14	13.2
5 days or more	28	26.4	Average/good	92	86.8
Training duration			Nutritional education status		
1 hour	21	19.8	Yes	23	21.7
2 hours	75	70.8	No	83	78.3
3 hours	7	6.6	The person from whom education on nutrition is taken		
4 hours or more	3	2.8	Coach	6	26.1
Educational level			Dietician	16	69.6
High school and equivalent	60	56.6	Doctor	1	4.3
Undergraduate	41	38.7	Nutrition knowledge (is it self-reported)		
Master's degree	3	2.8	Not sufficient	46	43.4
Doctoral degree	2	1.9	Sufficient	42	39.6
			No idea	18	17.0

The scale scores of the answers given by the individuals to the scales are given in **Table 2**. It was determined that the answers given by the individuals to the scales were generally at a high reliability level. When the average nutrition knowledge level of the sportspeople was examined, it was determined that they have poor knowledge (0-49%) and that they may be less prone to using doping and similar banned nutritional supplements in terms of their approach to sports nutritional supplements (**Table 2**).

No statistically significant difference was found in terms of the sports supplements belief scale and nutrition for sports knowledge questionnaire scores depending on sex, age, karate categories, training duration, and BMI classification of the sportspeople (p>0.05) (**Table 3**).

Table 2. Distribution of findings on scales					
Scales (n=106)	**Average**	**Standard deviation**	**Median**	**Min**	**Max**
The sports supplements belief scale	17.79	9.01	18.0	6.0	36.0
The nutrition for sports knowledge questionnaire	34.18	13.84	36.0	0.0	69.1

Table 3. Comparison of the sports supplements belief scale and nutrition for sports knowledge questionnaire scores depending on some characteristics of the karate athletes

Variable	n	The sports supplements belief scale		The nutrition for sports knowledge questionnaire	
		x̄±SD	Median [Min-Max]	x̄±SD	Median Min-Max]
Gender					
Female	54	17.68±8.51	18.0 [6.0-36.0]	33.55±14.06	36.0 [0.0-63.2]
Male	52	17.91±9.59	17.5 [6.0-36.0]	34.84±13.70	35.3 [0.0-69.1]
Analysis*		Z=-0.006		t=-0.478	
Possibility		p=0.995		p=0.633	
Age grades					
≤20	37	18.40±9.09	18.0 [6.0-36.0]	33.14±13.65	38.2 [0.0-63.2]
21-25	41	16.75±9.08	14.0 [6.0-36.0]	32.17±12.77	33.8 [0.0-52.9]
>25	28	18.50±8.98	19.0 [6.0-36.0]	38.49±15.07	38.9 [1.5-69.1]
Analysis		χ2=1.170		χ2=3.081	
Possibility		p=0.557		p=0.214	
Category					
Kata	17	15.59±9.07	12.0 [6.0-36.0]	37.19±8.36	36.8 [22.1-52.9]
Kata&kumite	28	19.11±9.71	20.5 [6.0-36.0]	35.24±16.11	36.8 [0.0-69.1]
Kumite	61	17.80±8.69	18.0 [6.0-36.0]	32.86±13.96	33.8 [0.0-63.2]
Analysis		χ2=1.416		F=0.761	
Possibility		p=0.493		p=0.470	
Training duration					
1 hour	21	17.95±8.36	19.0 [6.0-30.0]	29.97±15.92	30.9 [1.5-69.1]
2 hours	75	17.48±8.92	16.0 [6.0-36.0]	34.76±13.58	36.8 [0.0-64.7]
3 hours and more	10	19.80±11.63	22.5 [6.0-36.0]	38.68±9.33	38.2 [22.1-52.9]
Analysis		χ2=0.278		F=1.583	
Possibility		p=0.870		p=0.210	
BMI					
Underweight	8	18.80±8.67	18.0 [6.0-30.0]	26.18±16.09	29.4 [1.5-42.7]
Normal	80	17.96±9.15	19.0 [6.0-36.0]	34.50±12.28	35.3 [0.0-64.7]
Overweight/obese	21	16.90±8.90	14.0 [7.0-36.0]	34.88±18.46	38.2 [0.0-69.1]
Analysis		χ2=0.070		F=0.883	
Possibility		p=0.965		p=0.417	

*While the "Independent Sample-t" test (t-table value) was used to compare two independent groups with normal distribution in terms of the measurement values, the "ANOVA" test (F-table value) method was used to compare three or more independent groups. The "Mann-Whitney U" test (Z-table value) was used to compare two independent groups without normal distribution in terms of the measurement values, the "Kruskal-Wallis H" test (χ2-table value) method was used to compare three or more independent groups.

When the relationship between the answers given by the karate athletes participated in the study to the scales used in the study and the body mass index was examined, it was determined that there is no statistically significant relationship between the scales and BMI (kg/m²) values (p>0.05) (**Table 4**).

As a result of the Backward: LR logistic regression analysis conducted in the scale in which the sportspeople's nutrition knowledge was measured, using estimated parameters that may have any effect, based on poor knowledge level (0-49%), the optimal model is given in the table. In the current model, age (year) was determined to be an important parameter (p=0.004<0.05). As age increases by 1 unit, the risk of poor knowledge will decrease by 20.8% (OR=0.792). Karate duration (year) was determined to be an important parameter (p=0.004<0.05). As the time to practice karate (years) increases by 1 unit, the risk of poor knowledge will increase by 24.5% (OR = 1.245). The amount of water (liter) consumed during training was determined to be an important parameter (p=0.040). As the amount of water (liter) consumed during training increases by 1 unit, the risk of poor knowledge will decrease by 77.2% (OR=0.228) (**Table 5**).

Table 4. Investigation of the relationships between the scales and BMI (kg/m²) values

Correlation* (n=106)		BMI (kg/m²)
The sports supplements belief scale	r	-0.005
	p	0.956
The nutrition for sports knowledge questionnaire	r	0.050
	p	0.608

* The "Spearman" correlation coefficient was used to examine the relationship between two quantitative data that did not have a normal distribution.

Table 5. The Logistic Regression model formed on weak knowledge level

Variable	B	S.H.	Wald	sd	p	OR	95% Confidence Interval (OR)	
							Min	Max
Age (year)	-0.233	0.081	8.337	1	0.004	0.792	0.676	0.928
Karate duration (year)	0.219	0.090	5.887	1	0.015	1.245	1.043	1.486
Training water (liter)	-1.479	0.721	4.200	1	0.040	0.228	0.055	0.938
Fixed	7.810	1.950	16.044	1	0.000	24.216		

CCR=90.6%, χ2(8)=3.293; p=0.857

DISCUSSION

Genetic structure, appropriate training programs, and nutrition are the main factors affecting sportspeople's performance. The recent increase in interest in sports nutrition can be attributed to the fact that nutrition improves performance (22). For sportspeople, nutrition knowledge covers all of the nutrition-related practices and strategies specific to sports performance. A sportsperson should have sufficient nutrition knowledge by considering its importance on performance, recovery, and health while making daily food choices (23, 24). As a result of this study, it was determined that the nutrition knowledge level of karate athletes was poor (34.18±13.84). Additionally, it was determined that as the age of karate athletes increases, the level of nutrition knowledge increases; and as the duration of karate practice increases, the risk of poor knowledge increases. When the sportspeople in the study were evaluated according to the karate type (Kata and Kumite), it was determined that there was no difference in terms of nutrition knowledge. In addition to this, no relationship was found between the body mass indexes of the sportspeople and their nutrition knowledge levels. It was stated that the participants who received nutrition training received nutritional advice and information from a dietitian (69.6%), a trainer (26.1%) and a doctor (4.3%), respectively. In a study evaluating the nutrition knowledge levels of one hundred and ten professional sportspeople, it was determined that 5.4% of the participants had good and moderate nutrition knowledge, while 94.6% of the participants had insufficient nutrition knowledge. Additionally, similar to this study, no relationship was found between the body mass indexes of the sportspeople and their nutrition knowledge levels (25). In the study conducted by Devlin et al. (7) on 66 participants, the average nutrition knowledge score for all participants was 69.9 ± 11.9 (57%) out of 123 points. In a study conducted in Lebanon, the nutrition knowledge of basketball players was questioned and it was determined that only 20% of the sportspeople had sufficient nutrition knowledge (26). In a study conducted on football players, it was determined that most of the sportspeople (79.2%) had insufficient knowledge of sportspeople's nutrition (27). In a systematic review of 36 studies on the nutrition knowledge of sportspeople and coaches by Trakman et al. (14) supporting the results of this study, it was concluded that older participants had better general nutrition knowledge.

Sportspeople believe that more nutritional supplement consumption is necessary to manage compelling training efforts, maximize recovery, improve performance and/ or prevent disease and maintain overall health (28). Although the use of nutritional supplements is often preferred by sportspeople to increase performance,

the use of prohibited substances to increase physical performance is called doping. Doping use is ethically and legally wrong. For this reason, sportspeople should not use supplements that fall into the doping class (29). For this reason, it is important to increase the knowledge level of sportspeople about nutritional supplements. In a study involving 567 athletes in Canada, it was determined that 98% of the athletes used nutritional supplements (30). Creatine is in the category of nutritional supplements with performance-enhancing effects (31). Creatine is one of the most used supplements by sportspeople (32). Creatine's effects such as increasing exercise capacity, increasing adaptation to training, and providing faster recovery make it available as an ergogenic aid (33). Supporting this information, Claudino et al. (34) found that creatine supplementation prevented the training-induced gradual decrease in lower extremity performance during pre-season progressive training in professional elite football players. L-Carnitine is known as one of the supplements with supportive effects for body-weight loss. L-carnitine provides a decrease in body weight with the energy conversion it performs by contributing to fat oxidation and backing up muscle glycogen during exercise (35). In this study, 88.7% of professional karate athletes do not use any nutritional supplements. As a result of this study, it was determined that individuals are less prone to using doping and similar banned nutritional supplements in terms of their approach to sports nutritional supplements (17.79±9.01). It was determined that those who use nutritional supplements (n=11) prefer to use multivitamins, protein powder, creatine, and L-carnitine. Since the rate of use of nutritional supplements by the sportspeople is relatively low compared to the literature and it was determined that people may be less prone to the use of doping and similar prohibited nutritional supplements in terms of their approach to sports nutritional supplements, it is thought that the professionalism of the sportspeople and the sports branch may have affected this situation.

CONCLUSION

Adequate and balanced nutrition habits are very important for sportspeople to have good physical performance as well as health. Nutrition knowledge is one of the determinants of dietary behavior and affects individuals' food intake. The fact that sportspeople have adequate nutrition knowledge enables them to make informed decisions about their health. Inadequate nutrition knowledge of sportspeople, on the other hand, negatively affects their dietary habits and diet quality, leading to a decrease in their sportive performance. It is important to establish education on nutrition programs in order to increase sportspeople's knowledge in this

field and to positively affect their nutritional habits and accordingly to improve their sportive performance. In addition to determining the nutrition knowledge level of sportspeople, other issues that they lack should be determined and training should be given on these issues. Rational use of nutritional supplements can support the sportsperson in case of an insufficient nutrient intake. Incorrect or prohibited substance use can cause sportspeople to end their sports lives. For this reason, it is important for sportspeople to have sufficient knowledge about the correct use of nutritional supplements when needed. Sportspeople, need to be trained about nutrient intake and timing before, during, and after exercise to maximize their performance. Clubs, coaches and sportspeople seeking success in national and international fields should cooperate with a nutrition expert. At this point, it is very important to emphasize the role of qualified dietitians in sports clubs. This is a descriptive study investigating the nutrition knowledge levels of karate athletes and the evaluation of some data on karate sports. Longitudinal intervention studies are needed to see the impact of education on nutrition on individuals' attitudes and behaviors.

Limitations: As the study was carried out during the COVID-19 pandemic, the anthropometric measurements of the participants such as body weight and height were taken based on their own statements. Additionally, since there are not enough studies on karate athletes, the comparisons were made with other sportspeople. However, this study was conducted on karate athletes living in Ankara and cannot be generalized.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Medipol University, Noninvasive Clinical Researches Ethics Committee (Date: 03.12.2021, Decision No: 56).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: The author 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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Reciprocal activation changes of lower extremity muscles caused by the abdominal hollowing maneuver in patients with unilateral lumbar disc herniation: an electromyography study

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ABSTRACT

Aim: Decreased or delayed multifidus and transversus abdominis (TrA) activity, transition of the TrA from tonic to phasic activity, and increased activity in the more superficial erector spinae muscles are behaviors unique to people with lumbar disc herniation (LDH). This study investigates whether the abdominal hollowing maneuver (AHM), which activates the TrA, can improve the rates of impaired muscle reciprocal activation of the lower extremities due to unilateral LDH during walking, tandem walking, and stair climbing activities.

Material and Method: The healthy and affected lower extremities of 17 patients with unilateral LDH were analyzed. The participants performed three activities and three times without the AHM. For the walking activity, the participants took a total of eight steps without deviating from their normal gait pattern. For the tandem walking activity, the participants covered the eight-step distance by performing heel-to-toe walking. For the stair climbing activity, the participants climbed a total of four steps without support from their upper extremities. The researchers visually checked the postures of the participants during all stages of the activities. The ratio of tibialis anterior (TA) and medial gastrocnemius (MGC) electromyographic values that emerged during the activities to the maximum voluntary isometric contraction (MVIC) values of these muscles was called MVIC%. Then the MVIC% values of the TA and MGC were matched, and the muscle reciprocal activation ratio ("MVIC%"-TA/"MVIC%"-MGC) was determined. While the activities were being performed, the MVIC% values of both muscles were measured separately without and with the AHM.

Results: Reliability values ranged from 0.87 to 0.99, with an SEM of 2.22 to 11.98. The ICC3,1 was considered "good" or "excellent" for all muscle surface electromyography measurements. During the tandem walking activity performed with the AHM, the reciprocal activation rates of TA: MGC on the affected and healthy legs converged (p=0.010, d=0.71). However, TA: MGC reciprocal activation rates did not differ between the affected and healthy extremities in the walking (p=0.519, d=0.16) or stair climbing (p=0.180, d=0.35) activities performed with the AHM.

Conclusion: According to the results of the study, integration of the AHM into tandem walking activity brought the reciprocal activation rates of both legs closer to each other and enabled them to exhibit similar behaviors, even without adherence to any exercise protocol. Therefore, tandem walking can be selected as an appropriate activity to combine with spinal stabilization exercises performed by unilateral L4-L5 radiculopathy patients using the AHM along with the task.

Keywords: Abdominal hollowing maneuver, disc herniation, lower extremity muscles, surface electromyography, tandem walking

INTRODUCTION

Lumbar disc herniation (LDH) is a spinal degenerative disorder that can cause low back and leg pain, loss of muscle strength, and functional impairment in adult and middle-aged populations (1,2). In the symptomatic treatment of LDH, noninvasive or minimally invasive methods should be considered primarily (3). Physical and behavioral therapy, medication, and interventional therapy are noninvasive or minimally invasive methods used for the treatment of LDH (4). Physical and behavioral therapy techniques used in the management of LDH consist of exercise (5), traction (6), manual therapy (7), electrotherapy (8), and heat interventions (9), from a high to a low evidence level, respectively.

Spinal stabilization exercises are a commonly used method for low back pain (LBP) management (10). Muscular imbalance of the lumbopelvic region puts excessive pressure on the vertebrae and can lead to LBP Abdominal hollowing maneuver (AHM) and abdominal bracing maneuver (ABM) are commonly used to stabilize the trunk (11). AHM is a method of selectively contracting the transverse abdominis muscle and internal oblique abdominis (IO). ABM increases the stability of the vertebrae against sudden perturbations and reduces the movement of the lumbar spine (12). Decreased or delayed multifidus and transversus abdominis (TrA) activity, transition of the TrA from tonic to phasic activity, and increased activity in the more

Türkmen et al. Reciprocal activation changes of lower extremity muscles

J Health Sci Med 2023; 6(1): 59-65

superficial erector spinae muscles are behaviors unique to people with LBP (12, 13). The AHM is commonly used to increase spinal stability (14). The AHM activates the TrA and internal oblique muscles and minimizes the activity of the more superficial muscles (15).

In patients with unilateral LDH, phasic rather than tonic activity is observed in the TrA and multifidus muscles, which can be activated by the AHM (16). Activation of the TrA, multifidus, and pelvic floor muscles with the AHM provides lumbopelvic motor control by stabilizing the spine, hips, and lower extremities during movement. Thus, it is thought that deterioration in the reciprocal activation of the muscles working with agonist/antagonist activity due to muscle weakness in the lower extremity caused by LDH can be fixed by using the AHM alone, without any intervention in the related muscle. (12).

Previous studies have shown that AHM can affect lower extremity muscle activations. Kim et al. (14) suggested that AHM and ABM performed during side-lying hip abduction would increase pelvic stabilization. In another study, Lee examined the effects of three different abdominal maneuvers during different lower extremity activities and showed that both AHM and ABM increased lumbar stabilization (17). Enjuoo et al. (15) investigated the effect of the AHM on pelvic rotation during straight leg lifting activities and suggested that the AHM should be used to provide lumbopelvic stabilization. Although the current literature aims to examine the rotation angles of abdominal maneuvers to increase stabilization, it has been frequently assumed in recent years that these maneuvers can change the muscle activation rate of the trunk and lower extremities. Harput et al. (18) found that the rate of abdominal coactivation would affect lower extremity activation during functional exercises. Park et al. (19) similarly observed that AHM changed the activity of both trunk and quadriceps femoris muscles. Ennis et al. (20) investigated the effects of the ABM on gastrosoleus and tibialis anterior (TA) contraction rates and reported an increase in gastrosoleus activity on moving surfaces and tibialis anterior activity on stable surfaces. Although there has been an increase in studies examining the relationship between muscle activation rate and abdominal maneuvers in recent years, these studies are generally experimental studies performed in healthy individuals. There are no studies in the literature suggesting which exercise is appropriate for problems that prevent activation of lower extremity muscles, such as LDH, especially during the exercise stages where return to daily life activities are planned. Therefore, the aim of the present descriptive laboratory study was to investigate whether the isolated AHM could compensate for functional impairments caused by tibialis anterior muscle weakness due to unilateral L4-L5 disc herniation. We hypothesized that individuals with unilateral L4-L5 LDH would increase the reciprocal activation rate of the TA and medial

gastrocnemius (MGC) muscles on their affected sides with the isolated AHM during walking, tandem walking, and stair climbing activities and that it would become similar to the rate on their healthy sides. In this way, it will be revealed which of the activities of daily living will be more beneficial when combined with AHM.

MATERIAL AND METHOD

The study was carried out with the permission of Çankırı Karatekin University Faculty of Health Sciences Ethics Ethics Committee (Date: 09.11.2021, Decision No: 23). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Participants

Between February and June 2022, 17 subjects with LDH (6 females, 11 males; Age: 50.613.9 years BMI: 27.67.1) voluntarily participated in the study. (**Table 1**). The healthy and affected lower extremities of seventeen subjects with a diagnosis of unilateral LDH were analyzed. All measurements were performed at Çankırı Karatekin University, Physiotherapy and Rehabilitation Laboratory. The inclusion criteria were a diagnosis of unilateral protrusion by a neurosurgeon and a diagnosis confirmed by magnetic resonance imaging (MRI) reports. Disc herniations requiring surgical intervention and cases with bilateral herniations were excluded from the study. All participants provided informed consent before enrollment.

The sample size was calculated using G*Power 3.1 software (21). The effect size (dz) was calculated as 1.88 based on the changes in muscle activation patterns reported in a study by Nelson-Wong et al. (22) . The results of power analysis indicated that to detect improvement with $\alpha=.05$ and $\beta=90\%$, the estimated sample size was 16.

Procedures

Surface EMG (sEMG) Measurement Protocol: sEMG adjustments. The sEMG device (Noraxon USA, Inc., Scottsdale, AZ, USA) was used to measure the activation levels of the TA and MGC. The common-mode rejection ratio was greater than 80 dB and the input impedance was greater than 10 MΩ. The sampling rate for the sEMG data was 1000 Hz. Bipolar Ag/AgCl surface electrodes were placed at an interelectrode distance (center to center) of 2 cm. The electrode was 1 cm in width.

The electrode sites on the body were prepared by shaving the hair, abrading the skin with fine sandpaper, and cleaning the surface with 70% isopropyl alcohol to minimize skin impedance. Electrode placement was determined according to the SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) criteria (23). A licensed physical therapist who was trained and

J Health Sci Med 2023; 6(1): 59-65

Türkmen et al. Reciprocal activation changes of lower extremity muscles

experienced placed the fine-wire sEMG electrodes in accordance with a standardized protocol. The investigator placed the first sEMG electrode to measure the healthy TA and the second to measure the TA on the affected side due to protruded LDH. The electrodes were placed 1/3 proximal to the line formed by the proximal tip of the fibula and the medial malleolus for the TA. The researcher placed the third and fourth electrodes on healthy MGC muscles and MGC muscles affected by protruded LDH, respectively. The last two electrodes were placed on the most prominent bulge of both MGC muscles.

Determination of Maximum Voluntary Isometric Contraction (MVIC): For MVIC testing of the TA muscle, the researcher positioned the participant's ankle joint in dorsiflexion and inversion without extension of the big toe. The researcher then applied resistance to the dorsomedial surface of the foot in the direction of plantar flexion of the participant's ankle joint and eversion of the foot. The participants exerted maximum effort during the MVIC test. They performed 1 trial to understand the procedure. After that they performed 3 repetitions of 5-second duration. During the MVIC determination, the subjects received standardized verbal encouragement to produce maximum effort. A 2-minute rest was given between measurements.

Determination of MVIC% of TA and MGC. The ratio of TA and MGC values that emerged during the activities to the MVIC values of these muscles was called MVIC%. The MVIC% values of the TA and MGC were matched and muscle reciprocal activation values were TA: MGC ("MVIC%" TA / "MVIC%" MGC). During the activities, the MVIC% values of both muscles were measured separately when performing and not performing the AHM.

Activities
Activities performed without the abdominal hollowing maneuver. The researchers showed the patients how to perform the walking, tandem walking, and stair climbing activities. The participants performed all activities three times without the AHM. For the walking activity, the participants took a total of eight steps without deviating from their normal gait pattern. For the tandem walking activity, the participants covered the eight-step distance by performing heel-to-toe walking. For the stair climbing activity, the participants climbed a total of four steps without support from their upper extremities. The researchers visually checked the postures of the participants during all stages of the activities.

Activities performed with the abdominal hollowing maneuver. AHM was taught to the participants in three stages. Participants performed AHM training in both supine and standing positions. In the first stage, the correct breathing pattern for diaphragm activation was demonstrated. In the second stage, the participants applied the movement of pulling the lower abdominal wall slightly towards the spine. Researchers used a pressure biofeedback device for participants who had difficulty learning movement. In the final stage, the patients were taught how to contract the pelvic floor muscle. The training continued until the participants were able to continue the AHM they learned in three stages for 40 seconds. Then, they performed walking, tandem walking, and stair climbing activities with AHM.

sEMG Signal Processing
Noraxon MyoResearch XP Master Edition software (Noraxon, Scottsdale, AZ, USA) was used for the sEMG data processing. The sEMG signals were band-pass filtered (20–450 Hz) and smoothened using the root mean square with a 20-millisecond time window. The peak sEMG activity was measured during 5 s of walking, tandem walking, and stair climbing activities. Next, the internal consistencies between the three repeats determined using the sEMG signals were analyzed by statistical methods (**Figure 1**).
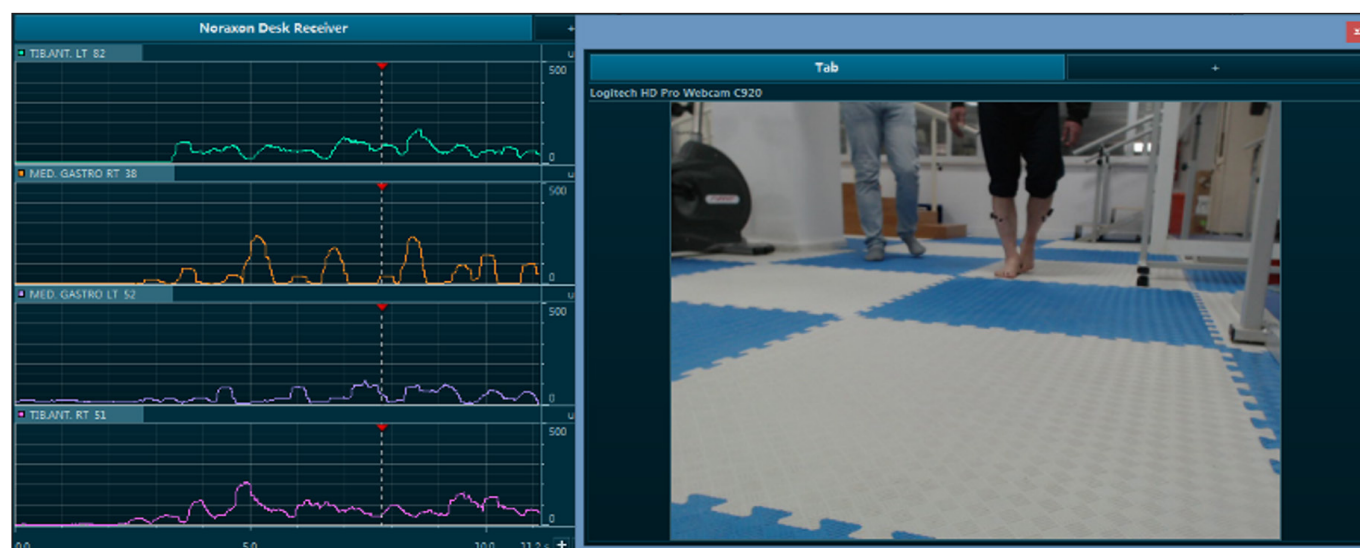
Figure 1. sEMG data recorded during tandem walking activity

Türkmen et al. Reciprocal activation changes of lower extremity muscles

J Health Sci Med 2023; 6(1): 59-65

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics v.28.0.1.1. (IBM, USA). All data were expressed as means and standard deviations for the descriptive data. The intraclass correlation coefficients (ICC3,1) and standard errors of measurement for the mmHg FS error value for the three replicates were used to determine the consistency between trials. The ICCs were classified as poor (< 0.50), moderate (0.50–0.75), good (0.75–0.90), and excellent (≥0.90) (24). The standard error of measurement (SEM) was calculated using the SEM formula (25). The paired samples t-test was used for time-dependent measurements to measure the effect of AHM on reciprocal activation, whereas the independent samples t-test was used for independent comparison of mean change differences.

RESULTS

Reliability values ranged from 0.87 to 0.99 with an SEM of 2.22 to 11.98 (**Table 1**). The ICC3,1 was considered "good" or "excellent" for all muscle sEMG measurements. The reliability of the measurements (ICC3,1) was considered "excellent" for healthy TA muscle sEMG measurements during walking (without-AHM: .94, with AHM: .92), tandem walking (without-AHM: .94, with AHM: .95), and stair climbing (without-AHM: .94, with AHM: .97) activities, both without and with AHM. Reliability for the TA muscle on the affected side ranged from good to excellent during walking (without-AHM: .88, with AHM:

.92), tandem walking (without-AHM: .91, with AHM: .96), and stair climbing (without-AHM: .92, with AHM: .92), activities. The reliability of sEMG measurements of the MGC muscle on the healthy side was good or excellent for walking (without-AHM: .88, with AHM: .97), tandem walking (without-AHM: .88, with AHM: .92), and stair climbing (without-AHM: .88, with AHM: .92) activities. Similarly, the reliability of measurements taken from the MGC muscle on the affected side was good or excellent during walking (without-AHM: .99, with AHM: .97), tandem walking (without-AHM: .87, with AHM: .96), and stair climbing (without-AHM: .99, with AHM: .99) activities (**Table 1**).

During the tandem walking activity performed with the AHM, the reciprocal activation rates of TA: MGC on the affected and healthy legs converged (p=0.010, d=0.71). However, TA: MGC reciprocal activation rates did not differ between the affected and healthy extremities in the walking (p=0.519, d=0.16) or stair climbing (p=0.180, d=0.35) activities performed with the AHM (**Table 2**, **Figure 2**). When the effect of AHM on the coactivation rates of healthy extremities during activities was examined, a change was observed during tandem walking activity (p=0.016, d=0.65), but no significant coactivation change was observed in walking (p=0.202, d=-0.32) and stair climbing (p=0.469, d=0.18). In the affected extremity, it was observed that AHM did not change the coactivation rate of TA and MGC during any activity (p>0.05).

Table 1. The mean percentage of maximum voluntary isometric contraction values. The intra-class correlation coefficient (ICC3,1), 95% confidence intervals (95%CI), and standard error of measurement (SEM) indicate reliability

%MVIC	Healthy Leg (n=17)			Affected Leg (n=17)		
	MeanSD	ICC3,1 (95%CI)	SEM	Mean±SD	ICC3,1 (95%CI)	SEM
Walking						
TA						
Non-AHM	34.2514.72	0.94 (0.83-0.99)	3.57	35.65±18.23	0.88 (0.70-0.97)	4.42
AHM	28.10±9.18	0.92 (0.78-0.98)	2.22	34.18±15.31	0.92 (0.82-0.97)	3.71
MGC						
Non-AHM	52.8119.35	0.88 (0.70-0.96)	4.69	68.70±40.98	0.99 (0.98-0.99)	9.93
AHM	44.19÷18.46	0.97 (0.92-0.99)	4.47	53.85±16.91	0.97 (0.91-0.99)	4.10
Tandem Walking						
TA						
Non-AHM	50.01±23.58	0.94 (0.82-0.99)	5.72	46.88±21.96	0.96 (0.89-0.99)	5.32
AHM	37.9015.12	0.95 (0.87-0.98)	3.66	40.53±12.31	0.91 (0.80-0.97)	7.60
MGC						
Non-AHM	42.12±16.37	0.95 (0.83-0.99)	3.97	54.16±34.72	0.87 (0.62-0.97)	8.42
AHM	47.2519.70	0.95 (0.88-0.98)	2.98	54.74±31.33	0.96 (0.90-0.99)	4.77
Stair Climbing						
TA						
Non-AHM	38.65±15.59	0.94 (0.87-0.98)	3.78	39.19±17.59	0.92 (0.83-0.97)	4.26
AHM	39.19±14.36	0.97 (0.91-0.99)	3.48	43.07±17.98	0.92 (0.82-0.97)	4.36
MGC						
Non-AHM	68.93±20.94	0.91 (0.78-0.97)	5.07	79.97±49.43	0.99 (0.97-0.99)	11.98
AHM	73.86±21.12	0.96 (0.89-0.99)	5.12	77.24±34.97	0.99 (0.98-0.99)	8.48

Abbreviations: %MVIC: percentage of maximum voluntary isometric contraction, TA: Tibialis anterior, MGC: Medial gastrocnemius, AHM: Abdominal hollowing maneuver, ICC: Intraclass correlation coefficient, SEM: Standard error of measurements, SD: Standard deviation.

J Health Sci Med 2023; 6(1): 59-65

Türkmen et al. Reciprocal activation changes of lower extremity muscles
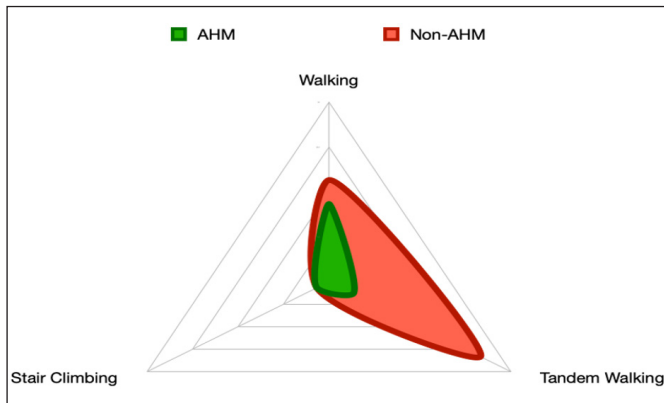
Figure 2. Mean differences in reciprocal activation (TA:MGC) ratios with and without AHM between both lower extremities during walking, tandem walking, and stair climbing activities.

Table 2. Reciprocal activation changes during activities due to the abdominal hollowing maneuver in healthy and affected legs.

Reciprocal Activation Ratio %MVIC (TA/MGC)	Non-AHM Mean±SD	AHM Mean ±SD	p	Effect size (d)
Walking				
Healthy Leg (n=17)	0.71±0.42	0.77±0.51	0.202	-0.32
Affected Leg (n=17)	0.54±0.19	0.64±0.22	0.111	-0.41
Mean Difference (Δ)	0.17±0.46	0.13±0.40	0.519	0.16
Tandem Walking				
Healthy Leg (n=17)	1.25±0.58	0.95±0.38	0.016*	0.65
Affected Leg (n=17)	1.00±0.49	0.99±0.42	0.851	0.04
Mean Difference (Δ)	0.25±0.42	0.04±0.49	0.010**	0.71
Stair Climbing				
Healthy Leg (n=17)	0.58±0.32	0.58±0.32	0.469	0.18
Affected Side (n=17)	0.56±0.31	0.60±0.30	0.191	-0.33
Mean Difference (Δ)	0.02±0.29	0.02±0.29	0.180	0.35

Abbreviations: %MVIC: percentage of maximum voluntary isometric contraction, TA: Tibialis anterior, MGC: Medial gastrocnemius, AHM: Abdominal hollowing maneuver, *paired samples t-test, *independent samples t-test.

DISCUSSION

The purpose of the present study was to examine the effect of the AHM on TA/MGC reciprocal activation and to observe whether there were changes in TA/MGC reciprocal activation during some activities performed. The results showed that, similar to the healthy leg, the AHM, when performed during tandem walking, regulates the reciprocal activation ratio of the TA: MGC affected by L4-L5 disc protrusion. While the extremity activation rates converged with the AHM during tandem walking, no similar activation changes were observed during walking or stair climbing. It is known that the reciprocal activation rates of agonist and antagonist muscles are more precisely adjusted to maintain the balance of the center of mass, especially during low-load tasks (26). It has been reported that while the TA plays a proprioceptive role in wide-stance activities such as walking, it contributes to body stabilization in narrow-stance activities such as tandem walking (27). According to the results of our study, the differences in the reciprocal activation rates between the healthy and affected legs are

greater in the activity of the TA to contribute to body stabilization. On the other hand, it is aimed to improve performance and regulate motor behaviors with TrA activation. In order to increase the cortical neuroplastic changes that occur after the AHM performed for this purpose, it is necessary to integrate functional tasks into motor learning stages (28).

The first stage of lumbar stabilization exercises performed on the basis of motor learning is the conscious contraction in the TrA activation (29). Considering that multiple repetitions and sensory feedback are required to increase the quality of contraction, it appears that even the initial AHM performed by LDH patients regulates the lower extremity motor activities somewhat (30). However, in our study, the most dramatic convergence between reciprocal activations of both extremities was seen in tandem walking. On the basis of motor learning, the cognitive and autonomous phases of movement should be performed by integrating various tasks into TrA activation in subsequent phases. According to the results of the present study, tandem walking may be the most appropriate option for tasks to be applied in the advanced stages of spinal stabilization exercises for patients with L4-L5 disc protrusion in which the TA is affected.

Although AHM was frequently used to provide low back and lower extremity motor control in previous studies, the ABM was also heavily preferred. (14, 17, 31, 32). Kim and Kim stated that ABM and AHM increase motor control by changing the rotation angles of the lower extremities in the side lying position and that ABM is more effective than AHM (14). One of the reasons why the ABM applied with functional activities is more effective than the AHM is thought to be that the ABM works more abdominal muscles than the AHM (33). The performance of the abdominal muscles is important to avoid unnecessary rotation of the lumbopelvic muscle. The external and internal oblique muscles, in particular, are important in controlling rotational force. However, intra-abdominal pressure increases significantly during ABM, and this maneuver may not be suitable for patients with LDH. The AHM selected in our study selectively contracts the TrA muscle (12). It has been suggested that the TrA muscle acts to control the translation and rotation of the lumbar spine. Even though AHM using only TrA isn't as good as ABM at giving motor control of the lower extremities, LDH patients may prefer it because it doesn't raise intra-abdominal pressure (17).

Finally, although the effectiveness of motor learning-based structured exercise programs for LDH patients with disc protrusion has been demonstrated in several studies (34-36), studies examining the effectiveness of the AHM integrated into the task from baseline without any exercise program being established are insufficient.

Türkmen et al. Reciprocal activation changes of lower extremity muscles

J Health Sci Med 2023; 6(1): 59-65

The results of our study showed that rapid integration of the AHM alone into tasks without a multistage designed exercise program can regulate the reciprocal activation rate in the lower extremities. However, as shown in our study, regulation of lower extremity reciprocal activation was only possible with tandem walking. Therefore, selection of the appropriate activity is also important when rapidly integrating the AHM into activities.

There were some limitations of the present study. The first is that the participants were enrolled in the study without a detailed sensory or pain examination. In addition, the results showed the benefit of integrating the AHM into activities in the early period, but 8-week AHM training was not conducted in order to compare these results. Therefore, another limitation of our study was that the TrA muscle, which is expected to be trained for at least 8 weeks, was trained only for 45 minutes and was not compared with a TrA muscle trained for 8 weeks.

CONCLUSION

Our study showed that the AHM, when done during tandem walking, regulates the reciprocal activation ratio of TA:MGC affected by L4-L5 radiculopathy in the similar way as a healthy leg. While the extremity activation rates converged with the AHM during tandem walking, no similar activation changes were observed during walking or stair climbing. In some activities, such as tandem walking, there are differences in reciprocal activation rates between the healthy extremity and the affected extremity due to L4-L5 unilateral radiculopathy caused by LDH. The present study showed that the integration of the AHM into tandem walking brought the reciprocal activation rates of both legs closer to each other and enabled them to exhibit similar behaviors, even without adherence to any exercise protocol. Therefore, tandem walking can be selected as an appropriate activity to combine spinal stabilization exercises performed by L4-L5 LDH patients using the AHM with the task. In addition to these results, it is still unclear to what extent the reciprocal activation rates in the lower extremities will change as a result of longer training of the AHM. In future studies, it will be useful to examine the effect of long-term motor learning-based training of AHM on daily living activities.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Çankırı Karatekin University Faculty of Health Sciences Ethics Ethics Committee (Date: 09.11.2021, Decision No: 23)

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

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The effect of thyroid hormone withdrawal performed to evaluate the success of I-131 ablation on quality of life and psychological symptoms in female patients with low-risk differentiated thyroid cancer

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ABSTRACT

Aim: There is a need to evaluate the treatment response in patients who have undergone radioiodine treatment (RIT) for differentiated thyroid cancer. Diagnostic tests that are used for this purpose include radioiodine whole-body scan (WBS) and serum thyroglobulin (Tg) measurement, which are most accurate during thyroid-stimulating hormone (TSH) stimulation. However, temporary discontinuation of thyroid hormone therapy to increase TSH (withdrawal) may be associated with the morbidity of hypothyroidism. The study aimed to show the effects of thyroid hormone withdrawal (THW) on quality of life and psychological symptoms in female patients with low-risk, well-differentiated papillary thyroid cancer.

Material and Method: We applied the short form-36 (SF-36) and Symptom Checklist-90-R (SCL-90-R) questionnaires to the patients in the euthyroid state who have referred a median of 9 months (6-13 months) after RIT to perform a dWBS and to evaluate stimulated Tg. We applied the same questionnaire again when thyroid-stimulating hormone (TSH) was > 30 μIU/mL 4 weeks after THW (hypothyroid state).

Results: 52 patients were evaluated (median age 48 years, range 23-65 years). There was a statistically significant worsening in anxiety, psychosis, additional items, and general symptoms of the SCL-90-R questionnaire. With the SF-36 questionnaire, we observed statistically significant worsening in physical functioning, role limitation due to physical health, energy/fatigue, emotional well-being, social functioning, and general health change.

Conclusion: THW worsened the patients' psychological symptoms and quality of life. To reduce the side effects of hypothyroidism, treatment response assessment with TSH stimulation should be used only in a selected group of patients.

Keywords: Thyroid cancer, hypothyroidism, life quality, psychological symptom

INTRODUCTION

The survival rate of patients with thyroid cancer is >90%, although it varies among disease subgroups. The standard treatment for DTC is total thyroidectomy, with cervical lymph node dissection if necessary, followed by radioiodine treatment (RIT) (1,2). Administration of iodine 131 (I-131) after total thyroidectomy has three main goals: (1) to destroy possibly benign residual thyroid tissue, which increases the specificity of serum thyroglobulin (Tg) measurement during follow-up; (2) to eliminate suspected but unidentified residual disease or known persistent or recurrent disease that may decrease disease-free survival (DFS) and overall survival (O.S.); and (3) to perform a highly

sensitive post-treatment whole-body scan (WBS) (3). In patients undergoing ablation, diagnostic whole-body scans (dWBS) with stimulated Tg test are performed 6-12 months after treatment to evaluate treatment success (4). Although the sensitivity of a dWBS in demonstrating residual normal thyroid tissue is high, its success in demonstrating metastatic disease is limited. For this reason, this method has steadily decreased, especially in low-risk patients. In some centers, stimulated Tg levels can be used alone for evaluation of the treatment response (5-8). However, both tests require TSH stimulation for optimal sensitivity (9). TSH stimulation can be performed by thyroid hormone withdrawal (THW) or administration of recombinant human thyrotropin (thyrotropin alfa) (10). THW is a cheap, readily available method of TSH stimulation. However, it is associated with clinical hypothyroidism, which has many side effects, including drowsiness, constipation, weakness, myalgia, emotional dysfunction, and physical discomfort (11,12). While improving survival is essential in cancer patient management, quality of life (QoL) preservation should also be one of the ultimate goals. However, temporary hypothyroidism reduces QoL (13,14). The 36-item Short-Form Health Survey (SF-36) is a validated questionnaire on general health and well-being. It has also been used in studies on thyroid diseases. The Symptom Checklist 90–Revised (SCL-90-R) is a questionnaire evaluating psychological symptoms and has been used in studies on hypothyroidism (15-17).

Our study aimed to determine the effects of THW, during the dWBS, on QoL and psychological symptoms by questionnaires in female patients with low-risk, well-differentiated thyroid cancer.

MATERIAL AND METHOD

The study was carried out with the permission of Recep Tayyip Erdoğan University Faculty of Medicine Non-interventional Clinical Researches Ethics Committee (Date: 28.11.2019, Decision No: 2019-191). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patient Selection

We included 52 female patients who underwent RIT between March 2017 and November 2017 (median age 48 years, range 23-65 years). (40465587-102.01-274). All patients provided written informed consent.

Inclusion Criteria

We included female patients aged 18-65 who underwent RIT for differentiated thyroid cancer.

After RIT, regular TSH, free thyroxine (fT4), Tg, and thyroglobulin antibody (TgAb) levels were followed up until the dWBS.

Exclusion Criteria

We excluded patients with diabetes mellitus, chronic kidney disease, chronic liver disease, chronic rheumatic disease, chronic musculoskeletal disease, and patients with non-thyroid cancer. We also excluded patients who were using active psychiatric drugs at that time. After RIT, some patients did not have regular TSH and fT4 level follow-up; hence, there were patients with overt hypothyroidism and hyperthyroidism, and we excluded them.

Treatment Protocol

Surgical treatment: 44% of our patients (n=23) were operated on in our center. 56% of them (n=29) were operated on in other centers and referred to our center for RIT. Total thyroidectomy (T.T.) was performed in 18 patients (34.6%), and total thyroidectomy plus central neck dissection (CND) was performed in 5 patients (9.6%). Near-total thyroidectomy (nTT) was performed in 15 patients (28.8%), and subtotal thyroidectomy (sTT) was performed in 14 patients (26.9%). The surgeon who performed the operations in our center (S.K.) is experienced in thyroid surgery (12 years of experience with endocrine surgery). The total number of patients in our center was 23 (18 patients TT, five patients TT + CND). Biopsy-proven lymph node metastases (n= 1) or suspicious findings were found preoperatively on neck ultrasound (n= 4) and were evaluated with CND. None of the patients underwent lateral neck dissection.

I-131 administration decision: Pathology results; TSH, Tg, and Tg-Ab levels; pre-ablation technetium-99m scintigraphy; and ultrasonography results of the patients were evaluated by the pathologists, endocrinologists, surgeons, radiologists, and nuclear medicine specialists, and a consensus decided the treatment. I-131 administration was initiated 2-4 months after surgery. Patients received 1.85 or 3.7 GBq of I-131 approximately four weeks after THW 2-to three weeks of a low iodine diet. At the time of I-131 administration, serum TSH levels were > 30 μIU/mL in all patients.

Research Protocol

A nuclear medicine specialist (O.K.) and two thyroid endocrinologists (Ş.A. and U.A.) reviewed each patient's staging and initial risk stratification based on the clinical, surgical, and pathological information and the post-ablation scintigraphy findings.

Kupik et al. Thyroid hormone withdrawal worsens life quality

J Health Sci Med 2023; 6(1): 66-72

Risk Stratification

We classified the tumors using TNM staging according to the American Joint Committee on Cancer (AJCC) 8th edition criteria. The risk classification of the patients and the RIT response were made according to the 2015 American Thyroid Association (ATA) criteria (10).

Clinical Outcome and Questionnaire Administration

After the I-131 administration, the patients were followed up in the endocrinology clinic. The patients were referred to the nuclear medicine clinic for the dWBS and evaluation of stimulated Tg at a median of 9 months (6-13 months) after RIT. We evaluated the TSH and fT4 levels of the patients. We informed the patients without overt hyperthyroidism and hypothyroidism about the questionnaire and administrated the SF-36 and SCL-90-R under the guidance of an expert (O.K.) (questionnaire 1: euthyroid state). We terminated the patients' LT4 use. After four weeks, the patients were given an appointment to receive 185 MBq in an oral I-131 capsule. Patients were recommended to consume a low-iodine diet for two weeks. After four weeks, we measured TSH, Tg, TgAb, and fT4 levels. The same specialist (O.K.) applied the SF-36 and SCL-90-R questionnaires again to the patients with serum TSH > 30 µIU/mL (questionnaire 2: hypothyroid state). Ultrasonography was performed on the patients by O.K. and H.G. We evaluated the absence of residue in the thyroid bed and the absence of pathological lymph nodes in the central/lateral neck compartment as a negative result. We then administered 185 MBq via an oral I-131 capsule to the patients. Two days after I-131 administration, a planar WBS was performed in anterior and posterior projections using a dual-head camera equipped with high-energy, parallel-hole collimators. The WBS was evaluated by the same specialist (O.K.), blinded to the patients' Tg, TgAb levels, clinical findings, and post-ablation WBS images. Radioiodine uptake in the thyroid bed, midline superior thyroglossal duct cyst/pyramidal lodge, and the central and lateral neck compartment were evaluated as a residual disease. RIT response assessments were made according to the 2015 American Thyroid Association (ATA) criteria (10).

Measurement of QoL and Psychological Symptoms

We applied the SF-36 to evaluate the patients' QoL. We also applied the SCL90-R psychological symptom screening test and calculated the total score of these tests for each patient.

The SF-36 scale was developed by Ware et al. (18). The validity and reliability study of the SF-36 in the Turkish population was performed by Koçyiğit et al. (19). We used this version of the scale in our study. The SF-36 consists of 36 items and provides eight symptoms: physical functioning, role limitation due to physical health, energy/fatigue, emotional well-being, social functioning, pain, general health, and health changes. The total score is obtained by adding the points for each sub-scale, with a total score of 100. A decrease in the numerical score indicates deterioration in health.

The SCL-90-R psychological symptom screening test is a self-assessment instrument. The psychopathological symptoms are scored from 0 to 4 for 90 items, and the sub-scale scores – somatization, anxiety, depression, obsession, intersensitivity, anger, paranoid, psychoticism, phobia, general symptom index, and additional items (symptoms related to sleep disorders, appetite disorders and guilt) – are calculated (20). The validity and reliability study of the SLC-90-R in the Turkish population was performed by Dağ et al. (21). We used this version in our study.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics of categorical variables are reported as frequency and percentages within the group (n, %). Continuous variables were subjected to normality analysis to determine their distributions. Changes between questionnaires 1 and 2 were evaluated with the paired-samples t-test or the Wilcoxon signed-rank test. The mean±standard deviation (S.D.) and t values of the normally distributed variables or the median (min-max) and Z values of the non-normally distributed variables are presented. The limit of significance was accepted as $p < 0.05$.

RESULTS

General Findings

Metastatic lymph nodes were detected in 5 patients who underwent CND (17 metastatic lymph nodes with a mean size of 8.4 mm [range 4-11 mm]). The mean time between surgery and RIT was 60.85±17.34 days. All patients had papillary thyroid carcinoma. Only one patient (2%) had stage 2 disease according to the TNM classification; all other patients had stage 1 disease. According to the ATA risk classification, forty-eight patients (92%) were in the low-risk group. Four patients (8%) were in the intermediate group. For treatment, 1850 MBq was administered to 7 patients, and 3700 MBq I-131 was administered to 45 patients. The main characteristics of the study population are shown in **Table 1**. Ablation success based on the dWBS only was 96.2%, on Tg only was 98%, and on TgAb only was 88.5%.

J Health Sci Med 2023; 6(1): 66-72

Kupik et al. Thyroid hormone withdrawal worsens life quality

Table 1. The characteristics of the study population.	
Parameter	**N (%)**
Histology	
Classical variant	33 (63.5)
Mixt+classical variant	3 (5.8)
Folicular variant	13 (25)
Tall cell variant	2 (3.8)
Oncositic variant	1 (1.9)
Multifocality	39 (75)
Tumor at surgery margin	6 (11.5)
Microcarcinoma	21 (40.4)
Vascular invasion	2 (3.8)
TNM stage 1	51 (98)
T1	37 (71.2)
T1a	20 (38.5)
T1b	17 (32.7)
T2	13 (25)
T3a	2 (3.8)
N0	46 (88.4)
N0a	10 (19.2)
N0b	36 (69.2)
N1a	5 (9.6)
Nx	1 (1.9)
M0	52 (100)
I-131 dose	
1850 MBq	7 (13.5)
3700 MBq	45 (86.5)
Treatment response	
Excellent	45 (86.5)
Indeterminate	7 (13.5)

SCL-90-R Analysis

Between questionnaire 1 and questionnaire 2, there was significant worsening in anxiety (Z=-2.052, p=0.040), psychoticism (Z=-2.187, p=0.029), additional items (Z=-2.306, p=0.021) and the general symptom index (Z=-2.086, p=0.037). None of the other symptoms showed a significant change (depression, t=-1.390, p=0.171; somatisation, Z=-1.453, p=0.146; obsession, Z=-0.700, p=0.484; intersensitivity, Z=-1.194, p=0.232; paranoia, Z=-0.261, p=0.794; anger, Z=-1.115, p=0.265; phobia, Z=-0.834, p=0.404) (**Figure 1**).

SF-36 Analysis

Between questionnaire 1 and questionnaire 2, there was significant worsening in physical functioning (t=2.588, p=0.013), role limitation due to physical health (Z=-2.677, p=0.007), energy/fatigue (Z=-2.502, p=0.012), emotional well-being (Z=-3.618, p < 0.0001), social function (Z=-3.179, p=0.001), general health (Z=-2.397, p=0.017) and health change (Z=-1.996, p=0.046). There was worsening of the pain symptom, but it was not statistically significant (t=1.234, p=0.223) (**Figure 2**). A summary of the questionnaire data is given in **Table 2**.
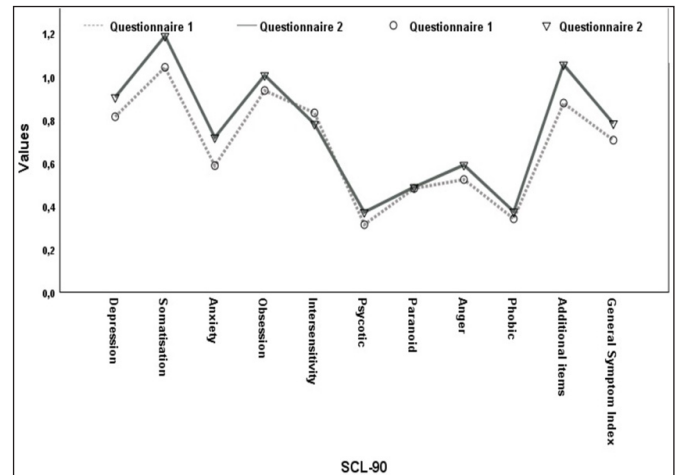
Figure 1. Graph showing the differences in The Symptom Checklist-90-Revised symptoms between questionnaires one and 2.

Table 2. The summary of SF-36 and SCL-90-R questionnaires.				
SCL-90 mean±SD/median (min-max)				
Sypmthoms	**Questionnaire 1**	**Questionnaire 2**	**p**	**t/Z**
Depression	0.814±0.580	0.903±0.605	0.171	-1.390
Somatisation	1.0 (0.25-2.75)	0.958 (0.083-3.0)	0.146	-1.453
Anxiety	0.450 (0.0-2.1)	0.600 (0.0-2.0)	0.04	-2.052
Obsession	0.850 (0.1-3)	0.900 (0.0-2.8)	0.484	-0.700
intersensitivity	0.666 (0.00-3.11)	0.666 (0.00-3.22)	0.232	-1.194
Psycotic	0.200 (0.00-2.00)	0.250 (0.00-1.7)	0.029	-2.187
Paranoid	0.333 (0.00-2.17)	0.333 (0.00-1.83)	0.794	-0.261
Anger	0.416 (0.00-1.67)	0.500 (0.00-1.83)	0.265	-1.115
Phobic	0.285 (0-1.28)	0.285 (0-1.42)	0.404	-0.834
Additional items	0.857 (0-2.57)	1.0 (0.0-2.571)	0.021	-2.306
General Symptom index	0.583 (0.06-2.02)	0.767 (0.78-1.82)	0.037	-2.086
SF-36 mean±SD/median (min-max)				
Sypmthoms	**Questionnaire 1**	**Questionnaire 2**	**p**	**t/Z**
Energy fatique	60 (0-100)	50 (5-100)	0.012	-2.502
Physical function	80 (10-100)	70 (5-100)	0.013	2.588
Phsical health (role limit)	83.350 (0-100)	50 (0-100)	0.007	-2.677
Emotional well-being	68 (12-100)	60 (24-92)	0.0001	-3.618
Social function	81.97±17.045	71.57±22.649	0.001	-3.179
Health change	62.50 (25-100)	50 (0-100)	0.046	-1.996
Pain	72.75±25.52	68.43±22.66	0.223	1.234
General health	60 (0-90)	55 (0-95)	0.017	-2.397

Kupik et al. Thyroid hormone withdrawal worsens life quality

J Health Sci Med 2023; 6(1): 66-72
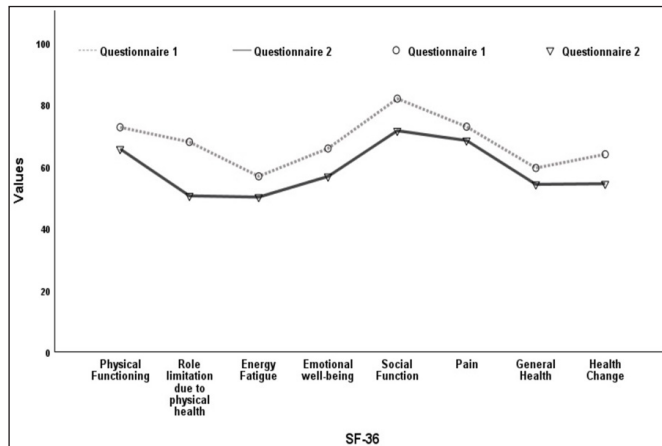
Figure 1. Graph showing the differences in Short form-36 symptoms between questionnaire one and questionnaire 2

DISCUSSION

There is a need to evaluate the treatment response in patients undergoing RIT for differentiated thyroid cancer. Treatment response evaluation is usually done by evaluating the dWBS or stimulated Tg. However, hypothyroidism that we created with THW causes some patient complaints and negatively affects QoL. Using questionnaires, we found that hypothyroidism adversely affected QoL (based on the SF-36) and worsened psychological symptoms (based on the SCL-90-R). With the SF-36, we found significant worsening in physical functioning, role limitation due to physical health, energy/fatigue, emotional well-being, social function, general health, and health change symptoms at the time of the dWBS. There was a worsening of the pain symptom, but it was not statistically significant. With the SCL-90-R, we detected significant worsening in anxiety, psychoticism, additional items, and general symptom index symptoms at the time of the dWBS. Although other symptoms worsened, the results were not statistically significant.

Banihashem et al. (22) investigated the psychological status and QoL of 150 patients who had undergone thyroidectomy for differentiated thyroid cancer. They evaluated the patients at four different times: 1 month before RIT, at the time of RIT, and one week and six months after RIT. Differently from our study, they used the Hospital Anxiety and Depression Scale (HADS) to measure the psychological state of the patients. The SF-36 was applied to determine QoL. According to the SF-36 survey, they determined that the most significant deterioration in QoL was during RIT. They stated that the reason for this was hypothyroidism caused by LT4 withdrawal. Botella-Carretero et al. (23) investigated psychometric functionality and QoL in patients with DTC. Fifty female patients with differentiated thyroid carcinoma were compared with 18 healthy females in the same age group. At the time of the

dWBS, when in the hypothyroid state, a comparison was made with healthy female patients in the control group, and impairment was found in QoL and cognitive performance. Tagay et al. (24) applied QoL assessments to 136 patients with thyroid cancer while in a hypothyroid state in preparation for radioiodine administration. The available results were compared with German population reference values. All values of the SF-36 were lower than the reference values of the population. In another study evaluating 61 patients with thyroid cancer who underwent LT4 withdrawal, quality of life was evaluated with a self-rating questionnaire and Hamilton depression scale. The scores were worse than the control group. Hamilton depression scale was found to be worse in women (14). Another study included 228 patients with DTC, the quality of life was evaluated separately with the SF-36 scale while using LT4, in the withdrawal period, and while applying tyrogen. It was determined that the quality of life was significantly worse in the withdrawal period (12). One of the differences from our study is that we have compared the QoL and psychological symptoms scores in the hypothyroid period with the QoL and psychological symptoms scores of the same patients in the euthyroid period four weeks before, not with the reference values of the population. We tried to evaluate the effects of deep hypothyroidism, which we developed in a short time, on QoL and psychological symptoms free from all factors. When comparing a patient's QoL with community reference values, it should be taken into account that there may be differences in QoL within the society due to social, cultural, economic, and regional reasons.

Thyrotropin alfa has been used for a long time to prepare thyroid cancer patients before radioiodine administration to reduce the adverse effects of LT4 THW on QoL, reduce the radiation dose to the body, and perhaps reduce the cost of the treatment by shortening the hospital stay (11,12,25-27). In two prospective studies (25,28) evaluating the ablation success of low-dose and high-dose I-131 administration in patients with low-risk differentiated thyroid cancer, QoL deteriorated in the group of patients who had undergone THW. The authors reported no deterioration in QoL in the group administered thyrotropin alfa, or the deterioration was much less compared with the LT4 withdrawal group, and this effect was independent of the applied radiation dose. The authors stated that thyrotropin alfa is superior to LT4 withdrawal in radiation exposure and side effects. Because our study aimed to evaluate whether the hypothyroidism we created adversely affected QoL, we did not administer thyrotropin alfa. In addition, thyrotropin alpha is imported into our country, access to it is not always possible, and

it is an expensive product, which is too high for our country and other developing countries. We can only administer thyrotropin alfa to select patients with comorbidities who cannot tolerate hypothyroidism. It has already been reported that in patients with a low-intermediate risk of well-differentiated thyroid cancer, basal thyroglobulin has an important prognostic value in predicting treatment response and prognosis, stimulating thyroglobulin may not be needed due to its cost and side effects (29-31).

One of the limitations of the study was that all surgeries were not performed in the same center, so a standard surgical procedure was not applied. Perhaps our most important limitation is why patients were not administered low-dose (1.1 GBq) I-131. However, in multicentre, randomized prospective studies (25,28), low-dose and high-dose I-131 administration did not differ in treatment success. Frankly, our treatment success is similar to theirs. However, we can explain the high dose I-131 administration; some of our patients had aggressive histological variants, tumors at the surgical margin, and lymph node metastases. In addition, since T.T. was not performed in all patients, the relatively high residuals in the pre-ablation evaluations were one of the reasons for our high dose administration. Multicentre, prospective, randomized studies were published in 2018 (32) and 2019 (33) on the association of low- and high-dose administration with recurrence in patients with low-risk differentiated thyroid cancer. The median follow-up was 6.5 and 5.4 years. As a result, it was stated that low-dose and high-dose administration did not have a statistically significant effect on recurrence, and low-dose administration caused fewer side effects. Our study group consisted of patients who received high-dose treatment in 2017. After these last two articles were published, low-dose administration in patients with low-risk thyroid cancer became more preferred in our center, as in many centers in our country. The number of our patients is not higher can be accepted as a limitation. Although some authors reported that the effects of hypothyroidism on quality of life were different in male and female patients (34), some authors stated that the gender factor was ineffective (35). We included only female patients in order to avoid suspicion of bias.

CONCLUSION

We have shown that THW adversely affects QoL and worsens psychological symptoms in female patients. In order to reduce the side effects of hypothyroidism treatment response assessment with TSH stimulation should be used only in a selected group of patients.

ETHICAL DECLARATIONS

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HEALTH SCIENCES MEDICINE

Treatment of distal femur fractures with retrograde intramedullary nailing utilizing a tibial nail

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ABSTRACT

Aim: Retrograde intramedullary nailing for the treatment of femur fractures is a sound option due to its advantages including ease of implant placement and better control of the distal segment, especially in distal femur fractures. Unfortunately, retrograde femoral nails are not available in most developing countries or rural areas. The primary aim of the study is to investigate the outcomes of an alternative treatment of distal femur fracture with retrograde intramedullary nailing using a tibial nail.

Material and Method: Patients who had distal femur fractures and underwent retrograde intramedullary nailing with a tibial nail between January 2020–March 2022 are retrospectively evaluated. Patients who were treated other than a tibial nail, aged below 18, had follow-up less than 6 months, multiple fractures, and open fractures were excluded. Patients' demographics, time to union, and complications were recorded. Functional outcomes included visual analogue pain score (VAS), Lysholm score, Tegner activity scale, Knee Society Score (KSS), and Short Form-36 (SF36) on the latest follow-up.

Results: A total of 15 patients (11 male, 4 female) met the inclusion criteria and included in the study. The mean age of the patients was 42.9±17.3 (range, 18 to 72) months. The mean follow-up period was 10.1±5.2 months (range, 6 to 18). There was no reduction loss and implant failure during follow-ups. One patient had nonunion. No intraoperative fracture occurred. Persistent knee pain was seen in a patient due to an intraarticular screw and removed arthroscopically. In the latest follow-up, the mean VAS was 0.73±0.70 (range, 0 to2), the mean Tegner score was 5.0±1.5 (range, 3 to 7), the mean Lysholm score was 90.9±9.9(range, 64 to 100), the mean KSS was 86.6±8.6 (range, 70 to 97), and the mean SF-36 score was 96.5±4.7 (range, 87 to 100).

Conclusion: The results of this study suggest that a tibial nail with retrograde intramedullary nailing has good functional outcomes in the treatment of distal femur fractures. The tibial nail should be considered as an alternative treatment option where retrograde femoral nails are not available.

Keywords: Femur, tibia, nail, plate, fixation, retrograde nail

INTRODUCTION

Distal femur fractures have a reported incidence of 8.7/100.000 and have bimodal age distribution including younger patients with high-energy traumas and older patients after low-energy traumas (1). In the surgical treatment of distal femur fractures; open reduction and internal fixation with a locking plate, minimally invasive plating, and intramedullary nailing after closed or open reduction are defined treatment options (2). Nonunion can occur due to distal femur fractures' inherent location at metaphyseal and metaphyso-diaphyseal regions (3).

Intramedullary nailing can be performed antegrade or retrograde in distal femur fractures. In the surgical treatment of distal femur fractures, the retrograde

intramedullary nailing (RIMN) technique is preferred more often than the antegrade nailing technique due to the ease of fracture reduction and its stability with multiple distal screws in varying angles (4). If the distal femur fracture does not extend to the knee joint, distal femoral retrograde nailing and locking plate fixation have similar union and complication rates but nailing provides better patient satisfaction (5, 6). However, in intra-articular comminuted fractures, a locking plate is preferred over nails, particularly in patients with low bone mineral density.

Due to the sagittal inclination of the femur, fixation cannot be performed with straight nails in the RIMN technique. For this reason, retrograde femoral nails

Ergişi et al. Tibial nail in distal femur fracture treatment

J Health Sci Med 2023; 6(1): 73-76

suitable for the anatomical inclination of the femur were introduced into the market. However, in developing countries, there may be difficulties in obtaining these retrograde nails produced for distal femur fractures. Despite being not manufactured for the treatment of distal femur fractures, the use of a tibial nail to benefit from the advantages of retrograde intramedullary nailing has been reported in these fractures with a limited patient population (7, 8). The aim of the study is to investigate the outcomes of an alternative treatment of distal femur fracture with retrograde intramedullary nailing using a tibial nail to take advantage of the intramedullary nailing technique.

MATERIAL AND METHOD

The study was carried out with the permission of Karabük University Non-interventional Clinical Researches Ethics Committee (Date: 23/09/2022, Decision No: 2022/1060). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants who participated in this study.

Patients treated with the RIMN technique using a tibial nail for distal femur fracture in our institution between January 2020 and March 2022 were retrospectively analyzed. Patients who were treated with other than a tibial nail, aged below 18, had follow-up less than 6 months, multiple fractures, and open fractures were excluded. Patients' demographics, time to union, fracture type according to AO classification, length of stay at the hospital, and complications were recorded. Functional outcomes included visual analog pain score (VAS), Lysholm score, Tegner activity scale, Knee Society Score (KSS), and Short Form-36 (SF36) on the latest follow-up. Absence of callus or bony bridge on at least three cortices at anteroposterior and lateral radiographs at six months follow-up was considered to be nonunion.

Surgical Technique

The patients were operated in supine position after standard skin preparation from the iliac crest to the foot and with a bump under the knee to position the 30 degrees of flexion of the knee joint. Articular reduction was achieved by performing medial paratendinous arthrotomy in fractures involving the joint. The patellar tendon split approach was preferred for fractures not extending the knee joint. The entry point of the nail was determined using the apex of the Blumensaat line on lateral imaging, and the femoral shaft axis in the middle or slightly medial of the trochlear groove in anterior-posterior imaging. Subsequently, after the determination of the entry point, the entrance hole was enlarged with a cannulated reamer over a guide wire. The ball tip guide wire was then passed through the fracture under fluoroscopy control and advanced to the level of the trochanter minor. The nail length was determined with an equal length ball tip guide wire. Nail thickness was determined by evaluating the reamer thickness at the isthmus by fluoroscopy during reamerization. The tibial nail was introduced into the intramedullary canal so that the Herzog angle of the tibial nail was in line with the sagittal inclination of the femur. Care was taken to ensure that the proximal end of the nail was proximal to the trochanter minor, and the distal end was a few millimeters above the joint level. Due to the short and wide metaphyseal region, two parallel and at least one divergent distal locking screws were placed over the guide. The nail was locked proximally with at least one bicortical screw using freehand technique (**Figure 1**).

Patients were allowed to bear full weight with double crutches immediately after the surgery, then with single crutches between 6-8 weeks, and without support after the 8th week. Knee and hip joint range of motion exercises were started immediately.

Figure 1. Anteroposterior (A) and lateral (B) radiographs of a 52-year-old male patient with a distal femur fracture treated with retrograde intramedullary nailing with a tibial nail (C). Anteroposterior (D) and lateral (E) radiographs demonstrating fracture healing

J Health Sci Med 2023; 6(1): 73-76

Ergişi et al. Tibial nail in distal femur fracture treatment

Descriptive statistics were expressed as mean±standard deviation for continuous numerical variables, categorical variables were expressed as the number of patients and percentage. Analyses of the data were performed using the IBM SPSS Statistics 23.0 (IBM Corporation, Armonk, NY, USA) program.

RESULTS

Of the 19 patients who were treated RIMN technique using a tibial nail for distal femur fracture, 15 patients met the inclusion criteria and were included in the study. The demographics of the patients are presented in **Table**. The mean follow-up period was 10.1±5.2 months (range, 6 to 18). Mean length of stay at hospital was 5.4±3.4 (range, 2 to 15) days. Mean time to union was 3.0±1.2 months (range, 2 to 6). No intraoperative fracture and neurovascular injury occurred. There was no reduction loss or implant failure during follow-up. One superficial wound infection was observed and treated with oral antibiotics. One patient had persistent knee pain after surgery which was due to an intraarticular screw and was treated with arthroscopic screw removal (**Figure 2**).

Table. Demographics of the patients.	
Mean age (years)	42.9±17.3 (range, 18 to 72)
Gender (M/F)	11/4
Side (R/L)	9/6
Mechanism of injury	
Traffic accident (n=4)	
Blount trauma (n=5)	
Simple fall (n=6)	
Fracture type (AO classification)	
A1 (n=6)	
A2 (n=6)	
A3 (n=2)	
C2 (n=1)	
M: male, F: female, R: right, L: Left	

In the latest follow-up, the mean VAS was 0.73±0.70 (range, 0 to2), the mean Tegner score was 5.0±1.5 (range, 3 to 7), the mean Lysholm score was 90.9±9.9(range, 64 to 100), the mean KSS was 86.6±8.6 (range, 70 to 97), and the mean SF-36 score was 96.5±4.7 (range, 87 to 100).

DISCUSSION

RIMN technique with a tibial nail allowed patients to give full weight immediately after surgery without any implant failure during follow-ups. Complication rates including nonunion, wound healing problems, neurovascular injuries seemed low in our series. RIMN with a tibial nail provided good functional outcomes as patients' pain levels were low, knee functions are quite good, and they can easily perform their daily activities.

The incidence of distal femur fractures has a bi-modal distribution, peaking in young men after high-energy trauma and in elderly women after low-energy trauma (9). The demographic data of the cases included in our study are consistent with the literature posing older women after energized trauma.

Orthopedic surgeons may have concerns regarding possible intraoperative fractures due to anatomical mismatch and possible implant failures after surgery as tibial nails are not designed for distal femur fractures. There were no intraoperative fractures or implant failures in our series. We believe that the Herzog angle of the tibial nail is in line with the sagittal alignment of the femur thus reduced potential anatomical mismatch. In addition, tibial nails have solid biomechanical compatibility and strength as well as anatomical retrograde femoral nails in the treatment of distal femoral fractures (7). One implant-related complication occurred in our series due to an intraarticular screw. The divergent screws have 90 degrees of angle in between. This narrow angle can cause
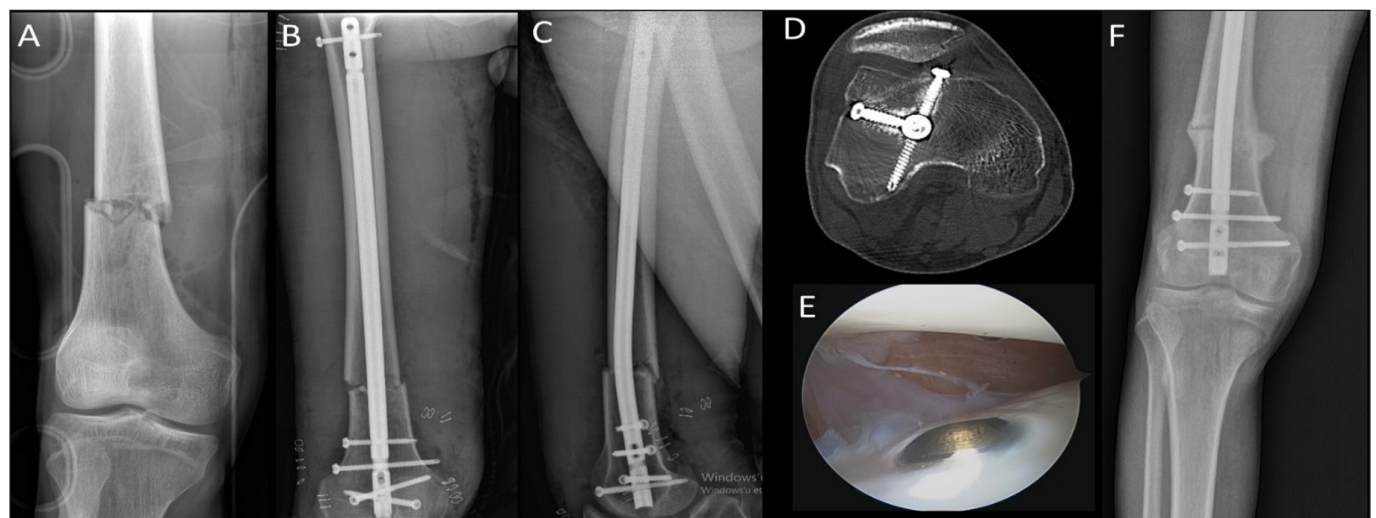
Figure 2. Anteroposterior (A) radiographs of a 30-year-old male patient with a distal femur fracture treated with retrograde intramedullary nailing with a tibial nail (B-C). The patient had persistent knee pain at four-week follow-up. Computed tomography showed an intra-articular screw (D). The patient underwent arthroscopic screw removal (E) and showed fracture healing at six months follow-up (F).

