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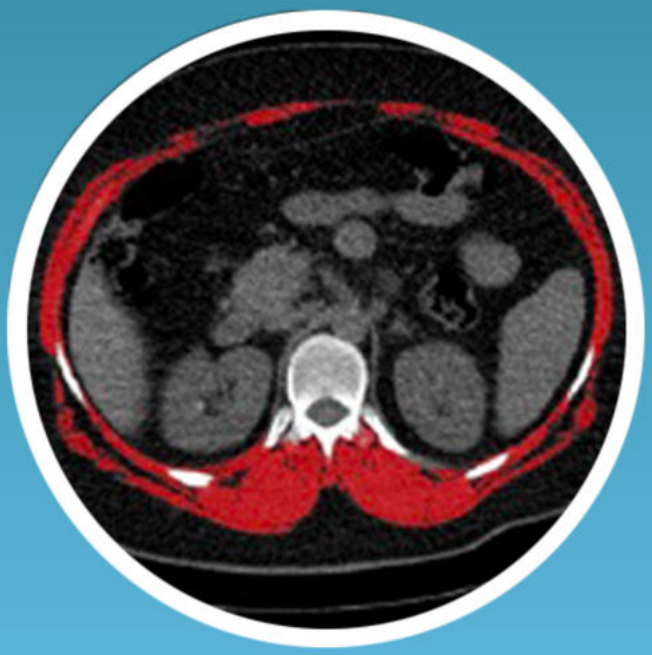

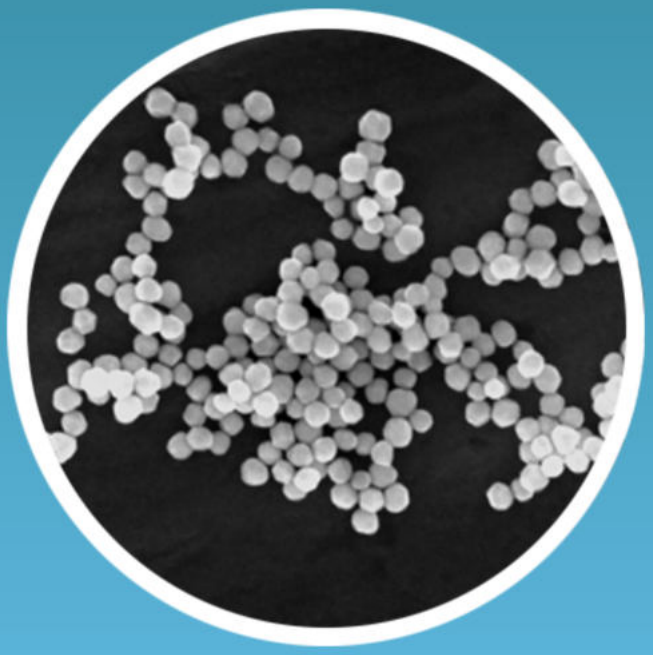

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Original Articles

Case Report

The European Research Journal 2023;9(1):1-7 Original Article

DOI: 10.18621/eurj.889233 *Hematology*

Evaluation of lymphoma patients after hematopoetic stem cell transplantation in terms of early period cardiotoxicity development

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ABSTRACT

Objectives: Autologous hematopoietic stem cell transplant (AHSCT) following high-dose chemotherapy in recurrent lymphomas has become the standard treatment. However, this method leads to various toxic side effects, including cardiotoxicity. This study aims to determine the factors that may cause post-transplant cardiotoxicity.

Methods: A total of 35 patients older than 18 years old, diagnosed with recurrent lymphoma, who underwent AHSCT at the Uludağ University Hematology Department, were included in the study. The patients were evaluated in two groups, with and without cardiotoxicity after AHSCT. We separated the frequency of cardiotoxicity that developed during hospitalization after transplantation and patients who developed and did not develop cardiotoxicity. We compare some parameters including gender, age, lymphoma type, stage, cardiac risk factors before transplantation, the number of chemotherapy cycles and the use of rituximab before the transplantation, radiotherapy before transplantation, Karnofsky performance scale, the amount of Dimethyl sulfoxide among these patients. Also, we evaluated patients with echocardiopraphy before transplantation and measured left ventricle ejection fraction (LVEF). We use CTCAE V 4.0 system for evaluating cardiotoxicity level from Grade I to Grade V.

Results: Nine patients developed cardiac events. One patient developed Grade V MI and died despite treatment. Other eight toxicities developed Grade III-IV and returned by treatment. In terms of risk factors, LVEF of the group with cardiotoxicity was found to be significantly low ($p < 0.05$). There is no statistical difference between the two groups with other parameters.

Conclusions: Cardiotoxicity is a frequent complication of autologous stem cell transplantation. A detailed pre-transplantation evaluation of all patents in terms of cardiac functions is essential to reduce cardiac morbidity. Therefore, patients should be evaluated cardiologically before transplantation and closely monitored for post-transplantation cardiac side effects. Some studies show that enalapril and carvedilol may be beneficial to prevent from cardiotoxicity. Although not in our routine, if approved by larger studies, it may be necessary to use agents such as enalapril and carvedilol in prophylaxis in reduce cardiotoxicity.

Keywords: stem cell transplantation, cardiotoxicity, lymphoma, chemotherapy

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Lymphomas are heterogeneous groups of hematological malignancies. Lymphomas result from various differentiation stages of lymphocytes and show different immunological and clinical manifestations. They constitute 3-4% of all cancers seen worldwide [1]. Despite the high treatment success rate, there is significant resistance to treatment or recurrence. Generally, autologous hematopoietic stem cell transplantation (AHSCT) following high-dose chemotherapy is the standard treatment regimen in all patients with recurrent lymphoma [2]. This treatment process can cause various toxic effects, including cardiotoxicity. Congestive heart failure or pericarditis at a late stage is the causes of morbidity and mortality due to radiotherapy or treatment regimens involving cardiotoxic chemotherapeutic agents [3, 4]. Acute cardiac toxicities after AHSCT include cyclophosphamide-induced acute cardiomyopathy or hemorrhagic pericarditis, acute myocarditis, and bradycardia associated with dimethyl sulfoxide (DMSO) used to protect progenitor cells, cyclophosphamide regimens [4-6].

The most frequently used chemotherapy regimen in AHSCT lymphoma is the BEAM (melphalan, cytosine arabinoside, etoposide, carmustine) protocol. Drug toxicities are briefly mentioned in various articles and each of these drugs are used for the treatment of different patient groups with other protocols. However, there are not enough publications evaluating the frequency of cardiotoxicity development (incidence, risk factors, cardiac toxicity results) clinically in patients with recurrent lymphoma with autologous hematopoietic stem cell transplantation using the BEAM protocol. This study aims to determine the factors that may cause post-transplant cardiotoxicity.

METHODS

A total of 35 patients older than 18 years old, diagnosed with recurrent lymphoma under AHSCT at the Bone Marrow Transplant Unit of Uludağ University School of Medicine, Hematology Department, between January 2009 and October 2015 were included in the study. The data of the patients were retrospectively analyzed for the research protocol.

We planned to screen some factors associated with cardiac risk through the automation system of the hospital. These factors include cardiac risk factors (hypertension, diabetes mellitus, hypercholesterolemia, obesity, family history of cardiovascular disease, smoking) pre-transplant echocardiography, electrocardiography (ECG), blood tests (Creatine kinase, Creatine kinase kinase-mb, troponin-I) [6].

Cardiotoxicites were evaluated according to the Common Terminology Criteria for Adverse Events Version 4.0 system and toxicities were classified in severity from grade I to grade V. Grade I is asymptomatic, grade II does not require urgent treatment, grade III is symptomatic, grade IV represents life-threatening case, grade V is death of all patients [7].

We designed this study to evaluate cardiotoxicity in early period after transplantation (during hospitalization). The patients were separated into two groups; the ones who developed cardiotoxicity after AHSCT and those who did not. Each group was compared in terms of some factors including gender, age, diagnosis, lymphoma, pre-transplant cardiac risk factors, chemotherapy cycles, using of rituximab, radiotherapy before transplantation, the karnofsky performance scale [8], the amount of DMSO, and the left ventricular ejection fraction (LVEF) values.

We use the BEAM (BCNU 300 mg/m^2, etoposide 800 mg/m^2, ARA-C, 1600 mg/m2, melphalan 140 mg/m^2) protocol as a standard chemotherapy regimen for patients with recurrent lymphoma [9].

DMSO is used in transplantation because it is a cryoprotective agent. We compared DMSO cassette numbers to see if there was a relationship with post-transplant cardiotoxicity.

Our study was approved by the Ethics Committee of Uludağ University Non-Interventional Clinical Studies on 16.02.2016 with the decision number 2016-3/7.

Statistical Analysis

Statistical Package for the Social Science (SPSS13.0) program was used for statistical analysis of the data. The data were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro–Wilk's tests) to determine their normal distribution. Differences between groups were evaluated using Student's t-test for parametric data and the Mann-Whitney U-test for non-parametric data. Relationships between categorical variables were analyzed using a chi-squared test. Data were expressed as frequency and percentage, mean

and standard deviation, median, minimum and maximum values. It was considered statistically significant when the p value was less than 0.05.

RESULTS

The characteristics of the patients were presented in Table 1. There were 35 patients, 19 of whom (54%) were male and 16 of whom (46%) were female. The median age of the patients was 46, the youngest patient was 22 while the oldest was 65.

Nine (26%) of the patients developed cardiotoxicity after transplantation. Post-transplant cardiotoxicities are summarized in Table 2.

We divided the patients into two groups: those who developed cardiotoxicity after transplantation (n = 9) and those who did not (n = 26). The group which didn't experience cardiac events is named control group. We found that the median age was 52 in the group with cardiotoxicity and 46.5 in the control group. There was no statistically significant difference in terms of age factor between the two groups (p = 0.661). When we analyzed the two groups in terms of gender distribution, there were 2 males, 7 females in the group of cardiotoxicity and 17 males and 9 females in the control group. No significant difference was found between the two groups in terms of gender (p = 0.050), either. When two groups were examined with respect to diagnosis, there were 6 NHL, 3 HL in the group of cardiotoxicity, 17 NHL and 9 HL in the control group. There was also no significant difference between the two groups related to the diagnosis of the disease (p = 1.000).

When lymphoma stages were evaluated as stage I, II, III, IV, a statistically significant difference was found between the group of cardiotoxicity and the control group (p = 0.047). It was determined that 5 (56%) of the patients who developed cardiotoxicity had stage IV lymphoma and 4 (44%) of them had stage II lym-

Table 1. Patient characteristics

	n = 35
Age (years), median (range)	46 (22-65)
Gender, n (%)	
Male	19 (54)
Female	16 (46)
Pre-transplant diagnosis, n (%)	
Non-Hodgkin Lymphoma	23 (66)
Hodgkin Lymphoma	12 (34)
Lymphoma stage, n (%)	
Stage I-II	13 (37)
Stage III-IV	22 (63)
Cardiovascular comorbidity, n (%)	
Yes	12 (34)
No	23 (66)
Pre-transplant rituximab using, n (%)	
Yes	20 (43)
No	15 (57)
Number of chemotherapy cycle, median (range)	10 (4-15)
Pre-transplant radiotherapy, n (%)	
Yes	6 (17)
No	29 (83)
Karnofsky Performance Scale, n (%)	
100	11 (31)
90	23 (66)
80	1 (3)
Left ventricular ejection fraction (%), median (range)	63 (56-74)
Hospitalization time (days), median (range)	28 (11-53)
Number of Cassette* (Stem cell aggregated product), median (range)	3 (1-8)

*Cassette: DMSO + autologous plasma + Stem cell product

Table 2. Post-transplant cardiotoxicities

Number of patients who developed cardiac events after transplantation	n = 9	%
Rhythm Disorders		
HVRAF	3	33
Sinus tachycardia	2	22
Bradycardia	1	11
VT, VF	1	11
Atrial extrasystole, atrial fibrillation	1	11
Ischemic Events		
Myocardial infarction	1	11

HVRAF: higher ventricular rate atrial fibrillation,
VT: ventricular tachycardia, VF: ventricular fibrillation

phoma. However, in terms of stage IV lymphoma, no significant difference was found concerning the development of cardiotoxicity in advanced stage patients compared to stage I-II-III lymphoma patients (p = 0.103). When examined in terms of cardiovascular comorbidities before transplantation, there was no significant difference in both groups. In the group of cardiotoxicity, comorbid disease was detected in only 1 (11%) patient, while in the control group, 11 (42%) patients had cardiac comorbid disease before transplantation. It was observed that pre-transplant using of rituximab in chemotherapy protocols did not make a statistically significant difference in both groups (p = 0.700). There were 6 (67%) patients in group of cardiotoxicity and 14 (54%) patients in the control group related to use of rituximab in pre-transplantation period. It was found that the number of chemotherapy cycles before transplantation did not make a statistically significant difference between the two groups (p = 0.590). Also there wasn't a significant difference between the two groups related to pre-transplant radiotherapy (p = 0.635). Two (22%) patients in the cardiotoxic group and 4 (15%) patients in the control group received pre-transplant radiotherapy.

It was observed that Karnofsky performance score (KPS) of the patients before AHSCT did not make a significant difference in the two groups (p = 0.083). It was determined that 1 (11%) of the patients had 100 KPS, 7 (78%) of them had KPS 90 1 (11%) of them had KPS 80 in the group of cardiotoxicity.

When the LVEF values calculated during the routine echocardiography performed before transplantation were compared, a significant difference was found between the two groups (p = 0.039). In the group of cardiotoxicity, the median LVEF value was 60, minimum 58 and maximum 65, while the median SVEF value was 65, minimum 56 and maximum 74 in the control group. When both groups were evaluated in terms of duration of hospitalization, there was no significant difference among them (p = 0.810). In

Table 3. Evaluation of patients with lymphoma who developed cardiotoxicity after AHSCT in terms of risk factors

	Cardiotoxicity (n = 9)	Control (n = 26)	*p value*
Age (years), median (range)	52 (23-64)	46.5 (22-65)	0.661
Male/female	2/7	17/9	**0.050**
NHL/HL	6/3	17/9	1.000
Stage			**0.047**
Stage I	0 (0%)	1 (4%)	
Stage II	4 (44%)	8 (31%)	
Stage III	0 (0%)	11 (42%)	
Stage IV	5 (56%)	6 (23%)	
Comorbidity	1 (11%)	11(42%)	0.121
Rituximab using	6 (67%)	14 (54%)	0.700
Number of chemotherapy cycle	11 (6-13)	11 (4-15)	0.590
Pre-transplant radiotherapy	2 (22%)	4 (15%)	0.635
Karnofsky performance scale			0.083
100	1 (11%)	10 (38.5%)	
90	7 (78%)	16 (61.5%)	
80	1 (11%)	0 (0%)	
LVEF (%), median (range)	60 (58-65)	65 (56-74)	**0.039**
Period of hospitalization (days), median (range)	30 (11-38)	27.5 (21-53)	0.810
Number of cassettes DMSO, median (range)	3 (1-6)	2 (1-8)	0.342

the group of cardiotoxicity, the median period of hospitalization is 30 days, at least 11 and at most 38 days. The median duration of hospitalization was found to be 27.5 days in the control group, with a minimum of 21 days and a maximum of 53 days. The number of cassettes infused during the transplant we used to indirectly measure toxicity of DMSO did not make a significant difference in either group ($p = 0.342$). The median value of the number of cassettes in the group with cardiotoxicity was found to be 3, minimum 1 and maximum 6. Considering the control group, the median value of the number of cassettes in this group was found to be 2, minimum 1 and maximum 8 cassettes.

Evaluation of patients with lymphoma who developed cardiotoxicity after AHSCT in terms of risk factors is summarized in Table 3.

DISCUSSION

AHSCT is used all over the world for recurrent lymphomas after the implementation of the standard chemotherapy and radiotherapy regimens [10]. Although this group of patients, whose number is increasing day by day, can develop a wide range of events especially early cardiac events may be an important cause of mortality and morbidity. It also requires urgent intervention [11]. In this single-center study, we tried to determine the characteristics, incidence, and risk factors of cardiotoxicities that occur in the post-transplant hospitalization process of the patients diagnosed with lymphoma.

When we review the literature on the frequency of cardiac events developing after transplantation, in the study of the first 100-day post-transplant study involving 2821 patients, published by Murdych and Weisdorf [12] in 2001, 26 (0.9%) patients developed major cardiac events and 13 were found to be mortal. In a study in which 249 patients with transplantation with NHL were evaluated retrospectively, 30 (13%) patients developed supraventricular tachycardia [13]. Also, it was mentioned in this study that patients with lymphoma developed more cardiac events after autologous transplantation than allogeneic transplantation. The cardiac event ratio of patients with allogeneic transplantation is 0.9% after transplantation. On the other hand, patients with autologous transplantation have higher risks of cardiac complications in comparison with allogeneic transplantation. Therefore, patients with lymphoma under autologous transplantation are very important in terms of cardiac events among patients with transplantation.

The cardiac events we observed during our study were frequently caused by rhythm disorders. MI was detected in only one patient. Especially the most frequently observed rhythm disorder appeared to be AF. In the study conducted by Hidalgo *et al*. [13], AF was found to be 91% among the supraventricular tachyarrhythmias. No patient developed heart failure in the early period. There are studies indicating that cardiotoxicity is observed as heart failure especially in the late period after transplantation [11, 14].

When we defined the patients as stage I-II-III-IV, we observed that there was a difference among these groups in terms of post-transplant cardiac event development. When we performed subgroup analysis, it was observed that patients in the group who developed cardiac events had stage II (44%) and stage IV (56%) diseases. In our study, as the disease stage increased, cardiotoxicity did not increase. Such a result may have derived from the fact that the number of patients in the cardiotoxicity group is relatively small.

The existence of diseases that cause comorbidities before the transplant (diabetes mellitus, hypertension, obesity, and hyperlipidemia) has been shown as a risk factor for the development of post-transplant cardiotoxicity in various studies [14, 15]. In our study, it was found statistically that the presence of pre-transplant comorbidity did not make a difference regarding post-transplant cardiac event development [$p = 0.112$]. If patients are selected correctly before transplantation, they may have chronical disease while they do not have complication about these diseases, patients would have lower risks of cardiac events after transplantation.

With the introduction of high doses of cyclophosphamide since the discovery of anthracyclines, there are hundreds of studies related to their cardiotoxic effects [16]. Because the intensity of chemotherapy exposed will increase as the number of cycles increases, we thought that this situation may have an effect on the development of cardiac events after transplantation. In our analysis, we found that the number of cycles was similar in both groups. When we examine the cardiac effects of cyclophosphamide and anthracyclines, it is observed that they cause congestive heart

failure by causing heart muscle damage in the long term [17]. The reason for the absence of any difference between the two groups in our study may be the fact that we examine the early effects after transplantation. There are some publications showing that a difference is detected when long-term follow-ups are made [16, 17].

It can be estimated that patients with low pre-transplant performance may develop more cardiac events after transplant. In our study, 1 patient with KPS was 80, and the others consisted of 90 and 100 patients. In our study, there was no difference between these groups. According to the results of our study, it can be thought that patients with a KPS of 80 and above are safe in terms of cardiac effects in the early post-transplant period.

Many studies using cardiotoxic chemotherapy agents have found that post-procedure cardiac events are common in patients with low LVEF before the procedure. For this reason, it is recommended to evaluate patients with ECO routinely to assess the cardiac functions of the patients before the transplant, since it is a routine cardiology consultation and noninvasive method and is often an affordable, cost effective method [18]. For this purpose, although various parameters are used in echocardiography, LVEF is often calculated. We also prefer patients with LVEF > 50 for transplantation in our department. When we evaluated the patients in this study in this respect, the median LVEF value of the ejection fraction in the group with cardiotoxicity was calculated as 60. LVEF value in the control group was 65 and there was a statistically significant difference between the two groups. In a progressive study evaluating patients with lymphoma who used BEAM and CBV chemotherapy regimens as a transplant preparation regimen 2-point decrease in LVEF values was found in the post-transplant patients compared to before the transplant [18]. In this study, we achieved a result that supports the results of many progressive and retrospective studies done on this subject worldwide. These results emphasize the importance of evaluation with echocardiography in the evaluation of patients before transplantation [18-20]. Thus, we eliminate one of the risk factors of a cardiac event likely to develop after transplantation.

DMSO related arythmias can be fatal. One of our patients developed bradycardia during infusion. Therefore, close cardiac monitoring during the transplant and especially in patients who are at risk in post-transplant period would be useful for the early detection of cardiac events.

Some studies investigating some anthracycline based chemotherapy protocols have concluded that prophylactic carvedilol and enalapril reduce the development of anthracycline-induced cardiotoxicity [21, 22]. Although there is no such practice in our routine, it is beneficial to carry out broader prospective studies.

Limitations

Restrictive factors of our study; the number of patients included in the study and the number of patients developing cardiotoxicity are small.

CONCLUSION

As a result, the risk factors of the patients should be determined in the pre-transplant period. Their cardiological examination and echocardiographic assessment should be made, and appropriate patients should be directed to the transplant. If cardiac complaints develop after transplantation, we should behave energetically and start treatment immediately after the diagnosis by performing ECG, echocardiography, cardiac marker examinations, so that we can reduce the mortality and morbidity that may develop. Although it is not in our routine, it may be beneficial to use agents such as enalapril and carvedilol in prophylaxis on the condition that it is approved by larger studies.

Authors' Contribution

Study Conception: SÇ, FY; Study Design: SÇ; Supervision: SÇ, VÖ, FÖ; Funding: N/A; Materials: SÇ, FY; Data Collection and/or Processing: SÇ, FY; Statistical Analysis and/or Data Interpretation: SÇ, FY; Literature Review: SÇ; Manuscript Preparation: SÇ and Critical Review: SÇ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(1):8-13 Original Article

DOI: 10.18621/eurj.886700 *Physical Medicine and Rehabilitation*

The effect of IL-17 blockage on the neutrophil to lymphocyte ratio in patients with axial spondyloarthritis: a comparative study with anti-TNF

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ABSTRACT

Objectives: This study aims to evaluate the neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) in patients with axial spondyloarthritis receiving different biologic treatments.

Methods: The study included 33 axial spondyloarthritis patients (20 males, 13 females) receiving anti-TNF or secukinumab therapy. Patients' age, disease duration, ongoing therapy duration, serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), neutrophil, lymphocyte and platelet counts, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores, NLR, and PLR were recorded retrospectively.

Results: The patients' mean age was 44 years, and the disease's mean duration was 121.55 months. Fourteen (42.4%) patients were receiving secukinumab, 57.6% of the patients were receiving anti-TNF agents. The male/female ratio was 6/8 in the secukinumab group and 14/5 in the anti-TNF group. The ongoing therapy duration was 8 ± 5.1 months for the secukinumab group and 27 ± 27.6 months for the anti-TNF group. Only NLR was significantly higher in the secukinumab group, and there was no other statistical difference between groups. There was no correlation between NLR, PLR, and CRP, ESR, BASDAI.

Conclusions: The results of our study show that only NLR is significantly higher in the secukinumab group compared to the anti-TNF group. Hence NLR may be a useful and sensitive parameter in terms of monitoring disease activity.

Keywords: .Axial spondyloarthritis, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, secukinumab

Spondyloarthritis (SpA) is a term used to describe a group of chronic rheumatological diseases such as ankylosing spondylitis (AS), psoriatic arthritis, reactive arthritis, and so on. It is classified clinically as peripheral SpA and axial SpA. Axial SpA is characterized by inflammatory back pain, sacroiliitis, spondylitis, and limited spinal motion [1]. There is no standard laboratory test defined to be used in the diagnosis or follow-up of this disease group. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), indicators of inflammation, and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), an indicator of the clinical activity of the disease, are used in follow-ups. Serum ESR and CRP levels are usually increased in SpA, especially in cases with peripheral joint involvement. Both tests have low sensitivity and

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specificity and do not reflect the disease process ultimately. Elevation of either one of them is observed in only 50% of patients with AS. BASDAI, which is routinely used, is only a reflection of the disease activity assessment, does not include the physician's disease evaluation, and is not a direct indicator of inflammation [2]. It is known that there are changes in the amount and composition of circulating blood cells in systemic inflammation. In studies based on this knowledge, it has been suggested that neutrophil-lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) increase in many rheumatologic diseases with inflammation and can be used as a marker of the inflammatory process [3, 4]. They were also shown as increased in most of the studies on AS [5-13]. A survey of patients with AS who received anti-tumor necrotizing factor (Anti-TNF) treatment concluded that NLR could be a useful marker for monitoring response to treatment [12]. Another study on AS found that NLR was lower in anti-TNF therapy patients than those followed-up with non-steroidal anti-inflammatory drugs [13]. It is known that interleukin-17 (IL-17) has synergistic effects with TNF-α and IL-1 on cytokine induction and tissue destruction. However, it has shown that it can act independently [14]. Therefore, secukinumab, an IL-17 inhibitor, may have different effects on inflammation and its markers than TNF inhibitors. There is no study evaluating the impact of various biological treatments on the NLR in the literature. Our research aims to compare the two groups' hematological parameters receiving different biological therapies in patients with axial SpA.

METHODS

Thirty-three patients (20 males, 13 females) classified as axial SpA within the scope of the 2009 ASAS (Assessment of Spondyloarthritis International Society) diagnostic criteria [15] were included in the study. Those with kidney disease, liver disease, and hematological disease were excluded. Age, gender, duration of illness, duration of treatment, serum CRP and ESR levels of last control visits, neutrophil, lymphocyte, and thrombocyte count, BASDAI scores (data on at least three months of biological therapy) were retrospectively recorded from the patients' electronic medical records. NLR and PLR values were calculated. Recorded laboratory results and clinical evaluations belonged to the same day. BASDAI assesses the patient's neck-back-waist-hip pain, pain and swelling in peripheral joints, and sensitivity levels with palpation at enthesis points. It is an index that evaluates the duration and level of morning stiffness with seven questions. The result is obtained by adding the mean value of the last two questions to the first five questions' total value. The score ranges from 0 to 10. Its validity and reliability in Turkish have been demonstrated [16].

This study was approved by Haydarpaşa Numune Research and Training Hospital Ethics Committee (approval code: HNHAH-KAEK 2021/26).

Statistical Analysis

Data were analyzed with the IBM SPSS Statistics 22 (IBM SPSS, Turkey) program. Whether the data was normally distributed was evaluated using the Shapiro Wilk test. Descriptive statistics were presented as mean (standard deviation) and median (min-max) or percentage and frequency. Chi-square test, Fischer Exact Test were used for qualitative variables, and the Mann-Whitney U test was used for quantitative variables in comparison of data between groups. Spearman Correlation test was used to analyze whether there was a correlation between the data. Statistical significance was accepted as $p < 0.05$.

RESULTS

The patients' mean age was 44 years (range: 23-67 years), and the mean disease duration was 121.55 months (range: 7-408 months). Serum ESR levels were recorded as 15.2 ± 13.6 mm/h, and CRP levels as 0.79 ± 0.9 mg/dl. Of the 33 patients included in the study, 42.4% (n = 14) were on secukinumab therapy, while 57.6% (n = 19) were on anti-TNF agents. There were no patients on synthetic disease-modifying drug therapy in both groups, but the patients temporarily took non-steroidal anti-inflammatory drugs during periods of activation. The male/female ratio was 6/8 in the secukinumab group and 14/5 in the anti-TNF group. There was no statistically significant difference between the two groups in terms of age, gender, or disease duration. 31.6% of the patients in the anti-TNF

group had used another anti-TNF drug before. The duration of treatment was 27 ± 27.6 months (range: 3-72 months) in the anti-TNF group. 71.4% (n = 10) of the patients receiving secukinumab had used at least one anti-TNF drug (n = 8) before, usually more than one. The duration of treatment was 8 ± 5.1 months (range: 3-19 months) in the secukinumab group. The duration of treatment in the secukinumab group was significantly shorter than in the anti-TNF group ($p = 0.001$). Only the NLR value was statistically significantly higher in the secukinumab group (Table 1). There was no correlation between NLR or PLR with CRP, ESR, or BASDAI values (Table 2).

DISCUSSION

In our study, NLR was higher in patients receiving secukinumab than those on anti-TNF therapy. In contrast, there was no significant difference between the two groups in ESR, CRP, BASDAI, and PLR.

ESR, CRP, and BASDAI parameters are widely used in follow-ups of patients with axial SpA for evaluating the response to treatment. In recent studies, it has been suggested that NLR and PLR can be used as easily accessible inflammation markers. These two values, which can be easily calculated as a result of hemogram studies, and are frequently applied in the routine, have been associated with systemic diseases

Table 1. Demographic data, clinical and laboratory findings of patients with axial SpA receiving anti-TNF or secukinumab treatment

	Anti-TNF mean ± SD median (min-max)	Secukinumab mean ± SD median (min-max)	*p**value
Age (years)	42.84 ± 10.39 42 (23-65)	45.57 ± 11.76 41 (30-67)	0.733
Disease duration (months)	140.84 ± 99.43 132 (8-312)	65.36 ± 102.42 69 (7-408)	0.152
Treatment duration (months)	27 ± 27.6 12 (3-72)	8 ± 5.1 7 (3-19)	**0.001**
ESR (mm/h)	12.42 ± 11.98 9 (2-44)	19.14 ± 15.17 9 (2-57)	0.142
CRP (mg/dl)	0.59 ± 0.77 0.2 (0.2-3.1)	1.08 ± 1.15 1.2 (0.2-3.7)	0.186
Neutophil count (10^3×cell/mm^3)	4.56 ± 1.62 3.9 (2.1-7.4)	5.53 ± 1.57 2.9 (3.6-8.6)	0.077
Lymphocyte count (10^3×cell/mm^3)	2.98 ± 0.75 2.8 (1.8-4.3)	2.62 ± 0.84 2.8 (1.5-4.2)	0.174
Platelet count (10^3×cell/mm^3)	250.37 ± 73.57 240 (104-450)	269.43 ± 55.83 240 (158-430)	0.174
NLR	1.56 ± 0.46 1.4 (0.6-2.4)	2.42 ± 1.29 1.4 (0.8-5.12)	**0.035**
PLR	88.40 ± 26.66 (94.7) (24-125.4)	111.52 ± 36.07 94.7 (56.4-129.2)	0.142
BASDAI	2.68.±.1.87 2.4 (0-6.6)	3.20 ± 1.90 3.50 (0.2-6.4)	0.353

NLR = neutrophil to lymphocyte ratio, PLR = platelet to lymphocyte ratio, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, BASDAI = Bath Ankylosing Spondylitis Disease Activity Index, SD = standart deviation, $p < 0.05$ statistically significant.

Table 2. Correlation results of NLR and PLR with ESR, CRP, and BASDAI

Parameters	r	*p* value
NLR-ESR	0.272	0.126
NLR-CRP	0.317	0.073
NLR-BASDAI	-0.248	0.195
PLR-ESR	0.130	0.471
PLR-CRP	0.234	0.190
PLR-BASDAI	-0.249	0.192

NLR = neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, BASDAI = Bath Ankylosing Spondylitis Disease Activity Index, $p < 0.05$ statistically significant.

such as malignancies, diabetes mellitus, and coronary artery disease [17-19]. In a study related to rheumatological conditions, NLR was significantly increased in polymyalgia rheumatica, polymyositis/dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, mixed connective tissue disease, primary sjögren's syndrome, osteoarthritis, and systemic sclerosis compared to the healthy population. It has been emphasized that PLR is higher in systemic lupus erythematosus and rheumatoid arthritis than in healthy individuals [3, 4]. NLR and PLR are generally higher in patients with AS. [5, 6, 8-13]. There are much fewer studies on axial SpA in this area [1, 7]. One showed that both NLR and PLR increased in patients with axial SpA than healthy controls [1]. On the other, only patients with axial SpA were evaluated, and there was no relationship between disease activity and NLR or PLR [7]. Since there was no healthy control group in our study and the patients were not grouped according to their disease activity levels, no conclusion could be reached on this issue.