Ergişi et al. Tibial nail in distal femur fracture treatment

J Health Sci Med 2023; 6(1): 73-76

screw malposition in the RIMN technique with a tibial nail. We recommend that if a second divergent screw is necessary for distal stabilization, it should be placed with direct visualization utilizing an arthrotomy.

The union rate of distal femoral fractures treated with an anatomical retrograde femoral nail has been reported as 84-92% (10). In this study, there was nonunion in one of the 15 patients with distal femur fractures who were operated RIMN technique with a tibial nail. This result is in line with the nonunion rates of previous literature.

RIMN technique interferes with the knee joint and may affect knee functions. However, it has been reported that locking plate fixation has no superiority over RIMN in the treatment of distal femur fractures in terms of knee scores (11). The KSS scores of the patients after RIMN with a femoral nail for distal femur fractures range between 70 to 80. The mean KSS score in this study was consistent with the previous literature (12) and the use of a tibial nail did not end up with an inferior outcome.

There are some limitations of our study. The generalizability of the study is limited as it is a retrospective and single-center study. The current study lacked a control group, but we were able to discuss our outcomes with existing literature.

CONCLUSION

The finding of this study suggests that the RIMN technique with a tibial nail in the treatment of distal femur fractures can provide good functional and radiographic outcomes. If a retrograde femoral nail cannot be accessed, the tibial nail can be used as an alternative fixation method in order to benefit from the advantages of intramedullary nailing.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Karabük University Non-interventional Clinical Researches Ethics Committee (Date: 23/09/2022, Decision No: 2022/1060).

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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Factors affecting the presentation time of patients with acute stroke to hospital and level of awareness of thrombolytic therapy

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ABSTRACT

Aim: In this study, it was aimed to reveal the factors affecting the time of presentation of patients with acute stroke to the hospital, determine the rates of benefiting from thrombolytic therapy and assess the thrombolytic therapy awareness of patients.

Material and Method: 276 patients with acute stroke were included in our study. Standard structured questionnaire was administered to the patients. Patients were asked about the time of onset of stroke, age, place of residence, level of education, whether they received thrombolytic therapy, what was done as the first intervention, and whether they had information about thrombolytic therapy. Stroke severity was also evaluated by applying the National Institutes of Health Stroke Scale (NIHSS) to the patients.

Results: Of the patients included in the study, 218 (79%) had ischemic stroke, 26 (9.4%) had intracerebral hemorrhage and 32 (11.6%) had TIA. The mean NIHH score of the patients was 6.7±6.2. Stroke onset time was mainly between 18.00-24.00 with a rate of 28.3%. It was understood that 83.1% of the patients came to the emergency department by ambulance and 16.9% came by their own vehicle. When the groups of patients who received and did not receive thrombolytic therapy were compared, no significant difference was found between who the patient lived with at home and the groups of the level of education. Statistical significance was found with symptom onset time (p<0.05). Significant differences were found when the time of presentation to the hospital was compared with the place of residence, type of stroke and symptom onset time (p<0.05).

Conclusion: When the results are evaluated, it is necessary to reduce the delay time of patients with acute stroke, especially outside the hospital. Increasing the awareness of the patients about the symptoms of stroke and early treatment techniques will help to reduce mortality and morbidity.

Keywords: Cerebrovascular disease, thrombolytic therapy, delayed treatment

INTRODUCTION

Stroke refers to a focal neurological syndrome temporarily or permanently located in a region of the brain that develops due to cerebrovascular disease (CVD) (1). Stroke is an important health problem in the world that causes death and disability and ranks second among the causes of death in developing countries and third among the disabilities caused by disease in developed countries. It also has an important place due to its preventability and treatability (2,3).

Stroke can occur in two forms as hemorrhagic and ischemic, 80-85% of which are ischemic and 15-20% are hemorrhagic (4).

Stroke is the second-ranking cause of death after ischemic heart diseases in the world and developing countries, and the third-ranking cause of the disabilities in developed countries (3). According to the 2018 data of the Turkish Statistical Institute, stroke is reported to be in the second place with 22.4% among the causes of death in our country after ischemic heart diseases (5).

In ischemic stroke, irreversible damage occurs in the cells at the center of the affected area in the brain tissue. However, the damage to the cells in the region called penumbra which is located in the periphery of this area can be reversed. The main goal of stroke treatment is to restore the blood circulation of the penumbra and to minimize the loss of functioning (6). Therefore, early presentation to the emergency department is of great importance from the onset of symptoms. Recognizing the symptoms of stroke and taking immediate actions are vital in the management of acute stroke.

With the emergence of treatment types such as thrombolytic and thrombectomy, the importance of rapid presentation of patients to the emergency department has increased due to the fact that these treatments have to be performed immediately and the benefit decreases as the treatment are delayed. In order to be able to administer thrombolytic and/or thrombectomy treatments, patients need to be delivered to the emergency department urgently and the pre-hospital delay should be minimized(7, 8).

The aim of this study is to show the factors affecting the time of presentation to the hospital in acute strokes, to determine the rate of patients' benefit from thrombolytic therapy and to investigate the thrombolytic therapy awareness of patients and their relatives.

MATERIAL AND METHOD

The study was carried out with the permission of Hatay Mustafa Kemal University Clinical Researches Ethics Committee (Date: 27.06.2022, Decision No: 02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study included 276 patients over the age of 18 who presented to Hatay Mustafa Kemal University Hospital with a stroke clinic, were diagnosed with stroke with a neurological examination and radiological imaging method, and followed up in the neurology clinic.After the necessary clarification was made about the evaluation test to be performed and informed consent was obtained, the patient (if possible) and his/her companion/relative were interviewed and the standard structured questionnaire was filled in for each patient.

All patients who came to the emergency department with stroke symptoms were evaluated by the on-call neurologist. A comprehensive clinical examination was performed. Cranial tomography, diffusion MRI and computed tomography angiography were performed in all patients. Stroke onset time was accepted as the time when the patient or an observer first noticed a neurological deficit. If symptoms were present during awakening, stroke was assumed to occur at night, and the onset time was considered to be when the patient last had no symptoms. Patients who needed thrombectomy were referred to the stroke center and were not included in the study.

The time of access to the hospital was marked on the questionnaire. Questions such as age, gender, place of residence, level of education, number of attacks, at what time the patients presented to the hospital after the onset of symptoms, whether they received thrombolytic therapy, what they did as the first intervention, type of stroke and symptom onset time were asked in the questionnaire. Thrombolytic therapy awareness of the patients was also questioned. Information was also obtained about the way of access to the hospital, the place of residence, whether the patients lived alone or if not with whom they lived at home, and the presence of any relatives or friends at the onset of the stroke. Neurological findings and stroke type were documented.

Stroke severity was documented using the National Institutes of Health Stroke Scale (NIHSS). Patients were divided into subgroups as those who came early (<4 hours) and those who came late (>4 hours) according to the duration of presentation to the hospital after the onset of stroke symptoms. Stroke start times were recorded according to four time intervals which are (6:00 - 12:00), (12:00 - 18:00), (18: 00-24:00) and (24: 00-06:00).

Statistical analyses were performed in IBM SPSS for Windows Version 22.0 package program. Numerical variables were summarized with mean±standard deviation and median [Min-Max] values, and categorical variables were summarized with numbers and percentages. The normality of the numerical variables was analyzed with the Kolmogorov Smirnov test; in the comparison of two groups, results were compared with t-test when the parametric test conditions were met, and the Mann Whitney U test was compared when they were not met. Significance level was taken as $p<0.05$.

RESULTS

276 patients were included in the study. 39.9% of the patients included in the study were female and 60.1% were male. The mean age of the patients was 72.4±12.6 years. The distribution of CVD was 218 (79%) ischemic stroke, 26 (9.4%) intracerebral hemorrhage and 32 (11.6%) TIA. The mean NIHHS score of the patients was 6.7±6.2. Of the patients who participated in the study, 43.5% had DM, 63% had HT, 12.3% had hyperlipidemia and 22.5% had coronary artery disease. Stroke onset time was mainly between 18.00-24.00 with a rate of 28.3%. When the way of access of the patients to the emergency department was evaluated, 83.1% came by ambulance and 16.9% came by their own vehicle.

As the first intervention of the patients, 37.7% waited to recover, 3.6% poured water on the patient's head and 58.7% called 112. 22.5% of the patients came to the hospital within the first 4.5 hours and 10.1% of the patients received thrombolytic therapy (**Table 1**).

When the patients who underwent and did not undergo thrombolytic therapy were compared, no significant difference was found between the watershed area, who they lived with at home and the level of education. Statistical significance was found with symptom onset time (**Table 2**).

Table 1. General Information

	Number (n=276)	%
Gender		
F/M	110/166	39.9/60.1
Place of Residence		
Village	26	9.4
District	36	13
Center	214	77.5
Presentation Center		
In Province	240	87
Out of Province	36	13
The time interval he/she presented after the stroke		
0-4.5	62	22.5
4.5-6	48	17.4
6-24	122	44.2
Over 24 hours	44	15.9
Presentation to another hospital	54	19.6
Waiting period		
0-4/hours	50	92.6
4 and over/hours	4	7.4
Presentation Department		
Emergency Department	248	89.9
Polyclinic (Not an Emergency Department)	28	11.1
Thrombolytic therapy recipient	28	10.1
Those Who Know Thrombolytic Therapy	26	9.4
First Action?		
I did not understand my disorder/Waited to Recover?	114	41.3
I called 112	162	58.7

Table 2. Comparison of groups with and without thrombolytic therapy

	Groups with thrombolytic therapy (n=28)	Groups without thrombolytic therapy (n=248)	p
Age	78.4±3.1	72.1±12.9	<0.001
Gender (F/M)	16/12 (57.1%-42.9%)	94/154 (37.9%-62.1%)	0.436
Watershed Area			0.601
Anterior	24 (85.8%)	210 (84.6%)	
Posterior	4(14.2%)	38 (15.4%)	
Who he/she lives with at home			0.494
Alone	8 (28.6%)	26 (10.4%)	
Spouse	12 (42.9%)	130 (52.4%)	
Extended Family	8 (28.6%)	92 (37.2%)	
Level of Education			0.055
Literate	24 (85.7%)	86 (34.6%)	
Primary School	4 (14.3%)	100 (40.3%)	
Secondary school	-	32 (12.9%)	
Highschool	-	30 (12.09%)	
Symptom Start Time			0.030
06-12	10(35.8%)	56(22.5%)	
12-18	12(42.8%)	49 (19.7%)	
18-24	6 (21.4%)	58 (25.8%)	
24-06	-	79 (31.8%)	

(p<0,05 statistically significant)

Significant differences were found when the time of presentation to the hospital was compared with the place of residence, type of stroke and symptom onset time (p<0,05) (**Tablo 3**). In addition, a significant difference was found between thrombolytic awareness and time of presentation to hospital.

Table 3: Time of presentation to the hospital

	0-4.5 (n=62)	4.5 -6 (n=48)	6-24 (n=122)	>24 (n=44)	p
Place of Residence					<0.001
Village	6 (9.7%)	-	8 (6.6%)	12 (27.3%)	
District	-	4 (8.3%)	14 (23%)	2 (9.1%)	
Center	56 (90.3%)	44 (91.7%)	86 (70.5%)	28 (63.6%)	
Type of the Stroke					<0.001
Minor (NIHSS 0-6)	18 (29%)	24 (50%)	48 (39.3%)	34 (77.3%)	
Major (NIHSS 7-24)	24 (38.7%)	22 (45.8%)	64 (52.5%)	10 (22.7%)	
TIA	20 (32.3%)	2 (4.2%)	10 (8.2%)	-	
Symptom Start Time					<0.001
06-12	30 (48.4%)	4 (8.3%)	12 (9.8%)	12 (27.3%)	
12-18	22 (35.5%)	8 (16.7%)	22 (18%)	14 (31.8%)	
18-24	6 (9.7%)	18 (37.5%)	44 (36.1%)	10 (22.7%)	
24-06	4 (6.5%)	18 (37.5%)	44 (36.1%)	8 (18.2%)	
Watershed Area					0.012
Anterior	60 (96.8%)	44 (91.7%)	96 (78.7%)	30 (68.2%)	
Posterior	2 (3.2%)	4 (8.3%)	26 (21.3%)	14 (31.8%)	
IV-TPA awareness	14 (22.6%)	4 (8.3%)	8 (6.6%)	-	0.021

(NIHSS: National Institutes of Health Stroke Scale, p<0,05 statistically significant)

DISCUSSION

In our study evaluating the factors affecting the hospital presentation times of patients receiving inpatient treatment in the Neurology clinic due to acute stroke, 28 (10.1%) patients received thrombolytic therapy. In a study conducted with 182 patients, 17% of the patients received thrombolytic therapy (9), while 25.3% of 301 patients received thrombolytic therapy in another study conducted in 2017, (10). In another study, only 36 of 469 patients were able to receive thrombolytic therapy (11).

In a survey investigating the thrombolytic therapy awareness of the patients conducted by telephone interview, 26.2% of the patients had information about thrombolytic therapy (12). In another study evaluating 173 patients, it was found that 11.5% of the patients were aware of thrombolytic, clot-busting treatment. Similarly, 9.4% of the patients had information about thrombolytic therapy in our study. Without awareness of treatment techniques and time sensitivity, it is unlikely that patients will rapidly take action despite they know the symptoms. In our study, although the patients realized that they had symptoms, 37.7% of them waited to recover and 3.6% poured water on their heads.

In the studies conducted, the duration of presentation of patients with a stroke to the emergency department in the first 3 hours varies between 21% and 48% (13,14). Similarly, in our study, 22.5% of the patients presented to the emergency department in the first 4.5 hours.

In the study conducted with 517 patients in Turkey, when it was evaluated whether the health institution to which the patients applied was the first center, it was determined that 26.9% of the patients had already presented to another health institution before (15). In our study, the rate of presentation to another hospital was 19.6%, and 92.6% of the patients were late between 0-4 hours after presentation to another hospital, while 7.4% of the patients were late for more than 4 hours. In order to increase the effectiveness of thrombolytic therapy, it will be beneficial to have emergency departments as the first place of presentation and to accelerate possible referrals.

In the study in which NIHSS scores were calculated when patients presented to the hospital according to their neurological dysfunction degrees, patients with NIHSS scores of 16 and below were found to be 82.92%, while 16 and above were found to be 17.08% (16). In a different study, the NIHSS total score ranged from 1-8 and the median value was found to be 4 (11). Studies have shown that patients with high NIHSS scores apply to the hospital earlier than others (17,18). In our study, similar to other studies, NIHSS total scores were between 2-9 and the median value was found to be 4. It was found that patients with low NIHSS scores presented to the emergency department less in the first 24 hours than patients with high NIHSS scores.

In a study evaluating stroke onset times, it was revealed that it started mainly between 12.00-18.00 with a rate of 31.6%, and in another study with 47.2% between 06.00-14.00 (16, 19). In our study, the most frequent presentation hours were found to be between 18.00-24.00 with 28.3%.

The longest delay in the treatment of acute stroke occurs outside the hospital (20). In a study comparing the means of transportation to the hospital, 68.63% of the patients and in another study, 62% of the patients used ambulance more than other means (16,21). In our study, it was found that a higher rate (83.1%) came to the hospital by ambulance compared to other studies. It has been revealed that the use of ambulance services is effective in early access to treatment (22) .

Known risk factors causing stroke include diabetes, hypertension, and high cholesterol (23,24). In our study, similar to other studies, these increased risk factors were observed and DM was detected in 43%, HT in 63%, and hyperlipidemia in 12.3% of the patients.

The study had some limitations. First of all, it is a single-center study with a small sample size. The stroke symptoms of the patients were not questioned. Multicenter studies involving larger patient groups are needed.

CONCLUSION

The major factors causing delays of the patients to the hospital were investigated in our study. Thrombolytic therapy awareness levels of the patients were evaluated. It has been concluded that patients need to be informed about the treatment and the importance of early presentation to the hospital. Reducing mortality and morbidity through raising awareness of society on stroke symptoms and early treatment benefits will lead to a decrease in social and economic burden.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hatay Mustafa Kemal University Clinical Researches Ethics Committee (Date: 27.06.2022, Decision No: 02).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Face-to-face assessment versus tele-assessment of chronic stroke patients: do the results meet the needs?

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ABSTRACT

Aim: Baseline evaluation of stroke patients is valuable to manage the treatment plan. As in the case of stroke evaluation and rehabilitation, in every aspect of healthcare, tele-medicine is growing gradually. The aim of this descriptive study was to explore whether initial tele-assessment of chronic stroke patients is similar to face-to-face assessment in terms of clinical scales.

Material and Method: Thirty-four chronic stroke patients (mean post-stroke duration 21.44±15.47 months; stroke etiology, 58.8% ischemic; hemiplegic side, 52.9% left; mean age 49.24±12.51; 22 males; 12 females) were included in this trial. Firstly, all the patients were evaluated online, and then at the same day they were evaluated face-to-face by the clinical scales including chair stand test, Berg balance scale, Stroke specific quality of life scale, Motricity index.

Results: The findings of the present study revealed that there was no statistically significant difference between tele-assessment and face-to-face assessment for all the scales (p>0.05) except chair stand test (mean time to stand: 9.41 secs vs 8.94 secs in tele-assessment and face-to-face respectively; p<0.013).

Conclusion: The authors think that tele-assessment could be performed conclusively as well as face-to-face assessment since the clinical scales used in this trial were gross motor and functional tests. These preliminary results may suggest that tele-assessment could be used for initial assessment of the post-stroke patients as a convenient tool in order to ameliorate the continuous care without disruption by location.

Keywords: Activities, daily living, balance, stroke, assessment

INTRODUCTION

Stroke influences many facets of nervous system function unfavorably (1,2) and may cause hemiparesis, other motor and sensory deficits, as well as balance and posture disturbances (1-5).

In order to develop a rehabilitation plan and evaluate the efficacy of treatment, a baseline evaluation of the neurological and physical condition of the post-stroke patient is important. Initial clinical evaluation often utilizes scales to quantify impairment, activity limitations, and participation restrictions (1,6,7). These scales are also useful for follow-up during and after neuro-rehabilitation (1).

Currently, there has been an increase in assessment and follow-up of patients via tele-medicine (8,9). Keeping up with times, there has been an increase in the use of tele-rehabilitation technologies for rehabilitation of patients with neurological problems, and tele-rehabilitation interventions have shown promising results in improving the health of post-stroke patients and supporting caregivers (10,11). Tele-assessment techniques can inform healthcare professionals about the clinical status, improvements of patients, and allows professionals to remotely collect real-time data on patients' progress. Various remote assessment systems have been developed for tele-assessment. Video conferencing system has been shown to be a convenient and reliable method for evaluating patients with neurological symptoms (12,13). In one study, healthcare professionals and participants reported high levels of satisfaction with tele-rehabilitation interventions in post-stroke patients (11).

The aim of this descriptive study was to explore whether initial tele-assessment of chronic stroke patients is similar to face-to-face physical assessment in terms of the above-mentioned clinical rating scales.

MATERIAL AND METHOD

The study was carried out with the permission of Üsküdar University Non-interventional Researches Ethics Committee (Date: 28.04.2021, Decision No: 2021-08/4). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Oral and written informed consent were obtained from the patients at the beginning of the study.

The number of people to be included in the study was determined as a minimum of 34 by using G*Power 3.1.9.2 software. A total of 34 post-stroke patients were assessed using tele-assessment and face-to-face examination. The inclusion criteria were as follows: 1) chronic stroke for more than six months in consequence of ischemic or hemorrhagic stroke; 2) Brunnstrom motor stage 3 or above; 3) technological competence; 4) volunteer for participation to the study; and 5) literacy. The exclusion criteria were incapacity to cooperate and lack of a person to assist the patient during the tele-assessment.

Clinical scales were initially administered online (tele-assessment) by video conferencing system followed by face-to-face administration on the same day. All assessments were made by the same physiotherapist qualified in neurology. During the tele-assessment, the patient was connected by phone via video conference and the phone was fixed so that the physiotherapist and the patient could see each other. A person was always present to assist the patient during the tele-assessment and helped the patient when needed. Before the assessment, the patients and their relatives were given education and information about the evaluation scales to be applied.

The clinical tests and scales that compared between tele-assessment and face-to-face administration were chair stand test (CST) (1,14-16), Berg balance scale (BBS) (1,17), Stroke specific quality of life scale (SS-QoL) (18), Motricity index (MI) (19).

CST evaluates a patient's locomotor ability and performance including balance, mobility, and risk of fall. The time taken by and individual to sit down and stand up from a chair is measured in seconds. Straight-backed, armless chair was used. The patient was to sit in the chair with non-paretic arm crossed over the chest with the knees at 90 degrees angle, feet on the floor and performed three sit to stands as quickly as possible safely without using arms with five minutes intervals. The mean of three trials used for data analysis (1,14-16).

The BBS assesses balance by having an individual complete fourteen diverse balance and locomotion tasks which are objectively scored by a qualified individual. Participants may obtain a maximum score of 56 indicating good balance (1,17).

SS-QoL evaluates the quality of life of stroke patients via a 49-item questionnaire with varied domains including energy levels, family roles, language, mobility, mood, personality, self-care, social roles, thinking, upper extremity function, vision and work productivity of the patient. Higher scores indicate better quality of life (18).

MI evaluates the motor ability of the upper extremity, lower extremity and trunk in post-stroke patients. Motion is evaluated at all major joints. The highest score of 33 indicates normal strength hile zero score means no movement at all (19).

Statistical Analysis

The analysis of the current data was calculated by PSPP (GNU PSPP 0.10.4-g50f7b7). Distribution of frequency, percentage, mean and standard deviation were utilized as statistical methods. Normality of the data was ensured by Kolmogorov-Smirnov test. Wilcoxon test was used for comparison between tele-assessment and face-to-face. The results were evaluated at the $p<0.05$ statistical significance level.

RESULTS

Relevant descriptive characteristics of chronic stroke patients in the current study are summarized in **Table 1**. The study included 34 post-stroke patients, among whom 22 (64.7%) were men, and 12 (35.3%) were women. Mean age of the participants was 49.24 years, mean Body Mass Index (BMI) was 26.02. Most of the participants were self-employed (32.4%), others were in management (11.8%), retired (11.8%), engineers (8.8%), lawyers (8.8%), shipping (8.8%), housewives (8.8%), students (5.9%), athletes (2.9%). More than half of the patients (52.9%) were university graduates. The stroke etiology was ischemia in 20 patients (58.8%), mean post-stroke duration was 21.44 months, and 52.9% of our participants had left hemiplegia.

Table 2 summarizes the results of the clinical scales of CST, BBS, SS-QoL and MI for tele-assessment and face-to-face assessment. MI of upper extremity, lower extremity and the trunk were similar for both assessments. Although there were numerical differences in BBS, and SS-QoL scores between the two evaluations, the values were not statistically significant ($p=0.18$ and $p=0.72$ respectively, **Table 2**). In contrast, CST score were statistically significant between the two evaluations (mean time to stand: 9.41 secs vs 8.94 secs in tele-assessment and face-to-face assessment respectively; $p < 0.013$, **Table 2**). Participants took longer to complete the chair stand test during tele-assessment.

Saral et al. Face-to-face assessment versus tele-assessment of chronic stroke patients

J Health Sci Med 2023; 6(1): 82-86

Table 1. Descriptive characteristics of the chronic stroke patients

Descriptive characteristics	Chronic stroke patients mean±SD
Age (years)	49.24 ±12.51
Gender – n (%)	
Male	22 (64.7%)
Female	12 (35.3%)
BMI (kg/cm2)	26.02 ±3.99
Height (cm)	171.5 ±8.5
Weight (kg)	76.76 ±13.99
Education level – n (%)	
Secondary school	5 (14.7%)
High school	13 (38.2%)
University	20 (52.9%)
Profession – n (%)	
Self-employed	11 (32.4%)
Employee/Managerial staff	4 (11.8%)
Retired	4 (11.8%)
Engineer/Technician	3 (8.8%)
Lawyer	3 (8.8%)
Sailor	3 (8.8%)
Housewife	3 (8.8%)
Student	2 (5.9%)
Athlete (Football player)	1 (2.9%)
Hemiplegia – n (%)	
Right hemiplegia	16 (47.1%)
Left hemiplegia	18 (52.9%)
Stroke etiology – n (%)	
Hemorrhagic	14 (41.2%)
Ischemic	20 (58.8%)
Post-stroke duration (months)	21.44 ±15.47
Brunnstrom upper extremity – n (%)	
Stage 3	14 (41.18%)
Stage 4	5(14.71%)
Stage 5	9(26.47%)
Stage 6	6 (17.65%)
Brunnstrom el – n (%)	
Stage 3	17 (50%)
Stage 4	6 (17.65%)
Stage 5	6 (17.65%)
Stage 6	5 (14.71%)
Brunnstrom lower extremity – n (%)	
Stage 3	10 (29.41%)
Stage 4	2 (5.88%)
Stage 5	8 (23.53%)
Stage 6	14 (41.18%)

Table 2. Results of clinical scales for tele-assessment and face-to-face assessment

Clinical scales	Face-to-face assessment (n=34) mean ±SD	Tele-assessment (n=34) mean ±SD	p value
CST	8.94±3.8	9.41±3.85	0.013
BBS	39.85±16.59	40.18±16.62	0.18
SS-QoL	149.35±39.77	149.09±37.2	0.72
MI			
Upper extremity	62.09±29.74	62.09±29.74	-
Lower extremity	67.88±23.18	67.88±23.18	-
Trunk kontrol test	52.76±10.81	52.76±10.81	-

DISCUSSION

The objective of this study was to explore whether clinical assessments for neurological rehabilitation of stroke patients administered telephonically are similar to performing them face-to-face. Our results show that tele-assessment was as good as face-to-face assessment when measuring BBS, SS-QoL and MI, but not for measuring CST. When the literature was searched, no study could be found on the effectiveness of tele-assessment in stroke. Studies investigating the effectiveness of tele-rehabilitation and comparing it with conventional rehabilitation are at the forefront. In situations when there is limited accessibility to health services, as in the case of the COVID-19 pandemic, tele-medicine including tele-assessment is a necessity, and the certainty about patients' assessment could lead to consistent and accurate follow-up and treatment plans.

Epidemiological studies have shown that the prevalence of stroke increases with age. Turk Boru U et al. (20) demonstrated that mean age of stroke patients was 64±14.8, percentage of male gender was 52%, and ischemic etiology was accounted for 80% of the stroke cases in Turkey in İstanbul. In concordance with this study, our study population of post-stroke patients was 49.24 ±12.51 years, had male predominance (64.7%), and ischemic etiology was accounted for 58.8%.

The recent studies point out the connection between educational level and stroke incidence, recurrence, and mortality. Che B et al. (21) concluded that low level of education gave rise to higher mortality from stroke. Xiuyun W et al. (22) demonstrated that high level of education was related to decline in stroke event. Contrary to this study, approximately 90% of the stroke survivors had high level of education in our study.

Tele-medicine has been used for evaluation, rehabilitation, treatment, diagnosis, and follow-up purposes for the last 20 years in most medical specialties (23-28). The key aim is to take care of the patients irrespective of their accessibility to health care services (23,26). Means of communication in tele-medicine include remote wireless dialog, web portals, mobile applications, and smartphones (23). In this study, video conferencing system was used.

Recent research by Park S et al. (29), has shown that tele-medicine is important for post-stroke patients due to their long-term recovery needs. In this meta-analysis, the authors evaluated the effectiveness of lower extremity-focused tele-rehabilitation interventions on clinical outcomes. This review aimed to both describe and quantitatively assess the effects of tele-rehabilitation interventions poststroke, therefore, they included randomised controlled trials (RCTs) as the primary study design and non-RCT interventional studies.

Observational or descriptive study designs, including cohort studies, case series, case reports and cross-sectional studies were excluded. As a result, authors concluded that function and ability to perform activities of daily living of the post-stroke patients could improve via tele-medicine. Similar to this trial, in our study we found that tele-assessment is comparable to face-to-face assessment in terms of ambulation, balance, mobility, and posture tests which are associated with locomotion.

In another recent study, researchers aimed to summarize and compare the effects of active rehabilitation assisted by new technologies (virtual reality, robot-assisted therapy and tele-rehabilitation) on upper limb function during the subacute and chronic phases of stroke (30). 15 meta-analyses were based on 189 randomized controlled trials, were included in the quantitative analysis. They concluded that rehabilitation assisted with technologies are at least as effective as face to face conventional therapy for patients with stroke. Although we used tele-medicine as an assessment or baseline diagnostic tool, in our study, we found that approximately the same results can be obtained via tele-assessment when compared to face-to-face evaluation in chronic stroke patients.

In biomedical engineering, literature research into tele-health has been rising since the early 2000s, and patterns of tele-health have been reported. Tele-health has been divided into three parts; tele-medicine, tele-healthcare, and e-health education, and tele-assessment is a subgroup of tele-medicine (31). Principally, tele-assessment is an interactive and real-time experience for the medical examiners and the inspected patients (31,32), allowing, visual, auditory, verbal, and even body language dynamics to be achieved by tele-assessment as in the case of face-to-face evaluation (31). Therefore, we were not surprised when we obtained similar results for the evaluation of the post-stroke patients when assessed telephonically or face-to-face in the current study. When we review the literature, tele-medicine studies in stroke patients are mostly tele-rehabilitation studies that include the effectiveness of exercise applications. From this point of view, our study can lead to future studies.

In a systemic review that aimed to summarized the effectiveness and safety of tele-medicine combined with usual care compared to usual care in neurological diseases, 25 RCTs (n=2335) were included: 11 (n=804) on stroke, four (n=520) on Parkinson's disease, three (n=110) on multiple sclerosis, two (n=320) on epilepsy, one (n=63) on dementia, one (n=23) on spina bifida, one (n=40) on migraine, one (n=22) on cerebral palsy and one (n=433) on brain damage (33). Types of tele-medicine assessed were online visits (11 studies), tele-rehabilitation (seven studies), telephone calls (three), smartphone apps (two) and online computer software

(two). In another cochrane systemic review by Laver KE et al. (34), aimed to determine whether the use of tele-rehabilitation leads to improved activities of daily living amongst stroke patients when compared with in-person rehabilitation; or no rehabilitation or usual care. They included 22 trials involving a total of 1937 participants, ranged in size from the inclusion of 10 participants to 536 participants. When we examine the studies, similar scales were used as outcome parameter in most of the studies such as Stroke Impact Scale, Quality of Life Scale, 6-Minute Walk Test, Balance and Mobility Scale, Fugl Meyer Assessment. However, it was not clear whether the assessments were carried out in the form of face-to-face or tele-assessment. Similarly BBS, SS-QoL, MI and CST were used as outcome parameters in our study.

We found significant difference in CST evaluation in favor of face-to-face assessment. Since patients stood up 0.47 faster in the face-to-face evaluation. We speculated that from the point of the examiner, there was eye contact separation from the patient to the time counter, disrupting the interactivity, and time elapsed during tele-assessment. Moreover, this may affect all manner of the patient during the movement. However, in the case of face-to-face evaluation, the examiner could find opportunity to closely follow the movement of CST while keeping track of the time. In other words it means that, while the gross motor tests used in tele-assessment give similar results with face-to-face assessment; more sensitive tests needing more attention, for example, requiring second account calculation may not give the same result.

There are some limitations in our study. Although power analysis was performed and the sample size was determined before the study, more reliable results may be obtained with a larger number of patients. Since clinical scales were administered twice on the same day, we did not control for fatigue or learning in the face-to-face assessment and, also the assessments were both time consuming and tiring for the patient. The high level of education of the patient population in our study may have a significant effect on the results. Conducting this study with groups of different socioeconomic status may lead to different results. Studies with groups of different socioeconomic status are recommended as they will increase the power of the study.

CONCLUSION

Initial tele-assessment of chronic stroke patients is comparable to face-to-face physical assessment in terms of the clinical rating scales; therefore, tele-assessment could replace face-to-face physical examination for the initial and follow-up evaluations of chronic stroke patients.

Saral et al. Face-to-face assessment versus tele-assessment of chronic stroke patients

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ETHICAL DECLARATIONS

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Fetal cavum septum pellucidum nomogram and its relationship with fetal Doppler: a prospective study of a Turkish population

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ABSTRACT

Aim: Septum pellucidum is a thin membrane with right and left leaves, and cavum septum pellucidum (CSP) is formed in the intermembrane region. This study investigates CSP nomogram dimensions for all trimesters in the Turkish population. In addition, the relationship between fetal Doppler flow and CSP size was investigated in this study.

Material and Method: This study was designed as a prospective cohort between 2019-2020. Pregnant women between 19-42 weeks who were followed up at University of Health Sciences Tepecik Training and Research Hospital, Department of Obstetrics and Gynecology were included in the study.

Results: A total of 517 fetuses meeting our criteria were included in this prospective study. In the second trimester (19-28 weeks) CSP width (4.12 ± 0.88 vs. 4.91 ± 1.42, $p<0.001$) and length (7.95 ± 1.04 vs. 9.48 ± 2.19, $p<0.001$) were significantly higher than in the third trimester (28-42 weeks). While the mean CSP width increased up to 32nd weeks, there was no clear increase-decrease pattern between 32nd-38th weeks, and it was observed to decrease after 38th weeks. The mean CSP length increased up to 29th weeks, while there was no clear increase-decrease pattern between 29th-38th weeks, but decreased after 38th weeks. While a significant correlation was observed between gestational week and CSP width ($r=0.118$, $p=0.010$), there was no significant correlation between CSP length ($r=0.086$, $p=0.062$). A significant correlation was observed between biparietal diameter (BPD) and CSP width ($r=0.180$, $p<0.001$) and length ($r=0.202$, $p<0.001$), but not with head circumference (HC). There was a significant correlation between middle cerebral artery (MCA) systolic/diastolic ratio (S/D) ($r=0.185$, $p<0.001$), pulsatility index (PI) ($r=0.210$, $p<0.001$) and resistive index (RI) ($r=0.233$, $p<0.001$) and CSP length, but not with CSP width.

Conclusion: Turkish population fetal CSP nomogram is presented in this study. Fetal middle cerebral artery Doppler measurements (S/D, PI, and RI) showing cerebral blood flow correlate with CSP length, but not with CSP width. There was no correlation between fetal umbilical artery Doppler measurements and CSP sizes. The results pave the way for population-based studies with much larger samples.

Keywords: Cavum septum pellucidum, nomogram, umblical artery, middle cerebral artery, Doppler

INTRODUCTION

In the anterior part of the brain, there are two midline cavities. These are the cavum septum pellucidum (CSP) and the cavum vergae (CV). These formations are cavities formed by the leaves of the midline septum pellucidum during brain morphogenesis. Septum pellucidum is a thin membrane with right and left leaves, and CSP is formed in the intermembrane region. The CV is the posterior extension of the CSP, associated with the CSP but lying behind the columns of the fornix (1). Various views of the embryogenesis of these cavities have been proposed. Although it is often suggested that they occur as a result of cavitation of the medial lower commissural plate (2), the exact mechanism of formation is not clear (3,4).

The septum pellucidum acts as an information transfer center: It transmits visceral information via the hypothalamic autonomic system to the hippocampus, amygdala, habenula, and reticular formation in the brainstem. Therefore, it is thought to play a role in functions such as mental process of self-care, competence, finding food, sexuality, attention and activity by playing a role in sleep-wake and emotional response to the environment.

The axial transthalamic plane is the standard plane in which biparietal diameter and fetal head circumference are measured (5). The symmetry of the CSP, thalamus, and cerebral hemispheres can be evaluated in this section, and many midline malformations associated with the absence of CSP can also be detected in this section (6,7). In its absence, preliminary diagnoses of corpus callosum agenesis, holoprosencephaly, septo-optic dysplasia, and lissencephaly come to mind (4).

The cavum septum pellucidum is a very important structure in the evaluation of the fetal neural axis, which is an important component of fetal anatomy screening, and prenatal CSP evaluation is important for correct planning of postnatal follow-up of anomalies (4,8). Measurement dimensions can affect prognosis, although studies are often based on its presence and absence. For this reason, nomograms are tried to be created. Today, nomograms for CSP measurement are often considered in second trimester screening, but size change is important in all trimesters. Population-based variations can also occur, and population-based nomograms are primary tools for reflecting racial measurements (9). Therefore, in this study, it was planned to examine CSP sizes and size changes according to gestational weeks. This study investigates CSP nomogram dimensions for all trimesters in the Turkish population. In addition, the relationship between fetal Doppler flow and CSP size was investigated in this study.

MATERIAL AND METHOD

The study was carried out with the permission of İzmir Tepecik Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date: 13.03.2019, Decision No: 2019/4-2). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

This study was designed as a prospective cohort between 2019-2020. Pregnant women between 19-42 weeks who were followed up at University of Health Sciences Tepecik Training and Research Hospital, Department of Obstetrics and Gynecology were included in the study. All participants were informed and informed voluntary consent was obtained.

Pregnant women using medication and those; with multiple pregnancies; with in vitro fertilization (IVF) pregnancies; with major or minor fetal anomalies; with known type 1 and type 2 diabetes mellitus; with gestational hypertension/preeclampsia; with fetal growth restriction; with hepatitis, gastroenteritis, pyelonephritis, or urolithiasis; and with incomplete records and/or whose records could not be reached were excluded from the study.

Ultrasound Examinations

Ultrasound assessment was performed (Toshiba AplioTM 500; Toshiba Medical Systems Inc., USA) equipped with an abdominal 3.5 MHz convex transducer. All measurements were performed by a single sonographer (B.B.) with the Doppler certificate of The Fetal Medicine Foundation in order to avoid differences. All examinations were performed in the supine position. First, biparietal diameter (BPD) and head circumference (HC) measurements of the fetus were taken. These measurements were made in the axial plane, with the calvarium symmetrical, in the plane of the thalamus and cavum septum pellucidum, and the cerebellum was not included in the image. BPD was measured in millimeters from outside to inside. The HC was measured in millimeters circumferentially from the outside edge of the calvarium. The probe was then rotated slightly to the cephalic part and the fluid-filled CSP was observed between the anterior horns of the lateral ventricles. The maximum anterior and posterior width between the inner boundaries of the CSP was measured in this plane as previously described by Jou et al. (10). Doppler measurements were made from both umbilical arteries and the middle 1/3 of the umbilical cord, and locations close to the fetus or placenta were avoided. In fetal middle cerebral artery (MCA) Doppler measurements, after determining the vascular structures of the Willis polygon with the help of color coding, measurements were made from the 1/3 beginning of the MCA near the origin from a. carotis interna. Traces in Doppler were followed until at least 3, at most 10 consecutive waves were obtained.

Statistical Analysis

Statistical Package for the Social Sciences version 26.0 (IBM Corporation, Armonk, New York, US) was used in the analysis of the data, and the significance level was taken as $p<.05$ in all analyses. Shapiro-Wilk test was used to determine the distribution of the data. Student's t-test was used for normally distributed data in comparison of the groups and the Mann-Whitney U test was used to compare the data that could not show normal distribution. Pearson's correlation coefficients analysis was performed to determine the relationship between parameters. For the number of samples, power analysis was performed with G-power 3.1.9.7 version. Accordingly, the minimum number of patients for each group was calculated as 33.

RESULTS

A total of 517 fetuses meeting our criteria were included in this prospective study. Demographic and clinical characteristics of the study participants are shown in **Table 1**. The mean age of the pregnant women was

28.1±5.7, and 236 (45.7%) were nulliparous and 281 (54.3%) were multiparous. The body mass index (BMI) was 28.4±4.8 kg/m² and 127 (24.5%) pregnant women were smoking. The mean CSP width was 4.77±1.37, and the CSP length was 9.21±2.11. (**Table 1**).

Table 1. Demographic and clinical characteristics of the study participants	
	n=517
Maternal age (year) (mean±SD)	28.1±5.7
Parity (n,%)	
Nulliparous	236 (45.7%)
Multiparous	281 (54.3%)
BMI (kg/m²)	28.4±4.8
Smoking (n,%)	127 (24.5%)
CSP width (mm) (mean±SD)	4.77±1.37
CSP length (mm) (mean±SD)	9.21±2.11
Abbreviations: BMI: body mass index, CSP: cavum septum pellucidum, SD: standard deviation	

The cavum septum pellucidum measurements according to trimesters were compared in **Table 2**. Accordingly, in the second trimester (19-28 weeks) CSP width (4.12±0.88 vs. 4.91±1.42, p<0.001) and length (7.95±1.04 vs. 9.48±2.19, p<0.001) were significantly higher than in the third trimester (28-42 weeks). (**Table 2**).

Table 2. Cavum septum pellucidum measurements according to trimesters			
	Second Trimester (19-28 weeks) n=358	**Third Trimester (28-42 weeks) n=159**	**p**
CSP width (mm) (mean±SD)	4.12±0.88	4.91±1.42	<0.001
CSP length (mm) (mean±SD)	7.95±1.04	9.48±2.19	<0.001
Abbreviations: CSP: cavum septum pellucidum, SD: standard deviation			

Mean width, length and standard deviation (SD) intervals according to gestational weeks were examined in **Table 3**. The mean CSP width ranged from 2.60 (19th week) to 5.80 (32nd week). The mean CSP length ranged from 7.30 (19th week) to 11.22 (29th week). The mean CSP width was between 2.06 (19th week) and 5.52 (32nd week) in -2 SD, and the mean CSP length was between 6.37 (19th week) and 10.42 (29th week) in -2 SD. The mean CSP width was between 3.13 (19th week) and 6.29 (35th week) in +2 SD, and the mean CSP length was between 8.22 (19th week) and 12.50 (33rd week) in +2 SD. (**Table 3**). While the mean CSP width increased up to 32nd weeks, there was no clear increase-decrease pattern between 32nd-38th weeks, and it was observed to decrease after 38th weeks. The mean CSP length increased up to 29th weeks, while there was no clear increase-decrease pattern between 29th-38th weeks, but decreased after 38th weeks (**Figure 1**).

Figure 1. Distribution of cavum septum pellucidum measurements according to gestational weeks

Table 3. Mean width, length and standard deviation intervals by gestational weeks							
Gestational age (weeks)	Number of cases	CSP width (mm) -2SD	Mean CSP width (mm)	CSP width (mm) +2SD	CSP length (mm) -2SD	Mean CSP width (mm)	CSP length (mm) +2SD
19	37	2.06	2.60	3.13	6.37	7.30	8.22
20	36	2.75	3.18	3.61	6.81	7.61	8.40
21	36	3.02	3.28	3.55	6.99	7.65	8.30
22	37	3.45	3.77	4.09	6.48	7.69	8.90
23	36	4.50	4.55	4.59	7.93	8.20	8.46
24	46	4.60	4.65	4.69	8.03	8.30	8.56
25	48	4.57	4.70	4.82	8.10	8.35	8.60
26	46	4.57	4.76	4.96	7.84	8.44	9.03
27	19	4.66	4.99	5.32	7.65	8.82	9.99
28	17	4.55	4.84	5.13	9.66	10.27	10.87
29	17	4.90	5.50	6.10	10.42	11.22	12.02
30	17	4.27	4.92	5.56	9.52	10.67	11.72
31	16	5.13	5.66	6.20	8.44	9.99	11.54
32	16	5.52	5.80	6.07	9.03	9.60	10.16
33	12	4.64	5.24	5.85	8.94	10.72	12.50
34	12	4.43	4.81	5.20	9.11	9.70	10.28
35	12	4.58	5.44	6.29	9.54	10.32	11.11
36	11	4.74	5.14	5.53	8.59	9.27	9.96
37	10	4.56	4.89	5.21	8.72	9.21	9.70
38	12	4.72	5.25	5.79	8.55	9.70	10.85
39	8	3.69	4.34	4.99	8.33	9.34	10.34
40	9	4.15	4.51	4.86	8.38	8.83	9.29
41	7	3.23	4.15	5.08	6.76	7.46	8.17
Abbreviations: CSP: cavum septum pellucidum, SD: standard deviation							

The correlation between gestational age, fetal head biometry and fetal Doppler values and CSP width and length is shown in **Table 4**. While a significant correlation was observed between gestational week and CSP width (r=0.118, p=0.010), there was no significant correlation between CSP length (r=0.086, p=0.062). A significant correlation was observed between BPD and CSP width (r=0.180, p<0.001) and length (r=0.202, p<0.001), but not with HC. There was a significant correlation between

MCA systolic/diastolic ratio (S/D) (r=0.185, p<0.001), pulsatility index (PI) (r=0.210, p<0.001) and resistive index (RI) (r=0.233, p<0.001) and CSP lenght, but not with CSP width. (**Table 4**).

Table 4. The correlation between gestational age, fetal head biometry and fetal Doppler values and CSP width and length		
	CSP width (mm)	**CSP length (mm)**
Gestational age (weeks)	r=0.118, p=0.010	r=0.086, p=0.062
BPD (mm)	r=0.180, p<0.001	r=0.202, p<0.001
HC (mm)	r=0.074, p=0.111	r=0.081, p=0.079
UA S/D	r=-0.012, p=0.802	r=-0.107, p=0.123
UA PI	r=-0.017, p=0.710	r=-0.082, p=0.077
UA RI	r=-0.022, p=0.635	r=-0.136, p=0.083
MCA S/D	r=0.064, p=0.198	r=0.185, p<0.001
MCA PI	r=0.025, p=0.583	r=0.210, p<0.001
MCA RI	r=0.062, p=0.183	r=0.233, p<0.001
Abbreviations: CSP: cavum septum pellucidum, BPD: biparietal diameter, HC: head circumference, UA S/D: umbilical arterial systolic/diastolic ratio, UA PI: umbilical arterial pulsatility index, UA RI: umbilical arterial resistive index, MCA S/D: middle cerebral arterial systolic/diastolic ratio, MCA PI: middle cerebral arterial pulsatility index, MCA RI: middle cerebral arterial resistive index		

DISCUSSION

The septum pellucidum is a structure containing translucent glial cells, neurons, nerve fibers and veins that associated with the choroid plexus and extending from the lamina terminalis to the splenium of the corpus callosum (3,4). The potential cavity between the leaves of the septum pellucidum is called the cavum septum pellucidum. The embryological development of CSP is related to the embryological development of the septum pellucidum. The development of CSP begins at 10-12 weeks of gestation and reaches its adult form at 17th weeks of gestation, simultaneously with the corpus callosum. CSP can often be seen on transabdominal ultrasonography between 18-37 weeks or when the biparietal diameter is between 44-88 mm (6). Its absence is well known and associated with severe anomalies, and the preliminary diagnosis of corpus callosum agenesis, holoprosencephaly, septo-optic dysplasia and lissencephaly comes to mind (4). However, literature about its dimensions is limited. It has been suggested that the anatomical differences detected in the septum pellucidum may also reflect possible embryonic developmental disorders in the adjacent anatomical structures (11,12). Large CSP in adults is thought to be associated with mental disorders. In this context, patients with schizophrenia are the most studied psychiatric patient group, and it has been argued that the higher rates of "wide CSP" in patients with schizophrenia than healthy patients are an anatomical finding that supports the neurodevelopmental hypothesis proposed for the etiology of schizophrenia. The dimensions of the CSP may reflect the evolution of associated structures. Therefore, CSP development nomogram consisting of healthy

fetuses is important. In this study, fetal CSP nomogram was planned in the Turkish population. To the best of our knowledge, this study is the study with the largest sample size investigating CSP nomogram dimensions for all trimesters in the Turkish population. In addition, for the first time in the literature, the correlation between fetal Doppler flow and CSP size was examined in this study.

Studies investigating the nomogram of all gestational weeks are limited in the literature. Falco et al. (13) examined the CSP width of 251 fetuses between 15 and 41 weeks in the Italian population in 2000. Overall, the average CSP widths were higher than our values. In their study, the mean CSP width was increasing by 31 weeks. On the other hand, we observed that CSP width increased until 32 weeks, and CSP length increased until 29 weeks. Tao et al. (14) studied CSP width in a total of 322 fetuses between 25 and 39 weeks in the Chinese population in 2013. In their study, the mean CSP width between the respective weeks was generally higher than in our study. CSP values at all gestational weeks were very close to each other, the range was very narrow, and no significant correlation was observed between CSP values and gestational weeks. On the other hand, we observed a significant correlation with the gestational week. Zhao et al. (15) examined 260 fetuses between 18-36 weeks in the Chinese population in 2019. In their study, CSP width and length increased up to 25 weeks. Similar to our study, CSP width was correlated with gestational week and BPD. We observed that CSP width increased until 32 weeks, and CSP length increased until 29 weeks. Ho et al. (16) investigated 503 fetuses between 15 and 39 weeks in the American population in 2020. Most of these measurements were made at 18 to 20 weeks at the time of the anatomy scan. CSP width was correlated with gestational week, similar to our study. However, only 1 fetus was examined at 38 and 39 weeks in this study, whereas we investigated 12 and 8 fetuses at 38 and 39 weeks, respectively. Yakubu et al. (17) examined the CSP width of 228 fetuses between 14-40 weeks in the Nigerian population in 2021. The mean CSP width was particularly high in the later weeks (36 weeks: 7.11±0.81, 37 weeks: 7.11±0.81, 38 weeks: 7.11±0.81, 39 weeks: 7.11±0.81 and 40 weeks: 9.30±0.28). However, they were able to examine only 4 fetuses at 39 weeks and only 2 fetuses at 40 weeks, whereas we investigated 8, 9 and 7 fetuses at 39, 40 and 41 weeks, respectively. In their study, CSP width was correlated with gestational week, similar to our study. We observed that CSP width increased until 32 weeks, and CSP length increased until 29 weeks.

As far as we know, there are only two studies investigating the measurement of CSP in the Turkish population. However, these studies had several limitations.

Serhatlioglu et al. (18) studied CSP sizes on 130 fetuses in 2003. This study included samples between 16-38 weeks. Accordingly, in the second and third trimesters, they found the mean CSP width to be 3.1±1.5 and 5.0±1.4, respectively, and the CSP length to be 7.7±2.6 and 11.7±2.5, respectively. In our study, second trimester and third trimester CSP widths were 4.12±0.88 and 4.91±1.42, respectively, and CSP length was 7.95±1.04 and 9.48±2.19, respectively. The difference in CSP according to trimesters was significant similar to our study. In the same study, they observed a correlation between gestational weeks, BPD and CSP width-length. We observed a significant correlation between BPD and CSP width and length, and between gestational week and CSP width. We did not observe correlation between gestational week and CSP length. The fact that our sample number was much higher may have caused this result. Again, they could not provide mean and standart deviation values in their studies, probably because the sample numbers were small. In addition, the correlation between fetal Doppler and CSP was not investigated in their studies. Arisoy et al. (19) retrospectively analyzed the nomogram of CSP width between 15-28 weeks in 2022. This study does not examine all gestational weeks, the nomogram is limited to the second trimester only. They found the second trimester mean CSP width to be 4.1±0.8. Our second trimester mean CSP width is 4.12±0.88, which is very close to this value. CSP width was correlated with gestational week and BPD, similar to our study. In their study, the 95th percentile values of CSP width between 15-28 weeks of gestation were found to be 3.7-7 mm. In our study, the +2SD CSP values were 3.13-5.13.

For the first time in the literature, we investigated the relationship between fetal Doppler values and CSP dimensions. We did not observe a significant correlation with fetal umblical artery Doppler measurements. However, fetal middle cerebral artery Doppler measurements (S/D, PI and RI) showing cerebral blood flow were correlated with CSP length, but not CSP width. In fact, the fetal cerebral artery is the main artery supplying the brain. The umbilical artery is not directly related to the brain. This may reflect the results. However, randomized controlled studies are needed on this subject.

This study had some limitations. Although it was higher than the literature, our sample number at the later gestational week was still limited. This study had also had strengths. For the first time in the literature, we investigated the relationship between fetal Doppler values and CSP dimensions. Also, to the best of our knowledge, this is the largest Turkish fetal CSP population study that includes all weeks of gestation.

CONCLUSION

Turkish population fetal CSP nomogram is presented in this study. Fetal CSP width increases up to 32 weeks of gestation and fetal CSP length increases up to 29 weeks. Third trimester CSP measurements are significantly higher than second trimester CSP measurements, which may be related to the growth of fetal head biometry. Fetal middle cerebral artery Doppler measurements (S/D, PI, and RI) showing cerebral blood flow correlate with CSP length, but not with CSP width. There is no correlation between umbilical artery values and CSP measurements. Population-based studies with much larger samples are required to establish clear values about fetal CSP measurements.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İzmir Tepecik Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date: 13.03.2019, Decision No: 2019/4-2).

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

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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Parallel changes in the promoter methylation of *voltage-gated T-type calcium channel alpha 1 subunit G* and histone deacetylase activity in the WAG/Rij model of absence epilepsy

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ABSTRACT

Objective: In the last two decades, research on epigenetic mechanisms has expanded dramatically. Recent studies demonstrated that epigenetic mechanisms regulate epilepsy and epileptogenic pathologies. In this study, we aimed to investigate changes in the promoter methylation status of the *voltage-gated T-type calcium channel alpha 1 subunit G* (*CACNA1G*) gene and total histone deacetylase activity in Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats which is one of the commonly used genetic absence rat models of epilepsy in the three different age groups (3, 6, and 9 months old) on both sexes.

Material and Method: Evaluation of changes in the spike-wave discharges (SWDs) was performed with electrocorticography (ECoG). The promoter methylation status of the *CACNA1G* gene was determined by methylation-specific PCR (MSP), and histone deacetylase (HDAC) activity was determined spectrophotometrically.

Results: Our results demonstrated that the number of SWDs increased age-dependent in WAG/Rij. Additionally, it was observed that *CACNA1G* promoter methylation decreased, and total HDAC activity increased with age in both sexes.

Conclusion: Our results provide further support for epigenetic regulation in the absence epilepsy phenotype and suggest that the underlying mechanism behind the increase in the number of SWDs with age in the WAG/Rij animals might be regulated by *CACNA1G* promoter methylation or HDAC activity.

Keywords: Epilepsy, voltage-gated T-type calcium channel, histone deacetylation, methylation, electrocorticography.

INTRODUCTION

Characterized by abrupt, comparatively brief lapses of consciousness, unusual electrographic spike-wave discharges (SWD) at 2,5-4 Hz, and involuntary movements, absence epilepsy is described as the generalized non-convulsive polygenic type of epilepsy (1). Psychiatric comorbidities and cognitive and mood impairments also accompany the clinical picture. Because the high incidence of pharmacoresistant, unresponsive patience and persistent comorbidities even following complete control of seizures indicate the unmet need for novel therapeutic interventions (2). Finding or identifying a novel approach is challenging, taking into consideration the large spectrum of absence seizure semiology and EEG features. Hopefully, the deficiency of biomarkers and high incidence around the world also motivate several groups to cope with this challenge. Recent decades also allowed improving experimental models that better mimic disease pathology and progression. Wistar Albino Glaxo/Rijswijk (WAG/Rij) is one of the strains that showed the crucial role of the localized cortex region in initiating the absence seizures (3). Several studies demonstrated that WAG/Rij is an appropriate model for absence seizures seen in humans (4). However, the molecular basis of absence epilepsy is not entirely understood (5).

Voltage-gated calcium channels manage the calcium entry into the cell in response to changes in membrane

Çelik et al. Epigenetic changes in absence epilepsy

J Health Sci Med 2023; 6(1): 93-98

potential in the heart, brain, and nervous system. As calcium has a crucial role in cell signaling, these channels are critical in regulating mechanisms such as muscle contraction, hormone release, synaptic transmission, and gene expression (6). As a member of the voltage-gated calcium channels family, the T-type calcium channels are low-voltage-activated ones and have a crucial role in the functioning of the nervous system. Therefore, research on whether T-type calcium channels can be drug targets has gained attention (7). In many studies, dysfunctions of these ion channels have been associated with many neurological diseases, but the most well-studied pathological implication is the absence epilepsy. Moreover, these channels have been demonstrated to be associated with peripheral neuropathic pain. More recently, T-type calcium channels have been revealed to take part in an important role in mitochondrial stress and apoptosis in dopaminergic neurons in Parkinson's disease (6).

In humans, T-type calcium channel isoforms are encoded by the *CACNA1H*, *CACNA1G*, and *CACNA1I* genes which express the Cav3.1, Cav3.2, and Cav3.3 isoforms. In addition to these isoforms, many splice variants have been identified for T-type calcium channel isoforms, enriching the functional and molecular diversity of the channels (7). In patients with the absence of epilepsy, genetic analyses indicated the *CACNA1G* gene, which is located on chromosome 19 and encodes the pore-forming α1A subunit of Cav2.1 channels, linked to the epileptic phenotype (8). Functional expression studies on *CACNA1G* demonstrated that the E147K mutation impairs calcium channel function and is related to abnormal neuronal firing (9). Further studies revealed that Cav2.1 channels are expressed in the cerebellar Purkinje and granule cells at high levels, directly related to the initiation point of absence seizures (10). However, another animal strain commonly used for absence seizure studies, Genetıc Absence Epileptic Rats From Strasbourg (GAERS), has *CACNA1G* mutation as a causative factor for phenotype of absence epilepsy; the WAG/Rij strain showed a polygenic background (11). Additionally, information about the possible role of *CACNA1G* channels in the phenotype of absence epilepsy in the WAG/Rij strain is limited. Although epileptic phenotype is mainly related to P-Q-type calcium channels in WAG/Rij rats, the other role of *CACNA1G*, another P-Q-type calcium channel, is still missing. In addition, the increase in seizures and SWDs also exacerbates in the aging WAG/Rij rats (12). Nevertheless, the possible mechanism behind that increase is also another gap now. Therefore, studies that investigate both genetic factors and cellular mechanisms opened new avenues for the further investigation of epigenetics to understand the possible relationships.

Studies in the recent decade also demonstrated that the absence epilepsy is affected by epigenetic mechanisms as well in humans and animals. The epigenetic mechanisms, such as DNA methylation, histone modifications, and microRNAs (miRNAs), establish the distinctive chromatin structure and modify the gene transcription without making alterations to the DNA sequence. DNA methylation, in mammals, usually takes place in clustered CpG dinucleotides located especially in the promoter regions of genes. The hypermethylation of the promoter blocks the binding of transcription factors to DNA and results in the silencing of gene expression, whereas in the hypomethylated state transcription factors can bind to DNA and activate the transcription (13). Additionally, covalent histone modifications, including acetylation and methylation, occur at histone tails as post-translational and control the chromatin state and gene transcription. Of these modifications, acetylation and methylation of histones have been the most widely studied. Histone acetyltransferases (HATs) transfer the acetyl groups to lysine residues and result in gene activation. On the other hand, histone deacetylases (HDACs) remove acetyl groups from histone tails and result in gene silencing (14). Especially methylation and histone acetylation, which have a strong effect on gene regulation, are shown to affect several causative genes related to epileptic phenotype. Thus, several studies investigated the impact of epigenetic modifications and tried to demonstrate possible interactions with neuronal excitability, which abnormally occurs in the absence of epilepsy.

In this study, we investigated possible epigenetic changes on *CACNA1G* in WAG/Rij rats. We focused on *CACNA1G* methylation status due to its relationship with the absence seizures seen in humans and looked for the total HDAC activity in the different age groups. However, most studies about the absence epilepsy in WAG/Rij studies conducted in male rats to avoid estrogen or other female sex-dependent effects; we investigated changes in methylation profile and total HDAC activity in both sexes.

MATERIAL AND METHOD

Animals

Experiments were conducted with eighteen female and eighteen male WAG/Rij rats. Ethical approval was obtained from the Sivas Cumhuriyet University Experimental Animals Local Ethics Committee (Date; 28.07.2022, Decision No: 540/2022). Animals were maintained in optimal laboratory conditions and fed ad libitum. All experimental procedures were performed following the principles of the Guide for the Care and Use of Laboratory Animals, and the Turkish legislation acts concerning animal experiments, according to the European Union Directive (2010/63/EU), protecting animal rights. All endeavors were done to minimize animal suffering, and experiments were conducted and recorded according to

J Health Sci Med 2023; 6(1): 93-98

Çelik et al. Epigenetic changes in absence epilepsy

the ARRIVE guidelines (15). Power analysis was carried out with G-power to establish the number of animals to examine the effects with 95% power.

Experimental Design and Animal Surgeries

Animals were selected according to their precise birthdate. In both sexes, animals that were 12 weeks old are included in 3-month groups, 24 weeks old are included in 6-month groups, and 36 weeks old are included in 9-month groups. After a one-week acclimation, animals were anesthetized with ketamine: xylazine (80:10 mg/kg, i.p.) and positioned in the stereotaxic apparatus. Hair above the skull was shaved, and a small incision was made to visualize the cranium under the subcutaneous tissue. Three burr holes were drilled carefully without disturbing the dura mater. Three screw electrodes were positioned according to the rat brain atlas (2 mm anterior, 3.5 mm right lateral for the first electrode, 4 mm right lateral, and 6 mm posterior, and for the second electrode) the earth electrode was positioned on the cerebellum) (16). Screw electrodes immersed and adjusted to the skull with dental cement. Animals were maintained in separate cages for at least three days for recovery. Following the recovery period, animals were placed in a plexiglass apparatus for observation and ECoG recordings (PowerLab, 16/SP, AD Instruments, Australia). Baseline electrocorticography (ECoG) recordings from each animal were collected for 3 hours at the same time (11:00 AM). The number of SWDs and the mean duration and amplitudes of SWDs were evaluated and determined every 20 minutes by LabChart software (v7 Pro, AD Instruments, Australia). ECoG recordings were performed after determining the estrous cycle as previously described to avoid the possible effects of sexual hormones (17).

DNA and Total Protein Isolation

Twenty-four hours after EcoG recordings, animals were anesthetized. Afterward, the animals were transcardially perfused with ice-cold phosphate-buffered saline, and then decapitated. Total brain tissues from each animal were carefully dissected, and total brain tissue was homogenized with liquid nitrogen. 50 mg of liquid nitrogen powdered brain tissues were suspended in RIPA (Radio-immunoassay precipitation buffer) solution for protein analysis and HDAC activity assay. Total protein amounts in the samples were determined by BCA assay. For DNA isolation, 50 mg of powdered brain tissues were resuspended in the 100 μl ice-cold PBS, and then DNAs were isolated with Pure Link® Genomic DNA Mini Kit (Invitrogen, USA), according to the manufacturer's protocols. The purity and concentration of DNA samples are measured by nanodrop (Jenway Genova Nano, England).

Determination of Total HDAC Activity and Methylation-specific PCR (MSP) Analysis

Total histone deacetylase (HDAC) activity was evaluated by a HDAC activity assay kit (#GTX85529, Genetex, California, USA) colorimetrically. Equal amounts of protein samples were treated with HDAC substrate (Boc-Lys(Ac)-pNA) assay buffer for 30 min at 37°C. Thereafter, the reaction was then completed with a lysine developer. Subsequently, optical densities were measured at 405 nm by a microplate reader (Tecan AG, Austria).

Methylation-specific PCR (MS-PCR) experiments were performed after bisulfite modification of all the DNA samples. The bisulfite modification was exerted by EpiJET Bisulfite Conversion Kit (#K1461, Thermo Fisher Scientific, USA), strictly following the manufacturer's instructions. Methylation-specific PCR was performed for *CACNA1G* promoter regions with methylation and unmethylation-specific primer pairs, designed via http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi, and sequences and annealing temperatures of primers were provided in **Table**. The MSP was carried out in a thermal cycler with DreamTaq™ Hot Start DNA Polymerase (#EP1702, Thermo Fisher Scientific, Lithuania) and methylation specific-primer pairs in a final volume of 25 μl at the following cycling condition for *CACNA1G*: initial denaturation at 95°C for 3 min, followed by 40 cycles at 95°C for the 40s; 50 °C for 40s and 72°C for 70s. Afterward, the final extension step was carried out at 72°C for 7 min. Then, maintained at 4°C. The promoter methylation status of *CACNA1G* was analyzed with agarose gel electrophoresis and UV transilluminator after the MSP. A DNA Ladder (#SM1193, Thermo Fisher Scientific, Lithuania) was used as a size marker. Then, the agarose gel results were analyzed using ImageJ software.

Table. The sequence of methylation-specific primers in this study.		
Primer Name	Sequences	Tm (°C)
CACNA1G MSP-F	5'-AGATGTTAAATATTTTGATTTTTCGAG-3'	49
CACNA1G MSP-R	5'-AAAACCACAACTAAAATCCGATTC-3'	50
CACNA1G USP-F	5'-ATTAGATGTTAAATATTTTGATTTTTTGAG-3'	49
CACNA1G USP-R	5'-AAAACCACAACTAAAATCCAATTC-3'	49
Abbreviation(s): MSP, methylation-specific primer; USP, unmethylation-specific primer; F, forward; R, reverse; Tm, melting temperature.		

Statistical Analysis

All the experimental data were recorded and then analyzed by GraphPad Prism (v9.0, USA). Data distribution was evaluated with Shapiro-Wilk's test. As the data were not normally distributed, statistical analyses were performed by non-parametric tests. The differences between male and female groups were assessed via the Mann-Whitney U test. For comparison among the different age groups, the Kruskal-Wallis test was used. P values less than 0.05 were taken into account as significant.

Çelik et al. Epigenetic changes in absence epilepsy

J Health Sci Med 2023; 6(1): 93-98

RESULTS

Total SWDs Increase with Age in Both Sexes

ECoG analysis was carried out to assess the seizure status of animals in all experimental groups (**Figure 1**). Our results demonstrated that the number of SWDs, which indicates the intensity of seizure, significantly increased in 6 (77.3±22.41) and 9 (154.25±34.89) months old male rats compared to 3-month-old males (30.50±13.30, p=0.07, p<0.001, respectively, **Figure 1**). Moreover, that rising at nine months is also more potent in the 9-month males than at six months (p<0.001, **Figure 1**). In parallel, numbers of SWDs also significantly increased in 6 (99,40±6,95) and 9 (116.20±24.99) months females compared to 3-month-old ones (59.66±7.57, p=0.046, p=0.004, respectively, **Figure 1**). In contrast to the difference between 6- and 9-month males, there were no significant differences between 6- and 9-month females (p>0.05, **Figure 1**).
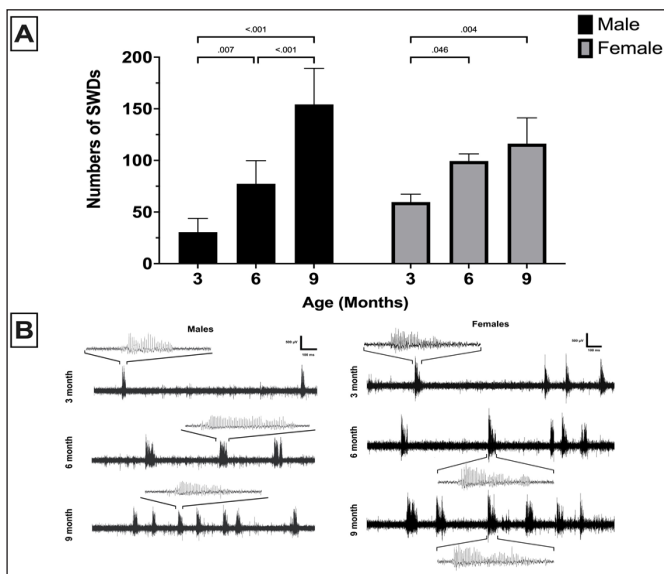
Figure 1. The number of SWDs was determined by ECoG in 3,6- and 9-month groups in both sexes. Significant increase in 6 and 9 months compared to 3-month-old seen in both experimental groups (A). ECoG recordings stand for six biological replicates for all groups at the 80th minute (B). All data were expressed as mean±SD.

The Promoter Methylation of *CACNA1G* Showed an Age-dependent Decreasing Profile in Both sexes

The MSP was carried out to the investigated promoter methylation status of the *CACNA1G* gene. In both sexes, the methylation percentage of the treatment groups was found to be decreased depending on the age (Fig 2). In males, although there was no remarkable difference in methylation profile between 3 (59.50±8.82) and 6 (56.16±6.55, p>0.05, **Figure 2**) months old animals, there was a significant decrease in the 9 (41.16±8.63, p<0.001, **Figure 2**) months old animals compared to the 3-month group. Moreover, that decrease was also significant when six-month and 9-month males were compared (p=0.007,

Figure 2). In female animals, methylation of *CACNA1G* significantly decreased 6 (47.16±9.01) and 9 (39.66±4.63) month old animals, compared to the 3 (58.83±8.54, p=0.040, p<0.001, respectively, **Figure 2**A) months old females.
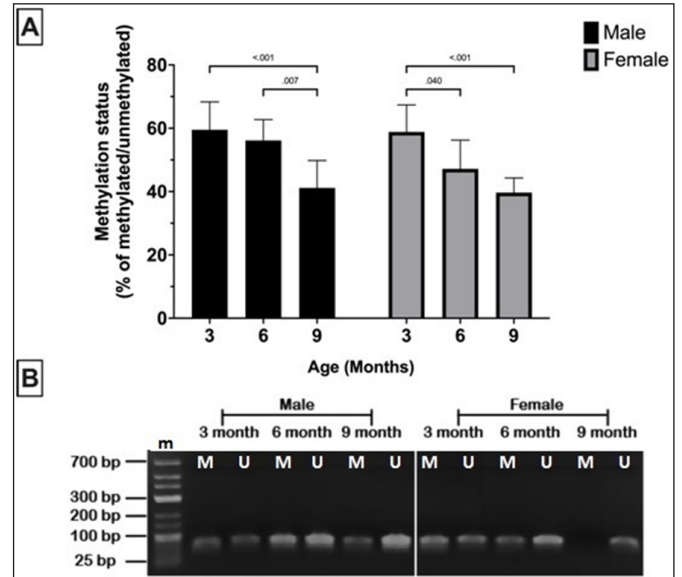
Figure 2. The MSP analysis of *CACNA1G* promoter region. Methylation status decreased with age in both sexes (A). The Gel picture stands as the representative of all groups (B). The methylated PCR product is 98 bp, and the unmethylated PCR product is 100 bp. Abbreviation(s): bp, base pairs; M, methylated; U, unmethylated; m, size marker.

HDAC Activity Increased in Both Sexes

Total HDAC activity was assessed in all experimental animals (**Figure 3**). In males, HDAC activity was significantly increased in 6 (7.81±2.81, **Figure 3**) and 9 (9.85±4.24) month old animals, compared to 3 months (2.03±0.68, p=0.005, p<0.001, respectively, **Figure 3**). There was no remarkable difference among 6- and 9-month-old males (p>0.05, **Figure 3**). In females, although both 6 (8.41±2.01) and 9 (7.51±3.79) months old animals showed increased HDAC activity, only 6-month animals were able to show a significant increase compared to the 3-month females (3.76±2.41, p=0.028, p>0.05, respectively, **Figure 3**).
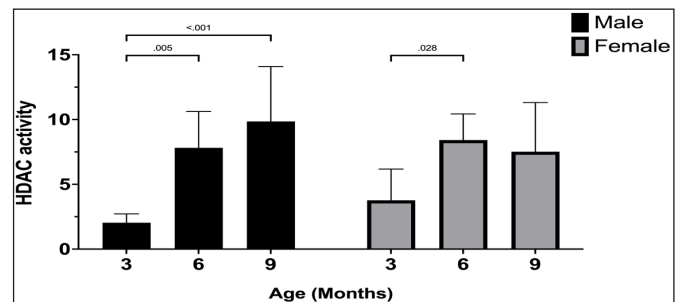
Figure 3. Total HDAC activity was assessed in all experimental groups. HDAC activity significantly increases in 6- and 9-month males. Although HDAC activity increased in 6-month females, there was no significant difference in 9-month females. All data were represented as mean±SD.

DISCUSSION

In this study, we have investigated possible changes in the promoter methylation profile of the *CACNA1G* gene, which is strongly related to the absence epileptic phenotype, in age and sex-dependent manner. Our results demonstrated that the number of SWDs increases in both sexes ages dependently. Also, the methylation status of *CACNA1G* in brain tissues was age dependently decreased in both sexes. Additionally, total HDAC activity also decreased time-dependent in male and female WAG/Rij rats.

Given the effects of T-type calcium channels on the function of the nervous system, it is not surprising that these ion channels are associated with numerous neurological diseases (7). Ion channel dysfunction is one of the accepted mechanisms underlying the mechanism of impaired synchronized circuits in the epileptic brain (18). In these circuits, P/Q-type calcium channels and *CACNA1* regulate membrane excitability in dendrites, dynamic oscillation balance in somas, and neurotransmitter recycling in the presynaptic terminals (19). It has already been shown that loss of function mutations reduces neurotransmitter recycle rates and causes an imbalance in the PQ-mediated exocytosis, leading to the absence of epilepsy in both humans and rodents (20). Additionally, with the increased use of sequencing technologies, studies demonstrated that inherited or childhood epilepsies also regulated epigenetic mechanisms (21). Because this P/Q alpha and regulatory subunit mutations demonstrate downstream rising in thalamic T-type calcium currents and disturb stable connection between brain areas, which is essential for expressing spike-wave rhythmicity, we selected WAG/Rij strain to investigate possible changes with epigenetic modification of *CACNA1G*. Recent studies demonstrated that WAG/Rij rats show age-dependent exacerbation of SWD profile (12). However, several studies were investigated to understand the age-dependent increase in SWD profile, and possible mechanisms remain elusive. We first examined the SWD profile in WAG/Rij rats in both sexes with that goal. Our results were in parallel with the previous studies, the number of SWDs were increased. The only difference was that females also had the same pattern as males, even though there was a conflict between males and females due to different brain metabolism.