The correlation analysis results in the studies mentioned above are also highly controversial. Correlation between NLR and PLR with laboratory parameters (ESR and CRP) was shown in most studies [1, 8, 9, 11, 12]. But the relationship with the clinical evaluation results (BASDAI, hand finger-to-ground distance, occiput-wall distance, modified Schober test) was not so common [1, 7, 11, 13]. In our study, there was no correlation between NLR or PLR with clinical or laboratory evaluations. We think that this result is related to the small number of patients included in the study and the fact that most of the patients included in the study were in remission.

Few studies investigate the relationship between NLR and treatment in AS in the literature. But there was no research on PLR. A survey carried out in 35 patients with AS evaluated the anti-TNF therapy effectiveness with BASDAI, ESR, CRP, and NLR on 3rd months; there was an improvement in all parameters with treatment [12]. In another study, BASDAI, ESR, CRP, NLR values were found to be higher in patients with AS receiving only non-steroidal anti-inflammatory drugs (n = 72) compared to the group receiving anti-TNF treatment (n = 24) [13]. In our study, among the parameters evaluated in patients with axial SpA, only the NLR was significantly higher in the secukinumab group. BASDAI, CRP, and PLR values were also higher in the secukinumab group, but the difference was not statistically significant. The probable reason for the difference between the two groups is that the secukinumab group consisted of relatively active patients who could not achieve remission even though they used at least one, and usually more anti-TNF drugs. The anti-TNF group consisted of patients whose medical treatment and clinical status have been stable for a long time. However, considering that the only difference between the two groups is related to NLR, these results can be interpreted as NLR may be a useful and sensitive parameter for disease activity monitoring. We think that further data on much more cases are needed to speculate about the relationship of NLR with the pathway affected by the treatment.

Limitations

Our study's limitations are the small sample size, the absence of a control group, or the fact that the pa-

tients were not grouped according to the disease activation criteria.

CONCLUSION

NLR may be a candidate parameter to be used for disease activity monitoring. In order to understand its effectiveness in evaluating the response to treatment, there is a need for studies in which comparisons are made in homogenized groups with larger numbers of patients, including the control group. Prospective studies would make valuable contributions in this area.

Authors' Contribution

Study Conception: EY, DGK; Study Design: EY, DGK; Supervision: EY, DGK; Funding: ŞEÖ, ŞE; Materials: EY, DGK; Data Collection and/or Processing: EY, DGK; Statistical Analysis and/or Data Interpretation: EY, DGK; Literature Review: EY, DGK; Manuscript Preparation: EY, DGK and Critical Review: EY, DGK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Could uric acid to high-density lipoprotein cholesterol ratio be used to predict late-stage saphenous vein graft disease after coronary artery bypass graft surgery?

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ABSTRACT

Objectives: Various vascular grafts are used in coronary artery bypass graft (CABG) surgeries, however, the most commonly used one is the saphenous vein graft (SVG). Various studies conducted on this topic have found a relationship between uric acid to HDL-C (UHR) and cardiovascular diseases. In this current study, we aimed to investigate the predictive importance of UHR value in predicting long-term SVG disease in patients who underwent CABG surgery.

Methods: Patients who had a history of CABG surgery at least one year prior, had stable angina pectoris, and underwent coronary angiography between January 01, 2016 and January 01, 2020, were included in the study retrospectively. Patients with open saphenous veins were defined as Group 1, and patients who had 50% or more stenosis in at least one SVG after coronary angiography, were defined as Group 2.

Results: The median age of the 204 patients included in Group 1 and 292 patients in Group 2 were 65 (38-77) years and 66 (45-79) years, respectively (p = 0.251). The two groups were similar in terms of gender, hypertension, chronic obstructive pulmonary disease rates, history of cerebrovascular events, ejection fraction, body mass index, and current medical treatments. In univariate analysis, SVG disease was found to significantly correlate with diabeter mellitus (odds ratio [OR]: 1.644, 95% confidence interval [CI]: 1.190-1.985, p = 0.008), current smoking (OR: 0.875, 95% CI: 0.669-0.940, p = 0.030), number of patients with target artery diameter < 1.5 mm (OR: 1.945, 95% CI: 1.221-2.398, p < 0.001), age of SVG (OR: 2.960, 95% CI: 1.980-4.168, p < 0.001), uric acid (OR: 1.241, 95% CI: 1.078-1.592, p = 0.004), triglyceride (OR: 0.780, 95% CI: 0.569-0.935, p = 0.044) and UHR (OR: 1.894, 95% CI: 1.384-2.896, p < 0.001).

Conclusions: In this study, we showed that we can predict saphenous vein graft occlusion with serum UHR value.

Keywords: Coronary artery bypass graft, inflammation, cholesterol, saphenous vein, occlusion

Today, coronary artery bypass graft (CABG) surgery is an indispensable treatment method for some patient groups despite advances in endovascular technology. Various vascular grafts are used in these

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surgeries, however, the most commonly used one is the saphenous vein graft (SVG) [1]. Although SVGs are widely preferred by surgeons due to their ease of preparation and practicality, patency difficulties do arise. About half of these vessels can be occluded in the 10th year after CABG operations. Surgical problems play a role in early occlusion but the main occlusion mechanism after the first year is atherosclerotic processes that begin with intimal hyperplasia. Inflammation also plays an important role in these processes [2, 3].

It has been shown that high uric acid blood levels decrease nitric oxide release and are associated with diseases such as endothelial dysfunction, atherosclerosis, and hypertension [4]. On the contrary, high-density lipoprotein cholesterol (HDL-C) is known for its anti-inflammatory and vasorelaxant properties and has protective properties from atherosclerosis [5]. It has been shown that there is a relationship between coronary artery plaque load and HDL-C [6]. Various studies conducted on this topic have found a relationship between uric acid to HDL-C (UHR) and metabolic syndrome and atherosclerotic diseases [7, 8].

The purpose of the current study was to look at the predictive importance of UHR value in predicting long-term SVG disease in patients who underwent CABG surgery.

METHODS

Patients who had a history of CABG surgery at least one year prior had stable angina pectoris and underwent coronary angiography between January 01, 2016, and January 01, 2020, were included in the study retrospectively. The information on the patients was obtained from the data system of the coronary angiography unit and the hospital data recording system. Demographic data of the patients and hemogram (white blood cell, platelet, hematocrit, etc.) and biochemical parameters [urea, creatinine, low-density lipoprotein-cholesterol (LDL-C), HDL-C collected at the time of admission were recorded. Patients with the acute coronary syndrome, heart failure, severe heart valve disease, malignancy, renal failure (creatinine > 1.5 mg/dL), gout disease, familial hyperlipidemia, and autoimmune disease, were all excluded from the study. Following the application of the exclusion criteria, 496 patients were included in the study.

Following a study of baseline clinical demographic variables, the following were identified: Chronic obstructive pulmonary disease was defined as having a post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70%. Use of at least one antihypertensive medication and/or arterial blood pressure greater than 140/90 mmHg were considered to be symptoms of hypertension. Diabetes mellitus was defined as the use of antidiabetic medications, and fasting blood glucose levels over 126 mg/dL or 200 mg/dL while being examined regularly. Finally, a neurological disability that has existed for at least 24 hours was considered a sign of a preoperative cerebrovascular event.

All coronary angiographies were performed after obtaining informed consent from the patients and were performed using the Judkin method via the femoral, radial, or brachial arteries. Selective images of all grafts were obtained with appropriate catheters. If the SVG did not display selectively, aortography was performed. All saphenous vein grafts were evaluated in at least two view planes. As a result of the evaluations made by two experienced cardiologists, patients with 50% or more stenosis in at least one SVG were defined as the SVG disease group. The patients were divided into 2 groups according to the presence of saphenous vein disease. Patients with open saphenous veins were defined as Group 1, and patients who had 50% or more stenosis in at least one SVG after coronary angiography, were defined as Group 2.

The blood parameters of all patients were evaluated from blood samples taken from peripheral venous vascular structures during hospitalization. These evaluations were performed by blood counts in EDTA-coated tubes. An automatic analyzer was used for the measurement of hematological parameters. Biochemical parameters were calculated using a molecular analyzer. In the measurements made, the UHR value was calculated as follows: UHR= Uric acid (mg/dL) / HDL- C (mg/dL).

Statistical Analysis

The Statistical Package for the Social Sciences was used to examine statistical data (IBM SPSS Statistic Inc. version 21.0, Chicago, IL). Nominal variables were expressed as frequency and percentage, whereas continuous and ordinal variables were ex-

pressed as mean standard deviation or median (minimum-maximum). Data distribution was determined using the Shapiro-Wilk and Kolmogorov-Smirnov tests for normalcy. When comparing two groups of continuous variables with a normal distribution, the Student's t-test was used. When there was no normal distribution for the continuous variables, the Mann-Whitney U test was used to compare the two groups. For nominal variables, the Chi-square test was employed to compare two groups. Binary logistic regression analysis was used to evaluate the predictors of SVG disease. For all tests, a p value of < .05 was accepted as statistically significant. The area under the curve (AUC) for UHR was computed using a receiver-operating characteristic (ROC) curve for the prediction of SVG disease.

RESULTS

The median age of the 204 patients included in Group 1 and 292 patients in Group 2 were 65 (38-77) years and 66 (45-79) years, respectively (p = 0.251). The two groups were similar in terms of gender, hypertension, chronic obstructive pulmonary disease rates, history of cerebrovascular events, ejection fraction, body mass index, and current medical treatments (Table 1). The rates of diabetes mellitus, current smoking rates, and number of patients with target diameter artery < 1.5 mm were higher in Group 2 (p = 0.007, p = 0.024, and p < 0.001 respectively). The age of SVGs was significantly higher in Group 2 (p < 0.001) (Table 1).

Table 2 lists the patient's entrance laboratory results. White blood cell, hematocrit, platelet, creatinine,

Table 1. Demographic and preoperative features of the patients

Variables	Group 1 (n = 204)	Group 2 (n = 292)	*p* value
Age (years)	65 (38-77)	66 (45-79)	0.251‡
Female gender, n (%)	38 (18.6)	61 (20.9)	0.535*
Hypertension, n (%)	165 (80.9)	226 (77.4)	0.348*
Diabetes mellitus, n (%)	37 (18.1)	84 (28.8)	**0.007***
Current smoker, n (%)	29 (14.2)	65 (22.3)	0.024*
COPD, n (%)	21 (10.3)	33 (11.3)	0.723*
Previous CVA, n (%)	17 (8.3)	29 (9.9)	0.546*
BMI (kg/m^2)	27.3 (24-40)	27.6 (25-39.8)	0.294‡
Ejection fraction (%)	45 (35- 5)	40 (30-55)	0.078‡
β- Blocker therapy, n (%)	178 (87.3)	260 (89)	0.542*
ARB/ACE-I therapy, n (%)	162 (79.4)	218 (74.7)	0.216*
DAPT, n (%)	32 (16.7)	35 (12)	0.236*
Acetylsalicylic acid, n (%)	133 (65.2)	185 (63.4)	0.674*
Clopidogrel, n (%)	19 (6.3)	21 (7.2)	0.393*
Oral anticoagulant, n (%)	17 (8.3)	19 (6.5)	0.440*
Statin use, n (%)	172 (84.3)	233 (79.8)	0.245*
LITA usage, n (%)	200 (98)	289 (99)	0.631*
Target artery diameter <1.5 mm, n (%)	27 (13.2)	79 (27.1)	**< 0.001***
Number of SVG, n (range)	3 (1-5)	3 (1-4)	0.217‡
Age of SVG (years)	3 (1-11)	7 (1-16)	**< 0.001‡**

*Chi-square test, ‡Mann Whitney U test (Data is expressed as median (minimum-maximum)) ACE-I = Angiotensin-converting enzyme inhibitor, ARB = Angiotensin receptor blocker, BMI = Body mass index, CVA = Cerebrovascular accident, COPD = Chronic obstructive pulmonary disease, DAPT = Dual antiplatelet therapy, LITA = Left internal thoracic artery, SVG = Saphenous vein graft

Table 2. Admission laboratory variables of the patients

Variables	Group 1 (n = 204)	Group 2 (n = 292)	*p* value‡
White blood Cell (10^3/μL)	7.8 (4.9-16.3)	8.1 (4.5- 5.1)	0.101
Hematocrit (%)	37 (33-47)	40 (34-46)	0.287
Platelet (10^3/μL)	234 (140-440)	241 (132-424)	0.446
Creatinine (mg/dL)	0.92 (0.8-1.5)	0.94 (0.7-1.5)	0.667
Urea (mg/dL)	18 (14-29)	16 (18-33)	0.192
Albumin (g/L)	37 (35-54)	39 (35-51)	0.794
Uric acid (mg/dL)	5.4 (3.2-8.1)	7.9 (3.5-9.8)	**0.002**
HDL-C (mg/dL)	37 (29-58)	34 (26-60)	0.076
LDL-C, mg/dL	114 (80- 226)	121 (85-210)	0.269
Total cholesterol (mg/dL)	187 (134-210)	191 (128-224)	0.118
Triglyceride (mg/dL)	161 (104-189)	169 (110-194)	**0.042**
UHR	0.15 (0.07-0.26)	0.24 (0.09-0.37)	**< 0.001**

‡Mann Whitney U test, LDL-C = Low density lipoprotein-cholesterol, HDL-C = High density lipoprotein-cholesterol, UHR = Uric acid to high density lipoprotein cholesterol ratio

urea, albumin, LDL-C, and HDL-C values did not significantly differ across the groups. Triglyceride levels, uric acid levels, and UHR levels were all significantly higher in Group 2 ($p = 0.042$, $p = 0.002$, and $p < 0.001$, respectively).

To determine the variables influencing SVG disease-occurring CABG surgeries, logistic regression analysis was carried out (Table 3). In univariate analysis, SVG disease was found to significantly correlate with diabetes mellitus (odds ratio [OR]: 1.644, 95% confidence interval [CI]: 1.190-1.985, $p = 0.008$), current smoking (OR: 0.875, 95% CI: 0.669-0.940, p =

Table 3. Logistic regression analysis to identify factors affecting development of saphenous vein graft disease

	Univariate analysis			Multivariate analysis		
Variables	***p value***	**Exp(B) Odds Ratio**	**95% CI Lower-Upper**	***p value***	**Exp(B) Odds Ratio**	**95% CI Lower-Upper**
Age	0.254	1.209	0.882-1.790	--	--	--
Hypertension	0.350	1.420	0.956-2.114	--	--	--
Diabetes mellitus	**0.008**	1.644	1.190-1.985	**0.035**	1.120	1.070-1.436
Current smoker	**0.030**	0.875	0.669-0.940	0.378	1.060	0.892-1.338
TAD < 1.5 mm, n (%)	**< 0.001**	1.945	1.221-2.398	**0.012**	1.344	1.110-1.742
Age of SVG	**< 0.001**	2.960	1.980-4.168	**< 0.001**	1.910	1.375-2.364
Uric acid (mg/dL)	**0.004**	1.241	1.078-1.592	--	--	--
HDL-C (mg/dL)	**0.081**	0.894	0.745-1.190	--	--	--
Triglyceride (mg/dL)	**0.044**	0.780	0.569-0.935	0.536	0.794	0.691- 1.010
UHR	**< 0.001**	1.894	1.384-2.896	**0.008**	1.290	1.060- 1.897

COPD = Chronic obstructive pulmonary disease, SVG = Saphenous vein graft, HDL-C = High density lipoprotein-cholesterol, UHR = Uric acid to high density lipoprotein cholesterol ratio, TAD = Target artery diameter

0.030), number of patients with target artery diameter < 1.5 mm (OR: 1.945, 95% CI: 1.221-2.398, p < 0.001), age of SVG (OR: 2.960, 95% CI: 1.980-4.168, p < 0.001), uric acid (OR: 1.241, 95% CI: 1.078-1.592, p = 0.004), triglyceride (OR: 0.780, 95% CI: 0.569-0.935, p = 0.044) and UHR (OR: 1.894, 95% CI: 1.384-2.896, p < 0.001). In multivariate analysis, diabetes mellitus (OR: 1.120, 95% CI: 1.070-1.436, p = 0.035), target artery diameter 1.5 mm (OR: 1.344, 95% CI: 1.110-1.742, p = 0.012), age of SVG (OR: 1.910, 95% CI: 1.375-2.364, p < 0.001) and UHR (OR: 1.290, 95% CI: 1.060-1.897, p = 0.008) were determined as independent predictors for SVG disease development.

ROC curve analysis revealed that the cutoff value for UHR was 0.19 (AUC: 0.790, 95% CI: 0.751-0.829, p < 0.001, sensitivity of 75.2% and specificity of 69.4%) (Fig. 1).

DISCUSSION

The incidence of coronary artery disease (CAD), which occupies a prominent place among atherosclerotic cardiovascular diseases, is increasing day by day. CABG surgery is one of the most important treatment methods of CAD and although the most important graft used in CABG operations is the left internal thoracic artery, SVG is also valuable because of its various advantages. A variety of blood parameters have been investigated in the literature to predict late (after one year) saphenous vein disease [9]. In this study, we are the first to reveal in the literature that serum UHR is associated with late SVG disease. In our multivariate analysis, in addition to known risk factors such as coronary artery structure and diabetes mellitus, we detected UHR as an independent predictor of SVG disease development.

Purine metabolism produces uric acid (UA), which is linked to poor metabolic health [10]. Uric acid penetrates the cell membrane, causing oxidation and inflammation. It also reduces nitric oxide release in the endothelium and inhibits cell proliferation and migration. In addition to these, aside from direct damage to vascular smooth muscle structures, the expression of proinflammatory cytokines also triggers the development of atherosclerosis [7].

In a recent study that included 15,843 (73.90% male) participants, a significant correlation was found between high UA values and carotid intima-media thickness [11]. In another recent study that included 369 people, a significant relationship was found between high UA values and metabolic syndrome [12]. In a study in which 814,804 (415,779 males and

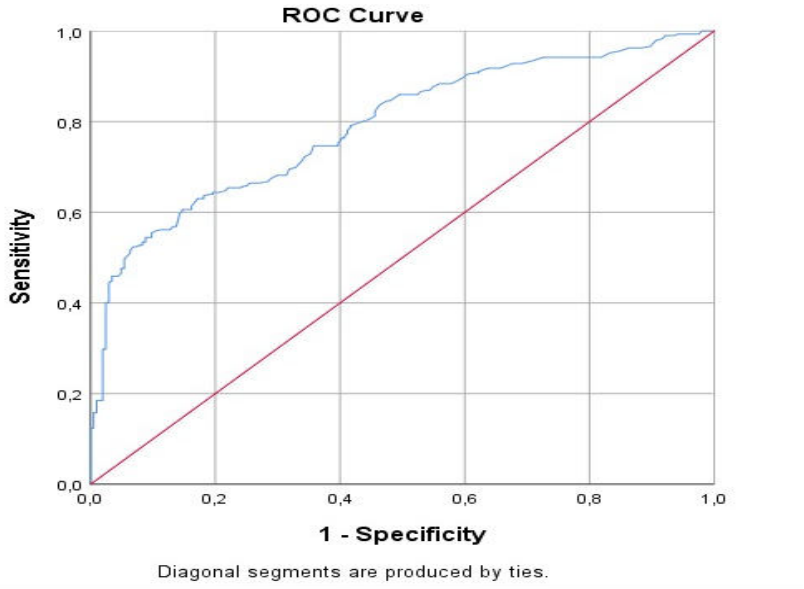

Fig. 1. ROC (Receiver operation characteristic) curve and AUC (Area under the curve) for admission uric acid to high-density lipoprotein cholesterol ratio for predicting saphenous vein graft disease. (Cut-off: 0.19, AUC: 0.790, 95% CI: 0.751- 0.829, p < 0.001, sensitivity of 75.2% and specificity of 69.4%).

399,025 females) volunteers were included, a linear relationship was revealed between serum UA values and atrial fibrillation [13]. In a study conducted by Tavil *et al*. [14], the relationship between serum UA levels and SVG disease was investigated in 192 patients, followed for an average of 5.6 years after CABG operation. At the conclusion of the study, a significant correlation was found between high serum UA values and SVG disease [14]. In our study, serum UA values were significantly higher in our patient group with SVG disease.

HDL-C is known for its protective effects from atherosclerosis. It has a composite structure consisting of lipid composites of different sizes. HDL-C has anti-inflammatory, anti-diabetic and antithrombotic effects along with cholesterol-mobilizing effects in tissues [15]. In a study conducted on these effects, it was shown that every 16 mg/dL increase in HDL-C levels leads to a decrease in CAD rates (OR of 0.95, 95% CI: 0.85-1.06) and diabetes mellitus rates (OR of 0.83 95% CI: 0.76-0.90) [16]. CAD was found in 60% of individuals with low HDL-C levels in cohort studies [17]. In a multicenter study by Jerzevski *et al*. [18], including 113 patients who underwent CABG and were evaluated at 12 months, the relationship between HDL-C and SVG disease was investigated. In their study, the HDL-C cut-off value was determined as 40 mg/dL, and low HDL-C values were shown to be mostly associated with SVG occlusion and increased intimal hyperplasia [18].

Considering this information available regarding uric acid and HDL-C, UHR values emerge as an important cardiovascular risk indicator. In a study conducted on the general population, UHR values were found to be significantly higher in patients with non-alcoholic fatty liver disease, compared to the control group [19]. In another study, the prognostic significance of UHR values in peritoneal dialysis patients was investigated. In their study, in which 1953 patients were included and followed up for an average of 61.3 months, high UHR values were found to be associated with cardiovascular and all-cause mortality [20]. In a retrospective cross-sectional cohort study conducted in the cardiovascular field, the effect of UHR values on hypertension control was investigated in 535 hypertension patients. Here, UHR values were significantly correlated with systolic ($r = 0.33$, $p < 0.001$) and diastolic ($r = 0.28$, $p < 0.001$) blood pressure. In addition, UHR value has been shown as an independent predictor of poor blood pressure control, and it has been determined that a unit increase in UHR value affects poor blood pressure control 7.3 times ($p < 0.001$, 95% CI: 3.9-13.63) [21]. In our study, high UHR values were also shown as an independent predictor of SVG disease.

After CABG surgery, SVGs can become occluded for several reasons. The cause of SVG disease occurring in the first month is thrombosis, and intimal hyperplasia for up to one year, followed by atherosclerosis [22]. In our study, at least 1 year had passed after CABG surgeries for all patients. Therefore, it is inevitable that atherosclerotic risk factors play a role in the development of the disease. Lipid-lowering therapies and antiplatelet therapy algorithms used by patients may play a role in the development of SVG disease. It has been shown that medical treatment strategies may affect the development of SVG disease [2, 23]. In our study, there was no significant difference between the postoperative medical treatments applied between the groups.

The age of the SVG and the coronary vascular structure and the diameter of the target vessel bypassed, are other important factors that may affect the development of SVG disease [24]. In a retrospective study by Bayam *et al*. [25] including 398 patients, SVG age was shown as an independent predictor of SVGD development (OR:1.18, 95% CI: 1.02-1.35, $p = 0.020$). In another recent study [26], the severity of coronary artery disease, calculated by the SYNTAX score, was shown as an independent predictor of SVG disease (OR: 0.978, 95% CI: 0.957-0.999, $p = 0.045$). And in a study in which SVGs were prepared with the no-touch technique in all patients, a target vessel diameter of less than 1.5 mm was shown as an independent predictor of SVG disease [26].

Limitations

There are some limitations in our study. Primarily, our study was a single-center retrospective study, therefore the number of patients was limited. In addition, only the diameter was taken as a variable for the coronary artery structure before CABG in our study group. It should be noted that the general disease status of the vessel, apart from the diameter, may also affect the development of SVG disease. In addition, a detailed lesion analysis of all saphenous veins could not

be performed. There is a need for multicenter prospective studies in which these analyzes are also carried out.

CONCLUSION

Although CABG surgeries constitute the prominent treatment option for atherosclerotic heart diseases, the atherosclerotic process continues as long as the patients are alive. Beyond the first year following CABG surgeries, atherosclerosis plays a leading role in the development of SVG disease. Therefore, clinical follow-up of these patients is particularly imperative. In this study, we showed that we can predict saphenous vein graft occlusion with serum UHR values; to the best of our knowledge, this is the first time this was done in the literature.

Authors' Contribution

Study Conception: OG, ME; Study Design: OG, ME; Supervision: OG, ME, ŞY; Funding: OG, FA, ÖFD; Materials: OG, ME; Data Collection and/or Processing: OG, ME, ÖFD; Statistical Analysis and/or Data Interpretation: OG, ME; Literature Review: OG, ME, ÖFD, FA, ŞY; Manuscript Preparation: OG, ME,ÖFD and Critical Review: OG, ŞY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(1):22-28 Original Article

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Evaluation of DNA versus collagen perception in scientific articles examining cancer and radiation therapy: implication for collagen-based approaches

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ABSTRACT

Objectives: Although radiation therapy has been used for more than a hundred years, its definitive mechanism of action is not known. Many studies indicate that radiation induces free radicals which damage DNA. However, irradiation should also affect the collagen connective tissue matrix. This database analysis aims to determine the extent of DNA versus collagen perception in scientific papers.

Methods: Journals indexed in PubMed were searched on March 3, 2021, using the medical keywords "cancer", "radiation therapy", "radiation therapy AND damage," radiation therapy AND mechanism AND damage, "radiation therapy AND clinical". The number of items found for each search was proportioned in terms of "DNA versus collagen" and the ratio was accepted as the perception shift coefficient.

Results: Results were tested with the p-value analysis to calculate the difference between the two proportions in both search items. Based on the main rule under the assumption that "all cells have DNA and all cells live in the collagen matrix". In the p1-p2 analysis of the data, a significant ($p < 0.001$) difference was obtained for all dichotomy scans.

Conclusions: This data analysis supports the argument that both cancer and radiation therapy perception is DNA-based rather than collagen, since the synthesis and degradation process of very slow; it is not possible to observe it in short-term studies. The effects of irradiation should be further analyzed in this manner for purpose of collagen matrix interaction.

Keywords: Radiation therapy, DNA, collagen, fuzzification

Radiation therapy is one of the main modalities in cancer treatment. Ionizing radiation had been used in the treatment of many diseases, even shortly after the discovery of its biological effects [1]. The first successful radiotherapy trials were performed in head and neck cancers, where surgical treatment was not possible, followed by other malignancies [2]. Based on the observations of the radiation tolerance of normal tissues, it was noticed that dividing the dose into fractions over time (fractionation) increased normal tissue tolerance, without a negative effect on tumor control, therefore, the concept of fractionated radiation therapy was emerged [3].

Although the general biological principles of radiation therapy had rapid progress, the mechanism of action continues to be a subject of debate even today.

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According to the radiobiological observations, the cells that divide faster have been affected than those that divide slowly; thus it has been generally accepted that the biological effect is related to the cell division rate [4, 5]. After the discovery that the genetic information is encoded in the DNA molecule, a consensus appeared that the control of cell division is related to DNA. This point of view led to the conclusion that the biological effects of radiation are directly related to DNA damage which is still valid today. According to this idea "radiation can cause single or double-strand breaks in DNA, resulting in fatal mutations or programmed cell death" [6].

However, in an intact organism, all cells are located in an extracellular matrix where the main component is collagen and the control of cell division is regulated by external stimuli. Although DNA encodes a complex synthesis process, it is structurally a simple molecule, that situation orientated the research toward DNA and facilitates DNA-based explanations for radiation effects. In contrast, the turnover of the collagen matrix is very slow and complex even to observe or explore. While cancer cells can be investigated under in vitro conditions, the collagen matrix production-destruction cycle is out of research scope due to its slow turnover and methodological limitations [7-9].

All these reasons have led to the establishment of a DNA-focused mainstream explanation of the biological mechanism of action for ionizing radiation. Thus the probable interaction of radiation with collagen seems to be overlooked. In contrast, it is not easy to determine how much an alternative second explanation overlaps the main explanation. One of the valid methods that can be applied for this purpose is the screening of associated medical subjects in this field and the testing of whether the difference between the matches is statistically significant, the approach is generally called fuzzification [10]. In this method, first, the main heading (nominator) is searched on the database and then their association (dichotomy) with the subheadings is researched. This approach produces numerical output for related subjects and they can be further analyzed.

Today, information technology enables access to large databases of peer-review scientific articles. The basic logic in this method is that the concepts studied are in the same system, but not directly related to each other. The result observed and the detected value in any data analysis is the sum of the actual, coincidental, and false results (bias). Although everything can be associated with another concept in living systems, choosing the right keywords will narrow the possibility of error. This study is a database analysis performed to determine the bias of "DNA versus collagen" perception based on cancer and radiation therapy research.

METHODS

The database of the United States National Library of Medicine encodes scientific publications with keywords Medical Subject Headings (MeSH) defined as medical titles [11]. This database structure gives a numerical value if any MeSH is used as a nominator. When a second MeSH keyword for dichotomy is added to the search (fuzzification), the numerical values obtained indicate the association of the nominator with the second concept. The ratio of the numerical result given by the same nominator with the two subconcepts obtained by dichotomy will determine the direction of research perception [12].

To evaluate the scientific perception of cancer retrospectively, the PubMed database was searched on March 3, 2021, using the medical keywords "cancer", "radiation therapy", "radiation therapy AND damage," radiation therapy AND mechanism AND damage, "radiation therapy AND clinical". In the second phase, the association of these key terms was searched by creating a dichotomy by adding "DNA" or "collagen" MeSH for each item. In order to test whether the "AND" logic shows a collocation relationship within the MeSH search results, a separate search was carried out by replacing the words used. It was observed that the obtained article order and numerical values completely overlapped, thus it was confirmed that the PubMed database was not affected by the keyword ranking.

The numerical sizes of the numerical numbers obtained with keywords were accepted as the "correlation value". No exclusion criteria were used in screening. Since the database contains a large number of articles, it was not possible to evaluate all the results, and samples were selected by considering the random numbers table. The accessed results with each search MeSH or combinations were randomly re-

viewed with 50 articles and the possibility of biases was refused. Later, the search was expanded by increasing the number of words that occur together; herewith the bias that the results contain search words together due to a random error was excluded.

The results obtained by each search nominator either with its DNA or collagen subtitles were rated to each other; the number obtained was called the perception shift ratio. Although the database search found a narrower set of results with each different option (DNA versus collagen) added to the nominator, the value of the perception shift coefficient remained in favor of DNA versus collagen.

Statistical Analysis

When interpreting a confidence interval that compares two population proportions, one should always be sure to use the words of the problem and to phrase the interpretation in terms of how much larger (or smaller) the first ratio compared to the second one. This procedure is valid because both samples were taken randomly and independently. So it is common to compare two independent groups with respect to the presence or absence of a dichotomous characteristic or attribute. When the outcome is dichotomous, the analysis involves comparing the proportions of successes between the two groups.

There are several ways of comparing proportions in two independent groups. One can compute a proportion difference, which is computed by taking the difference in proportions between comparison groups and is similar to the estimate of the difference in means for a continuous outcome. Generally the reference group (e.g. radiation therapy) is considered in the denominator of the ratio. The dichotomy ratio is a good measure of the strength of an effect (ie. DNA versus collagen) and therefore indicates a reason attributed. When the outcome of interest is relatively uncommon (e.g., < 10%), a dichotomy ratio has a good predictive value, confidence interval estimates for the dichotomous difference [13].