Epigenetic mechanisms are well-known to sustain long-lasting gene expression patterns and show tissue-specific differences (22). These mechanisms comprise diverse levels of regulation and affect different transcript regulators such as repressors and enhancers and the transcription machinery to manage the expression of specific genes. Recent knowledge supports the hypothesis that distinctive DNA methylation patterns can establish in response to environmental changes after birth and are long-lasting in rodents and humans (23). Additionally, excitatory neurotransmission in the hippocampus was demonstrated to be affected by DNA methyltransferases (24). Valproic acid, a commonly used antiepileptic drug, is also a histone deacetylase inhibitor and inducer of DNA demethylation in vitro, supporting the epigenetic mechanism hypothesis (25). Sarkisova and Gabova (3) showed that WAG/Rij rats with a high level of maternal care exhibit reduced depression-like comorbidity and less and shorter SWDs in adulthood by comparison with in adulthood by comparison with the WAG/Rij offspring with less maternal care. This study also reveals possible epigenetic changes affecting the epileptic phenotype of WAG/Rij rats. We demonstrated that *CACNA1G* promoter methylation decreased, which means an increase in *CACNA1G* signaling and indicates changes in the expression profile of the channels. Previous studies demonstrated that the *CACNA1* promoter is regulated by methylation in different pathologies (26-28). Therefore, our results indicate that methylation of the *CACNA1G* promoter might be another mechanism underlying age-dependent changes in the WAG/Rij animals.

Furthermore, our results also demonstrated that HDAC activity increased by age in both sexes. Several studies investigated the antiepileptic and antidepressant effects of histone deacetylase inhibitors. Additionally, a recent study demonstrated diminished acetylation of histone H3 and histone H4 protein in WAG/Rij rats at seven months of age than WAG/Rij before seizure onset (30 days old). Also, VPA and sodium butyrate (NaB), another histone deacetylase inhibitor, markedly increase these acetylation levels during co-administration (29,30). Therefore, our results also prove that increased HDAC activity may be another epigenetic mechanism behind the age-dependent deterioration of SWDs in WAG/Rij animals.

However, our study has two significant limitations. First, although we investigate the methylation status of *CACNA1G*, mRNA and membrane expression levels should also be demonstrated to confirm age-dependent decreased expression. Second, histone deacetylase activity is regulated by several histone deacetylase enzymes, which consist of four different classes. Therefore, possible changes in the activity of these enzymes also need to be demonstrated to provide further proof for our results. Besides, our study demonstrated that *CACNA1G* promoter methylation status and HDAC activity change with age in both sexes of WAG/Rij animals.

Çelik et al. Epigenetic changes in absence epilepsy

J Health Sci Med 2023; 6(1): 93-98

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Sivas Cumhuriyet University Experimental Animals Local Ethics Committee (Date; 28.07.2022, Decision No: 540/2022).

Referee Evaluation Process: Externally peer-reviewed.

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

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The perception and attitude of Turkish ophthalmologists related to the COVID-19 pandemic

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ABSTRACT

Aim: To investigate the level of perceptions and attitudes regarding COVID-19 among Turkish ophthalmologists in a tertiary eye care referral center.

Material and Method: A cross-sectional survey-based study including the sociodemographic form in addition to 52 multiple-choice questions was conducted in March 2021. The questions were to assess the perception of three concepts separately: The disease, the causes of COVID-19, and the control of COVID-19. Along with this, it also included questions to evaluate the attitudes of avoidance of COVID-19 and attitudes towards the COVID-19 vaccine.

Results: A total of 43 (15 males and 28 females) ophthalmologists completed the online survey. The perception of dangerousness and contagiousness was strong among ophthalmologists. The scores in the sub-dimensions of the perception of the causes of COVID-19 presented a moderate level. Statistically significant differences revealed between as follows: resident physicians and faculty in Macro Control (p=0.02), Controllability (p=0.38), and perception of the control of COVID-19 (p=0.022); males and females (p=0.009) along with resident physicians and faculty (p=0.023) in the behavioral avoidance attitudes from COVID-19; resident physicians and faculty in attitudes towards the COVID-19 vaccine (p=0.034).

Conclusion: COVID-19 was perceived as dangerous and contagious among ophthalmologists. The perception of the control of COVID-19 was stronger among faculty than resident physicians. Females and faculty developed higher behavioral avoidance attitudes from COVID-19. Faculty exhibited less negative attitudes than resident physicians towards the COVID-19 vaccine. These assessments could shed light on our path in combating the disease, both in the COVID-19 pandemic and in future outbreaks.

Keywords: Attitude, COVID-19, ophthalmologists, pandemic, perception

INTRODUCTION

Following the inception of atypical pneumonia cases of unidentified etiology on December 30, 2019, from the Hubei province of China; by January 7, 2020, a novel beta coronavirus was identified, while the disease has been named COVID-19 (1). Thereafter on March 11, 2020, The World Health Organization declared the COVID-19 outbreak a global pandemic (2).

The COVID-19 pandemic has considerably changed the way people live, act, and work in public and private life around the world. Measures such as hygiene and social behaviors were taken to contain the virus. Besides, more stringent and costly protection measures, such as school and store closures and stay-at-home orders were imposed by governments in most countries (3). Healthcare workers encounter a high risk of catching COVID-19, and their protection is vital for the supply of uninterrupted healthcare services together with averting the spread of the disease to other individuals. Ophthalmologists are no exception to this, and they are expressly susceptible (4). Nearness to the patients during the slit-lamp examination and ophthalmoscopy, the risk of spreading disease through asymptomatic individuals, and the possibility of contamination through the conjunctiva and tears are leading causes that pose a danger to ophthalmologists (5). In this regard; knowledge, perception of COVID-19 from different perspectives, and attitudes toward COVID-19 among ophthalmologists are the most crucial determinants in avoiding potentially lethal occupational hazards during the time of the pandemic.

Ekici et al. The perception and attitude of ophthalmologists

J Health Sci Med 2023; 6(1): 99-105

Dr. Li Wenliang who became aware of the impending disaster at the very beginning in China and alerted the local authorities to be taken the necessary actions was a young ophthalmologist. Unfortunately, he lost his life at age 34 due to respiratory failure thirty days after exposure (5). This example shows how ophthalmologists perceive the outbreak and develop an attitude that control and protection are precious in the management of the disease and the success of the fight against COVID-19. This study aimed to investigate the perception of the disease, causes of COVID-19, control of COVID-19 along with the attitudes of avoidance of COVID-19, and attitudes towards COVID-19 vaccine in Turkish ophthalmologists related to the pandemic in a tertiary eye care referral center.

MATERIAL AND METHOD

Study Design and Participants

A cross-sectional survey-based study was conducted in March 2021 in the course of partially eased COVID-19 restrictions and weekend curfews. The study population consisted of 43 ophthalmologists including academicians, specialists, and residents in a tertiary eye care referral center. Healthcare professionals other than ophthalmologists were excluded from the study.

Study Instrument

An online survey including the sociodemographic form in addition to 52 multiple choice questions divided into five scales regarding perception and attitudes (6) was conducted among ophthalmologists (please see supplementary material). The survey designed in the local language was constructed using Google Forms and the link of the survey was shared via WhatsApp groups or personally to participants in the contact lists of the investigators. The questions were to assess the perception of three concepts separately (please see supplementary tables S1, S2, and S3): The disease, the causes of COVID-19, and the control of COVID-19. Along with this, it also included questions to evaluate the attitudes of avoidance of COVID-19 and attitudes towards the COVID-19 vaccine (please see supplementary tables S4 and S5). Scales were in a five-point Likert structure. The expressions found were "Strongly disagree (1)", "Disagree (2)", "Undecided (3)", "Agree (4)", and "Strongly agree" (5)". Some expressions in the scales were reversely scored. This was taken into account in the analysis. Respondents had the option of adding their names and email addresses. However, this was not mandatory. The survey was available to participate in for 5 days.

The Perception of COVID-19 (P-COVID-19) scale had seven items and sub-dimensions of Dangerousness and Contagiousness. The high scores in both parts mean that the perception in that section is also higher. The

Perception of Causes of COVID-19 (PCa-COVID-19) consisted of fourteen items and three sub-dimensions of "Conspiracy", "Environment", and "Faith". High scores in each section reveal that the belief in that section is higher. The Perception of Control of COVID-19 (PCo-COVID-19) scale had twelve items and three sub-dimensions including the "Macro Control", "Personal (Micro) Control", and "Controllability". High scores indicate the belief that control may be accomplished at a good level, or that the disease may be controlled with precautions. Avoidance Attitudes from COVID-19 (AA-COVID-19) scale had ten items including sub-dimensions of "Cognitive" and "Behavioral". High scores from each section show higher avoidance. Attitudes Towards the COVID-19 Vaccine (ATV-COVID-19) scale had 9 items. High scores in each sub-dimension of "Positive" and "Negative" attitude indicate the attitude towards vaccination is ''positive'' or ''less'' respectively.

Ethics

The study was carried out with the permission of Ankara City Hospital Clinical Researches Ethics Committee (Date: 2021, Decision No: E1/1990/2021). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Informed consent details were obtained prior to the study commencement. Furthermore, a permit was also obtained for the study from the Directorate of Healthcare Services of the Ministry of Health. The survey of the study comprised an informed consent section declaring the aim of the study, the nature of the survey, study objectives, voluntary participation, declaration of confidentiality, and anonymity.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) 22.0 program was used for the data analysis. Descriptive statistics were shown in frequency, percentage, mean, and standard deviation values. Spearman's correlation test was used to measure the strength and direction of association between numeric variables. Mann–Whitney U and Kruskal-Wallis tests for paired comparisons and multiple comparisons respectively were used to analyze the non-parametric data. The statistical significance level was accepted as $p<0.05$ in analyses.

RESULTS

Demographic Profile of the Participants

A total of 43 (15 males and 28 females) ophthalmologists completed the online survey. The response rate of the survey was 61.4% (43 participated out of 70 invited ophthalmologists). The mean (SD) age of participants was 34.1 (9.05) years (range: 24–56 years). About 76.7% (n=33) were aged less than 40 years. Among the participants, 53.5% (n=23) were resident physicians, 34.9% (n=15) were

J Health Sci Med 2023; 6(1): 99-105

Ekici et al. The perception and attitude of ophthalmologists

ophthalmology specialists, and 11.6% (n=5) were faculty of ophthalmology. 46.5% (n=20) of the participants had more than 5 years of occupational experience in the field of ophthalmology. The demographic profile of participants is presented in **Table 1**.

Table 1. Demographic profile of the participants.	
Variables	**Frequency (%)**
n (%)	43 (100)
Age category (years)	
<25	1 (2.3)
25-30	20 (46.5)
31-40	12 (27.9)
41-50	6 (14.0)
51-60	4 (9.3)
Gender	
Male	15 (34.9)
Female	28 (65.1)
Experience in the field of ophthalmology (years)	
<5	23 (53.5)
5-10	5 (11.6)
11-15	8 (18.6)
16-20	2 (4.7)
>21	5 (11.6)
Current academic position	
Resident physician	23 (53.5)
Physicians & Attending physicians	15 (34.9)
Faculty	5 (11.6)

The Perception of the Disease, Causes of COVID-19, and Control of COVID-19

According to the survey, the disease was perceived as dangerous [13.1±(2.1) points (range: 7–15 points)] and contagious [16.1±(3.0) points (range: 4–20 points)] among ophthalmologists.

The mean±(SD) points of the perception of the causes of COVID-19 were 14.2±(5.2) points (range: 6–24 points) for "Conspiracy", 13.6±(3.9) points (range: 5–25 points) for "Environment", and 6.2±(2.5) points (range: 3–12 points) for "Faith" sub-dimensions respectively. The scores in the sub-dimensions of the perception of the causes of COVID-19 present a moderate level.

The mean±(SD) points of the perception of the control of COVID-19 were 10.7±(2.9) points (range: 4–16 points) for "Macro Control", 11.2±(2.9) points (range: 4–17 points) for "Personal (Micro) Control", and 14.1±(3.3) points (range: 8–20 points) for "Controllability" sub-dimensions respectively. According to academic status, there were statistically significant differences between resident physicians (mean rank=12.20 and 13.02) and faculty (mean rank=25.10 and 21.30) in Macro Control (p=0.02) and Controllability (p=0.38) sub-dimensions respectively along with the perception of the control of COVID-19 (p=0.022) in general (**Table 2**).

Table 2. Kruskal Wallis test analyses of COVID-19-related perceptions, attitudes, and sub-dimensions according to academic status.						
Group	**n= 23/15/5**	**Mean Rank**	**df**	**χ2**	**p Value**	**Significant Difference**
Dangerousness	1	20.93	2	1.439	.487	-
	2	21.67				
	3	27.90				
Contagiousness	1	21.83	2	.167	.920	-
	2	22.83				
	3	20.30				
Conspiracy	1	21.11	2	.681	.712	-
	2	21.97				
	3	26.20				
Environment	1	25.09	2	3.435	.180	-
	2	17.43				
	3	21.50				
Faith	1	21.50	2	1.105	.575	-
	2	20.97				
	3	27.40				
Macro Control	1	16.48	2	12.368	.002*	3-1**
	2	25.73				
	3	36.20				
Personal Control	1	21.70	2	.162	.922	
	2	21.77				
	3	24.10				
Controllability	1	22.20	2	6.517	.038*	3-1**
	2	17.67				
	3	34.10				
Cognitive Avoidance	1	22.41	2	4.786	.091	
	2	24.80				
	3	11.70				
Behavioral Avoidance	1	17.72	2	7.566	.023*	3-1**
	2	24.83				
	3	33.20				
Positive Attitude	1	20.78	2	2.926	.232	-
	2	20.90				
	3	30.90				
Negative Attitude	1	21.37	2	6.060	.048*	3-1**
	2	18.80				
	3	34.50				
P-COVID-19	1	21.46	2	.152	.927	-
	2	22.23				
	3	23.80				
PCa-COVID-19	1	22.11	2	1.162	.559	-
	2	20.13				
	3	27.10				
PCo-COVID-19	1	19.26	2	7.654	.022*	3-1**
	2	21.43				
	3	36.30				
AA-COVID-19	1	18.85	2	3.292	.193	-
	2	26.20				
	3	23.90				
ATV-COVID-19	1	21.07	2	6.784	.034*	3-1**
	2	18.97				
	3	35.40				

*p<0.05 . ** Paired comparisons were made using the Mann-Whitney U test to determine which groups had a significant difference. df: degrees of freedom, χ2: chi-square, P: Disease Perception of COVID-19, PCo: Perception of Control of COVID-19, PCa: Perception of Causes of COVID-19, AA: Avoidance Attitudes from COVID-19, ATV: Attitudes Towards the COVID-19 Vaccine. 1: Resident Physicians, 2: Physicians & Attending physicians, 3: Faculty.

Ekici et al. The perception and attitude of ophthalmologists

J Health Sci Med 2023; 6(1): 99-105

The Attitudes of Avoidance from COVID-19 and Towards COVID-19 Vaccine

The mean±(SD) points of the avoidance attitudes from COVID-19 were 11.02±(3.4) points (range: 5–20 points) for "Cognitive" and 20.3±(3.9) points (range: 5–25 points) for "Behavioral" sub-dimensions. There was a statistically significant difference (p=0.009) between males (mean rank=15.27) and females (mean rank=25.61) in the behavioral avoidance attitudes from COVID-19 (**Table 3**). The difference between resident physicians (mean rank=12.74) and faculty (mean rank=22.60) in the behavioral avoidance attitudes from COVID-19 was also statistically significant (p=0.023).

The mean±(SD) points of the attitudes towards the COVID-19 vaccine were 17.09±(3.3) points (range: 4–20 points) for "Positive" and 18.7±(3.6) points (range: 8–25 points) for "Negative" (higher scores in this sub-dimension signify that the negative attitude towards vaccination is less) sub-dimensions. Depending on academic status, there were statistically significant differences in the sub-dimension of negative attitude towards vaccination (p=0.048) along with the attitudes towards the COVID-19 vaccine (p=0.034) in general. Faculty (mean rank=22.40 and 21.30) exhibited less negative attitude than residents physicians (mean rank=12.78 and 13.02) towards the COVID-19 vaccine in general and the sub-dimension of "Negative" respectively.

The Correlations Between Sub-dimensions of the Scales

Spearman's correlation tests unveiled significant positive linear correlations as follows: Dangerousness and Controllability (r =0.363; p=0.017), Behavioral Avoidance Attitude (r =0.462; p=0.002), Positive Attitude towards COVID-19 vaccine (r =0.404; p=0.007), Negative Attitude towards COVID-19 vaccine (r =0.466; p=0.002); Contagiousness and Behavioral Avoidance Attitude (r =0.418; p=0.005), Positive Attitude towards COVID-19 vaccine (r =0.360; p=0.018); Faith and Personal (Micro) Control (r =0.375; p=0.013); Macro Control and Age (r =0.496; p=0.001); Controllability and Behavioral Avoidance Attitude (r =0.415; p=0.006); Behavioral Avoidance Attitude and Negative Attitude towards COVID-19 vaccine (r =0.355; p=0.019), Age (r =0.424; p=0.005) (**Table 4**).

Besides, significant negative linear correlations were revealed as follows: Conspiracy and Positive Attitude towards COVID-19 vaccine (r =-0.320; p=0.036), Negative Attitude towards COVID-19 vaccine (r =-0.305; p=0.046).

Table 3. Mann Whitney U-test result of gender according to perception, attitude, and sub-dimensions.

Scale	Group	Mean Rank (Male=15/Female=28)	p value
P			
	Dangerousness	19.17/23.52	.251
	Contagiousness	19.63/23.27	.359
PCa			
	Conspiracy	26.00/19.86	.125
	Environment	21.50/22.27	.847
	Faith	22.27/21.86	.917
PCo			
	Macro Control	18.13/24.07	.136
	Personal Control	20.30/22.91	.512
AA			
	Controllability	18.20/24.04	.144
	Cognitive Avoidance	22.87/21.04	.722
	Behavioral Avoidance	15.27/25.61	.009*
ATV			
	Positive Attitude	19.00/23.61	.245
	Negative Attitude	18.67/23.79	.200
Total Scores			
	P-COVID-19	19.30/23.45	.300
	PCa-COVID-19	24.83/20.48	.278
	PCo-COVID-19	17.40/24.46	.078
	AA-COVID-19	18.23/24.02	.147
	ATV-COVID-19	18.93/23.64	.238

*p<0.05. M: male, F: female, P: Disease Perception of COVID-19, PCo: Perception of Control of COVID-19, PCa: Perception of Causes of COVID-19, AA: Avoidance Attitudes from COVID-19, ATV: Attitudes Towards the COVID-19 Vaccine.

Table 4. Correlations Between Sub-dimensions of scales.

Scales and sub-dimensions		1	2	3	4	5	6	7	8	9	10	11	12	13
P	(1)Dangerousness	1												
	(2)Contagiousness	.647**	1											
PCa	(3) Conspiracy	-.290	-.236	1										
	(4) Environment	-.136	.082	-.014	1									
	(5) Faith	-.128	-.031	.561**	.133	1								
PCo	(6) Macro Control	-.068	-.066	.027	-.125	.244	1							
	(7) Personal Control	.009	-.186	.241	-.213	.375*	.42**	1						
	(8) Controllability	.363*	-.001	-.101	-.252	-.251	-.021	-.042	1					
AA	(9) Cognitive Avoidance	-.121	-.130	.140	-.077	-.077	-.071	.272	-.181	1				
	(10) Behavioral Avoidance	.462**	.418**	-.167	-.181	-.012	.204	.076	.415**	-.085	1			
ATV	(11) Positive Attitude	.404**	.360*	-.320*	-.068	-.088	.261	.098	.168	.004	.267	1		
	(12) Negative Attitude	.466**	.252	-.305*	.241	-.227	-.005	-.170	.292	-.147	.355*	.434**	1	
(13) Age (years)		.237	.152	-.059	-.067	.020	.496**	-.044	.002	-.067	.424**	.145	.161	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). P: Disease Perception of COVID-19, PCo: Perception of Control of COVID-19, PCa: Perception of Causes of COVID-19, AA: Avoidance Attitudes from COVID-19, ATV: Attitudes Towards the COVID-19 Vaccine.

J Health Sci Med 2023; 6(1): 99-105

Ekici et al. The perception and attitude of ophthalmologists

DISCUSSION

Our study revealed that the disease was perceived as dangerous and contagious among ophthalmologists. This result showed parallelism with the previous studies conducted on both COVID-19 patients (7) and healthcare workers (8). The fact that the disease is life-threatening, spreads rapidly in a very short time and causes a large number of casualties are the primary reasons that lead to this perception.

The results indicate the belief that the virus is a biological weapon or political game of developed countries to sell vaccines for "Conspiracy"; the pollution of clean water supplies, environment, and unhealthy lifestyle are the main causes of the pandemic for "Environment"; or the outbreak is a punishment of God for "Faith" was not strong. 'Conspiracy' sub-dimension can be explained by ophthalmologists' high levels of education, analytical thinking skills, and awareness of counter-arguments and refutations. Previous studies have concluded that the rise in education level and knowledge is inversely proportional to conspiracy beliefs (9,10). Some studies conducted in different parts of the world reported that continuous exposure to environmental pollution, particularly air pollution, was positively correlated with severity, transmission, and mortality in the COVID-19 outbreak (11,12). On the other hand, it is a fact that measures implemented such as lockdowns; restricted human activities; shutdowns of educational institutes, business centers, and other social interaction points; the decrease of industrial production and manufacturing; travel restrictions; and curfews also resulted in the reduction of environmental pollution and improved air and water quality across the world (13). Faith was also influenced by the COVID-19 pandemic in different ways. For example, in a study conducted among individuals belonging to Christianity in Poland, most participants stated that there was a strengthening of their faith with an increased risk of COVID-19, but there was no strengthening of their religious participation during the outbreak (14). Besides, research conducted on the Muslim generation in Indonesia has unearthed those participants adopted attitudes against the spread of the pandemic, the majority believed that the disease was generated by human error, and none agreed with the idea that the COVID-19 outbreak is a punishment from God (15). The current study, where all participants were Muslims, presented a similar result.

Our study suggested that the belief that control may be achieved by precautionary actions taken institutionally, publicly, and nationwide or cautious restraints may provide the control of COVID-19 was stronger in faculty members than residents. Moreover, behavioral avoidance attitudes from COVID-19 in females were higher than in males. This was also true of faculty and resident physicians. Males and resident physicians developed fewer avoidance attitudes from physical contact and collective public spaces. Our results bear a close resemblance to previous study results. Li et al. (16) and Yıldırım et al. (17) concluded that the perception of controllability increased in proportion to being female, having older age, a higher level of education or knowledge. More, Sobkow and colleagues observed that individuals with higher controllability exhibited increased intentions toward preventive behavior (18). Combating the COVID-19 pandemic has also led to the emergence of deprecating effects on the mental well-being of physicians. Providing adequate pre-job training and explaining accurate information on the ways of protection with clear guidelines may help relieve stress and increase occupational confidence (19).

Willingness to accept the COVID-19 vaccine was at a higher level in faculty than residents physicians. Besides, faculty showed fewer concerns than in resident physicians about the safety and effectiveness of the COVID-19 vaccine development process. This significant difference can be explained by the faculty's higher average age and protection behavior resulting from the higher level of knowledge regarding the safety and effectiveness of the COVID-19 vaccine. As a matter of fact, our results were in good agreement with earlier studies conducted in different geographies around the world (20-22).

Several statistically significant results were highlighted in the correlation analysis of our study. For example, high dangerousness perception was associated with high controllability and behavioral avoidance attitude along with highly positive approaches towards the COVID-19 vaccine. A similar relationship was observed between contagiousness perception and behavioral avoidance attitude together with a positive attitude toward the COVID-19 vaccine. The idea that the disease was dangerous and contagious strengthened the behavior of avoiding the disease and willingness to be vaccinated.

On the other hand, as the belief that the epidemic was our destiny or that the epidemic was God's wrath against social degradation increased, so did the perception of the effectiveness of personal measures taken to avoid contracting the disease. A similar positive linear correlation was also observed in the age parameter. As the age of participants increased, their belief in the effectiveness of preventive actions taken at the foundational, nationwide, or worldwide level also increased. A similar relationship was seen between age and behavioral avoidance attitude as well.

Ekici et al. The perception and attitude of ophthalmologists

J Health Sci Med 2023; 6(1): 99-105

Besides, there was a negative correlation between attitudes towards the Covid-19 vaccine and conspiracy beliefs about the causes of the disease taking place in the press or social media such as biological warfare or efforts to sell vaccines.

All these correlations can be clarified by the theory of reasoned action and the theory of planned behavior. According to these theories, the actual behavior is preceded by the intention to perform a particular behavior. As intentions become stronger, it is assumed that the effort made to perform the behavior also increases, and as a result, the probability that the behavior will be performed increases (23). Further research should be carried out in the future to understand in depth the potential factors underlying the insights and attitudes expressed by ophthalmologists.

We are aware that our research may have several limitations. First, this was an online survey. The correctness of the data depended on the ability of participants to feel encouraged to give accurate and honest answers. The second limitation was that our survey consisted of closed-ended questions. The fact that the validity rate of surveys containing closed-ended questions is lower than other question types can be considered a restricting factor in our study. Another limitation was the relatively small sample size in the study.

CONCLUSION

Our work has led us to conclude that COVID-19 was perceived as dangerous and contagious among ophthalmologists. The perception of the control of COVID-19 in "Macro Control" and "Controllability" sub-dimensions were stronger in faculty than in resident physicians. Females and faculty developed higher behavioral avoidance attitudes from COVID-19. Faculty exhibited less negative attitude than residents physicians towards the COVID-19 vaccine in general and the sub-dimension of "Negative" respectively. These assessments could shed light on our path in combating the disease, both in the COVID-19 pandemic and in future outbreaks.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital Clinical Researches Ethics Committee (Date: 2021, Decision No: E1/1990/2021).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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J Health Sci Med 2023; 6(1): 99-105

Ekici et al. The perception and attitude of ophthalmologists

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Bibliometric analysis of the most cited articles on congenital cataract from 1980 to 2022

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ABSTRACT

Aim: It was aimed to present a summary of the articles published between 1980-2022 on congenital cataract, to identify the most cited articles in the field, to analyze the most active journals and the development in countries by years.

Material and Method: Search was made using keywords "Congenital Cataract", "OR: Pediatric Cataract", "OR: Infantile Cataract", "AND: 1980-2022 (Year Published)", "AND: English (Language)" in Web of Science (WOS) database via Boolean operators (Access Date: 01.11.2022). Bibliometric analyzes were made using VOSviewer (ver.1.6.18), statistical analyzes were made using rstudio (ver.2022.02.1), other analyzes were made using Microsoft Excel.

Results: In the bibliometric analysis, 1383 articles were included between the dates determined. Over the past few decades, the total number of publications on congenital cataracts continually increased from 2 in 1980 to 68 in 2022 November. The most productive year was 2021 (n=93), while the most cited year was 2004 (1,184 citations, 32 publications). The most studied WOS categories were ophthalmology (n=900), pediatrics (183) and genetics (167). The most widely used keywords were congenital cataract (n=235), cataract (n=124) and pediatric cataract (n=75). The most cited paper in congenital cataract was "Pax6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central-nervous-system defects", which was published in Nature Genetics in 1994 and cited 562 times (impact factor: 8.78). In ophthalmology journals, the most cited article was published in Survey of Ophthalmology (267 times, 1996) and the Molecular Vision was the most attractive journal with 104 publications. The United States of America, England and Peoples R China had the highest total link strength (TLS), 226 (10,325 citations), 134 (3,621 citations) and 73 (3,871 citations), respectively.

Conclusion: These findings provide useful information on the status and trends of current clinical research on congenital cataracts. Our study can be used to identify areas of study and standard bibliographic references for better diagnosis and disease control.

Keywords: Bibliometric analysis, congenital cataract, infantile cataract, pediatric cataract.

INTRODUCTION

Congenital cataract is the most common cause of treatable childhood blindness. Worldwide, it is responsible for approximately 5% to 20% of all vision loss in children (1,2). The prevalence of congenital cataract has been reported as 3-4.5% per 10,000 live births (3). Since it affects the early period of vision development, it causes severe vision loss and amblyopia. Cataracts seen in childhood may be isolated, or they may present with systemic, genetic and infective diseases. Early diagnosis and appropriate surgery are very important for visual prognosis. If left untreated, social, economic and psychological negative effects on the child, family and society are observed.

Factors such as genetic structure, cultural and socioeconomic status of populations, access to health services and adequacy of screening programs cause great differences in the prevalence and morbidity of congenital cataracts between populations. These are important considerations when evaluating statistics.

About half of childhood cataracts are caused by mutations in genes that encode proteins involved in lens structure and transparency. While most of these genes are encoded in an autosomal dominant manner, a few of them are autosomal recessive or X-linked (4). In the last 20 years, advances in genetic testing, including next-generation sequencing, have allowed the genetic cause of most isolated congenital and syndromic cataracts to be determined (5).

The aim of our study was to provide a bibliographic-historical perspective by evaluating the studies on congenital cataract after 1980 and the most cited articles.

MATERIAL AND METHOD

The study was carried out in accordance with the Helsinki Declaration, which was revised in 2013. Because the study did not have human and animal research, ethics committee approval was not obtained.

Search was made using the keywords "Congenital Cataract", "OR: Pediatric Cataract", "OR: Infantile Cataract", "AND: 1980-2022 (Year Published)", "AND: English (Language)" in Web of Science database via Boolean operators (Access Date: 01.11.2022). The search was refined to include only research articles and reviews in ophthalmology and non-ophthalmology peer-reviewed journals, and only human studies. Case reports, letters to the editor and book chapters etc. were not included in the study. Impact factors of journals were obtained from Incites Journal Citation Reports (Clarivate Analytics, June 2021).

Bibliometric analysis is a method of analyzing research trends and knowledge structures in a field by statistical methods, first defined by Pritchard (6). It is widely used to describe the trending topics and contributions of academic studies, journals, countries and authors in quantitative terms. It also helps researchers understand current research trends, distribution and key issues in a given field.

Statistical Analyses

Bibliometric analyzes were performed using VOSviewer (Version 1.6.18) package program and statistical analyzes were performed using rstudio (Version 2022.02.1). Pearson correlation analysis was used for the relationship and significance was accepted as $p<0.05$.

RESULTS

A total of 1,383 publications on "congenital cataract", "infantile cataract", or "pediatric cataract" published from 1980 to 2022 in the English language were included in this study (**Figure 1**). The included publications were cited 24,531 times in total and 16,426 without self-citations. The average number of citations per item was 17.74 ranging from 1 to 1,383 citations. Over the past few decades, the total number of publications on congenital cataracts continually increased from 2 in 1980 to 68 in 2022 November. The most productive year was 2021 (n=93), while the most cited year was 2004 (1,184 citations, 32 publications)(**Figure 2**). The most prolific author in congenital cataracts research was Vanita Berry (n=10), the most studied WOS category

was Ophthalmology (n=900). One thousand fifty six (90.8%) publications were articles, and 57 (4.1%) were reviews. The most widely used keywords were congenital cataract (n=235), cataract (n=124) and pediatric cataract (n=75) as shown in **Figure 3**. The most cited paper in congenital cataracts was "Pax6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central-nervous-system defects" by Glaser, T. (Corresponding Author). It was published in Nature Genetics in 1994 and cited 562 times. The journal citation indicator was 8.78. The funding agency was United States Department of Health & Human Services National Institutes of Health (NIH) – USA NIH National Eye Institute (NEI). The most cited three paper in ophthalmology were 'Infantile cataract' review in Survey of Ophthalmology (240 citations), 'Good visual Function after neonatal surgery for congenital monocular cataracts' in American Journal of Ophthalmology (201 citations) and 'The critical period for surgical treatment of dense congenital unilateral cataract' in Investigative Ophthalmology & Visual Science journal (170 citations).

Figure 1. Current study flow diagram

Figure 2. Publications and citations from 1980 to 2022 November.

Figure 3. Most frequently used keywords in congenital cataract

The retrieved dataset was plotted for co-authorship visualization network mapping, and the minimum number of publications of a country was fixed at 5. A total of 44 countries were plotted. The USA, England and China had the highest total link strength (TLS), 226 (10,325 citations), 134 (3,621 citations) and 73 (3,871 citations), respectively, as shown in **Figure 4**. The minimum cluster size was selected at 5 and the document co-authored by a large number of countries was set at 25. A total of 5 clusters were formed, and each color represented a different cluster for years.

Figure 4. Co-authorship visualization network mapping, Countries

The university with the most studies was the University of London (84), followed by the University of College London (78) and Sun Yat Sen University (52). Infant Aphakia Treatment Study Group published highest number of studies on congenital cataract (5).

The top 200 most cited articles (top 100 most cited articles in Ophthalmology journals- top 100 most cited articles in non-Ophthalmology journals) were originated from 26 countries led by the United States (n=60; cited=5,959), England (n=28;cited=1,982) and China (n=28;cited=1,286) (**Figure 5**).

Figure 5. Distribution of Citation Numbers by Country in Ophthalmology and non-Ophthalmology journals

The most cited articles in the top 100 ophthalmology journals were published in Investigative Ophthalmology & Visual Science (n=15, 1376 citations), American Journal of Ophthalmology (n=12, 983 citations), and Ophthalmology (n=12, 822 citations). The most cited articles in the top 100 non-ophthalmology journals were published in American Journal of Human Genetics (n=11, 1067 citations), Nature genetics (n=3, 741 citations), and Human Molecular Genetics (n=3, 552 citations). The 20 journals with the highest citation frequency published in ophthalmology and non-ophthalmology journals are shown in **Table 1**.

Table 1. The most cited top 10 journals and impact factors (IF) of top 100 Ophthalmology and non-ophthalmology journals

T100 Ophthalmology Journals	N	Cititaion	If
Investigative Ophthalmology & Visual Science	15	1376	4.925
American Journal of Ophthalmology	12	983	5.488
Ophthalmology	12	822	14.277
Journal of Cataract and Refractive Surgery	9	568	3.528
Molecular Vision	8	521	2.711
British Journal of Ophthalmology	6	451	5.908
Archives of Ophthalmology	6	414	4.399
Journal of Aapos	5	323	1.325
Acta Ophthalmologica	4	245	3.988
Survey of Ophthalmology	1	240	6.197
T100 Non-Ophthalmology Journals			
American Journal of Human Genetics	11	1067	11.043
Nature Genetics	3	741	41.307
Human Molecular Genetics	3	552	5.121
Human Genetics	7	478	5.881
Journal of Medical Genetics	6	395	5.941
Human Mutation	7	330	4.700
Journal of Cell Biology	1	316	8.077
Seminars in Cell & Developmental Biology	1	268	7.499
Nature Biomedical Engineering	1	157	5.420
American Journal of Physiology-Cell Physiology	3	155	5.282

In both ophthalmology journals and non-ophthalmology journals, the number of citations increased as the age of publication increased (r=0,235; p=0,019 and r=0,205; p=0,041, respectively). Pearson correlation analysis showed that impact factor (IF) of both ophthalmology and non-ophthalmology top 100 journals did not have a significant effect on the total number of citations (p>0.05) (**Table 2**).

Table 2. Comparison of citations and impact factors (IF) by years				
	T100 non-ophthalmology journals		T100 ophtalmology journals	
	r*	p	r*	p
	YEARS (n=100)		YEARS (n=100)	
Times Cited	0.235**	0.019	0.205**	0.041
IF	0.149	0.145	0.054	0.595
*pearson correlation coefficient; statistically significant (p<0.05)				

DISCUSSION

Bibliometric analyzes in the health sciences and other fields are used to identify global research studies and trends, and to evaluate publication progress in a particular field. Such analyzes allow to evaluate the impact and effectiveness of scientific work by monitoring citations and other important bibliometric indicators (7-12). Although bibliometric studies are a point of reference for researchers, politicians and ophthalmologists, to the best of our knowledge, there is only one bibliometric analysis of congenital cataract indexed in the WOS database (13).

In our study, a significant increase was observed in the publications related to congenital cataract in the last 20 years. We thought that the reason for this might be related to technological advances, the increase in health investments of each country, easier access to science and information, or the emergence of new journals in these fields.

While USA was the most productive country in ophthalmology journals in congenital cataracts, China was the most productive in non-ophthalmology journals. As they invest more in scientific research and development, it is not surprising that the contribution of developed countries is higher than other countries in such studies (14). Compared to developed countries, fewer publications on cataract were produced in least developed countries. This may be attributed to the fact that countries with weak economies do not have adequate funding to support cataract research. For this reason, least developed countries should attach importance and support to more research on this issue, and the developed world should encourage more least developed countries with aid and cooperation programs to eliminate the visual impairment caused by cataracts.

Although China ranks first in terms of number of publications, it ranks 3rd in terms of citation and TLS, suggesting that the quality of research still needs to be improved. This discrepancy can be attributed to the lack of standardization of the academic evaluation system, unequal competence in clinical and scientific research among institutions, and the lack of high-quality multicenter randomized clinical trials.

The most studied areas have been ophthalmology, genetic inheritance, biochemistry and molecular biology. Epidemiology, clinical outcomes, complications and surgical techniques in the field of ophthalmology, and genetic etiologies and molecular mechanisms outside of ophthalmology have been the most researched topics (15-19). About half (47%) of the 100 most cited ophthalmology articles were on clinical outcomes and surgical technique. As it is known, the treatment of cataract is surgical, so it is inevitable that studies will be more about surgical treatment and its clinical outcomes. In the top 100 most cited studies in the field of ophthalmology, 20 journals came to the fore. The three journals with the highest number of articles were the Investigative Ophthalmology & Visual Science, the American Journal of Ophthalmology and the Ophthalmology, they were also the most cited journals (1376, 983 and 822, respectively). The most cited publications were in the Survey of Ophthalmology (240 citations) and the American Journal of Ophthalmology (201 citations). In our study, it was seen that the IF of the journals did not have a significant effect on the total citations, and a significant relationship was found between the year and the citations. As the year increased, there was a significant increase in the number of articles and citations. Especially the newest studies attracted more attention and receive more citations might cause the IF to remain in the background. The fact that ophthalmology journals target a very specific and limited audience and the articles are generally cited by this audience may partially explain the low IF values. Another explanation might be that the ophthalmology journals included in this study were quite successful in their own fields. The American Journal Of Human Genetics and Nature Genetics journals were non-ophthalmology journals with very high IF. Studies on basic science topics such as cell biology and molecular genetics have been published in journals with high IF (20). In our study, the citation numbers of ophthalmology journals were found to be higher when compared to non-ophthalmology journals, while non-ophthalmology journals had higher IFs. One study found that ophthalmology articles published in general medical journals with high IF had a lower risk of bias assessment compared to those published in ophthalmology journals with high IF. Another study

found that general medical journals had significantly lower self-citation rates (21,22). Bibliometric analyzes in different fields have revealed over time that IFs alone will not be a comprehensive indicator of the quality or impact of an article (23).

There were some limitations in our study, most importantly because we worked with the WOS database, which was frequently used in bibliometric studies, some highly cited articles in other databases had to be neglected. Our study was also limited by the title field, article language and document types in the search strategy. Since 2022 is not fully completed, data were included until November, so studies and citations for this year might be missing. Finally, self-citations were not included in the study, considering that some self-citations might be inappropriate and affect the analysis results.

In conclusion, significant advances were made in the genetic etiologies and surgical treatment of congenital cataracts in the last 20 years. As we can see from the studies, the contribution of developed countries to the literature cannot be ignored. Least developed countries should be supported in terms of both diagnosis and treatment and should be encouraged to share their results in peer-reviewed journals. Bibliometric analyzes can provide authors with useful information about the current situation and trends in the field of congenital cataracts.

ETHICAL DECLARATIONS

Ethics Committee Approval: Because the study did not have human and animal research, Ethics committee approval was not obtained.

Informed Consent: Because the study was not a human research, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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HEALTH SCIENCES MEDICINE

Evaluation of forensic cases admitted to the pediatric emergency department

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ABSTRACT

Aim: Pediatric forensic cases have an important share in pediatric emergency service admissions. These require immediate treatment as they can lead to pediatric morbidity and mortality. In this study, a retrospective analysis of pediatric patients admitted to the emergency department due to acute forensic events was performed, and it was aimed to contribute to the data collected in Turkey by evaluating the results along with the results of other studies.

Material and Method: All pediatric forensic cases admitted to the Pediatric Emergency Service of the Gülhane Training and Research Hospital between November 2016 and January 2018 were retrospectively analyzed. The demographic characteristics, forensic events, intoxication agents, hospitalization statuses and clinical follow-ups of the cases were recorded.

Results: Among the 819 pediatric forensic cases admitted to the Pediatric Emergency Service, 393 (48%) were male, and 426 (52%) were female. The cases were aged between 0 and 18, with a mean age of 7.75±6.87 years. The majority of the patients were in the 0-2 age group (n=349, 42.6%). Case admissions were more frequent in winter months (n= 240; 29.3%), and most admissions were made in December (n=92; 11.2%). The days of the week with the highest frequencies of admissions were Wednesday (n=138, 16.8%) and Monday (n=134, 16.3%). The hours of the day with the highest frequencies of admissions were between 18:00 and 24:00 (n=309; 37.72%). Drug intoxication (47.13%) was found to be the most frequently encountered reason for the admission of the cases. While 69.47% (n=569) of the cases were treated as outpatients, 29.42% (n=241) were treated as inpatients, and the mortality rate in the sample was 0.6%.

Conclusion: Most causes of pediatric forensic events are preventable. Therefore, a safe environment should be provided by taking effective protective measures, and strategies that include education in which families and children will actively participate should be developed.

Keywords: Child, intoxication, emergency, forensic event

INTRODUCTION

Forensic cases are important medical conditions that are very common in pediatric emergency departments. A forensic event is any external situation that intentionally or accidentally affects the physical or mental health of the patient, resulting in any health risk or death (1). Traumatic causes take the first place in forensic cases, while intoxication cases take the first place among non-traumatic causes. Forensic events, including intoxication cases, are among the leading causes of pediatric mortality (1). The Centers for Disease Control and Prevention (CDC) reported that in 2013, the most common cause of death in the pediatric age group was unintentional injury (2). The Statistical Institute of Turkey (TURKSTAT) stated in its reports of 2021 that external injuries and intoxication cases were the most common causes of

deaths in Turkish children in the age group of 1-17 (3). The aim of our study was to determine the profiles and analyze the outcomes of pediatric patients admitted to the emergency department due to acute forensic events. We also aimed to evaluate the variables that played a role in the admission of these cases to the pediatric emergency service.

MATERIAL AND METHOD

We retrospectively investigated the files and forensic incident reports of pediatric forensic cases, who were referred to the Emergency Department of Gülhane Training and Research Hospital, between November 2016 and January 2018. Cases whose medical records were not missing were included in the study. Cases with

missing data were excluded. The forensic incident form included questions developed to collect information on characteristics including age, sex of the patient, season, month, day, and time of admission, medical history, medical diagnosis, treatment, and prognosis. The study was carried out with the permission of Gülhane Training and Research Hospital Non-invasive Clinical Researches Ethics Committee (Date: 26.04.2018, Decision No:116). All procedures were carried out in accordance with ethical rules and the principles of the Declaration of Helsinki.

The statistical analyses were carried out using the IBM Statistical Package for the Social Sciences (SPSS) statistics software ([22]; IBM Corp., Armonk, NY, USA). The categorical data are presented with n and % values, and the numeric data are presented with mean ± standard deviation values.

RESULTS

During the study, 25,568 children presented to our department, and 819 of them were forensic patients (32.03 per 1000). Among these 819 patients, 393 (47.99 %) were males, and 426 (52.01%) were females. In this period, the total number of forensic cases including adults was 7513, and pediatric forensic cases constituted 10.9% of all forensic cases.

The mean age of the patients whose data were analyzed in this study was 7.75±6.87 years. While the mean age of the male patients was 6.78±5.23 years, the mean age of the female patients was 8.17±6.94 years. The majority of the patients were in the 0-2 age group (n=349, 42.6%). **Table 1** shows the demographic characteristics of the patients.

The admissions of forensic patients were more frequent in winter months (n=240; 29.3%), and most admissions were in December (n=92; 11.2%). The days of the week with the highest frequencies of admissions were Wednesday (n=138, 16.8%) and Monday (n=134, 16.3%). The times of the day with the lowest numbers of admissions were between 24:00 and 06:00 (n=104, 12.69%), while the highest numbers of admissions were found between 18:00 and 24:00 (n=309; 37.72%). The presentation times of the patients are shown in **Table 2**.

Table 2: Presentation times of the patients.

Variables	Time	Frequency	Percentage
Season			
	Winter	240	29.3
	Spring	184	22.46
	Summer	192	23.44
	Fall	203	24.78
Month			
	January	75	9.15
	February	73	8.91
	March	73	8.91
	April	58	7.08
	May	53	6.47
	June	51	6.22
	July	60	7.32
	August	82	10.01
	September	50	6.1
	October	75	9.15
	November	77	9.4
	December	92	11.23
Days			
	Monday	134	16.36
	Tuesday	107	13.06
	Wednesday	138	16.84
	Thursday	113	13.79
	Friday	113	13.79
	Saturday	113	13.79
	Sunday	101	12.33
	Total	819	100
Time period (Hour)			
	06-12	112	13.55
	12-18	294	35.89
	18-24	309	37.72
	24-06	104	12.69

It was determined that 511 (62.39%) of the patients presented to the pediatric emergency clinic by their own means, and 308 (37.61%) of them were brought by the emergency ambulance service. The mean time that elapsed between the occurrence of the forensic incident and their admission to the hospital was found to be 151.25±118.36 minutes (11-1050 minutes).

Drug intake was determined as the most common cause of presentation for the patients (n=386, 47.13%), and information on the types of intoxication cases such as accidental drug intake, oral corrosive substance

Table 1: Demographic characteristics of the patients.

Age group	Male		Female		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
0-2 years	192	55.02	157	44.98	349	42.61
3-6 years	89	53.3	78	46.7	167	20.39
6-10 years	29	53.71	25	46.29	54	6.59
10-14 years	28	33.34	56	66.66	84	10.25
14-18 years	55	33.34	110	66.66	165	20.14
Total	393	47.99	426	52.01	819	100

ingestion, carbon monoxide poisoning, food poisoning, suicidal drug intake, alcohol consumption, narcotic drug intake, inhaled gas exposure, trauma, and arrest are listed in **Table 3**. It was observed that nonsteroidal anti-inflammatory drugs were the most common among the cases of drug intoxication. We also present the forms of substance intake by the patients in **Table 3**. Oral intake was reported in 663 (80.9%) patients, whereas inhalation was reported in 135 (16.5%) patients. While 720 (87.9%) of toxic substance exposures occurred at home, 73 (8.9%) of them were in environments outside such as schools, dormitories, and shopping malls. Twenty (27.3%) of the aforementioned 73 patients obtained the substances from the street. Suicidal drug intake was observed in 75 (9.15%) of the patients, and 23 of them had consumed multiple drugs. Other substances taken with suicidal intent are presented in **Table 3**.

Table 3: Details of forensic cases and toxic substance types		
	Frequency	**Percentage**
Complaint		
Drug intake	386	47.13
Corrosive substance oral intake	216	26.37
Carbon monoxide intoxication	124	15.14
Food poisoning	29	3.54
Alcohol consumption	24	2.93
Inhaled gas exposure	11	1.34
Trauma	16	1.95
Narcotics	8	0.97
Arrest	5	0.61
Total	819	100
Drugs		
NSAIDs	174	45.07
Multiple drugs	41	10.62
Anticholinergics	26	6.73
Vitamins	24	6.21
SSRIs	24	6.21
Antihypertensives	23	5.95
TCA	21	5.44
Psychotics	15	3.88
Antibiotics	15	3.88
Antiepileptics	9	2.33
Iron supplements	6	1.55
LT4	5	1.29
Other drugs	3	0.77
Total	386	100
Suicide		
Multiple drugs	23	30.66
NSAIDs	17	22.66
SSRIs	10	13.33
Psychotics	7	9.33
Alcohol	4	5.33
Vitamins	4	5.33
Antibiotics	4	5.33
Antihypertensives	3	4
Corrosive substances	3	4
Total	75	100
NSAID: Nonsteroid Anti-Inflammatory Drug, SSRI: Selective Serotonin Reuptake Inhibitor, TCA: Tricyclic Antidepressants, LT4: Levothyroxine Sodium		

In clinical follow-up, 569 patients (69.47%) were treated as outpatients, 220 (26.86%) were hospitalized, 21 were (2.56%) referred to intensive care, 4 (0.48%) left against medical advice, and 5 (0.6%) died (**Table 4**). All intoxication cases were kept under observation, and the mean hospitalization period of the patients was found to be 31±19 hours. The patients who were transferred to the intensive care unit were unconscious and had low Glasgow Coma Scale scores, oral ingestion of a corrosive substance was found in 3, carbon monoxide intoxication was found in 2, and drug intoxication was found in the others. The patients who were reported to have died in our study were those who were brought to the emergency department as cardiac arrest cases and were found to have drug intoxication.

Table 4: Follow-up of the patients.		
	Frequency	**%**
Outpatient	569	69.47
Hospitalized	220	26.86
Referred to intensive care	21	2.56
Left against medical advice	4	0.48
Died	5	0.61

DISCUSSION

In this study, we evaluated the incidence of forensic cases in children, the demographic characteristics of these children, and the factors affecting their admission to our emergency department.

In four different studies conducted in Turkey, the rates of pediatric cases among all forensic cases who arrived at the emergency department were reported as 18%, 21.6%, 31%, and 70% (4-7). In our study, we found a rate of 10.9 pediatric forensic cases per 100 all forensic cases admitted to the adult and pediatric emergency clinics of our hospital. This rate in our study may be related to the socio-economic and cultural characteristics of the region where we conducted our study. Our forensic case rate, which was 3.2% among all pediatric cases, was different from those reported in previous studies which found forensic case rates such as 0.67%, 7.4%, 0.70%, and 0.95% (1, 8-10). The rate that was found in our study was close to the average of the rates reported in previous studies. The difference in rates found in these studies may be due to multiple factors affecting admissions to emergency services (11).

The mean age of our patient sample was 7.75±6.87. The age group of 0-2 was the most frequently encountered group, followed by the age groups of 3-6 and 14-18, respectively. We showed that the majority of our patients were younger than 5 years old, and this result was compatible with the results of previous studies (10,12). Özdemir et al. (13)

showed that the major reason for drug intake in this age group was that their families did not store medications and harmful substances under suitable conditions. It was reported that the reason for the decrease in forensic events after the age of 6 and increase again after the age of 14 is the intake of drugs for suicide (6). It was stated that intoxication-related diagnoses at these ages include taking suicidal drugs, and this is related to the higher suicidal tendency in children at this age (7,12).

In terms of sex, many studies have shown that those forensic cases were more common in boys than in girls (1,4-6,10). However, it has been reported that the rates of suicide attempts increase during adolescence, and most of these cases are drug-induced poisoning cases (12,14,15); therefore, forensic events are more common in female cases (16,17). We showed that female patients were more frequently admitted than male patients among the forensic pediatric cases. The main reason for this was that our study included fewer trauma patients than other studies which reported that male patients were more prone to trauma than females (1, 4-6). In contrast with our study, Gündüz et al. (8) found that female cases were more frequent than male cases, and trauma was the main cause of this difference.

Our patients were admitted most frequently during winter months, and the month with the most frequent admissions was December. Gündüz et al. (8) stated that January was the month with most admissions. On the other hand, previous studies have stated that the most common period of the year for pediatric forensic cases is summer, since trauma is more common in summer (5,10,12,13). This may be because children play outside longer during the school holidays and summer months, and they are more exposed to trauma. In our study, we found that the children were injured more often in winter, when they preferred to stay at home instead of playing outside, and this was the result of their intake of toxic substances at home.

In our study, similar to some previous studies (8,10), we found that the times of the day with most admissions were between 18.00 and 24.00 hours. On the other hand, other studies (6,18,19) have shown the most common admission times between 12.00 and 18.00, and trauma was the main difference between the results of our study and those of the aforementioned studies.

The rate of admission to the emergency department by an ambulance in our study was similar to the rates in the literature (10, 20).

When the time elapsed between the occurrence of the forensic incident and the admission of the case to the hospital was examined, it was found that this time in our study was similar to those in the literature (10, 21).

Most of our forensic patients were medically treated, they were mostly non-traumatic cases, and the most common complaint of our patients was drug intake. As in our study, drug intake has been reported as the most common forensic event in pediatric forensic cases (7-10, 12,14,22). It has been stated that analgesics/antipyretics are the most common cause of drug poisoning (14,23). We observed that nonsteroidal anti-inflammatory drugs were the most common among the cases of drug intake in our study. On the other hand, these results differed from the results of previous studies that reported higher trauma rates among forensic cases admitted to pediatric emergency departments (1,13). Drug intoxication was the main cause of non-traumatic forensic cases in these studies, where trauma was reported to be the most common among all pediatric forensic cases (1,13). The reason why our pediatric forensic patients were at a lower rate among all pediatric patients compared to previous studies may be that only non-traumatic medical cases are treated in our pediatric emergency department, and traumatic patients are referred directly to the adult trauma emergency department in our hospital for treatment. The traumatic forensic cases that we report in our study, who were mistakenly referred to our pediatric emergency department, were first treated in our pediatric emergency department and then referred to the trauma emergency department of our hospital.

While suicidal poisoning rates were found to be low at 9.15% in our study, Araz et al. (21) found these rates as 19.6%, Özdemir et al. (13) reported it as 31%, and Şen et al. (10) reported a rate of 9.5%. The reason for this low rate in our study may be that the suicide cases in question may have presented to other hospitals that are located in more central parts in our region.

The rates of outpatient treatment, hospitalization, and intensive care referral in our study were close to the average of the rates reported in previous studies (6,10,13). While the mortality rate (0.61%) in our study was found to be lower than those in some studies including traumatic forensic events (1,13), it was similar to those in other studies (6,7,10,18).

The limitations of our study were that this study was conducted as a retrospective and single-center study and that traumatic patients were referred directly to the adult trauma emergency department in our hospital, and so, pediatric trauma patients were not included in the study. Additionally, since there is no pediatric intensive care unit in our hospital, the follow-up of the patients who were referred to the intensive care unit could not be determined.

CONCLUSION

Most traumatic and non-traumatic forensic cases in childhood are preventable health problems. To prevent forensic incidents, measures should be taken at home and outside, and the necessary arrangements should be made in the environment where children live and at home.

To raise awareness, regular training should be given to children and parents by educators and healthcare professionals within the scope of prevention programs. Since this is a medical problem that can always be encountered, it is necessary to have sufficient knowledge and equipment in terms of emergency services.

Because of the limitations of our study, further studies should be conducted in the pediatric population to develop effective preventive strategies for forensic problems.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gülhane Training and Research Hospital Non-invasive Clinical Researches Ethics Committee (Date: 26.04.2018, Decision No:116).

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 has no conflicts of interest to declare.

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Author Contributions: The author declares that he has participated in the design, execution, and analysis of the paper, and he has approved the final version.

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Evaluation of the marginal fit of finish line designs of novel CAD/CAM restoration materials

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ABSTRACT

Aim: The purpose of this study is to compare the marginal fit of crowns manufactured using different CAD/CAM materials on 2 different types of finish line design.

Material and Method: Tooth preparations were made by creating 2 different finish lines (rounded shoulder, chamfer) on an acrylic mandibular second premolar model. Impressions were taken on each preparation using polyvinylsiloxane impression material, and blocks with three different compositions including lithium disilicate (LDS), zirconia-reinforced lithium silicate (ZLS), and monolithic zirconia (MZ) (UP.CAD, Celtra Duo, and VITA YZ HT) were produced using a CAD/CAM (computer-aided-design and computer-aided-manufacturing) milling device (VHF R5) (n=10). The marginal gap values of the crown restorations were measured by the same operator using a stereomicroscope (LEICA DVM6). Histogram plots and the Kolmogorov-Smirnov test were used to test the normality of the distributions of the variables. The non-normally distributed (nonparametric) variables were compared using the Mann-Whitney U test for two groups and the Kruskal-Wallis test for more than two groups.

Results: The marginal gap values were compared between finish line designs separately for each material. Accordingly, the marginal gap values of the rounded shoulder finish line were smaller than those of the chamfer finish line in all materials. The marginal gap values were also compared among the materials separately for each finish line type. Accordingly, the marginal gap values of the VITA YZ HT (MZ) material were smaller than those of the Celtra Duo (ZLS) and UP.CAD (LDS) materials for both finish line designs. There was no significant difference between Celtra Duo and UP.CAD.

Conclusion: The finish line design is a factor that affects marginal fit. Monolithic zirconia is more appropriate for clinical use as it shows a better marginal fit compared to LDS and ZLS.

Keywords: Finish line, glass ceramic, CAD/CAM, marginal fit, monolithic zirconia

INTRODUCTION

Metal-free all-ceramic restorations have become more prevalently used in recent years due to their high aesthetic properties and excellent biocompatibility. The most frequently preferred all-ceramic restorations are glass ceramic, zirconia-reinforced glass ceramic, and monolithic zirconia restorations (1,2). With the help of monolithic zirconia restorations, which have been developed to eliminate the chipping problem that is seen in cases of stress in porcelains with zirconia substructures, less preparation is needed, and thinner restorations can be produced (3).

The longevity of the survival of these restorations is dependent on many factors such as aesthetics, fracture strength, and marginal fit. The achievement of an ideal marginal fit is directly associated with the finish line design to be preferred in the preparation of the tooth and the material properties of the crown that will be placed on the top (4,5).

The prognosis of the restoration is dependent on the tight fit of the fixed denture on the finish line of the prepared tooth and the minimal gap between the material and the tooth. The microleakage that will occur in cases of inadequate marginal fit will lead to a failure in the treatment by causing caries directly or leading to caries and gingivitis by causing the accumulation of plaque and food in the tooth-crown interface (6-8).

Marginal fit can be defined as the vertical distance from the inner surface of the restoration margin to the outermost edge of the finish line of the preparation. McLean et al. (9) defined clinically acceptable marginal gaps to be in the range of 40 to 120 μm.

The finish line designs that are preferred for all-ceramic restorations are shoulder and chamfer (10-12). While the abutment margin in teeth prepared with chamfer finish line ends at a wide angle at the edge of the gingiva, there is a sharp 90° angle in those prepared with shoulder finish line. Rounded shoulder are a modification of shoulder finish line, and the marginal width in these finish lines is partially narrowed due to the rounding of the interior angle (13).

This study aimed to investigate the marginal fits of crown restorations produced out of different materials (lithium disilicate ceramic, zirconia-reinforced lithium silicate ceramic, and monolithic zirconia) to teeth prepared with different forms of finish lines (rounded shoulder and chamfer) under in-vitro conditions. The null hypothesis was determined as that the material type and finish line design do not affect the marginal fit.

MATERIAL AND METHOD

All procedures were carried out in accordance with the ethical rules and the principles. Ethics committee approval is not required as the study was not conducted on humans or animals. Sixty acrylic-based plastic mandibular second premolars (AG-3, Frasaco, Tettnang, Germany) were mounded in cold acrylic (Meliodent; Heraeus Kulzer, Hanau, Germany) up to the enamel-cement interface so that their long axes would be perpendicular to the ground plane by using plastic molds. Tooth preparations were performed by the same operator using diamond burs under water cooling to create equal numbers of preparations with rounded shoulder or chamfer finish lines (**Figure 1**). To achieve the standardization of milling amounts, using guide pin burs, 2 mm occlusal reduction was made, while an average of 1.2 mm was reduced from each of the other surfaces. Margin width of 1 mm were created around 30 of the teeth using a rounded shoulder bur with an interior angle of 90° and around the remaining 30 teeth using a conical chamfer bur with a rounded tip at a taper angle of 6°. The acrylic teeth were polished using a rotary rubber polishing tool to eliminate surface roughness. A master die was produced using polyvinylsiloxane impression material. After this, crown restorations were milled of UP.CAD (UpCera, China), Celtra Duo (Dentsply Sirona, USA), and VITA YZ HT (Vita Zahnfabrik, Germany) blocks using a CAD/CAM milling device (VHF R5, Germany). (**Figure 2**).
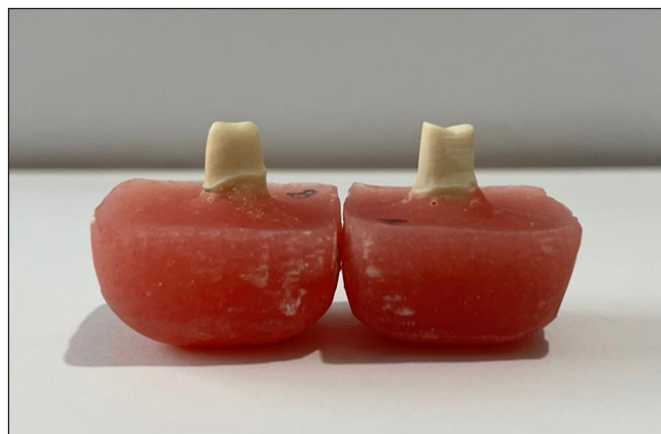
Figure 1. Chamfer and rounded shoulder finish line designs.

Figure 2. Crown restorations with different materials used in this study.

Standardization was established by measuring the thicknesses of the restorations using a digital caliper. The crowns that were produced were placed onto the prepared teeth, and the vertical distance from the inner surface of the restoration margin to the outermost edge of the finish line of the preparation was examined using a stereomicroscope (Leica microsystems, model DVM6, Germany) at X47 magnification (**Figure 3**). The measurements were carried out by the same operator from 4 points (buccal, palatal, mesial, and distal) (**Figure 4**).

Statistical Analysis

The statistical analyses were performed using the SPSS v.25.0 program. The normal distribution of the variables was tested based on histogram plots and the Kolmogorov-Smirnov test. Descriptive statistics were calculated and are presented as mean, standard deviation, median, and min-max values. The non-normally distributed (nonparametric) variables were compared using the Mann-Whitney U test for two groups and the Kruskal-Wallis test for more than two groups. In all analyses, $p < 0.05$ was considered statistically significant.

Altan et al. Marginal fit of CAD/CAM ceramic crowns

J Health Sci Med 2023; 6(1): 116-121

Figure 3. Specimen under stereomicroscope.

Figure 4. Marginal gap measurement under stereomicroscope.

Figure 5. Comparison of marginal fit between materials.
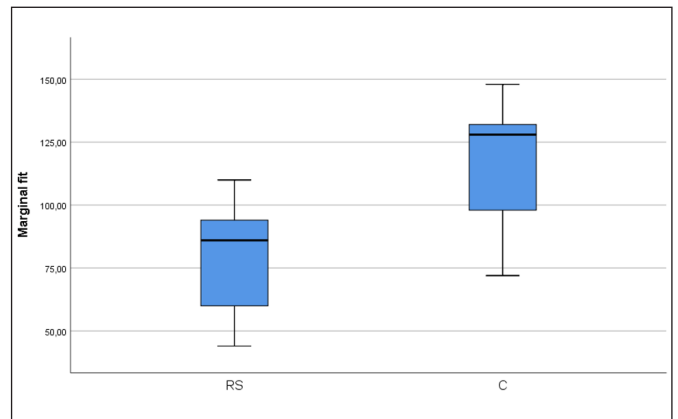
Figure 6. Comparison of marginal fit between finish line designs.

Table 1. Comparison marginal gap values between the materials and finish line design.			
	Mean±SD	**Median (Min-Max)**	**p**
Material			<0.001[1]
ZLS	111.65±22.97	114 (75-148)	
LDS	110.1±20.69	110 (83-134)	
MZ	72.55±21.38	71 (44-112)	
Finish line			<0.001[2]
RS	78.67±19.31	86 (44-110)	
C	117.53±21.16	128 (72-148)	
[1]Kruskal-Wallis Test [2]Mann-Whitney U Test			

RESULTS

The mean marginal gap values, standard deviations, median and min-max values for each group are presented in **Table 1**. It displays that the marginal gap values of the MZ material were smaller significantly than those of the ZLS and LDS materials while there was no significant difference between the ZLS and LDS. Additionally, the marginal gap values of the rounder shoulder finish line were smaller than those of the chamfer finish line. **Figure 5** and **6** show that comparisons of marginal fit among the materials and finish line designs.

Table 2 displays that the comparisons of marginal gap values between the finish line types separately for each material. According to **Table 2**, the marginal gap values of the rounded shoulder were smaller than those of the chamfer in all materials. The marginal gap values were also compared among the materials separately for each finish line. Accordingly, the marginal gap values of the MZ material were smaller than those of the ZLS and LDS materials for both finish line designs. However, there was no significant difference between ZLS and LDS for finish line types. **Figure 7** shows distribution of the marginal gap values of the materials according to the finish line designs while **Figure 8** shows the distribution of marginal gap values of finish line according to materials.

J Health Sci Med 2023; 6(1): 116-121

Altan et al. Marginal fit of CAD/CAM ceramic crowns
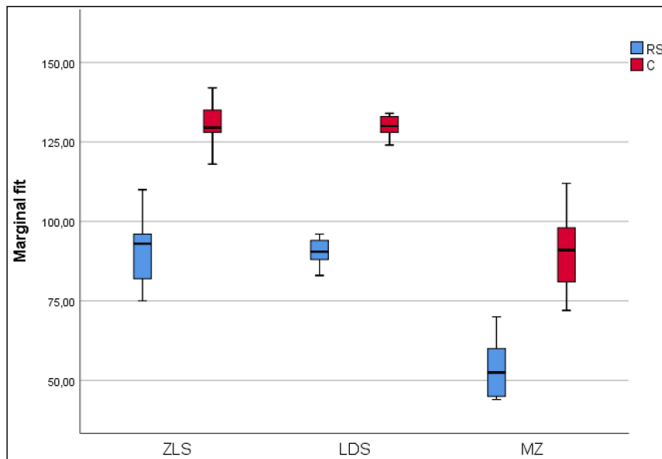
Figure 7. Distribution of the marginal gap values of the materials according to the finish line designs.
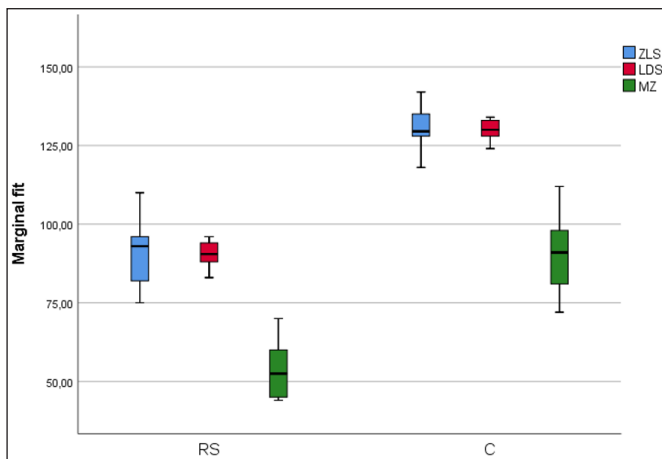
Figure 8. Distribution of marginal gap values of finish line according to materials.

Table 2. Comparison of marginal gap values between finish line types for each material.

	Finish line				p¹
	RS		**C**		
	Mean±SD	**Median (Min-Max)**	**Mean±SD**	**Median (Min-Max)**	
Material					<0.001
ZLS	91.2±10.70	93 (75-110)	132.1±8.37	129.5 (118-148)	
LDS	90.3±4.45	90.5 (83-96)	129.9±3.51	130 (124-134)	<0.001
MZ	54.5±9.64	52.5 (44-70)	90.6±12.17	91 (72-112)	<0.001
p¹	<0.001		<0.001		
¹Kruskal-Wallis Test ²Mann-Whitney U Test					

DISCUSSION

The long-term survival of restorations is closely related to their marginal fit. There are several factors that affect the marginal fit such as finish line design (4,14,15), abutment design (16,17), impression techniques (18,19,20), CAD/CAM systems (4,21,22), sintering time (23), type of material (5,25,26), cement space (26) and cementation (27,28). The literature review that was conducted for this study did not show any study on the marginal fit values of monolithic zirconia, lithium disilicate, and zirconia-reinforced lithium silicate ceramic crowns on

teeth prepared with different finish line designs. In this study, the effects of different finish line designs and different materials on marginal fit were investigated, and consequently, the null hypothesis was rejected.

While preparing teeth, abutments can be prepared with finish line designs such as the shoulder, rounder shoulder, and chamfer designs. While there are studies in the relevant literature that have concluded that different finish lines have an effect on marginal fit (15,29), there are also those that have stated the opposite (30,31). These differences can be attributed to the differences in methods of analyzing marginal fit, the production technique of restorations or the restoration material itself. In this study, independently of the materials, the marginal gap values of the crowns with rounded shoulder finish lines were smaller than those of the crowns with chamfer finish lines. The results of present study were in agreement with Euan et al. (4)' study.

The accuracy and dimensional stability of impressions taken from prepared teeth are important. For a fixed denture to be placed onto the existing tooth to have the ideal properties, an identical model of the preparation in the intraoral environment should be created in the laboratory environment. Impressions can be taken using digital methods (intraoral scanners) and conventional methods. Nevertheless, in the literature, there is no consensus on the superiority of both impression techniques to one another. More studies are needed to accurately evaluate the reliability, accuracy, repeatability, and scanning durations of intraoral scanners (32).

Previous studies have examined the marginal fit properties of restorations in cemented (33) or uncemented (19,23,29) forms. Because problems in cementation techniques such as disproportionate finger pressure or the overfilling of the crown with cement lead to loss of marginal fit, in this study, the marginal gap measurements were made before cementing (34).

In general, there is no definite evidence on the best methodology to assess the marginal fit accuracy of CAD/CAM crowns. In previous studies, marginal gap values have been measured using various devices including optical microscopes (15,22) scanning electron microscopes (SEM) (18,33) and stereomicroscopes (29,34). No statistically significant difference has been reported between these methods (35,36). A marginal fit measurement that is made using SEM may require cementation or the cutting of cemented crowns, which may lead to irreversible damage to the master die and limit the number and positions of measurements (37). Although marginal fit measurements can be made without cementing by micro-CT, it would be challenging to maintain a uniform pressure on the crown during measurements. For these reasons, a stereomicroscope was preferred in this study.

Altan et al. Marginal fit of CAD/CAM ceramic crowns

J Health Sci Med 2023; 6(1): 116-121

The marginal gap values obtained in different studies differ from each other. There is also an agreement among different researchers that marginal gap values smaller than 120 μm are clinically acceptable (9, 38). In studies in the relevant literature, the marginal gap values of zirconia crowns have been reported in the range of 36.56 to 70.94 μm (39,40) while the values of lithium disilicate crown restorations have been reported between 61.86 and 103.75 μm (40,41). Previous studies have shown smaller marginal gaps in zirconia material than in lithium disilicate material (35). On the other hand, Mohaghegh et al. (42) reported that monolithic zirconia crowns had better marginal fit values than zirconia crowns.

In the study where they investigated the post-cementing marginal fit values of conventional zirconia (Katana zirconia) and lithium disilicate (IPS e.max CAD) crowns by micro-CT, Riccitiello et al. (43) reported marginal gap values of 63 μm in zirconia crowns and 65 μm in lithium disilicate crowns. In our study, in the chamfer finish line measurements, the marginal gap values were found as 90.6±12.17 μm for monolithic zirconia (VITA YZ HT) and 129.9±3.51 μm for lithium disilicate (UP. CAD). The differences in the marginal gap values reported in previous studies and those in present study may be explained by the use of a stereomicroscope as the measurement instrument in our study and the fact that measurements was made before cementing.

In current study, while there was no statistically significant difference between the marginal gap values of the lithium disilicate (LDS) and zirconia-reinforced lithium silicate (ZLS) crowns, the marginal fit values of the monolithic zirconia crowns were found significantly superior to those of the LDS and ZLS crowns. Batson et al. (44) compared the marginal fit values of monolithic zirconia, lithium disilicate, and metal-ceramic restorations and concluded that the best fit was in the monolithic zirconia restorations.

In the study in which they compared the marginal fit values of lithium disilicate and hybrid ceramic CAD/CAM crowns, Azarbal et al. (45) found the mean marginal gap values of the lithium disilicate crowns as 132.25 μm. In present study, the marginal gap value of the lithium disilicate crowns was measured as 90.3 μm. This difference may have been caused by the abutment designs, finish line designs, and differences in milling systems.

A limitation of this study was the fact that the marginal fit of restorations was investigated only in second premolar teeth. Another limitation may be the fact that no thermal aging treatment was applied in our study. In future studies, thermal aging treatments can be performed to investigate whether the marginal fit of crown restorations is influenced by intraoral conditions such as saliva and humidity.

CONCLUSION

1. Clinically acceptable marginal gap values were obtained in all material groups prepared using rounded shoulder abutments.

2. Monolithic zirconia showed better marginal fit values compared to the LDS and ZLS materials in both finish line designs.

3. There was no significant difference between the LDS and ZLS materials in terms of marginal fit.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval is not required as the study was not conducted on humans or animals.

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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FeNO, systemic inflammation and other risk factors for osteoporosis in COPD

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ABSTRACT

Aim: The bone is one of the tissues that is adversely affected as a result of inflammation in COPD. The aim of this study is to examine the relationship between airway inflammation, systemic inflammation and osteoporosis in COPD.

Material and Method: A cross-sectional study was performed, including 50 patients with stable COPD. FeNO as a marker of airway inflammation; hs-CRP, WBC and fibrinogen as systemic inflammation markers; arterial blood gas, BMI and FEV_1 were measured. To identify osteoporosis, DEXA was performed.

Results: The prevalence of osteoporosis was 26%. Osteoporosis was associated with WBC and hs-CRP (OR: 1.80, 95% CI, 1.26-2.57 and OR: 2.32, 95% CI, 1.28-4.20, respectively). It was observed that the risk increased as FEV_1 and BMI decreased (OR: 0.94, 95% CI, 0.89-0.98 and OR: 0.75, 95% CI, 0.61-0.91, respectively). There was no relationship between osteoporosis and FeNO and fibrinogen (OR: 1.05, 95% CI, 0.99-1.12 and OR: 1.04, 95% CI 0.98-1.09, respectively). Arterial blood gases (PaO_2 and $PaCO_2$) were not effective on osteoporosis (OR: 1.01, 95% CI, 0.95-1.05 and OR: 1.04, 95% CI, 0.90-1.19, respectively). The cut-off values of hs-CRP and WBC for osteoporosis risk were 15.9 mg/L and 11.6 $\times 10^9$/L, respectively.

Conclusions: Osteoporosis was associated with low BMI, airway stenosis and high levels of hs-CRP and WBC.

Keywords: COPD, inflammation, osteoporosis, FeNO, arterial blood gas

INTRODUCTION

Osteoporosis is one of the systemic consequences of chronic obstructive pulmonary disease (COPD), which is often underdiagnosed and affects the prognosis. Inflammation may contribute to the initiation or worsening of comorbidities such as osteoporosis (1). However, the development of low-grade systemic inflammation in COPD is not as well understood as in other chronic diseases. Systemic inflammation is an important factor in the formation and progression of the decrease in bone mineral density. Several studies have shown changes in various circulating inflammatory cells, including neutrophils and lymphocytes, in COPD (2). White blood cells (WBC) are one of the most studied inflammatory markers in COPD (3). Fibrinogen is a soluble acute-phase plasma glycoprotein and is synthesized primarily in the liver. During coagulation, it is converted to fibrin by thrombin. It has been associated with the presence, exacerbations and mortality of COPD. Fibrinogen can be used as a biomarker for comorbidities in COPD. It may be useful in identifying individuals at higher risk of mortality, whether due to respiratory complications or inflammatory co-morbidities, within the COPD population (4). High-sensitivity C-reactive protein (Hs-CRP) is a sensitive marker of low-grade systemic inflammation (5). Hs-CRP has been associated with various prognostic factors in COPD (6). This parameter may be worth considering both in estimating the likelihood of extrapulmonary comorbidities such as osteoporosis and in determining the presence and severity of inflammation in COPD (7).

Studies attempting to explain the relationship between airway inflammation and a decrease in bone mineral density are limited. Fractional exhaled nitric oxide (FeNO) measurements can be useful for determining

J Health Sci Med 2023; 6(1): 122-127

Güngör et al. Effect of inflammation for osteoporosis in COPD

the level of airway inflammation in the clinical setting. Endogenous nitric oxide (NO) is a gaseous signaling molecule and is released from inflammatory cells in the airways and alveoli. It plays an important role in regulating airway and vascular function. In COPD patients, FeNO levels have been shown to increase in exacerbations (8). It appears to reduce levels in current smokers and those with severe diseases (9).

The aim of this study is to investigate the effect of airway inflammation and systemic inflammation on bone mineral density (BMD) in COPD. Another aim of this study is to examine other factors such as hypoxemia that may affect the bones in COPD. At the same time, we assessed which biomarkers can be used to identify individuals at risk of osteoporosis. Selection of patients for BMD scanning may allow early diagnosis and treatment.

MATERIAL AND METHOD

The study was carried out with the permission of Kırıkkale University Clinical Researches Ethics Committee (Date: 17.12.2012, Decision No: 12/14-02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Desing and Population

A total of 50 patients with COPD were recruited for the study. COPD was defined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. Patients with unstable COPD, history of smoking for the last four weeks; other metabolic, neoplastic and inflammatory diseases; any infection in the last two months; using inhaled steroids and those who received systemic steroid therapy in the last four weeks were excluded from the study. After informing the patients and signing the consent form, FeNO measurements were performed first. Blood collection for systemic inflammation biomarkers was performed after body mass index (BMI) measurements. An arterial blood gas was taken on the same day that an appointment was made for dual energy X-ray absorptiometry (DEXA). Two groups of participants were formed. Those with osteoporosis formed one group, while those with osteopenia and normal BMD formed the non-osteoporotic group. The pulmonary function test was performed concurrently with the DEXA. Written informed consent was obtained from all participants.

FeNO Measurement

In all groups, FeNO was measured with a NioxMino® analyzer (Aerocrine AG, Solna, Sweden) device at a speed of 50 ml/sec for 10 seconds and a constant flow rate of 10 cmH$_2$O, according to recommendations of American Thoracic Society/European Respiratory Society (ATS/ERS) Committee. The results of FeNO were represented as parts per billion (ppb).

Blood Inflammatory Biomarkers

For hs-CRP, venous blood samples were taken in two separate tubes without anticoagulation while the patients were in a sitting position. Hs-CRP samples were centrifuged at +4 degrees at 2000×g for 5 minutes with a cooled centrifuge in accordance with the cold chain, and then their serums were separated. Separated sera were transferred to Eppendorf tubes for the study and stored at -80 degrees until the working day. DiaSource® hs-CRP enzyme immunoassay was used. Fibrinogen was studied in a citrate tube using the SF-8100 series coagulation device of Succeeder® (Changping Science Zone, Beijing, China). The results of hs-CRP and fibrinogen were represented as mg/L. The upper limit of normal (ULN) were 15.23 mg/L and 40 mg/L, respectively. Hemogram was studied in an EDTA tube with Beckmancoulter® (Lakeview Parkway, Indianapolis, USA) in the LH780 series complete blood count device (autoanalyzer). The results of WBC were represented as ×10^9/L and the ULN was 12 ×10^9/L.

Arterial Blood Gases

While the patients were breathing room air, 2 ml of arterial blood was taken into heparinized syringes and blood gas analyses were performed. The Rapiblab 1265 series blood gas measurement device by Siemens® (Henkestrasse, Erlangen, Germany) was studied. The results were represented as mmHg.

Bone Mineral Density Measurement

The Hologic® Discovery QDR (Shelly Seaman Inc., Danbury, USA) series DEXA device was used to measure BMD. The T-score expresses how many standard deviations (SD) the BMD measurements are below or above from the mean of BMD measurements in young adults of the same gender. BMD was classified according to WHO guidelines. The T-score values above -1.0SD were normal, between -1.0 and -2.5SD were osteopenia, and below -2.5SD were osteoporosis. The diagnosis was determined by the lowest T-score at the lumbar or femoral neck.

Statistical Analysis

The SPSS for Windows 23.0 program was used to analyze all of the patients' data. The descriptive statistical methods (mean and standard deviation) and quantitative data comparison to compare parameters that did not show normal distribution between groups were calculated by the Mann Whitney-U test. The Spearman correlation test was used for continuous data. Univariate logistic regression analyses were performed to assess the impact of airway inflammation, systemic inflammation and other factors on the likelihood of having osteoporosis. The effect of confounders was examined using multivariate logistic regression analysis. Results were evaluated at 95% confidence interval and significance level of $p < 0.05$.

Güngör et al. Effect of inflammation for osteoporosis in COPD

J Health Sci Med 2023; 6(1): 122-127

The area under the receiver operating characteristic curve (AUC-ROC) was used to measure the ability to distinguish between the osteoporosis group and the non-osteoporosis group. The AUC-ROC is a useful metric for summarizing a diagnostic model's discriminative power, and it can vary from 0.5 (no discrimination) to 1.0 (complete discrimination) (perfect discrimination). A value of 0.7-0.8 is considered reasonable discrimination and a value of greater than 0.8 indicates good discrimination (10).

RESULTS

There were 50 COPD patients participating in the study and 10 (20%) were women. The mean age was 62.98 years, the mean duration of diagnosis was 6.87 years, the mean of smoking pack-years was 39.84, and the mean BMI was 26.29 m²/kg. The mean of FeNO, an airway inflammation marker, was 21.20 (ppd), and the means of fibrinogen, WBC and hs-CRP which are systemic inflammation biomarkers were 31.72 (mg/L), 8.23 (x109/L) and 13.93 (mg/L), respectively. The femur-T score average was -1.85 SD and the lumbar-T score average was -1.12 SD. The mean forced expiratory volume in 1 second (FEV_1) was 43.94, the mean partial oxygen pressure (PaO_2) was 62.75 (mmHg) and the mean partial carbon dioxide pressure ($PaCO_2$) was 42.34 (mmHg). The prevalence of osteoporosis was 26% (n=13) and the prevalence of osteopenia was 58% (n=29). The remaining 8 (16%) participants had normal BMD. Participant demographic information and inflammation biomarkers are presented in **Table 1**.

Table 1. Demographics of COPD patients (n=50)		
	Mean (sd)	**Min-max**
Age	62.98 (9.31)	44-88
Gender F/M, n (%)	10/40 (20/80)	
Body mass index (m²/kg)	26.29 (4.77)	17-41
Diagnosis year of COPD	6.87 (4.44)	1-20
Smoking (pack-years)	39.84 (19.58)	20-120
FEV_1 (%)	43.94 (19.22)	16-83
PaO_2 (mmHg)	62.75 (12.66)	38-91
$PaCO_2$ (mmHg)	42.34 (4.55)	35-51
FeNO (ppd)	21.20 (10.29)	5-47
Fibrinogen (mg/L)	31.72 (11.82)	13.00-74.30
WBC (×10⁹/L)	8.23 (3.05)	2.80-18.00
Hs-CRP (mg/L)	13.93 (1.01)	12.76-16.55
Osteoporosis, n (%)	13 (26)	
Osteopenia, n (%)	29 (58)	
Normal bone mineral density, n (%)	8 (16)	
Femur T-score (SD)	-1.85 (1.08)	(-0.10)-(-4.48)
Lumbar T-score (SD)	-1.12 (1.11)	(0.89)-(-3.43)

T-score less than or equal to -1 at either the hip or lumbar spine. FEV_1 (Forced expiratory volume in 1 second), PaO_2 (Partial oxygen pressure), $PaCO_2$ (Partial carbon dioxide pressure), FeNO (Fractional exhaled nitric oxide), WBC (White blood cells), Hs-CRP (High-sensitivity C-reactive protein)

While the osteoporosis group is compared with the non-osteoporosis group, there was no difference between age (p=0.14), year of diagnosis of COPD (p=0.57) and the pack-years of smoking (p=0.61). In the osteoporosis group, BMI and FEV_1 were significantly lower (p=0.004, p=0.003; respectively.) than the non-osteoporosis group. There was only one woman who had osteoporosis. There was no significant difference in PaO_2 values between the two groups (p=0.87). In addition, the PCO_2 values were similar (p=0.64). WBC and hs-CRP were significantly higher in those with osteoporosis (p=0.001, p=0.006; respectively.) but there was no significant difference between fibrinogen levels (p=0.10). FeNO values were higher in the osteoporosis group, but there was no statistical difference (p=0.12). The comparison of osteoporosis and non-osteoporosis groups is presented in **Table 2**.

Table 2. Comparison of osteoporosis and non-osteoporosis group			
	Normal BMD and Osteopenia group (non-osteoporosis) (n=37)	**Osteoporosis group (n=13)**	**p**
Age	61.76 (8.98)	66.46 (9.70)	0.14
Gender F/M	9/28	1/12	
Body mass index	27.51 (27.51)	22.80 (4.53)	0.004*
Diagnosis year of COPD	6.64 (4.41)	7.50 (4.65)	0.57
Smoking (pack-years)	38.92 (18.92)	42.46 (21.95)	0.61
FEV_1 (%)	48.24 (19.09)	31.69 (14.20)	0.003*
PaO_2 (mmHg)	62.57 (12.67)	63.23 (13.14)	0.87
$PaCO_2$ (mmHg)	42.14 (4.23)	42.92 (5.46)	0.64
FeNO (ppd)	19.68 (9.41)	25.54 (11.80)	0.12
Fibrinogen (mg/L)	30.11 (9.33)	36.28 (16.71)	0.10
WBC (x10⁹/L)	7.17 (2.06)	11.25 (3.45)	0.001*
Hs-CRP (mg/L)	13.69 (0.85)	14.60 (1.27)	0.006*

mean (sd), *p-value <0.05 significant, FEV_1 (Forced expiratory volume in 1 second), PaO_2 (Partial oxygen pressure), $PaCO_2$ (Partial carbon dioxide pressure), FeNO (Fractional exhaled nitric oxide), WBC (White blood cells), Hs-CRP (High-sensitivity C-reactive protein)

The association between the presence of osteoporosis with inflammation markers and other variables was examined using univariable logistic regression. Osteoporosis was not associated with age (OR: 1.06, 95% CI, 0.98-1.14; p=0.12). Although it was observed that being male increased the risk 3.85 times, it was not statistically significant (95% CI, 0.43-33.91; p=0.22). Lower BMI levels were associated with increased osteoporosis risk (OR: 0.75, 95% CI, 0.61-0.91; p=0.005). It was observed that the risk increased as FEV_1 decreased (OR: 0.94, 95% CI, 0.89-0.98; p=0.015). It was found that the presence of osteoporosis did not change with PaO_2 levels (OR: 1.01, 95% CI, 0.95-1.05; p=0.57). It was observed that the risk of osteoporosis did not increase with the year of diagnosis of COPD (OR: 1.04, 95% CI, 0.90-1.20; p=0.58). The risk was not affected by the smoking burden (OR: 1.01, 95% CI, 0.97-1.04; p=0.57).

Airway inflammation was not associated with the presence of osteoporosis (OR: 1.05, 95% CI, 0.99-1.12; p=0.85). Similarly, fibrinogen, which is one of the systemic inflammation markers, was not associated with the presence of osteoporosis (OR: 1.04, 95% CI, 0.98-1.09; p=0.12). WBC and hs-CRP were associated with the possibility of osteoporosis (OR: 1.80, 95% CI, 1.26-2.57; p=0.001 and OR: 2.32, 95% CI, 1.28-4.20; p=0.005). The risk of osteoporosis is shown in **Table 3**.

Table 3. The risk of osteoporosis in COPD		
	OR (95% CI)	**p**
Age	1.06 (0.98-1.14)	0.12
Gender (male)	3.85 (0.43-33.91)	0.22
Body mass index	0.75 (0.61-0.91)	0.005*
Diagnosis year of COPD	1.04 (0.90-1.20)	0.55
Smoking (pack-years)	1.01 (0.97-1.04)	0.57
FEV_1 (%)	0.94 (0.89-0.98)	0.015*
PaO_2 (mmHg)	1.01 (0.95-1.05)	0.87
$PaCO_2$ (mmHg)	1.04 (0.90-1.19)	0.58
FeNO (ppd)	1.05 (0.99-1.12)	0.85
Fibrinogen (mg/L)	1.04 (0.98-1.09)	0.12
WBC ($\times 10^9$/L)	1.80 (1.26-2.57)	0.001*
Hs-CRP (mg/L)	2.32 (1.28-4.20)	0.005*
*p-value <0.05 significant, OR>1 implies the probability of osteoporosis, FEV_1 (Forced expiratory volume in 1 second), PaO_2 (Partial oxygen pressure), $PaCO_2$ (Partial carbon dioxide pressure), FeNO (Fractional exhaled nitric oxide), WBC (White blood cells), Hs-CRP (High-sensitivity C-reactive protein)		

Multivariate logistic regression analyses were performed based on the risk factors that either reached significance in univariate analyses or that have been established as having a potential impact on osteoporosis. Considering that age and gender have an effect on bone mineral density, the effects of WBC and hs-CRP were examined by adjusting. While the effect of WBC was almost the same (OR: 1.83, 95% CI, 1.22-2.75), a slight decrease was observed in hs-CRP (OR: 2.54, 95% CI, 1.20-5.37). In a model that was adjusted with age, gender, FEV_1 and BMI, both WBC and hs-CRP significantly increased the likelihood of having osteoporosis (OR: 2.26, 95% CI, 1.19-4.30 and OR: 3.36, 95% CI, 1.22-9.22). The results are presented on an oddsplotty in **Figure 1**.

Using the AUC-ROC, predicted probabilities were calculated for the risk of osteoporosis by two systemic inflammation biomarkers that are significant in logistic models (WBC and hs-CRP). The AUC-ROC of the WBC was 0.84 (95% CI, 0.70-0.97) and the cut-off value was 11.6 $\times 10^9$/L (sensitivity 69% and specificity 89%). The AUC-ROC of the hs-CRP was 0.72 (95% CI, 0.53-0.89) and the cut-off value was 15.9 mg/L (sensitivity 69% and specificity 81%). The ROC curves and results are presented in **Figure 2**.
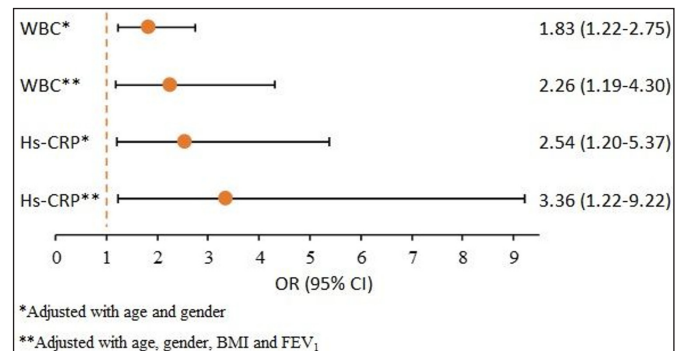
Figure 1. Multivariate logistic regression analysis for the risk of osteoporosis an oddsplotty
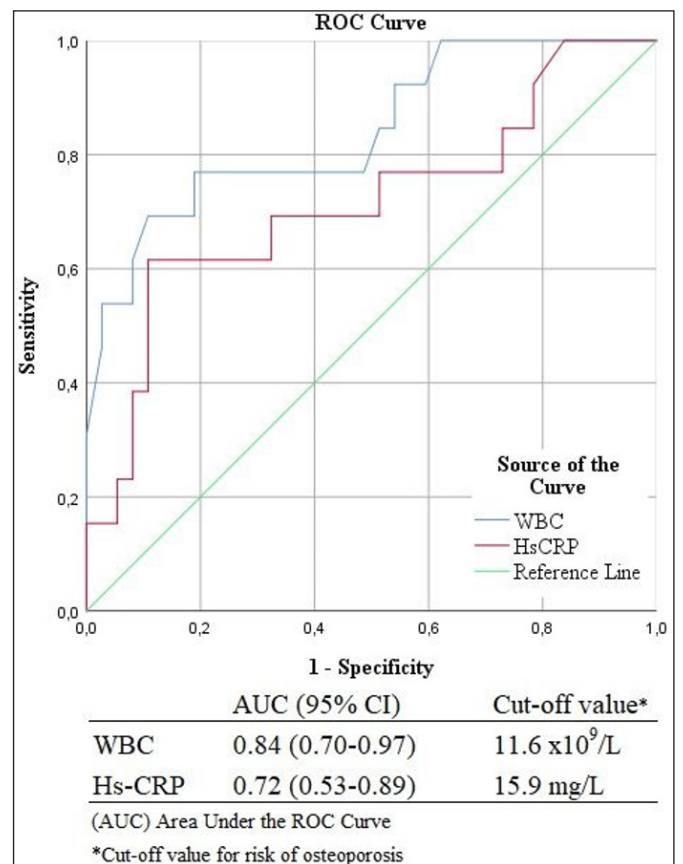
Figure 2. ROC Analysis for the risk of osteoporosis

DISCUSSION

Osteoporosis is a comorbidity with significant sequelae such as vertebral and hip fractures. The disability, which builds on patients who already have shortness of breath and respiratory failure, has extremely negative effects on their quality of life both in the short term and in the long term. At the same time, there is a serious financial loss when considering the treatment of fractures, inpatient stays and loss of workforce. The mechanism of osteoporosis in COPD is complex and multifactorial (11). This study examined the relationship of osteoporosis in COPD with both airway and systemic inflammation. We also examined other factors that may be influential. We found that systemic inflammation, WBC and hs-CRP were strongly associated with osteoporosis (OR: 1.80,

Güngör et al. Effect of inflammation for osteoporosis in COPD

J Health Sci Med 2023; 6(1): 122-127

95% CI, 1.26-2.57 and OR: 2.32, 95% CI, 1.28-4.20). We assessed that participants with a lower BMI are more likely to develop osteoporosis (OR: 0.75, 95% CI, 0.61-0.91) and found that the risk of osteoporosis increases with the severity of airway limitation (OR: 0.94, 95% CI 0.89-0.98). In the results of our study, it is known that although the risk of osteoporosis in COPD does not appear to be statistically significant in terms of age and gender, it is more common in the female gender and the risk increases with advancing age in the general population. Considering this situation, when we used age and gender as confounding factors, we observed that the effect of systemic inflammation continued (OR: 1.83, 95% CI, 1.22-2.75 and OR: 2.54, 95% CI, 1.20-5.37). The prevalence of osteoporosis in COPD has been reported to be between 36% and 60%, and osteopenia between 35% and 72% (12). In the present study, the prevalence of osteoporosis in COPD was slightly lower than other data (26%), and the prevalence of osteopenia was 46%. There was a statistically nonsignificant trend toward osteoporosis in males compared with females (OR: 3.85, 95% CI, 0.43-33.91). The reason for this tendency, which is in contrast to other studies (13,14) and the general population, may be that there was only one woman in the osteoporosis group. Our study is a prospective cross-sectional study and better results may be obtained in a larger study population.

FeNO has been shown to be associated with respiratory comorbidities in inflammatory and autoimmune diseases, especially psoriasis (15). Inflammation pathogenesis has also been considered in most comorbidities of COPD. Studies examining the relationship between FeNO and the comorbidities of COPD are rare. FeNO measurements may be useful clinically in COPD patients to determine the level of airway inflammation both at baseline and during therapy in relation to comorbidities such as pulmonary arterial hypertension (PAH) and cardiovascular diseases. The presence of decreased FeNO levels in patients with PAH or atherosclerosis suggests that serial monitoring of FeNO may be beneficial in these comorbidities. Decompensated heart failure was associated with higher FeNO levels (16). In our study, when the groups were compared, there was no statistical difference between FeNO levels and it was seen that FeNO did not affect the risk of osteoporosis (OR: 1.05, 95% CI, 0.99-1.12). However, the FeNO level was higher in the osteoporosis group. The pathophysiological changes found in the lungs in COPD are associated with an inflammatory response. This results in progressive airflow limitation, air trapping, and parenchymal tissue destruction (11). In a study conducted on patients with COPD, the relationship between osteoporosis and emphysema was examined (13). While emphysema was associated with a decrease in BMD, a common inflammatory response was thought to affect both the lungs and bones. Our findings support that systemic findings such as osteoporosis can be preserved in patients where inflammation can remain localized in the airway. We think that airway inflammation does not have a direct effect on osteoporosis. Better analysis of the effect of airway inflammation on systemic inflammation will give more insight into this issue.

There are specific and non-specific causes of the decrease in BMD in COPD. Systemic inflammation is a specific etiopathogenic factor (11). This effect of systemic inflammation on bones has also been shown in various rheumatic and endocrinological diseases (17,18). At the same time, its association with other comorbidities such as atherosclerosis, anemia and PAH in COPD can be attributed to systemic inflammation (19,20). In a study conducted on 102 stable male Thai COPD patients, hs-CRP was used as a systemic inflammation marker (21). Similar to our study, hs-CRP and BMI were found to be associated with osteoporosis. Patients with osteoporosis were compared with normal age matched participants and no difference was found between age, cigarette pack years and FEV_1. The cut-off value for hs-CRP was 2.3 mg/L and the mean value of hs-CRP was 5.9 mg/L. Our cut-off value for hs-CRP was 15.9 mg/L and it was above the upper limit value (15.2 mg/L). The reason for the different results is that the kits used for measurement and the patient populations were different. Another study of male COPD patients in Taiwan revealed that parameters such as increased hs-CRP and decreased body mass index are independent risk factors for the development of osteoporosis, similar to the current study, implying that increased systemic inflammation and possibly decreased muscle mass play important roles in bone loss (22). In a cohort study of 2164 patients with COPD followed for 3 years, high fibrinogen, IL-6 and IL-8 levels were associated with heart disease. Fibrinogen has not been found to be associated with osteoporosis (23). A study of 1634 COPD patients examined the correlation of computer tomography (CT) measured bone attenuation with clinical parameters and inflammatory biomarkers. While CT measured bone attenuation correlated positively with FEV_1, BMI and CRP after adjusting for age, sex and pack-years of smoking, it didn't correlate with fibrinogen and WBC (24). In our study, we also examined the effect of arterial blood gases on osteoporosis. Similar to a previous study (25), we found that PaO_2 and $PaCO_2$ had no effect on BMD (OR: 1.01, 95% CI, 0.95-1.05 and OR: 1.04, 95% CI, 0.90-1.19).

One of the limitations of this study is that it is based on a small group of patients with the characteristics specified in the study population. Although FeNO is a marker of airway inflammation, inflammation in the lung parenchyma may be different. The relationship

J Health Sci Med 2023; 6(1): 122-127

Güngör et al. Effect of inflammation for osteoporosis in COPD

between lung inflammation and systemic inflammation was not sufficiently investigated. Factors that may affect osteoporosis, such as vitamin D intake and physical activity, were not adequately questioned. Another limitation is that this study did not include other systemic inflammation markers such as IL-6 or IL-8. Our study was cross-sectional, and measurements were made only once. The situation might be different in a cohort design with repeated measurements.

CONCLUSION

Despite the increasing recognition of the prevalence of osteoporosis in COPD, the underlying pathophysiology and cause are still not fully understood. FeNO was not associated with osteoporosis. Our findings suggest that some biomarkers of systemic inflammation were related to osteoporosis in COPD independent of some predetermined risk factors. According to our findings, patients with high hs-CRP and WBC values may be suitable for osteoporosis scanning. Early treatment of these patients will result in better care and lower morbidity rates.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kırıkkale University Clinical Researches Ethics Committee (Date: 17.12.2012, Decision No: 12/14-02).

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

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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Is the magnesium phosphate ratio a predictor of arrhythmia in patients undergoing hemodialysis?

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ABSTRACT

Aim: Sudden death due to coronary artery disease, heart failure, arrhythmia or hyperkalemia constitutes the majority of cardiovascular causes in patients with end-stage renal disease. Magnesium plays an important role in many processes that regulate cardiovascular functions such as endothelial function, regulation of vascular tone and myocardial excitability. In addition, hyperphosphatemia is very common in patients with end-stage renal disease and is associated with an increased risk of mortality in hemodialysis patients. Our aim in this study; to determine the role of Mg/PO_4 ratio in predicting arrhythmia in patients with end-stage renal disease receiving hemodialysis.

Material and Method: A total of 103 consecutive patients admitted to the cardiology outpatient clinic and receiving hemodialysis for chronic renal failure were included in the study. Between January 2018 and October 2022, patients monitored with 24-hour rhythm holter ECG were recruited. Patients were analyzed by dividing into 2 groups as those with arrhythmia detected in 24-hour rhythm Holter ECG (group 1: 51 patients) and those without (group 2: 52 patients).

Result: The mean age of the patients in the study was significantly higher in group 1 compared to group 2 (66.96±10.27 and 62.21±10.50, p=0.02, respectively). When the 24-hour rhythm Holter ECGs of the patients were examined, the most common arrhythmia was ventricular extrasystole with a rate of 18.4% (n=19), and paroxysmal AF was the second with a rate of 9.7% (n=10). In the univariate regression analysis we performed for arrhythmia predictivity in patients receiving hemodialysis; age (OR: 1.046; 95%CI: 1.005-1.088, p=0.02), LVEF (OR: 0.941; 95%CI: 0.895-0.989, p=0.01), mid-severe MR (OR: 0.553; 95%CI: 0.215-1.424, p=0.22), Na (OR: 1.119; 95%CI: 0.967-1.294, p=0.13), Hemoglobin (OR: 0.872; 95%CI: 0.710-1.069, p=0.18), total cholesterol (OR: 1.006; 95%CI: 0.997-1.016, p=0.19), LDL (OR: 1.012; 95%CI: 0.998-1.026, p=0.10), Mg (OR: 0.117; 95%CI: 0.015- 0.941, p=0.04), PO_4 (OR: 1.664; 95%CI: 1.093-2.532, p=0.01), Mg/PO_4 ratio (OR: 0.002; 95%CI: 0.000-0.104, p=0.002) detected as arrhythmia predictors. In the multivariate regression analysis, independent predictors for the presence of arrhythmia were determined using 2 different models. In the model 1; age (OR: 0.993; 95%CI: 0.956 1.031, p=0.70), LVEF (OR: 0.955; 95%CI: 0.916-0.994, p=0.026), Mg (OR: 0.136; 95%CI: 0.014-1.308), p=0.08), PO_4 (OR: 1.545; 95%CI: 0.989- 2.414, p=0.056) (Table 6). In the model 2; age (OR: 0.988; 95%CI: 0.951-1.026, p=52), LVEF (OR: 0.955; 95%CI: 0.917-0.995, p=0.029), Mg/PO_4 ratio (OR: 0.002, 95%CI: 0.000-0.101 p=0.002) was detected independent predictors for the presence of arrhythmia. ROC analysis (Figure) showed that LVEF<54.5%, with 64% sensitivity and 53% specificity ([AUC]: 0.666, 95% CI: 0.560-0.772, p=0.004), Mg/PO_4 ratio<0.45, with 64% sensitivity and 65% specificity ([AUC]: 0.674, 95% CI: 0.570-0.778, p=0.002), predicts arrhythmia in hemodialysis patients

Conclusion: In our study, it was concluded that the Mg/P ratio, which can be calculated simply, is a predictor of arrhythmia in hemodialysis patients.

Keywords: Arrhythmia, hemodialysis, predictivity, Holter ECG

INTRODUCTION

According to the USA Renal Data System Report (1), the number of patients undergoing hemodialysis due to serious chronic renal failure tends to increase significantly. Despite the increasing technology and experience in hemodialysis (HD), the risk of death still remains high. Five-year life expectancy is 35% in the USA and 60% in Japan, lower than in the general population (2,3). Cardiovascular disease are the cause of death in approximately 40% of patients receiving hemodialysis (4). This situation may not be explained only by cardiovascular (CV) risk factors, but also by non-traditional risk factors such as inflammation,

oxidative stress, anemia and uremia (5). Sudden death from coronary artery disease (CAD), heart failure, or arrhythmia for the majority of CV causes in patients with ESRD (6).

The most important factors for arrhythmia in chronic renal failure patients entering HD are; left ventricular dysfunction, disturbance of electrolyte values such as potassium and magnesium (Mg), hypertension, diabetes mellitus (DM), presence of CAD. Many studies have investigated the effects of these factors on arrhythmia, but their relative importance is not fully known (7). Mg and phosphate (PO_4) minerals, which are electrolytes in the blood, are important minerals in the pathophysiology

J Health Sci Med 2023; 6(1): 128-133

Yurdam et al. Mg/PO4 ratio-predictor of arrhythmia ?

of atherogenesis. Mg plays an important role in many processes that regulate CV functions such as endothelial function, regulation of vascular tone and myocardial excitability (8,9). In the general population, a lower serum Mg level and/or lower dietary Mg intake is associated with an increased incidence of hypertension, Type 2 DM, metabolic syndrome, cerebrovascular disease, myocardial infarction, atrial fibrillation, and sudden cardiac death (10-12).

However, high serum PO_4 concentration, even within the normal range, has been reported to be predictive of the development of atherosclerosis and mortality in patients with normal renal function (13,14). Hyperphosphatemia is very common in patients with end-stage renal disease and is associated with an increased risk of mortality in hemodialysis patients. Patients in the high PO_4 group also had a non-significantly increased relative risk of death from other causes of cardiac and cerebrovascular death (14). Our aim in this study; to determine the role of Mg/PO_4 ratio in predicting arrhythmia in patients with end-stage renal disease receiving hemodialysis.

MATERIAL VE METHOD

Our study was designed as retrospective, observational. The study was initiated with the approval of the Bakırçay University Non-Invasive Clinical Researches Ethics Committee (Date: 2022, Decision No: 770). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 103 consecutive patients admitted to the cardiology outpatient clinic and receiving hemodialysis for chronic renal failure were included in the study. Between January 2018 and October 2022, patients monitored with 24-hour rhythm holter ECG were recruited.

Patients whose baseline ECG is not in sinus rhythm and known arrhythmia, younger than 18 years of age, and had malignancy, active infection, prosthetic valve disease, cardiac pacing, and whose transthoracic echocardiographic (TTE) measurements and 24-hours rhythm Holter ECG cannot be optimized were excluded from the study. In addition, diseases that may cause an arrhythmogenic condition such as amyloidosis and Fabry disease were excluded from the study. The results of blood tests, ECG and TTE findings, and 24- hours rhythm Holter ECG results were obtained from hospital records. The blood analysis results of these patients include the values after hemodialysis, and Holter ECG has been performed at least 1 day after receiving hemodialysis.

Patients were analyzed by dividing into 2 groups as those with arrhythmia detected in 24-hour rhythm Holter ECG (group 1: 51 patients) and those without (group 2: 52 patients).

Arrhythmia Definition in Rhythm Holter ECG

Non-sustained ventricular tachycardia (NSVT) was defined as ventricular-derived tachycardia with a wide QRS complex lasting more than 3 consecutive beats and lasting less than 30 seconds. Supraventricular tachycardia was defined as atrial origin tachycardia with narrow QRS complex and regular RR distance. Paroxysmal AF (Atrial Fibrillation) was defined as atrial origin tachycardia attack with narrow QRS complex and irregular RR distance in rhythm holter ECG. MAT (Multifocal Atrial Tachycardia) was defined as 3 or more different P waves in rhythm Holter ECG, variable P-P, P-R, R-R intervals and atrial origin tachycardia (atrial rhythm 100-180/min) attack.

Statistical Analysis

Analysis was done using IBM SPSS Statistics 24.0 program. The normality distribution of numerical variables was determined by the Kolmogorov-Smirnov test. Numerical variables were noted as mean and standard deviation using the independent Student's t test if they were normally distributed. Numerical variables were noted as IQR (Q (25-75)) using the mann whitney u test if they were not normally distributed. Chi-square and Fisher's exact tests were used to compare categorical variables and reported as number (n) and percentage (%). Logistic regression analysis was performed for the predictiveness of arrhythmia and statistical process was completed with ROC curve analysis. P values below 0.05 were considered statistically significant.

The number of individuals to be included in the study was made using the G*Power 3.1.9.7 program. Estimated sample size was calculated using an independent two-sample-t test, with 80% power, α=0.05 error level, and Cohen (d) effect size "medium"=0.5. Accordingly, it was found appropriate to complete the study with at least 102 patients. In the post hoc analysis the power (1-β err probe) was determined as 0.809 with alpha 0.05 error level, Cohen (d) effect size=0.5.

RESULTS

The mean age of the patients in the study was significantly higher in group 1 compared to group 2 (66.96±10.27 and 62.21±10.50, p=0.02, respectively). The most common comorbid conditions in the patients were hypertension and coronary artery disease, but there was no significant difference between the two groups (hypertension 49% in group 1 and 46% in group 2, p=0.77, coronary artery disease 39% in group 1 and 35% in group 2, p=0.62). When the gender was compared, the number of male patients was higher, but no statistically significant difference was found (Group 1: 61% and group 2: 50%, p=0.27). The baseline clinical features and comorbid conditions of the patients are shown in **Table 1**.

Table 1. Comorbid conditions and baseline clinical features of patients

Variables, n (%)	Group 1 (n=51)	Group 2 (n=52)	p
Age, year	66.96±10.27	62.21±10.50	0.02
Male sex	31 (61)	26 (50)	0.27
Hypertension	25 (49)	24 (46)	0.77
Diyabetes mellitus	14 (27)	14 (27)	0.952
Coronary artery disease	20 (39)	18 (35)	0.62
Hyperlipidemia	12 (24)	15 (29)	0.54
Peripheral artery disease	4 (7.8)	4 (7.7)	0.977
Thyroid disease	8 (16)	6 (12)	0.53
Pulmonary embolism history	3 (5.8)	3 (5.9)	0.652
Anemia	16 (31)	9 (17)	0.096
Chronic pulmonary disease	6 (11.8)	4 (7.7)	0.35
Smoking	16 (31)	13 (25)	0.47
Alcohol use	3 (5.9)	5 (9.6)	0.36
Hemodialysis time, hour	3.1±0.34	3.2±0.27	0.68

n: number of patients, Group 1: those with arrhythmia, Group 2: those without arrhythmia.

When the 24-hour rhythm Holter ECGs of the patients were examined, the most common arrhythmia was ventricular extrasystole with a rate of 18.4% (n=19), while paroxysmal AF was the second with a rate of 9.7% (n=10). Other arrhythmia types and rates are summarized in **Table 2**.

Table 2. Arrhythmias detected in rhythm Holter ECG inm hemodialysis patients

Arrhythmia type, n (%)	Hemodialysis patients (n=103)
Ventricular extrasystole	19 (18.4)
Paroxysmal AF	10 (9.7)
Supraventricular extrasystole	6 (5.8)
Supraventricular tachycardia	6 (5.8)
Atrial tachycardia	5 (4.9)
Non sustained VT	3 (2.9)
Multifocal atrial tachycardia	2 (1.9)

n: number of patient, AF: atrial fibrillation, VT: ventricular tachycardia

There was no statistical difference between the two groups in terms of body mass index (BMI), systolic blood pressure (sBP) and pulse rate (BMI; 23.80 (22.2-27.3) and 23.45 (21.87-28.25), p= 0.85, sBP; 140 (120-151) and 140 (127-159), p =0.48, heart rate; 83.05±15.79 and 82.98±14.41, p= 0.97, group 1 and 2, respectively). Among the transthoracic echocardiographic findings, LVEF (left ventricular ejection fraction) value was found to be significantly lower in group 1 than group 2 (50.00±8.92 and 54.42±8.48, p=0.01, respectively). When the blood analyzes of these patients were examined, the Mg, P and Mg/PO₄ ratios showed significant differences between the two groups (Mg; 1.92 (1.85-2.01) mmol/L and 2.01 (1.91-2.17), p=0.02, PO₄; 4.58 (4.1-5.18) mmol/L and 3.9 (3.6-4.87), p=0.003, Mg/PO₄ ratio; 0.43 (0.36-0.50) and 0.51 (0.41-0.61), p=0.002, group 1 and 2, respectively).

In **Table 3**, imaging, examination findings and blood analysis results of the patients are given in detail. The drugs they currently use are presented in **Table 4**.

Table 3. Imaging, examination findings and blood analysis results of patients

Variables	Group 1 (n=51)	Group 2 (n=52)	p
Body mass index, kg/m²	23.80 (22.2-27.3)	23.45 (21.87-28.25)	0.85
Pulse, beat/min	83.05±15.79	82.98±14.41	0.97
Systolic BP, mmHg	140 (120-151)	140 (127-159)	0.48
Diastolic BP, mmHg	80 (70-91)	80 (70-90)	0.87
LVEF, %	50.00±8.92	54.42±8.48	0.01
Mid-severe MR, n (%)	14 (27)	9 (17)	0.21
Mid-severe MS, n (%)	4 (7)	6 (11)	0.38
Mid-severe AR, n (%)	6 (11)	5 (10)	0.72
Mid-severe AS, n (%)	7 (14)	12 (23)	0.22
Mid-severe TR, n (%)	13 (25)	12 (23)	0.77
Fasting blood glucose, mg/dL	101 (93-123)	96 (88-119)	0.11
Urea, mg/dL	23.8 (12.3-32.1)	15.4 (11.77-34.92)	0.74
Creatinine, mg/dL	2.82 (2.14-3.7)	2.73 (1.91-2.99)	0.35
Na, mmol/L	139 (137-140.5)	138 (137-140)	0.12
K, mmol/L	4.33 (4.04-5.1)	4.38 (4-4.9)	0.49
Ca, mmol/L	8.39±0.77	8.44±0.64	0.68
Mg, mmol/L	1.92 (1.85-2.01)	2.01 (1.91-2.17)	0.02
PO₄, mmol/L	4.58 (4.1-5.18)	3.9 (3.6-4.87)	0.003
Mg/PO₄ oranı	0.43 (0.36-0.50)	0.51 (0.41-0.61)	0.002
Leukocyte, /mm³	8.8 (7.82-10.65)	8.91 (8.1-10.59)	0.76
Hemoglobin, g/dL	12.16±2.01	12.66±1.84	0.18
Thrombocyte, /mm³	244 (211-264)	239.5 (208-296)	0.68
TSH, mIU/L	1.38 (0.84-1.92)	1.52 (0.88-2.16)	0.55
B12 vitamin, pg/mL	211.4 (191-266)	220 (189-308)	0.73
Ferritin, ng/mL	41.53 (31.3-62)	45 (39.3-54.9)	0.46
Total cholesterole, mg/dL	211.37±41.76	198.92±46.76	0.19
Trygliseride, mg/dL	184±85.65	176±81.75	0.65
HDL-C, mg/dL	41.26±9.85	40.76±10.85	0.82
LDL-C, mg/dL	127.64±30.51	116.31±32.49	0.09
CRP, mg/dL	3.28±0.76	3.31±0.61	0.72

n: number of patient, BP: blood pressure, LVEF: left ventriclel ejection fraction, MR: mitral regurgitation, MS: mitral stenoz, AR: aort regurgitation, AS: aort stenoz, TR: tricuspit regurgitation, Na: sodium, K: potassium, Ca: calsium, Mg: magnesium, PO₄: phosphate, TSH: thyroid stimulant hormone, HDL-C: high density lipoprotein-cholesterole, LDL-C:low density lipoprotein-cholesterole, Grup 1: those with arrhythmia, Grup 2: those without arrhythmia. Those with normal distribution of continuous variables were presented as the mean±SD by using the Student's t test. Continuous variables that did not show normal distribution were presented as IQR (25-75) by applying the Mann Whitney U test.

Table 4. Medications of patients

Drugs, n (%)	Group 1 (n=51)	Group 2 (n=52)	p
Betablocker	18 (35)	11 (21)	0.11
Ca channel blocker (Dhp)	12 (24)	13 (25)	0.86
Ca channel blocker (Non-dhp)	7 (14)	7 (13)	0.96
Acetylsalicylic acid	18 (35)	16 (31)	0.62
Insulin	5 (10)	5 (10)	0.97
Statin	12 (24)	11 (21)	0.77
Oral iron preparation	9 (18)	9 (17)	0.96
Oral B12 vitamini	6 (12)	8 (15)	0.59
n: number of patient, Ca: calsium, Dhp: dihydropyridine, Group 1: those with arrhythmia, Group 2: those without arrhythmia.			

In the univariate regression analysis we performed for arrhythmia predictivity in patients receiving hemodialysis; age (OR: 1.046; 95%CI: 1.005-1.088, p=0.02), LVEF (OR: 0.941; 95%CI: 0.895-0.989, p=0.01), mid-severe MR (OR: 0.553; 95%CI: 0.215-1.424, p=0.22), Na (OR: 1.119; 95%CI: 0.967-1.294, p=0.13), Hemoglobin (OR: 0.872; 95%CI: 0.710-1.069, p=0.18), total cholesterol (OR: 1.006; 95%CI: 0.997-1.016, p=0.19), LDL (OR: 1.012; 95%CI: 0.998-1.026, p=0.10), Mg (OR: 0.117; 95%CI: 0.015- 0.941, p=0.04), PO₄ (OR: 1.664; 95%CI: 1.093-2.532, p=0.01), Mg/PO₄ ratio (OR: 0.002; 95%CI: 0.000-0.104, p=0.002) detected as arrhythmia predictors. In the multivariate regression analysis, independent predictors for the presence of arrhythmia were determined using 2 different models. In the model 1; age (OR: 0.993; 95%CI: 0.956 1.031, p=0.70), LVEF (OR: 0.955; 95%CI: 0.916-0.994, p=0.026), Mg (OR: 0.136; 95%CI: 0.014-1.308), p=0.08), PO₄ (OR: 1.545; 95%CI: 0.989- 2.414, p=0.056). In the model 2; age (OR: 0.988; 95%CI: 0.951-1.026, p=52), LVEF (OR: 0.955; 95%CI: 0.917-0.995, p=0.029), Mg/PO₄ ratio (OR: 0.002, 95%CI: 0.000-0.101 p=0.002) was detected independent predictors for the presence of arrhythmia. Logistic regression analysis is shown in **Table 5**. ROC analysis (**Figure**) showed that LVEF<54.5%, with 64% sensitivity and 53% specificity ([AUC]: 0.666, 95% CI: 0.560-0.772,

p=0.004), Mg/PO₄ ratio<0.45, with 64% sensitivity and 65% specificity ([AUC]: 0.674, 95% CI: 0.570-0.778, p=0.002), predicts arrhythmia in hemodialysis patients.
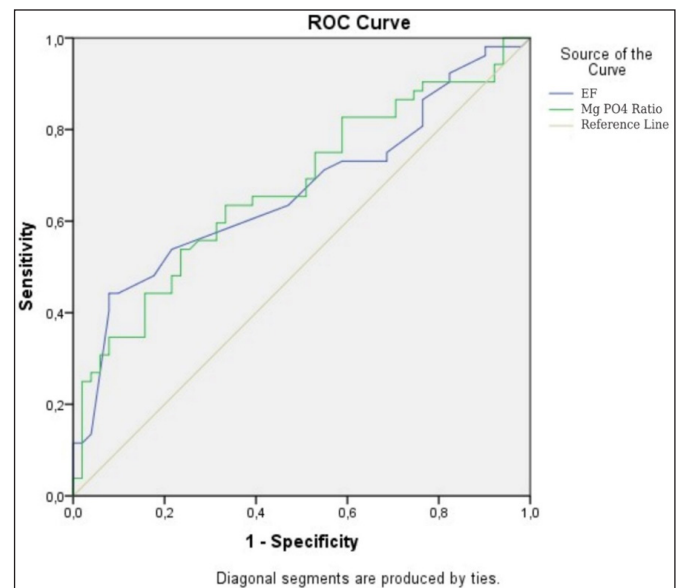
Figure. Receiver Operating Characteristic curve analysis for arrhythmia predictivity in hemodialysis patients.

DISCUSSION

The important finding of this study is that the Mg/PO₄ ratio determined by low magnesium and high phosphate values is a predictor of arrhythmia in patients receiving hemodialysis for chronic renal failure. After multivariate regression analysis, lower magnesium phosphate ratio and lower LVEF were potential risk factors.

Lu Wei et al. (15) found low serum Mg concentration to be a predictive factor of major adverse cardiac and cerebrovascular events (MACCE) in 290 hemodialysis patients. In the management of hemodialysis patients, it has been stated that a Mg level lower than 1.04 mmol/L can predict especially cardiovascular mortality.

Table 5. Logistic regression analysis for arrhythmia predictive in hemodialysis patients

Variables	Univarite Logistic Regression			Multivariate Logistic Regression Model 1			Multivariate Logistic Regression Model 2		
	OR	95 % CI	p	OR	95 % CI	p	OR	95 % CI	p
Age	1.046	1.005-1.088	0.02	0.993	0.956-1.031	0.7	0.988	0.951-1.026	0.52
LVEF	0.941	0.895-0.989	0.01	0.955	0.916-0.994	0.026	0.955	0.917-0.995	0.029
Mid-Severe MR	0.553	0.215-1.424	0.22						
Na	1.119	0.967-1.294	0.13						
Hemoglobin	0.872	0.710-1.069	0.18						
T. cholesterol	1.006	0.997-1.016	0.19						
LDL	1.012	0.998-1.026	0.10						
Mg	0.117	0.015-0.941	0.04	0.136	0.014-1.308	0.08			
PO₄	1.664	1.093-2.532	0.01	1.545	0.989-2.414	0.056			
Mg/PO₄ ratio	0.002	0.000-0.104	0.002				0.002	0.000-0.101	0.002
OR: odds ratio, CI: confidence interval, LVEF: left ventricular ejection fraction, MR: mitral regurgitation, Na: sodium, LDL: low density lipoprotein, Mg: magnesium, PO₄: phosphate.									

Magnesium is the most abundant ion in our body. A wide range of effects may occur with low Mg, ranging from vascular calcification, coronary artery calcification, and abdominal vascular calcification (16-18) to asthma development, a predisposing effect to chronic inflammation, myocardial remodeling, and electrophysiological abnormalities (19,20). In recent studies (21-23), higher Mg levels were associated with less arrhythmia and cardiovascular death after and during dialysis. In our current study, the Mg level was found to be significantly lower in the arrhythmia group.

In a study which data from two national randomized samples of hemodialysis patients (n=12,833) were used to test the hypothesis that high serum PO₄ contributes to the major causes of cardiac death, death from CAD, sudden death, infection and death from unknown causes were found to be higher in the high PO₄ group (>6.5 mg/day) than the lower PO₄ group (<6.5 mg/dl). This study (14) identifies strong associations between elevated serum PO₄ and causes of cardiac death in HD patients, particularly deaths from CAD and sudden death. More effective treatment measures to reduce the prevalence of these factors in HD patients may result in improved survival.

In previous studies (24,25), the relationship between coronary ischemia and endothelial functions and Mg/PO₄ ratio was investigated. The predisposition of magnesium and phosphate to thrombosis through atherosclerosis and inflammation is known. The association between low Mg levels and increased cardiac mortality has been noted by several investigators. Chipperfield et al. (26) found low myocardial Mg levels in cases of sudden cardiac death. Electrophysiological changes, albeit minimal, were recorded with Mg replacement and an antiarrhythmic effect was observed (27,28). Schwartz et al. (29) found a negative correlation between cardiac arrhythmia and phosphate level in early stage sepsis. Since both Mg and PO₄ can affect cardiac arrhythmia to a certain extent, we can think that their ratio to each other is also effective. In this study, we aimed to reveal the potential effect of Mg/PO₄ ratio on the development of arrhythmia.

The frequency of arrhythmias in patients undergoing hemodialysis is too high to be underestimated. In a study of 160 patients with end-stage renal disease receiving hemodialysis, 92% of arrhythmic events were detected. The frequency of these arrhythmias was mostly ventricular events with 81%, followed by supraventricular events with 51% (30). Similar to previous studies by Sforzini et al. (31), the most common arrhythmia in our study was ventricular events (37% among all arrhythmias).

The management of morbidity and mortality due to arrhythmia in dialysis patients is still difficult and opportunities to turn this situation in their favor are limited. Our most important diagnostic tool that facilitates this situation is ECG monitoring. In particular, it is possible to detect atrial fibrillation (paroxysmal), which we have identified as the cause of cerebrovascular disease with severe consequences, with 24-hour rhythm Holter ECG without creating both material and moral burden (32). Atrial fibrillation becomes chronic; it has adverse effects such as stroke, heart failure, and decreased LV systolic functions due to tachycardiomyopathy. Based on this, both a decrease in LVEF triggers atrial fibrillation and the development of atrial fibrillation enters a vicious circle with worsening LVEF (33). The DEFINITE (Significance of follow-up left ventricular ejection fraction measurements in the Defibrillators in Non-Ischemic Cardiomyopathy Treatment Evaluation trial) study (34) showed that mortality tends to decrease and the probability of experiencing arrhythmic events decreases in the group with left ventricular recovery. For this reason, it is expected that the frequency of ventricular arrhythmias will increase as the left ventricular EF value decreases in patients with ischemic or non-ischemic heart failure. In our current study, LVEF was lower in the group with arrhythmia.

Study Limitations

Our study has a few limitations, except that it is single-center and retrospective. Although the Holter ECG records of the patients were at least 24 hours after hemodialysis, we did not have access to the duration of the dialysis procedure, hemodynamic variables and fluid-electrolyte balance findings. Based on the assumption that the expected frequency of arrhythmias is higher in hemodialysis patients, it may be important to obtain rhythm recordings longer than 24 hours.

CONCLUSION

The Mg/PO₄ ratio, which can be calculated simply, is a predictor of arrhythmia in hemodialysis patients. It can be thought that our study can be a pioneer in this regard, supported by future multicenter and prospective studies..

ETHICAL DECLARATIONS

J Health Sci Med 2023; 6(1): 128-133

Yurdam et al. Mg/PO₄ ratio-predictor of arrhythmia ?

Author Contributions: The author declares that he has participated in the design, execution, and analysis of the paper, and he has approved the final version.

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Functional results of deltoid split minimally invasive osteosynthesis for neer type 3 proximal humerus fractures

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ABSTRACT

Aim: We aimed to evaluate the short-term functional and radiological outcomes of the minimally invasive fixation of three-part proximal humerus fractures without using calcar screws.

Material and Method: Twenty patients were treated with the minimally invasive approach using locking plate-screws. The relationship between the cephalo-diaphyseal angles and the functional outcomes were evaluated.

Results: The mean follow-up time were 22.7 months. The mean Constant-Murley score of the patients was 83.7. The modified Constant-Murley score was excellent in 16 and good in four patients. A statistically significant difference was detected between cephalo-diaphyseal angles.

Conclusion: Osteosynthesis with minimally invasive plate-screw and deltoid splitting application is encouraging with its satisfactory results in three-part fractures of the humerus; however, the total complication rate of 35% should not be ignored. In addition, in order to prevent a significant varus collapse and angular loss, the fracture subgroups should be studied in detail and additional measures should be taken based on the fracture type.

Keywords: Calcar screw, deltoid split, MIPO, proximal humerus fracture

INTRODUCTION

Fractures of the proximal humerus comprise 5% of all fractures. While the conservative treatment results of the non-displaced proximal fractures exhibit 100% of union and excellent functionality, the conservative treatment of the displaced three and four-part fractures result in complications in 48% of the patients, with avascular necrosis in 14% and varus-malunion in 23% (1).

The treatment of osteoporotic fractures with locking plate-screw systems is a popular approach among surgeons (2). Biomechanical studies have shown the superiority of locking plate-screws to intramedullary nails and conventional plates (3,4). However, the rate of complications due to the use of locking plates may reach as high as %48.8 (5). The high rates of complication and low functional scores were associated with the type of the fracture (6). In addition, fracture types without the medial cortex support have a higher risk of varus malunion (7-9). Therefore, the use of calcar screws has been asserted to decrease the secondary reduction loss (8).

In recent years, the deltoid splitting approach has gained popularity with minimally invasive plate-screw osteosynthesis (MIPO) applications (6,8). The deltoid splitting (DS) approach has been suggested as an alternative to the deltopectoral approach due to the lateral placement of the plate and easier management of the greater tuberosity (10,11). However, fixation with calcar screws along the axillary nerve is a technique problem with the DS-MIPO technique (12).

This study was designed with the aim of evaluating the functional and radiological results of the fixation of the three-part proximal fractures employing the DS-MIPO approach without using calcar screws. The cephalo-diaphyseal angular loss and its effect on the functional outcomes, malunion and complications in the postoperative early and late-term were investigated.

J Health Sci Med 2023; 6(1): 134-139

Bekmezci et al. Results of neer type 3 fractures

MATERIAL AND METHOD

The study was carried out with the permission of Ümraniye Training and Research Hospital Clinical Researches Ethics Committee (Date: 09/04/2021, Decision No: E-54132726-000-7920). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Three-part fractures of the proximal humerus treated between 2013 and 2015 were included in the study. Cases with accompanying injuries, fracture dislocations, splitting of the humeral head, the 'egg shell' appearance of the humeral head due to osteoporosis, and neurological problems were excluded as different methods had been employed in their treatment. A total of 20 patients (4 males, 16 females) with a mean age of 63.9 (range: 43 to 82) years were included in the study. Six patients had valgus-impaction fractures and 14 had procurvatum fractures on the sagittal or varus angulation fractures on the coronal plane.

Surgical Technique

All patients were operated in the beach chair position and under interscalene block and general anesthesia using an electromagnetic limb positioner (Spider 2; Smith & Nephew, Andover, MA, USA). The fluoroscope was sterilely placed to obtain a true anteroposterior view of the shoulder and kept stationary throughout the surgery. Deltoid splitting was performed 5 cm lateral of the proximal acromion. The axillary nerve exiting the quadrilateral space was palpated. First, the tubercle fragments were attached to the plate with sutures. In all cases, the plate was placed two millimeters behind the biceps tendon in the lateral plane and 5-8 millimeters distal to the upper end of the humerus in the sagittal plane. Plate placement was checked with fluoroscopy. An incision 3 cm to the distal of the plate was made to confirm the locking of the distal part of the plate on the sagittal plane. Alignment was achieved under fluoroscopy control with direct and indirect reduction methods. Then, temporary reduction was achieved with two Kirshner wires applied superiorly. In necessary cases, the continuity of the medial calcar was ensured with the help of a periosteal scraper applied from the fracture line. Reduction quality and continuity of medial calcar were confirmed by fluoroscopy in all cases. In patients with a metaphyseal defect, a cancellous graft of 15-30 cc was placed between the humeral head and greater tuberosity. A tunnel was created beneath the deltoid muscle group and axillary nerve and the anatomical 5-hole locking proximal humerus plate (Philos; DePuy Synthes, Oberdorf, Switzerland) was placed. Due to the location of the axillary nerve, 3.5 mm locking screws were used on the A1, A2, B1, B2, C1, C2 and D sections. The E section for calcar screws was left unused. 3.5 mm cortical, 4 mm spongious, and 3.5 mm locking

screws were used in the distal. Three cases with valgus impaction fractures and one case with varus-procurvatum fracture patient were treated with allografts while six cases with varus-procurvatum fractures were managed by the metaphyseal compression technique (**Figure 1, 2**).
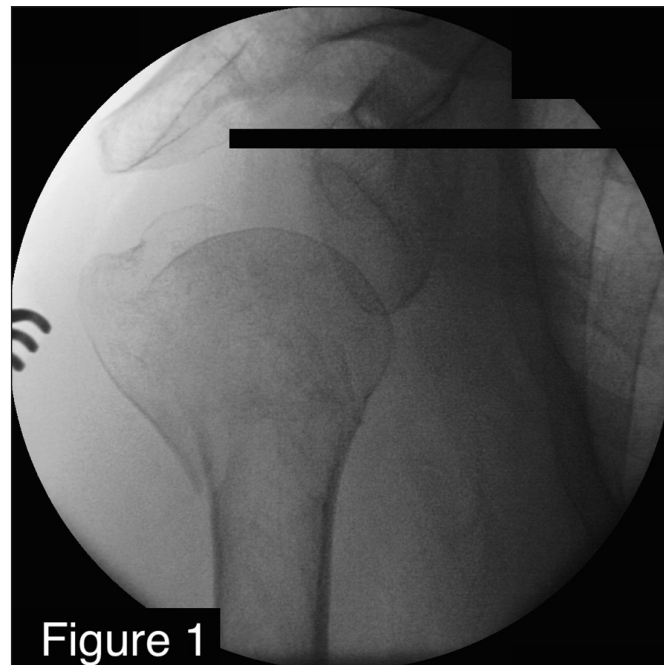
Figure 1. Valgus impaction fracture with displaced tuberculum majus was observed.
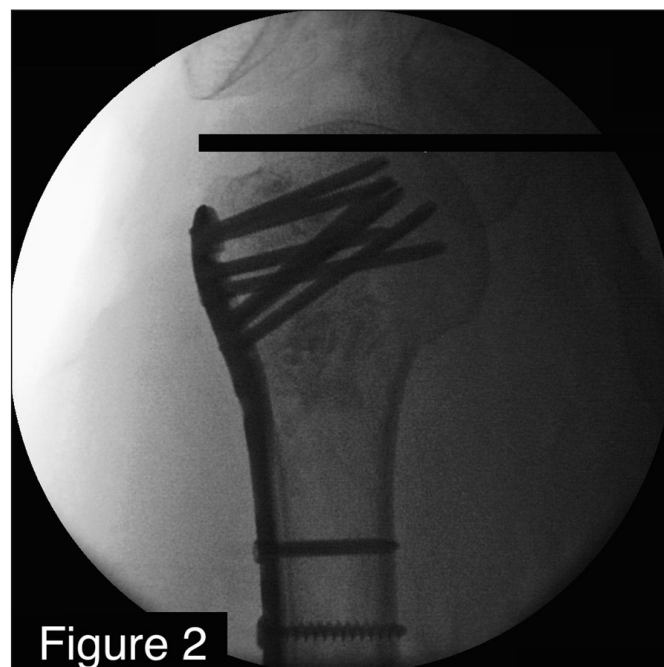
Figure 2. Fracture reduction and grafting was performed.

Follow-up After Surgery

The patients were given arm sling for three weeks postoperatively. Shoulder pendulum exercises, active assisted passive motion exercises and elbow-wrist ROM exercises were performed. The patients were examined at the 3rd, 6th, 12th, 16th week and final follow-up visits.

Bekmezci et al. Results of neer type 3 fractures

J Health Sci Med 2023; 6(1): 134-139

Assessment of the Results

Postoperative early-term and late-term cephalo-diaphyseal angles (CDA and LCDA) were measured for assessment of the radiological outcomes. Constant-Murley scores for the operated shoulder (CMS) and for the contralateral shoulder (CCMS) and the difference between two shoulders (DCMS) were noted for the assessment of functional outcomes (7,13). Normal distribution of the variables was checked with the Kolmogorov-Smirnov test. The relationship between the CDA and LCDA was assessed using the paired t-test. Pearson's correlation analysis was employed for evaluating the relationship between the CDA-LCDA and CMS-DCMS. The statistical significance level was set at $p<0.05$ (MedCalc Software Belgium 1993-2016).

RESULTS

The mean follow-up period and time to union were 22.7 (range: 8 to 44) and 3.2 (range: 3 to 8) months, respectively. No infection or non-union was observed in any patient. Migration of the B2 screw was observed in two patients (10%) in the early-term. Screw removal was performed in these two cases by reoperation. Four patients (20%) developed anterior deltoid deficiency which lasted up to six months. These patients were followed up with anterior deltoid training and electrostimulation.

Mean angular losses of 11° (range: 0° to 15°) in forward elevation, 12° (range: 0° to 15°) in abduction, and 8° (range: 0° to 10°) in adduction and external rotation versus the contralateral shoulder were observed (**Table 1**).

The mean CMS was 83.7 (range: 72 to 96), CCMS was 91.4 (range: 82 to 100) and DCMS was 7.25 (range: 0 to 19). The CMS and CCMS was found in correlation with age ($p=0.0004$ and $p=0.0001$) while DCMS showed no correlation ($p=0.6815$). Based on the DCMS, 16 patients had excellent and four had good results.

The mean CDA and LCDA were 139.25° (range: 134° to 149°) and 134.95° (130° to 145°), respectively. A statistically significant difference was detected between the CDA and LCDA ($p<0.0001$) (**Graphic**) One patient (5%) had 6° of varus. This patient did not encounter any nonunion or implant failure; however, developed migration of the B2 screw from the posterior.
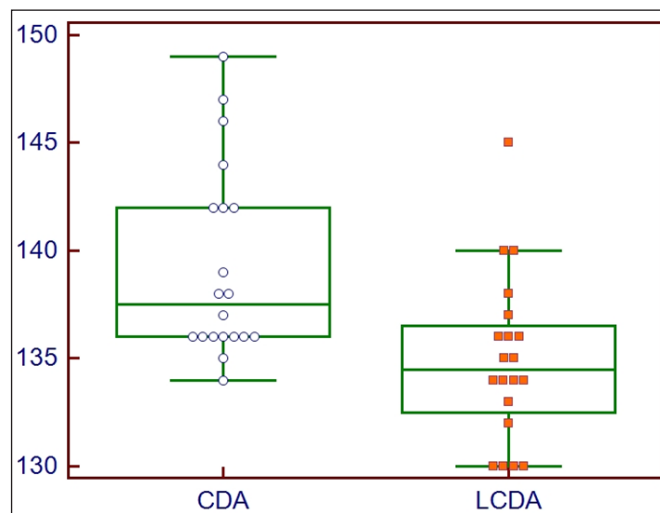
Graphic. A statistically significant difference was detected between the early cephalo-diaphyseal angle (CDA) and late cephalo-diaphyseal angle (LCDA).

In evaluation of the functional and radiological functions, neither the CMS nor the DCMS were found to correlate with the CDA or LCDA ($p=0.2474$) ($p=0.0882$) ($p=0.2600$) ($P=0.1520$).

DISCUSSION

The functional results were excellent in 16 and good in four DS-MIPO patients. Although their occurrences have been reported in the literature, complications of impingement, nonunion, malunion, or infection due superior positioning of the plate was not observed in our DS-MIPO series (14,15).

Table 1. Demographics & clinic results			
Sex	4 male	16 female	
Fracture type	6 Valgus impaction	14 Procurvatum varus	
Age	Mean 63.9	10.6 SD	Range 43-82
Follow	Mean 22.7	11.6 SD	Range 8-44
Union time	Mean 3.2	1.11 SD	Range 3-8
Forward flexion diffirence	Mean 11	2.88 SD	Range 0-15
Abduction diffirence	Mean 12	3.12 SD	Renge 0-15
External rotation diffirence	Mean 8	2.73 SD	Renge 0-10
Constant Murley score	Mean 83.7	7.94 SD	Renge 72-96
Contrlateral constant Murley score	Mean 91.4	5.60 SD	Renge 82-100
Delta constant Murley score	Mean 7.25	4.99 SD	Renge 0-19
Cephalo diaphysier angle	Mean 139.25	4.43 SD	Renge 134-149
Late Cephalo diaphysier angle	Mean 134.95	3.85 SD	Renge 130-145

J Health Sci Med 2023; 6(1): 134-139

Bekmezci et al. Results of neer type 3 fractures

The Constant-Murley scores of both the operated and healthy shoulders were found to decline with the age of the patients. The delta Constant-Murley score was an independent variable of age. In their series of 23 DS-MIPO patients, Barco et al. (14) observed that the CMS was lower in patients above 65 years of age in comparison to those younger than 65. However, the scores of the contralateral shoulder and their relationship with age were not investigated in their study.

The aim of this study was to assess whether the stability could be preserved with the use of calcar screws and to investigate the effects of instability (LCDA-CDA difference) on the clinical outcomes. The difference between the LCDA and CDA pointed out to the probability of small angular losses (mean: 4.4 °, range: 0° to 6°). However, the LCDA measurements in all patients were within the normal range (125° to 140°) (16,17). In addition, the functional scores did not correlate with this angular change. This disassociation might be explained with the distribution of the CDA and LCDA within physiological limits.

Few studies in the literature have compared the early and late-term CDA outcomes in DS-MIPO patients. Altman et al. (12) treated 21 patients employing the DS-MIPO technique without calcar screws and found no significant difference between the early and late-term CDA outcomes, a finding in contrast to ours. Their intraoperative measurements had a mean of 139° (range: 123° to 156°) and their follow-up measurements had a mean of 138° (range: 123° to 159°); with only 11 patients in their series with a Neer Type 3 fracture. Similarly, Sohn et al.(6) investigated the CDA changes in their 62-patient series, with Neer Type 3 fractures in 24, and observed no statistically significant difference. The angular loss in Type 4 fractures was more significant in comparison to Type 3 and Type 2 fractures. The mean early and late-term Neer CDA measurements in Type 3 patients were 131°±2° (range: 115° to 144°) and 138°±2° (range: 110° to 147°), respectively.

Although we did not use calcar screws in our study, we took other precautions we thought might be useful for a rigid fixation. One of them was grafting for the valgus impaction fractures and the other was metaphyseal compression for the varus procurvatum fractures.

The dead space technique with grafting is an important factor in rigidity of the fixation. Therefore, in order to increase the stability of the valgus impaction fractures, the use of allograft-autograft-tricalcium phosphate has been recommended (7,18-21). Thus, in order to increase the rigidity of the fixation, we used grafting in three patients with valgus impaction fractures.

Varus fractures possessing problem for the continuity of the medial cortex are also a challenge to providing balance. The use of calcar screws has been recommended especially in varus type fractures (8,9). Moreover, in addition to calcar screws, structural allografts are used in order to increase the rigidity of fixation (22,23). Fibular and femoral allografts are other options (1,24). In their study simulating a metaphyseal defect, Yang et al. (9) found that metaphyseal compression and/or calcar screws increased the rigidity of fixation. Six patients in the study had varus-procurvatum angular deformity and instability was observed due to metaphyseal destruction. Instead of grafting or using calcar screws, metaphyseal compression was performed, as suggested by Yang et al. (9).

Despite the presence of varus deformity, which could be interpreted as a radiological anomaly and to have clinical significance, one (5%) of our patients developed a varus angulation of 6° that led to a LCDA of 132°. Late-term screw migration was present as a secondary finding of deformity; however, we had good outcomes according to DCMS. We performed no metaphyseal compression in fixing the varus medial cortex defect of this patient and tried to increase the stability using allografts.

Due to its anatomical proximity, DS-MIPO surgery has a special significance for the axillary nerve. The distance between the axillary nerve and the lateral of the acromion, from the posterior edge of the acromion, varies between 79 (range: 65 to 90) and 72 (range: 60 to 85) mm. This distance has a statistically significant relationship with the humeral length (25). The EMG studies on the axillary nerve, performed following anterolateral fixations using the minimally invasive approach, presented subclinical findings which do not affect functionality (26). Some MIPO studies have recounted transient axillary nerve dysfunction rates of 2-5%. However, no permanent functional complications were reported and the criteria or definition for deltoid functional deficiency have not been defined in these studies (10,27). Axillary nerve dysfunction may go unnoticed as active movement is not recommended until 3 to 6 weeks after surgery and the patients try to avoid performing such moves. In our study, anterior deltoid deficiency, lasting up to six months in four (20%) patients, was observed. The patients had no difficulties with passive joint range of motion; however, they could not initiate abduction or forward elevation while on foot but perform forward elevation while lying down. These findings were considered clinically diagnostic factors for anterior deltoid deficiency. The patients were followed with anterior deltoid training and electrostimulation. Three patients showed signs of clinical recovery on the 12th, 12th and 16th weeks, respectively, and another one on the sixth month.

Bekmezci et al. Results of neer type 3 fractures

J Health Sci Med 2023; 6(1): 134-139

The screws in two patients (10%) were removed at the 6th and 8th months due to migration and joint mobilization was performed under anesthesia. DS-MIPO applications are prone to screw migration as in the deltopectoral approach (5,6,10). While early-term screw migration takes place during intraoperative screw insertion and is a key element for intraoperative radiological evaluation, late-term screw migration is secondary to the collapse of the fragment or varus deformity and points out to insufficient rigidity of the fixation (14,28).

One of the weaknesses of our study was the heterogeneity due to subgrouping of the three-part fractures under different characteristics. Grafting of the dead space in valgus impaction fractures in order to avoid the collapse of the head and fixation via metaphyseal compression in instable varus fractures where cortical continuity is failed have proved their support in achieving stability, however, this support could not be quantified. In this context, the consequences of not using calcar screws remain unclear. In addition, the lack of diagnosis and follow-up of the axillary nerve dysfunction via EMG can be considered another important weakness.

CONCLUSION

Fixation of the three-part proximal humerus fractures using the MIPO plate-screw approach without calcar screws returned excellent functional results in 80% and good results in 20% of the patients. Although varus angular losses within physiological limits did not affect the functional scores, the total rate of complications was 35%. Further studies are required to evaluate the support of grafting and metaphyseal compression, if any, on stability.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ümraniye Training and Research Hospital Clinical Researches Ethics Committee (Date: 09/04/2021, Decision No: E-54132726-000-7920).

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 has no conflicts of interest to declare.

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Author Contributions: The author declares that he has participated in the design, execution, and analysis of the paper, and he has approved the final version.

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The impact of SGLT2-inhibitor therapy on platelet function in type 2 Diabetes mellitus

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ABSTRACT

Aim: We consider mean platelet volume (MPV), a pointer of platelet activity related to type 2 diabetes mellitus (T2DM) and vascular complications, may have a role in patients using Sodium-Glucose Co-Transporter 2 inhibitors (SGLT2i). Therefore, we aimed to evaluate the MPV change after SGLT2i use in diabetic patients.

Matherial and Method: Hemogram parameters such as hemoglobin, hematocrit, and MPV in the 0th and 24th weeks of 102 patients with T2DM that received SGLT2i treatment added to their existing medications and of the control group in which participants are compatible in terms of age and gender factor were compared.