In this study the results obtained were tested with the p1-p2 analysis to calculate the difference between the two proportions in both search items. Based on the main rule under the assumption that "all cells have DNA and all cells live in the collagen matrix" the H0 hypothesis has been created for significance; H0: p1-p2 = 0 and H1: p1-p2 ≠ 0 as exclusion criteria. The numerical results were statistically analyzed for the fact of the H0 > H1 condition, $p < 0.01$ was considered significant. [14, 15].

SPSS was used in the calculations made during the study (to compare the effect of independent variables on dependent variables).

RESULTS

In the articles including "cancer", "radiation therapy", "radiation therapy AND damage," radiation therapy

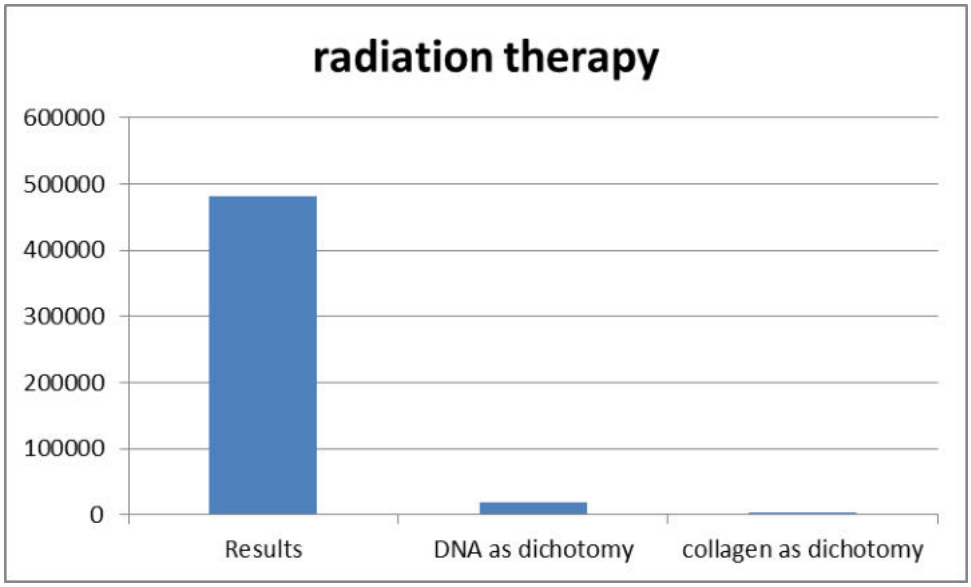

Fig. 1. Results for "radiation therapy" Medical Subject Headings, following bars demonstrate DNA versus collagen dichotomy.

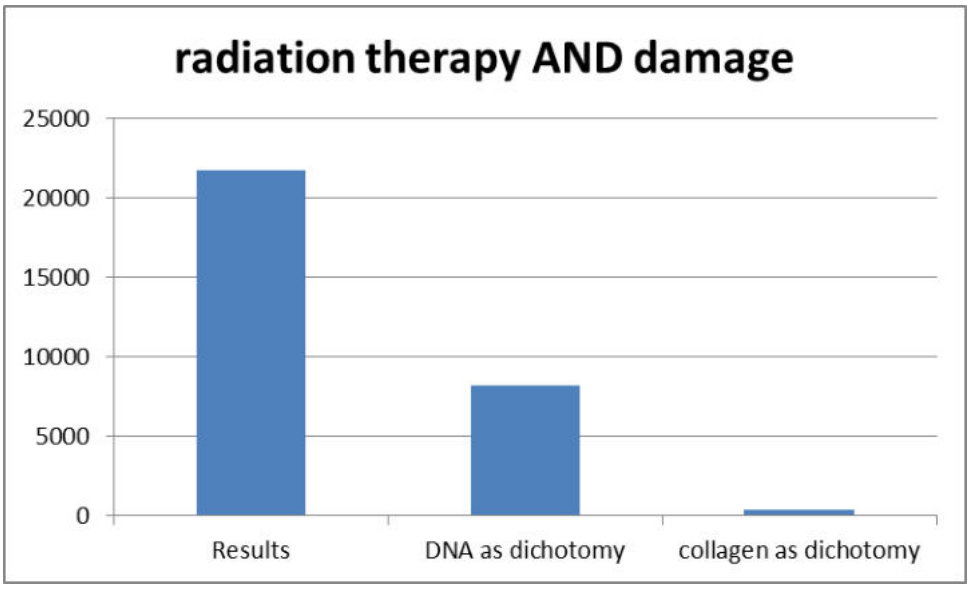

Fig. 2. Results for "radiation therapy AND damage" Medical Subject Headings, following bars demonstrate DNA versus collagen dichotomy.

AND mechanism AND damage, "radiation therapy AND clinical", the association with DNA was found higher than associating with collagen. Of a total of 4,569,302 articles with the MeSH cancer, 407,585 include DNA, whereas collagen was included in 30,087. If the search MeSH "radiation therapy" was used as nominator, 496,547 results were obtained, which resulted in 20487 articles when using "DNA"; and 2,846 using "collagen" for dichotomy respectively. According to these results dichotomy rate in the database favors DNA compared to collagen 7.2 to 30.4 times for all search MeSH items (Table 1). The detailed analyzes of the results in Table 1 are shown in Figs. 1, 2, 3 and 4.

In evaluating the significance of the difference between the groups compared in studies, the issue that is almost always taken into consideration is whether it is statistically significant. In other words, the p-value is less than 0.05. In our study, the *p* - value was statistically significant. However, since the p-value is affected by the sample size, the results were tested with the effect size factor to show that this significance was

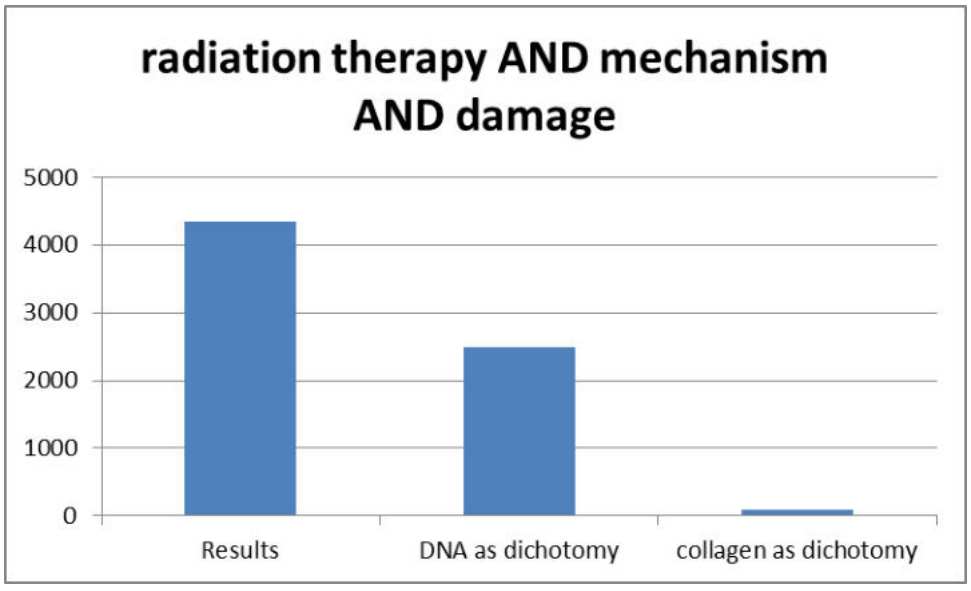

Fig. 3. Results for "radiation therapy AND mechanism AND damage" Medical Subject Headings, following bars demonstrate DNA versus collagen dichotomy.

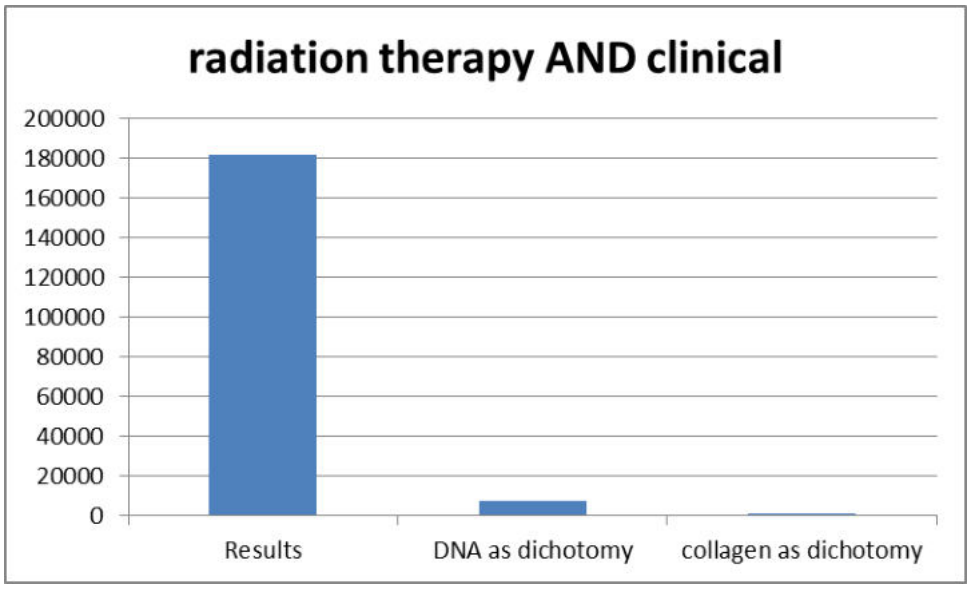

Fig. 4. Results for "radiation therapy AND clinical" Medical Subject Headings, following bars demonstrate DNA versus collagen dichotomy.

not accidental.

The effect size shows how much of the total variance in the dependent variable is explained by the independent variable or factor. The most widely used one in calculating the effect size is the calculation developed by Cohen (d). As a general recommendation, Cohen says that if the d value is less than 0.2, the effect size can be defined as weak, if 0.5 is medium, and if it is greater than 0.8, it can be defined as strong [16]. The data of the Cohen's d test, the results of which we tested, are given in Table 2.

When the effect size results were examined, it was confirmed that there were remarkable results greater than 0.8 and the p-value was not random.
Statistical evaluation of the data with p1-p2 analysis was found to be significantly different ($p < 0.001$) for all dichotomy results, thus ruling out the H0 hypothesis and confirming the H1 hypothesis. The results show that within the PubMed database the search terms "cancer", "radiation therapy", "radiation therapy AND damage," radiation therapy AND mechanism AND damage", and "radiation therapy AND clinical" have a very close association with DNA than associating them with collagen.

DISCUSSION

Today, information technology enables the search and analysis of large databases with relevant keywords. Based on this study, PubMed offers the opportunity to access millions of peer-review scientific articles online

Table 1. Output values and statistical analysis results obtained by each PubMed database search according to nominator and dichotomous Medical Subject Headings (MeSH) words.

Search MeSH	Results	DNA as dichotomy	Collagen as dichotomy	DNA / collagen	Z value	*p* value
Cancer	4,569,302	407,585	30,087	13.54	25448.476	**< 0.00001**
Radiation therapy	496,547	20,487	2,846	7.2	18760.6098	**< 0.00001**
Radiation therapy AND damage	22,660	8,645	410	21.1	463.0428	**< 0.00001**
Radiation therapy AND mechanism AND damage	4,627	2,676	88	30.4	143.5726	**< 0.00001**
Radiation therapy AND clinical	189,707	7,950	1,028	7.7	11366.6028	**< 0.00001**

Table 2. The effect size of the statistical analysis results of the values obtained according to the candidate and binary Medical Subject Headings words with each PubMed database search

Group 1: DNA Group 2: Collagen	Cohen's d	Effect-size r
Cancer	1.15	0.49
Radiation therapy	0.78	0.36
Radiation therapy AND damage	1.03	0.46
Radiation therapy AND mechanism AND damage	1	0.44
Radiation therapy AND clinical	0.85	0.39

[17, 18]. Since the PubMed database is very large, if valid keywords are plotted, it can be explored how much a concept had been associated with other related items (dichotomy). This database does not contain duplications; all journals within its scope feature peer-review and therefore allow objective data analysis.

The dichotomy subjects in this study are DNA and collagen, the reference groups used before the dichotomy are completely different, so it is not possible to interpret the results with bias. On the other hand, the fact that the database is very large, creates homogenization within itself. It can be argued that the journals published in different fields may also be the cause of bias, but selecting the keywords used in the search from the MeSH scope limits the bias possibility. One can use the p1-p2 hypothesis in statistical evaluation in the analysis of large databases. The PubMed, which covers knowledge of more than a century, supports the hypothesis that scientific opinion held DNA significantly more responsible than collagen in explaining cancer, radiation therapy, and its mechanism of action. The etiology of cancer is still unknown today. Cancer disease can be detected also in archaeological records [19]. However, it is generally accepted that cancer has increased in all countries of the world especially in the last decades [20-22]. Hundred years ago it was understood that the cell nucleus plays an ad hoc role in cell division, and it was proven in the second half of the last century that DNA encodes genetic information. This situation has attracted the attention through the genetic characteristics of diseases and put the microenvironment in which the cell is located to be ignored. All the environmental factors, which are claimed to play a role in cancer development, have been associated with DNA damage. This approach, which can also be called the Zeitgeist effect, lead to huge progress that enable DNA analysis with automatic devices in a short time and reinforced the shift of perception of cancer etiopathogenesis and treatment to the DNA-centric [23, 24]. However, even though the cell division is encoded in DNA, the stimulus that will initiate the division comes from the intercellular area, especially through extracellular matrix components, rather than DNA [25]. In contrast to DNA, the intercellular field is characterized by dynamics that cannot be easily investigated, and the slow turnovers do not allow observation.

Studies on the biological effects of radiation therapy on collagen are extremely limited. Although it has been known for a long period that radiation interacts with collagen, it has not been possible to test the effect in vivo. Even though early research indicated that radiation cause collagen damage, the Zeitgeist effect shifted the perception to DNA. Moreover, technical facilities limited the studies on interactions of radiation with the extracellular matrix, especially collagen [26, 27].

In contrast, current clinical experience confirms that radiation affects connective tissue (unlike DNA) in long term. Fibrosis occurring after radiation therapy is permanent even in conventional therapeutic doses; causing functional defects in the heart, bladder, or rectum [28-30]. Moreover, the lens, which is very sensitive to irradiation, is acellular and contains only cells in the boundary of the capsule, but loses its light transmittance even in very low doses [31].

CONCLUSION

This study confirmed that cancer, radiation therapy, damage, and mechanisms of action have been attrib-

uted to DNA significantly more than collagen, the main intercellular matrix component, in which the cell is located. Although the data in the literature are very limited, it is clear that collagen and extracellular matrix constitute a new and productive field for exploring the effects of radiation. Future studies could be very beneficial if objected to connective tissue instead of a DNA-based perception.

Authors' Contribution

Study Conception: ÖK; Study Design: ŞK; Supervision: ŞK; Funding: ŞK; Materials: YD; Data Collection and/or Processing: YD; Statistical Analysis and/or Data Interpretation: ÖK; Literature Review: ŞK; Manuscript Preparation: YD and Critical Review: ÖK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(1):29-38 Original Article

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Can 900 MHz and 2100 MHz radiofrequency radiation exposure induce endoplasmic reticulum stress and apoptosis in rat thymus?

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ABSTRACT

Objectives: Electrical appliances are source of radiofrequency radiation (RFR). The effects of RFR on the organism are not fully understood. Endoplasmic reticulum (ER) stress is appeared by the accumulation of misfolded proteins in ER lumen. The aim of this study was to investigate the effects of 900 and 2100 MHz RFR exposure on the ER stress pathway in rat thymus.

Methods: Rats were divided into six groups: 1 week (w) and 10 w Sham rats were kept in plexiglass tubes for 2 hours/day without RFR, experiment groups were created as 1-w (acute) and 10 w (chronic) rats which exposed to 900 and 2100 MHz RFR for 2 h/day. There were 20 male Wistar rats in each group. Immunohistochemistry stainings were performed GRP78, CHOP, Cleaved (Clv.) Caspase 3 and Caspase 12.

Results: Expressions of GRP78 and Clv. Caspase3 in RFR groups is significantly higher than sham groups ($p < 0.001$). In 900 MHZ-1 w rats, high levels of GRP78 expressions were at the cytoplasm of epithelial reticular cells. In other groups, GRP78 expressions were seen also at thymocytes. Expressions of CHOP in RFR rats were higher than sham rats (2100 MHz /Sham for 10 w; $p < 0.001$, 900 MHz /Sham for 10 w; $p = 0.004$, 900 MHz /Sham for 1 w; $p = 0.003$). Localization of CHOP expressions was at the nucleus membrane and cytoplasm. The expression of Caspase 12 in RFR rats was higher than sham rats (900 MHz /Sham for 1 w; $p = 0.006$, other groups; $p < 0.001$).

Conclusions: This study demonstrates RFR exposure could increase levels of ER stress pathway proteins and could cause apoptosis.

Keywords: Radiofrequency radiation, thymus, endoplasmic reticulum stress, apoptosis

The thymus is a primary lymphoid organ located behind the sternum, in the superior mediastinum. The thymus has an important role in the selection and maturation of T lymphocytes.

Endoplasmic reticulum (ER) is the biggest organelle in the cell. ER's essential functions are lipid synthesis, protein folding, and synthesis, Ca++ homeostasis, detoxification. Hypoxia, cancer, reactive oxidants, low Ca++ levels, and many other conditions can disrupt these functions [1]. Thus, unfolded, and dys-

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functional proteins accumulate in the ER and cause ER stress. ER stress initiates Unfolded Protein Response (UPR) [1, 2]. UPR tries to solve this ER stress problem in various ways. For instance, to increase ER protein turnover, to activate up-regulation of chaperons, etc... If the cell can not overcome ER stress, UPR initiates cell death programs [2].

UPR is controlled by three transmembrane sensors: PERK (protein kinase RNA-like endoplasmic reticulum kinase), IRE-1 (Inositol regulating enzyme-1) and ATF-6 (Activating Transcription Factor-6). Sensors detect unfolded proteins and activate the other steps [1, 2]. In normal conditions, a chaperon and calcium-binding protein; 78-kDa glucose-regulated protein (GRP78, or the other name Binding immunoglobulin protein-BiP) stabilizes the inactive state of sensors. But unfolded proteins send away GRP78, so UPR sensors become free and active [3].

Active PERK inhibits protein synthesis by phosphorylating eIF2α. PERK induces translation of some proteins, the most researched one is activating transcription factor 4(ATF4). ATF4 induces chaperon synthesis, antioxidant synthesis, C/EBP homologous protein (CHOP) synthesis, and autophagy genes. CHOP regulates BCL-2 family genes which are responsible for apoptosis. CHOP also induces proapoptotic genes and reduces antiapoptotic genes [1, 4]. PERK pathway also plays role in mitochondrial prosurvival signaling, mitochondrial autophagy, and mTOR-P3IK-Akt pathway [4].

IRE1 is an endoribonuclease localized in the ER membrane. If ER stress is present, IRE1 splice x-box binding 1 protein (XBP1) mRNA. XBP-1 induces UPR genes. Persistent IRE1 activation could trigger apoptosis via tumor necrosis factor receptor-associated factor (TRAF2) [5]. ATF6 induces XBP1 synthesis and chaperon synthesis like GRP78. Also, ATF6 induces genes that play a role in the degradation of misfolded proteins [6].

RFR is a power caused by the energy of electrical charges. It was classified by European Committee (Table1) [7]. As seen in the table mobile phones, tablets, electrical appliances, and others are radiofrequency radiation (RFR) sources. We are exposed to RFR intensely these days. Therefore, it is important to understand how RFR affects the organism. Some researchers have investigated RFR's effects on the thymus. In research published in 2011, it was demonstrated that 900MHz RFR increases superoxide dismutase (SOD) levels, glutathione peroxidase (GPx) activity, and decreases glutathione levels in rat thymus [8]. Hanci *et al*. [9] reported that exposure to 900 MHz RFR caused an increase in malondialdehyde (MDA)- an oxidative stress marker- and decreased glutathione levels in prenatal rat thymus. Also, it was reported that RFR could lead to pathological changes in prenatal rat thymus [9]. Another research showed 900 MHz RFR causes an increase in MDA levels and induces histopathological changes at mature rat thymus [10, 11]. Misa-Agustino *et al*. [12]. have applied 2.45 GHz (0-1-1.5-12-Watt power) RFR to rats. In this study heat shock protein (HSP) 90 decreased in only 12 W exposed animals and there were no differences in HSP70 levels. In exposed rats, glucocorticoid receptors presented more than control groups.

As shown in the studies, exposure to RFR could induce oxidative stress and make changes at thymus histopathology. There is no study about RFR's effects

Table 1. Electromagnetic fields and sources

Frequency Range	Frequencies	Some examples of exposure sources
Static	0 Hz	VDU (video display); MRI and other diagnostic/scientific instrumentation; Industrial electrolysis; Welding devices
ELF	0-300 Hz	Powerlines; Domestic distribution lines, Domestic appliances; Electric engines in cars, train and tramway; Welding devices
IF	300 Hz- 100 kHz	VDU; anti theft devices in shops, hands free access control systems, card readers and metal detectors; MRI; Welding devices
RF	100 kHz – 300 GHz	Mobile telephony; Broadcasting and TV; Microwave oven; Radar, portable and station radio transceivers, personal mobile radio; MRI

VDU = video display units for computers, videos, television using cathode ray tubes, ELF = extremely low frequency, IF = intermediate frequency, RF = radio frequency, MRI = magnetic resonance imaging, TV = television

on ER stress in the thymus. We investigate in this study that 900 and 2100 MHz RFR whether could cause ER stress in rat thymus.

METHODS

Animals and Study Design

This study was conducted on 120 male Wistar rats aged 3 months and weighing 250-300 gr. All animals were kept in a controlled environment with the artificial light-dark cycle of 12 h lights on and 12 h lights off and received food and tap water ad libitum. There were 4 animals in every cage. Sham rats were housed in separate rooms under the same conditions for an equal time. All experimenters were blind to animal experimental group memberships during the data collection and analysis. The animals were randomly divided into 6 groups like below.

Groups:

1. Sham 1w (acute)
2. Sham 10w (chronic)
3. Exposure to 900 MHz RFR for 1w (acute)
4. Exposure to 900 MHz RFR for 10w (chronic)
5. Exposure to 2100 MHz RFR for 1w (acute)
6. Exposure to 2100 MHz RFR for 10w (chronic)

Ethical Approval

Ethical approval for this work was obtained from Akdeniz University Local Committee on Animal Research Ethics (2013.05.01). All experimental protocols conducted on rats were performed under the standards established by the Institutional Animal Care and Use Committee of Akdeniz University.

Radio Frequency Radiation Exposure

The radio frequency (RF) values used in this study are the frequencies (900 and 2100 MHz) used in mobile phone communication. An RF generator (GSM Simulator; Everest Company, Adapazari, Turkey), which produces 900 and 2100 MHz RF radiations, was used to represent exposures of global systems for mobile communications (GSM). In the 900 MHz RFR experiment the carrier frequency was 900 MHz, the modulation frequency was 217 Hz, the pulse width was 0.577 msec, and the power range of the generator was 0-10 W. Besides in the 2100 MHz RFR experiment, the carrier frequency was 2100 MHz, the modulation frequency and the pulse width were the same as 900 MHz and the power range of the generator was 0-2 W.

The animals were placed in plexiglass tubes with holes where one single rat could fit, breathe comfortably, and not increase the body temperature. Rats in plexiglass tubes were placed radially at equal distances (in 900 and 2100 MHz experiments rats' noses were at 3.5 and 10 cm distances from the antenna, respectively) around the antenna (Fig.1). The applied carousel setup procedure of the present study was by the setup procedure of the other studies in the literature [13-15]. The tubes restrained the movement of the rats to such an extent as to follow for well-defined exposure conditions, yet without immobilizing them. RFR

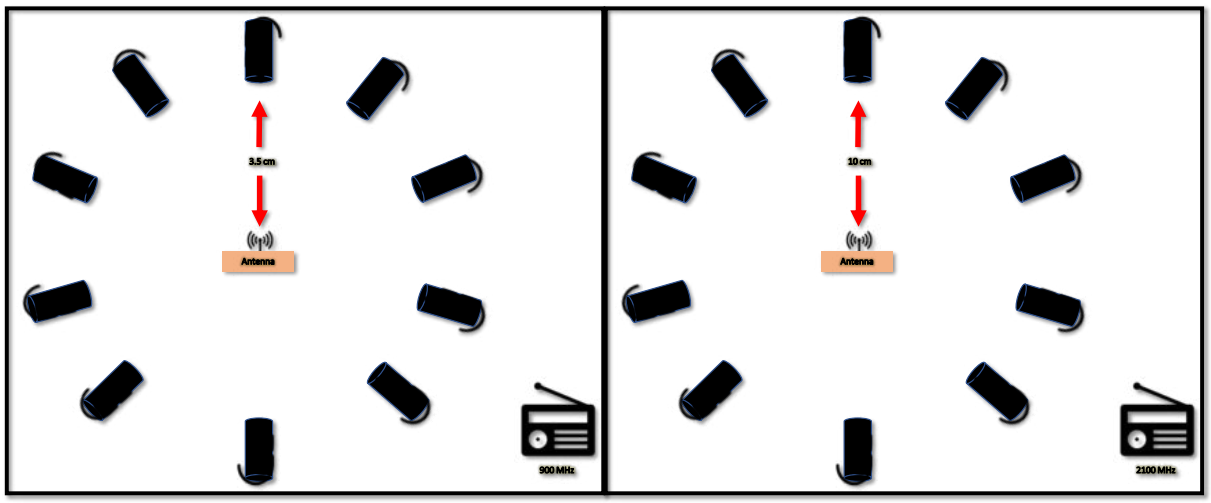

Fig. 1. The experimental setup for the exposure of 900 and 2100 MHz RFR.

application was held in a shielded room to prevent the rats from the effects of other electrical sources. For the used frequencies, the source output powers were selected by considering the power values emitted by the mobile phones (the output powers adjusted to 5 W (for 900 MHz) and 1.5 W (for 2100 MHz) during the exposure). During the experiment in the 'signal on' position, the measured electric-field strengths over the rat's heads were 35.5 and 35.2 V/m for 900 and 2100 MHz frequencies, respectively.

Group 3 rats were exposed to 900 MHz RFR 2 hours/day, 5 days/week for 1 week, and group 4 rats were exposed to the same frequency RFR 2 hours/day, 5 days/week for 10 weeks. Group 5 rats were exposed to 2100 MHz RFR for the same duration as group 3 and group 6 rats were exposed to 2100 MHz RFR for the same duration as group 4. Sham rats were kept under the same conditions and durations while the RFR generator was turned off for 1 week and 10 weeks.

Electric field strengths were measured by EMR300 (Narda, Germany) with a suitable probe in the experiments. The electric field background level was between 0.02 and 0.2 V/m in the shielded room. Also background magnetic fields were measured between 0.01 and 0.03 μT by Hioki 3470 Magnetic Field Hitester (Hioki E. E. Corp., Japan) with an appropriate probe.

Dosimetry simulations were carried out using a finite integration technique (FIT) based commercial software, CST Microwave Studio (3D EXPERIENCE®, Dassault Systemes, Hamburg). The FIT was introduced by Weiland [16]. Although the gridding can be applied as a finite difference time domain (FDTD) method, the FIT uses the integral form of Maxwell's equations [17]. In the present study, the rat model used in simulations has consisted of voxels with a resolution of 1.827×1.827×2.015 mm^3. The interaction between the incident electromagnetic wave and the biological tissue are explained by electrical properties that can be obtained by the dielectric properties of the interested tissue [18, 19]. In this study, each tissue of the simulated rats has its electrical properties at the operating frequencies. The average whole-body SAR values at 900 and 2100 MHz were 1.159 and 0.16 W/kg, respectively. Besides the SAR values for the thymus at 900 and 2100 MHz were on the average of 1.134 and 0.086 W/kg, respectively. Before and after all experimental sessions, the body temperatures of rats were monitored by rectal measurements. The RFR exposure did not lead to any rectal temperature raise.

Tissue Sample Obtaining and Processing

Thymus tissues were fixed by 10% formaldehyde solution. Tissues were processed through graded alcohols and xylene, then embedded in paraffin. Sections were cut 5-7 μm using a rotary microtome (Leica, Nussloch, Germany) and taken on Superfrost slides.

Immunohistochemistry

Prepared sections were stained immunohistochemically for GRP78, CHOP, Caspase 12, and Clv. Caspase3 proteins. Protein localization and levels of expressions were evaluated.

Thymus tissue samples were deparaffinized in xylene and dehydrated in ethanol series. Slides were boiled at 665 W microwave and waited 25 minutes at microwave's warm position, then kept at room temperature (RT) for 20 minutes in Tris-EDTA solution(ph = 9.0). They were incubated in 3% hydrogen peroxide (Sigma Aldrich18312), made with methanol, to block endogen peroxidase activity and washed with phosphate-buffer-saline (PBS). Then nonspecific binding sites were blocked with UV Blocking Solution (#TA-125-UB; Thermo Scientific/Lab Vision). Primary antibodies, prepared with an antibody diluent solution, were incubated for one night at +4oC GRP78 1/400 (ab21685; Abcam), CHOP :1/50 (ab11419; Abcam), Caspase 12 1/50 (ab62463; Abcam), and Clv. Caspase3 1/50 (9664; Cell Signaling) . After washing, secondary antibodies were incubated for 45 minutes at RT. They have washed again and incubated with streptavidin (TS-125-HR; Thermo Scientific/Lab Vision) for 30 minutes at RT. After washing, antibodies were identified with DAB(8050S; Cell Signalling) and then again washed. After Mayer's hematoxylin staining(Merck) and dehydration, mounting was done with Entellan (#1.07961.0100; Merck). The immunolocalization of the ER stress proteins was examined with bright-field microscopy (Zeiss). We have analyzed expression levels of proteins by using ImageJ software.

Statistical Analysis

Immunohistochemical stainings were analyzed by using Image J. The data were evaluated by using

Sigma Stat 3.5 One Way ANOVA/post hoc Tukey test. Values with $p < 0.05$ were considered statistically significant. The results were presented as mean ± SEM.

RESULTS

GRP78, an important protein of the UPR, increased in 900MHz-1 w relative to the Sham-1 w. But this increase was just in the cytoplasm of epithelial reticular cells. This increase was both at epithelial reticular cells and thymocytes in the 900MHz-10 w, 2100MHz-1 w, and 10 w groups (Fig. 2). Exposure to RFR can induce ER stress in thymocytes which have an important role in the immune system. Additionally, exposure duration of RFR and frequency of RFR can change this effect (Fig. 3).