Results: A significant increase was observed in the values of MPV and hemoglobin in the 0th and 24th weeks (9.3 (8.2-10.3) to 10.1 ± 1.3, $p < 0.001$, 13.9 ± 1.42 to 14.4 ± 1.5 $p < 0.001$, respectively). Similarly, the hematocrit value increased (42 ± 3.7 to 44.2 ± 3.8, $p < 0.001$). There was also a significant increase in both red blood cell (RBC) and platelet counts (5 ± 0.42 to 5.2 ± 0.47, $p < 0.001$, 252,000 (209,000-304,000) to 262,000 (221,000-322,000), $p = 0.007$, respectively). No correlation was identified in patients with T2DM between MPV and age and gender factors, diabetes duration, body mass index (BMI), fasting and postprandial blood glucose, and insulin use.

Conclusion: Contrary to the studies analyzing the relation between MPV and T2DM and its complications, we detected that a 24-week SGLT2i treatment led to an increase in MPV value.

Keywords: SGLT2 inhibitors, mean platelet volume, hematocrit

This study was published as an oral presentation at the "57th National Diabetes Congress" held in Bodrum on June 1-5, 2021.

INTRODUCTION

Type 2 diabetes mellitus (T2DM), a chronic complex disease appertaining to microvascular and macrovascular complications, develops secondary to hyperglycemia. Increased platelet activity plays a fundamental role in vascular complications in diabetes mellitus (DM) (1). Seventy-five percent of deaths depend upon platelet in diabetic patients is derived from cardiovascular problems, and a of them is due to peripheral vascular events as well as cerebrovascular complications (2).

Sodium-Glucose Co-Transporter 2 inhibitors (SGLT2i) are oral anti-diabetic agents decreasing blood sugar by inhibiting renal glucose reabsorption in the proximal tubule, and increasing urinary glucose excretion (3). SGLT2 inhibitors act independently from insulin production. In addition to glycemic activity, observational and cardiovascular studies have proven that SGLT2i lead lose weight and has cardiorenal protective effects compared to placebo and other anti-diabetic medications (4-5). Considering hemostasis and thrombosis, the role of platelets cannot be ignored (6). Large platelets usually display more metabolic and enzymatic activity compared to small platelets (7). To observe platelet function, mean platelet volume (MPV) takes an active role as a biological indicator. Increased MPV is shown to be associated with T2DM, myocardial infarction, atherosclerosis, and peripheral artery disease (8-9). Since patients with T2DM have several metabolic disorders, such as coronary diseases, hyperlipidemia, and hypertension, which can alter MPV levels independently, increased MPV levels in patients with T2DM is an expected result in comparison to the control group (10). For this reason, we aimed to evaluate MPV change after SGLT2i use.

MATERIAL AND METHOD

The study was carried out with the permission of University of Health Sciences Clinical Researches Ethics Committee (Date: 2021, Decision No: 115/18). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Our study is an observational retrospective study. Hemogram values of the the patients with T2DM that received SGLT2i treatment in addition to their existing medications and applied to the Endocrinology and

Metabolism diseases clinic, between January 2021 and 2022 were recorded retrospectively. HbA1c and hemogram levels of 432 patients, having started SGLT2i treatment, were determined. After evaluating these records, 102 patients that meet the requirements were enrolled in this current study, and they were treated with only insulin or with insulin and oral anti-diabetic agents except for SGLT2. In the 24-week follow-up period, patients whose treatment regimens were altered and those who had cardiovascular diseases, clotting disorders, malignity, liver and kidney failure, and/or hypertension were excluded from the study. Apart from these, the subjects receiving medications that could affect platelet and clotting systems and whose abnormal platelet count was less than 100 or more than 450 (<100 or > 450 platelet/L) were also excluded. The control group was formed by scanning the patients that applied to the internal diseases policlinic and had no additional diseases. Demographic features, onset examination findings, and laboratory results of both the patient and control groups were recorded.

Samples were taken through K-3 ethylenediaminetetraacetic acid tubules from the antecubital vein. The samples were tested in one hour to ensure that aging-related changes were minimized.

Statistical Analysis

SPSS software version 21 (Chicago, IL) was used to carry out statistical analysis. Visual (histograms and probability plots) and analytic methods (Shapiro-Wilk's test) were employed in order to investigate the variables to detect if they were normally distributed. The measurements at three-time points were compared via Paired Student's t-test and the Wilcoxon test (Baseline and the 6th month). Means and standard deviation were used for normally distributed variables, whereas medians and interquartile ranges (IQRs) for non-normally ones were used for descriptive analyses. In order to indicate statistically significant results, a p value below 0.05 was accepted. Correlations among variables were analyzed via the Spearman test. A 5% type-I error level was used to infer statistical significance.

RESULTS

One hundred and two patients, 50 (49%) males and 52 (51%) females, were included in this study.

The mean age was 55.5±9. The number of the patients using dapagliflozin and empagliflozin was 73 (71.6%) and 29 (28.4%), respectively. The mean duration of the disease was 12.7 (7.7-16) years. Diabetes-related complications, comorbid conditions, anti-diabetic medications, and smoking status of the patients are demonstrated in **Table 1**.

Table 1. Baseline characteristics, microvascular and macrovascular complications, and anti-diabetic medications of the patients

Number, n	102
Age, years	55.5±9
Female, n (%)	52 (51)
Duration of DM, years	12.7 (7.7-16)
Current smoking status, n (%)	30 (29.4)
Microvascular complications	
Nephropathy, n (%)	32 (31.4)
Neuropathy, n (%)	48 (47.1)
Retinopathy, n (%)	23 (22.5)
Anti-diabetic medications	
Metformin, n (%)	90 (89.1)
Gliclazide, n (%)	12 (11.8)
Insulin, n (%)	66 (64.7)
Dapagliflozin, n (%)	73 (71.6)
Empagliflozin, n (%)	29 (28.4)
Categorical data are demonstrated with numbers and percentages (%). Normally distributed variables are presented as mean and standard deviation and non-normally distributed variables are presented as median (interquartile ranges 25-75).	

Compared to 102 patients with T2DM and 116 subjects in the control group, MPV levels were found to be significantly higher in diabetic patients (9.3 (8.2-10.3) fl vs 9 (8.3-9.5) fl, P=0.048). There was not any statistical difference between the two groups concerning age and gender factors, BMI, platelet count, and Red Cell Distribution Width (RDW) (**Table 2**).

Table 2. Comparison of patients with T2DM and control group in pretreatment period.

	Patients under SGLT2i treatment (n=102)	Controls (n=116)	P value
Age, years	55.5±9	53.2±3	0.212
Female, n (%)	52 (51)	54 (46.5)	0.193
BMI (kg/m²)	29.3±2	28.1±3	0.112
Hemoglobin, g/dL	13.9±1.42	14±1.51	0.604
WBC, 10³/μl	7900 (7000-8900)	7400 (6300-9100)	0.092
RDW, %	13.65 (13-14.59)	13.45 (13-14.5)	0.667
MPV, fL	9.3 (8.2-10.3)	9 (8.3-9.5)	0.048
Platelet, 10³/μl	252000 (209000-304000)	252000 (224000-290000)	0.888
SGLT2i : Sodium-Glucose Co-Transporter 2 inhibitors, BMI: Body Mass Index, WBC: White Blood Cell, RDW: Red Cell Distribution Width, MPV: Mean Platelet Volume. Normally distributed variables are presented as mean and standard deviation and non-normally distributed variables are presented as median (interquartile ranges 25-75).			

Akhanlı et al. Change of mean platelet volume in SGLT2 inhibitors

J Health Sci Med 2023; 6(1): 140-144

There was a decrease in the levels of fasting blood sugar in patients with T2DM when compared to their 0th and 24th weeks (224 (161-257) to 170 (123-183), p<0.001). Likewise, it was observed that fasting plasma glucose (FPG) and HbA1c levels of the patients declined (312 (252-375) to 240 (180-280), p<0.001, 9.7 (8.6-11.8) to 8.6 (7.2-9.9), p<0.001, respectively).

In contrast, a significant increase was observed in MPV and hemoglobin values, and red blood cell (RBC) and platelet counts when evaluating the 0th and the 24th weeks of the patients (9.3 (8.2-10.3) to 10.1±1.3, p<0.001, 13.9±1.42 to 14.4±1.5, p<0.001, 5±0.42 to 5.2±0.47, p<0.001, 252,000 (209,000-304,000) to 262,000 (221,000-322,000), p=0.007, respectively). Similarly, hematocrit value increased (42±3.7 to 44.2±3.8, p<0.001).

Any significant changes were not observed in white blood cell (WBC) count as well as eosinophil and monocyte values (7900 (7000-8900) to 8190±1650, p=0.599, 150 (100-242) to 145 (100-200), p=0.379, 600 (500-700) to 550 (470-670), p=0.108, respectively). However, there was a significant increase in the number of basophils (25 (0-60) to 50 (30-70), p<0.001). No significant changes were observed in creatine levels along with the number of neutrophils/lymphocytes (p=0.796, p=0.819, respectively) (**Table 3**).

No correlation was identified in patients with T2DM considering MPV and age and gender factors, diabetes duration, body mass index (BMI), fasting and postprandial blood glucose, and insulin use.

DISCUSSION

We detected in our study that a 24-week SGLT2i use increased MPV level. Moreover, it was observed that there was an increase in the values of hemoglobin and hematocrit, and RBC count. Previous studies revealed that MPV levels had decreased with a reduction in HbA1c levels in diabetic patients after anti-diabetic treatment. However, in our study, an increase in MPV level was observed since there was an improvement in HbA1c levels.

Platelets play an essential role in hemostasis and thrombosis. When activated by vascular injury, they excrete several substances, which are necessary for clotting, thrombosis, inflammation, and atherosclerosis (6). MPV is a platelet size pointer detected with ease and routinely obtained in automatic hemograms at a low cost. Larger platelets are more active owing to high prothrombic content such as thromboxane A2, thromboxane B2, platelet factor 4, serotonin, and platelet-derived growth factor (PDGF) (11). Insulin resistance and hyperglycemia are significant agents feading to increased platelet reactivity in patients with DM. Platelet hyperreactivity is a well-established contributing factor to the prothrombotic state in diabetic patients and therefore causes increased clotting, impaired fibrinolysis, and endothelial dysfunction. These hyperactive platelets have a vital role in the pathophysiology of thrombotic events causing diabetic complications (12). Osmotic swelling depend upon increased blood sugar and its metabolites is regarded as a potential mechanism when it comes to MPV (10).

Table 3. Hemogram parameters and other laboratory test results of the patients at the baseline and in the 6th month					
	Baseline	**6th month**	**AC**	**CI**	**P value**
FPG, mg/dL	224 (161-257)	170 (123-183)	-56±83.6	-74, -38.1	<0.001
PPG, mg/dL	312 (252-375)	240 (180-280)	-67±0.91	-93, -41	<0.001
HbA1c, %	9.7 (8.6-11.8)	8.6 (7.2-9.9)	-1.2 (-2, 0.2)	-1.5, -0.75	<0.001
Creatinin, mg/dL	0.82 (0.69-0.98)	0.84 (0.68-0.97)	0.01 (-0.08, 0.08)	-0.04, 0.03	0.796
Hemoglobin, g/dL	13.9±1.42	14.4±1.5	0.5±1.17	0.27, 0.73	<0.001
Hematocrit, %	42±3.7	44.2±3.8	2.3±3.55	1.6, 3	<0.001
RBC, 10^6/µl	5±0.42	5.2±0.47	0.25±0.42	0.16, 0.33	<0.001
WBC, 10^3/µl	7900 (7000-8900)	8190±1650	840±162	2300, 4000	0.599
Monocytes, 10^3/µl	600 (500-700)	550 (470-670)	-10 (-60, 100)	-20, 40	0.379
Eosinophils, 10^3/µl	150 (100-242)	145 (100-200)	-5 (-3, 70)	-10, 20	0.108
Basophils, 10^3/µl	25 (0-60)	50 (30-70)	20 (0, 50)	0.01, 100	<0.001
Neutrophils/lympocytes, %	1.75 (1.35-2.45)	1.92 (1.45-2.6)	0.05 (-0.33, 0.33)	-0.12, 0.15	0.819
MCV, fL	85.4 (81.6-88.1)	84.9 (81.7-88.3)	0.2 (-2.6, 2)	-0.7, 0.7	0.782
RDW, %	13.65 (13-14.59)	13.65 (13-14.72)	-0.05 (-0.7, 0.5)	-0.3, 0.1	0.341
MPV, fL	9.3 (8.2-10.3)	10.1±1.3	0.3 (-0.4, 1.9)	0.08, 1.45	<0.001
Platelet, 10^3/µl	252000 (209000-304000)	262000 (221000-322000)	12500 (-15000, 35700)	-1500, 17000	0.007
FPG: Fasting Plasma Glucose, PPG: Post-prandial Glucose, RBC: Red Blood Cell, WBC: White Blood Cell, MCV: Mean Corpuscular Volume, RDW: Red Cell Distribution Width, MPV: Mean Platelet Volume Normally distributed variables are presented as mean and standard deviation and non-normally distributed variables are presented as median (interquartile ranges 25-75)					

J Health Sci Med 2023; 6(1): 140-144

Akhanlı et al. Change of mean platelet volume in SGLT2 inhibitors

In literature, there are several studies analyzing platelet activity and its association with diabetes complications in diabetic patients and/or comparing it to a control group. In studies (13–15) comparing MPV values between diabetic patients and non-diabetic ones, MPV values in diabetic patients are found to be significantly higher than non-diabetic patients. Furthermore, that MPV is related to the microvascular and macrovascular complications of diabetes has been identified (9-11-12). Nevertheless, the number of studies comparing the level of platelet activity before and after treatment is limited. In a study (10) diabetic patients with poor glycemic control and with no specified agents for treatment were evaluated after 52 weeks, and those with an improved glycemic values presented a significantly decreased MPV value. Another study (16) in which no agents were specified revealed a significant decline in MPV value after a 12-week treatment in patients with T2DM whose glycemic control was poor. Similarly, MPV value decreased significantly at the end of a 24-week metformin treatment administered in 60 patients with DM diagnosed recently (17). That there was no positive correlation between anti-diabetic medication used in the patient group and MPV was proven in another study (9).

After a 12-week SGLT2i treatment, we observed an increase in MPV value. A larger study in which 6354 patients were included proved that MPV increase is associated with peripheral arterial disease (8).

SGLT2i treatment may be related to a mild increase in hematocrit when compared to placebo. Volume depletion due to diuresis and hemoconcentration are considered to be among the mechanisms, which cause such an increase (18). Additionally, that SGLT2 inhibitors increase hematocrit, thereby augmenting erythropoiesis can be suggested as another mechanism (19). It is also suggested that hematocrit elevation after initiation of SGLT2i treatment in patients with T2DM is an indicator of decreased metabolic stress in the proximal tubules or adjacent interstitium of the kidney (18). In a similar way, we detected that a 24-week SGLT2i treatment leads to an increase in the values of hemoglobin and hematocrit.

However, some of the shortcomings of this paper can be discussed. First, this study is a retrospective study. Second, oral and anti-diabetic medications along with insulins have not been classified separately. Finally, the diet and exercise status of the patients during the study is unknown.

CONCLUSION

Consequently, we revealed an increase in MPV values with SGLT2i treatment. The most interesting aspect of this study was that MPV value rised unlike other studies, although blood sugar regulation was achieved with the treatment. To comprehend how SGLT2 inhibitors lead to MPV increase and what possible impacts occur, large-scale research is required to be conducted.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Sciences Clinical Researches Ethics Committee (Date: 2021, Decision No: 115/18).

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 has no conflicts of interest to declare.

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Akhanlı et al. Change of mean platelet volume in SGLT2 inhibitors

J Health Sci Med 2023; 6(1): 140-144

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HEALTH SCIENCES MEDICINE

Nutritional indices may have prognostic value in elderly critically ill patients with sepsis

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ABSTRACT

Aim: Nutritional indicators are associated with adverse outcomes in critically ill elderly patients. In this study, we aimed to evaluate the prognostic potential of prealbumin and albumin in the prediction of mortality in elderly patients with sepsis.

Material and Method: A total of 108 patients who developed intensive care unit-acquired sepsis were divided into two groups: Survivors (n=72) and Non-survivors (n=36).

Results: Patients in the Non-survivors group were often older (68 vs 74) and presented lower prealbumin (15.1 vs 11), and higher Charlson index (4 vs 6), Sequential Organ Failure Assessment (SOFA) score (8.5 vs 10), C-reactive protein (CRP) (68.8 vs 91) and procalcitonin (PCT) (6 vs 8.4) (p< 0.05). The area under the curve of PCT was the highest at 0.74. Prealbumin presented the best sensitivity (75%) and 12 mg/dL cut-off value, while PCT had the best specificity (75%) and a cut-off value of 7 ng/mL. Although prealbumin was negatively correlated to SOFA score in a significant way (r=-0.226, p=0.019), White blood cells, CRP, and PCT were positively correlated to SOFA score (r=0.198, p=0.040; r=0.233, p=0.015; r=0.286, p=0.003, respectively). In addition, a weak negative correlation was observed between prealbumin and CRP and PCT (r=-0.203, p=0.037; r=-0.215, p=0.026, respectively). Multivariate analysis showed that a reduction in serum prealbumin levels compared to steady prealbumin greater than 4 mg/dL increased the risk of death by 85% (aHR: 1.85, 95% CI: 1.05-2.56, p=0.029).

Conclusion: Changes in serum prealbumin in the acute phase of sepsis may assist in determining the risk of mortality and in the administration of specific treatment in critically ill elderly patients.

Keywords: Elderly, inflammation, mortality, prealbumin, sepsis

INTRODUCTION

Infection and changes in the immune response in the elderly lead to a severe systemic response to infection in septic patients, severe and often irreversible damage to cells and tissues, and ultimately a life-threatening clinical picture (1). In addition, severe infections in the elderly are sometimes difficult to diagnose due to atypical clinical appearance causing adverse outcomes. Therefore, the incidence of sepsis increases in elderly patients, and advanced age is associated with an increased risk of mortality (2).

Elderly patients, who constitute the majority of critically ill patients in the intensive care unit (ICU), are prone to exposure to acutely stressful clinical situations that add to their chronic illness. Nitrogen losses increase in these patients due to hypermetabolism status, anorexia, and developing malabsorption. Prealbumin (transthyretin) levels can help determine the risk of malnutrition at an early stage and provide nutritional support (3). However, in addition to malnutrition, serum prealbumin levels decrease in conditions such as inflammatory response and ageing (4). The fact that the hepatic synthesis rate of prealbumin is higher compared to that of albumin, its half-life is shorter, and its catabolic rate is predictable suggests that it may be a more reliable indicator than albumin (5). Although nutritional indicators such as prealbumin and albumin have been shown to be associated with adverse outcomes in critically ill elderly patients (3,4,6), whether they have prognostic significance in elderly patients with ICU-acquired sepsis should be further investigated.

In our study, therefore, we aimed to evaluate the relationship of nutritional parameters with clinical outcomes and compare their predictive values with conventional inflammatory markers (White blood cells (WBC), C-reactive protein (CRP) and procalcitonin (PCT)).

MATERIAL AND METHOD

Study Design

In this cohort study, elderly (age ≥65 years) patients who developed microbiologically proven sepsis at least 48 hours after admission to the General Intensive Care Unit of Konya Numune Hospital were retrospectively analyzed. This study was carried out in accordance with the Declaration of Helsinki between August 2020 and August 2022, with the approval of the ethics committee of Necmettin Erbakan University Non-Interventional Clinical Researches Ethics Committee (Date: 18.11.2022, Decision No: 4054). Written consent was not obtained due to the retrospective design.

Inclusion and Exclusion Criteria

Patients who, according to the definition of SEPSIS-3, developed new-onset sepsis (7) during their treatment in the ICU and who received enteral, parenteral, or both nutritional support were included in the study.

Oral alimentation (as total protein and calories may not be measured properly), malnutrition, chronic inflammatory disease, corticosteroid usage, severe immunosuppression, chronic hepatic failure, estimated glomerular filtration rate (eGFR)< 15 mL/kg/m² or renal replacement therapy, missing clinical data, patients who were re-admitted to the intensive care unit and those previously included in the study were excluded from the study.

Demographic and clinical features at the onset of sepsis, comorbidities, biochemical parameters, inflammation profile, disease severity determined by Sequential Organ Failure Assessment (SOFA) score, empirical antimicrobial therapy (initiation of appropriate antimicrobial drugs, including possible pathogens at adequate doses), and microbiological results were evaluated along with the data obtained from medical records.

Study Protocol

Patients admitted to the ICU were screened for sepsis, and routine treatment, including fluid replacement, positive inotropes, antimicrobial agents, and surgery, was applied according to current guidelines to all patients who developed sepsis (8). The nutritional needs of adult critically ill patients were evaluated according to the SCCM/ASPEN guidelines (9). Nutritional parameters (prealbumin, albumin) and inflammation markers (WBC, CRP, PCT) were determined by an AU5800 automated analyzer (Beckman Coulter, California, USA). Serum prealbumin levels were reevaluated in blood samples taken 4 days later.

Sepsis developing 48 hours after ICU admission was defined as ICU-acquired sepsis. Death from any cause within 30 days of sepsis onset was considered 30-day ICU mortality.

The prognostic values of nutritional indicators in elderly sepsis patients were the primary outcome measures, and their associations with systemic inflammation were the secondary outcome measures.

Sample Size

The sample size analysis performed using the G*Power version 3.1 program was based on a similar study by Xie et al. (10). To detect a significant difference in serum prealbumin levels between Survivors and Non-survivors groups, a power analysis was accomplished with the 2-sided Independent Samples t-test using a 0.61 effect size, maximum 5% type I error and 80% power. Considering the 10% drop out rate, the sample size was found to be 72 patients in the Survivors group and 36 patients in the Non-survivors group (108 patients in total).

Statistical Analysis

SPSS software version 26 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Continuous variables were given as mean (SD) or median (IQR), while categorical variables were given as numbers and ratios. Pearson's chi-squared or Fisher's exact tests were used for categorical variables in group comparisons. The Independent Samples t-test was used for continuous variables when normal distribution was shown, and the Mann-Whitney U test was used if not. Mortality predictability of significant laboratory parameters in the univariate analysis was determined by Receiver operating characteristic (ROC) analysis and compared with the values of the area under the curve (AUC). Youden criterion was used to distinguish cut-off values. The SOFA score and the relationship between prealbumin and inflammatory biomarkers (r values) were evaluated with Spearman's correlation coefficient. The association of prealbumin changes with the risk of mortality was analyzed with Cox proportional hazard regression models adjusted for age, sex, and SOFA score. The associations were calculated as unadjusted and adjusted hazard ratios (aHR) with a 95% confidence interval (CI). $p< 0.05$ values were regarded as statistically significant.

RESULTS

A total of 150 patients with clinical signs of sepsis were registered during the study period. The microorganism responsible for the primary infection was detected in 120 (81%) of these 150 patients. Twelve patients died within 4 days of the onset of sepsis. The nutritional indicators and clinical outcomes of 108 patients with microbiologically proven ICU-acquired sepsis were analyzed.

The clinical features of septic patients in the Survivors and Non-survivors groups are shown in **Table 1**. Hypertension, observed in 47% of patients, was the most

common primary underlying disease. Patients in the non-survivors group were often older (68 vs 74), with lower prealbumin (15.1 vs 11), and higher Charlson index (4 vs 6), SOFA score (8.5 vs 10), CRP (68.8 vs 91) and PCT (6 vs 8.4) (p< 0.05). There was no significant difference in clinical outcomes and identified microorganisms between the groups of patients.

ROC analysis was performed to evaluate the predictability of mortality of prealbumin, CRP, and PCT, which are significantly correlated with mortality in sepsis patients. PCT had the highest AUC value, 0.74. Prealbumin with the best sensitivity (75%) and a cut-off value of 12 mg/dL and PCT with the best specificity (75%) and a cut-off value of 7 ng/mL are eligible for ICU mortality predictors. (**Table 2**, **Figure 1**)

The prognostic values of laboratory parameters that in single measurements did not have adequate predictability of mortality due to the relatively low (< 0.80) AUC values were analyzed in their combinations and pairwise comparisons. When these three parameters were evaluated together, although there was no improvement in specificity (70.8%), sensitivity (86.1%), and AUC (0.84) values increased.

The pairwise comparison with ROC curves of these parameters showed no significant difference between the prognostic values of all three parameters (**Table 3**). However, when the combination of the three parameters was compared pairwise with prealbumin, CRP and PCT, the prognostic value was significantly higher (p=0.001, p=0.010, p=0.042, respectively) (data not shown).

Table 1. Clinical characteristics of elderly patients with ICU acquired sepsis				
Variables	Total (n=108)	Survivors (n=72)	Non-survivors (n=36)	p value
Age, year	69 (67-76)	68 (66-74)	74 (67-81)	0.028
Gender, male, n (%)	53 (49)	34 (47)	19 (53)	0.586
Underlying condition, n (%)				
Diabetes mellitus	31 (29)	22 (31)	9 (25)	0.547
Hypertension	51 (47)	35 (49)	16 (44)	0.683
Renal disease	17 (16)	10 (14)	7 (19)	0.455
Koroner arter hastalığı	21 (19)	13 (18)	8 (22)	0.606
COPD	23 (21)	14 (19)	9 (25)	0.506
Stroke/serebral hemoraji	19 (18)	11 (15)	8 (22)	0.372
Charlson comorbidity index	4 (3-7)	4 (3-5)	6 (4-8)	<0.001
SOFA score	8.5 (6-10)	8.5 (7-10)	10 (8-11)	<0.001
Mechanical ventilation, n (%)	49 (45)	28(39)	21 (58)	0.056
Vasopressor use, n (%)	57 (53)	34 (47)	23 (64)	0.172
Identified microorganisms, n (%)				
Gram negative bacilli	69 (64)	43 (60)	26 (72)	0.202
Gram positive cocci	55 (51)	39 (54)	16 (44)	0.341
Fungi	11 (10)	5 (7)	6 (17)	0.175
Laboratory findings				
WBC, mm3	12.8±5.4	12.1±5.1	14.2±5.7	0.053
Prealbumin, mg/dL	13.7±5.8	15.1±5.8	11.0±4.8	<0.001
Albumin, g/dL	3.0±0.6	3.1±0.6	2.8±0.5	0.061
C-reactive protein, mg/dL	78.5 (50-99)	68.8 (45-94)	91 (73-114)	0.001
Procalcitonin, ng/mL	7 (4-10)	6 (3-9)	8.4 (6-14)	<0.001
Lactate, mmol/L	2.7±0.9	2.6±0.9	2.9±1.0	0.133
Clinical outcomes				
Appropriate initial treatment, n (%)	66 (61)	48 (67)	18 (50)	0.094
ICU length of stay (days)	14.5±7.4	13.7±7.2	16.2±7.7	0.107
Duration of ICU stay prior to sepsis (days)	7.2±3.5	6.4±3.1	7.8±4.1	0.202
Data shown as mean ± standard deviation, median (interquartile ranges) or n (%). COPD: chronic obstructive pulmonary disease; SOFA: sequential organ failure assessment; WBC: White blood cells; ICU: intensive care unit.				

Table 2. Performance of significant parameters in predicting ICU mortality						
Variable	Cut-off	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PPV	NPV
PAB	12 mg/dL	0.72 (0.62-0.80)	75.0	65.3	51.9	83.9
CRP	84 mg/dL	0.70 (0.61-0.79)	66.7	69.4	52.2	80.6
PCT	7 ng/mL	0.74 (0.65-0.82)	66.6	75.0	57.1	81.8
PAB+CRP+PCT		0.84 (0.76-0.90)	86.1	70.8	59.6	91.1
PAB: prealbumin; CRP: C-reactive protein; PCT: procalcitonin; ICU: intensive care unit; AUC: area under the receiver-operating-characteristic curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.						
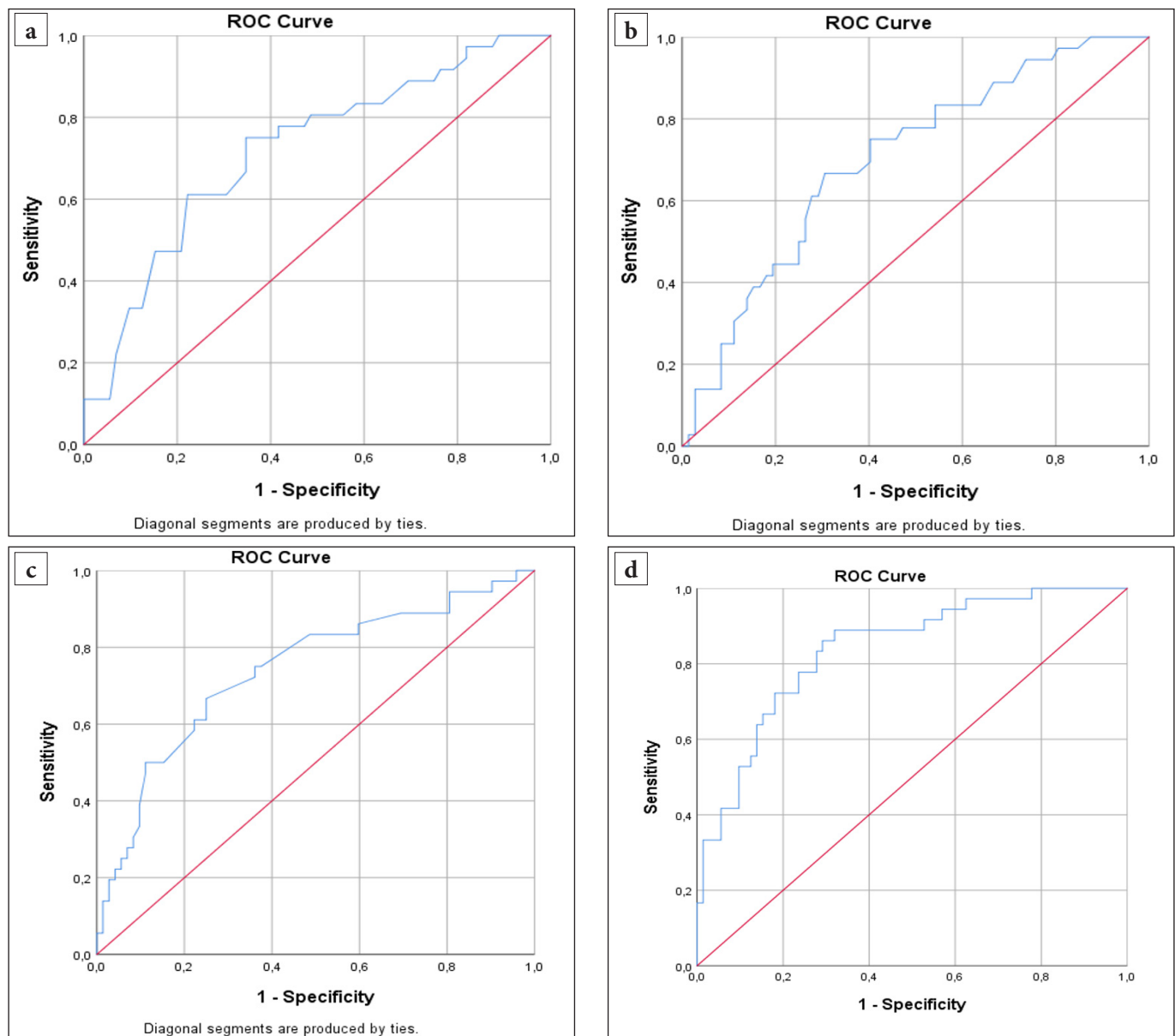
Figure 1. Receiver operating characteristic curves for a) PAB (AUC: 0.72, 95% CI, 0.62-0.80), b) CRP (AUC: 0.70, 95% CI, 0.61-0.79), c) PCT (AUC: 0.74, 95% CI, 0.65-0.82) and d) the combination of PAB, CRP and PCT (AUC: 0.84, 95% CI, 0.76-0.90) in septic patients to predict mortality.

Table 3. Comparison of ROC curves			
	PAB vs. CRP	**PAB vs. PCT**	**CRP vs. PCT**
Difference between areas	0.019	0.022	0.041
Standard error	0.079	0.073	0.077
95% confidence interval	-0.136 to 0.173	-0.121 to 0.165	-0.111 to 0.192
Z statistic	0.235	0.301	0.524
Significance level	p = 0.814	p = 0.764	p = 0.600
PAB: prealbumin; CRP: C-reactive protein; PCT: procalcitonin; ROC: Receiver operating characteristic analysis			

The relationship in sepsis patients between inflammatory markers and the SOFA score, which is an indicator of disease severity, was evaluated by bivariate analysis. Prealbumin was significantly negatively correlated with SOFA score (r=-0.226, p=0.019), while WBC, CRP, and PCT were positively correlated with SOFA score (r=0.198, p=0.040; r=0.233, p=0.015; r=0.286, p=0.003, respectively). Compared to other biomarkers, the correlation between PCT and SOFA score was more clear (**Table 4**). In addition, a weak negative correlation was observed between prealbumin and CRP and PCT (r=-0.203, p=0.037; r=-0.215, p=0.026, respectively) (data not shown).

Table 4. The relationship between SOFA scores and inflammatory biomarkers							
		WBC	**Prealbumin**	**Albumin**	**CRP**	**PCT**	**Lactate**
SOFA	rs*	0.198	-0.226	-0.145	0.233	0.286	0.121
	p	0.040	0.019	0.134	0.015	0.003	0.213
*Spearman correlation. SOFA: sequential organ failure assessment; WBC: White blood cells; CRP: C-reactive protein, PCT: procalcitonin							

Table 5. Risk relationship between categories of change in prealbumin and mortality

Categories (mg/dL)	Unadjusted		Model 1*		Model 2†	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
< -4 (n=25)	1.89 (1.09-2.58)	0.026	1.76 (1.03-2.51)	0.044	1.85 (1.05-2.56)	0.029
-4 to < 4 (n=55)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
≥ 4 (n=28)	0.70 (0.37-1.21)	0.244	0.65 (0.32-1.11)	0.512	0.67 (0.30-1.19)	0.282
*Model 1 adjusted for age and gender. †Model 2 adjusted for age, gender and Sequential Organ Failure Assessment score. HR: hazard ratio; CI: confidence interval						

Prealbumin changes associated with mortality were calculated by Cox hazard regression. The multivariate analysis, adjusted for age, sex, and SOFA score, showed that, compared to steady prealbumin levels, a decrease in serum prealbumin above 4 mg/dL increased the risk of death by 85%, (aHR: 1.85, 95% CI: 1.05-2.56, p=0.029) (**Table 5**).

DISCUSSION

In this study, the observed reductions in serum prealbumin levels in critically ill patients with sepsis were associated with adverse outcomes. In elderly patients with ICU-acquired sepsis, prealbumin values demonstrated similar prognostic significance to conventional inflammatory markers such as CRP and PCT.

Although the nutritional parameters prealbumin and albumin were previously defined as indicators of nutritional intake, subsequent studies in acutely stressed patient groups have shown that they are also affected by conditions such as inflammatory response, surgery, trauma, and ageing (4,11,12). The decrease in nutritional markers in acute stress situations can be explained by several mechanisms independent of nutrition. First, cytokines such as tumour necrosis factor (TNF), interleukin-1, and interleukin-6 secreted in case of inflammation (e.g., infectious disease) increase the synthesis of acute phase proteins and decrease hepatic synthesis of other proteins, including prealbumin and albumin (12,13). Second, in the other cytokine-related mechanism, TNF and secondary eicosanoid metabolites cause capillary leakage, causing hepatic proteins to pass into the extravascular compartment (14). Third, by playing a nonspecific role in host defence, it ensures the clearance of toxic metabolites induced by infection and is consumed during this process (15). In our study, the higher levels of CRP and PCT, which are established inflammatory markers in the Non-survivors group, and the negative correlation of prealbumin with these markers were consistent with the aforementioned mechanisms. In conclusion, inflammation in the early stage of sepsis in critically ill patients may be more effective on nutritional markers, which are negative acute phase reactants, than nutrient intake or replacement therapy (16,17).

Compared to albumin, with a short metabolic half-life (2 days), stronger correlation with the inflammatory response, and, due to its higher affinity for ligands, increased detoxification capacity, prealbumin is considered a better indicator than serum albumin in assessing malnutrition and predicting mortality in critically ill patients. (5,18-20). Our multivariate analysis, including the SOFA score, which is positively associated with the mortality of septic patients, sheds light on the interaction of disease severity with prealbumin levels in the elderly population (21). In our study, a more significant decrease in serum prealbumin was noticed in patients who died, and this relationship remained statistically significant even when adjusted for disease severity. In addition, the correlation analysis observed a weak negative correlation between prealbumin levels and disease severity. This suggests that, in addition to being an indicator of disease severity, prealbumin levels in critically ill patients may also be significantly affected by other factors in their prognostic potential.

Malnutrition is common in hospitalized elderly patients. Kubrak et al. (22) demonstrated in a study on acute care patients that the incidence of malnutrition in hospitalized elderly patients was between 42% and 91%. The increased risk of malnutrition and inflammation in elderly patients with decreased prealbumin levels adversely affects the prognosis (4,6). In our study, it can be thought that the potential effects of malnutrition on immune dysregulation were limited by providing the necessary nutritional support during the ICU stay (23). Therefore, the older age and increased comorbidity index of the patients in the Non-survivor group may contribute to adverse outcomes by leading to deterioration in immune functions related to ageing, underlying disease and frailty.

Li et al. (6) found that serum prealbumin was associated with mortality in a study conducted on elderly patients with severe pneumonia. Qin et al. (24) indicated that CRP and PCT have prognostic significance in elderly patients with sepsis caused by pulmonary infection. In our study, serum PCT presented the highest AUC (0.74) and specificity (75%), while serum prealbumin had the highest sensitivity (75%). The AUCs of all three biomarkers had a fair discriminative ability (< 0.80) and similar prognostic value in differentiating Survivors and Non-survivors groups. However, the combination of prealbumin, CRP, and PCT, in which we evaluated the prognosis of the septic patients, reached a significantly higher AUC (0.84) than the individual measurements.

These findings suggest that prognostic predictability in elderly patients with ICU-acquired sepsis may be increased by multimarker evaluation of the parameters.

The present study showed a significant negative correlation between serum prealbumin and the established inflammatory markers CRP and PCT. In addition, the correlation of prealbumin with PCT, which is a superior biomarker compared to CRP in the diagnosis of sepsis, was more evident (25). Therefore, prealbumin, in addition to a nutritional protein, may be an inflammatory marker for infections in elderly patients. These results showing the potential effects of the acute phase response in the early phase of sepsis on nutritional markers are consistent with previous studies.

In addition to baseline measurements, changes in prealbumin levels are also used in the assessment of mortality risk. Rambod et al. (26) studied the relationship between prealbumin levels and mortality in outpatients receiving hemodialysis treatment. In patients with initial prealbumin levels between 20-40 mg/dL, decreases in prealbumin levels above 10 mg/dL over a 6-month period were independently associated with mortality. Nichols et al. (27) evaluated the relationship between serum prealbumin and clinical outcomes in critically ill patients. In the study in which the median baseline prealbumin levels were 11.6 mg/dL, a decrease of 1.3 mg/dL in prealbumin levels evaluated with measurements made with 3-day intervals was associated with mortality. In our study, the mean prealbumin level at the onset of sepsis was 13.7 mg/dL, and reductions above 4 mg/dL measured at 4-day intervals increased the mortality risk by 85%, which was consistent with the previous study. However, the smaller absolute decrease due to lower baseline prealbumin compared to the study of Rambod can be explained by the higher severity of the disease in critically ill patients and the significantly shorter time between the measurements of prealbumin.

This study had several limitations. First, due to the single-centre nature of our study, our results cannot be generalized to other ICUs. Second, advanced age, comorbidities, and increased disease severity in sepsis patients in the ICU may cause differences in prognosis-related cut-off values of inflammatory parameters. Third, the retrospective design of the study may affect the serum levels of prealbumin and albumin, resulting in the inability to evaluate the nutritional status of patients when enrolled in the study due to the lack of data such as total protein or body mass index. Fourth, because of examining the prognosis of patients in the ICU only, our data do not include information on the association of nutritional indicators with long-term clinical outcomes.

CONCLUSION

In elderly patients, prealbumin values at the onset of sepsis have a similar and moderate prognostic ability as CRP and PCT. Prealbumin changes may help identify the increased risk of mortality in elderly patients with nosocomial infections and receive specific treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Necmettin Erbakan University Non-Interventional Clinical Researches Ethics Committee (Date: 18.11.2022, Decision No: 4054).

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 has no conflicts of interest to declare.

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Medicolegal evaluation of geriatric deaths in Bursa, Türkiye

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ABSTRACT

Aim: Differentiation of natural and forced deaths observed in this age group is very important in the forensic medicine practice. According to the address based population registration system, population rate of 65 years and older has been 9.5% of the total population in 2020. In the present study, we aimed to medicolegal evaluation of demographic data and autopsy findings of judicial geriatric deaths in Bursa and around.

Material and Method: Autopsy reports of the cases who have been taken to Morgue Specialization Office of Council of Forensic Medicine Institution and at 65 years and over, crime scene and deceased examination reports and data in the prosecution documents were assessed retrospectively between 2003-2008 in our study.

Results: During 6-year period, totally 5155 autopsies have been investigated in Morg Specialization Office of Bursa Council of Forensic Medicine Institution. Among such cases, 870 (16.8%) were 65 years old and older. Cases who were 65 years old and older included 640 (73.6%) were male, 230 (26.4%) were female. In terms of death origin, 434 (49.9%) were natural deaths, 267 (30.7%) were accidents, 102 (11.7%) were suicides and 52 (6%) were homicides. Origin of death of the remaining 15 (1.7%) could not be detected. According to the information obtained from the statements of the witnesses in crime scene investigation and dead body examination reports, a previous disease existed in 336 (38.6%) cases before death. Diseases which have been existing before death includes cardiac diseases, hypertension, central nervous system pathologies, diabetes mellitus, respiratory system pathologies, undefined psychiatric disorders, gastrointestinal system pathologies and malignancies, respectively to frequency.

Conclusion: In line with prolongation of life expectancy and increase in the geriatric population, it is observed according to our outcomes that an increase is detected in judicial geriatric death cases due to neuropsychiatric disease, leading a solitary life and psychomotor imbalance.

Keywords: Forensic autopsy, death, elderly.

INTRODUCTION

According to World Health Organization, 65 years is accepted as a limit for senility (1). Such increase detected in the geriatric population have brought different problems to the agenda. According to the address based population registration system, population rate of 65 years and older has been 9.5% of the total population in 2020 (2). In line with the increase in the geriatric population, multiple disease rates, multiple drug use, abuse and negligence become important. Especially, significant increase in frequency of patients with dementia bring many medical, social and judicial problems along. In conjunction with increased geriatric population, an increase in judicial

deaths has also been observed in the group who were 65 years old and older. In a study presented by Collins et al. (3) and in the other study presented by Collins (4), most of the geriatric deaths have appeared due to natural causes whereas an increase in unnatural deaths have also been reported.

Except for cases of origin of death with an accident, suicide or homicide, cases with a recent history of trauma before death, injuries detected in external examination, contradictions and inconsistencies in the history, deaths in detention and prison, cases with accusations and allegations regarding the cause of death,

J Health Sci Med 2023; 6(1): 152-157

Çetin et al. Medicolegal evaluation of geriatric deaths

cases with unknown identy and cases with suspicion in the determination of the cause of death are also defined as 'forensic cases'. However, it is also considered as a 'forensic case' in case of suspicion or claim of neglect and abuse, especially in the newborn, childhood and elderly deaths.

Differentiation of natural and forced deaths observed, detection of time of deaths and definite causes of deaths in this age group are very important in the forensic medicine practice. Determination of cause and origins of deaths contribute to exclude the factors causing to death or determine the possible preventions for the cause. Considering the fact that there was not any study conducted about geriatric deaths in Bursa and around before, to assess the demographic data and autopsy findings of judicial geriatric death cases in Bursa and around medico-legally.

MATERIAL AND METHOD

After obtaining ethics committee approval, parameters such as age, gender, date of death, cause and origin of death, event that has caused death, whether the deceased has drunk alcohol,taken hypnotic-drug and other toxicological analysis results, whether the deceased was unidentified person, place where the deceased was found dead, whether the deceased has been treated, presence of a disease before death, drugs used for such disease, people or partner who live with the deceased on geriatric cases who were 65 years old and older and taken to Morgue Specialization Office of Bursa Council of Forensic Medicine Institution between 2003-2008 were assessed retrospectively from electronic medical records and patient files (Date: 13.07.2010, Decision No: B.03.1.ATK.0.01.00.08/466). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analysis: Statistical analysis were performed through SPSS for Windows ver.15.0 package program. Date were presented as mean ± Standard deviation or median and interval. Distribution characteristics of dependent variables were assessed via Kolmogorov-Smirnov and Shapiro-Wilk tests. Change of continuous variables according to the case groups were assessed by Student's T test; and comparison between categorical variables were assessed by Ki-square and Fisher's Exact tests. Continuous variables between the groups were assessed by one-way analysis of variance (ANOVA) and Student's T test. Statistically significance was accepted as $p<0.05$.

RESULTS

During the 5 year-period, totally 5155 autopsies have been performed in Morgue Department of Bursa Council of Forensic Medicine Institution. Among the cases, 870 (16.9%) have been 65 years old and older and they were accepted as geriatric death. Male cases were 640 (73.6%), female cases were 230 (26.4%) and male – female ratio is 2.8:1. While age average of all cases was 73.45±6.26, age distribution varied between 65 and 93 in male patients and between 65 and 101 in female patients (**Table 1**). Average age of the male patients were detected 72.77±5.82 whereas it was 75.33±7.03 in female patients. Higher age average values in females than males was found significant ($p<0.001$, t=5.411). There was not any significant difference between genders of our cases in terms of years ($p=0.163$, χ2=7,886) and months ($p=0.288$, χ2=13,081). A significant difference was not detected in average of age in terms of months ($p=0.285$) and years ($p=0.178$).

Deaths of 870 geriatric individuals which was evaluated in terms of origin of death were detected as natural death by 49.9% (n=434), accident by 30.7% (n=267), suicide by 11.7% (n=102), homicide by 6% (n=52). Origin of death of the remaining 15 cases (1.7%) could not be determined according to the current data referred by prosecution offices.

Among the causes for natural deaths, cardiovascular system pathologies were in the first place (n 290, 66.8%). Respiratory system pathologies follow cardiovascular system diseases by 12.4% (n=54). Other causes for natural deaths included central nervous system pathologies (n=28, 6.5%), gastrointestinal system pathologies (n=14, 3.2%) and urinary system pathologies (n=6, 1.4%) according to the frequency. For remaining 42 (9.7%) cases, after exclusion of all external causes that might cause death, cause of deaths for such cases was considered natural. Detection of cardiovascular system pathologies which was the most common among natural causes of death frequent in males was found significant ($p=0.002$, χ2=10.030).

Table 1. Age distribution according to the gender						
	Age groups					**Total n (%)**
	65-69 n (%)	**70-74 n (%)**	**75-79 n (%)**	**80-84 n (%)**	**85≤ n (%)**	
Gender						
Female	57 (20.4%)	51 (21.2%)	56 (28.6%)	45 (41,3%)	21 (46.7%)	230 (26.4%)
Male	222 (79.6%)	190 (78.8%)	140 (71.4%)	64 (59,7%)	24 (53.3%)	640 (73.6%)
Total	279 (100%)	241 (100%)	196 (100%)	109 (100%)	45 (100%)	870 (100%)

Çetin et al. Medicolegal evaluation of geriatric deaths

J Health Sci Med 2023; 6(1): 152-157

According to the information obtained from the statements of the witnesses in crime scene investigation and dead body examination reports, a previous disease existed in 336 (38.6%) cases before death. Diseases which have been existing before death includes cardiac diseases, hypertension, central nervous system pathologies, diabetes mellitus, respiratory system pathologies, undefined psychiatric disorders, gastrointestinal system pathologies and malignancies, respectively to frequency. It was reported that 72 (8.3%) cases had not any disease known whereas there was not any information on 462 (53.1%) cases if they have any disorder previously. It was recorded among the cases who have died because of natural causes that 41.7% (n=181) of them have a previous disease, 41.7% (n=181) of them were unclear for any previous disease and 16.6% (n=72) of them had not any disease. Cardiovascular system pathologies which is the first in natural causes have been detected in 27.4% of the cases only whereas only 27.3% (n=79) of these cases have been treated because of their diseases. However, no data existed on 17.2% (n=34) of the cases who had a previous disease and have died due to natural causes if they have been treated for their current disease in crime scene investigation and deceased examination reports.

Blunt and penetrating traumas are the most common cause of forced deaths by 43.9% (n=187). Asphyxia cases follow this by 30.8% (n=131). Other causes of forced death include CO poisoning by 14.3 (n=61), burn by 5.6% (n=24), other intoxications (organic phosphate, drugs etc.) by 3.1% (n=13), hypothermia by 1.6% (n=7) and electric shock and lightning strike by 0.7%. Detection of deaths occurred as a result of CO poisoning and burn as

more common in women was found significant (p<0.001, $\chi2$==23.972, p<0.001, $\chi2$=14.909, respectively).

For blunt and penetrating traumas which was the first cause of forced deaths included traffic accidents by 35.2% (n=66), falling by 27.8% (n=52), firearm injuries by 13.4% (n=25), sharp object injuries by 9.6% (n=18), crushing object injuries by 4.8% (n=9), pounding by 3.2% /n=6) and blunt trauma by 5.9% (n=11).

It was recorded for asphyxia cases which was the cause following blunt and penetrating traumas that 55% (n=72) have died because of hanging, 38.9% (n=51) have died due to drowning in water, 4.6% (n=6) have died because of smothering by hands or a tie and 1.5% (n=2) have died because of foreign body aspiration. It was detected in all cases who have died because of asphyxia as a result of hanging were suicide and 94.15 of the cases who have drowned in the water were accident (p<0.001, $\chi2$==582.092, p<0.001, $\chi2$=102.465, respectively). Detailed information about the cause for death and origin of death were showed in **Table 2**.

It was recorded that 427 (49.1%) cases have died at home or were found death. This is followed by being found death in open field with 156 (17.9%) cases, death during hospitalization by 107 (12.3%) cases, being found death in water (lake, river, sea etc.) by 52 (6%) cases and being found death on or near a road by 40 cases. Among the remaining cases, 30 (3.4%) cases have died in social spaces (thermal spring, hotel, café etc.), 27 (3.1%) have died in a vehicle, 18 (2.1%) cases have dies in the workplace and 12 (1.4%) cases have died in the senior house or jail. There was not any data detected about the place where remaining 1 (0.1%) case have been found death.

Table 2. Origins and manners of death						
Manner of death	**Origin of death**					**Total**
	Natural	**Accident**	**Suicide**	**Homicide**	**Undetermined**	
Natural causes	434	-	-	-	-	434
Traffic accident	-	66	-	-	-	66
Falling from height	-	45	5	1	1	52
CO poisoning	-	60	-	-	-	60
Burning	-	23	1	1	-	25
Drowning	-	48	3	-	-	51
Hanging	-	-	71	-	-	71
Lightning attack, electrocution	-	4	-	-	-	4
Freezing, hypothermia	-	7	-	-	-	7
Drug intoxication	-	5	8	-	-	13
Firearm injury	-	-	14	11	-	25
Sharp object injury	-	-	-	18	-	18
Strangulation	-	-	-	6	-	6
Foreign body aspiration	-	2	-	-	-	2
Pounding	-	-	-	9	-	9
Crusher object injury	-	-	-	6	-	6
Other blunt traumas	-	7	-	-	6	13
Unknown	-	-	-	-	8	8
Total	434	267	102	52	15	870

J Health Sci Med 2023; 6(1): 152-157

Çetin et al. Medicolegal evaluation of geriatric deaths

It was detected that 217 (24.9%) cases have lived alone, 288 (33.1%) have lived with their families (spouse and/or children), 11 (1.3%) cases have lived with their friends and 11 (1.3%) have stayed in the jail or senior house during the death. It was detected that 3 (0.3%) cases have lived with their caregiver or helper whereas there was not any information about accompany of 340 (39.1%) cases. It was learned that 174 (20%) cases were married and lived with their spouses, 157 (18%) cases were widow, divorced or single and 8 (0.9%) cases have died in the same incident with their spouses. There is not any data about marital status of 531 (61%) cases.

While putrefaction findings in different stages were detected in 164 (18.9%) of the cases, it was detected that statistically significant majority of these cases have lived alone (n=87, 53%) (p<0.001, χ2=85.277). Furthermore, it was detected that statistically significant majority of putrefacted cases have been found death at home (n=104, 63.4%) (p<0.001, χ2=16.615). Besides, 52 (6%) of 871 autopsies were assessed as negative autopsy and deterioration in different stages were observed in 49 (94.2%) of them (p<0.001, χ2=205.435).

Ethanol in different levels were detected in 58 (6.7%) of 870 cases. The highest blood level of ethanol detected was 320 mg/dl and data about blood ethanol level was presented in **Table 3**. Also, carboxyhemoglobin was detected in 65 (7.5%) cases including the highest level in 3.7% of the cases and the lowest in 81% of the cases (**Table 3**). Methanol was detected in 2 cases whereas formaldehyde was detected in 1 case and insecticides were detected in gastric content of 6 cases. Metabolites of benzodiazepine, tricyclic antidepressant and barbiturate group drugs were detected in 19 cases; opiate metabolites were detected in 5 cases; tetrahydrocannabinol (TCH), active agent of marijuana and amphetamine was detected in urine analysis of 1 case.

Table 3. Ethanol and carboxyhemoglobin levels detected in blood samples			
Ethanol (mg/dl)	n (%)	COHb* (%)	n (%)
0-50	15(26.3%)	1-10	1 (1.5%)
51-100	23 (40.3%)	11-30	12 (18.5%)
101-150	12 (21.1%)	31-50	29 (44.6%)
151≤	7 (12.3%)	50≤	23 (35.4%)
Total	57(100%)	Total	65 (100%)
*COHb: Carboxyhemoglobin.			

DISCUSSION

Majority of 870 geriatric death cases in our study consisted of males. The most common origins of death were natural causes. In association with medical histories and social life styles of the cases, crime scene investigation and dead body examination reports including first assessment data

were quite insufficient. However, being found death at home and in open field because of neuropsychiatric problems such as dementia and Alzheimer's developed in line with loneliness and aging have significantly contributed to judicial situation of death case. On the other hand, it was detected that violence and abuse was substantially frequent in the geriatric population which becomes an easy target as well as emotional and physical incapacity.

According to Hilal et al. (5), the average expected life period has prolonged and a dramatic increase was observed in the geriatric population worldwide. Lachs et al. (6) and Kossberg et al. (7) reported that as a result of decreased fertility rates, increased socioeconomic level and prolonged life period, ratio of the geriatric population to the total population was reported as 11.4% in developed and as 3.9% in developing countries. However, different problems have appeared in this age group in line with developments. Changes in the family and social life styles and technological developments gradually doom geriatric population to loneliness. In parallel with this, Ince et al. (8) indicated that being found death at home and traffic accidents are the most common causes of death for geriatric population rebounded to the courts in the world as well as in our country.

It was detected in the study carried out in Istanbul by Ince et al. (8) that 7.8% of the autopsies evaluated were geriatric deaths, this rate was reported as 8.1% in the study conducted in Adana by Hilal et al. (5) whereas 12.4% in the study of Canturk et al. (9) in Ankara. Also Turkoglu et al. (10) reported this rate as 23.9%. In our study, 16.8% of 5155 judicial death cases consists of deaths over 65 years and over. This high rate detected may be explained by higher geriatric population rate to total population in the cities which are affiliated with Bursa Council of Forensic Medicine Institution. This rate was determined as 5.1% in Istanbul and affiliated cities, 6.3% in Ankara and affiliated cities and as 3.9% in Adana and affiliated cities. According to Address Based Population Registration System, ratio of the geriatric population to total population in specified regions was obtained through 2000 data (2). It was reported in a study carried out in USA by Shokrani et al. (11) that 772 medical autopsies have been performed and 23% of these autopsies consisted of cases who are 70 years old and over. Cause for high rate in such study is a hospital experience including medical autopsies.

In our study, results obtained on gender, age range and average were consistent with other studies conducted in Türkiye. Differently, it was reported in the study performed by Shokrani et al. (11) that 45% and 55% of 180 medical autopsies were male and female, respectively. In the study carried out in Istanbul by Uzun et al. (12), it

Çetin et al. Medicolegal evaluation of geriatric deaths

J Health Sci Med 2023; 6(1): 152-157

was specified that 69.2% of 77 suicide cases who were 60 years old and older were male and 30.8% of these cases were female. In a different study conducted in Austria by Berzlanovich at al. (13), 70.3% of 1886 cases who were 85 years old and older were female. High female rates obtained in the study may be explained due to the fact that average life expectancy is more in females and includes the age group at 85 years and over. On the other hand, higher male rates in judicial autopsies supports the idea that man is still dominant in the social life in the world and in our society in each period of life.

It was reported in two different studies carried out in Adana by Hilal et al. (5) and in Istanbul by Ince et al. (8) that natural causes were the first among causes of geriatric deaths whereas accidents were the second. In an other study presented by Akar et al. (14) 48.5% of presented elderly deaths were derived from unnatural causes, followed by natural deaths as 42.4% of cases. Also, in a study conducted in Ankara by Canturk et al. (9), it was reported on traumatic death cases that 21.6% of the cases originated from suicide, 18.1% of them were homicide and 60.2% were accident. In line with this, in our study, the first cause was detected as deaths due to natural causes and this was followed by accidents. In a large study conducted by Collins et al. (3) including 1985 and 2004, 70.5% of the death cases were natural, 16% were accident, 6.5% were suicide and 6% were homicide. In our study, a similar grading was detected as natural causes by 49.9%, accident by 30.7%, suicide by 11.7% and homicide by 6%. Since natural death causes were the first in judicial autopsy studies and information related with medical history of the deceased was insufficient in crime scene investigation and dead body examination reports, the first evaluation performed by physicians or prosecutors in geriatric deaths is especially important for deaths to have judicial dimension and autopsy decision ratios. From another point of view, this first evaluation should be performed carefully so that a forced death should not be missed in geriatric death cases.

According to the statistical data obtained in Portugal in 2006 (15), 62.8% of the geriatric population have lived with their spouses (only spouse or spouse and children), 20.7% have lived alone, 16.5% have lived with their children without their spouses. Timur et al. (16) reported that 82.95% of the decedents, had been living with family and 13.48% of the decedents lived alone. It was detected in our study that 24.9% of the cases (30% were female and 70% were male) have lived alone, 34.7% have lived with their spouses and/or children and 1.3% have stayed in senior house or jail. There was not any information about person who live together with the remaining 39.1% of the geriatric death cases. We believe that the

difference between the values depends on including the geriatric population in the study conducted in Portugal and assessment of judicial cases in our study only and lack of information on 39.1% of the cases. However, living alone seems to be effective to provide death a judicial situation. It was reported in a study conducted in northern Portugal by Coelho et al. (17) that unlike the accepted fact in geriatric population, majority (45%) of the victims were married.

Ethanol was detected in 6.7% of our cases as a result of chemical analysis of blood samples collected from the deceased during autopsy. Similar with our study, ethanole was detected in 8% of the cases in the study conducted in Istanbul by Ince et al. (8) and 6.4% of the cases in the study carried out in Ankara by Canturk et al. (9). In the study carried out on geriatric homicides in USA by Collins et al. (3), it was reported that ethanol was detected in the blood in 17% of the cases as well as in 49% of the cases in the study reported by Coelho et al. (17) which included geriatric homicide cases in Portugal. Ethanol was detected in 3.8% of the homicide cases in our study. According to these data, we see that alcohol intake rate of geriatric population in our country is less than other communities. However, deterioration findings were detected in 58.6% of our cases whom alcohol was detected in blood samples. Bacterial activity which is the most important component of deterioration process as well as endogenous alcohol production is in question. Therefore, it should not be concluded that exogenous alcohol might not have been taken in each case whom alcohol was detected in onset of deterioration. On the other hand, in the studies conducted in such countries, evaluation of homicide cases only and high rates due to this evaluation may be one of the significant factors for selection of victims whether they were intoxicated by the attacker. Because, reduction in logical thinking and defense capabilities due to the alcohol makes the attacker's violence easier.

CONCLUSION

When studies conducted in our country and in the world are reviewed, place of the geriatric population during the daily life and against developments and reflection of these facts to the judicial death reports base on similar reasons. These causes include absence of elder people in the family concept defined by the modern life style, condemning geriatric population to live alone due to this definition, poor social relationships and increased neuropsychiatric pathologies. Consequently, it should be emphasized that crime scene investigation and deceased examination reports are insufficient in terms of content and this makes to interpret the findings obtained in the studies difficult.

J Health Sci Med 2023; 6(1): 152-157

Çetin et al. Medicolegal evaluation of geriatric deaths

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ministry of Justice Forensic Medicine Institute Education and Scientific Research Commission (Date: 13.07.2010, Decision No: B.03.1.ATK.0.01.00.08/466).

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

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Author Contributions: The author declares that he has participated in the design, execution, and analysis of the paper, and he has approved the final version.

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Association between atherogenic index of plasma and in-hospital mortality in patients with STEMI undergoing primary percutaneous coronary intervention

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ABSTRACT

Aim: Dyslipidemia is an established risk factor for cardiovascular disease. Increased triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) levels, and decreased high lipoprotein cholesterol (HDL-C) levels were associated with increased cardiovascular risk. Recently, comprehensive lipid profile indices derived from these conventional parameters have attracted to attention. Atherogenic index of plasma (AIP) is one of the indices calculated as the logarithm of TG/HDL-C levels and it is accepted as an alternative and simple marker of plasma atherogenicity. Although various studies demonstrated that their relationship with these lipid indices and clinical outcomes in patients with acute coronary syndrome, this situation is not yet clear in acute ST-elevation myocardial infarction (STEMI) patients undergoing primer percutaneous coronary intervention (pPCI). In this study, we aimed to investigate the relationship of AIP with early mortality in STEMI patients undergoing pPCI.

Material and Method: This is a retrospective, single center, hospital-based study carried out between January 2019 and April 2021. A total of 873 consecutive STEMI patients (705 men; median age 59 years), whose undergoing pPCI, were enrolled. The patients were divided into two groups according to in-hospital mortality status namely survivors vs non-survivors. Conventional lipid values were measured and non-traditional lipid indexes including non-HDL-C [Total cholesterol minus HDL-C], Total cholesterol/HDL-C, LDL-C/HDL-C, atherogenic index (AI) [non-HDL-C/HDL-C], lipoprotein combine index (LCI) [Total cholesterol*TG*LDL-C/HDL-C] and atherogenic index of plasma (AIP) [Log(TG/HDL-C)] were calculated. Angiographic images of the patients were evaluated through the hospital automation system.

Results: AIP was significantly higher in non-survivors compared to survivor group (0.59, 0.47, p=0.006, respectively). AI, non-HDL-C, Total cholesterol/HDL-C ratio, LDL-C/HDL-C ratio and LCI measurements were similar between two groups. The cut-off value of the AIP (0.50) was associated with 70% sensitivity and 52% specificity for predicts in-hospital mortality. Multivariate logistic regression model indicated AIP (OR: 3.77, 95% CI: 1.34–10.6, p < 0.012) as independent predictor of in-hospital mortality in STEMI patients undergoing pPCI.

Conclusion: AIP predicts in-hospital mortality in patients with STEMI undergoing pPCI. AIP, which can be calculated easily by complete blood can be beneficial in evaluating the prognosis of these patients.

Keywords: Atherogenic index of plasma, acute myocardial infarction, dyslipidemia

INTRODUCTION

Cardiovascular diseases are among the most common causes of death worldwide and are responsible for 1/3 of all deaths in individuals over the age of 35 (1). Cardiovascular diseases refer to different clinical conditions originating from the cardiovascular system. The incidence and prevalence of atherosclerotic coronary artery disease in this group has increased in recent years (2). Although there are advances in primary and secondary prevention, mortality and morbidity still remain high (3). Atherosclerotic cardiovascular disease, a common condition involving plaque formation on arterial walls, is directly related to high levels of low-density lipoprotein cholesterol (LDL-C) (2-4). Atherosclerosis, a lipid-induced chronic inflammatory disease, is an important mechanism in the pathogenesis of coronary artery disease. This phenomenon facilitates the formation of clinical scenarios such as plaque rupture and erosion occurring in the continuation of intravascular plaque formation. As a result of this pathophysiological change in the coronary arteries, coronary occlusion, which prevents the blood supply to the myocardium, develops and acute coronary syndrome (ACS) clinic emerges (5). ACS may present with different clinical presentations. Situations such as ST elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), unstable angina and sudden cardiac death can be seen. While ACS cause death, they also carry a high risk for recurrence in surviving patients. Although improvements have been

J Health Sci Med 2023; 6(1): 158-164

Kasapkara et al. Atherojenic index of plasma and coronary artery disease

achieved in the treatment of ACS over time, factors such as diabetes, hypertension, hyperlipidemia, obesity, sedentary life and stress, which are risk factors for cardiovascular diseases, have important effects on prognosis (6, 8). This is associated with both early and long term mortality and morbidity (9). In the Asian population, each 1 mmol/L (39 mg/dl) increase in total cholesterol (TC) causes a 35% increase in the risk of coronary death (10). For this reason, preventive approaches constitute the key point in the treatment of coronary artery disease.

To date, different parameters, biomarkers and indices have been proposed to assess cardiovascular disease (CVD) risk and to administer preventive drugs. Therefore, low-cost, rapid and specific tools are used to identify high-risk cases. In this context, individual lipid risk factors such as triglyceride (TG), , high-density lipoprotein cholesterol (HDL-C), TC, LDL-C and non-HDL cholesterol are used. It has been shown that indices based on lipid profile over time can be used as better predictors for CVD (6). Atherogenic index of plasma (AIP) is one of the indices calculated as the logarithm of TG/HDL. Based on the significant positive association of AIP with cholesterol levels, it is considered an alternative and simple marker of plasma atherogenicity. Epidemiological studies have shown that AIP is significantly associated with diabetes mellitus (DM), hypertension (HT) and other risk factors for coronary artery disease (CAD) (11,12). In addition, there is strong evidence that AIP is a superior predictor of other lipid profile indicators in predicting all-cause mortality in CVD (13).

ACS are a group of diseases with high mortality. The prognosis of the disease is affected by the patient's current risk factors and angiographic findings. Treatment approaches vary according to the patient's presentation. In STEMI patients, the mainstay of the treatment approach is primary percutaneous coronary intervention (pPCI) or immediate reperfusion with fibrinolytic therapy if it cannot be performed on time (14). Evaluating the risk factors after coronary intervention in this patient group and developing a treatment strategy accordingly may affect the early and late prognosis of the patient. This study was planned to investigate the association of AIP with early mortality in STEMI patients undergoing pPCI.

MATERIAL AND METHOD

The study was carried out with the permission of Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 09.06.2021, Decision No: E1-21/1571). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This is a retrospective, single-center, hospital database-based study. From the hospital data system, a total of 873

consecutive patients who were diagnosed with STEMI and underwent pPCI between January 2019 and April 2021 were included in the study. The criteria for inclusion in the study were used for the diagnosis of STEMI and the STEMI guideline criteria published in 2017 by the European Society of Cardiology for the indication of pPCI (15). Electrocardiography (ECG), clinical data and laboratory values of the study patients were obtained by scanning the hospital data system and patient files. The patient population to be included in the study was created by examining the available data. Patients with familial hypercholesterolemia, systemic inflammatory diseases, metabolic diseases, malignancies, renal failure requiring hemodialysis, pulmonary edema, cardiogenic shock and patients requiring inotropic support and/or mechanical circulatory support were excluded from the study

Age, gender, medical history, cardiovascular risk factors, medications and all other demographic data of all participants included in the study were recorded. Complete blood count, routine biochemical parameters and cholesterol panel data of the patients were examined and recorded. Calculated indices were calculated based on these data. ECG data required for diagnosis were evaluated through the system. The 12-lead ECG data of all patients taken within 10 minutes of admission were analyzed. Percutaneous coronary intervention procedures were viewed and examined through the hospital data system, and patient procedure reports were evaluated. The clinical follow-up and mortality data of the patients in the hospital were obtained by using data from the patient file and hospital automation system.

Study Design

Routine blood samples were taken for examination during the first admission to the patient for complete blood count, cardiac markers and routine biochemical parameters. Whole blood samples are made with Symex K-1000, Kobe, Japan device. Separate blood samples are taken for follow-up for detailed biochemical and hormonal examinations. These samples are studied with the COBAS C-501 (Roche, Manheim, Germany) device. Another sample is taken for the cholesterol panel, such as TC, TG, fasting blood glucose (FG), HDL-C and LDL-C after 10-12 hours of fasting, and these data were used in the study. The lipid panel measurements and the calculated indices with the values to be obtained from it were calculated as indicated. AIP was calculated as log10 of the ratio of plasma concentration of triglycerides to HDL-C. Coronary angiographic procedures of the patients were performed with General Electric (GE) INNOVA IGS 620, Rye de la Miniere, France and GE OPTIMA IGS 320 001, Milwaukee, Wisconsin model devices in the catheter laboratory. Image data were analyzed through the hospital automation system. Comorbid conditions were defined as

Kasapkara et al. Atherojenic index of plasma and coronary artery disease

J Health Sci Med 2023; 6(1): 158-164

indicated. HT was defined as systolic blood pressure ≥ 140 mmHg on at least three office measurements or diastolic blood pressure ≥ 90 mmHg or receiving antihypertensive therapy. DM was defined as a fasting blood glucose level of ≥126 mg/dl or a glucose level of ≥200 mg/dl measured at any time or concomitant use of antidiabetic medication. Body mass index (BMI) was calculated as body weight (kg)/height(m)2. Regardless of the amount, those who smoked actively in the last 6 months were defined as smokers. Dyslipidemia was defined as triglyceride>150 mg/dl and/or total cholesterol (TC)>200 mg/dl and/or low-density lipoprotein cholesterol (LDL-C)>130 mg/dl and/or high-density lipoprotein cholesterol (HDL-C) <40 mg/dl according to the adult treatment panel III (NCEP ATP III) criteria of the national cholesterol education program.

Statistical Analysis

Statistical analyses were carried out using IBM SPSS Statistics for Macintosh, Version 25.0 (IBM Corp., Armonk, New York, USA). Kolmogorov-Smirnov (K-S) test was used in order to determine the distribution of continuous variables. Chi-square test was used for categorical variables and presented as percentages. The Mann-Whitney-U test was used for the abnormal distributed variables and the findings were presented as median with interquartile range. Receiver operating characteristic (ROC) curve analyses were carried out in order to determine the cut-off values for the sensitivity and specificity of AIP for predicting in hospital mortality. The area under the ROC curve (AUC) was given with 95% confidence interval (CI) in addition to sensitivity, specificity value. The multivariate logistic regression analyses were used in order to identify independent predictors of mortality. Variables, which might be a possible confounding factor for mortality such as age, gender, diabetes mellitus, hemoglobin, creatinin and AIP were included in multivariate analysis. A p value <0.05 was considered statistically significant in all analyses.

RESULTS

A total of 873 patients diagnosed with STEMI and undergoing pPCI were included in this study. The basic demographic, comorbid diseases and procedural data of the patients included in the study are given in **Table 1**. The median age of the patients was 59 (51-67) years and the number of male patients in the group was 705 (81%). Demographic characteristics of the patients were evaluated. When the non-survivors and survivor groups were compared, the mean age of the non-survivor group was found to be higher [69 / 58 years, p<0.05] and the rate of previous cerebrovascular disease (13% / 2%, p<0.05) was different, respectively. Considering the laboratory findings of the patients, creatinine (mg/dl) value was higher [1.29 (0.83-1.71), 0.83 (0.72-0.99) mg/dl, p<0.05] and GFR (ml/min) value was lower [59 (32-95), 95 (79-104) ml/

min, p<0.05], in the non-surviving group respectively. The clinical diagnoses of the patients, the localization of the infarction and the ratio of infarct-related arterial distributions were found to be similar in both groups. Similarly, there was no significant difference between the groups in the stent length and diameter values used during percutaneous coronary intervention.

When traditional and non-traditional lipid values were examined, total cholesterol levels [177 (154-207), 162 (135-180) mg/dl, p=0.05] and HDL-C values [34 (29-40), 31 (25-38) mg/dl, p<0.005] were found to be statistically significant in the survivor group, respectively. LDL-C and triglyceride levels were similar between both groups. When non-traditional lipid parameters were examined, AIP [0.47 (0.26-0.72), 0.59 (0.46-0.83), p=0.005] was found to be higher in the non-survivor group. Other parameters such as atherogenic index, total cholesterol/HDL-C, LDL-C/ HDL-C and non-HDL cholesterol were not significantly different between two groups. These data are shown in **Table 2**. Comparison of median AIP values between nonsurvivor and survivor groups are shown in **Figure 1**.
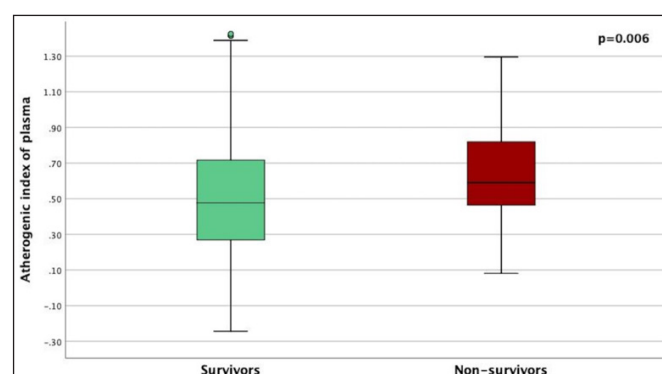
Figure 1. Comparison of median AIP values between survivor and non-survivor groups. P values are given according to Mann-Whitney U test.