If cells cannot handle ER stress, CHOP levels will increase, and CHOP induces proapoptotic genes and by the way apoptosis. In 10-w groups, an increase of CHOP expression was observed, and this increase was in the nuclear membrane and cytoplasm. Caspase12 showed a significant increase depending on RFR exposure and frequency levels. CHOP and Caspase12

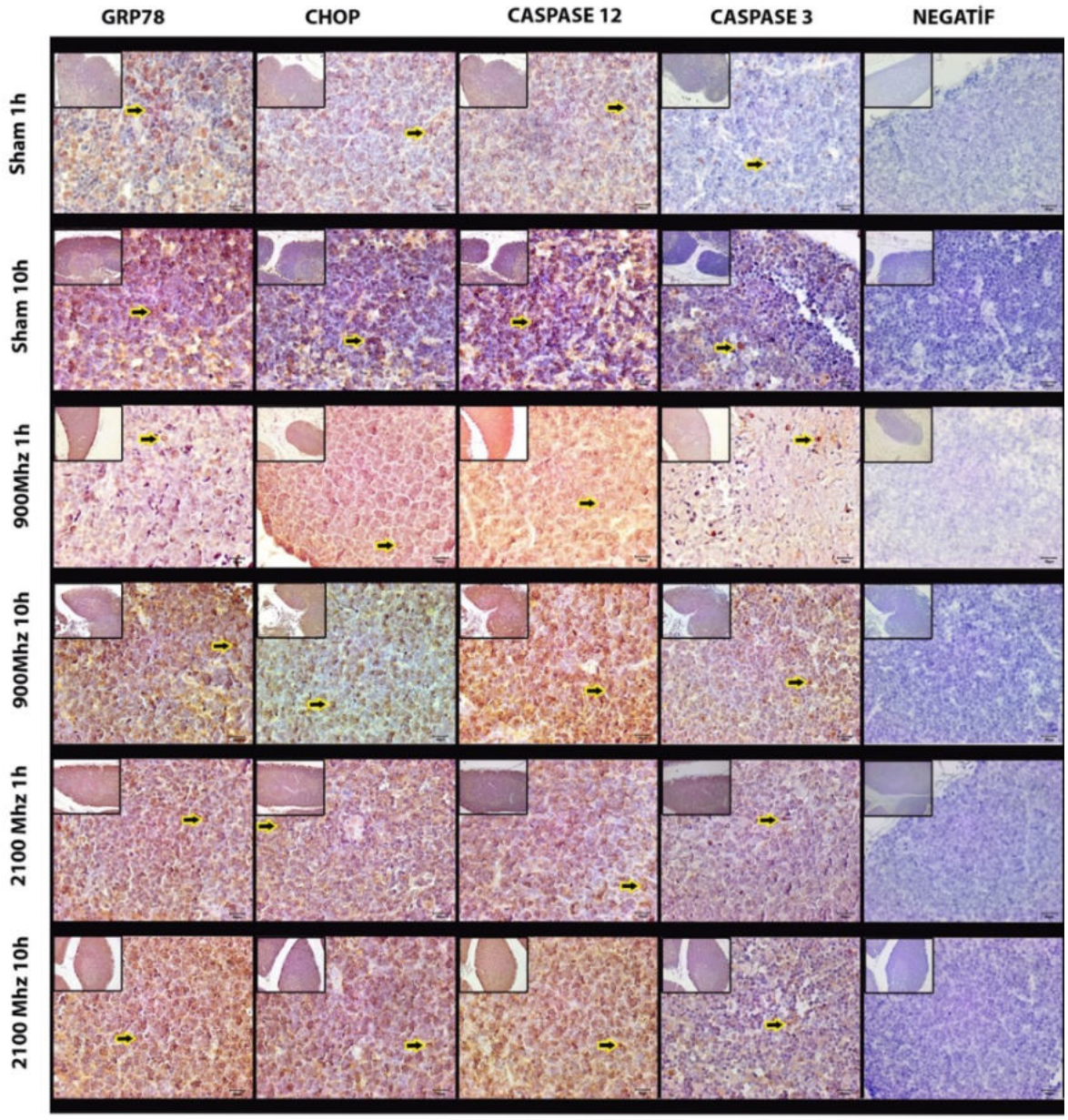

Fig. 2. Hematoxylin staining for all groups and immunohistochemistry staining for GRP78, CHOP, Clv. Caspase3 and Caspase12,10× and 40×, bar 50µm.

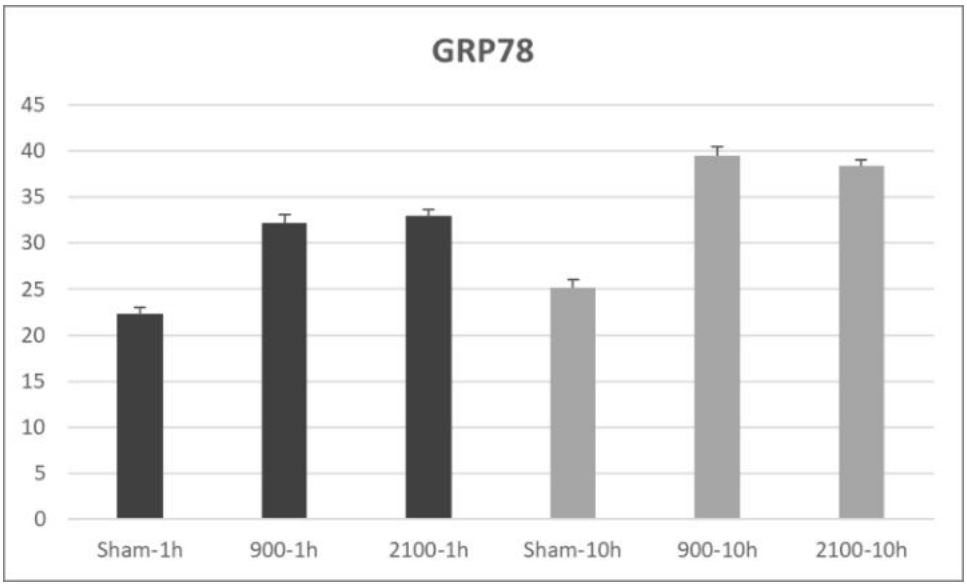

Fig. 3. ImageJ analyze for the immunohistrochemistry staining GRP78. 900 MHz 10 w vs. Sham 1 w, 900 MHz 10 w vs. Sham 10 w, 900 MHz 10 w vs. 900 MHz 1 w, 900 MHz 10 w vs. 2100 1 w, 2100 MHz 10 h vs. Sham 1 w, 2100 MHz 10 h vs. Sham 10 w, 2100 MHz 10 w vs. 900 MHz 1 w, 2100 MHz 10 w vs. 2100 1 w, 2100 1 w vs. Sham 1 w, 2100 MHz 1 w vs. Sham 10 w, 900 MHz 1 w vs. Sham 1 w and 900 MHz 1 w vs. Sham 10 w ($p < 0.001$).

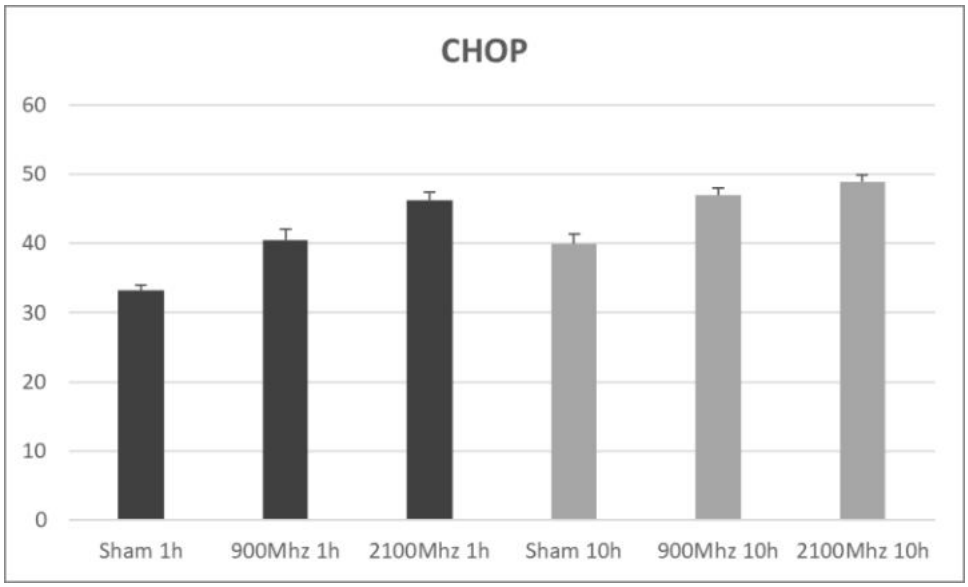

Fig. 4. ImageJ analyze for the immunohistrochemistry staining CHOP. 10 w vs. Sham 1 w, 2100 MHz 10 w vs. Sham 10 w, 2100 MHz 10 w vs. 900 MHz 1w, 900 Mhz 10 w vs. Sham 1 w and 2100 MHz 1w vs. Sham 1 w ($p < 0.001$), 900 MHz 10 w vs. Sham 10 w ($p = 0.004$), 900 MHz 10 w vs. 900 MHz 1 w ($p = 0.008$), 900 MHz 10 w vs. 2100 MHz 1 w ($p = 0.999$), 2100 MHz 1 w vs. Sham 10 w ($p = 0.01$), 2100 MHz 1 w vs. 900 MHz 1 w ($p= 0.02$), 900 MHz 1 w vs. Sham 1 w ($p = 0.003$), Sham 10 w vs. Sham 1 w ($p = 0.007$).

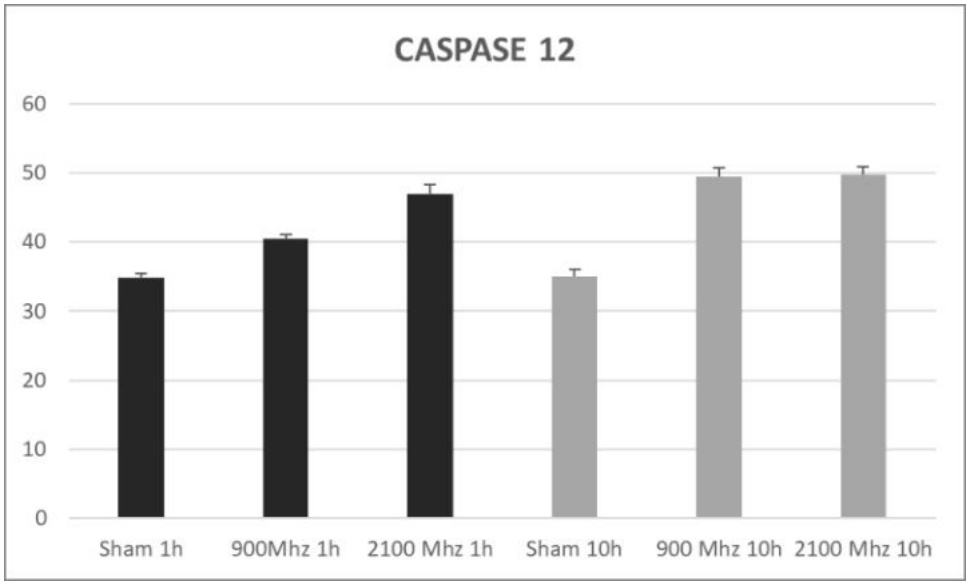

Fig. 5. ImageJ analyze for the immunohistrochemistry staining Caspase 12. 2100 MHz 10 w vs. Sham 1 w, 2100 MHz 10 w vs. Sham 10 w, 2100 MHz 10 w vs. 900 MHz 1 w, 900 MHz 10 w vs. Sham 1 w, 900 MHz 10 w vs. Sham 10 w, 900 MHz 10 w vs. 900 MHz 1 w, 2100 MHz 1 w vs. Sham 1 w and 2100 MHz 1 w vs. Sham 10 w ($p < 0.001$), 2100 MHz 1 w vs. 900 MHz 1 w ($p = 0.001$), 900 MHz 1 w vs. Sham 1 w ($p = 0.006$), 900 MHz 1 w vs. Sham 10 w ($p = 0.009$).

levels were also high in sham groups, but the increases in RFR groups were significantly higher (Figs 2, 4 and 5).

Clv. Caspase 3 levels were higher in RFR groups than sham groups. But the change of frequency did not affect meaningfully Clv. Caspase 3 levels. Interestingly, the expression of Clv. Caspase 3 in 1w RFR groups were higher than 10w RFR groups (Fig. 6). These three markers were observed in epithelial reticular cells and thymocytes (Fig. 2).

DISCUSSION

With the developing technology, RFR has become an important part of human life. Unfortunately, the effects of RFR on ER stress and apoptosis have not been clarified yet. There are too many studies about the effects of RFR, in different frequency levels, on the organism. We will discuss here only the studies about exposure to 900 MHz and 1950-2450 MHz RFR. We have investigated the effects of RFR on ER stress and apoptosis in rat thymus in our study.

As known oxidative stress is a possible trigger of ER stress and apoptosis. There are many studies on different tissues about this subject. In a study on the sciatic nerve of adolescent rats, 900MHz RFR increased MDA, SOD, and catalase (CAT) levels and also, induced apoptosis [11]. 1950 MHz RFR exposure for 48h, not 12 or 24 h, could induce apoptosis on rat astrocytes according to Liu *et al*. [20]. In another study, no difference was detected between 900 MHz 0 h and 24 h groups of rats [21]. As can be seen, exposure duration is important for the effects of RFR. There are also several studies in the urogenital system. There are studies for male or female rats, prenatal or natal exposure, different exposure durations, and frequencies. These studies [22-33] demonstrated that RFR could induce oxidative stress and apoptosis in testis and kidney tissues. Further in another study, 900 MHz RFR increased oxidative stress in the ovary [34]. In different studies on the prenatal and adolescent rats, exposure to 900 MHz RFR caused oxidative stress [35, 36]. Exposure to 2450 MHz RFR caused oxidative stress to the heart, but there was no difference in the blood tissue [37].

As for the immune system, studies on the liver suggested that RFR could induce oxidative stress and decrease antioxidant levels in the rat liver [38-40]. Ohtani et al. [41]. exposed the rats to 2.14GHz RFR

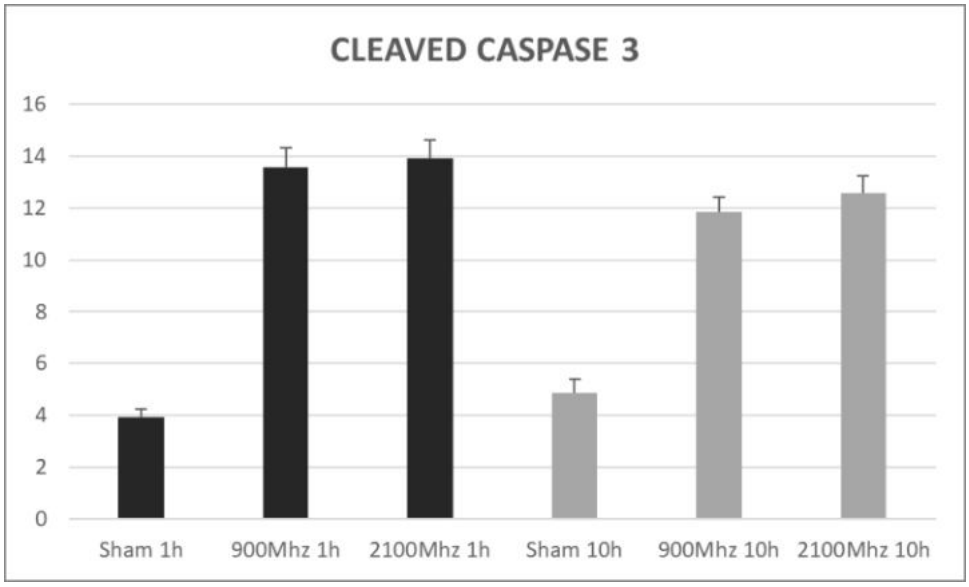

Fig. 6. ImageJ analyze for the immunohistrochemistry staining Clv. Caspase 3. 2100 MHz 1 w vs. Sham 1 w, 2100 MHz 1 w vs. Sham 10 w, 900 MHz 1 w vs. Sham 1 w, 900 Mhz 1 w vs. Sham 10 w, 2100 MHz 10 w vs. Sham 1 w, 2100 MHz 10 w vs. Sham 10 w, 900 MHz 10 w vs. Sham 1 w and 900 MHz 10 w vs. Sham 10 w ($p < 0.001$).

for 9 weeks which was included in utero, lactation, and juvenile period. They presented IL4-IL5 and IL23a genes in the thymus and just IL5 gene in spleen up-regulated. But there was no effect on the growth of T cells [38, 41]. In another study, rats were exposed to 900 MHz RFR during the prenatal period. In this study increased MDA levels, decreased GSH levels, and not changed SOD levels were found in the exposed thymus. Also for the spleen, increased MDA and GSH, but decreased SOD levels were found [9]. For the natal exposure in the thymus, researchers found high MDA levels in exposed groups (900MHz) [10]. Exposure to 900 MHz RFR in mature or immature rats causes increased SOD levels, decreased CAT-GPx activity, and GSH levels in all lymphoid organs according to Aydin et al. [8]. Misa-Agustino et al. [12]. applied 2.45 GHz (0-1-1.5-12-Watt power) RFR to rats and studied on thymuses. In this study heat shock protein (HSP)90 decreased in only 12W exposed animals and there were no differences in HSP70 levels. Also, in exposed rats, glucocorticoid receptors presented more than control groups [12].

In the mentioned studies, researchers used 900 MHz or 1950-2100-2450 MHz RFR like our study. The exposure durations and frequencies were different in these studies and findings could be different due to this. As shown, exposure to 900-2100 MHz RFR, prenatal or natal, can cause oxidative stress and apoptosis in different tissues. But in some studies, it was found that there is no specific effect of RFR [21, 38, 39]. In our study, rats were exposed to RFR at least 1w (2h/day). Caspase12 and Clv. Caspase3 were evaluated for apoptosis, GRP78 and CHOP were evaluated for the oxidative stress relevant to ER stress. Exposure to 900-2100 MHz RFR for 1 w or 10 w increased CHOP and GRP78 expressions in the thymus (for CHOP: 2100/Sham 10 w; $p < 0.001$, 900/Sham 10 w; $p = 0.004$, 900/Sham 1 w; $p = 0.003$ and for GRP78; $p < 0.001$). Also, these exposures increased Clv. Caspase 3 and Caspase 12 expressions (Caspase 12: 900 MHz 1 w/Sham 1 w; $p = 0.006$, 900 MHz 10 w/Sham 10 w, $p = 0.01$, 2100 Mhz/Sham; $p < 0.001$ and cl Caspase 3; $p < 0.001$).

CONCLUSION

We demonstrated that exposure to RFR -acute or chronic, 900 MHz or 2100 MHz- causes ER stress and apoptotic markers in rat thymus. These findings are compatible with the literature. The effects of RFR on the thymic cells of rats, one important part of the im-

mune system, suggest that RFR may cause various pathological diseases and weaken our immunity via cell death.

Authors' Contribution

Study Conception: ErK, EsK; Study Design: ErK, EsK, HE, AOO, ŞÖ, ND; Supervision: EsK, ND; Funding: TUBITAK; Materials: ErK, EsK, HE, AOO, ŞÖ, ND; Data Collection and/or Processing: ErK, EsK, HE, AOO, ŞÖ, ND; Statistical Analysis and/or Data Interpretation: ErK, EsK; Literature Review: ErK, EsK; Manuscript Preparation: ErK, EsK and Critical Review: ErK, EsK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Comparison of the performances of parametric k-sample test procedures as an alternative to one-way analysis of variance

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ABSTRACT

Objectives: The performances of the Welch test, the Alexander-Govern test, the Brown-Forsythe test and the James Second-Order test, which are among the parametric alternatives of one-way analysis of variance and included in the literature, to protect the Type-I error probability determined at the beginning of the trial at a nominal level, were compared with the F test.

Methods: Performance of the tests to protect Type-I error; in cases where the variances are homogeneous and heterogeneous, the sample sizes are balanced and unbalanced, the distribution of the data is in accordance with the normal distribution and the log-normal distribution, how it is affected by the change in the number of groups to be compared has been examined on simulation scenarios.

Results: The Welch test, the Alexander-Govern test and the James Second-Order test were not affected by the distribution and performed well in situations where variances were heterogeneous. The Brown-Forsythe test was not affected by the distribution, it performed well when the variance was homogeneous and the sample size in the groups to be compared was not equal.

Conclusions: The Welch test, the Alexander-Govern test and the James Second-Order test are the tests that can be recommended as an alternative to the F test.

Keywords: Analysis of variance, conformity of normal distribution, parametric k-sample tests

Data analysis methods that will be allied to the data obtained from research with at least interval scale; variance varies according to sample size, distribution of data, and the number of groups to be compared. One of the most critical steps of statistical data analysis is to decide whether the test procedure to be used to analyze the data will be a parametric or non-parametric test. Parametric tests are statistical methods that require data to be measured on an interval or ratio scale, which can be applied due to certain assumptions. Non-parametric test procedures are alternatively preferred when the necessary assumptions are not met for performing parametric tests.

One-way analysis of variance (ANOVA) or F-test, which is a parametric test, is used to compare the mean of more than two populations and is one of the most frequently used and most important statistical methods for this purpose [1]. The assumptions for the F test include that the data is normally distributed, the sample variances are equal, and the samples are independent [2].

Pearson [3], Glass *et al*. [4], and Wilcox [5] exam-

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ined the effect of the normality assumption violation on the Type-I error. Wilcox [5] concluded that samples that do not conform to normal distribution have some impact on the Type-I error rate, but the effect is minimal if the variances are homogeneous. Glass *et al*. [4] reported similar results to Wilcox [5] in their studies if the variances were homogeneous. In his study, Buning [6] examined the performances of the Kruskal-Wallis test, the normal score test and the Welch test, which he included as an alternative to the F test and the F test, in terms of Type-I error and power. He evaluated the performances of the tests under various simulation scenarios in terms of whether the variances are homogeneous or not in equal and unequal sample sizes if the data show normal distribution or not. In his study, Moder [2] stated that the location parameters of the groups should be investigated in detail when there are unbalanced sample sizes.

In our study, we compared the performances of the Welch test, the Alexander-Govern test, the Brown-Forsythe test, the James Second-Order test, which are among the parametric alternatives of the F test, to protect Type-I error under various simulation scenarios.

Table 1. Sample sizes of the groups

Number of groups	Balanced Sample	Non-balanced Sample		
		Observation combinations where the number of sample sizes are not equal	Observation combinations where the number of sample sizes differs excessively	Observation combinations with inverse matching between variance and number of sample sizes
3	3:3:3 5:5:5 10:10:10 15:15:15 20:20:20 25:25:25 30:30:30 50:50:50 80:80:80 100:100:100	3:5:7 5:10:15 20:25:30 50:60:70 65:75:85 70:90:100	3:25:30 3:80:80 5:20:100	7:5:3 15:10:5 30:25:20 70:60:50 85:75:65 100:90:70
5	3:3:3:3:3 5:5:5:5:5 10:10:10:10:10 15:15:15:15:15 20:20:20:20:20 25:25:25:25:25 30:30:30:30:30 50:50:50:50:50 80:80:80:80:80 100:100:100:100:100	3:5:7:9:11 5:7:9:12:15 20:22:24:28:30 50:55:60:65:70 55:65:75:85:95 60:70:80:90:100	3:20:25:80:100 3:5:30:80:100 5:10:20:25:80 3:5:10:15:100	7:5:3 15:10:5 30:25:20 70:60:50 85:75:65 100:90:70
8	3:3:3:3:3:3:3:3 5:5:5:5:5:5:5:5 10:10:10:10:10:10:10:10 15:15:15:15:15:15:15:15 20:20:20:20:20:20:20:20 25:25:25:25:25:25:25:25 30:30:30:30:30:30:30:30 50:50:50:50:50:50:50:50 80:80:80:80:80:80:80:80 100:100:100:100:100:100:100:100	3:5:7:9:11:12:14:15 20:22:24:25:26:28:29:30 50:55:60:65:70:75:80:85 60:65:75:80:85:90:95:100	3:5:10:20:25:30:80:100 5:10:20:20:25:80:90:100 3:5:10:80:80:90:100:100 20:25:30:80:90:90:100:100	15:14:12:11:9:7:5:3 30:29:28:26:25:24:22:20 85:80:75:70:65:60:55:50 100:95:90:85:80:75:65:60

METHODS

In our study, the Welch test, the Alexander-Govern test, the Brown-Forsythe test, the James Second-Order test in terms of maintaining the probability of Type-I error determined at the beginning of the experiment were compared with the F test. Simulation scenarios run under the R program [7].

The performance of the tests was evaluated as a result of comparisons between three, five, and eight groups for simulation scenarios involving balanced/non-balanced sample sizes (Table 1), normal distribution or log-normal distribution, homogenous or heterogeneous variances (Table 2). In addition to the specified simulation conditions, observation combinations are also included, where the number of units varies excessively among the group with higher variance is assigned a lower number of observations, and the group with a lower variance is assigned a higher number of observations and inverse matching between variance and sample size.

In comparisons made to determine Type-I error, group means were taken equally. The Type-I error probabilities for each of the simulation scenarios were obtained after the numbers of H0 hypotheses were determined, which were rejected at the end of 50000 repetitions. In our study, the evaluation criterion proposed by Peterson [8] was adopted and it was concluded that the performance of the tests with a probability of the Type-I error between 4.49% and 5.49% was sufficient to maintain Type-I error.

Table 2 shows the variance rates of the groups that are suitable for normal distribution and the scale parameter values of the groups that are suitable for log-normal distribution.

The F Test

One-way analysis of variance (ANOVA) or F-test is used to compare the mean of more than two populations. It is one of the most important and frequently used methods of applied statistics [1]. The null hypothesis H_0: $\mu_1=\mu_2=\ldots=\mu_k$ versus alternative H_1: at least one μi (i= 1, 2, . . ., k) is different. The F test statistic,

$$F = \frac{\sum_{i=1}^{k} n_i(X_{i.}-\bar{X}_{..})^2/(k-1)}{\sum_{i=1}^{k}\sum_{j=1}^{n_i}(X_{ij}-\bar{X}_{i.})^2/(N-k)} \sim F_{1-\alpha;k-1;N-k} \quad (1)$$

In Equation, k is the number of groups, N is the total number of observations, X_{ij} is the *j*th observation (j = 1, 2, . . ., *ni*) in the *i*th group (i = 1, 2, . . . , *k*) , $N = \Sigma\, n_i$, $\bar{X}_{..}$ is the overall mean, $\bar{X}_{i.}$ is the sample mean for the *i*th group. The F is more powerful if the assumptions of normality and variance homogeneity hold. The null hypothesis, H: $\mu_1=\mu_2=\ldots=\mu_k$, should then be rejected at the α level of significance when $F \geq F_{1-\alpha;k-1,N-k.}$

The Welch Test

The Welch test is a robust test against the violation of the assumption of variance homogeneity, which is considered as an alternative to the F test [9]. The null hypothesis H_0:$\mu_1=\mu_2=\ldots=\mu k$ versus alternative H_1: at least one μ_i (i= 1, 2, . . ., k) is different. The formula for the Welch test is

$$F_W = \frac{\sum_{i=1}^{k} w_i(\bar{x}_i-\hat{\mu})^2/(k-1)}{1+[2(k-2)/k^2-1]\sum h_i} \quad (2)$$

where

$$(w_i) = n_i/s_i^2;\ \hat{\mu} = \sum_{i=1}^{k} w_i x_i/W;\ W = \sum_{i=1}^{k} w_i;\ \ h_i = (1-w_i/W)^2/(n_i-1)$$

The null hypothesis should then be rejected at the α level of significance when $F_W > F_{\alpha;k-1,\,f.}$

The Alexander Govern Test

The Alexander-Govern (AG) test is another alternative to the F test developed by Alexander and Govern [10]. This test is used when the sample sizes in the groups are not equal. It is a parametric test that can be used instead of the F test if the data conform to normal distribution. To calculate the test statistic, the t statistic is first calculated for each group.

$$t_i = \frac{\bar{X}_i - X^+}{S_{\bar{X}_i}} \quad (3)$$

where

$$S_{\bar{X}_i} = \sqrt{\frac{\sum_{j=1}^{n_i}(X_j-\bar{X}_i)^2}{n_i(n_i-1)}};\ W_i = \frac{1/S_i^2}{\sum_{i=1}^{k}(1/S_i^2)};\ X^+ = \sum_{i=1}^{k} W_i\bar{X}_i.$$

Calculated t values are converted to the standard normal distribution Z using the normalization ap-

Table 2. Variance rates of groups (k)

Number of groups	Normal distribution		Log- normal distribution	
	Homogeneous variance	Heterogeneous variance	Homogeneous scale parameter (b)	Heterogeneous scale parameter (b)
3	1:1:1 2:2:2 4:4:4 8:8:8 10:10:10	1:1:2 1:2:2 1:1:4 1:4:4 1:1:8 1:8:8 1:1:10 1:10:10 1:4:8 2:1:1 2:2:1 4:1:1 4:4:1 8:1:1 8:8:1 10:1:1 10:10:1 8:4:1	0.1:0.1:0.1 0.2:0.2:0.2 0.3:0.3:0.3 0.4:0.4:0.4 0.5:0.5:0.5 0.6:0.6:0.6 0.7:0.7:0.7 0.8:0.8:0.8	0.10:0.10:0.20 0.10:0.20:0.20 0.10:0.30:0.50 0.10:0.40:0.50 0.10:0.10:0.50 0.10:0.50:0.60 0.10:0.60:0.80 0.20:0.10:0.10 0.20:0.20:0.10 0.50:0.30:0.10 0.50:0.40:0.10 0.50:0.10:0.10 0.60:0.50:0.10 0.80:0.60:0.10
5	1:1:1:1:1 2:2:2:2:2 4:4:4:4:4 8:8:8:8:8 10:10:10:10:10	1:1:2:2:2 1:1:4:4:4 1:1:8:8:8 1:1:10:10:10 1:2:4:8:10 2:2:2:1:1 4:4:4:1:1 8:8:8:1:1 10:10:10:1:1 10:8:4:2:1	0.1:0.1:0.1:0.1:0.1 0.2:0.2:0.2:0.2:0.2 0.3:0.3:0.3:0.3:0.3 0.4:0.4:0.4:0.4:0.4 0.5:0.5:0.5:0.5:0.5 0.6:0.6:0.6:0.6:0.6 0.7:0.7:0.7:0.7:0.7 0.8:0.8:0.8:0.8:0.8	0.1:0.1:0.2:0.2:0.2 0.1:0.1:0.4:0.4:0.4 0.1:0.1:0.5:0.5:0.5 0.1:0.1:0.6:0.7:0.8 0.1:0.3:0.5:0.7:0.8 0.2:0.2:0.2:0.1:0.1 0.4:0.4:0.4:0.1:0.1 0.5:0.5:0.5:0.1:0.1 0.8:0.7:0.6:0.1:0.1 0.8:0.7:0.5:0.3:0.1
8	1:1:1:1:1:1:1:1 2:2:2:2:2:2:2:2 4:4:4:4:4:4:4:4 8:8:8:8:8:8:8:8 10:10:10:10:10:10:10:10	1:1:1:1:1:1:1:2 1:1:1:1:1:1:1:4 1:1:1:1:1:1:1:8 1:1:1:1:1:1:1:10 1:1:1:2:2:2:4:4 1:1:1:1:4:4:4:4 1:1:1:1:8:8:10:10 2:1:1:1:1:1:1:1 4:1:1:1:1:1:1:1 8:1:1:1:1:1:1:1 10:1:1:1:1:1:1:1 4:4:2:2:2:1:1:1 4:4:4:4:1:1:1:1 10:10:8:8:1:1:1:1 10:10:8:8:4:4:2:1	0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.2:0.2:0.2:0.2:0.2:0.2:0.2:0.2 0.3:0.3:0.3:0.3:0.3:0.3:0.3:0.3 0.4:0.4:0.4:0.4:0.4:0.4:0.4:0.4 0.5:0.5:0.5:0.5:0.5:0.5:0.5:0.5 0.6:0.6:0.6:0.6:0.6:0.6:0.6:0.6 0.7:0.7:0.7:0.7:0.7:0.7:0.7:0.7 0.8:0.8:0.8:0.8:0.8:0.8:0.8:0.8	0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.2 0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.3 0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.5 0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.7 0.1:0.1:0.1:0.3:0.3:0.3:0.5:0.5 0.1:0.1:0.1:0.1:0.6:0.6:0.8:0.8 0.2:0.3:0.4:0.5:0.6:0.7:0.7:0.8 0.2:0.2:0.2:0.4:0.4:0.8:0.8:0.8 0.2:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.3:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.5:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.7:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.5:0.5:0.3:0.3:0.3:0.1:0.1:0.1 0.8:0.8:0.6:0.6:0.1:0.1:0.1:0.1 0.8:0.7:0.7:0.6:0.5:0.4:0.3:0.2 0.8:0.8:0.8:0.4:0.4:0.2:0.2:0.2

proach [11].

$$Z_i = c + \frac{(c^3+3c)}{b} - \frac{(4c^7+33c^5+240c^3+855)}{(10b^2+8bc^4+1000b)} \quad (4)$$

where

$a_i = v_i - 0.5, b = 48a^2, c = \left[a * ln\left(1 + \frac{t_i^2}{v_i}\right)\right]^{\frac{1}{2}}$ and $v_i = n_i - 1$.