Table 2. Traditional and non-traditional lipid parameters of the patients with STEMI

Variables	Non-survivor (n= 53)	Survivors (n= 820)	P Value
Traditional lipid profiles			
TC, mg/dL	162 (135-180)	177 (154-207)	.005
TG, mg/dL	144 (83-165)	103 (70-164)	.033
HDL-C, mg/dL	31 (25-38)	34 (29-40)	.007
LDL-C, mg/dL	104 (81-135)	118 (93-143)	.123
VLDL-C, mg/dL	24 (15-32)	21 (14-33)	.300
Non-traditional lipid profiles			
AIP	0.59 (0.46-0.83)	0.47 (0.26-0.72)	.006
Atherogenic index	4.71 (3.11-5.84)	4.13 (3.19-5.22)	.168
Non-HDL-C, mg/dL	130 (109-173)	143 (119-170)	.125
TC / HDL-C ratio	5.5 (4.1-6.5)	5.1 (4.2-6.2)	.533
LDL-C/HDL-C ratio	3.59 (2.60-4.67)	3.42 (2.68-4.21)	.519
Lipoprotein combine index, ×10³, mg/dL	649 (430-1121)	632 (335-1139)	.567

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL: very low-density lipoprotein cholesterol. TC: Total cholesterol, TG: Triglyceride,

Table 1. Baseline demographic, comorbid, laboratory characteristics and procedural features				
Variables	All (n=873)	Non-survivors (n= 53)	Survivors (n= 820)	P Value
Demographic and comorbid features				
Age, years	59 (51-67)	69 (61-76)	58 (51-66)	<.001
Gender, male	705 (81%)	45 (85%)	660 (80%)	.429
Diabetes mellitus	224 (26%)	21 (40%)	203 (25%)	.016
Hypertension	329 (38%)	27 (51%)	302 (37%)	.040
Prior coronary artery bypass grafting	36 (4%)	4 (8%)	32 (4%)	.196
Prior percutaneous coronary intervention	135 (15%)	11 (21%)	124 (15%)	.272
Prior cerebravascular disease	27 (3%)	7 (13%)	20 (2%)	<.001
Atrial fibrillation	34 (4%)	5 (9%)	29 (4%)	.031
Laboratory findings				
Urea, mg/dL	34 (28-43)	45 (39-75)	34 (28-41)	<.001
Creatinin, mg/dL	0.84 (0.73-1.00)	1.29 (0.83-1.71)	0.83 (0.72-0.99)	<.001
Glomerular filtration rate, ml/min	94 (76-104)	59 (32-85)	95 (79-104)	<.001
Albumin, mg/dL	41 (38-43)	37 (33-42)	41 (38-43)	<.001
Alanin aminotransferase, U/L	35 (24-58)	49 (25-170)	34 (24-59)	.003
Aspartate aminotransferase, U/L	99 (44-243)	150 (52-525)	97 (43-237)	.007
Hemoglobin, g/L	14.2 (13.0-15.3)	13.2 (11.5-15.1)	14.2 (13.0-15.3)	.011
Hematocrit, %	42 (39-46)	42 (35-46)	42 (39-46)	.360
White blood cells, ×10³	11.8 (9.6-14.5)	15.6 (11.1-20.8)	11.8 (9.5-14.3)	<.001
Neutrophils, ×10³	9.5 (7.0-12.1)	12.5 (8.9-18.1)	9.4 (6.9-11.8)	<.001
Lymphocyte, ×10³	1.5 (1.1-2.1)	1.4 (0.7-2.3)	1.5 (1.1-2.1)	.116
Monocytes, ×10³	0.5 (0.4-0.7)	0.6 (0.4-0.9)	0.5 (0.4-0.7)	<.001
Platelets, ×10³	256 (215-303)	285 (213-346)	255 (216-302)	.071
Procedural features				
Infarct location				.341
Anterior	398 (46%)	26 (49%)	372 (45%)	
Inferior	428 (49%)	25 (47%)	403 (49%)	
Isolated posterior	16 (2%)	2 (4%)	14 (2%)	
Isolated lateral	31 (4%)	0	31 (4%)	
Infarct-related artery				.992
Left anterior descending artery	420 (48%)	26 (49%)	393 (48%)	
Circumflex artery	105 (12%)	6 (11%)	99 (12%)	
Right coronary artery	348 (%40)	21 (40%)	327 (40%)	
Total stent length, mm	30 (23-43)	33 (28-48)	29 (23-41)	.057
Stent diamater, mm	3.0 (2.75-3.0)	3.0 (2.75-3.0)	3.0 (2.75-3.0)	.576
Hospital stay duration, days	3 (2-5)	2 (1-5)	3 (2-5)	.001

ROC analysis for the AIP to predict inpatient hospital mortality showed an AUC of 0.61 (95% CI: 0.55-0.68, P=0.006). The cutoff value of AIP (0.50) was associated with 70.0% sensitivity and 52% specificity. It is shown in **Figure 2**.

AIP (OR: 3.77, 95% CI: 1.34–10.6, p <0.012), age (OR: 1.07, 95% CI: 1.03–1.10, p <0.001) in multivariate analysis as an independent predictor of inpatient clinic mortality found by logistic regression analysis, creatinine (OR: 2.02, 95% CI: 1.26–3.24, p < 0.005) was found to be statistically significant. Logistic regression analysis and mortality predictors are given in **Table 3**. In addition, when we evaluated in terms of AIP, the mortality rate was significantly higher in patients with high AIP values compared to patients with low AIP values (28% vs. 72%, p=0.001).

Figure 2. ROC curve analysis for AIP to predict in-hospital mortality demonstrated an AUC value 0.61 (95% CI: 0.55–0.68, p=0.006). The cutoff value of AIP (0.50) was associated with 70.0% sensitivity, 52% specificity.
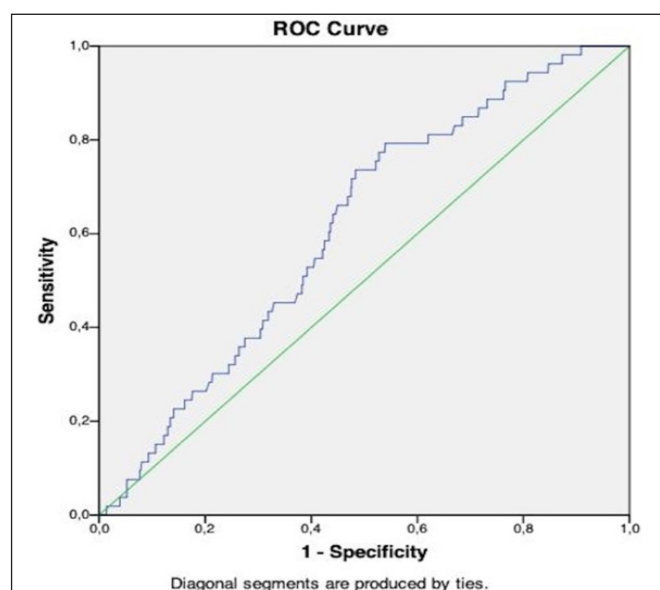
Kasapkara et al. Atherojenic index of plasma and coronary artery disease

J Health Sci Med 2023; 6(1): 158-164

Table 3. Independent predictor of in-hospital mortality by logistic regression analysis

Variables	Multivariate analysis		
	Odds Ratio	95%CI	P value
Age	1.07	1.03-1.10	<.001
Gender	0.88	0.39-1.98	.752
Diabetes mellitus	1.43	0.71-2.90	.316
Hemoglobin	0.99	0.91-1.10	.988
Creatinin	2.02	1.26-3.24	.004
AIP	3.77	1.34-10.6	.012

DISCUSSION

Atherosclerosis may present with variable clinical presentations as a result of different pathophysiological processes such as intravascular plaque formation, plaque erosion and plaque rupture. It is known that CAD is generally associated with dyslipidemia, HT, DM, sedentary life, obesity, stress and chronic proinflammatory conditions (6,8).

Various risk factors and indices are used to assess cardiovascular risk and implement preventive treatments. Therefore, efforts are being made to find low-cost, rapid, specific, non-invasive and predictive tools to identify high-risk cases. In this context, lipid parameters such as TG, TC, HDL-C, LDL-C have been suggested. However, indices that are better predictors of CVD based on lipid profile have also been used (6).
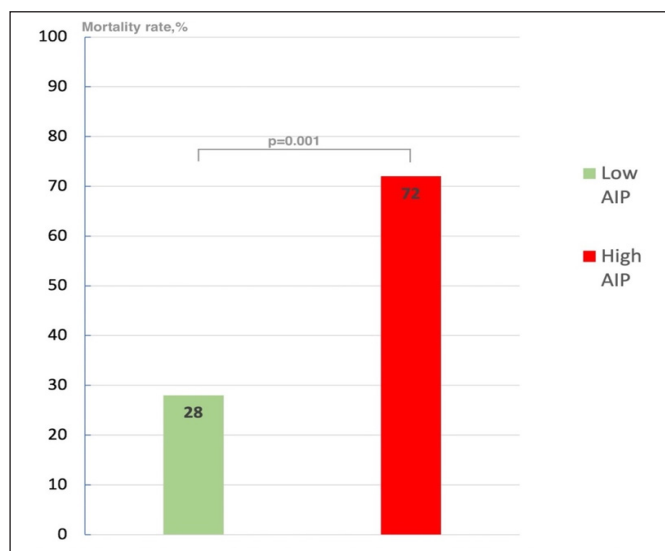
Figure 3. Comparison of mortality rate according to AIP value of study patients. It was significantly higher in patients with high AIP as compared to those with low AIP (28% vs 72%, p=0.001)

AIP is one of the indexes calculated as the logarithm of TG/HDL. Epidemiological studies show that AIP is significantly associated with obesity, HT, DM and other risk factors for CAD (11,12). Similarly, it has been suggested to be a valuable marker for the prediction of atherogenicity and CAD (16,17). In addition, it is claimed that AIP is a predictor of all-cause mortality in CVD and is superior to other lipid indices (13).

When the studies investigating the relationship between AIP with CVD and risk factors are examined, it is seen that there is a positive relationship in general. Considering all these data, we investigated the relationship between AIP and early mortality in patients with STEMI who underwent pPCI. ACS are diseases with high mortality and early diagnosis, treatment planning and risk determination are important in these patients. Especially identifying high-risk groups and planning treatment strategies are important in terms of prognosis.

Two main findings from our study are (a) AIP values were found to be higher in STEMI patients who underwent pPCI who died in-hospital (b) AIP was an independent predictor of mortality determined by multivariate regression analysis. It is considered an alternative and simple marker of plasma atherogenicity. Positive correlation observed between AIP and cholesterol levels. Recently, it has been shown that AIP is associated with cardiovascular outcomes in the general population and in different patient groups (18,19). TG and HDL-C are two lipid parameters routinely measured in clinical practice, but neither are markers that consistently reflect plasma atherogenicity. HDL-C is associated with anthropometric indices such as weight, BMI, waist and hip circumference, and metabolic indices including glycemic status (20).

A new lipid index defined as AIP is accepted as a better indicator for atherosclerosis (21). In our study, it was observed that TC, LDL-C and HDL-C levels were lower in the mortality group. There are contraversies in the data here and in their relationship with CVD in general. Different results have been suggested in studies on this subject. A number of large population-based studies from Scandinavian countries have shown that hyperlipidemia is inversely related with mortality, especially in older adults (22,23). A prospective observational study found that low LDL-C at admission was associated with a lower 3-year survival in patients admitted to hospital for non-ST-elevation myocardial infarction (24). Another study suggests that the risk of death from hyperlipidemia decreases with increasing age (25). However, when the AIP value between groups is considered, it is seen that it is more consistent in showing cardiovascular adverse outcomes. LDL-C has been used for a long time as a target for prevention and treatment of cardiovascular diseases. However, the importance of other conventional lipid parameters in atherosclerotic patients with normal LDL-C levels has been understood. Because of this contradiction, indices calculated from these parameters have come prior to conventional lipid parameters.

In a prospective cohort study including 2676 middle-aged adults, it was reported that the group with high AIP significantly predicted the probability of CAD determined by age, in both genders especially in women

(26). AIP has been shown to be associated with mortality in elderly patients and dialysis patients. Edwards MK et al. (13) showed that AIP was positively and independently associated with mortality risk and was a better predictor for mortality than individual cholesterol risk factors. Bendzala M et al. (17) they also found that AIP was positively associated with the risk of all-cause mortality in older women with hypertension. The predictive value of AIP was also investigated in ACS patients. Cai G et al. (29) they divided the patients into 2 groups in a study that included 1478 patients who had retrospectively undergone coronary angiography. They showed that AIP was independently associated with the presence and severity of ACS in a gender-dependent manner, and as the AIP quartiles increased, the prevalence of ACS, acute myocardial infarction, unstable angina pectoris and Gensini score also increased. Qin Z et al. (30) retrospectively enrolled 2356 patients with DM who underwent percutaneous coronary intervention and were followed up for 4 years. They found that AIP was an independent predictor of major cardiovascular and cerebrovascular adverse events, including cardiac death, myocardial infarction, repeated revascularization and stroke, regardless of clinical presentation.

In a study showing that AIP is associated with adverse cardiovascular events, it was shown that the AIP formula consisted of HDL-C and triglycerides. Low HDL-C and high triglyceride levels were associated with adverse cardiovascular events after ACS, independent of diabetic status (31,32). In addition, moderate-to-vigorous physical activity, increased duration of aerobic exercise and decreased sedentary life have been reported to be inversely correlated with AIP, meaning that a healthy lifestyle helps to reduce the risk of cardiovascular disease (33,34). When the results of these studies are examined, it can be argued that AIP is a similar but stronger predictor of the relationship between conventional lipid parameters and CVD. In our study, the results obtained in logistic regression analysis showed that AIP is a strong independent predictor of mortality and supports these studies.

CONCLUSION

AIP may be a good predictor of early mortality in patients with STEMI undergoing pPCI. AIP can be a good marker with its advantages like inexpensive, accessible and easy to apply to reduce mortality in STEMI patients, to categorize risk and to determine treatment strategy and intensity in the early period. It would be more appropriate to support these results with larger-scale studies and a prospective study in which other factors that may affect mortality in acute coronary syndromes are taken into account.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 09.06.2021, Decision No: E1-21/1571).

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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J Health Sci Med 2023; 6(1): 158-164

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The effect of some family characteristics on the relationship between mental symptoms and levels of serum serotonin and salivatory cortisol

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ABSTRACT

Aim: Psychological symptoms (PSs) are also seen in healthy individuals. The aim of this study is to examine some familial features in the relationship between PSs and serum serotonin (5-HT) and salivary cortisol (CTS) levels in healthy subjects.

Materials and Method: Sociodemographic data form and psychiatric symptom screening questionnaire (SCL-90-R) were given to 320 healthy individuals (156 males, 164 females) aged 18-65 without any mental illness. Blood and saliva samples were duly taken and evaluated by ELISA method. Informed consent and local ethics committee approval were obtained from the participants before starting the study. Statistical analyzes were done with SPSS 15.0 program. Descriptive statistical data (number, percentage, mean, standard deviation, minimum and maximum), independent sample t-test, One-way ANOVA test, Pearson Correlation analysis were used in the analyzes. For statistical significance, p<.05 was accetted significant.

Results: The mean age of the participants was 29.19±8.41. According to family characteristics, PSs were found to be higher in those whose parents were separated, whose parents had a low educational level, and those with a family history of psychiatric illness. Depressive symptoms were statistically significantly higher in those whose families were separated. Somatization, anxiety, obsessive symptoms, depressive symptoms, hostility and additional symptoms were statistically significantly higher in those with a family history of psychiatric illness. Although it was not statistically significant, the 5-HT levels were highest in those living in an extended family, those whose parents lived together, those whose parents were primary school graduates, and those who did not have a family history of psychiatric disease. CTS levels were statistically significantly higher in those who did not have a family history of psychiatric disease. There was a negative correlation between 5-HT and CTS levels and PSs excluding additional symptoms. There was a positive correlation between 5-HT and CTS levels.

Conclusion: It was concluded that familial characteristics have an effect on the relationship between psychological symptoms and serotonin and cortisol levels in healthy individuals, but these characteristics alone are not effective in this relationship. There is a need for studies investigating other stressor factors that are thought to be effective in the relationship between mental symptoms and serotonin and cortisol levels.

Keywords: Family characteristic, psychological symptom, healthy subject, serotonin, cortisol

INTRODUCTION

The two most frequently focused biomarkers in the etiology of mental disorders and the emergence of psychological symptoms (PSs) are serotonin (5-HT) and cortisol (CTS). Fluctuations in 5-HT levels and the diurinal rhythm of CTS play an important role in the formation of these symptoms. 5-HT is a monoamine neurotransmitter that regulates mood level, sleep-wake behavior and appetite in the central nervous system, and regulates various physiological functions (gastrointestinal system, cardiovascular system, immune system, endocrine system, etc.) in the periphery (1). Deficiencies in the serotonergic system, may cause various mental disorders, including anxiety disorders (2), depression (3), obsessive compulsive disorder (4), schizophrenia (5), mood disorders and autism (6)

Changes in the activity of the hypothalamo-pituitary-adrenal axis, especially hypercortisolemia, are associated with depression. In addition, generalized anxiety disorder, panic disorder, and phobias may be associated with increased and prolonged activation of the hypothalamo-pituitary-adrenal axis (HPAA). In social phobias, normal basal CTS levels and hypersensitivity of the adrenal cortex can be observed during psychosocial stressors. Early stressful life events may predispose individuals to develop psychopathology

Maden et al. Mental symptoms, serotonin, corstisol and family characteristics

J Health Sci Med 2023; 6(1): 165-173

by causing changes in the stress response and thus in the HPAA during adulthood (7). In a study conducted by Walker et al. (8) in young adults, CTS levels were followed for one year in those who met the criteria for psychosis risk, and they found that individuals who developed psychosis had significantly higher CTS levels than the control group who were not at risk. CTS levels were also found to be high in anxiety disorders (9), depression (10) and specific phobias (11).

In recent studies, it has been stated that the interaction between 5-HT and CTS levels has an important place in the etiology of mental disorders (12). Chronic CTS excess in the brain can also lead to 5-HT deficiency due to decreased availability of tryptophan, the substrate for 5-HT production. Moreover, it reduces the density and reactivity of 5-HT receptors (13). Currently used antidepressants not only affect neurotransmitters and activate amine receptors, but also normalize the HPAA activity, reduce cortisol-releasing hormone levels and therefore adrenocorticotropic hormone and cortisol (14).

In addition to the effects of 5-HT and CTS, sociodemographic characteristics such as age, gender, educational status, occupational status, marital status, medical and mental illness burden also play a role in the etiology of mental disorders. Independent of these stressors, familial factors can be effective in the emergence of mental disorders and PSs.

The family is essential in providing a support system for individuals to overcome the difficulties they face. Family processes include many interactions that occur family life, such as supporting each other, sharing love, communicating, problem solving, aggression, and neglecting one another. These processes occur in family systems, and the cognition, emotions, genetics, and physiology of family members are inextricably intertwined with these processes (15). On the other hand, various stressor factors such as family type, education level of parents, and a family history of mental illness may cause PSs and mental disorders in individuals. In a study by Park et al. (16), it was stated that the children of mothers with less than secondary school education had a higher risk of developing depression in early adulthood than those whose mothers had more education, and this was not related to father education. The French longitudinal study suggested that low level of parental education not only causes mental health problems such as anxiety and depression in childhood, but also continues to affect mental health in adulthood (17). It has been reported that a family history of psychiatric illness is associated with generalized anxiety disorder (18), depression (19), and psychotic disorders (20).

In the light of this information, it is seen that randomized controlled studies on mental symptoms mostly focus on anxiety and depressive symptoms. In addition to these symptoms, PSs such as somatic symptoms, obsessive symptoms, and psychotic symptoms can also be seen in healthy individuals. In our study, PSs were evaluated according to the PSs (somatization, anxiety, depression, obsession, interpersonal relationships, psychotic symptoms, paranoid symptoms, hostility, phobic symptoms and additional symptoms) included in the Symptom Check-List-90-R (SCL-90-R; 21,22).

In most of the literature, studies on 5-HT, CTS and stressors, which are claimed to play a role in the etiology of mental disorders and the emergence of PSs, have been examined as sample group, patient and health control groups. The main feature of these studies is that they reveal the changes in the patient groups compared to the healthy control group. The results of these studies focused significantly on the patient group. Therefore, changes in 5-HT and CTS levels in healthy individuals and in the levels of PSs due to stressors have often been neglected due to the original design of the studies. The effects of the stressor factors that play a role in the emergence of these symptoms in healthy individuals have not been emphasized much. Likewise, studies on this subject are limited in number. In addition, among these stressors, familial factors have a very important role in the development of healthy individuals, interpersonal relationships and the emergence of mental symptoms. We developed the hypotheses of our study in order to fill the gap in this regard. According to these hypotheses, in this study, we aimed to determine whether there is a relationship between PSs and 5-HT levels, and whether there is a relationship between PSs and CTS levels, and whether familial characteristics are effective in the relationship between PS and 5-HT levels, and whether familial characteristics are effective in the relationship between PSs and CTS levels, and whether there is a relationship between serotonin and cortisol levels, in healthy subjects.

MATERIAL AND METHOD

The study was carried out with the permission of Clinical Researches Ethics Committee of Ankara Keçiören Training and Research Hospital (Date: 11.07.2012, Decision No: B.10.4.İSM.4.06.68.49). The healthy individuals included in the study were informed about the study and their written consent was obtained. All procedures were performed in accordance with ethical rules and the principles of the Declaration of Helsinki.

This study was carried out with 320 participants aged between 18-65 years, between December 2012 and March 2013 at the Gülhane Military Medical Academy Mental Health and Diseases Clinic. This cross-sectional and

descriptive study included healthy personnel working in various units of the Gülhane Military Medical Academy and without any psychiatric disorders or psychiatric complaints, and healthy subjects who examined to the mental health and diseases polyclinic for various reasons and did not receive any psychiatric diagnosis.

The selection of the participants was made using the purposive sampling method. Before the study, the sample size was obtained by calculating the number of people to be included in the study. Accordingly, the number of people to be included in the study was determined as 313. The sample of the study was 320 people, and when this number was reached, the data collection process was terminated. As a result of an interview with a specialist psychiatrist, those whose physical and cognitive health level is suitable to answer the applied forms, those who volunteer to participate in the research, and those who do not have a chronic medical disease (hypertension, heart disease, systemic disease such as endocrinological, neurological, physical therapy, etc.), and those who do not use drugs (birth control pills, hormonal drugs, etc.) that affect psychotropic and cortisol levels, and non-pregnant, non-premenstrual period and non-menstrual period women were included in the study. Volunteer participants were briefed about the study by the researchers and a questionnaire was applied by face-to-face interview technique. After filling out the data forms, they were checked by a specialist psychiatrist to ensure that there were no missing or erroneous data, and the participants were asked to answer the missing parts. Thus, it was ensured that there were no missing and incorrect data. Individuals who did not agree to participate in the study were excluded from the study.

Participants were asked not to eat after 22.00 pm, as blood would be drawn the next morning. Regarding the collection of saliva samples, necessary warnings were given to the participants that they should not brush their teeth in the morning the next day, that the smoking participants should not smoke, that they could only drink water and not take any liquid food. Blood and saliva samples were taken the next day.

Data Collection Utensils

Sociodemographic data form: The information form was developed by the researchers to determine the sociodemographic characteristics of individuals (age, education level, marital status, family type, mother's education level, father's education level, mother-father cohabitation status, and psychiatric illness in the family).

The Symptom Check-List-90-R (SCL-90-R): It is a 90-item self-assessment scale developed by Derogatis (21) that provides a five-point Likert-type measurement between "not at all" and "too much". Each item is scored between 0-4. This scale, which measures psychiatric symptoms and stress

response, consists of nine subscales and one additional scale: Somatization, anxiety, obsession, depression, interpersonal sensitivity, paranoid thought, psychotism, hostility, phobic anxiety. Turkish adaptation studies of SCL-90-R were performed by Dağ et al. (22). Although the cut-off score varies from researcher to researcher, it is generally accepted as 1. The Cronbach α internal consistency coefficient of the scale was 0.97, and the test-retest reliability coefficients were between 0.65-0.87 according to the subscales, found to be .90 in total. Construct validity and criterion-related validity of the scale were also studied.

Collection and Analysis of Samples

Blood samples taken from the brachial vein were put in an EDTA blood tube and centrifuged at 2500 rev for 8 minutes. After centrifugation, approximately 2 cc plasma sample from the "buffer", where platelets are concentrated, was transferred to capped Epandorf tubes with a Pasteur pipette. Participants were asked to chew sugar-free gum for 3-4 minutes to increase salivation and then spit 8-10 times (approximately 2 cc) into sterile urine collection cups. Saliva samples collected in the urine collection cup were transferred to capped Epandorf tubes with another Pasteur pipette. The names of the participants were written on the capped tubes from which both samples were taken, and the samples were stored in the refrigerator at - 80°C until the target sample number was reached. When this number was reached, they were analyzed in the biochemistry laboratory of the hospital. Serotonin and cortisol levels were measured by ELISA method.

Statistical Analyzes

Descriptive statistical data were given as numbers, percentages, mean, standard deviation, minimum and maximum. The correspondence of the continuous data to the normal distribution was made by examining the skewness and kurtosis values. Values between -1.50 and +1.50 for skewness and kurtosis were evaluated as normal distribution (23). In the analysis of normally distributed data, independent sample t-test was used to compare two groups, and one-way ANOVA test was used to compare multiple groups. Pearson correlation coefficients was prefered to evaluate the relationship between variables. For statistical significance, $p<.05$ was accepted as significant at the 95% confidence interval.

RESULTS

In the study, in which 320 healthy individuals participated, the mean age of the participants was determined as 29.19±8.41. 51.2% (n=164) of the participants were female, 55.0% (n=176) were 13-16 years educated, 54.4% (n=174) were single. Some data on familial characteristics are given in **Table 1**.

Maden et al. Mental symptoms, serotonin, corstisol and family characteristics

J Health Sci Med 2023; 6(1): 165-173

Table 1. The cases of socio-demographics characteristics and familial characteristics		
Parameter	Frequency (n=320)	Percent (%)
Sex		
Men	156	48.8
Women	164	51.2
Age		
18-30 years	179	55.9
31-40 years	105	32.8
41 years and upper	36	11.3
Years of Education		
5-8 years	28	8.8
9-12 years	43	13.4
13-16 years	176	55.0
17 years and/or more	73	22.8
Marital status		
Married	139	43.4
Single	174	54.4
Divorced	7	2.2
Income		
High	68	21.3
Middle	211	65.9
Low	41	12.8
Family type		
Nuclear family	263	82.2
Extended family	37	11.6
Living apart from family	20	6.3
Cohabitation of parents		
Parents live together	267	83.4
Parents live separately	53	16.6
Father's education status		
Illiterate	22	6.9
Primary school	174	54.4
High school and upper	124	38.8
Mother's education status		
Illiterate	53	16.6
Primary school	206	64.4
High school and upper	61	19.1
Family history of psychiatric illness		
Yes	41	12.8
No	279	87.2

According to familial characteristics, PSs were found to be higher in those whose mothers and fathers were separated, those whose parents had a low education level, and those with a family history of psychiatric disorder. Depressive symptoms were found to be statistically significantly higher in those with separated families, and somatization, anxiety, obsessive symptoms, depressive symptoms, hostility, and additional symptoms in those with a family history of psychiatric disorder. PSs levels were statistically the highest in the participants whose parents had a low education level (**Table 2**).

According to the familial characteristics of the participants in the study, the 5-HT levels were found to be highest in those living in an extended family, those whose parents lived together, those whose parents were primary school graduates, and those who did not have a family history of psychiatric illness, although it was not statistically significant (**Table 3**). It was determined that the CTS levels were statistically significantly higher in those who did not have a family history of psychiatric disorder.

Table 3. Distributions of serotonin and cortisol levels according to familial characteristics of the participants			
	n=320	Serotonin	Cortisol
Family type† (x̄±SD)			
Nf	263	201.11±150.73	5.73±2.12
Ef	37	242.41±133.30	6.26±2.35
Lf	20	223.70±147.87	6.05±1.54
F		1.381	1.161
p*		>.050	>.050
Cohabitation of parents (x̄±SD)			
Pl-t	267	212.91±150.01	5.87±2.14
Pl-s	53	179.06±140.91	5.54±2.06
t		1.515	1.035
p*		>.050	>.050
Father's Education Status† (x̄±SD)			
Illiterate	22	220,41±174,42	6.21±2.48
Prim.sch.	174	203,18±147,26	5.73±2.04
H. sch/upp.	124	210,76±147,32	5.85±2.17
F		.185	.558
p*		>.050	>.050
Mother's Education Status (x̄±SD)			
Illiterate	53	216.58±153.67	6.14±2.11
Prim.sch.	206	199.04±146.87	5.72±2.11
H. sch/upp.	61	227.13±151.44	5.84±2.20
F		.916	.811
p*		>.050	>.050
Family history of psychiatric illness (x̄±SD)			
Yes	41	176.63±149.02	5.12±1.62
No	279	211.81±148.57	5.92±2.17
t		-1.415	-2.803
p*		>.050	.007

*p<.05. x̄=Mean, S.D.=Standard Deviation, χ2=Chi-Square value, t=Independed Samples T-test value, F=Oneway ANOVA value, Nf=Nuclear family, Ef=Extended family, Lf=Living apart from family, Pl-t=Parents live together, Pl-s=Parents live separately, Prim.sch.=Primary school, H. sch/upp.=High school and upper.

According to the results of Pearson correlation coefficients, a statistically significant negative correlation between 5-HT levels and PSs excluding additional symptoms was determined in **Table 4**. There was a statistically significant negative correlation between CTS levels and PSs excluding additional symptoms. There was a statistically significant positive correlation between the 5-HT and CTS levels of the participants.

Table 2. Distributions of psychiatric symptom sub-dimension mean scores according to familial characteristics of the participants

PSYCHIATRIC SYMPTOMS	n=320	Somatization	Anxiety	Obsession	Depression	Interpersonal sensitivity	Psychotism	Paranoid idea	Hostility	Phobic symptoms	Additional symptoms	GSI
Family type† (x̄±SD)												
Nf1	263	.61±.56	.48±.54	.91±0.66	.71±.67	.74±.70	.34±.47	.66±.64	.55±.60	.21±.40	.64±.64	.60±.52
Ef2	37	.67±.49	.51±.46	.92±.59	.67±.52	.72±.46	.41±.30	.81±.50	.67±.60	.27±.26	.78±.66	.64±.40
Lf3	20	.59±.69	.55±.73	.99±.74	.89±.90	.83±.94	.55±.75	.88±1.00	.62±.83	.30±.46	.87±.74	.72±.73
F		2.017	1.601	.228	.395	.825	7.021	5.065	2.159	7.130	4.188	.588
p*		>.05	>.050	>.050	>.050	>.050	>.050	>.050	>.050	>.050	>.050	>.050
Cohabitation of parents (x̄±SD)												
Pl-t	267	.58±.52	.48±.51	.89±.62	.68±.65	.72±.67	.34±.45	.67±.64	.57±.62	.21±.37	.65±.64	.59±.49
Pl-s	53	.78±.72	.55±.67	1.08±.78	.90±.79	.86±.78	.45±.57	.79±.73	.56±.57	.29±.50	.77±.70	.73±.62
t		-1.818	-.707	-1.685	-2.114	-1.320	-1.435	-1.215	.024	-1.065	-1.269	-1.522
p*		>.05	>.05	>.05	.035	>.05	>.05	>.05	>.05	>.05	>.05	>.05
Paternal education status† (x̄±SD)												
Illiterate4	22	.95±.70	.70±.71	1.20±.60	.95±.81	1.14±.82	.65±.62	1.11±.84	.70±.65	.33±.47	.94±.85	.87±.66
Prim.sch.5	174	.62±.57	.53±.55	.94±.66	.77±.70	.79±.71	.40±.48	.73±.63	.64±.66	.26±.42	.70±.64	.65±.52
H. sch/upp.6	124	.55±.51	.39±.48	.84±.64	.60±.60	.61±.61	.21±.41	.56±.62	.44±.52	.16±.30	.57±.61	.51±.46
F		6.740	8.968	7.756	6.281	12.826	19.033	15.968	8.795	9.481	6.949	6.066
p*		.010	.016	.044	.027	.001	<.001	.001	.014	.042	.029	.003
S/d		4-5, 4-6	4-6, 5-6	4-5, 4-6	4-6, 5-6	4-5, 4-6, 5-6	4-5, 4-6, 5-6	4-5, 4-6,5-6	4-6, 5-6	4-6, 5-6	4-6, 5-6	4-6, 5-6
Maternal education status (x̄±SD)												
Illiterate7	53	.73±.64	.55±.63	1.04±.66	.88±.76	.93±.78	.51±.52	.91±.76	.65±.58	.27±.39	.81±.70	.74±.59
Prim.sch.8	206	.65±.57	.53±.53	.94±.65	.73±.65	.77±.68	.37±.49	.70±.62	.60±.64	.25±.41	.71±.66	.63±.51
H. sch/upp.9	61	.40±.39	.29±.46	.76±.63	.52±.62	.50±.59	.21±.34	.50±.61	.37±.52	.12±.26	.39±.46	.42±.44
F		5.811	5.152	3.008	4.387	6.170	5.772	5.542	3.907	3.025	7.605	6.337
p*		.003	.006	.050	.013	.002	.003	.004	.021	.050	.001	.002
S/d		7-9, 8-9	7-9, 8-9	7-9	7-9	7-9, 8-9	7-9, 8-9	7-9	7-9, 8-9	-	7-9, 8-9	7-9, 8-9
Family history of psychiatric illness (x̄±SD)												
Yes	41	.84±.75	.73±.74	1.12±.75	.97±.80	.90±.76	.48±.56	.84±.72	.91±.77	.36±.56	.94±.77	.80±.62
No	279	.58±.53	.45±.50	.89±.64	.68±.65	.72±.68	.34±.46	.67±.64	.52±.57	.21±.36	.63±.62	.58±.50
t		2.108	2.351	2.146	2.548	1.510	1.444	1.538	3.096	1.728	2.454	2.477
p*		.041	.023	.033	.011	>.050	>.050	>.050	.005	>.050	.018	.014

*p<.05, x̄=Mean, SD=Standard Deviation, χ2=Chi-Square value, t=Independed Samples T-test value, F=Oneway ANOVA value, S/d=Significant difference, Nf=Nuclear family, Ef=Extended family, Lf=Living apart from family, Pl-t=Parents live together, Pl-s=Parents line separately, Prim.sch.=Primary school, H. sch/upp.=High school and upper.

Maden et al. Mental symptoms, serotonin, corstisol and family characteristics

J Health Sci Med 2023; 6(1): 165-173

Table 4. The relationship between the participants' mean scores of psychological symptoms sub-dimensions and serotonin and cortisol levels

	1	2	3	4	5	6	7	8	9	10	11	12
1. Global symptom index	-											
2. Somatization	.825**	-										
3. Anxiety	.909**	.802**	-									
4. Obsession	.872**	.677**	.744**	-								
5. Depression	.925**	.708**	.834**	.804**	-							
6. Interpersonal sensitivity	.889**	.652**	.749**	.789**	.821**	-						
7. Psychotism	.872**	.646**	.790**	.744**	.790**	.789**	-					
8. Paranoid idea	.843**	.621**	.719**	.718**	.794**	.795**	.778**	-				
9. Hostility	.792**	.655**	.739**	.634**	.714**	.684**	.657**	.708**	-			
10. Phobic symptoms	.769**	.661**	.816**	.612**	.689**	.642**	.703**	.582**	.534**	-		
11. Additional symptoms	.831**	.729**	.736**	.674**	.767**	.688**	.692**	.662**	.646**	.609**	-	
12. Serotonin	-.181**	-.209**	-.184**	-.136*	-.209**	-.134*	-.168**	-.176**	-.145**	-.116*	-.095	-
13. Cortisol	-.174**	-.156**	-.177**	-.133*	-.194**	-.146**	-.147**	-.160**	-.098	-.187**	-.086	.487**

*p<.05.

DISCUSSION

Psychiatric symptoms can be seen in individuals with mental disorders as well as in healthy individuals. However, these symptoms may not be as pronounced as in a mental disorder and may not affect their level of functioning. For this reason, the PSs levels in healthy individuals have been neglected in most of the studies. On the other hand, serotonin and cortisol are the two most emphasized biomarkers in the emergence of PSs. In addition to these, stressor factors also play a role in this process. In this study, the effect of some family characteristics of healthy individuals on the relationship between PSs and 5-HT and CTS cortisol levels was investigated.

According to the findings of our study, PSs were found to be higher in those whose parents were separated, those whose parents had a low educational level, and those with a family history of psychiatric disorder. In addition, depressive symptoms were found to be higher in those whose parents were separated than those whose parents lived together. Parental separation, or parental divorce, has a long-term impact on individuals' mental health, family relationships, and education. Sands et al. (24) found a significant relationship between parental divorce and depression in adult children; stated that this relationship was not related to anxiety levels. However, they were unable to prove that the length of time the divorces occurred had any effect on this relationship. When exposed to chronic stress such as parental separation, it has been suggested that there is a loss of spines and a dendritic reduction in the hippocampus and medial amygdala, while dendrites enlarge in the basolateral amygdala due to hypothalamo-pituitary-adrenal axis imbalance and structural and functional changes in the amygdala and hippocampal regions. (25). Disruptions in frontoamygdal connectivity have been associated with a variety of mental health problems, including depression (26) and anxiety (27). The findings of our study support

these results, which show that there is a relationship between PSs and parental divorce experience in those whose parents are separated.

Psychological symptom levels of those whose parents were illiterate were found to be higher than those with primary and high school education or higher. In a study by Park et al. (16), it was stated that the children of mothers with less than secondary school education had a higher risk of developing depression in early adulthood than those whose mothers had more education, and this was not related to father education. In a study conducted in France, it was suggested that low level of parental education not only causes mental health problems such as anxiety and depression in childhood, but also continues to affect mental health in adulthood (17). On the other hand, it has been reported that the children of university graduate parents are more likely to have positive psychological health than children of non-university graduate parents (28). The findings of our study, in line with these findings, indicate that parent education plays an important role in the emergence of PSs.

Another finding of our study is that somatization, anxiety, obsession, depression, anger-hostility, phobic symptoms, additional symptom levels and GSI levels were significantly higher in patients with a family history of psychiatric disoredr. In studies, having a family history of psychiatric disorder has been associated with generalized anxiety disorder (18), depression (19), and psychotic disorders (20). On the other hand, it has been stated that positive family history and model learning do not play an important role in the development of psychogenic non-epileptic seizures (29). Serretti et al. (30) pointed out that there was no difference in general depressive symptoms between patients with and without major depression in their family. Although there are conflicting data in the literature, the findings of our study support the view that the presence of a psychiatric disorder in the family is a risk factor for the emergence of PSs.

In our study, it was found that 5-HT levels were highest in those with extended families, those whose parents lived together, those whose parents were primary school graduates, and those who did not have a family history of psychiatric disorder, and this result was not statistically significant. In a recent review study, it was declared that 5-HT plays a positive moderator role between negative childhood experiences such as functional disorders at home (parental incarceration, divorce, parental substance abuse and maternal physical abuse) and the risk of depression, and may also be effective in poor treatment outcomes in adulthood (31). Muck-Seler et al. (32) reported that platelet 5-HT concentration was higher in schizophrenic patients than in depressed patients or healthy controls, and it was lower in depressed patients compared to healthy controls and schizophrenic patients. In recent studies, it has been suggested that there is no difference between platelet 5-HT levels between patients with depressive symptoms and healthy individuals (33,34). Although the findings of our study show that 5-HT levels are affected by familial stressors, they do not provide sufficient evidence.

Another finding of our study is that the salivary CTS levels in patients with a family history of psychiatric disorder are significantly lower than those without a family history of the disease. Yıldırım et al. (20) found that CTS levels in wakefulness were higher in schizophrenia patients compared to their first-degree relatives, and CTS levels in first-degree relatives compared to healthy controls. In another study, it was reported that patients with newly diagnosed bipolar disorder had higher hair CTS levels than their unaffected first-degree relatives and healthy controls (35). CTS levels were found to be lower in the sons of fathers who had behavioral disorders in childhood and later developed antisocial personality disorder, compared to fathers who did not develop any axis 1 disorder or antisocial personality disorder (36). In a study conducted with patients with major depression, it was emphasized that there was no difference between the CTS levels of the patients and their unaffected first-degree relatives (19). The findings of our study suggest that CTS may play a role in pathological processes in individuals with genetic predisposition. In addition, these findings may contribute to the view that CTS levels, which are high at the beginning of the disease, may decrease during the long-term disease process, as they adapt to the stress load arising from the disease as a result of changes in the function of the HPAA.

In our study, a negative and significant relationship was found between 5-HT levels and somatization, anxiety, obsession, depression, interpersonal sensitivity, psychoticism, paranoid symptom, anger-hostility, phobic symptom and GSI subscale scores. Decreased 5-HT levels may cause PSs to occur in mental disorders such as anxiety disorders (2), depression (3), obsessive-compulsive disorder (4), schizophrenia (5), mood disorders and autism (6). In a recent study, a negative correlation was found between platelet 5-HT levels and impulsivity levels (37). The findings of our study support the findings in the literature.

Another finding of our study is that there is a negative and significant relationship between CTS cortisol levels and somatization, anxiety, obsession, depression, interpersonal sensitivity, psychoticism, paranoid symptom and phobic symptom and GSI subscale scores. It has been reported that the blood levels of CTS, which is a stress hormone, are high in patients with anxiety disorder (9), depression (10), psychotic disorder (8) and specific phobia (11) while it does not differ in those with social phobia (38). Muck-Seler et al. (32) found that plasma CTS levels increased significantly in both schizophrenia patients and depressed patients when compared to the values in healthy controls. The fact that CTS levels were low in individuals with PSs in our study supports the view that CTS level alone was not effective in the emergence of PSs.

In our study, a positive and significant relationship was found between 5-HT and CTS levels. Affecting the hippocampal region as a result of prolonged exposure to stressors can cause modulation of various neural pathways, including serotonergic input from the Raphe nucleus (39). Muck-Seler et al. (32) suggested that there was a significant correlation between platelet 5-HT and plasma CTS concentrations in healthy controls, but they stated that there was no such relationship in schizophrenia or depressed patients. On the other hand, CTS may be effective in the emergence of PSs such as depressive symptoms by affecting 5-HT levels (40). Evidence for an interaction between 5-HT and CTS in the occurrence of other PSs other than anxiety and depressive symptoms is insufficient. The findings of our study confirm the interaction between 5-HT and CTS.

Our work has some strengths. First, we believe that this is the first study to examine the effects of certain familial factors on the PSs and their relationship with 5-HT and CTS levels in a healthy population in Turkey. Secondly, SCL-90-R and two biomarkers (5-HT and CTS) that are effective in the etiology of mental disorders were used together instead of other diagnostic tests. Thus, the use of the SCL-90-R made it possible to evaluate not only depression and anxiety in healthy individuals, but also the GSI as well as other PSs such as somatization, obsessive symptoms, interpersonal sensitivity, anger-hostility, phobic anxiety, psychotic symptoms and additional psychological symptoms. Third, by considering the relationship between PSs and 5-HT and CTS levels

Maden et al. Mental symptoms, serotonin, corstisol and family characteristics

J Health Sci Med 2023; 6(1): 165-173

separately, we show that although PSs are highly correlated with these two components, they may actually have different effects and require different prevention and intervention strategies. Finally, we show that it is critical to acknowledge cross-context variability in the relationship between familial factors and adult mental health outcomes.

There are some limitations in our study. First, a cross-sectional design was applied and therefore no cause-effect relationship could be determined between the research variables. Secondly, it is considered to be partially sufficient in terms of sample size. Since it is a descriptive study, the sample size was kept large. Third, while self-report measures are used for familial characteristics and PSs, the gold standard for psychiatric diagnosis is a structured/semi-structured clinical interview. Fourth, few studies have examined associations between PSs and 5-HT and CTS levels, making it difficult to compare these results in our study with other studies. Fifth, it was limited to collecting a single blood sample, which did not take into account the circadian rhythm of CTS secretion. Finally, it is unclear whether the level of 5-HT in the plasma reflects the level in the brain (41), but some studies have shown that circulating 5-HT is related to brain tissue serotonin (42).

CONCLUSION

Psychological symptoms diverged from person to person, place of residence, socio-cultural characteristics, work life and stressful events. It can be said that familial characteristics, the effect of serotonin and cortisol, as well as assorted life events, personal characteristics and the reactions of individuals to these events are potent in the emergence of these symptoms. Evaluating the relationship between psychological symptoms, which are highly related to these two biomarkers, and serotonin and cortisol levels separately may require different prevention and intervention strategies due to different effects. More research is needed on how these determinants affect serotonin and cortisol levels.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Clinical Researches Ethics Committee of Ankara Keçiören Training and Research Hospital (Date: 11.07.2012, Decision No: B.10.4.İSM.4.06.68.49)

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

Referee Evaluation Process: Externally peer-reviewed.

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

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J Health Sci Med 2023; 6(1): 165-173

Maden et al. Mental symptoms, serotonin, corstisol and family characteristics

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HEALTH SCIENCES MEDICINE

Effects of stone density on alteration in renal resistive index after extracorporeal shock wave lithotripsy for non-obstructed kidney stones

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ABSTRACT

Aim: The doppler-based renal resistive index is a recently proposed technique for measuring changes in renal perfusion and predicting acute kidney damage. The purpose of this study was to look at the influence of stone density on the renal resistive index (RI) after extracorporeal shock wave lithotripsy (ESWL) in patients with non-obstructed kidney stones.

Material and Method: Between May 2020 and July 2021, 48 consecutive patients with unilateral renal calculi of ≤ 20 mm were treated with ESWL monotherapy. The patients' non-contrast computed tomography (NCCT) images were processed and grouped into two groups using Hounsfield units (HU) (Group 1, n=27, ≤ 1000 HU; Group 2, n=21, > 1000 HU). The same radiologist performed Doppler ultrasonography on all cases before, one hour, and one week following ESWL. Measurement of the RI taken in the remote region (at least 20 mm from the stones). Patient age, gender, BMI, stone laterality, stone size, and stone position were investigated as potential predictors.

Results: The average stone size for Group 1 was 11.7±3.3 mm and 12.1±2.8 mm for Group 2. The mean RI values before ESWL for Group 1 and Group 2 were 0.54 and 0.53, respectively. On comparing the pre-treatment data with the 1 hour after ESWL, a statistically significant increase was recorded in the RI value for both groups. However, there was no significant difference in RI values between groups 1 and 2 1 hour and 1 week following lithotripsy therapy. After one week, the mean RI returned to pretreatment levels, according to a follow-up doppler investigation. There was no association between stone density and RI (p > 0.05).

Conclusion: High stone densities detected with NCCT were not associated with a significant change in RI. Post-ESWL therapy alterations are present and reversible one week after the treatment.

Keywords: Resistive index, stone, kidney

INTRODUCTION

Urinary stones are one of the most frequent urological disorders, affecting millions of people globally and putting a substantial strain on the healthcare system. Extracorporeal shock wave lithotripsy (ESWL) is a well-established minimally invasive therapy for stones in the upper urinary system (1). It employs an extracorporeal lithotripter to repeatedly target and generate shock waves to break up the stones and pass through the urine. The global SARS-COV-2 pandemic may promote ESWL use since it does not require general anesthesia and so avoids its potential repercussions in COVID-19 patients (2).

Although radiological imaging is effective at locating the kidney stone, there can still be localized problems with ESWL. The main vascular hemorrhage is the most common ESWL-related damage (3, 4). After ESWL, 29% of patients developed renal hematomas, which was initially expected to be 1% (5-7).

Renal trauma also triggers an inflammatory response, leading to tissue remodeling and scar tissue formation (7). According to research, canine subjects undergoing ESWL experienced dose-dependent renal fibrosis (8). Renal tissue fibrosis can result in a loss of function in

the affected area, either partially or completely. The resistive index (RI) is a non-invasive approach for assessing changes in renal vascular resistance caused by vascular compliance (9). The RI, which is independent of transducer angle and position and determines the ratio of peak systolic velocity (PSV) to end-diastolic velocity (EDV) in peripheral vessels (RI=(PSV - EDV)/PSV), enables accurate and repeatable measurements (10). Because the RI is evaluated at an artery in the renal parenchyma, it is raised in tubulointerstitial or vascular disorders.

It has been reported that one of the factors affecting the success of ESWL is the average stone density. Stone density >1000 Hounsfield unit (HU) was accepted as predictive for ESWL failure (11). To our knowledge, there are not enough studies in the literature evaluating the relationship between the mean stone index and the resistive index formed after ESWL. Therefore, this study aimed to determine whether there was a correlation between changes in stone density and RI levels in patients receiving ESWL for the treatment of kidney stones.

MATERIAL AND METHOD

The study was carried out with the permission of Fırat University Non-interventional Clinical Researches Ethics Committee (Date: 26.05.2022, Decision No: 2022/07-40). Data from 48 patients who had ESWL for kidney stones between May 2020 and July 2021 were evaluated retrospectively. All procedures were carried out by the ethical rules and the principles of the Declaration of Helsinki. The patient's age, gender, stone dimension, HU, and stone positions were all analyzed. Stones were determined by computed tomography before treatment. According to their HU, patients were classified into two groups (HU) (Group 1; 1≤ 1000 HU, Group 2; > 1000 HU). The study included total patients with normal renal functioning and no hydronephrosis. Patients with ureteral stones, hypertension, parenchymal disease, urinary system infections, diabetes, renal masses, or a record of kidney operation were excluded from the study. Patients were examined on the 1 hour and 7th days after the ESWL process. The RI is a modern, non-invasive diagnostic tool for assessing circulatory system alterations. The benefit of the RI is that it is not impacted by the doppler angle.

Extracorporeal Shock Wave Lithotripsy Procedure

ESWL was performed with an EMD E-1000 electro-hydraulic lithotripter. The device includes X-ray fluoroscopic focus systems. The patients received one to four (mean: 1.65) sessions of ESWL. The mean number of shocks per patient was 1000 to 2000, with shock severity ranging from 10 to 20 kV. (mean, 16 kV).

Doppler Assessment of Kidneys

All US examinations were performed separately by the same radiologist before, one hour after, and one week after ESWL procedures. The ultrasonograph EsoateMyLab 70 was used to assess intrarenal blood vessels using doppler. Each kidney was evaluated in three projections: superior, middle, and inferior. Doppler tests used a pulse wave method with a convex-type head. In all measurements, the Doppler angle was less than 60 degrees. The flow spectrum in the renal interlobular arteries the RI was calculated. In enrolled patients, the flow in the interlobular arteries of the ipsilateral kidney was measured.

Statistical Analyses

In both groups, the paired t-test, a parametric test, was employed to compare RI values one hour and one week after ESWL to pre-ESWL values. The RI values for the two groups were contrasted using the Mann-Whitney U-test. The outcomes are displayed as means SD.

RESULTS

We included 10 (20.8%) women and 38 (79.1%) men in the study. Group 1 (4 women, 17 men) had a mean age of 42.9±13.7, a mean stone size of 11.5±3.35 mm, 2580±1414 (1000-4500) shocks, and a mean number of sessions of 2.1±0.8. In Group 2 (6 women, 21 men), the mean age was 44.3±9.8, the stone size was 12.1±2.8 mm, the number of shocks was 2375±1103 (1200-3750) and the number of treatments was 2.3±0.6. The stones were anatomically placed in the upper calyx (20), mid-calyx (19), and inferior calyx (9). Evaluation of the success rates after 1 month did show that patients in group 1 had a higher rate of stone-free status when compared with group 2 (87.4 vs 67.1%, p=0.043). There were no significant differences in process amount, pre-ESWL RI value, and sessions between groups 1 and 2 (p=0.886).

The mean RI value for Group 1 (0.54±0.13 to 0.64±0.18) and Group 2 (0.53 ±0.11 to 0.66±0.19) significantly increased 1 hour after ESWL treatment. One hour after the RI value, there are no significant differences in Group 1 compared with Group 2 (p=0.93), (**Figure 1**). Although both groups had a significant increase in the RI after the ESWL there is no difference in RI values at 1 week after ESWL between groups (p= 0.92). In both groups, the 1-week ESWL RI values were considerably lower than the 1-hour values. Furthermore, no statistically significant difference in RI values was seen before and after ESWL (**Table**).
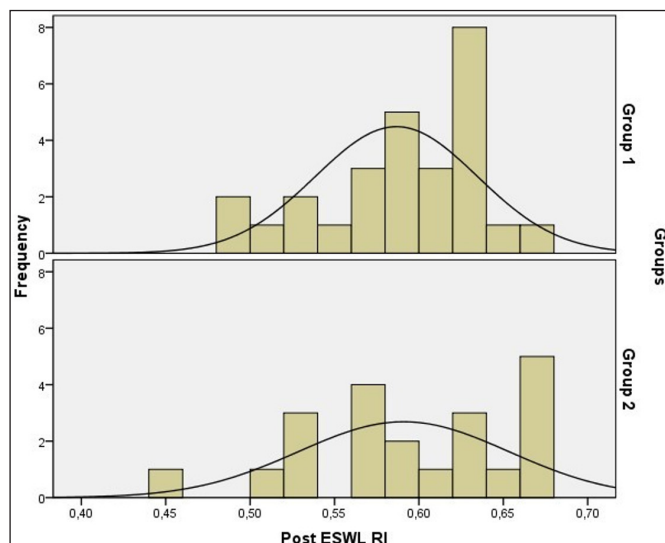
Keleş et al. Effects of stone density on alteration in renal resistive index

J Health Sci Med 2023; 6(1): 174-177

Figure. The number of cases that showed Resistive Index changes before and 1 hour after Extracorporeal shock wave lithotripsy

Table. Comparison of demographic characteristics and renal resistive index changes of study groups

Variables	Groups	Mean	Std. Deviation	Std. Error Mean	P value
Age (years)	Group 1	42.96	13.7	2.63	0.643
	Group 2	44.33	9.81	2.14	
Height (cm)	Group 1	171	8.17	1.57	0.974
	Group 2	170.23	10.21	2.23	
Weight (kg)	Group 1	82.14	10.03	1.93	0.735
	Group 2	85.38	7.32	1.59	
Stone diameter (mm)	Group 1	11.73	3.35	0.64	0.854
	Group 2	12.19	2.8	0.61	
Stone density (HU)	Group 1	643.48	193.81	37.3	**<0.001**
	Group 2	1217	152.71	33.32	
ESWL session (n)	Group 1	2.1	0.8	0.14	0.131
	Group 2	2.3	0.6	0.08	
Pre ESWL RI	Group 1	0.54	0.13	0.1	0.886
	Group 2	0.53	0.11	0.11	
Post ESWL 1-hour RI	Group 1	0.64	0.18	0.11	0.935
	Group 2	0.66	0.19	0.13	
Post ESWL 1 week RI	Group 1	0.58	0.18	0.009	0.928
	Group 2	0.59	0.12	0.136	

The significance of the differences in the values before and after ESWL was evaluated with the help of the Student's paired t-test. ESWL – Extracorporeal shock wave lithotripsy, RRI – Renal resistive index, HU – Hounsfield Unit,

DISCUSSION

European Association of Urology Guidelines recommends ESWL or endourology treatments for stones less than 2 cm as first-line therapy. Even though ESWL is a safe and effective treatment for kidney stones, it might induce problems because shock waves can damage the renal parenchyma (12). Several studies have been conducted to study the morphologic and functional alterations that occur due to ESWL, as well as the underlying causes and potential protective measures. It is thought that the change in RI after ESWL is due to cellular infiltration and edema occurring around the peripheral branches of the renal artery and swelling of the perivascular tissue, which in turn causes an increase in vascular resistance (13). Most investigators have found that these changes resolve rapidly. RI is a non-invasive method that can be used to evaluate changes in renal vascular resistance caused by vascular compliance after ESWL.

According to Derchi et al. (14), although the increase in RI values in calyx stones was not statistically significant 1 hour after ESWL, it decreased to the pre-ESWL level. Knapp et al. (15) suggested utilizing color doppler ultrasonography as a non-invasive approach for determining changes in renal vascularisation after ESWL. They reported an increase in RI, particularly among the elderly (those over the age of 60). Similarly, Janetschek et al. (16) (Age groups: <40 years, 40–59 years, and >60 years) found that RI values were significantly higher in the region around the stone within 3 hours after the ESWL procedure in the third group, but this was not observed in the first two groups. It was thought that this condition developed due to sclerosis of the renal vessels and loss of elasticity. It has been stated that due to the low tolerance of elderly patients, changes related to the same amount of energy may be greater than in younger patients. Nazaroglu et al. (17) found a transient increase in RI in both kidneys within hours after the procedure in patients undergoing ESWL for kidney stones. They stated that this increase was highest in the vicinity of the stone and that it was the least in the healthy kidney and returned to normal values within 2 weeks.

As evaluated by HU on CT, stone density has been examined as a possible predictor of treatment effects in various ways. Indeed, stone density >1000 HU on CT was accepted as a predictor for ESWL failure (17). However, to date, no study has evaluated the relationship between stone density and RI changes. Our findings revealed that there is no relationship between RI alterations and stone density. The current investigation shows a transient increase in RI values in the treated kidney after ESWL. Within a week, RI levels in the surrounding area revert to normal. A comparison of the findings with those of other studies confirms that ESWL therapy did not affect RI value in the long term. Because some studies have indicated that untreated kidneys had no substantial RI alterations, we did not plan to measure RI in the contralateral untreated kidney (18).

The most significant drawback of our study was the small number of patients included in subgroup comparisons. We could attain more solid findings by splitting the cases into density groups and stone site subgroups if we had access to a large enough number of patients. Secondly, renal tissue changes following ESWL were not histopathologically verified.

CONCLUSION

In our study, the stone density implication of RI changes induced by electro-hydraulic lithotripter remains to be answered. However, the clinical significance of our findings should be investigated further.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Fırat University Non-interventional Clinical Researches Ethics Committee (Date: 26.05.2022, Decision No: 2022/07-40).

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

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Determination of the frequency of influenza-A and B antigens in swab samples in differentiating the diagnosis of influenza infection from other causes of upper respiratory tract infection

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ABSTRACT

Aim: The present study aimed to determine the frequency of influenza-A and B antigens in swab samples and to examine their potential changes at the time of initial diagnosis in differentiating the diagnosis of influenza infection from other causes of upper respiratory tract infection by physical examination and vital clinical signs in the emergency room.

Material and Method: This retrospective cross-sectional descriptive research analyzed 113 patients with Influenza-A (n:8) and B (n:15) over the age of 18 who applied to the emergency department in the last three years, were diagnosed with acute upper respiratory tract and underwent nasopharyngeal swab sampling were included in the study. The data of the patients were accessed digitally from the University hospital database.

Results: The headache score was higher, while there was no difference between vital clinical signs and those with positive or negative swab tests (p>0.05). White blood cells in blood count parameters were lower in the Influenza test-positive group (p<0.0001), platelet distribution width (p=0.006), and monocyte counts (p=0.008) were significantly higher in Influenza positive patients than negative ones. The influenza swab test was not positive in any patient with tonsillar crypt (p>0.05).

Conclusion: Influenza infections should be examined in detail in terms of costs to both public health and social security institutions, considering the burdens of diagnosis and treatment.

Keywords: Influenza-A, influenza-B, swab sample, upper respiratory tract infection

INTRODUCTION

Influenza, a single-stranded RNA virus of the Orthomyxoviridae family that can affect human and animal populations, frequently causes acute respiratory tract infections (1). Although difficult to assess, it is estimated to generate more than 1 billion yearly cases, including severe disease (2). After a short incubation period, sudden onset of weakness, cough, fever, runny nose, sore throat, muscle pain, and frequent symptoms of this disease, whose course is more severe in children, pregnant women, those with chronic conditions, and immunodeficiency (3).

Considering the development of resistance due to antibiotics, rapid and accurate diagnosis of the Influenza virus is essential in starting early treatment, reducing contagiousness, preventing unnecessary antibiotic use, and obtaining epidemiological data (4). Although methods such as antigen testing, polymerase chain reaction, or culture are most commonly preferred in the diagnosis of Influenza virus infections, nasopharyngeal swabs and rapid antigen tests are used in the diagnosis of Influenza virus infections due to immediate results and ease of sampling in emergency medicine practice (5,6). These tests, which are successful in distinguishing between Influenza A and B, are insufficient to distinguish subtypes of Influenza A (7). We observe that Influenza rapid antigen tests are more sensitive than Influenza-B in detecting Influenza A infection, and the preference for nasopharyngeal swab samples increases this sensitivity. Determining the frequency of Influenza A and B antigens in swab samples is of clinical importance in differentiating the diagnosis of Influenza infection from other causes of upper respiratory tract infection by physical examination and vital clinical findings (6).

This study aims to determine the frequency of Influenza-A and B antigens in swab samples and to examine their potential changes at the time of initial diagnosis in differentiating the diagnosis of Influenza infection from other causes of upper respiratory tract infection by physical examination and vital clinical signs in the emergency room. In addition, it is to explain the access to healthcare resources in the most appropriate way in terms of the health status, number, and disability of patients in future Influenza pandemics.

MATERIAL AND METHOD

The study was carried out with the permission of İstinye University Clinical Researches Ethics Committee (Date: 07.11.2022, Decision No: 3/2022.K-85). All procedures were carried out following the ethical rules and the principles of the Declaration of Helsinki.

Study Design

This retrospective cross-sectional descriptive research analyzed 113 patients with Influenza-A (n:8) and B (n:15) over 18 years old who applied to the emergency department in the last three years, were diagnosed with acute upper respiratory tract and underwent nasopharyngeal swab sampling were included in the study. The data of the patients were accessed digitally from the University hospital database. Patients' complaints, vital clinical signs, physical examination findings, antibiotic use in the last week, complete blood count and blood biochemistry values, and Influenza swab test reports were retrospectively analyzed from the database.

Inclusion and Exclusion Criteria

Inclusion criteria covered the cases over 18 years of age diagnosed with acute upper respiratory tract infections and underwent nasopharyngeal swab sampling in the emergency department. Exclusion criteria covered any cases whose files cannot be accessed or are missing, patients with acute upper respiratory tract diagnosis who have used antiviral drugs in the last week, patients whose tests have not been performed, and patients who refused treatment.

Laboratory Assessment

The parameters were analyzed within one hour after being taken on the second day of hospitalization with the standard tubes of routine sampling in the morning after 24-hour fasting. Laboratory measurements included leukocyte, neutrophil, lymphocyte, monocyte, and platelet count (SYSMEX Hemogram Autoanalyzer) as well as serum albumin and CRP (Cobas6000 Biochemistry Autoanalyzer).

Statistical Analysis

We retrospectively collected data, including demographics, laboratory, and clinical findings, and analyzed the windows-based software of IBM-SPSS v26.0. GraphPad Prism v9.4.1 software draws scatter plots or column bar graphs. We collected routine blood count data. The Chi-square test analyzed the categorical data. Categorical data was given as n (%), while all the continuous data were expressed as mean±standard deviation. The Kolmogorov-Smirov test was used to analyze the normality. We used Mann-Whitney U for abnormally distributed data (such as monocyte and lymphocyte count), while the independent Sample T-test was used for normally distributed data. The study accepted P<0.05 significance based on a two-way test.

RESULTS

Patients' Characteristics

Influenza swab test was positive in 23 of them (8 Influenza-A / 15 Influenza-B). **Table 1** showed no significant difference between the two groups when comparing kidney function tests, electrolytes, and CRP levels (p>0.05). While headache scores were higher and close to significant, there was no difference between vital clinical signs and those with positive or negative swab tests (p>0.05). WBC (white blood cell) counts in complete blood count parameters were lower in the Influenza test positive group (10.4±2.8 vs. 6.1±2.1; p<0.001), platelet distribution width (10.1±1.6 vs.13.8±2.3; p=0.006), and monocyte counts (8.9±3.8 vs. 11.2±4.1; p=0.008) were found to be significantly higher in Influenza positive patients than negative ones.

Table 1. Complaints and laboratory values			
Features	**Influenza (-)** (n:90)	**Influenza (+)** (n:23)	**P value**
Body Heat, °C	37.6±0.9	37.4±0.8	0.516
Heart Rate, min	96.7±19.7	91.0±16.8	0.521
CRP, mg/dL	5.1±11.4 (5)	3.6±5.6 (4.8)	0.887
WBC, 10³/mm³	10.4±2.8	6.1±2.1	p<0.001
PDW, fL	10.1±1.6	13.8±2.3	0.006
Platelet, 10³/mm³	220.1±50.9	217.6±63.4	0.867
Neutrophile, 10³/mm³	69.4±17.6	67.6±10.9	0.204
Lymphocyte, 10³/mm³	19.5±39.3 (27.3)	19.5±9.8 (26)	0.129
Monocyte, 10³/mm³	8.9±3.8 (8.1)	11.2±4.1(11.7)	0.008
Abbreviations. CRP: C-reactive protein, PDW: Platelet distribution width, WBC: White Blood Cell * Abnormally distributed data, including CRP, Monocyte, and Lymphocyte, were analyzed with Mann Whitney-U test and median values were given in brackets, while other data were analyzed with the Independent Student T-test. All data were presented as mean±standard deviation.			

A history of cough was higher in the group with a positive test result. The influenza swab test was not positive in any patient with tonsillar crypt. Submandibular tenderness was significantly less in the group with a positive

Influenza test. The striking part is that antibiotic use in the last week was significantly higher in those with positive test results (**Table 2**).

Table 2. History and physical examination findings			
Variables	Influenza (-) (n:90)	Influenza (+) (n:23)	P value
Cough	48.9% (44)	87% (20)	0.004
Nausea	32.3% (29)	39.1% (9)	0.531
Vomiting	11.1% (10)	4.3% (1)	0.091
Feeling of fever	77.8% (70)	82.6% (19)	0.613
Sneezing	18.9% (17)	30 4% (7)	0.454
Hoarseness	34.4% (31)	52.2% (12)	0.118
Eye redness	14.4% (13)	17.4% (4)	0.724
Tonsillar crypt	37.8% (34)	0% (0)	p<0.001
Tonsillar erythema	42.2% (38)	30.4% (7)	0.221
Joint pain	68.9% (62)	78.3% (18)	0.583
Continuous drug use	13.3% (12)	8.7% (2)	0.725
Cervical LAP	8.9% (8)	4.3% (1)	0.473
Submandibular tenderness	56.7% (51)	26.1% (6)	0.009
Postauricular LAP	4.4% (4)	0% (0)	0.303
Pharyngeal petechiae	2.2% (2)	4.3% (1)	0.571
Throat Kx strep	6.7% (6)	0% (0)	p<0.001
* All categorical data were analyzed with the chi-square test. All data were given as n (percent).			

DISCUSSION

It was exciting that leukocyte was lower in the influenza test positive group, while the PDW and monocyte counts were higher in the positive influenza group. While those with a history of cough were significantly higher in the group with positive test results, the influenza swab test was not positive in any patient with tonsillar crypt. As a remarkable result, antibiotic use in the last week was significantly higher in those with positive test results. Our results in diagnosing patient differentiation may benefit physicians.

Depending on the virus and host characteristics, Influenza typically consists of malaise, fever, chills, headache, and myalgia, with clinical presentation ranging from asymptomatic infections to severe illness (8, 9). Headache and myalgia involving the extremities or back muscles are often the most bothersome symptoms, and Respiratory symptoms are also present at the onset of the disease (10, 11). Although dry cough, pharyngeal pain, and runny nose are prominent, they are usually overshadowed by the systemic symptoms that distinguish Influenza from other upper respiratory tract infections (12). Fever usually lasts for three days continuously but may remain high for up to 8 days, and systemic symptoms subside upon remission (13). Additional findings on physical examination are a flushed face, warm, moist skin, clear runny nose, hyperemic nasal and throat mucosa, and small, tender cervical lymphadenopathies. In our study, while

headache scores were higher and close to significant, there was no difference between vital clinical signs and those with positive or negative swab tests. White blood cells in complete blood count parameters were lower in the Influenza positive group, and platelet distribution width and monocyte were higher in Influenza positive than in the negative ones.

Leading cause of influenza-related mortality, two pulmonary complications are frequently associated with Influenza (14). Primary influenza pneumonia begins with typical influenza symptoms and is followed by rapid respiratory decompensation with severe dyspnea, cyanosis, and hypoxemia (15, 16) a. Secondary bacterial pneumonia develops biphasic; symptoms resolve after the first presentation of typical Influenza and are associated with shortness of breath, productive cough, and consolidation in chest imaging (17). The similarities in these upper respiratory tract infections increase the importance of the distinction. In our study, a history of cough was higher in the group with a positive test result. The influenza swab was not positive in patients with tonsillar crypt. Submandibular tenderness was found less in the group with a positive Influenza test.

High creatinine was observed in more than 50% of hospitalized Influenza A patients. While symptoms usually resolve after 4-6 weeks, CNS involvement causes higher morbidity in adult patients (18). Influenza can also be complicated by altered kidney function, including acute kidney injury, glomerulonephritis, and tubulointerstitial nephritis (19). Liver damage has also been associated with Influenza, as patients may experience elevated AST and ALT, more commonly exacerbating underlying cardiac disorders (20). Our study found no significant difference between the two groups when comparing kidney function tests, electrolytes, and CRP levels.

Research results indicate that the preferred methods for influenza B antigen detection exhibit lower sensitivity than those for Influenza A detection (21). Influenza B virus-associated antigen and secretion levels are lower than for A virus infections. This may explain the low diagnostic levels for the influenza B virus due to the prevalence of anti-influenza antibodies in nasal secretions, as nasal secretions in influenza A and B virus infections likely contain similar amounts of anti-influenza virus immunoglobulins (22). Influenza swab test was positive in 23 of them (8 Influenza-A / 15 Influenza-B). There was no difference between vital clinical signs and those with positive or negative swab tests. The essential part is that antibiotic use in the last week was significantly higher in those with positive test results.

In primary health care, identifying the pathogen causing respiratory diagnosis should preferably be made during the visit. An integrated multianalyte testing panel is needed to make the laboratory diagnosis cost-efficient. Such a testing panel would comprise reagents for six to nine different bacterial and viral pathogens, sufficient to cover a significant proportion of respiratory infection cases. The use of such a diagnostic product in primary health care would enable the differentiation between bacterial and viral infections, promote correct treatment, and decrease the unnecessary use of antibiotics.

The study's main limitations are its retrospective design with limited cases and the routine variability of physiological conditions. Overcoming the limitations and making generalizable presentations will only be possible with a large population in a prospective design.

CONCLUSION

In a remarkable conclusion, antibiotic use in the last week was significantly higher in those with positive test results. Leukocyte was lower in the influenza-positive group, while the PDW and monocyte were higher in the influenza-positive group. While those with a history of cough were significantly higher in the group with positive test results, the influenza swab test was not positive in any patient with tonsillar crypt. Influenza infections should be examined in detail in terms of costs to both public health and social security institutions, considering the burdens of diagnosis and treatment on the patient and society. The fact that health institutions collect polyclinic and emergency service data healthily and present them to the authorized units for interpretation will contribute to the work and long-term planning of health policies, social services, and social security institutions on a regional basis, regionally, and on a country basis in general.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstinye University Clinical Researches Ethics Committee (Date: 07.11.2022, Decision No: 3/2022.K-85).

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 has no conflicts of interest to declare.

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Prognostic role of primary tumor metabolic-volumetric parameters of ¹⁸F-fluorodeoxyglucose positron emission tomography in tongue squamous cell carcinoma

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ABSTRACT

Aim: It was aimed to evaluate the prognostic role of primary tumor metabolic-volumetric parameters of ¹⁸F-Fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography/computed tomography (PET/CT) in resectable tongue squamous cell carcinoma (TSCC).

Material and Method: The imaging findings of 44 TSCC patients (23 females, 21 males, mean age: 58±12 years) with resectable tumors who underwent ¹⁸F-FDG PET/CT imaging for primary staging before surgery between 2010-2021 were evaluated retrospectively. Maximum standardized uptake value (SUVmax), total lesion glycolysis (TLG), metabolic tumor volume (MTV) of primary tumors were acquired from PET/CT. Histopathological risk factors (pathological tumor and nodal stage, perineural and lymphovascular invasion, depth of invasion, surgical margin positivity) obtained from surgical resection material of primary tumors were also recorded. The prognostic values of imaging and histopathological parameters were assessed by Cox proportional hazards regression models. Survival curves were estimated by using the Kaplan-Meier analysis.