The null hypothesis H_0:$\mu_1=\mu_2=\ldots=\mu_k$ versus alternative H_1: at least one μ_i (i= 1, 2, . . ., k) is different. The test statistic is calculated as [10],

$$AG = \sum_{i=1}^{k} Z_i^2 \quad (5)$$

The null hypothesis should then be rejected at the α level of significance when AG > X_{k-1}^2.

The James Second-Order Test

The James Second-Order (JSO) test, developed by James [12] as an alternative to the F test, is a robust test against violating the assumption of variance homogeneity. To calculate the test statistic, the t statistic is first calculated for each group.

$$t_i = \frac{\bar{X}_i - \bar{Y}}{S_i^2} \quad (6)$$

where

$$\bar{Y} = \sum_{i=1}^{k} a_i \bar{X}_i, a_i = \frac{1/S_i^2}{\sum_{i=1}^{k}(1/S_i^2)}.$$

The null hypothesis H_0: $\mu_1=\mu_2=\ldots=\mu_k$ versus alternative H1: at least one μ_i (i= 1, 2, . . ., k) is different. The test statistic (J) is calculated as [9],

$$J = \sum_{i=1}^{k} t_i^2 \quad (7)$$

The null hypothesis should then be rejected at the α level of significance when J > CV_α. The test statistic, J, is compared to a critical value, CV_α, where

$$CV_\alpha = C + \frac{1}{2}(3\chi_4 + \chi_2)T + \frac{1}{16}(3\chi_4 + \chi_2)^2\left(1 - \frac{I-3}{C}\right)T^2 + \frac{1}{2}(3\chi_4 + \chi_2)\left[(8R_{23} - 10R_{22} + 4R_{21} - 6R_{12}^2 + 8R_{12}R_{11} - 4R_{11}^2) + (2R_{23} - 4R_{22} + 2R_{21} - 2R_{12}^2 + 4R_{12}R_{11} - 2R_{11}^2)(\chi_2 - 1) + \frac{1}{4}(-R_{12}^2 + 4R_{12}R_{11} - 2R_{12}R_{10} - 4R_{11}^2 + 4R_{11}R_{10} - R_{10}^2)(3\chi_4 - 2\chi_2 - 1)\right] + (R_{23} - 3R_{22} + 3R_{21} - R_{20})(5\chi_6 + 2\chi_4 + \chi_2) + \frac{3}{16}(R_{12}^2 - 4R_{23} + 6R_{22} - 4R_{21} + R_{20})(35\chi_8 + 15\chi_6 + 9\chi_4 + 5\chi_2) + \frac{1}{16}(-R2_{22} + 4R_{21} - R_{20} + 2R_{12}R_{10} - 4R_{11}R_{10} - 4R_{11}R_{10} + R_{10}^2) \times (9\chi_8 - 3\chi_6 - 5\chi_4 - \chi_2) + \frac{1}{4}(-R_{22} + R_{11}^2)(27\chi_8 + 3\chi_6 + \chi_4 + \chi_2) + \frac{1}{4}(R_{23} - R_{12}R_{11})(45\chi_8 + 9\chi_6 + 7\chi_4 + 3\chi_2) \quad (8)$$

with C denoting the 1 − α quantile of a X_{k-1}^2 distribution and with

$$T = \sum_{i=1}^{k} \frac{(1-a_i)^2}{n_i - 1}, \chi_{2s} = \frac{X_{k-1,\alpha}^{2s}}{[(k-1)(k+1)\ldots(k+2s-3)]}, R_{st} = \sum_{i=1}^{k} \frac{a_i^t}{(n_i-1)^2}.$$

The JSO test was accepted as the best option for both data with normal distribution, heterogeneous variance [10], and situations that do show the non-normal distribution and heterogeneous variance [13]. The disadvantage of this method is the complexity of the computation of critical values [14].

The Brown-Forsythe Test

One of the parametric alternatives to the F test is the Brown-Forsythe test. It is a robust test if the sample size is small, the population heterogeneous variance, and the normality assumption is provided. The null hypothesis H_0: $\mu_1=\mu_2=\ldots=\mu_k$ versus alternative H1: at least one μ_i (i= 1, 2, . . ., k) is different. The test statistic is calculated as [15],

$$BF = \frac{\sum_{i=1}^{k} n_i(\bar{X}_i - \bar{X})^2}{\sum_{i=1}^{k}(1-n_i/N)(S_i)^2} \quad (9)$$

The null hypothesis, H_0: $\mu_1=\mu_2=\ldots=\mu_k$, should then be rejected at the α level of significance when F > $F_{\alpha;k-1,\,f.}$

BF statistic has an approximately F distribution with *k-1* and *f* degrees of freedom, where *f* is obtained with

$$f = \left(\sum_{i=1}^{k} \frac{C_i^2}{n_i - 1}\right)^{-1} \quad (10)$$

C_i used in calculating degrees of freedom f is calculated with the Satterthwaite [16] approach.

$$C_i = \frac{\left(1-\frac{n_i}{N}\right)s_i{}^2}{\left[\sum_{i=1}^{k}\left(1-\frac{n_i}{N}\right)s_i{}^2\right]} \quad (11)$$

RESULTS

In this study, the tests were compared with the help of simulation scenarios in terms of the Type-I error protection. Simulation scenarios were performed under the R program [7].

Comparisons in which sample size is balanced, the group variances are homogeneous, and the data follow to the normal distribution

Considering all simulation scenarios given in Table 3, it was observed that the F test and the JSO test were able to maintain the Type-I error level (α = 0.05) determined at the beginning. When *Supplementary* Table 1 is examined, it has been observed that the AG test can maintain the Type-I error level initially determined. The F test is the test that shows the most successful performance in estimating the Type-I error level determined at the beginning, according to the alternative parametric tests (*Supplementary* Table 2).

Comparisons in which the sample size is not balanced, the group variances are homogeneous, and the data follow to the normal distribution

The F test and the BF test are the tests that show the most successful performance in estimating the Type-I error level determined at the beginning. The F test tended to maintain the Type-I error in all simulation scenarios given in the tables. The BF test estimated the Type-I error level as deviant only in a simulation scenario (*Supplementary* Table 3, *Supplementary* Table 4, and *upplementary* Table 5). The F test was also not affected by excessive differences of sample size in groups and tended to maintain the Type-I error level initially determined in all simulation scenarios according to the Peterson criterion (*Supplementary* Table 6, *Supplementary* Table 7, and *Supplementary* Table 8).

Comparisons in which the sample size is balanced, group variances are heterogeneous, but the data follow to the normal distribution

As expected, when the simulation scenarios were examined according to the Peterson criterion, the F test was highly affected by distortion in-group variance and failed to maintain the Type-I error at a nominal level and gave highly deviant results. When the simulation scenarios given in *Supplementary* Table 9, *Supplementary* Table 10, and *Supplementary* Table 11 are examined, it is seen that the AG test is the best alternative to the F test. Among the other tests included in the study, the alternatives of the F test after the AG test in this trial can be seen as the Welch test and the JSO test.

Comparisons in which the sample size is not balanced, group variances are heterogeneous, but the data follow to the normal distribution

When the combinations of observations in which the sample size in the groups are not equal are examined (*Supplementary* Table 12, *Supplementary* Table 13, and *Supplementary* Table 14), it is seen that the AG test and Welch tests are the best alternative of the F test respectively. Although the performance of the JSO test is negatively affected by the increase in the number of groups compared, it can be seen as an alternative test after the AG test and the Welch test. When the simulation scenarios (*Supplementary* Table 15, *Supplementary* Table 16, and *Supplementary* Table 17) are examined, it has been seen that the tests included in the study generally give deviated results in terms of protecting the Type-I error, and their performance was not found sufficient. When the simulation scenarios in which the assumption of homogeneity of variances were not met, a lower number of observations was assigned to the group with high variance, and a higher number of observations was assigned to the group with a low variance (*Supplementary* Table 18, *Supplementary* Table 19, and *Supplementary* Table 20), it was seen that the Welch, the AG test and the JSO test were alternatives to the F test.

Comparisons in which the sample size is balanced, group variances are homogeneous, and the data follow to log-normal distribution

As expected, the F test is the test that shows the most successful performance to estimate the level of Type-I error determined at the beginning when considering the parametric alternatives available. The JSO test tends to preserve the Type-I error in all simulation scenarios in three group comparisons. The AG test

Table 3. Type-I error rates (%) for k=3 groups where $\sigma_1^2 : \sigma_2^2 : \sigma_3^2 = 1:1:1 \sim 10:10:10$, $\mu_1=\mu_2=\mu_3=0$, sample size is balanced ($n_1=n_2=n_3$)

σ^2	n	F	Welch	AG	JSO	BF
1	3	4.70%	3.44%	3.91%	4.99%	3.10%
	5	4.98%	4.47%	4.39%	4.94%	4.19%
	10	5.03%	4.92%	4.79%	5.08%	4.83%
	15	5.09%	5.11%	4.99%	5.20%	5.01%
	20	5.07%	5.04%	4.95%	5.11%	5.01%
	25	5.08%	5.09%	4.99%	5.12%	5.04%
	30	4.93%	4.90%	4.83%	4.92%	4.91%
	50	5.11%	4.94%	4.90%	4.95%	5.09%
	80	5.07%	5.06%	5.03%	5.07%	5.06%
	100	5.04%	5.06%	5.05%	5.06%	5.04%
2	3	4.82%	3.64%	3.65%	4.85%	2.92%
	5	4.93%	4.51%	4.45%	4.99%	4.12%
	10	4.88%	4.80%	4.66%	4.96%	4.68%
	15	5.03%	5.07%	4.97%	5.16%	4.95%
	20	4.80%	4.79%	4.69%	4.85%	4.74%
	25	5.15%	5.17%	5.09%	5.19%	5.12%
	30	5.03%	5.15%	5.04%	5.16%	5.01%
	50	4.93%	4.93%	4.89%	4.94%	4.93%
	80	4.99%	5.01%	4.99%	5.02%	4.99%
	100	5.00%	5.02%	5.00%	5.03%	4.99%
4	3	5.08%	3.90%	3.75%	4.84%	2.92%
	5	4.96%	4.36%	4.40%	4.93%	4.17%
	10	5.06%	4.99%	4.79%	5.07%	4.83%
	15	4.77%	4.70%	4.85%	5.02%	4.91%
	20	5.10%	5.13%	4.95%	5.11%	5.01%
	25	4.95%	4.91%	4.94%	5.07%	5.04%
	30	5.21%	5.20%	4.69%	4.78%	4.74%
	50	5.10%	5.06%	4.80%	4.86%	4.90%
	80	4.89%	4.88%	4.94%	4.97%	5.02%
	100	4.86%	4.83%	4.87%	4.92%	4.88%
8	3	4.97%	3.71%	3.78%	4.84%	3.00%
	5	4.78%	4.33%	4.32%	4.77%	3.98%
	10	5.07%	4.98%	4.85%	5.17%	4.87%
	15	5.15%	5.12%	4.99%	5.22%	5.06%
	20	4.90%	4.81%	4.73%	4.86%	4.86%
	25	5.01%	4.94%	4.87%	4.98%	4.98%
	30	5.00%	4.94%	4.89%	4.98%	4.97%
	50	5.08%	5.06%	5.02%	5.07%	5.07%
	80	5.21%	5.27%	5.25%	5.27%	5.20%
	100	5.09%	5.09%	5.07%	5.09%	5.09%
10	3	5.10%	3.80%	3.81%	5.03%	3.17%
	5	4.99%	4.49%	4.48%	4.92%	4.09%
	10	4.88%	4.73%	4.61%	4.88%	4.67%
	15	5.01%	4.88%	4.76%	4.99%	4.89%
	20	5.02%	4.92%	4.81%	4.99%	4.97%
	25	5.08%	5.00%	4.92%	5.05%	5.05%
	30	4.90%	4.90%	4.85%	4.93%	4.88%
	50	5.06%	5.00%	4.90%	4.96%	5.01%
	80	5.08%	5.09%	4.97%	5.02%	5.05%
	100	5.09%	5.12%	5.10%	5.12%	5.09%

tends to preserve the Type-I error in all simulation scenarios in five group comparisons (*Supplementary* Table 21, *Supplementary* Table 22, and *Supplementary* Table 23).

Comparisons in which the sample size is not balanced, group variances are homogeneous, and the data follow to log-normal distribution

When the simulation scenarios are examined, the F test and the BF test are the tests that show the most successful performance in estimating the Type-I error level determined at the beginning (*Supplementary* Table 24, *Supplementary* Table 25, and *Supplementary* Table 26). The F test tended to maintain the Type-I error in all simulation scenarios given in the tables. The BF test has given biased estimates in only two simulation scenarios in three group comparisons, in only one simulation scenario in five group comparisons. It tends to preserve Type-I error in all simulation scenarios for eight groups. It was observed that the other tests included in the study were negatively affected by the imbalance of the number of units in the groups, and their performance in maintaining the Type-I error level determined at the beginning was not considered sufficient. When the simulation scenarios involving observation combinations in which the sample size in the groups differ excessively (*Supplementary* Table 27, *Supplementary* Table 28, and *Supplementary* Table 29), it was observed that the Welch test, the AG test, the BF test, the JSO test were affected by the extreme differences in the sample size.

Comparisons in which the sample size is balanced, group variances are heterogeneous, and the data follow to log-normal distribution

The F test was highly affected by the deterioration of group variances and failed to maintain the Type-I error at the nominal level. Considering the performances determined according to Peterson criteria, it was seen that the AG test is the best alternative of the F test. Among the other tests included in the study, the alternatives of the F test in these simulation scenarios after the AG test can be accepted as the JSO test and the Welch test (*Supplementary* Table 30, *Supplementary* Table 31, and *Supplementary* Table 32).

Comparisons in which the sample size is not balanced, group variances are heterogeneous, and the data follow to log-normal distribution

When the simulation scenarios (*Supplementary* Table 33, *Supplementary* Table 34, and *Supplementary* Table 35) are examined, as expected, the F test was highly affected by the deterioration in group variances and failed to protect the Type-I error at the nominal level. Among the other tests included in the study, the alternatives of the F test in these simulation scenarios after the AG test can be accepted as the JSO test and the Welch test. When the simulation scenarios (*Supplementary* Table 36, *Supplementary* Table 37, and *Supplementary* Table 38) are examined, it has been seen that the tests included in the study generally give deviated results in terms of protecting the Type-I error, and their performance was not found sufficient. When the simulation scenarios (*Supplementary* Table 39, *Supplementary* Table 40, and *Supplementary* Table 41) are examined, the alternatives of the F test in these simulation scenarios after the AG test can be accepted as the JSO test and the Welch test respectively.

DISCUSSION

The F test is the test that shows the most successful performance as expected in cases where the conformity to the normal distribution and the homogeneity of the variances are provided. When the simulation scenarios where the assumption of homogeneity of variances are not met, as expected, the F test was highly affected by the deterioration in group variances and failed to maintain the Type-I error at the nominal level ($\alpha = 0.05$). The results of our study reach similar results to the studies conducted by Buning [6] and Moder [2]. It is the test that shows the most successful performance compared to other alternative tests in cases where the data conform to the log-normal distribution, and the variances are homogeneous. Blanca *et al*. [17], Clinch and Keselman [18], Gamage and Weerahandi [19], Lantz [20] and Schmider *et al*. [21] reported that the F test tends to protect the Type-I error in cases where the assumption of conformity to the normal distribution is violated. It was observed that the effect of violation of the homogeneity of variances on the performance of the F test was more than the violation of the assumption of conformity to normal distribution. Bishop and Dudewicz [22], Blanca *et al*. [17], Brown and Forsythe [23], Buning [6], Debeuck-

elaer [24], Lee and Ahn [25], Li *et al*. [26], Lu and Mathew [27], Markowski [28], Keselman *et al*. [29], Tomarken and Serlin [30] concluded that the F test is highly affected by the deterioration in group variances.

In this study, the Welch test, the AG test and the JSO test were not affected by the distribution of the data, and in cases where the variances were not homogeneous, they tend to protect the Type-I error. Penfield [31], Lix *et al*. [32] and Hartung *et al*. [33] found that the Welch test is not affected by the distribution of data and performs better in simulation scenarios where variances are heterogeneous. Bishop and Dudewicz [22], Brown and Forsythe [23], Buning [6], DeBeuckelaer [24], Keselman *et al*. [29], Markowski [28], Rafinetti [34], Tomarken and Serlin [30], Wilcox *et al*. [35] similar results in their work; They found that the Welch test performed better in cases where both assumptions were not provided. In their studies, Alexander and Govern [10], Myers [36], Oshima and Algina [13] concluded that the performance of the AG test was sufficient in terms of protecting Type-I error in cases where the data conformed to normal distribution, but the variance was not homogeneous. They stated that in cases where the assumption of conformity to normal distribution and homogeneity of variances is not realized, the sample size should be considered in order to use the AG test. Alexander and Govern [10] and Myers [36] stated that the JSO test is a good alternative to the F test when the distribution of the data is symmetrical and the assumption of homogeneity of variances is not met. Oshima and Algina [13] and Wilcox [37] found that the JSO test performed better in cases where both assumptions were not provided.

It has been concluded that the BF test shows an adequate performance in cases where the data show normal and log-normal distribution, the assumption of homogeneity of the variances is met, and the sample size in the groups to be compared are not equal. DeBeuckelaer [24], found that the BF test gives better results than the F test when one or both of the assumptions of normality and variance homogeneity cannot be achieved. Gamage and Weerahandi [19], Roth [38], Steel *et al*. [39], when group variances were not homogeneous, Wilcox *et al*. [35], stated that in cases where groups with large variances have small sample sizes, Oshima and Algina [13] stated that in cases where the homogeneity and normality assumption of variances cannot be achieved, the BF test can be used to make comparisons between groups.

CONCLUSION

As a result as stated in the literature, it was determined that the F test tends to maintain its robustness in case of violation of the normal distribution, however, it is more affected by the violation of the homogeneity assumption of variances. The Welch, the AG test and the JSO test are tests that can be recommended as an alternative to the F test because they are less affected by the sample size in the groups, the distribution of the data or the number of groups to be compared, if the homogeneity of the variances is neglected.

Authors' Contribution

Study Conception: GO; Study Design: GO; Supervision: GO; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ACM; Statistical Analysis and/or Data Interpretation: GO, ACM; Literature Review: ACM; Manuscript Preparation: GO, ACM and Critical Review: GO, ACM.

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Supplementary Tables 1 to 41

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The European Research Journal 2023;9(1):49-56 Original Article

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Intra-abdominal cystic lesions after ventriculoperitoneal shunting

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ABSTRACT

Objectives: Definitive diagnosis is essential for the medical and surgical management of pediatric patients with ventriculoperitoneal (VP) shunt. In patients with a VP shunt, abdominal complications have been well described, among which abdominal pseudo cysts are uncommon. In this report, we present our experience in terms of the multi-disciplinary management of intra-abdominal cystic lesions associated with the VP shunt procedure.

Methods: From 2016 to 2021, 245 VP shunt procedures were performed in our institution. Intra-abdominal cystic lesions were recorded as intra-abdominal complications (abdominal pseudocyst, intestinal subserosal bowel cyst, and scrotal cyst) in 3 patients. For these patients we retrospectively collected data on medical history, complaints, diagnosis, treatment procedure, and postoperative results. The study was performed on 2 male and 1 female patients. The average patient age was 11.6 months (5 months to 1.5 years). The most common complaint was that of abdominal distention with ileus symptoms. The average time of admission after the catheterization of VP shunt was 1 month; laparotomy was performed for 2 patients in whom treatment was needed for high ligation.

Results: A VP shunt operation is followed by abdominal complications in about 5%-47% of all cases. These complications are manifested as ileus symptoms, such as vomiting, abdominal distension, and abdominal pain with intestinal obstruction.

Conclusions: In pediatric patients with VP shunts, a shunt catheter-induced abdominal cystic formation should always be considered a complication. Management of these cystic lesions requires the use of a multi-disciplinary approach with neurosurgery and pediatric surgery for treatment.

Keywords: Intra-abdominal cyst, ventriculoperitoneal shunt, hydrocephalus

Placement of a ventriculoperitoneal (VP) shunt often includes complications and is the most commonly applied treatment for hydrocephalus. Shunt complications are noted in 45%–59% of all patients undergoing VP shunting [1]. The most frequently observed complications are mechanical failure, dysfunction, and infection [2]. Mechanical complications are malposition, blockage, and fracture of the shunt [2]. Infection is the most common complication, comprising 8-12% of all shunt complications. It occurs mostly within the first 6 months [2]. Other complications may also be present, including overdrainage, underdrainage, subdural hematoma, hemorrhage, obstruction, displacement, and abdominal complications [2].

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The peritoneum is the most common site for the placement of a VP shunt catheter. Complications include obstruction, peritoneal pseudocysts, ascites, bowel perforations, disconnection, infection and hernias [3]. Although these complications are rarely observed in patients (0.25%-10%), their symptoms and clinical manifestations (seizures, vomiting, abdominal pain, fewer and ileus) are very serious [3]. Under these conditions, a multi-disciplinary approach is required for managing the complications. As the management of intra-abdominal lesions for children is in the purview of the pediatric surgery department, with regard to the benefits in pediatric patients, the treatment protocol should be co-administrated by the neurosurgery and the pediatric surgery departments. The present study aims to critically discuss the management of intra-abdominal VP shunt complications in the context of the literature.

METHODS

For our research, approval was obtained from Bursa Yüksek İhtisas Training and Research Hospital, Clinical Research and Ethics Committee. (Decision number: 2011-KAEK-25 2021/02-20). During the period from 2016 to 2021, 247 patients underwent VP shunt catheterization at our institution (Fig. 1). Among these,

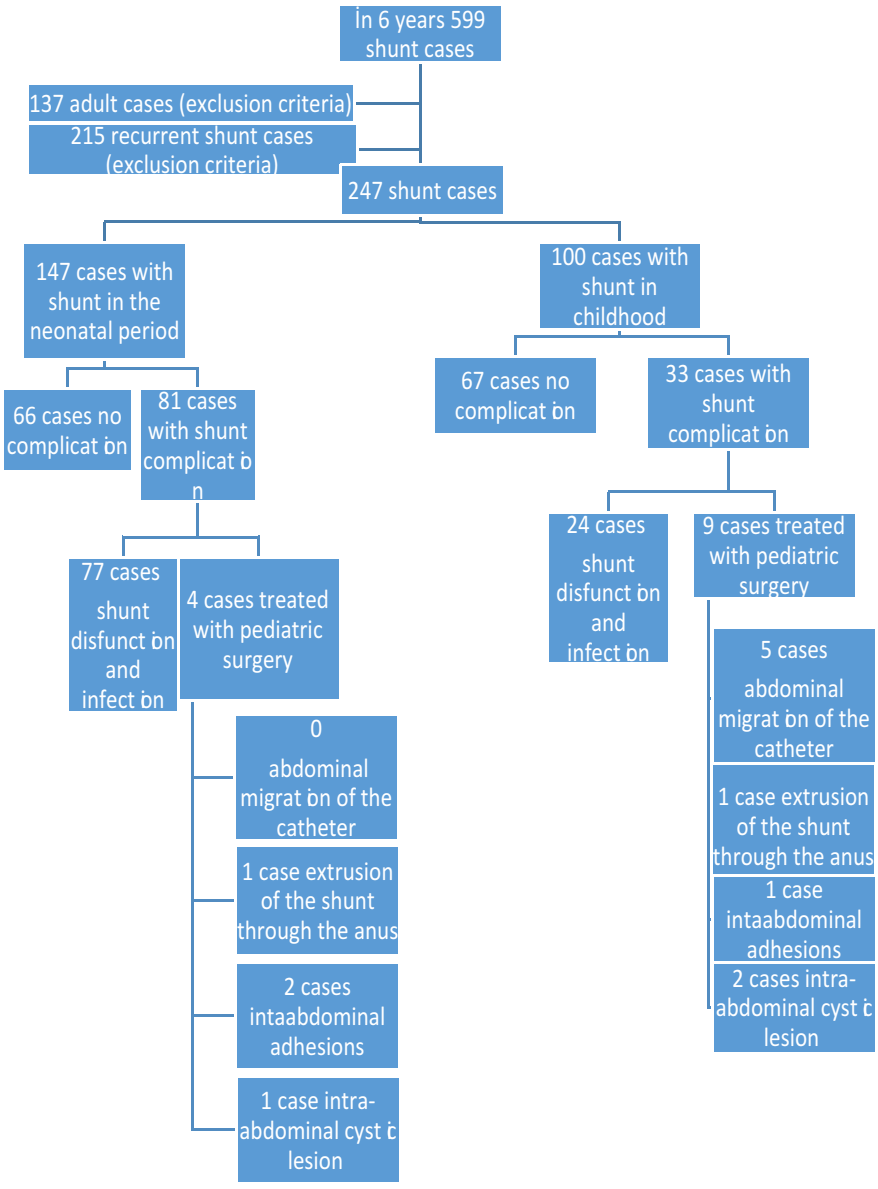

Fig. 1. During the period from 2016 to 2021, ventriculoperitoneal shunt catheterization at our institution.

Table 1. Shunt complications

Shunt complications (n = 114)	Treated with pediatric surgery								Shunt disfunction and infection	
	Abdominal migration of the catheter		Extrusion of the shunt through the anus		Intra-abdominal adhesions		Intra-abdominal cystic lesion			
	n	%	n	%	n	%	n	%	n	%
Neonatal period	0	0	1	0.87	2	1.75	1	0.87	24	21.05
Childhood period	5	4.38	1	0.87	1	0.87	2	1.75	77	67.54

37 had a shunt infection, 60 had a shunt dysfunction, 4 had overdrainage, 5 had an abdominal migration of the catheter, 2 had an extrusion of the shunt through the anus, 3 had intra-abdominal adhesions, and 3 had an intra-abdominal cystic lesion (abdominal pseudocyst, subserosal bowel cyst and scrotal cyst) related to a VP shunt catheterization and were evaluated (Table 1). This descriptive study is based on the retrospective analysis of 3 patients who were treated at the department of neurology and pediatric surgery at the Bursa Health Sciences University (Figs. 2, 3 and 4). The required data were obtained from the electronic database of our institution. Information regarding the patients such as clinical features, diagnostic methods, surgical approaches and postoperative results was recorded. The intra-abdominal cysts were treated with both laparotomy and neurosurgical interventions. All the samples were subjected to histopathological examination.

Of these 3 cases, the first one was a 5-month-old female, the second one was a 1.5-year-old boy and the third case was a 1-year-old boy. These cases presented again with abdominal symptoms (2 cases after 1 month and 1 case after 2 months of the last revision of the shunt). 1 case presented with abdominal distention, vomiting, loss of appetite, and an inability to pass gas and stool, 1 case presented with abdominal distension, vomiting and constipation, and the last one showed tenderness in the right lower abdominal quadrant.

Radiologically, the first case control magnetic resonance imaging showed an abdominal cystic lesion extending from the subhepatic area to the right inguinal region, sized 10 × 5 × 10 cm. Her abdominal

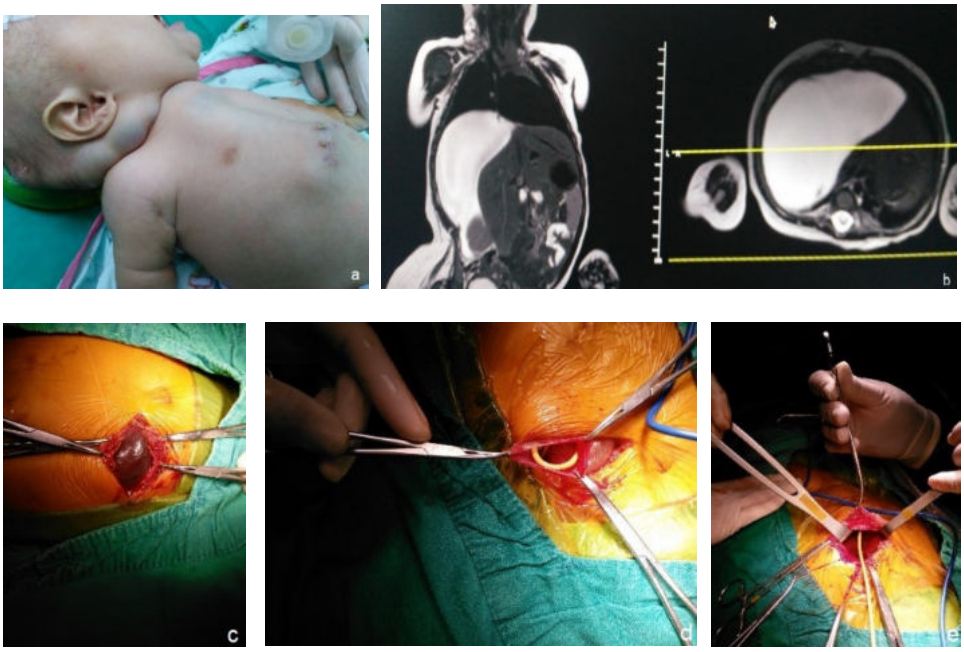

Fig. 2. (a) Preoperative view showed swelling along the shunt, (b) The MRI planning indicate intra-abdominal cystic lesion, (c) Laparatomy performed by median insicion and (d and e) VP shunt catheter, placed subserosal cystic lesion.