Results: The median follow-up period after diagnosis was 24 months (range: 2-152 months). The univariate and multivariate regression analyses demonstrated that MTV was the only parameter which was significantly related to prognosis for PFS and OS. The patients with higher MTV (> 3.13 cm³) had lower PFS and OS rates compared to those with lower MTV (≤ 3.13 cm³) (p<0.001, p=0.002, respectively).

Conclusion: Primary tumor MTV is an independent prognostic factor in resectable TSCC. PET volumetric features can be used as prognostic biomarker to predict patients with poor prognosis.

Keywords: FDG, metabolic tumor volume, PET/CT, survival, tongue squamous cell carcinoma

INTRODUCTION

Tongue squamous cell carcinoma (TSCC) is the most common malignancy in oral cavity (1). Despite improvements in diagnostic tools and treatment modalities, the mortality rate of TSCC has not diminished in recent years and 5-year survival rate for TSCC is still below 50% (2,3). Tumor-node-metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC) is the most commonly used method to determine treatment modalities and to predict patient prognosis. Nevertheless, the prognosis of patients within the same TNM stage may vary (4). In addition to TNM staging system, various histopathological risk factors (depth of invasion, perineural invasion [PNI], surgical margin status, lymphovascular invasion [LVI],) in resectable

TSCC can be used to predict patient prognosis (5-9). However, histopathological prognostic markers can only be obtained after surgical resection. New prognostic biomarkers which can be obtained in the preoperative period and allow individualized risk stratification are needed in order to improve patient survival.

Fluorine-18 fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography / computed tomography (PET/CT) imaging is a hybrid molecular imaging system used in primary staging of head and neck cancers for detection of lymph node and distant metastasis (10-13). FDG PET imaging can also have prognostic role in TSCC patients. The previous studies demostrated that the PET quantitative features of primary tumors, such

Aydos et al. Prognostic role of FDG PET in TSCC

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as maximum standardized uptake value (SUVmax), total lesion glycolysis (TLG), metabolic tumor volume (MTV), could contribute to the prediction of survival in TSCC (14-17). However, the studies on this subject are still few and the validation of the previous results with new studies is needed.

The main aim of the study was to investigate the prognostic role of ^{18}F-FDG PET/CT metabolic-volumetric parameters of primary tumor in resectable TSCC. It was also aimed to examine the association between histopathological risk factors and quantitative FDG PET parameters of primary tumors.

MATERIAL AND METHOD

The study was carried out with the permission of Gazi University Clinical Researches Ethics Committee (Date: 05.12.2022, Decision No: 889). Because the study was designed retrospectively, no written informed consent form was obtained from patients. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

Patients with diagnosed resectable TSCC, who underwent primary staging whole body ^{18}F-FDG PET/CT before surgical resection between April 2010 and November 2021 in our hospital, were reviewed retrospectively. Patients who (a) had distant metastasis, (b) were inoperable, and (c) received neoadjuvant therapy were excluded. Finally, 44 patients (21 males, 23 females, mean age: 58±12 years) were included. Histopathological risk factors (pathological tumor and nodal stage, PNI and LVI, depth of invasion, surgical margin positivity) obtained from surgical resection material of primary tumor were recorded. The median time between preoperative PET/CT and surgical resection was 10 days (range, 2-28 days). After surgical resection, TNM stages were determined by using the AJCC staging guidelines (8th edition) (18).

^{18}F-FDG PET/CT

All patients fasted for 4 to 6 hours prior to the PET/CT scan. Blood glucose levels were less than 150 mg/dl in all patients before ^{18}F-FDG injection (3,7 MBq/kg). PET/CT was performed on a GE Discovery ST PET/CT system (General Electric Medical Systems, Milwaukee, WI, USA) with a spatial resolution of 5 mm, 60 minutes after radiotracer injection. A low dose CT scan without contrast injection (120 keV, 10-90 mA) was acquired. Following the completion of CT scan, a PET scan (from vertex to mid-thigh) with an axial field of view of 15.7 cm was acquired. The CT data were used to obtain the attenuation corrected PET emission data. PET scan duration was 3 minutes per bed position.

Image Analysis

PET/CT images were evaluated quantitatively by one nuclear medicine specialist who was blinded to the patient outcomes. Tumor SUVmax, TLG and MTV were obtained on a dedicated workstation (GE Healthcare, AW Workstation Volume Share 2). The volume of interest (VOI) was drawn on primary tumor by using the software with 42% threshold of SUVmax. TLG was obtained as the product of SUVmean and MTV.

Statistical Analysis

The relationship of PET parameters with histopathological risk groups were analyzed by using the Kruskal-Wallis test and the Mann-Whitney U test. Spearman's rank correlation was used to analyze the correlation levels between FDG PET parameters and histopathological risk factors. Progression-free survival (PFS) time was the time from the diagnosis to the date of disease related clinical relapse, or to the date of last follow-up visit. Overall survival (OS) time was the time from the diagnosis to the date of death or to the date of the final follow-up visit. The prognostic significances of the variables were evaluated by univariate an multivariate analyses using Cox proportional hazards regression models. The optimal cut-off levels of quantitative PET parameters were determined in the receiver operating characteristic (ROC) curve analysis by using the Youden index (sensitivity + specificity-1). Survival curves were estimated by using the Kaplan-Meier analysis and for comparison the log-rank test was used. SPSS 23.0 (IBM, New York) software was used for statistical analyses. For all analyses, p values of <0.05 were considered statistically significant.

RESULTS

Patients

The patient characteristics were shown in **Table 1**. 21 patients were male (47.7%) and the mean age was 58.0±12.0 years at the time of diagnosis. The median pathological tumor size was 2.6 cm. The LVI, PNI, surgical margin were positive in 10 (22.7%), 22 (50%) and 7 (15.9%) patients, respectively. The median depth of invasion was 10.3 mm and median distance from the closest surgical margin was 3 mm. Elective neck dissection was performed in all patients and revealed cervical LN metastasis in 21 patients. According to the AJCC staging system, 6 patients (13.6%) had stage I, 10 (22.7%) had stage II, 14 (31.8%) had stage III and 14 (31.8%) had stage IV disease. All primary tumors had ^{18}F-FDG uptake. The median SUVmax, MTV and TLG were 10.5 (range: 3.5-23.3), 4.3 (range: 0.88-38.2) and 24.9 (2.3-371.3), respectively.

J Health Sci Med 2023; 6(1): 183-189

Aydos et al. Prognostic role of FDG PET in TSCC

Table 1. The characteristics of patients	
Age (mean±SD)	58.0±12.0 years
	Median (minimum-maximum)
Maximum standardized uptake value (SUVmax)	10.5 (3.5-23.3)
Metabolic tumor volume (MTV, cm³)	4.3 (0.88-38.2)
Total lesion glycolysis (TLG)	24.9 (2.3-371.3)
Histopathological tumor diameter (cm)	2.6 (0.3-7.2)
Depth of invasion (mm)	10.3 (2.0-45.0)
Distance from closest surgical margin (mm)*	3.0 (1.0-8.0)
	N (%)
Gender	
Female	23 (52.3)
Male	21 (47.7)
Pathological tumor stage (pT)	
T1	8 (18.2)
T2	14 (31.8)
T3	16 (26.4)
T4	6 (13.6)
Pathological nodal stage (pN)	
N0	23 (52.3)
N1	9 (20.5)
N2	4 (9.1)
N3	8 (18.2)
AJCC stage (8th)	
I	6 (13.6)
II	10 (22.7)
III	14 (31.8)
IV	14 (31.8)
Tumor differentiation	
Well-differentiated	29 (65.9)
Moderately differentiated	13 (29.5)
Poorly differentiated	2 (4.5)
Lymphovascular invasion	
Negative	34 (77.3)
Positive	10 (22.7)
Perineural invasion	
Negative	22 (50.0)
Positive	22 (50.0)
Surgical margin status	
Negative	37 (84.1)
Positive	7 (15.9)
Tumor Recurrence	
Negative	22 (50.0)
Positive	22 (50.0)
Mortality	
Yes	19 (43.2)
No	25 (56.8)
*In patients with negative surgical margins	

The median follow-up period was 24 months (range: 2-152 months). 22 patients (50%) had tumor recurrence and 19 patients (43.2%) had died during the follow-up.

Relationship Between PET Quantitative Parameters and Histopathological Risk Factors

TLG and MTV were significantly higher in the pT stage T3-T4 group compared to the pT stage T1-T2 group (TLG: 51.6 vs 13.1, respectively, p=0.003; MTV: 6.0 vs 2.4 cm³, respectively, p=0.001). Tumor SUVmax was not significantly different among pT stage categories (p=0.336). FDG PET parameters did not demonstrate significant differences among pN stage categories (p>0.05 for all).

Tumor SUVmax, MTV, TLG had significantly higher values in the patients with LVI than those without LVI (SUVmax: 14.4 vs 9.3, respectively, p=0.028; MTV: 8.0 cm³ vs 3.3 cm³, respectively, p=0.01; TLG: 76.3 vs 18.2, respectively, p=0.005).

Tumor SUVmax, MTV, TLG had significantly higher values in the patients with PNI than those without PNI (SUVmax: 11.9 vs 8.7, respectively, p=0.031; MTV: 5.9 cm³ vs 2.8 cm³, respectively, p=0.005; TLG: 51.6 vs 12.7, respectively, p=0.003).

MTV and TLG had significantly higher median values in patients with positive surgical margin compared to patients with negative surgical margin (MTV: 15.2 cm³ vs 4.0 cm³, respectively, p=0.041; TLG: 91.2 vs 19.3, respectively, p=0.015). However, tumor SUVmax was not significantly different among surgical margin categories (p=0.125).

Tumor SUVmax was significantly but moderately correlated with histopathological tumor diameter (r=0.47, p=0.001) and depth of invasion (r=0.453, p=0.006). However, MTV and TLG had significant stronger positive correlations with histopathological tumor diameter (r=0.70 and 0.717, respectively, p<0.001 for both) and depth of invasion (r=0.70 and 0.727, respectively, p<0.001 for both). While the correlation between tumor SUVmax and the distance from the closest surgical margin was not significant (p=0.37), MTV and TLG had significant moderate negative correlations with the distance from the closest surgical margin (r=-0.408 and -0.465, p<0.05 for both).

Survival Analysis for PFS

The median PFS in all study population was 24 months. On univariate analysis, TLG, MTV, pathological T stage, AJCC stage, LVI and PNI were significant predictors of PFS (p<0.05 for all; **Table 2**). On multivariate analysis, MTV of primary tumor was identified as the only significant parameter for PFS (hazard ratio [HR], 9.2; 95% confidence interval [CI], 1.53-55.2; p=0.015; **Table 2**).

The Kaplan-Meier analysis demonstrated that patients with higher tumor MTV (> 3.13 cm³) had significantly lower PFS rate than the patients with lower MTV (28.6% vs. 87.5%; p<0.001; **Figure 1**).

Aydos et al. Prognostic role of FDG PET in TSCC

J Health Sci Med 2023; 6(1): 183-189

Table 2. Results of univariate and multivariate Cox regression analyses of progression-free survival (PFS)

Variables	Categories	HR (95% CI) (Univariate)	P	HR (95% CI) (Multivariate)	P
Gender	Male vs Female	1.62 (0.69-3.8)	0.270	-	-
Age	-	1.03 (0.99-1.07)	0.125	-	-
SUVmax	≤ 9.86 vs>9.86	2.07 (0.84-5.12)	0.114	-	-
MTV	≤ 3.13 vs>3.13	8.84 (2.1-38.1)	**0.003**	9.2 (1.53-55.2)	**0.015**
TLG	≤ 18.15 vs>18.15	3.34 (1.23-9.1)	**0.018**	2.1 (0.5-8.54)	0.316
Pathological tumor diameter (cm)	≤ 2.4 vs>2.4	2.12 (0.89-5.1)	0.091	-	-
Pathological T stage	T1-T2 vs T3-T4	3.28 (1.33-8.1)	**0.01**	1.15 (0.36-3.65)	0.813
Pathological N stage	N0 vs N1-N2-N3	1.78 (0.76-4.16)	0.183	-	-
AJCC Stage	I-II-III vs IV	3.28 (1.39-7.72)	**0.007**	2.68 (0.92-7.81)	0.072
Tumor differentiation	Well vs Moderate-Poor	1.77 (0.76-4.11)	0.183	-	-
Lymphovascular invasion	Negative vs Positive	3.53 (1.42-8.76)	**0.006**	1.58 (0.53-4.7)	0.413
Perineural invasion	Negative vs Positive	3.54 (1.42-8.83)	**0.007**	2.24 (0.7-7.17)	0.173
Surgical margin	Negative vs Positive	2.6 (0.95-7.13)	0.062	-	-

The bold entries indicate a significant result. Abbreviations: HR: hazard ratio, CI: confidence interval, SUV: standardized uptake value, MTV: metabolic tumor volume, TLG: total lesion glycolysis, AJCC: American Joint Committee on Cancer
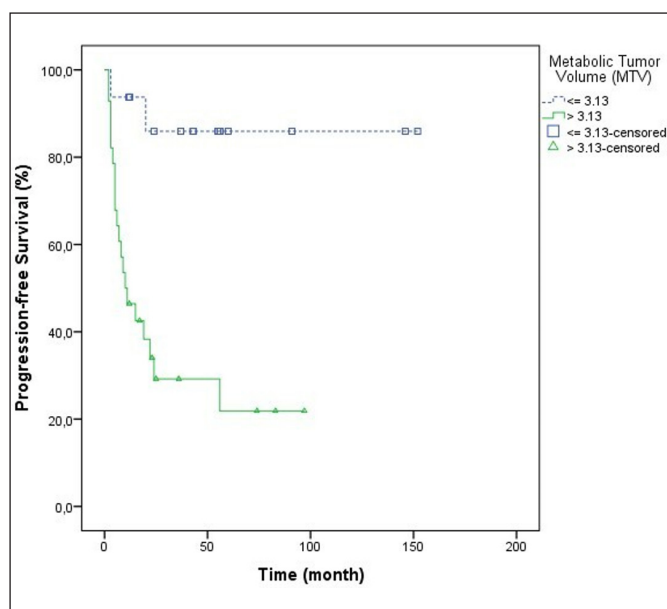
Figure 1. Kaplan-Meier curve of progression-free survival (PFS) of study subjects (n= 44) according to the MTV of primary tumors (Log Rank p<0.001). Patients with higher tumor MTV (> 3.13 cm³) had significantly lower PFS rates compared to those with lower MTV (28.6% vs. 87.5%, respectively).

Survival Analysis for OS

The median OS in all study population was 44 months. On univariate analysis, TLG, MTV, pathological T stage, pathological N stage, AJCC stage, LVI, PNI and surgical margin positivity were significant predictors of OS (p<0.05 for all; **Table 3**). On multivariate analysis, MTV of primary tumor was found as the only significant prognostic parameter for OS (HR, 9.5; 95% CI, 1.5-60.6; p=0.017; **Table 3**).

The Kaplan-Meier curves demonstrated that patients with higher tumor MTV (> 3.13 cm³) had significantly lower OS rate than the patients with lower MTV (39.3% vs. 87.5%; p=0.002; **Figure 2**). PET/CT images of two patients with TSCC were shown in **Figure 3** and **Figure 4**. Tumor SUVmax, MTV and TLG of the patient in **Figure 3** were 21.3, 1.37 cm³ and 20.1, respectively. The patient had no tumor recurrence and is alive at 146 months after cancer diagnosis. Tumor SUVmax, MTV and TLG of the other patient in **Figure 4** were 13.5, 10.37 cm³ and 88.9, respectively. This patient had tumor recurrence at 24 months and had died at 33 months after diagnosis.

Table 3. Results of univariate and multivariate Cox regression analyses of overall survival (OS)

Variables	Categories	HR (95% CI)(Univariate)	P	HR (95% CI)(Multivariate)	P
Gender	Male vs Female	2.58 (1.0-6.6)	0.056	-	-
Age	-	1.03 (0.99-1.1)	0.142	-	-
SUVmax	≤ 9.86 vs>9.86	2.62 (0.94-7.32)	0.066	-	-
MTV	≤ 3.13 vs>3.13	7.5 (1.72-32.7)	**0.007**	9.5 (1.5-60.6)	**0.017**
TLG	≤ 18.15 vs>18.15	3.7 (1.23-11.2)	**0.02**	2.47 (0.49-12.5)	0.276
Pathological tumor diameter (cm)	≤ 2.4 vs>2.4	1.96 (0.77-5.0)	0.157	-	-
Pathological T stage	T1-T2 vs T3-T4	3.1 (1.17-8.2)	**0.023**	1.41 (0.37-5.36)	0.615
Pathological N stage	N0 vs N1-N2-N3	2.84 (1.11-7.3)	**0.03**	1.25 (0.33-4.7)	0.743
AJCC Stage	I-II-III vs IV	5.16 (2.04-13.1)	**0.001**	2.88 (0.64-13.0)	0.169
Tumor differentiation	Well vs Moderate-Poor	2.37 (0.96-5.84)	0.062	-	-
Lymphovascular invasion	Negative vs Positive	4.4 (1.65-11.7)	**0.003**	1.5 (0.38-5.8)	0.565
Perineural invasion	Negative vs Positive	4.87 (1.71-13.9)	**0.003**	2.95 (0.73-11.9)	0.130
Surgical margin	Negative vs Positive	3.46 (1.22-9.8)	**0.019**	2.83 (0.65-12.4)	0.167

The bold entries indicate a significant result.Abbreviations: HR: hazard ratio, CI: confidence interval, SUV: standardized uptake value, MTV: metabolic tumor volume, TLG: total lesion glycolysis, AJCC: American Joint Committee on Cancer
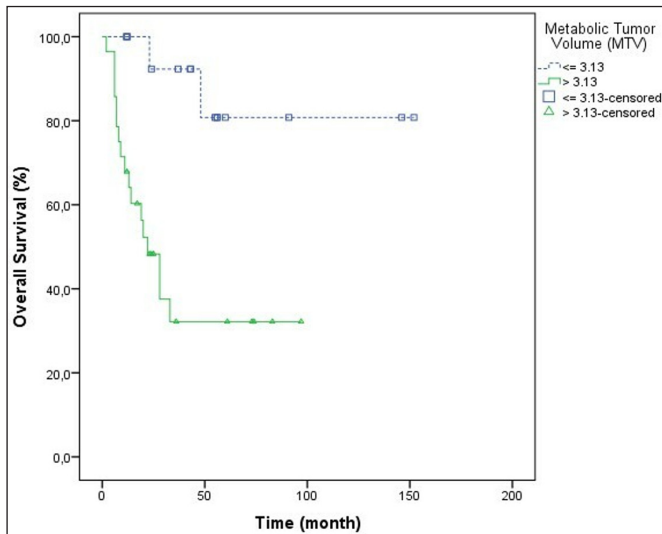
J Health Sci Med 2023; 6(1): 183-189

Aydos et al. Prognostic role of FDG PET in TSCC

Figure 2. Kaplan-Meier curve of overall survival (OS) of study subjects (n= 44) according to the MTV of primary tumors (Log Rank p = 0.002). Patients with higher tumor MTV (> 3.13 cm³) had significantly lower OS rates compared to those with lower MTV (39.3% vs. 87.5%, respectively).
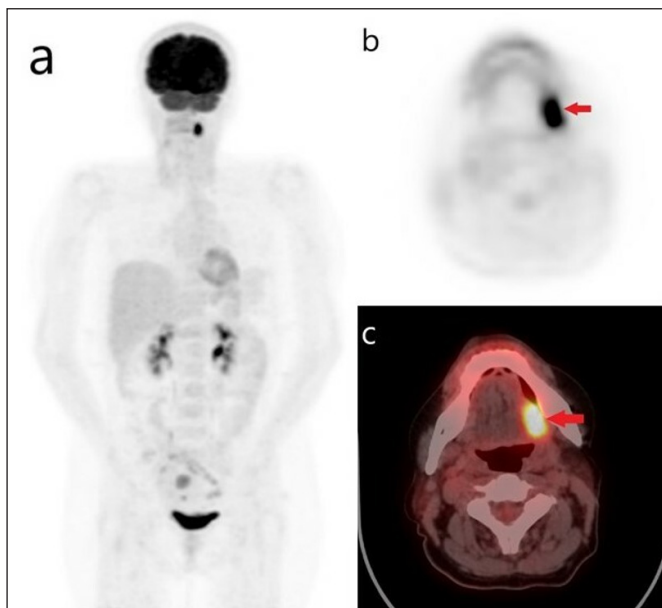

Figure 3. Maximum intensity projection (a), axial ¹⁸F-FDG PET (b) and PET/CT images (c) of a 54-year-old female patient with TSCC. Primary tumor was seen on the left lateral part of the tongue (red arrows). She had pT2-pN0 tumor with LVI and PNI. The surgical margin was positive and the depth of invasion was 5 mm. Tumor SUVmax, MTV and TLG were 21.3, 1.37 cm³ and 20.1, respectively. The patient had no tumor recurrence and is alive at 146 months after initial cancer diagnosis.

DISCUSSION

In this study, it was demonstrated that primary tumor TLG and MTV had significant associations with higher pathological tumor stage (pT3-T4), LVI, PNI and positive surgical margin status. Moreover, MTV and TLG showed higher correlations with histopathological tumor diameter and depth of invasion compared to SUVmax. In patients with negative surgical margins, MTV and TLG showed significant negative correlations with the distance from the closest surgical margin, while SUVmax
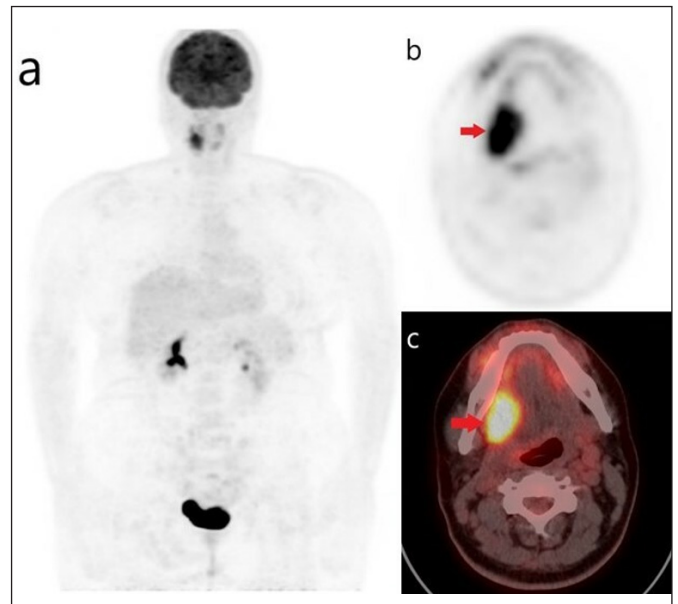
Figure 4. Maximum intensity projection (a), axial ¹⁸F-FDG PET (b) and PET/CT images (c) of a 33-year-old female patient with TSCC. Primary tumor was seen on the right lateral part of the tongue (red arrows). She had pT2-pN0 tumor with PNI but without LVI. The surgical margin was negative, the distance from the closest surgical margin was 2 mm and the depth of invasion was 6 mm. Tumor SUVmax, MTV and TLG were 13.5, 10.37 cm³ and 88.9, respectively. The patient had tumor recurrence at 24 months and had died at 33 months after initial cancer diagnosis.

did not have significant correlation. This study also demonstrated that MTV, as a PET metabolic-volumetric feature, was the only independent prognostic parameter for PFS and OS.

Histopathological tumor and nodal stages, LVI, PNI, surgical margin status and depth of invasion were shown as poor prognostic histopathological risk factors in TSCC (5-9, 19-21). In our study, TLG and MTV were significantly higher in higher pT stage (T3-T4) tumors, in the presence of LVI, PNI and positive surgical margin. While tumor SUVmax had significantly higher values in the presence of LVI and PNI, it did not have a significant association with pathological tumor stage and surgical margin status. These findings suggest that the intensity of FDG uptake in primary tumor may be associated with the tumor aggressiveness and histopathological risk factors. In the study by Yonezawa et al. (15), it was found that tumor SUVmax was higher in patients with the LVI and PNI, similar to the findings of our study. However, while Yonezawa et al. (15) found a significant relationship between tumor SUVmax and advanced T stages, we did not find a similar relationship between SUVmax and T stage groups. This difference between the results of two studies may be related to the use of clinical T stage in this previous study and the use of pathological T stage in our study. In another previous study by Lin et al. (16), it was reported that tumor SUVmax had significant and positive association with depth of invasion. Similar to this finding, we also found a positive correlation between

Aydos et al. Prognostic role of FDG PET in TSCC

J Health Sci Med 2023; 6(1): 183-189

tumor SUVmax and depth of invasion. Unlike these two previous studies, we also evaluated the relationship of tumor MTV and TLG with histopathological risk factors. While MTV and TLG showed significant relationship with LVI and PNI similar to SUVmax, these parameters also showed significant relationship with pathological T stage and positive surgical margin unlike SUVmax. Furthermore, MTV and TLG showed higher correlations with histopathological tumor diameter, depth of invasion and the distance from the closest surgical margin compared to SUVmax. These findings may be related to the fact that MTV and TLG are metabolic-volumetric quantitative parameters which provide metabolic data about the entire tumor. SUVmax is the single voxel value with highest FDG uptake in tumors, therefore SUVmax may not represent the total tumor metabolic activity.

The present study evaluated the prognostic role of histopathological risk factors and quantitative PET parameters. Our study showed that advanced pathological T stage (pT3-T4), advanced AJCC stage (stage IV), neck lymph node metastasis, LVI, PNI and positive surgical margins were important prognostic factors in the univariate survival analyses. These findings were in accordance with previous studies (14-16). In these previous studies, tumor SUVmax had also significant assocations with patient survival. Unlike these studies, a significant relationship between survival and tumor SUVmax was not found in our study. Although tumor SUVmax did not show significant association with survival, tumor TLG and MTV were also found to have prognostic value in the univariate analyses for both PFS and OS. This result may be related to the fact that SUVmax may not represent the total tumor metabolic activity. On the contrary, TLG and MTV could reflect the total tumor metabolic activity.

Our study demonstrated that tumor MTV was the only significant parameter in the multivariate analyses for PFS and OS. Tumor MTV was also found as independent prognostic parameter of OS in a previous study by Lee et al. (14). We demonstrated that tumor MTV had a prognostic value not only for OS, but also for PFS. Lee et al. used absolute threshold (SUV 2.5) method for the measurement of tumor MTV. Unlike this method, we used relative percentage threshold method (42% of tumor SUVmax) for the measurement of MTV. Despite the differences in the measurement methods, the result of tumor MTV as an independent prognostic factor in both studies suggests that this metabolic-volumetric FDG PET parameter may be an important preoperative imaging biomarker to predict the prognosis in resectable TSCC.

TLG is an important FDG PET parameter that combines SUV and MTV, providing information about the total glycolytic activity of the tumor mass. In our study, tumor TLG had significant associations with survival in univariate analyses. However, TLG did not have independent prognostic role in multivariate analyses. This result was in accordance with the study of Lee et al (14). This result in our study may be associated with the relatively small patient sample size in the multivariate analyses. Further studies with larger patient populations are needed to evaluate the prognostic role of tumor TLG in TSCC.

This study has several limitations. First, this study was a single-center, retrospective study with a small patient size. Second, a single threshold method was used for the measurement of metabolic tumor volume. Prospective multicenter studies with larger sample size are needed to validate the prognostic role of FDG PET metabolic-volumetric parameters in TSCC.

CONCLUSION

Tumor MTV was the only independent prognostic factor for PFS and OS. MTV may be a preoperative prognostic tool to identify patients with poor prognosis in resectable TSCC. The FDG PET metabolic-volumetric features may be used as an indicator for preoperative risk stratification of patients. The validation of these results is necessary in multicenter prospective studies..

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gazi University Medical Faculty Clinical Researches Ethics Committee (Date: 05.12.2022, Decision No: 889).

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.

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

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

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J Health Sci Med 2023; 6(1): 183-189

Aydos et al. Prognostic role of FDG PET in TSCC

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HEALTH SCIENCES MEDICINE

Comparison of Tritube™ tube and Evone® ventilator use with traditional narrow-lumen tube use in microlaryngeal surgery cases

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ABSTRACT

Aim: To evaluate the feasibility and safety of the Tritube™ tube and Evone® ventilator and compare patients intubated using Tritube™ and ventilated with flow-controlled ventilation (FCV) using Evone® (TT–FCV group) to those intubated using a traditional microlaryngeal intubation tube and ventilated with volume-controlled ventilation (MLT-VCV group) in terms of perioperative parameters and outcomes during microlaryngeal surgery (MLS).

Material and Method: A prospective observational design was conducted. This study was carried out in 6 (six) months between February 2022 and September 2022. After receiving their informed consent, 18 patients were randomly assigned to two groups. Patients older than 18 years, who were scheduled for elective MLS were included in the study. The closed opaque envelope method was used for randomization. The same standard anesthesia protocol was applied to all patients. The patients' demographic parameters, American Society of Anesthesiology physical status (ASA), Mallampati and Cormack-Lehane scores, duration of ventilation, duration of surgery, hemodynamic parameters, ventilation parameters, and complications were recorded.

Results: Twenty-one patients recruited for the study. Three of them regretted to participate to study. At the end 18 patients were analyzed. The mean age of population was 53(43-62). When the intraoperative respiratory and hemodynamic parameters of the patients were compared between the two groups, the regional cerebral oxygen saturation ($rScO_2$) (p=0.020), tidal volume (p=0.005), compliance of the respiratory system (p=0.001), and post-extubation $rScO_2$ (p=0.001) values were statistically significantly higher in the TT-FVC group compared to the MLT-VCV group. Right $rScO_2$ (p=0.038), left $rScO_2$ (p=0.047), and time to extubation (p=0.021) were statistically significantly lower in the TT-FVC group compared to the MLT-VCV group.

Conclusion: According to the findings we obtained, utilizing Tritube™ and Evone® during MLS surgeries appears to be effective in terms of achieving safe airway management.

Keywords: Microlaryngeal surgery, ventilation, hemodynamics

Our research's data was presented in 6th European Airway Management Congress as 'Oral Presentation' on September 2022.

INTRODUCTION

Head and neck surgery is usually challenging due to its anatomical nature for both anesthesiologist and surgeon (1,2). Therefore, perioperative management of it is difficult and open to complications. The restricted tracheal lumen produced by any mass, the wide area covered by difficult airway devices, the need to utilize a small-diameter tube to expose the surgical field, and the resulting continual increase in pressure are potential hurdles during these procedures. (3). Tritube™ is an ultra-thin tracheal tube with an outer diameter of 4.4 mm and an inner diameter of 2.4 mm, designed for adult patient ventilation (4). It consists of three lumens: a ventilation lumen, a cuff lumen, and an intratracheal pressure measurement lumen (5). The ventilation lumen has a cross-sectional area equal to that of a tube with an internal diameter of 2.4 mm and is designed to be connected to devices using EVA technology (such as Ventrain) through a Luer connector. The ventilation lumen has an eye of Murphy. The lumen for measuring intratracheal pressure can be attached to a manometer for continuous monitoring of intratracheal pressure. With Ventrain, inflation of the cuff will both protect the airway and optimize ventilation. The ventilation lumen of the tube contains a pliable stylet with centimeter markings.

J Health Sci Med 2023; 6(1): 190-194

Sayın Kart et al. Different ventilation techniques in microlaryngeal surgery

Evone® (Ventinova Medical, Eindhoven, the Netherlands) is a ventilator designed for use with the Tritube™ intubation tube and offers two different ventilation modes: jet ventilation and flow-controlled ventilation (FCV) (6-8). When in the FCV mode, Evone® creates negative suction pressure during the expiratory phase to actively remove air from the lungs. In volume-controlled ventilation (VCV), there is passive air outflow during the expiratory phase, and an exponential decrease occurs in the airway pressure (Paw) graph, which appears as a curved curve. In contrast, in the FCV mode, since there is an active expiratory phase, Paw decreases linearly, which indicates that the outflow of gas from the alveoli is constant throughout the expiratory phase (6). This new ventilation strategy has been used in many experimental animal studies, and it has been shown to improve lung recruitment and oxygenation (5, 9, 10). Difficult airway management has been successfully performed in patients intubated with Tritube™ tube and ventilated with Evone® (Ventinova Medical, Eindhoven, the Netherlands) ventilator, and the benefits of these devices during laryngeal surgery have been reported (11-14). In this study, our primary aim was to compare microlaryngeal surgery patients intubated using a traditional narrow-lumen intubation tube and ventilated with VCV using a standard ventilator to those intubated using the Tritube™ tube and ventilated with FCV using the Evone® ventilator in terms of hemodynamics, perioperative respiratory parameters and complications. The secondary objectives of our study are the duration of anesthesia and surgery in minutes and the length of hospitalization in days.

MATERIAL AND METHOD

The study was carried out with the permission of University of Health Sciences, Kartal Dr. Lütfi Kırdar City Hospital Ethics Committee (Date: 28.01.2022, Decision No: 2022/514/218/26). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study had a prospective observational design. It was carried out in 6 (six) months between February 2022 and September 2022. Written informed consent from the participants, we randomly assigned the patients into two groups. The closed opaque envelope method was used for randomization. The first group was intubated using the Tritube™ tube and ventilated with FCV using the Evone® ventilator (TT-FVC group), and the second group was intubated using a traditional microlaryngeal tube and ventilated with VCV (Primus IE; Dräger Medical, Germany) (MLT-VCV group). Patients aged older than 18 years, who were scheduled for elective microlaryngeal surgery were included in

the study. The patients' demographical parameters, American Society of Anesthesiology physical status, Mallampati and Cormack-Lehane scores, duration of surgery, hemodynamic parameters (cardiac apex beat, mean arterial pressure, oxygen saturation, end tidal carbon dioxide pressure), ventilation parameters (end expiratory pressure, positive inspiratory pressure, compliance of the respiratory system) and complications were recorded. To standardize intubation conditions, 0.6 mg/kg rocuronium was administered. Total intravenous anesthesia was induced and maintained with 10 mg/kg/hour propofol and 0.5-1 mcg/kg/min remifentanil. At the end of surgery, the cuff was deflated, high-frequency jet ventilation (HFJV) was applied, and the value of tidal volume after extubation was recorded.

Statistical Analysis

Statistical analyses were performed using SPSS version 26.0. Categorical groups were examined using the Pearson test and Fisher's exact test. The effect size was taken to be the same as the medium (medium=0.50), with a confidence interval (CI) level of 80% and alpha value of 5%, and the values showed the Laplace distribution. The total sample size was found to be 18 using the Mann-Whitney U test as a reference model (G Power 3.1.9.2, Windows 10). The averages of the values used in the perioperative patient follow-up were taken.

RESULTS

Twenty-one patients recruited for the study. Three of them regretted to participate to study. At the end 18 patients were analyzed. No unexpected intensive care stays or complications were observed in the patients. No significant difference was observed between the two groups in terms of demographic and clinical data (**Table 1**). The mean age for all study participants were 53 (43-62). Gender distribution of population was 16 (88.8%) male, 2 (11.2%) female. There was no statistical significance difference between the groups on age, gender, smoking status and Body Mass Index (BMI) (**Table 1**). (p> 0.05)

In the TT-FVC group, the regional cerebral oxygen saturation (rScO₂) (p=0.020), tidal volume (p=0.005), compliance of the respiratory system (p=0.001), and post-extubation rScO₂ (p=0.001) values were statistically significantly higher, and right rScO₂ (p=0.038), left rScO₂ (p= 0.047), and time to extubation (p=0.021) were statistically significantly lower compared to the MLT-VCV group. Intraoperative respiratory and hemodynamic variables were summarized in **Table 2**.

Sayın Kart et al. Different ventilation techniques in microlaryngeal surgery

J Health Sci Med 2023; 6(1): 190-194

Table 1. Demographic and clinical data of the patients				
Variables	All patients n=18	TT-FCV n=9	MLT-VCV n=9	p value
Age (year)	53 (43-62)	45 (41-59)	56 (51-63)	0.122
Gender (male)	16 (88.8)	8 (44.4)	8 (44.4)	1.000
Smoking status (smoker)	13 (72.2)	7 (38.9)	6 (33.3)	1.000
BMI (kg/cm²)	22.7 (22.4-25)	25 (21.1-27.9)	22.7 (22.4-23)	0.170
Comorbidity (present)	8 (44.4)	4 (22.2)	4 (22.2)	1.000
ASA score				
1	3 (16.7)	2 (11.1)	1 (5.6)	0.812
2	13 (72.2)	6 (33.3)	7 (38.9)	
3	2 (11.1)	1 (5.6)	1 (5.6)	
Cormack score				
1	9 (50)	3 (16.7)	6 (33.3)	0.347
2	9 (50)	6 (33.3)	3 (16.7)	
Mallampati score				
1	7 (38.9)	3 (16.7)	4 (22.2)	1.000
2	11 (61.1)	6 (33.3)	5 (27.8)	
Duration of surgery (minute)	35 (30-46)	33 (25-45)	40 (32-47)	0.425
Duration of anesthesia (minute)	45 (40-60)	41 (32-53)	50 (40-61)	0.268
Length of hospital stay (day)	1 (1)	1 (1)	1 (1-1.5)	0.539

Values given as frequency (percentage) and median (interquartile range). TT-FCV: TritubeTM tube intubation and flow-controlled ventilation with Evone®, MLT-VCV: traditional microlaryngeal tube intubation and volume-controlled ventilation, ASA: American Society of Anesthesiology, BMI: body mass index

Table 2. Intraoperative respiratory and hemodynamic variables				
Variables	All patients n=18	TT-FCV n=9	MLT-VCV n=9	p
Post-intubation $rScO_2$ (%)	99 (99-100)	99 (99-100)	99 (98-100)	0.416
CAB (1/minute)	71 (63-86)	63 (57-81)	83 (70-86)	0.063
OAB (mmHg)	91 (89-100)	94 (88-103)	90 (89-95)	0.330
$rScO_2$ (%)	99 (99-100)	100 (99-100)	99 (99-100)	0.020
$EtCO_2$ (kPa)	37 (36-38)	36 (36-40)	37 (37-38)	0.893
Tidal volume (ml)	499 (467-547)	546 (520-623)	484 (456-494)	0.005
EPP (cmH_2O)	5 (5)	5 (5)	5 (5)	0.317
Respiratory rate (1/minute)	12 (11-13)	11 (9-13)	12 (12-14)	0.170
PIP (cmH_2O)	18 (16-20)	16 (14-20)	19.4 (18-20)	0.085
CRS (ml/cmH_2O)	39 (33-56)	55 (47-63)	33 (32-37)	0.001
Right $rScO_2$ (%)	75 (72-77)	72 (70-76)	76 (74-77)	0.038
Left $rScO_2$ (%)	75 (72-76)	74 (71-75)	76 (73-77)	0.047
Time to extubation (minute)	6.5 (5.75-7.25)	6 (4-7)	7 (6-8.5)	0.021
Post-extubation $rScO_2$ (%)	99 (98-100)	100 (99-100)	98 (97-98)	0.001

Values given as median (interquartile range). TT-FCV: TritubeTM tube intubation and flow-controlled ventilation with Evone®, MLT-VCV: traditional microlaryngeal tube intubation and volume-controlled ventilation, CAB: cardiac apex beat, MAP: mean arterial pressure, $rScO_2$: regional cerebral oxygen saturation, $ETCO_2$: end tidal carbon dioxide pressure, EPP: end expiratory pressure, PIP: positive inspiratory pressure, CRS: compliance of the respiratory system

DISCUSSION

Microlaryngeal surgical operations involve a number of challenges for both anesthesiologists and surgeons. The aim should be to ensure adequate ventilation throughout the operation and provide optimal surgical conditions while maintaining a safe and secure airway (15, 16).

The Tritube™ intubation tube was designed to be used with the Evone® ventilator, and it has been reported that it may be a good alternative to traditional small-diameter tubes since it provides adequate gas exchange, and its outer diameter of 4.4 mm allows for a sufficient working space for the surgeon (17, 18). In this study, we compared the respiratory and hemodynamic parameters of the patients intubated using Tritube™

and ventilated using Evone® (TT-FCV group) to those intubated using a traditional small-diameter intubation tube and ventilated using VCV (MLT-VCV group). We obtained a lower airway peak pressure and more stable hemodynamic data from the TT-FCV group and did not observe any complication in the perioperative period. Similarly, Meulemans et al. (19), reported that the FCV mode provided adequate oxygenation, with the end-tidal CO_2 values being within the normal range and no complications being observed related to the ventilation mode. In another study using Tritube™, Schmidt et al. (11) found that this tube could be clogged with secretions and stated that tube dislocation could occur due to coughing and retching. We encountered no such complication in any of our patients.

Mora et al. (20), referred to the risk of blood aspiration following the switch to HFJV due this ventilation mode requiring the lowering of the cuff of the intubation tube. In the current study, after the surgical bleeding control of the patients, intravenous sugammadex was administered to eliminate neuromuscular blockade, and we observed that the patients were hemodynamically stable and softly spontaneously breathing, and we extubated them by lowering the cuffs of the Tritube™ intubation tube and switching them to the HFJV mode. We did not encounter any complications related to blood aspiration in any of our patients.

As stated in many studies, in upper airway operations, especially in microlaryngeal surgery, the use of an ultra-thin Tritube™ tube offers a great advantage by creating sufficient working space for the surgeon (3, 5, 19).

Throughout our study, we observed that this tube provided sufficient working space in the surgical field, and there was no airway pressure or ventilation problem during the operation, resulting in high surgical team satisfaction and even shortening the operation time relatively. The Evone® ventilator takes time to set up and calibrate, and its use requires experience, which are factors limiting its use in emergency operations (11, 19).

Although we had some reservations concerning the use of a ventilator and ventilation mode that we were not accustomed to when we first started the study, we did not encounter any ventilation problems in the patients. For the Tritube™ intubation tube, the higher cost compared to standard intubation tubes can be considered as a drawback (3, 21).

Tritubes' advantages in terms of airway management and surgical settings are combined with its ventilation advantages. FCV mode can result in more homogeneous lung aeration, higher ventilation efficiency, and enhanced gas exchange compared to VCV and PCV modes (22-25). Recently a crossover research comparing FCV and VCV found that FCV provides superior ventilation efficiency (26). In addition, compared to HFJV, FCV minimizes the risk of air entrapment, hyperinflation and barotrauma (27,28). Similarly, we did not observe any complications in any of the trial participants. In accordance with the literature, this supports the safety of Tritube and FCV.

This study does have some limitations. First, our research was limited to a specific patient group. Second, our study was conducted on a small number of patients in a single-center, highly specialized surgical procedure. Future multicenter, multiple-method surgical trials will aid in determining the efficacy of Tritube™ intubation tube and Evone® ventilator use.

CONCLUSION

Although the literature on the Tritube™ intubation tube and Evone® ventilator is still limited, safe airway management in microlaryngeal surgery seems to be advantageous in terms of ventilation and extubation, and we consider that the data obtained from the current study will be supported by future studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Sciences, Kartal Dr. Lütfi Kırdar City Hospital Ethics Committee (Date: 28.01.2022, Decision No: 2022/514/218/26).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Evaluation of risk factors for pelvic and paraaortic lymph node metastasis in endometrioid type endometrial cancer

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ABSTRACT

Aim: Determining the relationship between clinical and pathological features in endometrial cancer is essential for both prognostic and potential therapeutic benefits. In this study, we aimed to investigate the relationship between pelvic and paraaortic lymph node (PLN and PALN) metastasis and prognostic factors in patients with endometrial cancer (EC).

Material and Method: Medical records of patients who underwent primary surgery for EC in our gynecological oncology center between the 2016 and 2018 were reviewed retrospectively. The relationship between pelvic and paraaortic lymph node metastasis was evaluated with data such as patient age, body mass index, serum CA 125 level, macroscopic tumor diameter, and patients' risk groups.

Results: Fifty-seven patients with EC were evaluated. Lymph node involvement was detected in 10 patients (17.5%). Acording to Modified Mayo criterias ; the patients with grade 3 EC had a higher risk of metastasis compared to other grades (p=0.025). Patients with lymph node metastases had a greater depth of invasion (p=0.001). There was no relationship between tumor size and lymph node metastasis (p=0.494). In the logistic regression analysis, the depth of invasion was found to be an independent risk factor for lymph node metastasis. There was no significant relationship between the presence of PLN and PALN metastases in patients with high-risk endometrial cancer, but the presence of PALN metastasis was significant in patients with low-risk endometrial cancer with PLN metastasis (p=0.002).

Conclusion: These findings support the idea that routine evaluation of tumor invasion depth during endometrial cancer surgery may be useful in predicting lymph node metastasis and guiding the operation.

Keywords: Endometrial cancer, metastasis, lymph node

INTRODUCTION

Endometrial cancer is the fourth most common type of cancer in women after breast, lung and colorectal cancers, and is the most common gynecological malignancy in our country (1,2). Age, obesity, parity, caucasian race, endocrine diseases, early menarche-late menopause, tamoxifen use and family history are risk factors for endometrial cancer (1). The most common pathogenetic type is associated with exposure to endogenous or exogenous unopposed estrogen, and the tumor starts as a hyperplastic endometrium and progresses to cancer. The other endometrial cancer is the type that develops spontaneously without an estrogen source and generally has a worse prognosis than estrogen-dependent cancer. The mainstay of surgical treatment is bilateral salpingo-oophorectomy, paraaortic and pelvic lymphadenectomy, and total hysterectomy, including examination of the

abdominal cavity fluid. Laparoscopy has been associated with fewer postoperative complications than laparotomy.

In addition to the surgical staging recommended by the International Federation of Gynecology and Obstetrics, histological features, size, degree of myometrial invasion, serum tumor marker levels, lymphovascular area invasion, peritoneal cytology and lymph node (LN) involvement are also of prognostic importance (1,3-5).

LN involvement is important in terms of initiating postoperative adjuvant therapy and determining the area of radiotherapy. There is still no definitive method used to detect the presence of perioperative LN metastases. Routine pelvic and paraaortic lymph dissection (lymphadenectomy) is controversial in patients with early stage endometrial cancer. Although there are studies in

Özdemir et al. Lymph node metastasis in endometrial cancer

J Health Sci Med 2023; 6(1): 195-200

the literature reporting that survival is associated with improvement, there are also research results suggesting that it is not necessary (6-9).

In this study, it was aimed to determine the risk factors for pelvic and paraaortic lymph node metastasis by examining the clinical and surgical characteristics of endometrial cancer patients who underwent surgical staging and to compare them with the literature data.

MATERIAL AND METHOD

In this study, 57 endometrioid-type endometrial cancer patients who had complete medical records (excluding non-endometrioid cancer and/or extrauterine involvement) and underwent endometrial cancer surgery in the Gynecology Clinic of the University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital between January 2016 and December 2018 were evaluated retrospectively. Written consent was obtained from all patients for treatment and analysis of scientific data. The study was carried out with the permission of Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Researches Ethics Committee (Date: 06.12.2021 Decision No: 06.12.2021/421). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Information about age, body mass index (BMI), International Federation of Gynecology and Obstetrics (FIGO) stage, myometrial invasion, menopause status, tumor size, type of surgery (laparoscopy/laparotomy) and serum CA 125 level were collected by examining the relevant medical records. Lymph node regions were classified as pelvic (PLN) and paraaortic lymph node (PALN). All patients underwent lymphadenectomy according to total hysterectomy, bilateral salpingo-oophorectomy and perioperative frozen result. Lymphadenectomy was performed in the presence of grade 3 and/or more than 50% myometrial invasion and/or cervical invasion and in the presence of tumors larger than 2 cm. The FIGO 2009 staging system was used. Prognostic factors determining lymph node metastasis distribution and metastasis were determined. The data and statistical results obtained from the literature regarding the prognostic factors determining lymph node metastasis were taken into consideration. In the study conducted by Mariani et al. (10) in Mayo clinic in 2000, they determined the low and high risk groups of patients with endometrial cancer. Accordingly, patients with tumor size ≤ 2 cm, stage 1 or 2 tumors and invasion depth <50% are considered low-risk. Therefore, in our study, patients with low risk characteristics were defined as "endometrioid type endometrial cancer, FIGO grade 1 or 2 histology, myometrial invasion < 50%" and other patients were classified as high-risk.

The inclusion criteria are listed below:

1. Patients with pathologically proven endometrial cancer.
2. Patients over 18 years of age.
3. Patients whose detailed medical records can be accessed, including the patient's history, clinical findings, laboratory and pathology test results, treatment results, etc.

The exclusion criteria are as follows:

1. Patients with no definitive pathological diagnosis.
2. Patients with secondary cancer.
3. Patients with conservatively treated endometrial cancer.

Statistical Analysis

In this study, Statistical Package for Social Science (SPSS) version 23.0 was used. The normal distribution of the data was evaluated with the Shapiro-Wilk-W test, and the continuous variables were evaluated with the Student-t test or Mann-Whitney U test. Categorical variables were evaluated by chi-squared test or Fisher's exact tests. Logistic regression and ROC curve analysis were used to evaluate lymph node metastasis and associated clinical conditions. A value of $p<0.05$ was considered statistically significant.

RESULTS

In the three-year period, 57 female patients were operated with laparotomy or laparoscopy methods applied in the gynecological oncology unit of our hospital due to endometrial cancer. The mean age of the patients was 60.72±9.12 years. In-hospital mortality rate was 0%. The clinical and surgical characteristics of patients with and without pelvic and paraaortic nodal metastasis are given in **Table 1**. Nodal metastasis was not seen in 47 (82.5%) patients, while it was present in 10 (17.5%) patients. There was no significant relationship between age, weight, height, body mass index (BMI), preCA125 level, menopause status and nodal metastasis (p>0.05). More PALNs were excised in patients with nodal metastasis (p=0.006). The presence of nodal metastasis does not have a significant effect on the duration of the operation (p=0.643).

The characteristics of the risk groups in the presence of nodal metastasis are shown in **Table 2**. According to the modified Mayo criteria, nodal lymph metastasis was found to be significantly lower in patients with lower risk than those with higher risk, that is, patients with grade 3 had a higher risk of metastasis than other grades (p=0.025), patients with lymph node metastasis had a higher depth of invasion (p=0.001), and there was no relationship between tumor size and lymph node metastasis (p=0.494).

J Health Sci Med 2023; 6(1): 195-200

Özdemir et al. Lymph node metastasis in endometrial cancer

Table 1. Clinical characteristics of patients with and without nodal metastasis

Presence of nodal metastasis	Not available n=47 (82.5%)	Available n=10 (17.5%)	p-value
Age	59.08±11.33	61.30±8.11	0.561 *
Weight (kg)	73.34±5.45	74.90±6.70	0.433 *
Height (cm)	165.70±4.13	166.30±4.40	0.683 *
Body mass index (BMI) (kg/m2)	31.20±31.16	26.84±1.76	0.663 *
Serum CA125 level	16.60±13.26	47.60±64.42	0.071 **
Menopause Status			0.671 ****
Menopause	36 (%76.6)	9 (%90.0)	
Premenopausal	11 (%23.3)	1 (%10.0)	
Surgical technique			0.034 ***
Laparoscopy	23 (%48.9)	1 (%10.0)	
Laparotomy	24 (%51.1)	9 (%90.0)	
Number of excised pelvic lymph nodes	22.72±10.92	23.30±10.67	0.880 *
Number of excised paraaortic lymph nodes	5.62±11.33	11.10±7.50	0.006 **
Operation_duration (minutes)	162.98±41.69	151.50±17.96	0.643 **
* t-test, **Mann-Whitney U test, *** Chi-Square test, **** Fisher's Exact test			

Table 2. Characteristics of risk groups in the presence of nodal metastasis

Presence of nodal metastasis	Not available n=47 (82.5%)	Available n=10 (17.5%)	p-value
Grade			0.025 *
1 & 2	43 (91.5%)	6 (60.0%)	
3	4 (8.5%)	5 (40.0%)	
Invasion			0.001 *
<50	36 (76.6%)	2 (20.0%)	
≥50%	11 (23.3%)	8 (80.0%)	
Tumor Size			0.494 **
<2 cm	21 (44.7%)	3 (30.0%)	
≥2 cm	26 (55.3%)	7 (70.0%)	
* Fisher's Exact test, ** Chi-Square test			

The characteristics of the patients according to the low and high risk patient groups are given in **Table 3**. The number of low-risk patients was 20 (35.1%) and the number of patients with high-risk endometrial cancer was 37 (64.9%). There was no statistically significant relationship between age, weight, height, BMI, serum CA125 level, menopause status and duration of surgery and risk groups (p>0.05). When the surgical technique was examined, it was found that laparotomy technique was used more in high-risk patients (p=0.044). PLN and PALN were excised more frequently in high-risk patients (p=0.022; p=0.06).

Table 4 shows the surgical characteristics of the low and high-risk patient groups. Grade 3 cancer was significantly higher in the high-risk group (p=0.000). The higher invasion depth of 50% in the high-risk group was significantly higher (p=0.001). There was no significant relationship between the high-risk group

and lymph node metastasis (p=0.467). There was no significant relationship between the presence of pelvic and paraaortic lymph node metastasis and patient risk groups (p>0.05) (**Table 5**).

Table 3. Characteristics of patients by risk groups

	Low, n=20 (35.1%)	High,n=37 (64.9%)	p-value
Age	58.45±11.36	60.03±10.61	0.603 *
Weight (kg)	74.00±6.55	73.41±5.19	0.708 *
Height (cm)	166.40±4.12	165.49±4.18	0.432 *
Body mass index (BMI) (kg/m2)	37.27±47.75	26.74±1.82	0.182 *
Serum CA125 level	15.95±15.09	25.32±36.48	0.186 **
Menopause Status			1.000 ****
Menopause	16 (%80.0)	29 (%78.4)	
Premenopausal	4 (%20.0)	8 (%21.6)	
Surgical technique			0.044 ***
Laparoscopy	12 (%60.0)	12 (%32.4)	
Laparotomy	8 (%40.0)	25 (%67.6)	
Number of excised pelvic lymph nodes	18.25±8.11	25.30±11.33	0.022 **
Number of excised paraaortic lymph nodes	2.00±5.42	9.05±12.32	0.006 **
Operation_duration (minutes)	167.25±47.75	157.57±33.10	0.449 **
* t-test, **Mann-Whitney U test, *** Chi-Square test, **** Fisher's Exact test			

Tablo 4. Tumoral characteristics of risk groups

	Low n=20 (35.1%)	High n=37 (64.9%)	p-value
Stage			0.000 *
1	16 (80.0%)	3 (8.1%)	
2	4 (20.0%)	26 (70.3%)	
3	0 (0.0%)	8 (21.6%)	
Invasion			0.001 *
<50	19 (95.0%)	19 (51.4%)	
≥%50	1 (5.0%)	18 (48.6%)	
Tumor Size			0.000 *
< 2 cm	19 (95.0%)	5 (13.5%)	
≥ 2 cm	1 (5.0%)	32 (86.5%)	
Nodal Metastasis			0.467 **
Available	2 (10.0%)	8 (21.6%)	
No	18 (90.0%)	29 (78.4%)	
* Chi-Square test * * Fisher's Exact test			

Table 5. Evaluation of the risk of pelvic and paraaortic nodal metastasis according to risk groups

	Low, n=20 (35.1%)	High, n=37 (64.9%)	p-value
Pelvic lymph node metastasis			0.699 *
Not available	18 (90.0%)	31 (83.8%)	
Yes	2 (10.0%)	6 (16.2%)	
Paraortic lymph node metastasis			0.697 *
Not available	19 (95.0%)	33 (89.2%)	
Yes	1 (5.0%)	4 (10.8%)	
* Fisher's Exact test			

Özdemir et al. Lymph node metastasis in endometrial cancer

J Health Sci Med 2023; 6(1): 195-200

When high-risk patients were examined in two groups as those with and without PLN metastasis, no relationship was found between them and PALN metastasis. However, in patients with low-risk a patient with PLN metastasis (n=1), PALN metastasis was also present and this finding was statistically significant (p=0.002) (**Table 6**).

Table 6. The relationship between pelvic and paraaortic nodal metastases

Risk Group	Pelvic lymph node metastasis	Paraaortic lymph node metastasis		p value
		Not available	Available	
High-risk patients*				0.052 *
	Not available	29 (87.9%)	2 (50.0%)	
	Available	4 (12.1%)	2 (50.0%)	
Low-risk patients				0.002 **
	Not available	18 (94.7%)	0 (0.0%)	
	Available	1 (5.3%)	1 (100.0%)	
* Chi-Square test, ** Fisher's Exact test				

In the logistic regression evaluation, the depth of invasion was found to be significantly correlated with the presence of PALN and PLN metastases (p=0.02) (**Table 7**). **Figure** shows the ROC curve, according to which the cut-off value was 0.526 and the area under the curve was 0.809.
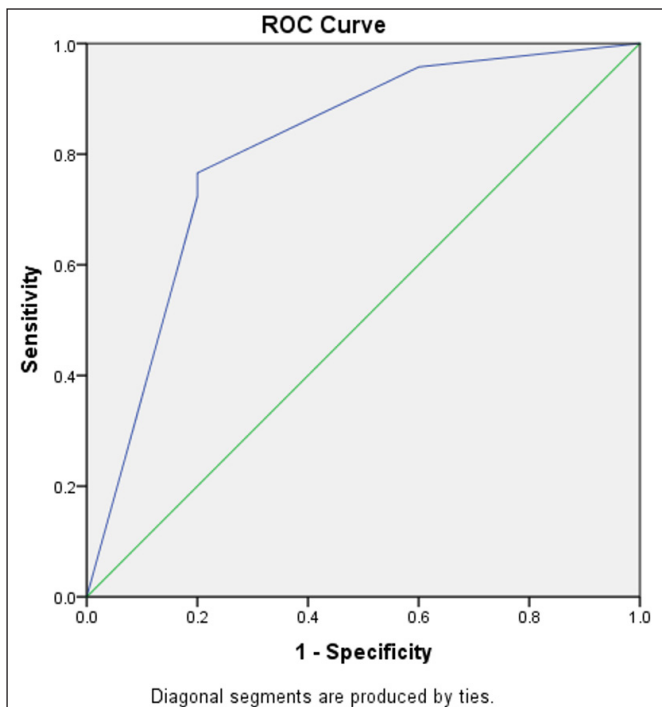

Figure. Cutoff Value: 0.526, Roc Curve and Area Below Curve (Auc): 0.809

Table 7. Logistic regression assessment

	Odds Ratio	P value
Invasion	2.218	0.020
Grade	0.908	0.184

DISCUSSION

In this study, it was aimed to determine the clinical and pathological risk factors for pelvic and paraaortic lymph node metastases in patients with endometrial cancer. In addition to determining important risk factors for LN metastases, the effect of nodal involvement was also evaluated. According to the results of our study, laparotomy procedure was performed more in patients with nodal metastasis. The presence of nodal metastasis did not affect the duration of the operation. Tumoral invasion depth was higher in patients with lymph node metastasis. There was no relationship between tumor diameter and the presence of lymph node metastasis. Clinical features such as age, weight, height, BMI, serum CA125 level, menopause status and duration of surgery were not statistically significantly correlated with patient risk groups. PLN and PALN were excised more in high-risk patients. There was no significant relationship between the presence of lymph node metastasis and patient risk groups. However, the depth of invasion is associated with the presence of PALN and PLN metastasis, and the depth of invasion may be useful in predicting lymph node metastasis.

The need for paraaortic (PA) lymphadenectomy in terms of survival in the treatment of endometrial cancer is controversial. In addition, the incidence of finding metastatic lymph nodes in the PA region is not very high in patients undergoing systematic pelvic and PA lymphadenectomy. In a study conducted by Fotopoulou et al. (11) on 62 patients with moderate and high-risk endometrial cancer and aimed to determine the predictive factors for pelvic and aortic lymphadenectomy, pelvic node involvement was reported in 21% of patients and both pelvic and PA lymph nodes were affected in 12% of this patient group. In our sample, lymph node involvement was present in 17.5% of the patients. Since low stage patients were also evaluated in our study, different results may have been obtained. According to the results of their study using logistic regression analysis to determine independent prognostic factors for PALN metastasis, Karube et al. (12) revealed that PLN and ovarian metastasis were associated with PALN metastasis. In this study, no significant relationship was found between lymph node metastasis and high-risk endometrial cancer. According to the results of a large patient population retrospective study comparing the relationships between paraaortic lymph node metastasis and various clinicopathological factors to evaluate whether paraaortic lymph node dissection is necessary in the treatment of endometrial cancer, the researchers emphasized that pelvic lymph node status should be taken into consideration when deciding whether to perform PALN dissection in patients with endometrial

J Health Sci Med 2023; 6(1): 195-200

Özdemir et al. Lymph node metastasis in endometrial cancer

cancer and that PALN dissection is not required if PLN metastasis is not present (13). In our study, the presence of PALN metastasis was also found to be statistically significant in patients with PLN metastasis, even in low-risk patients. In this study, which we set out to identify high-risk patients who will benefit from paraaortic lymphadenectomy and to create risk groups, we showed the importance of invasion depth. As a result, with the help of risk models, the status of PALN can be determined more clearly and unnecessary dissection of the PA region and related morbidity can be prevented. However, these models should be usable in routine practice. For this reason in our study, it was aimed to divide the patients into endometrial cancer risk groups and evaluate the presence of nodal metastasis. However, prospective studies with a larger number of cases are needed to define the risk groups more clearly and to standardize the treatment. In this study, involvement in the PA region was detected in 8.7% of patients who underwent systematic lymphadenectomy up to the level of the renal vein. It is possible to prevent procedure-related morbidity by evaluating independent risk factors such as depth of invasion.

Unlike cervical cancer, lymphatic spread in endometrial carcinoma does not occur in regional order. This is due to the variability of tumor localization and the apparent incidence of adnexal metastasis in endometrial carcinoma. Therefore, the lymphatic chain pattern is not associated with predictable lymphatic spread in endometrial carcinoma (14). Turan et al. (14) aimed to define a high-risk group for PALN metastasis in patients with endometrial cancer and showed that PALN involvement significantly increased in patients with high-risk endometrial cancer. Although PLN and PALN were excised more in high-risk cases evaluated in this study, no significant relationship was found between the presence of lymph node metastasis and patient risk groups. This can be explained by the low number of patients.

In their study evaluating the risk factors for lymph node metastasis in patients with endometrial cancer, Taş et al. (15) found lymph node metastasis in 9.1% of the patients participating in the study [pelvic only in 3.5%, paraaortic only in 2.1%, both pelvic and paraaortic lymph node involvement in 3.5%] and a significant relationship between lymph node metastasis and deep myometrial invasion (≥50% invasion depth), lymphovascular space invasion, positive peritoneal cytology and tumor size. According to the results of this study, there was a positive correlation between tumor size and lymph node metastasis. Similarly, our results show that paraaortic lymph node involvement is less than pelvic node involvement. Similarly, we found a relationship between lymph node metastasis and deep myometrial invasion.

In a study by Yokoyama et al. (16) in 1997 investigating the importance of pelvic and paraaortic lymphadenectomy in endometrial cancers, they found that both PLN and PALN metastases in 10% of patients with Stage I disease according to the FIGO 1988 classification, and reported that there was no significant relationship between the location or number of PLN and PALN metastases. In multivariate analysis, it was reported that low-grade and deep myometrial invasion had an independent relationship with PALN metastases, whereas vascular cavity invasion and cervical invasion were independently associated with PLN metastases. According to the advanced statistical evaluation of our data, it was observed that the depth of invasion was independently correlated with nodal lymph involvement. Furthermore, the survival of patients with PALN metastasis was significantly worse compared to patients with PLN metastasis alone (44.4% and 80.0%, respectively, p< 0.05). These results reveal that PLN and PALN metastases occur frequently even in early stage endometrial cancer, and PLN metastases, especially PALN metastases, have a serious effect on patient survival. In our study, survival was not evaluated because it was not the main purpose of the study.

In a study evaluating the effects of PALN in endometrial cancer patients without pelvic lymph node metastasis, it was reported that those without PLN involvement and those with PALN involvement constituted only 2.4% of all cases included in the study (9). We only had 5%. The probability of isolated PALN metastasis is considered low enough. The effects of a PLN-PALN lymphatic propagation model should therefore be considered by gynecological oncologists when determining patient management strategies in endometrial cancer.

Our study has some limitations such as being single-center and retrospective. However, our results are important in terms of showing that the depth of invasion is an important risk factor in the risk of increased LN metastasis in patients with endometrial cancer. Therefore, considering the depth of invasion before the operation when making the PALN decision may affect the lymph node sampling decision and the degree of LN sampling in high-risk patients.

CONCLUSION

According to our study, the only independent risk factor for lymph node metastasis in endometrial cancer cases is the depth of invasion. There was no linear relationship between tumor size and lymph node metastasis. Systemically concurrent pelvic and paraaortic lymphadenectomy may be useful for providing prognostic information, selecting appropriate postoperative treatment, and performing accurate figo staging in all patients with low and high endometrial cancer, except

Özdemir et al. Lymph node metastasis in endometrial cancer

J Health Sci Med 2023; 6(1): 195-200

those with stage Ia stage 1 and stage IV. Further studies are needed to determine other risk factors for lymph node metastasis. Randomized prospective publications evaluating systemic lymphadenectomy over disease-free survival times are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Researches Ethics Committee (Date: 06.12.2021 Decision No: 06.12.2021/421).

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 has no conflicts of interest to declare.

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HEALTH SCIENCES MEDICINE

The relationship of dietary antioxidant capacity with laboratory and anthropometric measurements in hemodialysis patients

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ABSTRACT

Aim: Dietary antioxidant intake correlates with blood antioxidant content and protects against oxidative damage and related inflammatory complications. This study was conducted to examine the relationship between total antioxidant capacity of diet and effective factors with laboratory and anthropometric parameters in patients undergoing hemodialysis.

Material and Method: The present case-control study consisted of 62 cases and 59 controls individuals who received hemodialysis treatment between the ages of 35-75. Dietary intake, sociodemographic data, medical history, and anthropometric measurements were collected from participants using a validated questionnaire.

Results: Examining the association between dietary components and diet's total antioxidant capacity (dTAC) reveals a positive correlation between dietary protein (kg/avg), beta carotene (mcg/day), vitamin C (mg/day), vitamin E (mg/day), and polyunsaturated fatty acids (PUFA) (g/day) (p=0.002). The serum albumin, serum neutrophil to lymphocyte ratio and HDL-cholesterol have been reported to have a positive relationship with dTAC. And body mass ındex (BMI) and other anthropometric parameters were found to have a negative connection with dTAC (p=0.007).

Conclusion: Total dietary antioxidant capacity is effective on anthropometric measurements and serum laboratory values. Increasing the antioxidant capacity of the diet in hemodialysis patients is important to prevent complications related to inflammation.

Keywords: Hemodialysis, diet total antioxidant capacity, anthropometric parameters

INTRODUCTION

There is a balance between oxygen radicals and antioxidant defense mechanisms in healthy humans. Oxidative stress (OS) is the condition that arises when this equilibrium is disrupted in favor of oxygen radicals. Uncontrollable reactive oxygen products (ROS) cause tissue damage and dysfunction in the presence of oxidative stress. Oxidative stress is involved in the pathogenesis of numerous disorders, including atherosclerosis, stroke, diabetes, preeclampsia, heart failure, cancer, and chronic kidney failure (1). Oxidative stress, which is causally related to chronic renal failure, increases with chronic renal failure and hemodialysis and increases mortality and morbidity by promoting metabolic risk factors (2). In hemodialysis patients, inflammation and oxidative stress indicators are positively correlated (3, 4). Uremia and the exposure

of blood to dialysate and dialysis membrane during dialysis stimulate proinflammatory cytokines in the extracorporeal circulation (4). Free oxygen radicals (SOR) produced from polymorphonuclear cells stimulated by cytokines generates a vicious spiral between cytokines and SOR by activating nuclear factor kB (NF-kB), the transcription factor of cytokines. Activation of NF-kB induces the production of ınterleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), ınterleukin-6 (IL-6), and C-reactive protein (CRP) (5). Due to existing renal parenchymal damage and decreased glomerular filtration rate in chronic renal failure (CRF), studies have revealed that serum antioxidant capacity diminishes, free oxygen radical levels rise, and inflammation increases (6). In end-stage renal disease patients, inflammation is associated

Alataş et al. Hemodialysis patients and diet total antioxidant capacity

J Health Sci Med 2023; 6(1): 202-208

with insulin resistance, oxidative stress, endothelial dysfunction, and vascular calcification, and it raises cardiovascular risk factors (7). Dietary antioxidant intake correlates with blood antioxidant content and protects against oxidative damage and related inflammatory complications. Finding a correlation between dietary diversification and total antioxidant capacity is crucial for preventing serum oxidative stress and inflammatory diseases (8, 9). This study was conducted to examine the relationship between total antioxidant capacity of diet and effective factors with laboratory and anthropometric parameters in patients undergoing hemodialysis.

MATERIAL AND METHOD

Study Settings and Participants

All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The study comprised patients who got treatment at the Hemodialysis Unit of the Malatya Turgut Özal Training and Research Hospital as well as individuals who matched the patient group in terms of age and gender and were deemed healthy by the doctor.

Exclusion Criteria

Individuals who smoke and drink alcohol, have acute inflammatory disease, use anti-inflammatory drugs, have chronic inflammation such as active hepatitis, HIV(+), have heart disease detected and symptoms by electrocardiography in the past three months, and have diabetes mellitus with a history of ischemic heart disease are at increased risk for developing ischemic heart disease. Patients who have received hemodialysis treatment for less than one month and for more than ten years, those who are scheduled for transplantation and who have recently undergone transplantation and re-entered dialysis, those who have previously received peritoneal dialysis, those who use fish oil and other antioxidant drugs, those with a cancer diagnosis, and pregnant and lactating patients who refuse to participate in the study and healthy volunteers were excluded.

Inclusion Criteria

The study included a total of 41 male and female patients who received hemodialysis treatment (6 months-10 years), entered dialysis at least 2 days a week, did not meet the exclusion criteria, aged between 35 and 75 years, and voluntarily agreed to participate in the study. The control group consisted of 41 healthy male and female volunteers aged between 35 and 75 years, who were matched with the patient group in terms of age and gender, and who visited the internal medicine outpatient clinic for routine check-ups.

Data Collection

The objectives of the study and the contents of the data collecting form were explained to the individuals who voluntarily participated in the study. In addition, they were advised that no additional measures would be taken and that they would not be required to pay any costs. By signing a consent form, individuals who consented to participate in the study were included in the study's scope.

Demographic Data: After determining the patients to be included in the study, the demographic parameters (age, gender, education level, and marital status) and health status of the patients were collected via face-to-face interviews with a questionnaire.

Laboratory Index: Blood glucose, high density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglyceride (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, sodium, and potassium values were extracted from the hemodialysis unit records at the Malatya Turgut Özal University Training and Research Hospital. Blood samples from healthy individuals were tested in the biochemistry laboratory of Malatya Turgut Özal University Training and Research Hospital with the physician's clearance.

Anthropometric Measurements

Body weight (kg), body fat mass (kg), lean body mass (kg), body fat percentage (percent), water mass (kg), and visceral adiposity index (VAI) are all measurements of body composition. Using bioelectric impedance technology, the Tanita BC 545N portable body analyzer (bioelectric impedance device -BIA) was manufactured. The device operates on the basis of the differential in electric current permeability between lean tissue mass and fat. The impedance is measured against the method-specified weak electric current (50 kHz). The weight measurements were recorded with 0.1 kg of precision in kilograms (9). Individuals should be informed that they must fast for at least four hours prior to the measurement, not consume liquids (water, tea, coffee), not be congested with urine, refrain from engaging in strenuous physical activity for 24 hours prior, and not have any metal objects in contact with their skin during the measurement. has been made.

The height was measured using a portable stadiometer (height meter) of the Leicester brand, with the head in the Frankfurt plane, the feet next to the heels, and the back, hips, and heels touching the wall, while taking deep breaths. The measurements were taken in cm with an accuracy of 0.1 cm. Body mass index (BMI) was calculated using the formula "body weight/height2 (kg/m²)" with data of body weight and height. The resulting BMI values were assessed using the WHO classification system (10).

The waist circumference was measured with a rigid tape measure halfway between the lowest rib and the cristailiac. Before measuring the waist circumference, respondents were requested to remove any items or clothes that could obstruct the measurement. In order for the correct measurement to be taken, the individual was positioned face-to-face with the person to be measured, standing upright with a relaxed abdomen, arms at both sides, and feet together. The measurements were taken in centimeters with an accuracy of 0.1 cm. Consequently, a waist circumference of more than 80 cm in women and 94 cm in men was deemed dangerous, and a waist circumference of 88 cm or more in women and 102 cm or more in males was deemed obese (11). The individual's hip circumference was measured from the highest point on the side of the body. The waist-to-hip ratio has been linked to chronic disorders in adults. A waist-to-hip ratio of 0.85 or higher in women and 0.90 or higher in men was deemed obese (12). This measurement also determines the obesity of androids and gynoids. The waist-height ratio was calculated by dividing the waist circumference by the height, and risk classification was determined. (Waist-height ratio risk classification: for men and women; Care should be taken <0.4, normal= 0.40-0.50, precaution should be taken= 0.50-0.60, ıntervention should be done >0.60 (13).