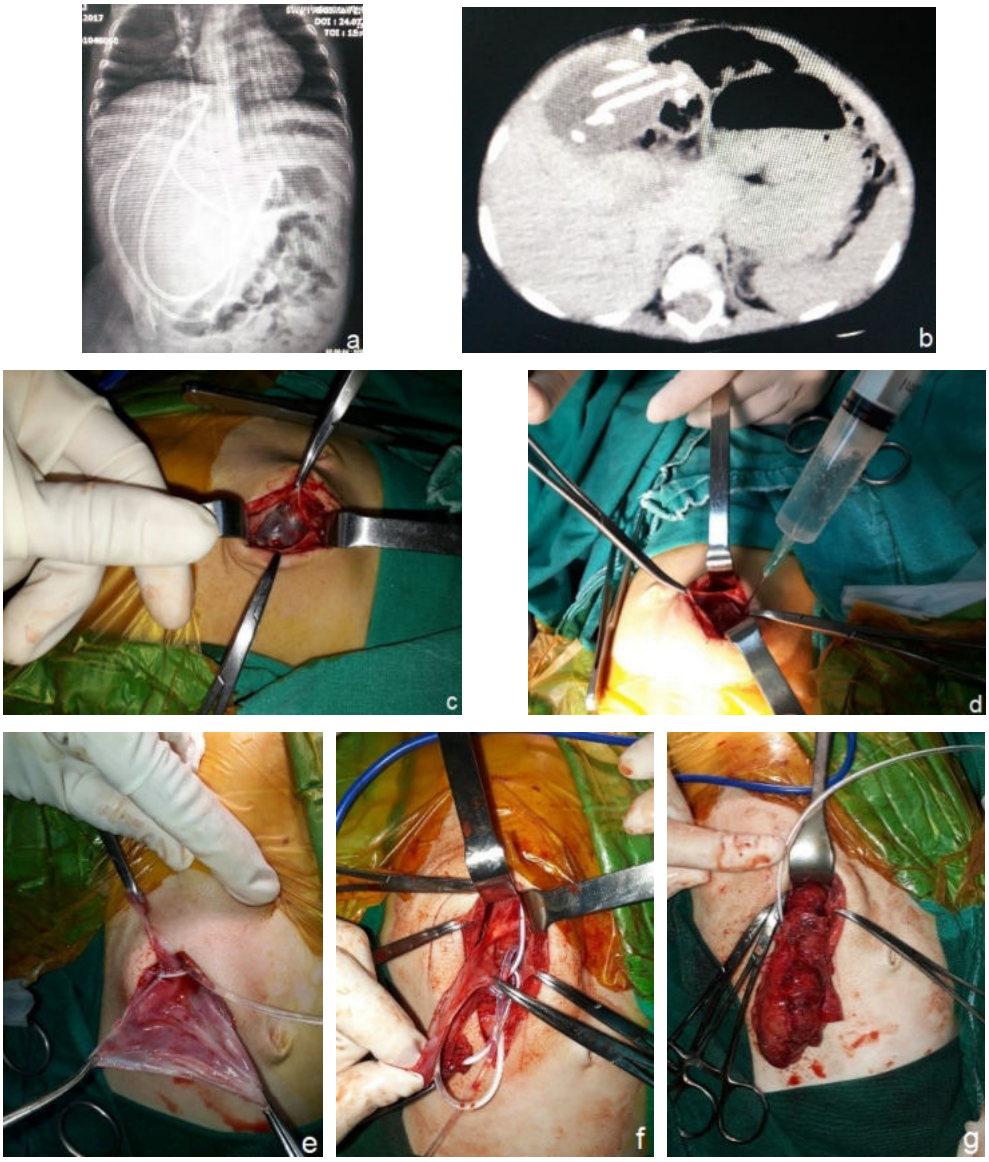

Fig. 3. (a and b) The VP shunt catheter was showed on X ray and CT sagittal image, (c) Laparatomy performed by median insicion and large pseudocyst seen, (d) cyst aspiration and (e, f, and g) cyst excision and adesiolysis performed.

radiography also confirmed ileus (Fig. 2b). In the second case, X-ray and computed tomography (CT) demonstrated a cystic mass in the abdominal cavity, measuring about 10 × 5 × 5 cm in size (Figs. 3a and 3b). In the third case, on X-ray, the size of the cyst was found to be 1 × 1.5 cm toward the shunt line to the scrotal area (Fig. 4a).

Regarding surgical intervention, in the first two cases of abdominal cyst, laparotomy and excision of the cyst and adhesiolysis were performed, then the shunt was repositioned in the right inguinal space (Figs. 2c, 2d and 2e) (Figs. 3c, 3d, 3e, 3f and 3g). In all 3 cases, a right inguinal incision was performed to approach the cystic lesion for hernia repair and high ligation procedure was performed for the cystic hernia sac; the left upper quadrant of the peritoneum was chosen for placing the new shunt catheter with laparotomy (Figs. 4b, 4c, 4d and 4e). There was no problem in the 2-year follow-up for all 3 cases.

RESULTS

A total of 3 intra-abdominal cystic formation repairs were performed in 247 patients. The patient age ranged from 5 months to 1.5 years (median, 11.6 month). One neurosurgeon and 1 pediatric surgeon individually performed all the procedures. All the cases

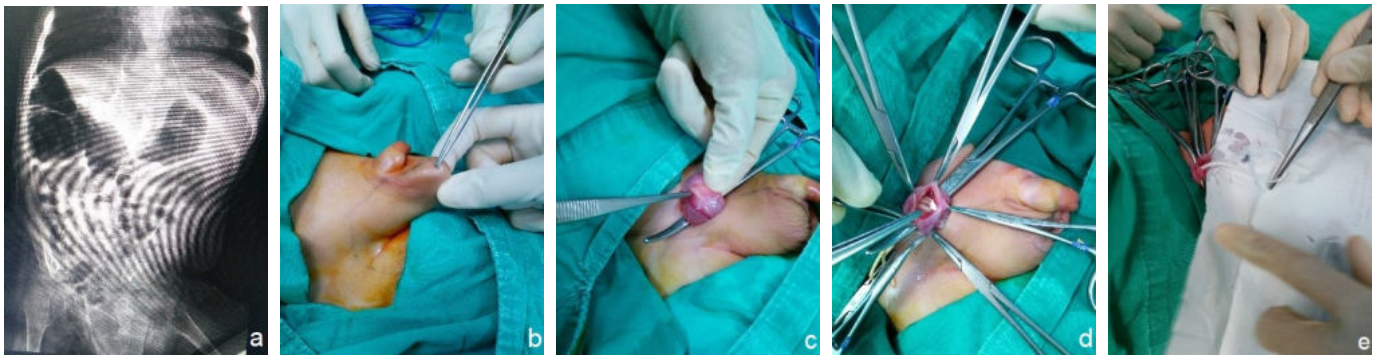

Fig. 4. (a) The VP shunt catheter was showed on X ray in the scrotal area, (b) Right inguinal incision was performed, (c) The cystic hernia sac dissected from spermatic cord and (d and e) The shunt catheter was seen and removed.

were complicated with bowel obstruction. The patients presented with increased intracranial pressure, abdominal distention and ileus symptoms. All the patients initial evaluation was performed with abdominal X-ray. After ileus was detected, cystic lesions were first determined by ultrasound. CT was performed on 1 patient to assess an identified cystic mass and make a treatment decision. A unilateral high inguinal approach was used on 1 patient, and laparotomy was performed on 2 patients for cystic lesion treatment. The histopathological evaluation revealed a mesothelial cyst in 2 patients and a simple cyst in 1 patient.

DISCUSSION

In our clinic, a VP shunt is commonly used for hydrocephalic patients who are ineligible for a third ventriculostomy. VP shunts' complications are common in pediatric and adult patients with a reported incidence rate of 45% and 59%, respectively [1]. The complication rate seen in our cases was 46%. Several predisposing factors, including infection, multiple shunt revisions, obstruction or dislodgement and a peritoneal foreign body reaction have been suggested; however, the pathophysiology remains unclear [4].

The most common intra-abdominal complications with VP shunt are infection, malfunction, disconnection and catheter migration. Infection is the most common complication, comprising 8-12% of all shunt complications. It occurs mostly within the first 6 months [2]. Infection was observed in 37 of our 247 cases (this includes cases of shunt infection referred from other institutions). The most common agent was Staphylococcus epidermidis, which is consistent with the literature.

In addition, complications related to equipment failure, such as extraperitoneal retraction of the shunt catheter, subcutaneous collection of CSF and peritoneal pseudocyst formation, are noted in patients with VP shunt [5, 6]. Intestinal perforation, CSF ascites, inguinal hernia and intestinal volvulus are rare complications that are associated with a high morbidity rate [5]. Of our 247 cases, 147 shunts were inserted in the neonatal period and 100 in the post-neonatal period. In 13 (5.2%) of 247 cases, abdominal complications that required the intervention of pediatric surgery developed, and no additional complications developed after the intervention. Of 247 cases, 5 had an abdominal migration of the catheter (in cases of subduroperitoneal shunt), 2 had extrusion of the shunt through the anus, 3 had intra-abdominal adhesions, and 3 had an intra-abdominal cystic lesion. The rate of intra-abdominal complications, that recovered with the combined effort of neurosurgery and pediatric surgery, was 5%.

An abdominal pseudo cyst is an uncommon manifestation of VP shunt catheterization [6]. Causes of VP shunt-related intestinal obstruction vary from intestinal perforation to mechanical obstruction caused by the mass effect of the cystic structure. Intestinal volvulus is the most common cause in the pediatric population with a VP shunt, a mechanical obstruction due to the twisting of the shunt catheter is the second most common cause. In some cases, obstruction occurs when a loop of the shunt catheter is tightened around a bowel loop during removal [7-9]. Peritoneal CSF pseudo cysts are a rare but significant complication of VP shunt surgery, with a reported incidence rate ranging from 10% to 0.25% [3]. Based on the data of our patients, the annual incidence rate of pseudocysts

is 1.21%.

Dabdoub *et al*. [10] reported that 295 of their 393 patients presented with abdominal pseudocysts related to the VP shunt procedure. Further, 33% of these patients were < 10 year-old, and the recurrence ratio of pseudocyst formation due to VP shunt was 19.8%. In our patients, no recurrence was observed at the 2-year follow-up.

In abdominal pseudocyst formation, the time till the occurrence of complications and symptoms ranges from 3 weeks to 5 years [5]. However, as per some case reports on pseudocyst formation of CSF, the pseudocyst developed between 5 days to 25 months after the VP shunt procedure. The average duration between VP shunt operation and abdominopelvic CT was 11 months (range, 1 week to 115 months) and the average number of VP shunt procedures was 1.4 (range, 1-6) [5]. The period till the development of the pseudocyst was 1 month in 2 of our patients and 2 months in the third patient.

CSF pseudocysts have a variable appearance and may impair CSF absorption [11]. Bowel obstruction is a rare complication. Larger CSF pseudocysts tend to be noninfectious, while smaller cysts or multiloculated cysts may cause an infection [11]. Pathi *et al*. [12] reported no significant relationship between pseudocyst size and infection. As per Ersahin *et al*. [13], Staphylococcus epidermidis may be present in pseudocysts that develop in the first year. Our patients had no infective process, and they each had a single cyst.

From a clinical perspective, concerning patients with a VP shunt, pediatric patients mainly present with symptoms of elevated intracranial pressure, such as a headache and nausea, while adults predominantly exhibit local abdominal symptoms [14]. Clinical symptoms are similar to those in acute abdomen, such as abdominal pain with/without a palpable mass, abdominal distension with/without tenderness, nausea and/or vomiting. In addition, decreased appetites, constipation, fever, and signs of shunt malfunction, such as lethargy and headache may be present [6]. In keeping with previous findings, our patients exhibited the symptoms of abdominal distention; vomiting; appetite loss and ileus symptoms, such as the inability to pass gas and stool.

A diagnosis of intra-abdominal shunt complication is based on clinical findings and imaging diagnostic studies (abdominal radiography, US and CT). Abdominal radiographs help rule out other causes of acute abdomen and enable a decision regarding the continuity of the catheter tube. Although the results of these modalities may be normal, ileus should be considered in the differential diagnosis [15].

Although abdominal radiography is a first-line radiological tool that is useful for evaluating patients with a VP shunt, US is important for the initial diagnosis of abdominal CSF pseudocyst [6]. US easily reveals cystic lesions in terms of the distances, localization and surrounding tissues. US may also help detect the distal end of the VP shunt catheter, in a similar way to radiography [15-17].

CT is the best noninvasive imaging method that enables the treatment decision procedure of APC by visualizing complete abdominal anatomy [18]. Thus, it provides diagnostic information about pseudocyst localization and may help identify the type of cystic lesion [6, 18]. In the present study, the initial evaluation for all the patients was performed using abdominal radiography and US. Thereafter, all cystic structures were confirmed on abdominal CT because of the treatment procedure.

As stated by Hamid *et al*., patients with intra-abdominal cysts after VP shunt catheterization should be managed individually [14]. The treatment options include percutaneous drainage and both surgical approaches, laparotomy and laparoscopy [3].

Percutaneous drainage of the cyst initially resolves the obstruction; however, this involves a high recurrence risk [3]. In particular, in patients with ileus, an urgent surgical procedure must be initially considered for both VP shunt removal and cyst excision. In general practice, it is not uncommon to encounter an abundance of adhesions between cystic formation and abdominal viscera and/or between the viscera themselves due to VP shunt catheterization. Thus, in most cases, the laparoscopic approach described by Kim *et al*. [19] for the management of a CSF pseudocyst is not routinely preferred, and depends on the experience of the surgeon and adhesions.

Considering visceral damage or perforation during adhesiolysis, laparotomy is safer than laparoscopy. Laparotomy provides either repositioning or exteriorization of a VP shunt catheter simultaneously during operation and adhesiolysis can be performed with minimal damage [14]. Thus, cyst excision was per-

formed using laparotomy in our cases. In addition, the shunts of all of our patients were repositioned in the peritoneal cavity with ease during laparotomy.

The authors believe that these complications are attributable to the fact that peritoneal catheters are longer than needed; however, previous literature shows contradictory findings. To our knowledge, this is the first study to demonstrate that the use of 90-cm-long peritoneal catheters in neonates and infants is safe and effective. It does not increase the incidence of abdominal complications and prevents the need for revision for insufficient length of the peritoneal catheter [20].

The cysts of CSF are surrounded by a wall of non-epithelial tissue, such as intestinal serosa, peritoneum, or intra-abdominal organ surfaces [14]. Histopathological evidence demonstrates the presence of inflamed serosal lining, fibrous tissue, inflammatory cells and granulomatous tissue consisting of fibroblasts and collagen with inflammatory cells [14]. Our histopathological results were consistent with previous findings in the literature.

CONCLUSION

Abdominal cysts are rare complications of VP shunt. In 5 years, 3 of our 247 patients developed abdominal complications, including an abdominal pseudocyst, subserosal bowel cyst and a scrotal cyst. There was no relevant infection. Abdominal complications should always be considered when a patient with a VP shunt presents with abdominal symptoms.

Ethical Approval

The study was initiated after obtaining approval from the Bursa Yüksek İhtisas Training and Research Hospital local ethics committee (Decision number:2011-KAEK-25 2021/02-20). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants in the study.

Authors' Contribution

Study Conception: EBG, EÖ; Study Design: EBG, EÖ; Supervision: EBG, EÖ; Funding: N/A; Materials: N/A; Data Collection and/or Processing: EBG; Statistical Analysis and/or Data Interpretation: EBG, EÖ; Literature Review: EBG; Manuscript Preparation: EBG, EÖ and Critical Review: EBG, EÖ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;(1):57-65 Original Article

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Postdischarge pain, fatigue severity and quality of life in COVID-19 survivors

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ABSTRACT

Objectives: Fatigue and pain symptoms were common complaints among post-COVID-19 patients, and these lead to impaired quality of life (QoL). We aimed to evaluate severity of pain and fatigue 3 months after disease onset in discharged COVID-19 patients.

Methods: Patients were contacted by phone at their third month following disease onset. Demographic data of the patients such as weight, height, body mass index (BMI), gender, smoking history, comorbidities, length of hospitalization, duration of stay in the intensive care unit, were recorded. The patients' pain and fatigue severities were evaluated by visual analog scale (VAS). QoL was questioned with the EuroQol Group Association five-domain, three-level questionnaire (EQ-5D-3L).

Results: A total of 392 participants enrolled into the study. At admission, 94.6% of the participants had fatigue and 73.7% of them had pain. A high proportion of them still reported fatigue (55.1%) and pain (41.3%) at third month. The mean value of pain-VAS score was 5.37 ± 3.85, and it was 7.58 ± 2.82 for fatigue-VAS at admission. VAS scores of pain and fatigue at third month were 1.44 ± 2.11 and 2.04 ± 2.40 respectively. While 66.6% of the patients reported moderate-severe pain at disease onset, the rate was 18.1% at the third month. And also almost half had severe pain at admission (48%), it was 2.8% at third month. At disease onset 89.6% of the patients reported moderate-severe fatigue (severe:48%). Aproximately one third of them had moderate-severe fatigue (27.9%) at third month (severe: 5.1%). The mean value of EQ-VAS score was 26.76 ± 20.26 at admission, and it was 78.84 ± 16.15 at third month. Statistically significant differences were recorded between the disease onset and third month in terms of pain-VAS fatigue-VAS, and EQ-VAS scores ($p < 0.001$). Female gender, ICU admission, long duration of hospitalization, older age, higher BMI scores, multiple comorbidities, fatigue and pain severity were related to the decrease in QoL scores.

Conclusions: Hospitalized COVID-19 survivors need ongoing support for pain, fatigue complaints and impaired QoL after discharge. The factors that cause poor QoL should be taken into account during post-COVID-19 follow up.

Keywords: COVID-19, pain, fatigue, visual analog scale (VAS) , quality of life, EQ-5D-3L

Coronavirus disease 2019 (COVID-19) which has become a worldwide pandemic, affects people in different ways, including asymptomatic infection, mild respiratory illness and severe pneumonia with acute respiratory failure and even death [1, 2]. Some patients do not show a rapid recovery after COVID-

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19 treatment and their symptoms may persist up months, if the symptoms persist beyond 12 weeks, it is called post-COVID-19 syndrome [3]. Highly heterogeneous clinical manifestations were found in post-COVID-19 syndrome, such as fatigue, pain, hair loss, dyspne cough, memory loss, sleep disorder, anxiety etc. [2, 4-7]. Fatigue and pain symptoms were common complaints among post covid patients [7, 8]. However, it is not known to what extent fatigue and pain intensity continue. We aimed to assess severity of pain, severity of fatigue and quality of life (QoL) of hospitalized COVID-19 patients 3 months after disease onset.

METHODS

Study Design and Participants

This was a single-center, cross-sectional study carried out in a tertiary hospital in İstanbul, Turkey during February 15, 2021 to May 15, 2021.

The study protocol was approved by the Ethics Committee of University of Health Sciences Turkey, Prof. Dr. Cemil Tascioglu City Hospital, Istanbul, Turkey (No. 2021/21). It was performed by the principles stated in the Declaration of Helsinki.

A total of 1921 patients hospitalized with the diagnosis of COVID-19 (positive COVID-19 PCR test, or presence of COVID-19 based on clinical and radiological criteria) between 15 November 2020 and 15 February 2021 were recruited from the hospital's database. Patients that were death, older than 65 years and younger than 18 years, prisoners and foreign nationalities were excluded. A total of 392 of the remaining 894 eligible patients were contacted at 3rd month following disease onset. Trained physicians called the patients by phone, explained the aims of the study, and asked patients to answer the questionnaire. Informed consent was given verbally over the phone and recorded by the investigators.

Demographic data of the patients such as weight, height, body mass index (BMI), gender, smoking history, comorbidities, length of hospitalization, duration of stay in the intensive care unit (ICU), were recorded either from their files or by phone visit. The World Health Organization (WHO) classification criteria were used for BMI classification. The patients' pain and fatigue severity and QoL at the time of the phone call and at the hospital admission were questioned. Pain and fatigue severities were evaluated by visual analog scale (VAS). QoL was questioned with the EuroQol Group Association five-domain, three-level questionnaire (EQ-5D-3L).

EQ-5D-3L

OoL was assessed using the EQ-5D-3L, which consists of two sections: the descriptive system and the VAS. The descriptive system assesses five domains: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The participant describes the severity levels of each dimension in a categorical scale without arithmetic properties from 1 to 3, where 1 indicates no problem, 2 indicates some problem and 3 indicates extreme problem. The second part includes the VAS in which respondents evaluate their current health status between 0 (worst imaginable health) and 100 (best imaginable health) [9].

VAS

Patients score their pain or fatigue on a pain scale with 0 representing 'no pain or no fatigue' and 10 representing 'most severe pain possible or most severe fatigue possible [10].

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 25 (SPSS Inc., Chicago, IL, USA). Categorical data were presented as numbers and percentages, while continuous data were reported as means ± SD. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to examine the normal distribution, data were normally distributed so means for continuous variables were compared using unpaired or paired t-tests. Wilcoxon test was used for categorical data. Also, Spearman's correlation was used to find the correlation between symptoms' score in acute and post COVID-19 stages. The results were evaluated bilaterally at 95% confidence interval, significance level at $p < 0.05$ and $p < 0.01$.

RESULTS

A total of 392 participants completed the survey by telephone call. Of the patients, 50% were male and 50% were female. The mean age was 51.27 ± 11.13

Table 1. Demographic and clinical charactersitics of hospitalized COVID-19 patients

	Total (n = 392)
Age (years), mean ± SD	51.27 ± 11.13
Gender, n (%)	
Male)	196 (50)
Female	196 (50)
BMI, mean ± SD	30.11 ± 5.30
Normal (BMI < 25kg/m^2), n (%)	51 (13.0)
Overweight (BMI = 25-30kg/m^2), n (%)	177 (41.8)
Obese (BMI > 30kg/m^2), n (%)	164 (37.2)
Comorbidities, n (%)	
No comorbidities	130 (33.2)
One comorbidity	106 (27.0)
> 1 comorbidity	156 (39.8)
HT	144 (36.7)
DM	134 (34.2)
Hyperlipidemi	53 (13.5)
Cardiac	60 (15.3)
Pulmoner	49 (12.5)
Renal	22 (5.6)
Malignancy	23 (5.9)
Others	21 (5.4)
Smoking status, n (%)	
Non-smoking	288 (73.5)
Current-smoking	35 (8.9)
Previous-smoking	69 (17.6)
Symptoms at hospital admission, n (%)	
Fever)	70 (17.9)
Dyspne	198 (50.5)
Cough	77 (19.6)
GIS symptom	39 (9.9)
Pain	289 (73.7)
Fatigue	371 (94.6)
Inpatient day, mean ± SD	10.36 ± 6.71
< 10 days, n (%)	213 (54.3)
≥ 10 days, n (%)	179 (45.7)
ICU day, mean ± SD	0.85 ± 3.93
Present, n (%)	25 (6.4)
No, n (%)	367 (3.6)

Data are shown as mean ± standard deviation or n (%). BMI = body mass index, HT = hypertension, DM = diabetes mellitus GIS = gastro intestinal system, ICU = intensive care unit, SD = standard deviation

years, the mean BMI was 30.11 ± 5.30 kg/m2, and most of them were overweighted (41.8%). 73.5% were non-smokers. Most of the patients had at least one comorbidy (66.8 %). There were no comorbidity in 33.2% of the patients.

Among 392 patients, 6.4% of them had required intensive care treatment at some point throughout hospital stay. The median length of hospital stay was 10.36 ± 6.71 days, although it ranged from 1 to 50 days. The characteristics of the study population are summarized in Table 1.

Pain and Fatigue Severity

At the time of the survey (90 days post-disease onset), participants were asked to describe current pain and fatigue severity by VAS and to recall pain and fa-

Table 2. Pain, fatigue and quality of life of hospitalized covid-19 patients at hospital admission and 3 months after disease onset

	Hospital admission	3 months after COVID-19	*p value*
Pain symptom/moderate-severe pain, n (%)	282 (71.9)/261 (66.6)	162 (41.3)/71(18.1)	**< 0.001**[1]
Fatigue Symptom/moderate-severe fatigue, n (%)	371 (94.6)/351(89.6)	216 (55.1)/109 (27.9)	**< 0.001**[1]
Pain VAS, mean ± SD	5.37 ± 3.85	1.44 ± 2.11	**< 0.001**[2]
Fatigue VAS, mean ± SD	7.58 ± 2.82	2.04 ± 2.40	**< 0.001**[2]
QOL (EQ-5D-3L)			
Mobility, n (%)			**< 0.001**[1]
No problems	100 (25.6)	311 (79.3)	
Some problems	201 (51.3)	78 (19.9)	
Extreme problems	91 (23.2)	3 (0.8)	
Self-care, n (%)			**< 0.001**[1]
No problems	118 (30.1)	352 (89.8)	
Some problems	170 (43.4)	35 (8.9)	
Extreme problems	104 (26.5)	5 (1.3)	
Usual activities, n (%)			**< 0.001**[1]
No problems	93 (23.7)	342 (87.2)	
Some problems	168 (42.9)	46 (11.7)	
Extreme problems	131 (33.4)	4 (1.0)	
Pain or discomfort, n (%)			**< 0.001**[1]
No pain or discomfort	105 (26.8)	292 (74.5)	
Some pain or discomfort	125 (31.9)	94 (24.0)	
Extreme pain or discomfort	162 (41.3)	6 (1.5)	
Anxiety or depression, n (%)			**< 0.001**[1]
Not anxious or depressed	113 (28.8)	330 (84.2)	
Moderately anxious or depressed	126 (32.1)	53 (13.5)	
Extremely anxious or depressed	153 (39.0)	9 (2.3)	
EQ-VAS, mean ± SD	26.76 ± 20.26	78.84 ±16.15	**< 0.001**[2]
EQ-5D index score, mean ± SD	0.346 ± 0.318	0.86 ± 0.193	**< 0.001**[2]

Data are shown as mean ± standard deviation or n (%). QOL (EQ-5D-3L) = quality of life (EuroQol five-domain, three-level) VAS = visual analog scale, SD = standard deviation

[1]Wilcoxon, [2]Paired samples t test

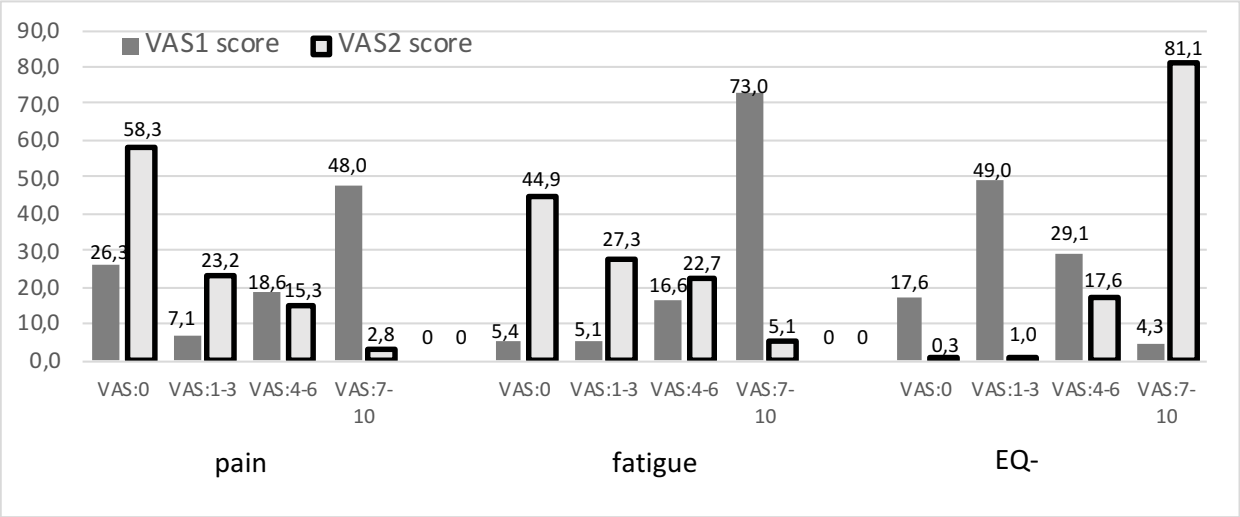

Fig. 1. VAS score frequencies of pain, fatigue and EQ of hospitalized Covid-19 patients at hospital admission and 3 months after disease onset. VAS1 = visual analog scale at hospital admission, VAS2 = visual analog scale at 3 months after disease onset, EQ-QoL = Euro Quality of Life

tigue severity at disease onset (Table 2).

At admission, 94.6% of the participants had fatigue and 73.7% of them had pain. A high proportion of individuals still reported fatigue (55.1%) and pain (41.3%) at the time of the questionnaire. The mean value of pain VAS score was 5.37 ± 3.85 for pain, and 7.58 ± 2.,82 for fatigue VAS at admission. 3 months after COVID-19 onset the VAS scores of pain and fatigue was 1.44 ± 2.11, 2.04 ± 2.40 respectively. A statistically significant decrease was found both in pain and fatigue VAS scores at 3rd month of COVID-19 according to disase onset ($p < 0.001$). VAS scores of pain and fatigue of the patients at hospital admission and 3 months after disease onset were shown in Fig. 1.

Quality of Life

The mean value of EQ-VAS score was 26.76 ± 20.26 at admission, and it was 78.84 ±16.15 3 months after COVID-19 onset. The EQ-5D index score was 0.346 ± 0.318 at admission, and it was 0.86 ± 0.193 at 3rd month. A statistically significant improvement was found both in EQ-VAS and EQ-5D index scores at 3rd month of COVID-19 according to disase onset ($p < 0.001$). Although the QOL of the patients according to EQ-5D-3L were better at third month, 25.5% had still pain and discomfort complaints and 15.8 % felt anxious and depressed, 21.7% had problems with mobility (Table 2).

Demographic and clinical variables can affect pain, fatigue and QoL. So we examined the relation between the parameters. A statistically significant relationship was found between female gender and 3rd month pain VAS ($p = 0.033$), fatigue VAS ($p = 0.018$), EQ-VAS ($p = 0.002$). ICU admission and longer inpatient day affected 3rd month VAS pain, VAS fatigue, EQ-VAS scores negatively ($p < 0.05$) (Table 3). According to the correlation analysis, a statistically significant relationship was found between EQ-VAS at 3rd month and female gender, older age, higher BMI scores, having multiple comorbidities, ICU admission, and length of hospital stay, fatigue VAS and pain VAS (Table 3). Fatigue-VAS had a correlation with female gender, older age, higher BMI, having multiple comorbidities, length of ICU stay, and length of hospital stay. Pain-VAS had a correlation with female gender, ICU admission, and length of hospital stay

DISCUSSION

Post-COVID-19 syndrome is a debilitating problem for those who recovered from COVID-19. Highly heterogeneous clinical manifestations were found in post-COVID-19 syndrome [2, 4-7]. Aproximately 64% - 76% of the patients reported persistence of at least 1 symptom for > 12 weeks [2, 11]. At post acute period (> 4 weeks) the rate of persistant symptomps was 87%

Table 3. Relationship between the parameters of hospitalized covid-19 patients 3 months after disease onset

	Inpatient day	ICU day	Pain-VAS2	Fatigue-VAS2	EQ-VAS2
Age					
r	.140**	0.038	0.094	.119*	-.176**
p value[2]	**0.006**	0.455	0.063	**0.019**	**< 0.001**
Female	10.40 ± 7.42	0.96 ± 4.36	1.66 ± 2.18	2.32 ± 2.45	76.33 ± 16.60
Male	10.32 ± 5.92	0.73 ± 3.46	1.21 ± 2.01	1.75 ± 2.31	81.35 ± 15.32
p value[1]	0.904	0.564	**0.033**	**0.018**	**0.002**
BMI					
r	0.085	.122*	0.060	.115*	-.134**
p value[2]	0.091	**0.015**	0.237	**0.023**	**0.008**
Non-smoking	10.48 ± 7.08	0.79 ± 3.92	1.52 ± 2.15	2.10 ± 2.44	78.68 ± 15.56
Current/previous smoking	10.02 ± 5.57	1.01 ± 3.97	1.20 ± 1.96	1.87 ± 2.31	79.28 ± 17.74
p value[1]	0.549	0.629	0.168	0.399	0.747
Number of comorbidities					
r	.141**	.147**	0.093	.237**	-.271**
p value[2]	**0.005**	**0.004**	0.067	**< 0.001**	**< 0.001**
Inpatient day					
r	1	.644**	.158**	.141**	-.178**
p value[2]		**< 0.001**	**0.002**	**0.005**	**< 0.001**
ICU admision					
yes	25.32 ± 10.76	1	2.32 ± 2.15	1.96 ± 2.39	70.00 ± 15.20
No	9.34 ± 4.91		1.38 ± 2.09	3.16 ± 2.17	79.44 ± 16.05
p value[1]	**0.001**		**0.030**	**0.015**	**0.005**
Pain-VAS2					
r	.158**	. 125*	1	.597**	-.439**
p value[2]	**0.002**	**0.013**		**< 0.001**	**< 0.001**
Fatigue-VAS2					
r	.141**	. 130**	.597**	1	-.567**
p value[2]	**0.005**	**0.010**	**< 0.001**		**< 0.001**

BMI = body mass index, ICU = intensive care unit, EQ-5D = Euro Quality of Life five-domain, VAS2 = visual analog scale at 3 months after disease onset,

[1]Chi square, [2]Pearson, **Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

[7, 8, 11].