Food Consumption Diary

In order to determine the nutritional status of the patients, 3-day food intake records were taken on successive days: the day before dialysis, the day of dialysis, and the day following dialysis. In order to determine the nutritional status of healthy individuals in the control group, retrospective 3-day food intake records were acquired. The oxygen radical absorption capacity value (ORAC) of that food was calculated by multiplying the previously reported (14) ORAC value for each food and the grams of the consumed food. The diet's total antioxidant capacity (dTAC) was estimated by adding the ORAC values calculated for each food consumed during the day. Individuals' daily calorie, macronutrient, and micronutrient intakes were determined using the "Nutrition Information System (BEBIS)" software developed for Turkey (15).

Statistical Analysis

SPSS software version 22.0. (SPSS; IBM Corp., Armonk, NY, USA). As descriptive statistics, the number, percentage, mean, standard deviation, minimum and maximum values were determined. In analytical evaluations, scale scores were analyzed using the t-test and analysis of variance. A Bonferroni analysis was performed to establish which group was responsible for the difference. As a multivariate analysis, the forward

linear regression method was utilized to determine the factors influencing dORAC. Using multiple linear regression, the connections between independent variables and dORAC were determined. In binary and multivariate linear analysis, only variables with $p<0.05$ were added to the model, and only variables with $p<0.05$ were included.

RESULTS

This study was conducted with 121 people, 62 patients with a mean age of 54.8±12.60 years and 59 individuals in the control group with a mean age of 53.9±11.77 years. It was determined that hemodialysis patients received dialysis treatment for 4 years, 93.55% were married, 35.48% were primary school graduates, and 51.61% were working (**Table 1**)

Table 1. General characteristics of individuals			
	Hemodialysis (n=62)	**Control (n=59)**	**p**
Age (years)	54.8±12.60	53.9+11.77	0.134
	n (%)	n (%)	
Marital status			
Married	58(93.55)	47(90.24)	0.125
Single	4(6.45)	12(9.76)	
Educational status			
Illiterate	28(45.16)	13(22.03)	0.003
Literate	4(6.45)	5(8.47)	
Primary school	22(35.48)	15(25.42)	
High school	4(6.45)	14(23.72)	
University	4(6.66)	17 (28.81)	
Working status			
Working	32(51.61)	34(57.62)	0.001
Not working	30(48.39)	25(42.37)	
CRF (years)	10.8±5.88	-	
HD (years)	4.8±3.12	-	
Abbreviations: CRF: Chronic Renal Failure, HD: Hemodialysis			

Table 2 compares the laboratory results of hemodialysis and control groups according to dietary ORAC quartiles. Serum ferritin levels were found to vary between quartiles in both HD and control groups, with levels being lowest in the quartile with the greatest ORAC value (p<0.05). LDL-C and HDL-C values were found to differ between quartiles in the HD group. The group with the highest mean blood HDL-C level was in the quartile range with the greatest ORAC level, and the distribution of T-cholesterol levels between quartiles was significantly different in the HD group (p<0.05). The group with the lowest mean ORAC level in their diet had the highest total cholesterol level (p<0.05). In the control group, the group with the greatest ORAC level had the highest serum total cholesterol level (p<0.05). In the HD and control groups, the group with the highest serum ORAC level also had the greatest mean total protein level.

Alataş et al. Hemodialysis patients and diet total antioxidant capacity

J Health Sci Med 2023; 6(1): 202-208

Table 2. The Laboratory Results Of Hemodialysis And Control Groups According To Dietary ORAC Quartiles								
	Hemodialysis				Control			
	1.Quartile	2.Quartile	3.Quartile	p1	1.Quartile	2.Quartile	3.Quartile	p2	p3
CRP (mg/dL)	0.7±0.65	1.0±0.78	0.5±0.54	0.345	0.4±0.72	0.4±0.35	0.4±0.41	0.165	0.001
Lymphocyt to monocyte ratio	3.0±0.92	3.1±1.54	2.9±1.38	0.260	3.8±1.08	4.7±1.60	4.2±1.16	0.138	0.031
Neutrophil to lymphocyte ratio	3.2±2.70	3.0±1.29	3.1±1.63	0.156	2.1±0.76	1.5±0.54	1.5±0.57	0.091	0.051
Platelet to lymphocyte ratio	137.8±66.52	148.5±108.64	146.8±69.91	0.785	123.1±47.10	114.0±24.04	113.9±36.12	0.265	0.003
Ferritin (mg/dL)	344.7±222.27	460.2±259.35	302.1±181.33	0.011	109.8±90.87	111.6±95.12	98.9±62.42	0.001	0.004
Albumin (mg/dL)	3.4±0.19	3.5±0.36	3.2±0.31	0.219	3.9±0.27	4.0±0.28	4.0±0.25	0.368	0.001
LDL-Cholesterol (mg/dL)	89.0±32.33	71.4±26.59	67.5±21.41	0.001	105.9±31.35	123.4±22.30	116.1±29.40	0.001	0.009
HDL-Cholesterol (mg/dl)	42.8±11.99	45.4±11.78	57.1±25.66	0.002	48.6±9.09	50.2±13.25	52.8±14.03	0.001	0.001
Triglyceride (mg/dl)	227.2±118.39	135.1±81.98	138.3±78.27	0.001	146.7±68.21	105.3±55.90	135.9±67.46	0.002	0.001
Total Cholesterol (mg/dl)	172.0±37.15	146.7±34.98	169.7±23.94	0.003	185.5±37.50	191.9±39.21	196.2±28.42	0.001	0.029
Glucose (fasting) (mg/dl)	110.5±23.17	98.1±14.90	97.6±16.90	0.103	93.5±7.59	92.9±9.89	93.0±13.33	0.387	0.001
Hemoglobin (g/dL)	10.8±1.54	10.9+1.47	10.4+0.98	0.871	14.7±1.51	13.9±1.86	14.3±1.72	0.962	0.041
Total protein (g/dL)	6.6±0.46	6.8±0.49	6.8±0.47	0.221	7.0±0.53	7.1±0.38	7.1±0.39	0.035	0.081

p1: difference between quartiles of the patient group, p2: difference between quartiles of the control group, p3: difference between the patient and control groups' laboratory findings.
Abbreviations: CRP: C-Reactive Protein, HDL: High Density Lipoprotein, LDL: Low-Density Lipoprotein

By use of linear regression, univariate and multivariate analyses of parameters associated with dietary total antioxidant capacity in hemodialysis patients were conducted (**Table 3**). In the regression analysis, it was determined that gender played a major role in all models. BMI and other anthropometric parameters were found to have a negative connection with dTAC (p=0.007).

The biochemical measures HDL-C, serum albumin, and serum N/L ratio have been reported to have a positive relationship with dTAC. Examining the association between dietary components and dTAC reveals a positive correlation between dietary protein (kg/avg), beta carotene (mcg/day), vitamin C (mg/day), vitamin E (mg/day), and PUFA (g/day) (p=0.002).

Tablo 3. Univariate And Multivariate Analyzes Of Parameters Associated With Dietary Total Antioxidant Capacity İn Hemodialysis Patients							
Anthropometric variables	β	p	95% CI EXP(B)		βAj	CI 95%	p
			Lower	Upper	-0.078	-0.145; 0.033	0.007
BMI (kg/m²)	-0.074	0034	-0.259	0.430			
TSFT(mm)	-0.022	0.073	-0.187	0.640			
MUAC (cm)	-0.043	0.113	-0.109	-0.008			
Waist/Hip Ratio	-0.084	0.238	-0.101	0.421			
Waist/Height Ratio	-0.050	0.091	-0.239	0.019			
Biochemical variables					0.059	0.255; 0.023	0.005
CRP(mg/dL)	-0.077	0.026	-0.098	-0.005			
LDL-Cholesterol (mg/dL)	-0.091	0.001	-0.213	-0.059			
HDL-Cholesterol (mg/dL)	0.021	0.076	-0.101	0.039			
Albumin (mg/dL)	0.757	0.050	0.202	1.254			
Neutrophil/Lymphocyte Ratio	0.242	0.072	-0.105	0.331			
Lymphocyte/Monocyte Ratio	-0.223	0.039	-0.393	-0.016			
Platelet/Lymphocyte Ratio	-0.301	0.821	-0.307	0.109			
Dietary variables					0.054	0.126; 0.013	0.002
Dietary Protein (kg/avg)	0.404	0.009	0.303	1.012			
Beta carotene (mcg/day)	0.505	0.007	0.0121	1.022			
Vitamin C (mg/day)	0.807	0.001	0.232	2.028			
Vitamin E (mg/day)	0.511	0.004	0.401	2.228			
PUFA (g/day)	0.845	0.006	0.677	2.013			

Note: β: β value simple linear regression analysis; βAj: adjusted value of β for covariates; p value: multiple linear regression analysis significance value p<0.05; gender, educational status, socioeconomic status variables were maintained constant in the final model. Abbreviations: BMI: Body Mass Index, TSFT: Triceps Skinfold Thickness, MUAC: Mid-upper Arm Circumference, CRP: C-Reactive Protein, HDL: High Density Lipoprotein, LDL-C: Low-Density Lipoprotein, PUFA: Polyunsaturated Fatty Acids

J Health Sci Med 2023; 6(1): 202-208

Alataş et al. Hemodialysis patients and diet total antioxidant capacity

DISCUSSION

End-Stage Renal Disease (ESRD) is a complex pathology characterized by an increase in free radicals and a decrease in antioxidant defense. All patients with chronic renal failure, regardless of whether or not they receive renal replacement therapy, experience elevated oxidative stress (16). Moreover, inflammation manifests in the early stages of conical kidney failure. Nguyen–Koha et al. (17), demonstrated that the presence of inflammation and the length of time on dialysis are the most significant causes of OS in HD patients. In this study, it was determined that individuals had CRF for 10.8±5.88 years and received hemodialysis treatment for approximately 4.8±3.12 years. According to reports, low socioeconomic status and low education are risk factors for CRF (18). According to studies, hemodialysis patients have a low level of education, are married, have a moderate or low income, and are typically retired (19-21). In this study, it was established that the majority of participants were married, 53.65% of the hemodialysis patients had completed elementary school, and 41.46% of the control group had completed college.

While uremic toxins are eliminated during hemodialysis, trace elements and hydrophobic, non-protein-bound low molecular weight molecules also enter the dialysate fluid, resulting in a fall in serum levels. Trace elements having antioxidant qualities, such as vitamins C and E, copper, zinc, and selenium, enter the dialysate fluid, increasing oxidative damage in the plasma. Plasma activity of antioxidant enzymes such as glutathione-reductase (GSSG-Red), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) is decreased by hemodialysis treatment (22, 23) This decrease is caused by a decrease in the synthesis of these enzymes in the kidneys or by uremic toxins that alter the protein structures of these enzymes. In hemodialysis, the loss of hydrophilic free small molecular weight substances including vitamin C, trace elements, and regulating enzyme compounds, as well as the existence of malnutrition and malnutrition, are nutrition-related factors that exacerbate oxidative stress.

Serum antioxidant level is correlated with dietary antioxidant intake, and it is recognized that antioxidants protect against oxidative damage and associated inflammatory consequences (24). The intake of nutrients with antioxidant properties is very important for dietary antioxidant intake (25). Fruits and vegetables are high in fiber, bioactive compounds, and micronutrients (26). Vitamins C and E, carotenoids, and flavonoids are the most typical antioxidants present in vegetables (27). Flavonoids are the most prevalent polyphenols found in plants, and they are abundant in tea, coffee, juices, fruits, vegetables, and grains (28). However, medical nutrition therapy and restrictions in food and fluid consumption to reduce minerals such as potassium, sodium, and phosphorus cause hemodialysis patients to consume fewer of these antioxidant-rich food categories than healthy individuals (29). In addition, calcium, magnesium, and potassium intakes of CRF patients are claimed to be low, while dietary cholesterol intake is high (30). This circumstance has a negative impact on dietary antioxidant consumption and increases serum oxidative stress. Consuming foods with a high glycemic index simultaneously promotes oxidative stress and inflammation in hemodialysis users (31). In this study, the connection between dietary components and dTAC was found to be positive for dietary protein (kg/avg), beta carotene (mcg/day), vitamin C (mg/day), vitamin E (mg/day), and PUFA (gr/day) (p=0.002). In addition, this study demonstrated that these nutrients boost antioxidant capability.

There is a positive correlation between inflammation and oxidative stress marker values in dialysis patients, according to studies (32, 33). Indicators of inflammation in the hemodialysis and control groups were found to differ significantly in this investigation. In the highest quartile dTAC of serum ferritin, an essential biomarker of inflammation, was shown to be low. In addition, the inverse association between dTAC, CRP, and the ratio of lymphocytes to monocytes has been established. In HD patients, dyslipidemia and cardiovascular events are more prevalent than in the general population. Therefore, cardiovascular illnesses observed in dialysis patients are linked to antioxidant activity, and inadequate antioxidant capacity was discovered (32).

In these cases, an increase in oxidative stress in the CRF is responsible for the alteration of LDL cholesterol and the development of atherosclerosis. According to reports, oxidized LDL inhibits PON-1 by interacting with its sulfhydryl group (34). LDL cholesterol, HDL cholesterol, TG, and total cholesterol levels in the HD group were found to differ between quartiles in this investigation. It was determined that the group with the highest serum mean HDL cholesterol level was also in the quartile range with the highest dTAC level, and that a strong positive association existed between HDL cholesterol and dTAC. At the same time, it was revealed that the group with the lowest antioxidant capability had the highest overall cholesterol levels. Serum levels of total protein and albumin are significant indications of malnutrition and oxidative stress and inflammation. In the group with the greatest dTAC level, the HD and control groups had the highest mean total protein concentration. In addition, a link between serum albumin concentration and dTAC has been established.

Alataş et al. Hemodialysis patients and diet total antioxidant capacity

J Health Sci Med 2023; 6(1): 202-208

Mild obesity and obesity have been observed to lessen the risk of mortality in hemodialysis patients (35), but the opposite has also been recorded (36). The paradoxical relationship between obesity as a risk factor for CRF and positive survival results in advanced CRF is known as the "obesity paradox." According to this paradox, overnutrition was analyzed as a long-term and undernutrition as a short-term hazard to death, and it was concluded that obesity is not a desirable model for a high survival rate in CRF patients (37, 38). Despite the obesity paradox, high BMI levels may raise the risk of atherosclerosis and cardiovascular disease, which are prevalent in CRF patients. In order to prevent obesity in patients, it is necessary to guarantee enough and balanced nutrition, encourage regular physical exercise, maintain a normal ratio of body fat, and protect muscle mass.

In a study that split dietary TAC values into five categories from least to greatest, a rise in dietary TAC value was related to a drop in BMI (39). In another study, dietary TAC values were separated into two groups based on the median of dietary TAC and BMI. There were statistically significant associations between ($p<0.05$) (40). Levels of dietary antioxidants are inversely linked to body fat percentage. In addition, larger intakes of fiber and phytochemicals, waist circumference, BMI, and plasma lipid peroxidation are inversely proportional (41). This study demonstrated that BMI and other anthropometric parameters were negatively correlated with dTAC in hemodialysis patients ($p=0.007$).

CONCLUSION

Oxidative stress, which results from an imbalance between oxidants and antioxidants, leads to the development of several problems in CRF patients. To improve serum antioxidant capacity in patients on dialysis, it is necessary to prevent uremic condition, bacterial contamination of the dialysis membrane and dialysate, and dietary inadequacies. The relationship between dietary antioxidant intake and serum antioxidant content is strong. In order to prevent complications in these people, a diet rich in antioxidants is necessary. In circumstances when enough antioxidant consumption cannot be reached by diet alone, antioxidant support therapy should be considered.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Malatya Turgut Özal University Clinical Researches Ethics Committee (Date: 01.12.2022, Decision No: 2022/61).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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The effect of the COVID-19 pandemic on the perceived stress levels and psychological resilience of healthcare professionals

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ABSTRACT

Aim: It is aimed to contribute to the literature with the broad support of participants actively working in the field during the COVID-19 pandemic in Turkey. This study was conducted to examine the effect of the COVID-19 pandemic on the perceived stress levels and psychological resilience of healthcare professionals.

Material and Method: A total of 856 healthcare professionals, actively working in the COVID-19 pandemic process across Turkey, participated in the research. The data in the study were collected using the "11 Demographic Questions", the "Four-Item Perceived Stress Scale", developed by Cohen and friends, and the "Six-Item Brief Resilience Scale", developed by Smith and friends to measure psychological resilience levels. The statistical analysis of the study was performed by using SPSS 23. The data, which were not normally distributed, were compared using the Mann Whitney U test and the Kruskal Wallis test. Correlation between the variables was examined via Spearman's correlation analysis and the data, which were not normally distributed, were presented as median.

Results: It was found that the mean score of the perceived stress scale was 12.7±2.9 and the mean score of psychological resilience was 17.8±4.9. It was determined that there was a moderately negative significant correlation between perceived stress and psychological resilience (r:-0.542 p:<0.001).

Conclusion: The results suggested that COVID-19, whose impacts have been felt globally, increased the stress level of healthcare professionals and decreased their psychological resilience.

Keywords: Perceived stress, work stress, psychological resilience

INTRODUCTION

The new coronavirus which first emerged in Wuhan city, China in the last days of 2019 and quickly spread across the globe in these days in the middle of 2020, has been announced by the World Health Organization as a pandemic. Putting individuals at risk in the global sense with Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS), COVID-19 poses serious threats for the physical-mental health and life of individuals (1).

The protection of healthcare professionals who are accepted to have the highest risk in terms of infection is evaluated as one of the most important priorities (2,3). It is known that the epidemic causes fear, helplessness, and anxiety in people and these feelings affect people's behavior negatively (5). Doctors, nurses, and all employees in medical institutions who get exposed to the stress of pandemics at the highest level and try to cope with their psychological results to a long time, comprise a group that is mostly affected by all pandemics and has a risk of suffering (6,4).

Today, stress and job stress negatively affect the life of the individual and cause health problems, but also negatively affect the quality of life of the individual and the support needs of each individual vary according to their personality traits (8,9,11,12). A study conducted with firefighters working under high stress such as healthcare workers found that emotional social support in the workplace is related to occupational health (10). On the other hand, the participation of employees is important for managing stress and psycho-social risks, especially in the workplace, and it will increase the general morale and make the precautions adequate and effective (13).

The Perceived Stress Scale is a short and manageable measure of a person's degree of rating the stressful situations in her/his life. It has been proven to have significant validity and reliability (14,15). Uncontrollable uncertainties of the pandemic period, high-stress and intensive working environment, and the thought that healthcare professionals as well as their relatives also face the risk of infection and incorporate a physical, mental and spiritual imbalance (7). Resilience is directly related to adapting to all these difficult and traumatic conditions and emerges as an individual's ability to continue his life without losing his sense of control over events (17).

Healthcare professionals encounter a heavy virus load the struggle and also their mental health is seriously affected due to working intensely and insecurely missing opportunities under high risk without taking adequate rest (20). Also, the quarantine applications may lead to an increase in stress level and emotional problems (21,22).

MATERIAL AND METHOD

The research questions of the study are as follows;

1. What is the perceived stress level of healthcare professionals during the COVID-19 pandemic?
2. What is the psychological resilience level of healthcare professionals during the COVID-19 pandemic?
3. Is there a relationship between the perceived stress level and the psychological resilience of healthcare professionals during the COVID-19 pandemic?

The study was carried out with the permission of Dokuz Eylül University Non-interventional Clinical Researches Ethics Committee (Date: 01.06.2020, Decision No: 2020/11-41). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This descriptive and cross-sectional study was conducted between May-June 2020. A total of 856 healthcare professionals could be reached by applying online questionnaires to healthcare services employees (such as doctors, nurses, and health technicians), general administrative services employees (such as clerks, and data record officers) and assisted services employees (such as cleaning staff, caregivers, servants, dead body bathers) working during the pandemic. In this study with many participants, an online questionnaire was chosen because of curfews, social isolation rules, and risk of infection and to reach people working actively across Turkey (23). The questionnaire form link was shared via Whatsapp and other social networks and also by voluntary supporters. With this method, it became possible to reach broad participants working in different institutions across Turkey. A sample group with demographically different qualities voluntarily contributed to the study and spread the study. Data collection difficulties and time hardships of the pandemic period were thus minimized and 856 people were reached.

The data in the study were collected using the "11 Demographic Questions", prepared by the researchers to determine demographic characteristics, the "Four-Item Perceived Stress Scale", developed by Cohen et al. (14); and the "Six-Item Brief Resilience Scale", developed by Smith et al. (18) to measure psychological resilience levels. The reason for applying the short forms of the scales used is to encourage the participants to answer the questions in the shortest time possible. The questionnaire comprises three sections.

1. The section including the personal characteristics of healthcare professionals comprises 11 questions about age, gender, educational level, etc.
2. The section aimed at determining the perceived stress of healthcare professionals comprises four questions.
3. The section aimed at determining the psychological resilience of healthcare professionals comprises six questions.

Developed by Cohen et al. (14), the Perceived Stress Scale comprises a total of 14 items. Along with the 14-item form, the Perceived Stress Scale also has two other forms with ten and four items. The Turkish adaptation of the four-item form of the Perceived Stress Scale, was indicated to have adequate reliability. The internal consistency coefficient was found to be 0.66. The four-item Turkish version of the Perceived Stress Scale was indicated to be useful as a valid and reliable measurement tool for measuring the stress perceptions of individuals in their lives (16).

Developed by Smith et al. (18), the Brief Resilience Scale (BRS) was conducted on a sample comprising university students to examine psychometric properties (18) and to adapt the scale to Turkish. The results acquired as a result of this study showed that the scale was valid and reliable enough to measure the psychological resilience level. In the Turkish adaptation of the Brief Resilience Scale by Dogan (19), the relevant internal consistency coefficient was found to be .83.

To evaluate the data, statistical analyses were analyzed by the researchers via the IBM SPSS V23 in the computer environment and the convenience for normal distribution was examined using the Kolmogorov Smirnov. The data, which were not normally distributed, were compared using the Mann Whitney U test and the Kruskal Wallis test. Correlation between the variables was examined via Spearman's correlation analysis and the data, which were not normally distributed, were presented as median (minimum – maximum). The categorical data were compared using the chi-square test and were presented as frequency (percentage). The significance level was taken as $p < 0.050$.

Özçelik Kaynak et al. Stress levels and psychological resilience in COVID-19

J Health Sci Med 2023; 6(1): 208-214

RESULTS

When evaluating the demographic characteristics of the healthcare professionals comprising the sample; 75% of 856 participants were in the age range of 30-50 years, 74.9% were female, and 72.1% were married. When examining the title distribution, 27.8% were nurses, 23.2% were health technicians, 20.4% were doctors, 11.3% were administrative and white-collar workers and 17.3% were support personnel. Among the participants, 36.3% had a professional seniority of 20 years and above, 20.7% 15-19 years, 19.9% 10-14 years, and 11.1% 0-4 years. 83.1% of the participants were employees of the public medical institutions. 82% of them stated that there were patients diagnosed in the institution. 27.6% of the participants often contacted positive patients on duty and 27 healthcare professionals (3.2%) were diagnosed with COVID-19. Among the factors creating stress for the participants during the pandemic period, which were a multiple-choice questions, the most important factor was the fear of not having the family when needed (83.2%), which was followed by the anxiety of infecting the family and immediate circle with the virus (81.3%) and the thought of having a loss in the family (61.9%). Among the participants; 63.6% met their family every day during the pandemic period, but 18% did not (**Table 1**).

Perceived stress varied according to gender (p=0.002). The perceived stress median value was found to be 12 for males and 13 for females. Perceived stress varied according to age groups (p=0.003). The perceived stress score median value was found to be 13 for the age groups of 20-30 and 31-40 years and 12 for the age groups of 41-50 and 51 years and over. The difference was caused by the higher perceived stress level in the age group of 20-30 years compared to the age group of 41-50 and 51 years and over. The age group of 31-40 years was not different from other age groups (mean rank values of 479.1, 444.1, 402.7, and 379.5, respectively). Perceived stress varied according to a title (p=0.006). The perceived stress score was 12 for the doctor group, 13 for the nurse, administrative, and office services, and health technician groups and 12 for other groups. The difference was associated with the lower perceived stress score in doctors compared to the health technician group (mean rank values of 397.2, 418.5, 472.9, 469.7, and 396.7, respectively). Perceived stress varied according to having close contact with COVID-19 patients (p<0.001). The perceived stress median value was found to be 13 for individuals who occasionally and frequently have close contact with COVID-19 patients and 12 for those having no close contact. The perceived stress score was found to be lower for individuals having no close contact than those occasionally and frequently having close contact. Perceived stress varied according to the frequency of meeting the family during the pandemic period (p<0.001). It was found

to be 14 for individuals meeting their family every other day, 13 for those meeting their family once a week, 12 for those meeting their family every day, and 13 for those never meeting their family. The perceived stress score was found to be lower for individuals meeting their family every day than those meeting their family every other day and those never meeting their family (mean rank values of 488.2, 431.2, 401.1, and 491.2, respectively). Perceived stress did not vary according to other variables (p>0.050).

Table 1: Nurses' sociodemographic characteristics (n=856)		
Characteristics	**n**	**%**
Gender		
Male	215	25,1
Female	641	74,9
Age Groups		
20-30 ages	141	16,5
31-40 ages	313	36,6
41-50 ages	331	38,7
51 ages and above	71	8,3
Marital Status		
Single	239	27,9
Married	617	72,1
Title		
Doctor	238	27,8
Nurse	199	23,2
Administrative and White-Collar Worker	175	20,4
Health Technician	97	11,3
Other	147	17,3
Duration of working		
0-4 years	95	11,1
10-14 years	170	19,9
5-9 years	103	12,0
15-19 years	177	20,7
20 years and above	311	36,3
Health institution worked		
University Hospital	365	42,6
Ministry of Health	347	40,5
Other	85	9,9
Private Hospitals	59	6,9
COVID-19 positive diagnosis		
Yes	27	3,2
No	829	96,8
Contact with a COVID-19 patient at close range		
Sometimes	394	46,0
Never	226	26,4
Often	236	27,6
Are there patients diagnosed with COVID-19 in the healthcare institution?		
Yes	702	82,0
No	154	18,0
Frequency of meeting with the family during the pandemic period		
Every other day	75	8,8
Once a week	74	8,6
Everyday	544	63,6
Never meet	163	19,0
Total		
	856	100

J Health Sci Med 2023; 6(1): 208-214

Özçelik Kaynak et al. Stress levels and psychological resilience in COVID-19

Psychological resilience varied according to gender (p=0.002). The psychological resilience median value was found to be 18 both for males and females. The difference was associated with the lower mean rank value in males compared to females (mean rank values of 383.1 and 443.7, respectively). Psychological resilience varied according to age groups (p=0.023). The psychological resilience score median value was found to be 17 for the age group of 20-30 years and 18 for other age groups. The difference was associated with the lower psychological resilience score in the age group of 20-30 years compared to the age group of 41-50 years (mean rank values of 375.1, 426.9, 451.5, and 434.3, respectively). Psychological resilience varied according to a title (p=0.047). The psychological resilience median value was found to be 17 for the health technician group and 18 for the other groups. The difference was caused by a higher psychological resilience score in the doctor group than in the health technician group (mean rank values of 467, 439.9, 412.9, 396.9, and 413.7, respectively). Psychological resilience varied according to the frequency of meeting the family during the pandemic period (p=0.004). It was found to be 16 for individuals meeting their family every other day, 18 for those meeting their family once a week, 18 for those meeting their family every day, and 17 for those never meeting their family. The psychological resilience level was found to be higher for individuals meeting their family every day than those never meeting their family (mean rank values of 384.5, 453.6, 446.7, and 376.5, respectively). Psychological resilience did not vary according to the other variables (p>0.050) (**Table 2**).

It was found that the mean score of the perceived stress scale was 12.7±2.9 and the mean score of psychological resilience was 17.8±4.9. It was determined that there was a moderately negative significant correlation between perceived stress and psychological resilience (r:-0.542 p:<0.001). As the perceived stress scale score of healthcare professionals increased, their psychological resilience score decreased (**Table 3**).

Table 3: The correlation between the mean scores of perceived stress and psychological resilience	
	Psychological Resilience (17.8±4.9)
Perceived Stress (12.7±2.9)	r:-0,542 p:<0,001
r: Spearman correlation coefficient	

Table 2. Comparisons according to perceived stress and psychological resilience scores (n=856)	Perceived stress median (min-max)*	Psychological resilience median (min-max)*
Gender		
Male	12 (4-20)	18 (6-30)
Female	13 (4-20)	18 (6-30)
Test statistics	U=78676	U=59059.5
p	0.002	0.002
Age Groups		
20-30 ages	13 (4-20)[a]	17 (6-30)[a]
31-40 ages	13 (5-20)[ab]	18 (6-30)[ab]
41-50 ages	12 (4-20)[b]	18 (6-30)[b]
51 ages and above	12 (5-20)[b]	18 (10-30)[ab]
Test statistics	χ^2=13.741	χ^2=9.528
p	0.003	0.023
Marital Status		
Single	13 (4-20)	18 (6-30)
Married	12 (4-20)	18 (6-30)
Test statistics	U=67642	U=77142.5
p	0.059	0.292
Title		
Doctor	12 (5-20)[a]	18 (6-30)[a]
Nurse	13 (4-20)[ab]	18 (6-30)[ab]
Administrative and White-Collar Worker	13 (4-20)[ab]	18 (6-30)[ab]
Health Technician	13 (4-20)[b]	17 (6-30)[b]
Other	12 (4-20)[ab]	18 (6-30)[ab]
Test statistics	χ^2=14.453	χ^2=9.647
p	0.006	0.047
Duration of working		
0-4 years	13 (4-20)	18 (6-30)
10-14 years	13 (5-20)	18 (6-30)
5-9 years	13 (4-19)	18 (6-30)
15-19 years	12 (4-20)	18 (6-30)
20 years and above	13 (5-20)	17 (6-30)
Test statistics	χ^2=14.288	χ^2=5.414
p	0.050	0.247
Health institution worked		
Private Hospitals	13 (4-18)	18 (6-30)
Ministry of Health	13 (4-20)	18 (7-30)
University Hospital	13 (4-20)	18 (6-30)
Other	13 (6-20)	18 (6-29)
Test statistics	χ^2=8783.5	χ^2=12396.5
p	0.855	0.062
COVID-19 positive diagnosis		
Yes	14 (10-18)	17 (8-26)
No	13 (4-20)	18 (6-30)
Test statistics	U=8783.5	U=12396.5
p	0.055	0.339
Contact with a COVID-19 patient at close range		
Sometimes	13 (4-20)[b]	18 (6-30)
Never	12 (4-20)[a]	18 (6-30)
Often	13 (4-20)[b]	18 (6-30)
Test statistics	χ^2=20.700	χ^2=2.666
p	<0.001	0.264
Are there patients diagnosed with COVID-19 in the healthcare institution?		
Yes	13 (4-20)	18 (6-30)
No	12 (4-20)	18 (6-30)
Test statistics	U=49204	U=55967.5
p	0.079	0.490
Frequency of meeting with the family during the pandemic period		
Every other day	14 (4-20)[b]	16 (6-30)[ab]
Once a week	13 (7-19)[ab]	18 (9-30)[ab]
Everyday	12 (4-20)[a]	18 (6-30)[a]
Never meet	13 (4-20)[b]	17 (6-30)[b]
Test statistics	χ^2=21.858	χ^2=13.382
p	<0.001	0.004
U: Mann Whitney U test, χ^2: Kruskal Wallis test a-b: There is no difference between groups with the same letter, * Median (minimum-maximum)		

Özçelik Kaynak et al. Stress levels and psychological resilience in COVID-19

J Health Sci Med 2023; 6(1): 208-214

DISCUSSION

The pandemic period that we experience has included all people within the context of risk groups and has become alarming for the masses. Individuals have begun to worry about themselves, their relatives, and the future. They also suffer from physical and psychological difficulties due to the stress created by uncertainty. Individuals' perception of diseases and the behaviors they display in line with this perception, play a key role in the transmission rate of the pandemic and loss of lives. Therefore, it is crucial to analyze the psychological state of individuals in the struggle against the pandemic and to develop applications accordingly (24). As is known, pandemics create traumatic effects and also increase anxiety and stress levels. In the studies, it has been determined that mood changes related to COVID-19 cause worsening effects on psychological cases to psychiatric cases (25). COVID-19 concern appears as common symptom of anxiety and depression (26).

This study which was conducted with the broad support of participants actively working in the field during the COVID-19 pandemic in Turkey and is considered to make a higher contribution to the literature is important. The fact that a total of 856 healthcare professionals across Turkey could be reached online, is crucial despite the social limitations brought by the COVID-19 pandemic period. It was determined that the participants were usually unable to control important things in their lives and their power of coping with personal problems decreased. They had a sense that things were not right. This is a reflection of the uncontrollable and unpredictable properties of pandemic periods. The data aimed at determining the perceived stress level during the pandemic period revealed that the stress means value (12.7±2.9) was high (**Table 3**). Similarly, it was determined that health issues caused by the COVID-19 pandemic and having a higher possibility of resulting in death as well as the limitations imposed by quarantine applications increased depression and anxiety levels and had a risk for permanent problems with psychological crises triggered (27,28).

In the face of the compelling conditions of the pandemic period, individuals have to confront these difficulties and stretch their reactions to pressures. Developing the self by confronting compelling conditions, which is an indicator of the individual's strength is a dynamic structure affected by personal, familial, and environmental features. Healthcare professionals' fear of not being with their family when needed and their anxiety about infecting their family increase their stress levels and reduce their resilience. In their study, Smith et al. (29), found that healthcare professionals had stress due to the possibility of infecting their relatives as much as for them.

In line with the results acquired from the study, gender was found to be effective on perceived stress and psychological resilience. Stress and psychological resilience were found to be lower in males than females, which might be associated with a higher number of female participants than male participants in the study. In regards to age, younger individuals (20-30) had higher perceived stress and lower psychological resilience. The fact that they have faced such a global pandemic risk for the first time and the limitations imposed by the precautions taken explain the psychological outcomes on young people. The life experience of especially individuals above 51 years, has increased their resilience.

Nurses and intermediary medical personnel who professionally take active and intensive charge in the care process also had lower perceived stress levels and higher resilience. It is possible that doctors felt more confident and had lower stress levels and higher resilience thanks to their knowledge level regarding the disease and their conscious behaviors related to preventive measures. On the other hand, healthcare professionals having contact with patients diagnosed with COVID-19 had higher perceived stress levels. In the study conducted by Zhang et al. (30) to determine whether healthcare professionals had psycho-social problems during the COVID-19 pandemic or not they determined that especially healthcare professionals having direct contact with patients had higher anxiety and depression levels than those not having any contact; which compatible with the result of the present study.

Healthcare professionals who meet with their family regularly have lower perceived stress levels and higher psychological resilience, although they fear that they might infect with the virus. The support individuals give to one another and the comfort of socializing will relieve individuals in the face of social isolation difficulties of the pandemic period. In Turkish society; the mother, father, siblings, and other members of a family have an important place and provide social and psychological support for individuals. Controlling stressful situations and knowing not alone in the face of difficulties will enhance the individual's psychological resilience and help her/him embrace life more positively and collect herself/himself more quickly. A family which has a very important place in Turkish culture and Islamic belief as a basis of society, advises individuals to support each other both materially and spiritually at the hardest times (31). The results demonstrated the positive effects of family relationships on the majority of the healthcare professionals who participated in the study from different parts of Turkey.

As the perceived stress scale score of healthcare professionals increased, their resilience score decreased.

J Health Sci Med 2023; 6(1): 208-214

Özçelik Kaynak et al. Stress levels and psychological resilience in COVID-19

The moderately negative significant correlation determined between these two is compatible with studies examining the correlation between psychological resilience and perceived work stress and determining that as the psychological resilience level of nurses decreased, their depression and stress levels increased (32).

CONCLUSION

Infectious diseases threatening human health worldwide such as plague, cholera, AIDS, and influenza, were experienced in the past years and resulted in the death of many people by spreading among the large masses. Uncontrollable aspects of pandemic periods such as unforeseen transmission rate, area of influence, and time of ending affect the physiological and psychological condition of individuals. While individuals over the age of 60 years with chronic diseases are involved in a high-risk groups, it is inevitable for healthy individuals to be affected psychologically due to the risk of getting infected, infecting their relatives, and experiencing loss as well as the changes caused by limitations in the routine.

Healthcare professionals who have non-negligible importance in the struggle against COVID-19 as in other pandemic, are under physical and psychological threat because of their key role. The high responsibility imposed by working during the pandemic period, heavy workload, the obligation of keeping away from the family environment and the threat perceived regarding health caused by infection risk, may cause them to experience symptoms such as stress, anxiety, depression, sleep problems, anger, and fear. Therefore, healthcare professionals must be protected and reinforced for an effective struggle against the pandemic. It will not be possible to stop COVID-19 without protecting healthcare professionals. It is necessary to create an environment where working conditions are safe, personal protective equipment is accessed easily, workload is distributed fairly, the health system is employee-based, deficiency experienced in the family and social circle is supported, and negative effects of the stress that might be caused the pandemic are minimized and resilience is enhanced.

Knowing the effect of the COVID-19 pandemic on the stress and psychological resilience of healthcare professionals and making arrangements in this direction will reinforce the struggle of leading actors in the process and be effective in the continuity of healthcare service delivery.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dokuz Eylül University Non-interventional Clinical Researches Ethics Committee (Date: 01.06.2020, Decision No: 2020/11-41).

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 has no conflicts of interest to declare.

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

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Regulatory immune cells: a review of the novel paradigm of primary Sjogren's syndrome

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ABSTRACT

Primary Sjögren's Syndrome (pSS) is an autoimmune disease that mostly affects women. Patients with pSS experience dry mouth and eyes in addition to signs of systemic disease. pSS was considered a Th1 autoimmune disease for many years. However, in various studies, it has been shown that dysregulation of regulatory cells play critical role in the pathogenesis of the disease. This review focuses on studies supporting this view and answers questions about the role of regulatory cells in the pathogenesis of pSS.

Keywords: Arthritis, rheumatoid, regulatory T-cells, regulatory B-cells, primary Sjogren's syndrome, follicular regulatory T-cells

INTRODUCTION

Primary Sjögren's syndrome (pSS) is an exocrinopathy that is associated with lymphocyte infiltration in exocrine glands which results in progressive inflammation and tissue destruction. The glands generally affected are the lacrimal glands and salivary glands. However, lung, heart, kidney , nervous system, and lymphatic systems may be affected as part of extra-glandular pattern (1). Environmental factors, especially viruses, activate glandular vascular endothelial cells, the glandular epithelial cells and dendritic cells (DCs) in the stromal area. Along with the activation of DCs, type I and type II IFN pathways are activated and further IL-12 production results in IFN-gamma production by NK and TH1 cells (2). Vascular endothelial cells also secrete nitric oxide (NO) which decrease the secretion of exocrine glands (3). As a result of decrease in secretions, clinical ocular symptoms (keratoconjunctivitis sicca) and oral symptoms (dry mouth) are seen in pSS. There is no etiology directed treatment of pSS for today. Symptomatic therapies such as glucocorticoids, cyclophosphamide, conventional disease-modifying antirheumatic drugs (DMARDs), rituximab make up the backbone of therapy (4).

Disease mechanism through IFN-gamma pathway is also supported by genetic, epigenetic and immunological alterations. Interaction of IFN-gamma pathway with both innate and acquired immunity takes shape via the B lymphocytes. In addition to B lymphocyte custody, T cells also contribute to immunopathogenesis of pSS. Recent studies have shown that regulatory cells have critical functions in the evolution of pSS pathology as effector immune cells (2,3-5). In this review we summarized the functions and proofs of regulatory cells and in pSS.

REGULATORY B CELLS

B cells take part a major place in the pSS pathogenesis by production of autoantibodies, cytokines which cause apoptosis in epithelial cells in exocrine glands (3). Regulatory B (Breg) cells are immunosuppressive cells that promote immunological tolerance. Breg cells suppress immunopathology by inhibiting the expansion of helper T cells, and another proinflammatory lymphocytes through the production of interleukin-10 (IL-10), IL-35 and transforming growth factor β (TGF-β) (3,6). The suppression mechanisms of Breg cells are shown in **Figure 1**. Interleukin 10 (IL-10) production classically defines a Breg, but the stability and/or plasticity of this population is not yet well understood by studies. It is known that more studies are needed to understand Breg plasticity (7).
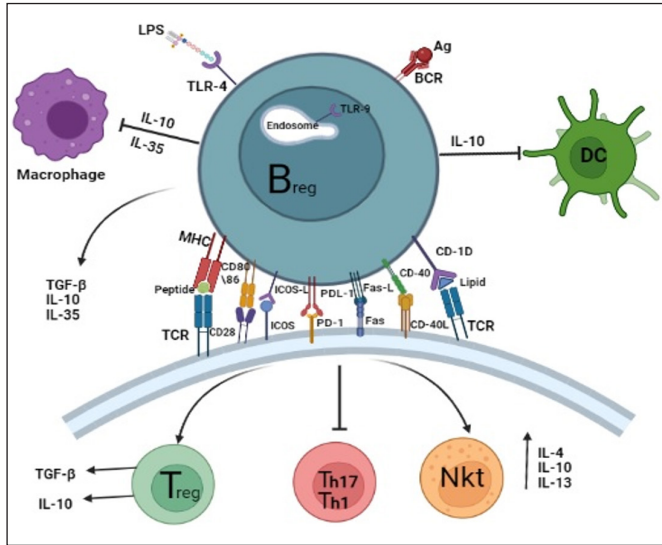
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Figure 1. Breg cells suppression mechanisms

Breg cells form 0.2 to 0.6% of B cells and this ratio reveals that its regulatory properties are quite strong. The phenotype of Bregs is CD19+CD24hiCD38hi. Three different types of Bregs identified in pSS. IL-10-producing Bregs which suppress Th17 and Tfh cells augmenting the autoimmune inflammation are found to be decreased in pSS (2). GrB-producing Bregs that cause apoptosis in target cell via perforin, are increased in pSS. IL-35 producing Bregs act as IL-10 producing Bregs that both suppress Th1 and Th17 and also induce Tregs. It is not clear whether IL-35 producing Bregs increased or decreased in pSS.

Various studies have shown that regulatory B cells are effective in the pathogenesis of pSS and in disease control. In one study, the percentage of regulatory B cell subsets showed significant differences in pSS patients with active and inactive disease. CD19+CD24hiCD38hiIL-10+ cells were found to be strikingly higher in the whole pSS group crosschecked to healthy controls which suggests that they are effective in disease regulation and pathogenesis (8). In another study 21 pSS patients with low disease activity and 21 healthy controls were compared and IL-10 producing regulatory B cell frequency is not decreased and these cells are functional (9). There is opposite findings that do not support this view, for example Lin et al. (10) showed that CD24hiCD38hi Breg cells from pSS patients could not effectively suppress follicular helper T cell differentiation due to reduced IL-10 production, arguing that Bregs are dysfunctional. The reason for the ineffectiveness of the regulatory properties was affirmed that the selected patients were inactive patients and used various immunosuppressive drugs. Overall, it can be resulted in that the levels of regulatory B cell phenotypes studied are different in patients with clinically inactive pSS and active pSS. All these results suggest that Breg regulatory functions are important in pSS progress.

Currently approved treatments of pSS is mainly symptomatic treatment and the treatment of organ involvement are antirheumatic drugs or targeted therapies adapted from other rheumatologic diseases. Earlier studies on pSS focusing on targeting the B cells have been reported to fail in efficacy but recent phase IIb study of anti-B cell-activating factor (BAFF) receptor antibody ianalumab showed positive findings in subjects with moderate to severe pSS (11). BAFF has an inhibitor role on Bregs (12). BAFF inhibition inversely has potential to influence the effector B cells negatively, and Bregs cell function positively, thus, while autoantibodies secreted by effector B cells would decrease, immunsupression by Bregs will increase which results in a double hit to autoimmune pSS.

REGULATORY T CELLS

Regulatory T cells (Tregs), were originally identified by the high surface expression of the alpha chain of the IL-2 receptor (IL-2Rα, CD25) and contain forkhead box protein P3 (FoxP3) transcriptional factor as a marker. The phenotype of Tregs is CD4+CD25hiFOXP3+. Their ability to inhibit autoimmunity originates from suppressive activity against autoreactive lymphocytes through cell-to-cell contact or the soluble mediators including IL-10 and transforming growth factor β (TGF-β) (13). Most of the peripheral Treg cells originate from the thymus and these cells are called "thymus-derived Treg (tTreg)" cells (14-17). In addition, Treg cells can differentiate from naive CD4+ T cells in the periphery, and these Treg cells are called "peripherally derived Treg (pTreg)" cells. Also, it can differentiate after stimulation in the presence of TGF-β and IL-2 in the periphery in vitro and these cells are called "iTreg cells" (18,19). FoxP3 expression plays an important role in Treg plasticity. it is also known that intracellular metabolites and metabolic pathways regulate the expression of Foxp3 and the functional plasticity of Treg cells. (20-22).

The immunopathogenesis of pSS has not yet been fully clarified. There are many studies on the role of Tregs in the pathogenesis of pSS. In one study of pSS patients, Tregs were investigated by immunohistochemical staining (IHC) from lip salivary gland and by flow cytometric assay from peripheric blood. Also mRNA expression of Foxp3 is analysed by real-time polymerase chain reaction (rt-PCR) in salivary gland tissue. At result Tregs relative expresions in peripheric blood and Foxp3 genetic expression in salivary gland tissues of patients with pSS were significantly decreased when compared with healthy controls (23). Other studies also confirmed these findings (24,25). Also, there are contrasting studies which showed that

circulating Tregs were increased (26,27) or remained similar in pSS when compared to healthy controls (8,28,29). Alunna et al. (30), found higher relative expression of CD4+CD25lowGITR+ cells in milder Sjögren's syndrome patients than in healthy controls. Clonal expansion of this subset of Treg cells attenuated the activity of effector T cells that results in milder disease. Christodoulou et al. (28) reported also Treg frequency in salivary gland tissue positively correlated with disease inflammation grade. In another study, CD161+CD25+CD4+ Treg subpopulation, which have regulatory properties with IL-17 production was increased when compared with healthy controls. Furthermore, the function of this regulatory subset in SS patients is related to the clinical severity of the pathogenesis of pSS (31).

Studies have shown that Treg cells help in the regulation of pathogenesis in pSS patients. It is thought that the presence of these cells may help the development of new diagnostic techniques or treatment methods in pSS patients.

FOLLICULAR REGULATORY T CELLS (TFR)

Lately defined follicular regulatory T (Tfr) cells, a subset of Treg cells, are known to control the function of T follicular cells and Germinal Center (GC) reactions by regulating T follicular helper (Tfh) cell mediated B cell responses after antigenic exposure. Tfr cells inhibit B cells via CTLA4, an inflammatory cytokine producion via Tfh cells (32). Tfr cells have the properties of Treg cells, regulating Germinal Center responses and antibody production. The regulating Germinal Center responses and antibody production shown schematically in **Figure 2**. They are identified as FoxP3+ CD4+ T cells that express chemokine receptor CXCR5 (33,34). Tfr cells have lower expression of CXCR5 when compared to the Tfh cells (32).
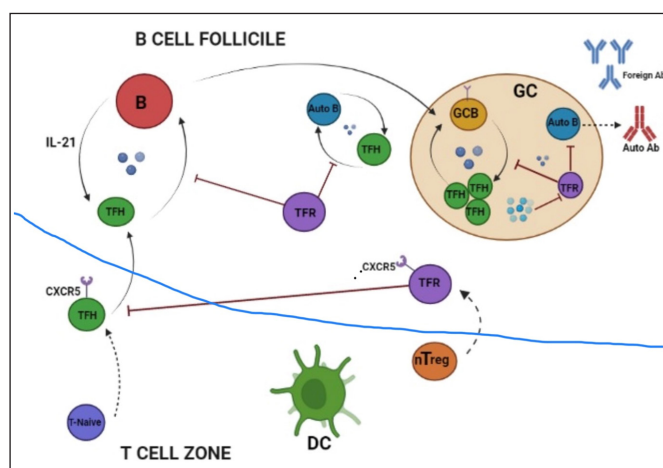

Figure 2. Schematic view of regulating Germinal Center responses and antibody production.

CXCR5 expression on Tregs is dependent on Bcl-6. These CXCR5(+) Bcl-6(+) T(reg) cells are absent in the thymus, but CXCR5(-) Foxp3(+) can be regenerated from natural T(reg) precursors. (33-35) Upregulation of CXCR5 results in TFH cells and mature TFR allows migration to germinal centers. Also, Tfr cells can migrate to GC independently of CXCR5. Tfr cells that migrate to the germinal center become equipped to regulate larger germinal center reactions, including affinity maturation of antibodies and differentiation of plasma cells (36,37). It is reported that humoral immunesuppression capacity of Tfr was inferior compared to conventional Treg cells (38).

Fonseca et al. (39) reported increased levels of Tfr cells and Tfr/Tfh ratio in peripheral blood of pSS subjects. In another study Fonseca et al. also reported strong correlation between circulating Tfr cells and lymphocytic infiltration in minor salivary glands of pSS subjects. They stated that the ratio of cTfr/cTfh as a marker for the diagnosis of pSS could be a biomarker for the diagnosis of pSS (40). Contrary to Fonseca's study Verstappen et al. (41) found circulating Tfr/Tfh ratio was increased in pSS patients but this increase was not associated with gland inflammation. Kim et al. (42) investigated subsets of Tfh and Tfr cells in the blood and relation of these subsets with disease activity, glandular inflammation, and autoantibody responses in 18 pSS patients compared to HCs. They found that blood Tfr and Tfh cell ratios were increased in pSS patients compared to HCs. These data prove the presence of Tfhr cells with regulatory functions in the peripheral blood and salivary gland tissues of pSS subjects. Nevertheless, the role of Tfr cells in the pathogenesis of pSS is still controversial. However, the functional capabilities of Tfr cells in SG tissue and peripheral blood requires more research.

CONCLUSION

In the pathogenesis of pSS, damage to salivary and lacrimal gland epithelium results in pro-inflammatory cytokine secretion, organised B and T cell infiltration. Autoreactivity exocrine gland antigens drives more T cell activation and many inflammatory cytokines which trigger more T cells and B cells. Increased B cell activation and autoantibody production causes progressive tissue damage. This cycle of activation and reaction that repeats itself over and over again. Regulatory cells are critical brakes for this endless activation mechanisms. Tregs, Bregs and fTr cells control the overactivated immune response. Dysregulation or deficiency in Treg cells expansion and differentiation contribute Th1-like or Th17 inflammatory phenotype. It has been shown that the density of Treg cells in the blood and exocrine glands of pSS patients depends on the disease activity,

and the disease activity increases in cases where the density is low or Treg cells are dysfunctional. The Tfr cells as a subset of Tregs, extend the suppression effect of Tregs into GC. They are antagonistic to Tfh cells that additionally inhibit autoantibody production, somatic hypermutation, and class switch recombination of B cells. Bregs attenuate cytokine production from monocytes and T cells. They inhibit Th1, Th17 and CD8+T cell responses, transfom naive CD4+ cells into Tregs and suppress inflammation by soluble mediators IL-10, TGF-β and IL-35.

It is important to systematically evaluate the correlation among the count of circulating Treg, Breg and Tfr cells and markers of the diagnosis, severity of disease, treatment efficacy, and prognosis of pSS. Also, the correlation among circulating - tissue regulatory cells should be clarified in pSS. Phenotypically and functional distinct regulatory cells cooperate in pSS in order to limit inflammation and overbuild immune homeostasis.

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Persistent trigeminal artery incidentally found in a patient with brain posterior system infarction: a rare case report

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ABSTRACT

The persistent trigeminal artery (PTA) is a persistent, primitive, embryonic anastomosis that provides the blood flow between the vertebro-basilar system and the carotid system. The presence of PTA anastomosis may create an advantage by providing collateral circulation during a stroke as in cases of leptomeningeal collaterals, external-internal carotid artery anastomoses, and the polygon of Willis. However, patients with this variation are more susceptible to the development of non-occlusive posterior arterial system infarction when they experience hypotension attacks. In the literature, there are only few case reports on non-occlusive posterior system infarction associated with PTA variation. In this case report, we present to an 81-year-old patient with posterior system infarct findings and persistent trigeminal artery variation.

Keywords: Persistent trigeminal artery, posterior arterial system infarction, hypotension

INTRODUCTION

Carotid-vertebrobasilar anastomoses, which provide blood supply to the vertebrobasilar system from the internal carotid artery (ICA) in the embryonic period, are called presegmental arteries. Presegmental arteries that are present in the embryonic period disappear by involution during the intrauterine period, but if there is a problem during this process, they do not show regression and can still be seen in the adult period (1). The most common presegmental arteries in adulthood are the persistent trigeminal artery (PTA), persistent hypoglossal artery, persistent otic artery, and persistent proatlantal intersegmental artery (1). PTA accounts for approximately 85% of all persistent presegmental arteries (2). In digital subtraction angiography (DSA), PTA is observed at a rate ranging from 1 in a 1,000 to 6 in 1,000, and it is usually unilateral (2, 3). In radiological examinations, almost all cases are detected incidentally. Therefore, the number of actual cases is considered to be above this rate (2). The PTA variation was angiographically classified by Saltzman (3) and Wollschlaeger (4). In Saltzman type 1, PTA opens to the distal of vertebral artery. The posterior communicating artery (PCOM) and the distal end of the basilar artery are absent, or the vertebral and basilar arteries are hypoplastic. In Saltzman type 2, PTA supplies the superior cerebellar arteries (SCAs) through the posterior cerebral arteries (PCA) supplied by PCOM.

In Saltzman type 3 (rare), PTA supplies bilateral SCA and contralateral PCA (4).

PTA is mostly asymptomatic. However, in the literature, pathological conditions, such as aneurysms, vascular-nerve compression, trigeminal cavernous fistulas, and thromboembolic ischemia, as well as associated neurological conditions, palsies of the third sixth cranial nerves, trigeminal neuralgia, vertigo, ataxia, migraine, and headache have been described in patients with this variation (2,4). In this report, we present a case of PTA variation with multiple foci of infarction in the posterior arterial system irrigation area and no arterial occlusion.

CASE REPORT

An 81-year-old female patient presented to the emergency department in April 2022 with the complaints of sudden onset of dizziness, visual impairment, imbalance, and ataxia. She stated that she had previously experienced these symptoms occasionally. There was no exacerbation of symptoms with head rotation. According to her anamnesis, she had a history of surgery due to aortic valve stenosis but no previous history of loss of consciousness or seizures. She also stated that she occasionally had attacks of a sudden drop in blood pressure. There was no suspicion of recent toxin exposure or medication use. No significant neurological deficit was detected in the physical examination.

J Health Sci Med 2023; 6(1): 220-222

Aytaç et al. Non-occlusive infarction and persistent trigeminal artery variation

During the follow-up of the patient in the emergency department, millimetric foci showing diffusion restriction consistent with acute infarction were observed in the left cerebellar hemisphere, left occipital lobe, and both thalami in the diffusion-weighted magnetic resonance imaging examination performed as the initial imaging method (**Figure 1a, 1b, 1c**).
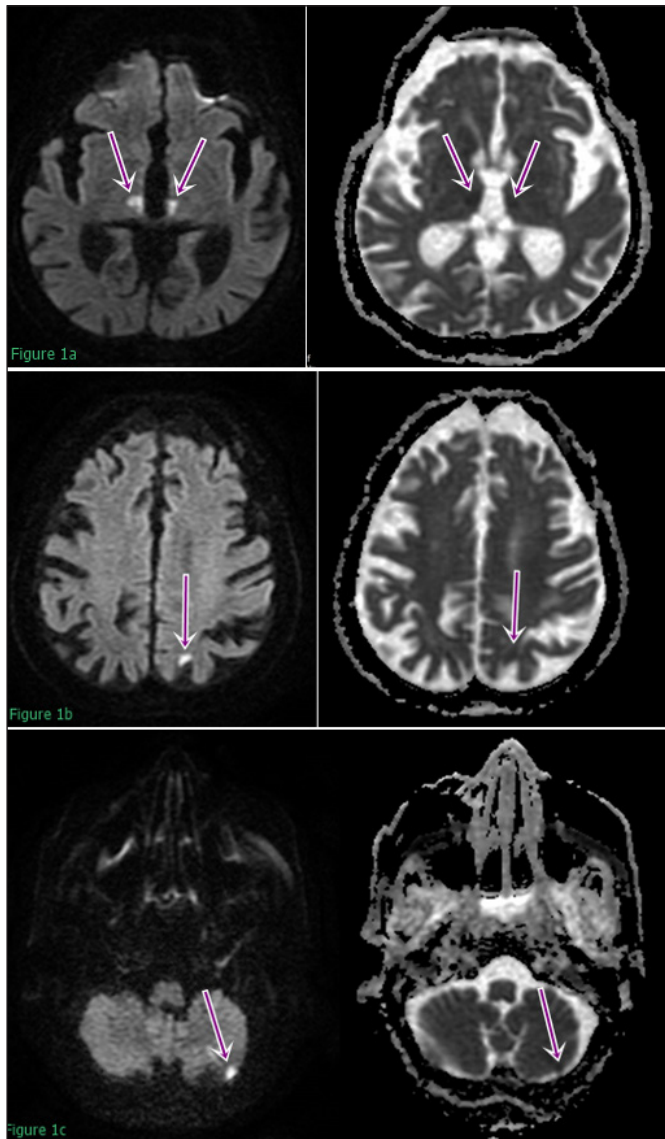

Figure 1: Diffusion-weighted magnetic resonance images showing millimetric foci showing diffusion restriction consistent with acute infarction in a) both thalami, b) left occipital lobe, and c) left cerebellar hemisphere

The patient was followed up in the neurology service with the diagnosis of stroke. During this period, brain magnetic resonance angiography was performed and showed hypoplasia in the distal parts of both vertebral arteries and proximal part of the basilar artery. In addition, it was observed that the basilar artery was filled by a collateral artery originating from the right ICA (**Figure 2a, 2b**).

In the subsequent diagnostic DSA examination, there was no contrast filling in the basilar artery after the right

and left vertebral artery injection. However, after the right common carotid artery injection, a collateral artery consistent with PTA was observed to originate from the right ICA and fill the basilar artery (**Figure 3a, 3b, 3c, 3d**).
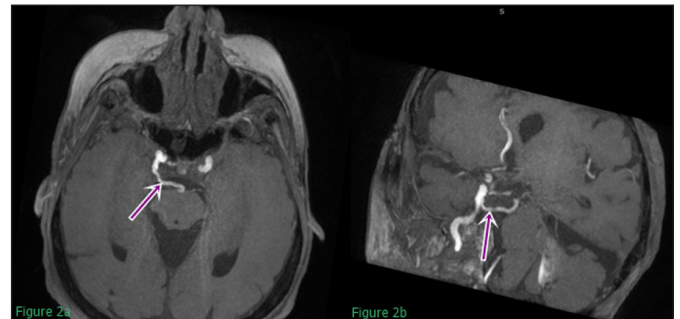

Figure 2: Magnetic resonance angiography images showing the hypoplasia of the distal of both vertebral arteries and proximal of the basilar artery. The distal of the basilar artery is filled by the collateral artery originating from the right internal carotid artery. Arrows in a and b indicate the collateral artery.
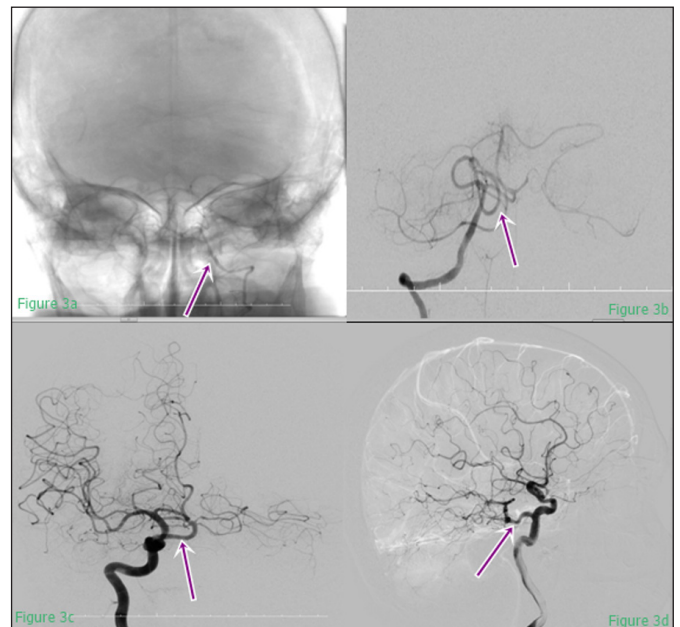

Figure 3: Diagnostic digital subtraction angiography images of the patient: a) There is no contrast filling in the basilar artery after both vertebral artery injections. The left vertebral artery is hypoplastic (arrow). b) The right vertebral artery ends in the posterior inferior cerebellar artery (arrow), with no contrast filling. c) and d) There is a collateral artery (arrow) originating from the right internal carotid artery after the right common carotid artery injection, which fills the basilar artery and is consistent with the persistent trigeminal artery. The posterior system shows contrast filling from the anterior system via the persistent trigeminal artery.

The patient was monitored with the use of anticoagulants in the neurology ward. The patient's vitals were observed to be stable during this period. She was discharged with clinical recovery after one week of follow-up.

DISCUSSION

PTA is the most common variation of the four well-known embryological primitive anastomoses between the carotid and vertebrobasilar systems and can be accompanied by vascular pathologies, such as stenosis,

Aytaç et al. Non-occlusive infarction and persistent trigeminal artery variation

J Health Sci Med 2023; 6(1): 220-222

occlusion, and dissection and vascular variations, such as the congenital absence of ICA (2,5,6). In addition, this variation may be a cause of vertebrobasilar insufficiency (2). Considering the conditions accompanying this variation and associated pathologies, there may also be a relationship between the presence of PTA and the formation and volume of infarction (5).

In non-occlusive ischemic strokes, the effect of collateral circulation on brain perfusion has been rarely reported in the literature. According to Da-Ping et al. (5), it is unclear whether PTA is associated with ischemic cerebrovascular disease. However, Ferreira et al. (3) reported that patients with PTA and basilar hypoplasia had hypoperfusion in the posterior fossa, and therefore were more susceptible to ischemic events. Engelhardt et al. (4) stated that as the number of collaterals increased, infarction became smaller.

In patients with the PTA variation, the steal phenomenon may occur in case of anterior system stenosis, which can cause vertebrobasilar insufficiency and lead to hemodynamic posterior system infarction. In some cases, the PTA variation can also be an advantage. For example, in case of basilar artery occlusion before the PTA entry point, PTA may have a preventive effect on the development of infarction by providing blood flow to the distal basilar artery (3).

In our patient, there was a Saltzman type 1 PTA variation, in which the posterior system irrigation area was supplied blood from the anterior arterial system via PTA. The patient had conventional imaging findings indicating posterior arterial system infarction, and clinical signs were consistent with the ischemic lesions of the cerebellar hemisphere and occipital cortex. Given the absence of large vessel occlusion and presence of a history of surgery due to aortic valve stenosis, it was considered that the infarct areas had developed in the posterior arterial system irrigation area secondary to hypoperfusion caused by a hypotension attack.

CONCLUSION

A PTA anastomosis is a rare variation. The presence of PTA anastomosis may create an advantage by providing collateral circulation during a stroke as in cases of leptomeningeal collaterals, external-internal carotid artery anastomoses, and the polygon of Willis. However, patients with this variation are more susceptible to the development of non-occlusive posterior arterial system infarction when they experience hypotension attacks..

ETHICAL DECLARATIONS

Ethics Committee Approval: Institutional approval was obtained for the use of the images.

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ARTICLE WRITING RULES

Manuscripts are double-spaced with Microsoft Word, and titles (Abstract, Introduction, Material and Method, Results, Discussion, References, etc.) are written in 12 pt. 2.5 cm space should be written at the top and bottom. The writing style should be Times New Roman. "System International" (SI) units should be used. Figures, tables and graphs should be referenced in the text. Abbreviations should be given in parentheses where the word first appears. Review articles and research articles should not exceed 4000 words, case reports 2000 words, letters to the editor should not exceed 500 words (This limits to all article types are excluding Abstract and References section). Pages should be numbered from the abstract page.

SECTIONS OF MANUSCRIPT

1. Presentation to the Editor

This is the article that the author of the article sends to the editor of the journal. In this section, it should be noted that part or all of the article is not published elsewhere and is not in the process of being evaluated in another journal at the same time, "**Material Support and Interest Relationship**" status, language and statistical checks are made.

2. Title Page

The category of the article submitted at the beginning of the page should be indicated (clinical analysis, research article, experimental study, case report, review, etc.). The names and surnames of all authors should be numbered after the superscript and numbered from 1, and they should be added under the names of the institutions, clinics, cities and countries. On the title page, each author's **Orcid ID** should be his/her e-mail address. This page should include the Authorized Author (s), name, full address, telephone and **e-mail** (address information should be indicated in English. Oral or Poster presentations presented at congresses should be indicated on the title page by giving the name, place and date of the congress.

3. Article File

There should be no names of authors and institutions, only this information should be on the title page.

Title: There should be a short and clear title. It should not contain abbreviations.

Abstract: English abstracts should be written. In research articles; It should be divided into sections of Aim, Material and Method, Results, Conclusion and should not exceed 400 words. In the review, case reports and the like.

Keywords: A minimum of 3 and a maximum of 6 keywords should be written. Words should be separated by semicolons. Keywords should be submitted in accordance with Subject **Medical Subject Headings (MESH)** (www.nlm.nih.gov/mesh/MBrowser.html).

Figures, Photographs, Tables and Graphics: It should be indicated at the end of the sentence where it is mentioned in the text, should not be placed in the text, and should be added to the end of the text after the references. Abbreviations used should be indicated in the description below. If previously printed figures, pictures, tables and graphics are used, written permission must be obtained and this permission should be stated in the description of figures, pictures, tables and graphics. The article should be passed by the authors for academic plagiarism prevention program. The picture/photo should be in jpeg and at least 300 dpi resolution.

Text Sections: The text samples to be sent for publication are as follows.

Editorial Comment/Discussion: It is the evaluation of the original research articles published by the expert other than the authors. It is published before the articles in the journal.

Research Article: Prospective-retrospective and all kinds of experimental studies can be published. Introduction, Materials and Methods, Results, Discussion, Conclusion. Abstract (approximately 400 words; aim, material and method, results and conclusion sections), Introduction, Material and Method, Results, Discussion, Conclusion, Acknowledgments, References.

Review: Can be prepared by invited authors or directly. It can be prepared to include the latest medical literature for any subject that has medical characteristics. Abstract (about 300 words, unpartitioned), titles, references.

Case Report: These are rare or different articles in diagnosis and treatment. It should be supported with sufficient number of photographs and diagrams. Abstract (about 250 words; no section), Introduction, Case report, Discussion, Conclusions.

Letter to the Editor: The articles that are published in the journal within the last year include a maximum of 500 words containing various opinions, experiences and questions of the readers. There are no Title and Abstract sections. The number of references is limited to 5 (Max: 10). It should be indicated which article (number, date) is dedicated and at the end there should be the name, institution and address of the author. The answer to the letter is given by the editor or the author (s) of the article and published in the journal.

Education: Scientific articles supported by the latest clinical and laboratory applications that send messages to readers on current issues within the scope of the journal. Abstract (about 200-250 words; no section), related titles, references.

Book Evaluations: Evaluations of national or internationally accepted books of current value within the scope of the journal.

WHAT SHOULD BE INDICATED BEFORE THE RESOURCES

ETHICAL CONSIDERATIONS

Ethics Committee Approval: The study was carried out with the permission of …………... Ethics Committee of ……………………. (Date: ………., Decision no: …………).

Informed Consent: All patients signed the free and informed consent form. (If retrospective study; **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.

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.

Acknowledgements: If any, it should be written before references.

References: References should be written according to the order of arrival. If the number of authors in the source is 6 or less, all authors (surname and first name should be the first letter, the names of the authors should be separated by commas) should be specified; ("et al "), the name of the article (only the first letter of the sentence and the first letter of the special names will be capitalized), short journal name, year, volume, short page number (15-8, not 15-18) and a space between the punctuation marks. The format used for the manuscript submission should be as specified in Index Medicus (www.icmje.org). The list of references should only include studies that have been published or accepted for publication or have a Doi number. Journal abbreviations should follow the style used in **Cumulated Index Medicus** (http://www2.bg.am.poznan.pl/czasopisma/medicus.php?lang=eng.). The number of references should be limited to 40 in research articles, 60 in reviews, 20 in case reports and 5 (max. 10) in letter to the editor. References should be given in parentheses at the end of the sentence just before the period. For example (4,5). The author (s) is responsible for the accuracy of the references. Importance should be given to the synthesis of domestic and foreign sources.

4. Figures and Table Titles

Titles should be written after the references. Each must be submitted as a separate image file (at least 300 dpi resolution, jpg).

After the article is accepted for publication, the first copy of the string will be sent to the responsible author by e-mail. In this text, only the spelling errors will be corrected and no additions or substitutions will be made. The responsible author will notify the editorial center by e-mail of the corrections within 2 days.

SOURCE WRITING EXAMPLES

Excerpt from journals;

Cesur S, Aslan T, Hoca NT, Cimen F, Tarhan G, Cifci A. Clinical importance of serum neopterin level in patients with pulmonary tuberculosis. Int J Mycobacteriol 2014; 3: 15-8 (not 15-18).

Excerpt from the book;

Tos M. Cartilage tympanoplasty. 1st ed. Stuttgart-New York: Georg Thieme Verlag; 2009.

Excerpt from the book, which is the only author and editor;

Neinstein LS. The office visit, interview techniques, and recommendations to parents. In: Neinstein LS (ed). Adolescent Health Care. A practical guide. 3rd ed. Baltimore: Williams & Wilkins; 1996: 46-60.

Excerpt from the book with multiple authors and editors;

Schulz JE, Parran T Jr.: Principles of identification and intervention. In: Principles of Addicton Medicine, Graem AW. Shultz TK (eds). American Society of Addiction Medicine, 3rd ed. Baltimore: Williams & Wilkins; 1998: 1-10.

If the editor is also the author of the chapter in the book;

Diener HC, Wilkinson M (editors). Drug-induced headache. In: Headache. First ed., New York: Springer-Verlag; 1988: 45-67.

Excerpt from PhD/Undergraduate Thesis;

Kilic C. General Health Survey: A Study of Reliability and Validity. phD Thesis, Hacettepe University Faculty of Medicine, Department of Psychiatrics, Ankara; 1992.

Excerpt from an internet site;

Site name, URL address, author names, access date should be given in detail.

Giving a Doi number;

Joos S, Musselmann B, Szecsenyi J. Integration of complementary and alternative medicine into the family market in Germany: Result of National Survey. Evid Based Complement Alternat Med 2011 (doi: 10.1093/ecam/nep019).

For other reference styles, see "ICMJE Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Sample References".

Eder I hereby declare that all or part of the material in this study has not previously been published in any place and is not currently being evaluated elsewhere for publication. electronic submissions and all kinds of pre-declarations.

Sponsorship Statement

Authors should declare, if any, the roles of sponsors of the study:

1. Design of the study 2. Data collection, analysis and interpretation of the results 3. Writing the report

CHECKLIST/CONTROL LIST

The checklist must be complete.

What should be in the article;

—Editor to Presentation Page

—Title Page

- Ethical Status,
- "Conflict of Interest"
- Orcid numbers and author information should be on this page.

—Main Text

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3. **Main pages of the article:** Article Titles/Short Titles, Abstract and Keywords, Article Text, References, Table and Figure Titles, Tables. **This page will not contain author names or institution information.**

4. **Font:** Titles should be "Times New Roman 12 and 12 pt, with 11 pt, double-spaced line spacing and 2.5 cm indentation in all areas.

5. **Abstract**: Abstract should begin with the title ABSTRACT and include the sections "**Introduction/Aim, Material and Method, Findings/Results, Conclusion**".

6. **Keywords** should be added under the abstract in "**Keywords**", under "**Abstract**". Keywords should be at least 3, at most 6 words, separated by commas, and should be MeSH-compliant.

7. **M**aterial and Method section should indicate the approval of the **Ethics Committee** (it is recommended to include the place, date, ethics committee number). In articles that do not require Ethics Committee Approval, it should be stated that the Approval/Permission of the Institution has been obtained (in order to avoid Conflict of Interest). Related documents should be sent on request. It should be noted that the author (s) is responsible for ethical problems.

8. **S**tatistical terms (such as p, r, α) should **not** be used in the discussion.

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10. **References Representation;** should be as detailed in the spelling rules. Journal's number number "(2)" **is not** in bibliography. In articles with up to six authors, the names of all authors should be written (with the first letter of surname and first name), and for articles with seven or more authors, the first three authors should be cited as et al. (et al.). The name of the manuscript should be in the form of sentence usage (**except for special names and first letter**). **The journal should be given a short name.** A space must be left between the punctuation marks after the journal name.

11. **T**ables, Graphs, Pictures and Figures should be placed under a separate title after the bibliography. **Figures/ Images** (at least 300 dpi resolution, must be **jpeg** file) and **Table**s should be submitted as one or more separate files.

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