Musculoskeletal pain was one of the most reported complaint in acute infection (70% - 89.5%) [7, 8, 12, 13]. As a persistant symptom post covid pain was found in a range of 27-61% [5-8, 14, 15]. We found 41.3% pain symptom at the time of the questionnaire, while it was 73.7% at disease onset. We also measured the severity of pain with the VAS scale both at acute period and at the 3rd month. While 66.6% of the patients reported moderate to severe pain at disease onset, the rate was 18.1% at the third month. And also almost half had severe pain at the onset of the disease

(48%), it was 2.8% at third month.

In a study using only the initial VAS scores it was found that 68% of patients had severe myalgia complaints at disease onset [12]. Murat *et al*. [12] found that 96.2% of the patients had moderate pain symptom according to VAS during acute period.

Since there was no pain severity measurement at third month, we investigated EQ-5D results. Pain/discomfort problems were reported in a range of 39.5-81.1%, with 4.5-12.4% of severe problems at third month [11, 16]. At sixth month after COVID-19 33-48.4% of the patients had still pain/discomfort problems (8.8% with severe problems) [17, 18].

We also assesed the corelation of pain with demographic and clinical parameters. According to our results female gender presented higher frequencies of pain in agreement with previous studies [5]. In addition to this we found that ICU requirement, and longer length of hospital stay caused more pain complaints. According to ED-5Q results Halpin *et al*. found worsened pain/discomfort ratio 14.7% among wards patient, 28.1% among ICU patients [196]. On the other hand an association was found between higher BMI and myalgia [5]. However we couldn't confirm this association.

Post-COVID fatigue has been found to be the most frequent symptom either at acute or post-COVID period [2, 4-8, 12-15, 20] and also our prevalence of fatigue in the acute period was 94.6%. In previous studies initial fatigue symptom range was 80 - 85% [7, 8, 12, 13] and as a persistant symptom the range was 55-85% [2, 4-6, 14, 15, 20].

Tuzun *et al*. [13] evaluated fatigue with Chalder Fatigue Score CFS at the acute period and found 85.3% of the patients had severe mean fatigue VAS scores, but they didn't have postcovid period results. Townsend *et al*. [21] also examined the prevalence of fatigue with CFS at 10 weeks after initial COVID-19 symptoms. Half of the patients met the criteria for fatigue with half of them reporting severe fatigue, but they didn't have acute period results. Sykes *et al*. [5] found extreme fatigue 39.6% at forth month after post-discharge.

We didn't use a specific fatigue scale but according to VAS scores 55% of our patients reported fatigue at 3rd month. Aproximately one third of them had moderate-severe fatigue (27.9% including severe of 5.1%). At disease onset 89.6% of the patients reported moderate-severe fatigue (severe: 48%).

We examined corelation of fatigue with demographic and clinical parameters. According to our results fatigue-VAS had an assosiation with female gender, older age, higher BMI, having multiple comorbidities, ICU recuirement, and longer length of hospital stay. Unlike our results Townsend *et al*. [21] didn't find a relation with fatigue and ICU or ward admission. On the other hand Halpin *et al*. [19] investigated hospitalized patients and found that ICU group were more likely to report fatigue than wards group (72% vs 60.3%). Similar to our results Sykes *et al*. [5] also found that female gender and higher BMI was associated with fatigue.

There are different results according to hospitalization or evaluation time after disease onset. There had been still persistant pain and fatigue complaints with higher rates than ours 4 months after disease onset [2, 5, 14] persistent symptoms such as pain and fatigue often lead to impaired quality of life [7, 14, 15].

The EQ-5D data showed that patients had worse QoL at disease onset and the impaired QoL lasts on months after discharge [11, 15, 16]. Restriction of daily activities was found at 84.9% of patients at disease onset [7]. Among our patients 76.3% of them reported problems with usual activities and 69.9% with self care at disease onset.

Studies showed that 23.2-67% of the patients reported poorer QoL at post covid period [6, 8, 17, 18]. Restriction of daily activities (57%) was the most frequently seen [7]. Shah *et al*. [11] found that 79.5% of patients had problems with usual activities at third month (severe: 26.3%). Among our patients 12.7% of them reported problems with usual activities and 10.2% with self care at third month.

Todt *et al*. [16] found pain/discomfort and anxiety/depression as the most affected domains at third month after discharge. According to our results pain-discomfort domain was the most affected (25.5% of the patients had problems). Unlike their results we found that 21.7% of the patients had mobility problems. Perhaps it was because of ongoing dyspne complaints, it would be better if we had asked persistant dyspne symptom.

In our study 15.8 % of the patients felt anxious and depressed at third month. But the rates in previous studies were higher than ours (24-53%) [2, 7, 19]. Six

months after symptom onset, Anxiety or depression was reported among one forth of patients [2]. In addition to these psychological distress was also higher in ICU discharged patients. (46.9% in ICU group and 23.5% in ward group) [19].

Similar to our results Garrigues *et al*. [4] found that post-discharge mean EQ-VAS was 70.3% and mean EQ-5D index was 0.86, there were no difference between ICU and ward patients. Female gender, being hospitalized, ICU requirement, longer length of hospital stay and comorbidity presence were associated with decreased QoL scores in agreement with previous studies [11, 16, 22]. In addition to these findings we also found that older age, higher BMI scores, fatigue and pain severities caused decrease in QoL scores. Unlike our results Tabooda found male sex was associated with poor QoL [18].

Sixth months after disease EQ-5D scores worse than ours was also detected. The results according to EQ-5D questionaire were as follows: 33-56% of the patients had problems with mobility, 35-37% with usual activities, 13-17% with self-care, 33-48% with pain/discomfort and 26-46% with anxiety/depression [17, 18]. Perhaps it was because of older patients or ICU discharged patients that included into these studies.

Limitations

A limitation of the present study is that it was a phone survey because of pandemic conditions. Another limitation is that it included only hospitalized patients and the number of ICU patients that we had reached was very low. Our results were based on patients' self-reported data. Also we could assess anxiety/depression and sleep with a disease spesific questionaire since these exacerbate fatigue and impact QoL negatively. Regardless, our results show either pain or fatigue lasts long periods of time altough the treatment is over.

CONCLUSION

Post-COVID-19 syndrome is a debilitating problem. Pain and fatigue were the most common post-COVID-19 syndromes. Our findings are in agreement with other previous studies, different from others we evaluated the severity of pain and fatigue. Half of the patients had severe fatigue and pain symptoms at COVID-19 onset. Despite the end of the treatment, it was determined that moderate-to-severe pain and fatigue complaints continued at 3rd month in some patients, and these worsened QoL. Patients which were female, older or overweighted, who had multiple comorbidities, who required ICU or stayed at hospital for a long time had higher fatigue severity and poor QoL. These findings may be important for planning the ongoing support for these patients. The factors that cause poor QoL should be taken into account during post-COVID-19 follow up.

Authors' Contribution

Study Conception: ED, SA, GE, İO, ÖK; Study Design: ED, SA, GE, İO, ÖK; Supervision: ED, SA, GE, İO, ÖK; Funding: ED, SA, GE, İO, ÖK; Materials: ED, SA, GE, İO, ÖK; Data Collection and/or Processing: ED, SA, GE, İO, ÖK; Statistical Analysis and/or Data Interpretation: ED, SA, GE, İO, ÖK; Literature Review: ED, SA, GE, İO, ÖK; Manuscript Preparation: ED, SA, GE, İO, ÖK and Critical Review: ED, SA, GE, İO, ÖK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Evaluation of age-related changes in the vitreous using magnetic resonance imaging

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ABSTRACT

Objectives: Age-related liquefaction of vitreous humor may result in posterior vitreous detachment, retinal tear, and detachment. The purpose of this study is to determine the normative values of age-related changes in the vitreous in the normal population using different MRI sequences.

Methods: A total of 180 eyes of 90 healthy cases were enrolled in this retrospective study. Patients were divided into nine groups according to age, and each group was of equal size with 10 patients(5 male and 5 female). The T1, T2, standardized T1, standardized T2 signals and ADC values determined for each vitreous humor of each eye. MRI parameters of the vitreous were compared within and between age groups.

Results: No difference was detected within the decadic age groups for mean T1W for the right and left (p = 0.912 and p = 0.903, respectively), T2W for the right and left (p = 0.966 and p = 0.983, respectively), standardized T2W for the right and left (p = 0.915 and p = 0.899, respectively), and ADC for right and left values (p = 0.622 and p = 0.524, respectively). A significant difference was found between decadic age groups in terms of the standardized T1W values for right and left ($p < 0.001$ and $p < 0.001$, respectively). Standardized T1W values of vitreous fluid show a moderate degree of correlation with age for the right (r = 0.514, $p < 0.001$) and left eyes (r = 0.534, $p < 0.001$).

Conclusions: This study provides comprehensive normative data on the different MRI signal properties of the human vitreous and its change with age. Using MRI, especially with standardized T1 measurements, age-related changes in the vitreous humor can be revealed non-invasively.

Keywords: Aging, eye, diffusion-weighted imaging, MRI, vitreous humor

The vitreous makes up 80% of the globe and is the largest structure of the eye. Essentially, it consists of water, collagen, and hyaluronic acid groups and it is in a homogeneous gelous form during childhood. Deterioration of this gel structure and resulting inhomogeneity is termed liquefaction and this condition increases with increasing age. Albeit the mechanism of liquefaction is not known precisely, increased amounts of collagen and increased collagen bond ratio are considered among potential factors [1, 2].

Age-related liquefaction of vitreous humor is clinically important as it may result in posterior vitreous detachment, retinal tear and detachment. Therefore, assessment of the vitreous can provide early diagnosis

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and treatment of pathologies in the vitreous and adjacent structures [3]. The vitreous can be evaluated using invasive techniques such as vitrectomy or vitreous fluid sampling, but aside from being invasive, this involves several risks such as hemorrhage, retinal rupture and detachment [4]. Magnetic Resonance Imaging (MRI), allows protein and water content to be evaluated noninvasively. Increased protein and decreased water percentage, which occurs especially with aging, can be shown as an increase in signal on T1 (spin-lattice interaction) weighted sequences on MRI [5].

The objective of this study is to reveal the normative values of age-related changes in the vitreous in the normal population on different MRI sequences and to explore its correlation with age.

METHODS

Patient Selection

This retrospective study was approved by the local Ethics Committee (Decision no: 09.2020.1072). Patients who underwent brain MRI between January 1, 2020, and November 1, 2020 and demonstrated no abnormality on imaging formed our study population. Patients with any history of neurological or ophthalmological disease that might impact cerebrospinal fluid (CSF) or vitreous humor and those with a history of malignancy and chronic disease were excluded from the study. MRI examinations which contained artifacts or were non-diagnostic were also excluded from the study. From this population, 90 patients were randomly selected according to their age. Patients were divided into 9 age groups based on the decade of their age, each consisting of 10 individuals (groups 1-9, respectively). Equal gender distribution was achieved, with 5 females and 5 males in each group.

Equipment and MRI Examination

All images were obtained on 1.5 T MRI devices (Ingenia; Philips Healthcare, Cleveland, Ohio). Routine brain sequences were acquired using 8 and 16-channel head coils. In the examinations, T1-weighted (T1W), T2-weighted (T2W), Diffusion-weighted images (DWI) and Apparent Diffusion Coefficient (ADC) maps were obtained (SE T1 axial, TR/TE 324/9, FA: 90, slice thickness 5.5 mm, gap 1.6 mm; TSE T2 axial, TR/TE 3790/89, slice thickness 5.5 mm, gap 1.6 mm). The b0 and b1000 values were used for diffusion imaging (SE FS DWI axial, TR/TE 3230/85, FA: 90, slice thickness 5 mm, gap 1 mm).

Image Interpretation

The brain MRI images were transmitted to the workstation and picture archiving and communication system (Infinitt PACS; Infinitt Healthcare, Seoul, South Korea). Images were evaluated by a radiologist with 5 years of experience, blinded to the patient information. The axial T1W and T2W sequences and ADC maps where the globe was seen at its largest diameter were chosen for evaluation. Measurements were performed by drawing the largest oval or round "region of interest" (ROI) possible in the vitreous, without extending beyond the globe. Three measurements were taken for each image sequence and for each orbit and the mean signal value was recorded. The minimum, maximum, median values and standard deviations of each measurement were recorded. In order to normalize differences between individuals due to local magnetic inhomogeneities, standardized T1W and T2W values were computed separately for the right and left by proportioning the vitreous T1W,

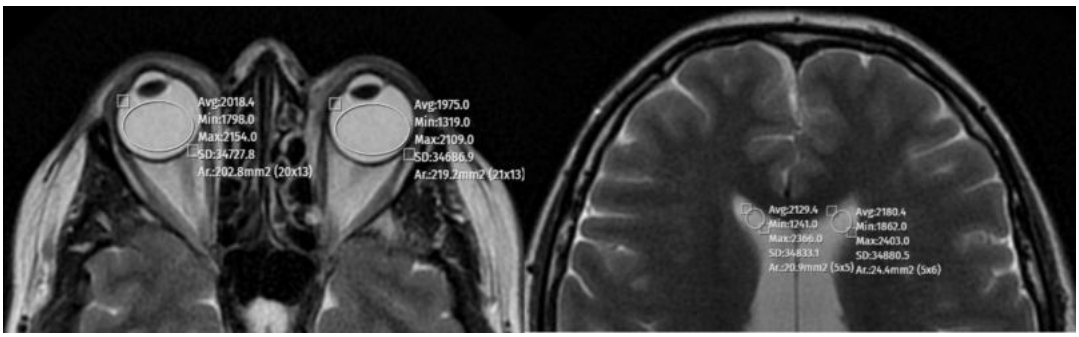


Fig. 1. An example of measurement of T2 values of vitreous and cerebrospinal fluid in axial sections.

T2W signal values to the values of the CSF at the level of the ipsilateral lateral ventricular frontal horn (Fig. 1).

Statistical Analysis

Whether the data conformed to the normal distribution was analyzed via the Kolmogorov–Smirnov test. Data not showing normal distribution were analyzed with the Pearson correlation test, and r coefficients and p values were calculated. The regression coefficient was calculated by conducting regression analysis between age and vitreous values, and the regression equation was established. Vitreous humor values between decades were compared with the ANOVA test and post hoc analysis was made. The results were considered statistically significant at $p < 0.05$. SPSS 17.0 (IBM Inc., Armonk, NY, USA) was used for the statistical analysis.

RESULTS

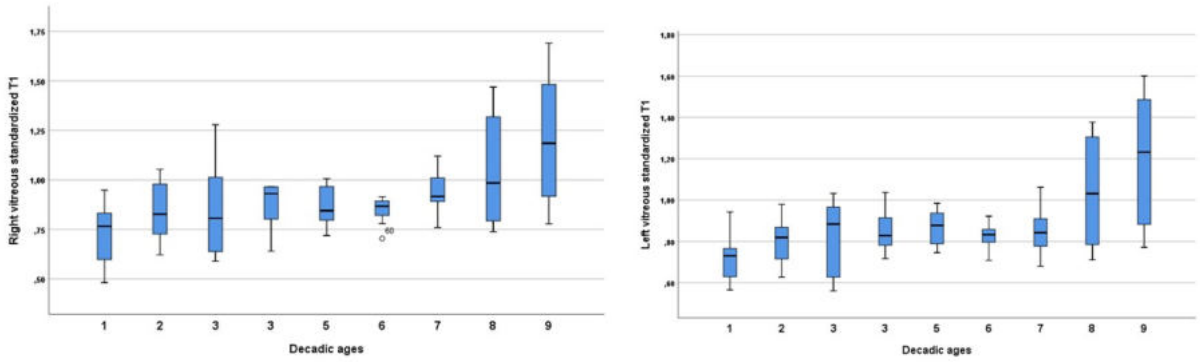
A total of 90 patients, 45 male, and 45 female, aged between 0 months and 87 years were included in the study. Each of the 9 decadic age groups consisted of 5 males and 5 females. The mean age of all patients was 43.6 ± 25.5 years.

The mean T1W value of the vitreous fluid was measured as 149.9 ± 55.1 in the right eye and 146.9 ± 52.1 in the left eye, and no difference was detected between the decadic age groups in terms of the mean T1W values for the right and left ($p = 0.912$ and $p = 0.903$, respectively). Likewise, the mean T2W value of the vitreous fluid was measured as 839.9 ± 349.1 in

Tables 1. Standardized T1, standardized T2 and ADC values of the cases according to decades for right and left eyes

	Right vitreous standardized T1				Right vitreous standardized T2				Right vitreous ADC			
Decadic Age Groups (year)	**Mean**	**SD.**	**Min.**	**Max.**	**Mean**	**SD.**	**Min.**	**Max.**	**Mean**	**SD.**	**Min.**	**Max.**
1 (0-10)	.736	.150	.48	.95	.844	.126	.58	.98	3291.10	294.89	2741.46	3695.15
2 (11-20)	.845	.151	.62	1.05	.785	.194	.52	.99	3443.38	175.82	3159.45	3716.12
3 (21-30)	.846	.223	.59	1.28	.824	.152	.57	.96	3395.18	318.27	3037.15	3905.89
4 (31-40)	.867	.126	.64	.97	.815	.167	.61	1.11	3310.01	237.68	2972.73	3620.99
5 (41-50)	.865	.098	.72	1.01	.878	.154	.60	1.01	3280.94	222.95	3054.48	3594.87
6 (51-60)	.849	.065	.70	.92	.832	.144	.64	1.01	3222.87	286.51	2882.11	3675.43
7 (61-70)	.942	.107	.76	1.12	.848	.139	.65	.97	3325.18	300.01	2885.52	3734.04
8 (71-80)	1.059	.284	.74	1.47	.835	.186	.60	1.10	3218.05	222.76	2966.31	3521.52
9 (81-90)	1.208	.340	.78	1.69	.914	.318	.60	1.70	3254.59	327.67	2834.66	3815.86
	Left vitreous standardized T1				**Left vitreous standardized T2**				**Left vitreous ADC**			
Decadic Age Groups (year)	**Mean**	**SD.**	**Min.**	**Max.**	**Mean**	**SD.**	**Min.**	**Max.**	**Mean**	**SD.**	**Min.**	**Max.**
1 (0-10)	.726	.120	.57	.94	.820	.108	.64	.96	3301.39	292.52	2873.09	3642.84
2 (11-20)	.807	.118	.63	.98	.805	.172	.56	1.04	3484.87	183.73	3261.11	3793.10
3 (21-30)	.821	.179	.56	1.03	.834	.125	.62	.99	3390.15	309.98	2948.89	3825.00
4 (31-40)	.849	.103	.72	1.04	.782	.121	.60	.95	3316.97	241.84	2957.06	3650.26
5 (41-50)	.866	.085	.75	.98	.860	.135	.60	1.02	3286.67	262.00	2984.86	3630.66
6 (51-60)	.825	.064	.71	.92	.833	.110	.67	.96	3232.75	305.25	2855.52	3716.48
7 (61-70)	.849	.109	.68	1.06	.839	.140	.61	.98	3341.04	300.27	2907.75	3738.90
8 (71-80)	1.047	.268	.71	1.38	.790	.162	.60	1.01	3210.80	232.12	2957.10	3667.89
9 (81-90)	1.186	.322	.77	1.60	.888	.289	.62	1.64	3331.24	306.06	2850.79	3873.54

SD = standard deviation, Min. = minimum, Max. = maximum



a b

Fig. 2. Box-line plot of standardized T1 values of vitreous humor according to decades for right (a) and left (b) eyes.

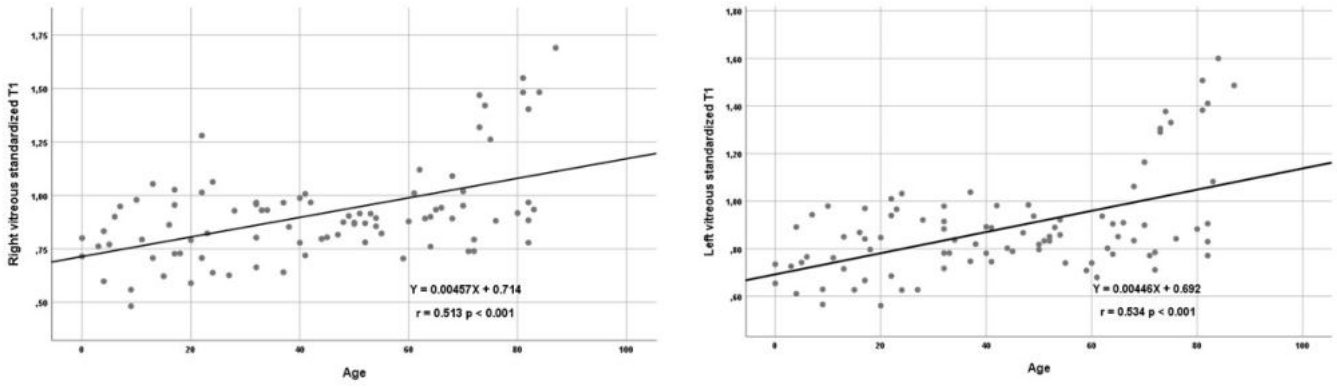
the right eye and 836.7 ± 358.7 in the left eye, and no difference was determined between the decadic age groups regarding the mean T2W values for the right and left ($p = 0.966$ and $p = 0.983$, respectively).

Standardized T1W values of vitreous fluid obtained by dividing T1W values of vitreous fluid by CSF T1W values are presented in Table 1 for all patients according to decadic age groups. Standardized mean T1W values of vitreous fluid were measured as 0.913 ± 0.226 in the right eye and 0.887 ± 0.213 in the left eye, and a significant difference was determined between decadic age groups in terms of the standardized T1W values for right and left ($p < 0.001$ and $p < 0.001$, respectively). Post hoc analysis of mean standardized T1W values revealed that group 9 (81-90 years) values were significantly different for both eyes from all age groups except group 8 (71-80 years) ($p < 0.05$) (Fig. 2). The mean standardized T1W values observed in group 8 (71-80 years) were found to be different from group 1 for both eyes ($p < 0.05$).

The standardized mean T2W values of the vitreous were measured as 0.842 ± 0.179 in the right eye and 0.828 ± 0.157 in the left eye, and no difference was determined between the decadic age groups regarding

Table 2. Correlation of different magnetic resonance imaging (MRI) variables with ages for rigth and left eyes

Variables		Age (Right Vitreous)	Age (Left Vitreous)
Vitreous T1	r	.070	.075
	p value	.511	.481
Cerebrospinal fluid T1	r	-.165	-.167
	p value	.120	.115
Standardized T1	r	.515	.534
	p value	**< 0.001**	**< 0.001**
Vitreous T2	r	-.042	-.060
	p value	.694	.576
Cerebrospinal fluid T2	r	-.070	-.067
	p value	.514	.530
Standardized T2	r	.130	.091
	p value	.220	.393
Vitreous ADC	r	-.171	-.140
	p value	.107	.188



a **b**

Fig. 3. Pearson linear regression plot between age and vitreous humor standardized T1 values for right (a) and left (b) eyes.

the standardized T2W values for the right and left (p = 0.915 and p = 0.899, respectively).

Mean ADC values of vitreous were found to be 3304.6 ± 266.9 mm^2/s for the right eye, and 3321.7 ± 272.2 mm^2/s for the left eye, and no significant difference was found between decadic age groups in terms of ADC value for right and left (p = 0.622 and p = 0.524, respectively).

Standardized T1W values of vitreous fluid demonstrated moderate correlation with age for both the right (r = 0.514, $p < 0.01$) and left eyes (r = 0.534, $p < 0.01$). Other measurements did not correlate with age (Table 2). Linear regression models, showed that every one year increase in age corresponded to an increase of 0.0045 units in standardized T1 value of vitreous for the right and left eyes (CI: 0.632 to 0.796; $p < 0.001$ and CI: 0.617 to 0.768; $p < 0.001$, respectively) (Fig. 3).

DISCUSSION

In this study, normative values of age-related changes in the vitreous in the normal population were calculated for different MRI sequences, and standardized T1W values were found to be correlated with age.

This age dependent change in the vitreous can be explained by ongoing liquefaction with aging [6]. The first theory regarding the gelous structure of the vitreous, was put forward by Duke-Elder and is widely accepted today. According to the theory, 'the structure of the vitreous consists of loose and sensitive filaments surrounded by liquid' [7]. Subsequently, in the 17th-18th centuries, different morphological and histological characteristics of the vitreous were described by Alveolar theory, Lamellar theory, Radial sector theory, and Fibrilar theories [8]. Accordingly, it was suggested that the vitreous was homogeneous in volunteers under 30 years of age, while macroscopic fibers were located centrally in the middle-aged group. With aging, thickening of the fibers and development of tortuosity has been observed, and a decrease in vitreous volume and collapse have been described with the surrounding of the fibers by the vitreous [9]. Balazs *et al*. [10] reported that the fibrils degenerated and liquid lacunae formed around them in the 80-90 age group, and that half of the vitreous was liquid. Likewise, in our study, standardized T1W values increasing with growing age also support this reorganization in the fibrillar structure.

Collagen is a key component in the fibrillar vitreous structure and with aging, the collagen component on the fibril surface decreases. Collagen fibrils, which break up and form small fragments due to age-related liquefaction, form aggregates to preserve the non-liquefacted gelatinous vitreous structure [11]. Hyaluronic acid is another key ingredient in the vitreous and contributes to its viscosity. The decrease of hyaluronic acid and proteoglycans with aging leads to liquefaction, which could be associated with an increase in standardized T1 values, as in our study [12].

In addition to conventional and modern histological examinations, microscopic and ophthalmoscopic examinations are also used in the evaluation of the vitreous [13]. MRI, however, allows non-invasive examination of the entire the globe structure, the vitreous,

as well as the extra-orbital area. Currently, 1.5 T and 3 T devices can be used for orbital imaging, and image resolution improves with 3 T MRI due to its stronger magnetic field. However, as the magnet power increases, the sensitivity to magnetic inhomogeneity also increases and it becomes more susceptible to possible artifacts. Orbit is a region where MRI artifacts are frequently seen because of the air-filled paranasal sinuses and bone structure around it. As a consequence of this, distortion due to magnetic susceptibility artifact is more common in images obtained on 3T devices. Although this situation sometimes reduces the detectability of lesions, artifacts that may occur can be prevented with the use of appropriate coils and extraction techniques [14]. Lesions can be identified in sequences such as T1W, T2W, and DWI, and pathological contrast enhancement can be demonstrated in the lesion after intravenous contrast agent (IVCM) administration [15]. Since it does not require ionizing radiation, it can be used in different age groups, and signal changes due to aging can be evaluated using MRI. Revealing these changes with MRI could allow early diagnosis and treatment.

In MR examinations, the vitreous signal was is seen as hyperintense in T2W examinations due to its high (98%) water content, while it is hypointense in T1W examinations compared to the extraocular muscles [15]. The studies in the literature on age-related changes in the vitreous are limited. In the study conducted by Kupeli *et al*. [16] on aging and changes in the vitreous, a positive correlation was found between the increasing age and ADC values after the third decade, whereas no significant difference was found between ADC values in the first three decades, showed a significant difference. In the study by Meral *et al*. [17], a significant difference was reported between pediatric and adult groups regarding ADC values.However, there was no regular increase in ADC values with age. Similarly, no significant correlation was found between ADC values and decadic age groups in our study. This situation can be explained by vitreous heterogeneity and disorganized diffusion environment as a result of liquefaction occurring with aging.

In the literature, there is no detailed information on T1W and T2W MRI signal changes that occur in the vitreous with aging. In our study, an increasing trend was observed in the standardized T1W values of decadic age groups, and a moderate positive correlation was determined between standardized T1W values and age. This can be explained by the structural change of protein groups in the vitreous with aging and the relative increase due to the decreasing amount of free water. On the other hand, no significant difference was found between decadic age groups in terms of T2W signals. The lacunae that form due to liquefaction over time might not sufficiently alter the already high T2W signal of the healthy vitreous.

Limitations

The limitations of the study include the relatively small number of patients included in the study and the inability to calculate interobserver compliance due to the evaluation of imaging by a single radiologist. Furthermore, the images were obtained from a 1.5T scanner and results might vary or additional correlations might be found on higher field strength scanners.

CONCLUSION

In conclusion, our study provides comprehensive normative data on the different MRI signal properties of the human vitreous and its change with age. Aging-related changes in the vitreous can be non-invasively assessed by MRI, particularly with standardized T1W values. MRI could provide an opportunity for earlier diagnosis and treatment of the pathological conditions of the vitreous. The information obtained from our study could serve as a basis for further studies aimed specifically at identifying vitreous MRI signal properties.

Authors' Contribution

Study Conception: BNK, OB; Study Design: BNK, TYK; Supervision: OB, FE; Funding: N/A; Materials: N/A; Data Collection and/or Processing: BNK; Statistical Analysis and/or Data Interpretation: TYK; Literature Review: BNK, TYK; Manuscript Preparation: BNK, TYK and Critical Review: OB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any

grant during conduction or writing of this study.

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The European Research Journal 2023;9(1):73-80 Original Article

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Sarcopenia is associated with mortality in patients with COVID-19 independent of other demographic risk factors

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ABSTRACT

Objectives: To investigate whether sarcopenia had an effect on in-hospital mortality independent from other demographic characteristics in patients with Coronavirus disease 2019 (COVID-19), and to determine a reliable cut-off value for sarcopenia if there is such a relationship.

Methods: A total of 302 patients with COVID-19 were included in the study. Sarcopenia was assessed by indexed skeletal muscle mass at T12 vertebrae level (T12-SMI) on initial chest computed tomography (CT). A receiver operating characteristic (ROC) curve analysis was performed to detect a cut-off value of T12-SMI for mortality prediction. Then, sarcopenia was diagnosed by this value. Multivariable logistic regression analysis was used to detect independent variables for mortality.

Results: Patients were separated into groups; 26 (8.6%) patients in the mortality group and 276 (91.4%) patients in the no-mortality group. In ROC analysis, cut-off values of 34.06 cm²/m² (sensitivity: 70%, specificity: 77%) in males and 29.36 cm²/m² (sensitivity: 67%, specificity: 69%) in females for T12-SMI were computed for mortality prediction. There were 110 (36.4%) patients with sarcopenia. Sarcopenia was more frequent in the mortality group than the no-mortality group (73.1% vs 33%, $p < 0.001$). In multivariate analysis age, previous cardiovascular and respiratory disease, and sarcopenia were independently associated with mortality in COVID-19 patients.

Conclusions: A cut-off value of 34.06 cm²/m² in males and 29.36 cm²/m² in females for T12-SMI can be used to diagnose sarcopenia in patients with COVID-19. Sarcopenia is clearly associated with mortality in these patients.

Keywords: COVID-19, sarcopenia, mortality, computed tomography, skeletal muscle index

Coronavirus disease 2019 (COVID-19) caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) has led to a worldwide outbreak [1]. Until today, millions of people have been infected with the disease and still tens of thousands of people continue to caught the disease every day. Its clinical course varies from patient to patient and from asymptomatic situation to severe respiratory collapse

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and even death. Health care services in many countries have faced serious difficulties in responding to heavy patient burdens. Therefore, anticipating the people who have increased mortality rate is especially crucial to form an individual treatment plan and to manage healthcare resources efficiently. Some baseline demographic features such as older age, diabetes mellitus (DM), cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD) as well as laboratory parameters including elevated D-dimer have already been recognized as major determinants of worse prognosis in patients with COVID-19 [2-4].

Sarcopenia is diagnosed by the presence of low muscle quantity or quality, low muscle strength, and impaired physical performance [5]. It is known that sarcopenia is associated with impaired immune resistance and susceptibility to infectious diseases such as pneumonia [6, 7]. In addition, sarcopenia is associated with heart and respiratory diseases, DM, renal disease and increases the risk of hospitalization, and death [8-12]. These clinical problems are also firmly related with poor prognosis in patients with COVID-19 [2-4]. In the literature, there are a few studies with conflicting results regarding the impact of sarcopenia on adverse outcomes in patients with COVID-19 [13-15]. Moreover, there are no standardized values for the evaluation of sarcopenia in this population. Therefore, we had two purposes in this study; first, to investigate whether sarcopenia had an effect on in-hospital mortality independent from other demographic characteristics, and second, to determine a reliable cut-off value for sarcopenia if there is such a relationship.

METHODS

Patient Selection

Study population consisted of consecutive adult patients who applied to the COVID-19 outpatient clinic between December 1, 2020 and December 30, 2020 and were diagnosed with COVID-19 by reverse transcription-polymerase chain reaction (RT-PCR) test. Patients who underwent unenhanced chest computed tomography (CT) examination were included in the study but those with no or inadequate CT imaging were excluded from the study. In addition, those with no follow-up data for intensive care unit (ICU) admission, intubation, and mortality were also excluded from the study. Hypertension (HT), DM, CVD (coronary heart disease or heart failure), and COPD were diagnosed by the presence of the previous history and/or drug use.

The study was conducted in accordance with the guidelines in the Declaration of Helsinki. The study protocol was approved by our Local Research Ethics Committee and written informed consent was obtained for all of the participants. (the report number: 2011-KAEK-25 2020/11-11 of University of Health Sciences, Bursa Yüksek Ihtisas Training and Research Hospital Ethics Committee).

Clinical Examination

Patients were classified into four groups according to their clinical symptoms, signs, and chest imaging manifestations as being mild, moderate, severe, or critical COVID-19 cases:

1. Mild cases: mild or minimal clinical symptoms, no sign of pneumonia on chest imaging
2. Moderate cases: fever and respiratory symptoms, pneumonia on chest imaging
3. Severe cases: severe respiratory distress and/or increased respiratory rate ≥ 30 breaths/min and/or decreased oxygen saturation (SpO_2) on room air with ≤ 93 % and/or arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ≦ 300 mmHg
4. Critical cases: respiratory failure and requiring mechanical ventilation and/or septic shock and/or other organ failure requiring ICU admission [16].

Assessment of Sarcopenia and Chest Computed Tomography Imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) are accepted as gold standard methods to evaluate muscle quantity and quality [17]. Sarcopenia was assessed by chest CT obtained during the routine initial evaluation of patients with COVID-19. Chest CT images were acquired using a 64 slice multi-detector CT scanner (Somatom Sensation, Siemens, Germany). Initially, cross-sectional area (CSA, in cm^2) of all skeletal muscles at the level of the twelfth vertebrae (T12) were measured by using OsiriX Lite software (version 7.0.2, Pixmeo SARL, Bernex, Switzerland). The Hounsfield Units (HU) of -29 to +150 were used to isolate the skeletal muscle. Thus, the exact skeletal muscle area (T12-SMA, cm^2) was calculated based on HU, excluding vasculature

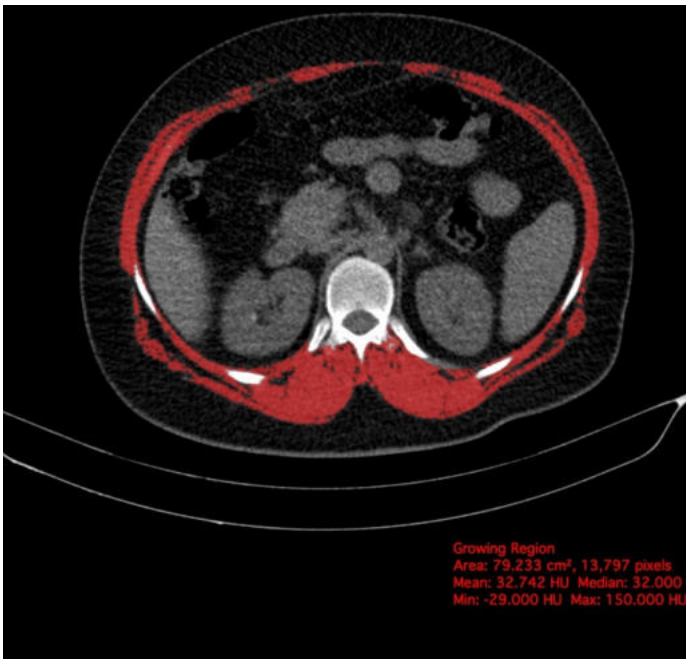

Fig. 1. T12-SMA measurement on chest CT image at the T12 vertebra level. Red zone indicating the crosssectional skeletal muscle area identified using a threshold of -29 to + 150 HU (T12-SMA 79.23 cm², T12-SMI 30.07 cm²/m²).

and fat infiltration (Fig. 1). Then, CSA measurements were normalized to patient size by dividing CSA with height in square meters to provide the skeletal muscle index (T12- SMI; cm2 /m2) [13, 14].

Study Endpoints

The main endpoint of the study was in-hospital mortality caused by COVID-19. The study population was separated into two groups as mortality (+) and mortality (-). In addition, the presence of ICU admission and intubation were determined second line endpoints of the study. Lastly, we created a combined end-point including mortality, ICU admission, and intubation.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation and categorical variables were expressed as numbers and percentages. Normal distribution of continuous variables was evaluated by using the Kolmogorov-Smirnov test and histogram. Continuous variables were analyzed by independent-sample t-test or Mann-Whitney U test according to normal distribution. Chi-square and Fisher's exact tests were used for categorical variables. Receiver operating characteristic (ROC) curve analysis was performed to detect a cut-off value for sarcopenia diagnosis. Then, multivariable logistic regression analysis was used to detect independent demographic variables for mortality. Intra- and inter-observer variability was computed for reproducibility of T12-SMA by predefined Bland-Altman method [18, 19]. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were carried out by the SPSS 21 statistical software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

A total of 302 patients were included in the study. There were 26 patients in the mortality group (8.6%) and 276 patients in the no-mortality group (91.4%). The baseline demographic and laboratory parameters of the study population were demonstrated in Table 1. DM, HT, CVD, and COPD were more frequent in the mortality group than the no-mortality group. T12-SMA, T12-SMI, age, C-reactive protein (CRP), white blood cell (WBC), and creatinine were higher but SpO_2 was lower in the mortality group than the no-mortality group. Intra- and inter-observer variability were assessed for T12-SMA and were found 4.9% and 6.8%, respectively.

In ROC analysis, cutt of values of 34.06 cm2/m2 (AUC = 0.758, $p = 0.002$, sensitivity 70%, specificity 77%) in males and 29.36 cm^2/m^2 (AUC = 0.772, $p = 0.001$,sensitivity 67%, specificity 69%) in females were detected for T12-SMI in association with mortality (Figs. 2 and 3). Then, patients with sarcopenia were determined in accordance with this cut-off value. Fifty (34.2%) of males and 60 (38.5%) of females had sarcopenia in the study population. Sarcopenia was more frequent in the mortality group than the no-mortality group (73.1% vs 33%, $p < 0.001$). Demographic features and end-points of patients with- and without sarcopenia were presented in Table 2. The patients with sarcopenia had more severe COVID-19 disease than those without sarcopenia. Hospitalization (53.6% vs 31.8%, $p < 0.001$), ICU admission (20.9% vs 7.3%, $p = 0.001$), intubation (18.2% vs 5.2%, $p < 0.001$) and combined end-point (21.8% vs 7.3%, $p < 0.001$) as well as mortality (17.3 vs 3.6%, $p < 0.001$) were more frequent in patients with sarcopenia than those without.

Table 1. Baseline demographic and laboratory variables of study population

	Mortality (+) (n = 26)	Mortality (-) (n = 276)	*p value*
Female gender, n (%)	13 (50)	143 (51.8)	1.0
Diabetes mellitus, n (%)	10 (38.8)	55 (19.9)	**0.04**
Hypertension, n (%)	16 (61.5)	72 (26.1)	**< 0.001**
CVD, n (%)	13 (50)	26 (9.4)	**< 0.001**
COPD, n (%)	7 (26.9)	18 (6.5)	**< 0.001**
Age, years	69.7 ± 11.1	51 ± 16.2	**< 0.001**
SpO_2 (%)	80.7 ± 12.6	95.9 ± 40.1	**< 0.001**
CRP (mg/L)	95.9 ± 58.8	29.9 ± 44.6	**< 0.001**
WBC ($\times 10^9$/L)	9.3 ± 6.8	6.5 ± 2.6	**0.01**
Creatinine (mg/dL)	1.3 ± 1.0	0.9 ± 0.7	**< 0.001**
T12-SMA (cm^2)	80.4 ± 23.5	98.1 ± 21.6	**0.001**
T12-SMI (cm^2/m^2)	28.7 ± 7.0	34.6 ± 6.3	**< 0.001**
Sarcopenia, n (%)	19 (73.1)	91 (33)	**< 0.001**

Data are shown as mean ± standard deviation or n (%). COPD = chronic obstructive pulmonary disease, CRP = C-reactive protein, CVD = coronary artery disease and heart failure, SpO_2 = arterial blood oxygen saturation, T12SMA = spinal muscle area at T12 level, T12SMI = spinal muscle index at T12 level, WBC = white blood cell

The results of the univariate and multivariate analysis were shown in Table 3. In univariate analysis, age, DM, HT, COPD, CVD, and sarcopenia were associated with mortality. In multivariate analysis, age (OR: 1.051, 95% CI: 1.010-1.093, $p = 0.014$), COPD (OR: 5.731, 95% CI: 1.683-19.522, $p = 0.005$), CVD (OR: 5.246, 95% CI: 1.796-15.318, $p = 0.002$) and sarcopenia (OR: 6.091, 95% CI: 1.945-19.078, $p = 0.002$) were independently associated with mortality in patients with COVID-19.

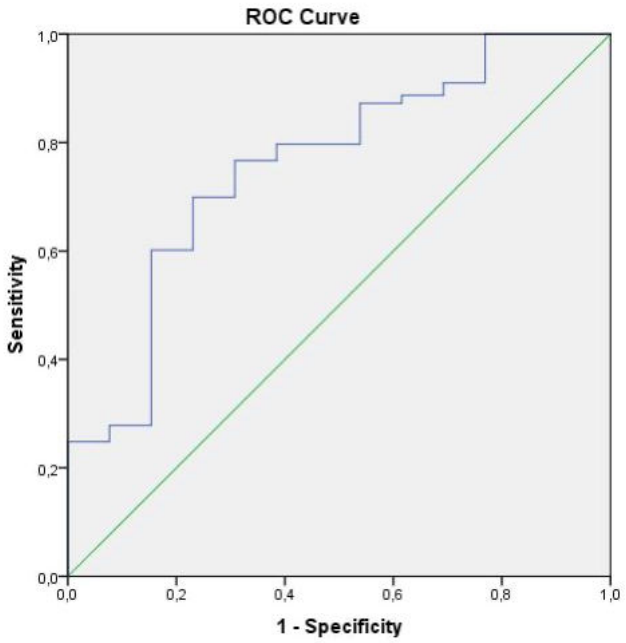

Fig. 2. ROC analysis for cut-off value of T12-SMI in male patients. AUC: 0.758, $p = 0.002$. Sensitivity 70%, spesicifity 77% for 34.06 cm^2/m^2 of T12SMI.

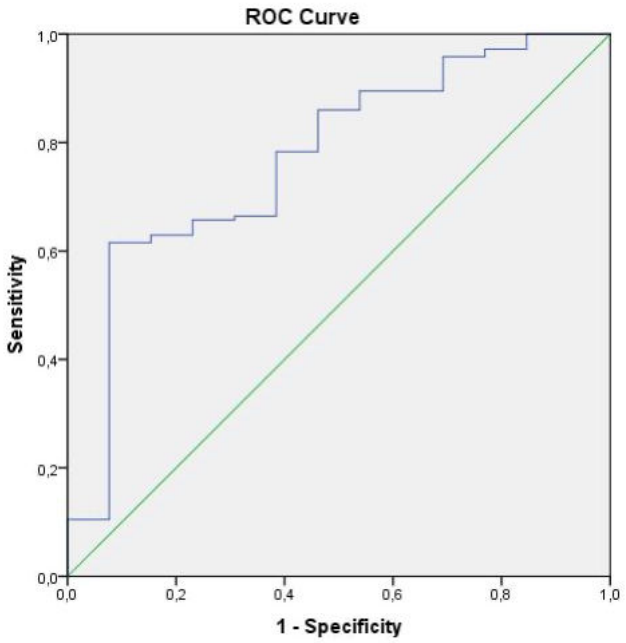

Fig. 3. ROC analysis for cut-off value of T12-SMI in female patients. AU: 0.772, $p = 0.001$. Sensitivity 67%, spesicifity 69% for 29.36 cm^2/m^2 of T12SMI.

Table 2. Baseline demographic and laboratory variables of patients with- and without sarcopenia

	Patients with sarcopenia (n = 94)	Patients without sarcopeina (n = 208)	*p value*
Age (years)	56.7 ± 19.3	50.3 ± 14.5	**0.003**
Female gender, n (%)	60 (54.5)	96 (50)	0.45
CRP (mg/L)	50.5 ± 61.9	27.0 ± 38.4	**0.025**
WBC ($\times 10^9$/L)	7.14 ± 3.5	6.5 ± 3.1	0.145
Ferritin (ng/mL)	416.7 ± 981.7	174.0 ± 172.9	**0.007**
D-dimer (µg/mL)	1.4 ± 2.6	0.7 ± 0.9	**0.06**
Diabetes mellitus, n (%)	22 (20)	43 (22.4)	0.63
Hypertension, n (%)	34 (30.9)	54 (28.1)	0.61
CVD, n (%)	16 (14.5)	12 (23)	0.52
COPD, n (%)	8 (7.3)	17 (8.9)	0.63
Disease severity, n (%)			
Mild	26 (23.6)	59 (30.7)	**< 0.001**
Moderate	45 (40.9)	106 (55.2)	
Severe	26 (23.6)	22 (11.5)	
Critical	13 (11.8)	5 (2.6)	
Hospitalization, n (%)	59 (53.6)	61 (31.8)	**< 0.001**
Pneumonia on CT, n (%)	84 (76.4)	133 (69.3)	0.19
Mortality, n (%)	19 (17.3)	7 (3.6)	**< 0.001**
Intubation, n (%)	20 (18.2)	10(5.2)	**< 0.001**
ICU admission, n (%)	23 (20.9)	14 (7.3)	**0.001**
Combined endpoint [&], n (%)	24 (21.8)	14 (7.3)	**< 0.001**

Data are shown as mean ± standard deviation or n (%). COPD = chronic obstructive pulmonary disease, CRP = C-reactive protein, CT = computed tomography, CVD = coronary artery disease and heart failure, ICU = intensive care unit, WBC = white blood cell

[&] Combination of mortality, intubation and ICU admission

Table 3. Demographic predictors of mortality in patients with COVID-19

	Univariable analysis				Multivariable analysis			
	OR	95% CI		*p value*	OR	95% CI		*p value*
		Upper	Lower			Upper	lower	
Age	1.086	1.051	1.123	**< 0.001**	1.051	1.010	1.093	**0.014**
Gender	1.075	0.481	2.403	0.86				
DM2	2.51	1.080	5.838	0.03	1.049	0.360	3.057	0.930
HT	4.53	1.968	10.443	**< 0.001**	1.405	0.445	4.441	0.562
COPD	5.28	1.963	14.206	**0.001**	5.731	1.683	19.522	**0.005**
CVD	9.615	4.035	22.914	**< 0.001**	5.246	1.796	15.318	**0.002**
Sarcopenia	5.518	2.239	13.602	**< 0.001**	6.091	1.945	19.078	**0.002**

COPD = Chronic obstructive pulmonary disease, CVD = Coronary artery disease and heart failure, DM2 = Diabetes mellitus type 2, HT = Hypertension

DISCUSSION

In this study, we stated that sarcopenia is more frequent in patients with COVID-19 who died and it is independently associated with mortality in this population. In addition, sarcopenic COVID-19 patients had more severe disease and increased rate of hospitalization, ICU admission, and intubation than those without sarcopenia.

COVID-19 pandemic has led to millions of cases and death around the world. The clinical picture during COVID-19 disease is very variable that changes asymptomatic situation to life-threatening respiratory collapse because of pulmonary involvement. Moreover, the clinical course can show very sudden changes. Unfortunately, there is no effective and specific treatment method to quickly control the disease. Because of these factors, the patients were usually followed up in the hospital especially during the inital time of the pandemic. This pushed health care systems to their limits in many countries. Therefore, identifying individuals at high risk of poor prognosis has become crucial in managing health system resources effectively. Some demographic features such as older age, DM, CVD, COPD were quickly reported as worse prognosis indicators in patients with COVID-19 [2-4]. However, we still need to expand our knowledge about risk factors that have a negative effect on mortality because the COVID-19 pandemic is still continuing and health systems in some countries are facing serious challenges to compensate patient burden.

Sarcopenia is a relatively novel issue that reflects the presence of low muscle quantity or quality, low muscle strength, and low physical performance [5]. It is not only a part of musculoskeletal system disease but also is closely connected with other organ system disorders. Additionally, clinical evidence has shown that sarcopenic patients have impaired immune responses to infectious diseases [6]. These patients have a higher incidence of community-acquired pneumonia and nosocomial infection, and increased risk of infectious complications and poor prognosis [7, 20-22]. Some mechanisms were proposed to reveal the relationship between sarcopenia and susceptibility to infections and immune compromise. Skeletal mucle is currently considered as an organ breeding several solvable components (myokines) such as Interleukine (IL)-15 and IL-7 affording autocrine and paracrine reactions [23]. Beside promoting muscle regeneration and physiology, myokines also regulate immune reactions. For example, IL-15 promptes the reproduction and activity of natural killer (NK) cells and CD8+ (cytotoxic) T lymphocytes, also triggers stimulation and scavenging function of neutrophils [24, 25]. Likewise IL-7 is secreted from skeletal muscle cells and it provides the development and maintenance of immature lymphocytes [26]. Since NK cells and lymphocytes yield crucial protection against viral agents, the deficient of IL-15 and IL-7 expression might bring ineffective immunity for viral infections and SARS-CoV-2.

In addition, inflammation may play another pivotal role in the relationship between sarcopenia and negative results of COVID-19. It is known that sarcopenia is associated with chronic inflammation detected by increased blood level of CRP, Tumor Necrosis factor-α, and IL-6 [27]. Similarly, it is obvious that COVID-19 is caused by a hyperinflammatory response induced by SARS-CoV-2. In this study, both CRP and ferritin were higher in patients with sarcopenia than those without. Therefore, we thought that sarcopenia-related chronic inflammation may have a role in the development of adverse results in patients with COVID-19.

There are a few studies that investigated the relationship between sarcopenia and adverse end-points of COVID-19 [13-15]. Kim *et al*. [13] investigated prognostic effects of sarcopenia in patients with COVID-19 patients. They asserted that sarcopenia assessed by chest CT at T12 level was an independent predictor for delayed hospital discharge but not for mortality. Moctezuma-Velázquez *et al*. [14] also studied the relationship between sarcopenia and negative outcomes in patients with COVID-19. They reported that sarcopenia was not related to in-hospital mortality, need for intubation, and ICU admission. However, they diagnosed sarcopenia by using a predefined cut-off value which was established for patients with aortic valve stenosis. We thought that this led to a generalizability bias that affected their results. In other research, Feng *et al*. [15] evaluated the effects of sarcopenia detected by paraspinal muscle measurement on chest CT on composite end-point including death, ICU admission, and intubation in patients with COVID-19. They used median values of paraspinal

muscle index and density to assess sarcopenia and found its robust association with composite end-point including death, ICU admission, and intubation [15]. Our study has some differences from previous ones. First, we aimed to assess the prognostic effects of sarcopenia in association with only demographic features that can offer a simple and objective prognostic tool during this patients' initial clinical evaluation. Second, we computed a cut-off value for T12-SMI to diagnose sarcopenia in this population, specifically. There was no spesific cut-off value for patients with COVID-19 in the literature. And, every study used a different value (ie. median value or smallest quarter of T12-SMI) to evaluate the presence of sarcopenia that led to uncertainty. Thus, according to our results, we thought that this study may present a simple prognostic parameter for these patients' initial triage.

Limitations

This study has some limitations. First, this was not a randomized study. However, it should be accepted that a randomized study of COVID-19 in the case of the pandemic has some ethical issues. Therefore, we had to do this research observationally. Second, in the multivariable model, we used only demographic parameters as well as sarcopenia. However, we initially intended to seek the prognostic effect of sarcopenia as an imaging-based demographic feature and intended to evaluate a possible effect of sarcopenia on mortality as a demographic feature. Third, we could not assess the possible long term-effect of sarcopenia on mortality, because we did not have a long-term follow-up after hospital discharge. Therefore, further studies are needed to investigate this issue.

CONCLUSION

Sarcopenia is independently associated with mortality in patients with COVID-19. Hospitalization, ICU admission, and intubation encounter frequently in patients with COVID-19 who have sarcopenia. A cut-off value of 34.06 cm2/m2 in males and 29.36 cm2/m2 in females for T12-SMI can be used to diagnose sarcopenia in this population.

Authors' Contribution

Study Conception: ME, DA, HES; Study Design: ME, DA, HES, MG; Supervision: DT, MG; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ME, MG; Statistical Analysis and/or Data Interpretation: DA, HES; Literature Review: ME, DA, HES; Manuscript Preparation: ME, DA, MG and Critical Review: ME, DA, DT, MG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(1):81-86 Original Article

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Disability assessment due to stroke

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ABSTRACT

Objectives: The main purpose of the study is to determine the frequency of stroke in patients who applied to the disability board. The secondary aim of the study is to determine the factors affecting disability rates in patients who applied to the disability board due to stroke.

Methods: Patients over the age of 18 who applied to the physical nedicine and rehabilitation outpatient clinic for disability assessment were included in the study. Disability board files of all patients were scanned retrospectively and the information obtained was recorded.

Results: It was found that 9.1% of patients who were evaluated for disability and were found to have any level of disability experienced disability due to stroke. A statistically significant relationship was found between stroke-related disability score and advanced age, female gender, and the presence of aphasia and dementia in the patients ($p < 0.05$). However, no statistically significant relationship was found between the type of stroke, the affected side and the stroke-related disability scores ($p > 0.05$).

Conclusions: Stroke patients constitute a remarkable part of disability assessment. When evaluating stroke patients in terms of their disability, comorbid diseases and demographic characteristics of the patients should also be taken into consideration.

Keywords: Stroke, disability, community health

Disabled individual; it refers to the individual who is affected by the attitudes and environmental conditions that restrict his full and effective participation in society on equal terms with other individuals due to the loss of his physical, mental, spiritual and sensory abilities at various levels. If the disability is; these are the ratings and classifications that determine the disability of the individual due to tissue, organ and/or function and psychiatry diagnosis and related loss of reasoning ability, based on international methods. Disability status assessment; it covers the evaluation of disease severity, organ or function loss with the aim of determining disability [1]. It is important that disability is evaluated in a standard way according to these ratings, so that disabled people can benefit from the rights provided by social support systems.

Stroke is defined as the sudden loss of brain functions as a result of cessation of blood flow to the brain [2]. Stroke is one of the most common causes of death worldwide and a leading cause of permanent and acquired disability in adults [3]. Every year, 15 million people worldwide have a stroke, resulting in 5 million deaths and permanent disability in 5 million [4]. A review noted that stroke, listed as the most common cause of disability, ranks second after arthritis and back pain in its impact on functional limitations [5]. In another study, stroke was associated with the highest disability-adjusted life expectancy loss of all dis-

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eases in China, with more than 2 million new cases per year [6]. Studies on stroke-related disability rates and associated risk factors in our country are limited. The rate of severe disability was found to be 57% in stroke patients who applied to the health board for disability assessment, and severe disability was associated with right-sided involvement, advanced age, dementia, and aphasia [7]. In another study, disability was found in 145 (4.2%) people out of 3443 selected by stratified sampling method in Kayseri province, and disability was found to be more common especially in women [8].

The main purpose of the study is to determine the frequency of stroke in patients who applied to the disability board. The secondary aim of the study is to determine the factors affecting disability rates in patients who applied to the disability board due to stroke.

METHODS

The study was carried out retrospectively and cross-sectionally in the physical medicine and rehabilitation outpatient clinic of Bursa Uludağ University. In this context, the medical records of 620 patients who applied to the outpatient clinic for disability status evaluation between November 15, 2019 and November 15, 2020 were retrospectively reviewed. Patients younger than 18 years of age were not included in the study because they did not comply with the regulations on disability assessment for adults. Apart from this, patients who applied for disability assessment but were not found to be disabled and patients whose disability board files could not be accessed were excluded from the study. When the patients were classified, those who had a stroke due to causes other than vascular stroke were considered to be admitted due to non-stroke causes. Among the disabled patients, the proportion of patients who applied to us for disability assessment due to stroke was determined. In addition, patients who applied for stroke were grouped separately and the factors affecting the disability rates of these patients were tried to be determined. The study was conducted in accordance with the principles of the

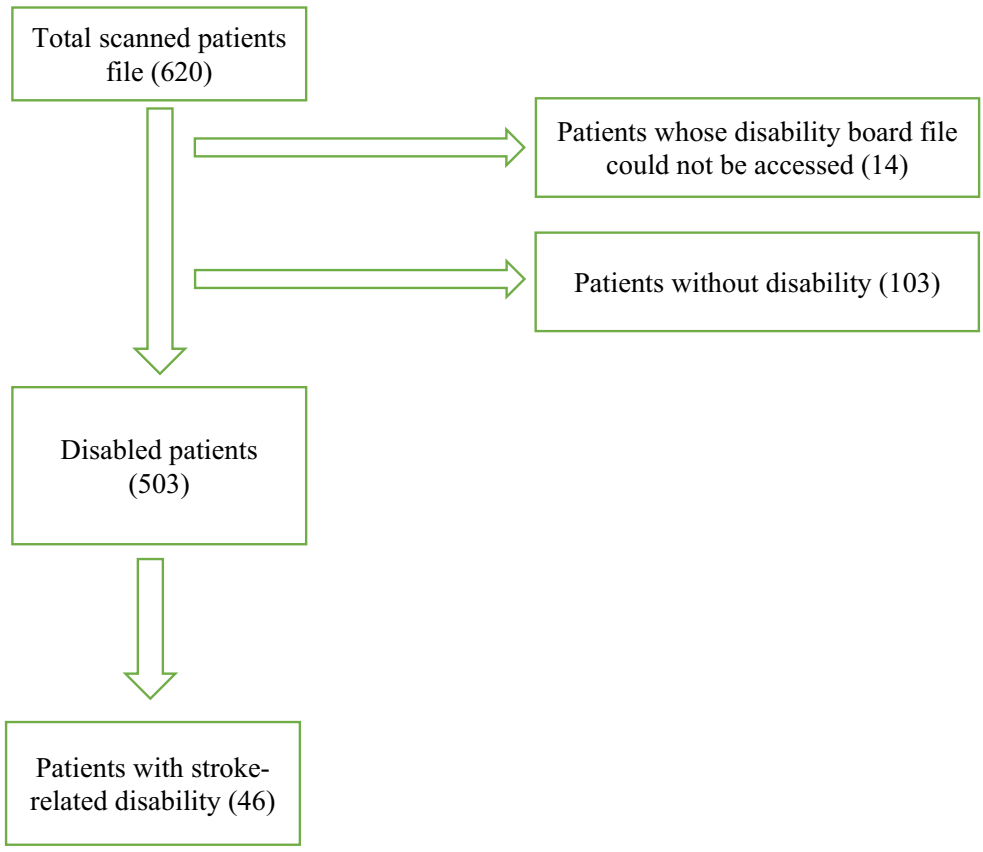

Fig. 1. Study flow chart.

declaration of Helsinki, with the approval of Bursa Uludağ University School of Medicine Clinical Research Ethics Committee (decision no: 2020-21/13, date: 25/11/2020).

Statistical Analysis

Analysis of the study was performed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) statistical analysis program. Conformity of continuous variables to normal distribution was examined with the Shapiro Wilk test, and the Mann Whitney U test was used for comparisons between groups according to the test result. In addition, Spearman correlation analysis was performed to evaluate the relationship between numerical data. Descriptive statistics are given as unit number (n), percentage (%) and median (minimum-maximum). Statistical significance was taken as 0.05 in the analysis.

RESULTS

Medical records of 620 patients over the age of 18 who applied to the disability board and were evaluated in the outpatient clinic were reviewed retrospectively. 503 of these patients were included in the study. The patients who were not included in the study were excluded because the disability board files could not be reached in the system or the disability was not detected. Stroke-related disability was detected in 46 patients. The flow chart of the study is given in Fig. 1. The status of stroke patients among the disabled patients is given in Table 1. A strong positive correlation was found between stroke-related disability score and age ($p < 0.001$, r = 0.676) (Table 2). In addition, a statistically significant relationship was found between stroke-related disability score and female gender, dementia and aphasia coexistence ($p < 0.05$) (Table 3).

Table 1. General distribution of patients with disability

	n	%
Stroke-related disability	46	**9.1**
Disability due to non-stroke causes	457	90.9

However, there was no statistically significant relationship between stroke-related disability score and the affected side and type of stroke ($p > 0.05$) (Table 3).

DISCUSSION

Stroke is one of the leading causes of disability associated with a neurological disease. Yang *et al*. [9] found the long-term disability rate after stroke to be 45%. Bensenor *et al*. [10] found the prevalence of post-stroke disability to be 29.5% in men and 21.5% in women. In the study of Teh *et al*. [11], it was determined that the dependence of elderly individuals who had a stroke in Singapore to another individual increased due to stroke. Another study, it was found that the Rankin Disability scores of 67.6% of stroke patients were negatively affected [12]. In general, we can say that stroke causes significant disability. We did not look at the prevalence of stroke-related disability in our study. We aimed to review the status of patients with stroke-related disability among other disabling musculoskeletal diseases. 9.1% of the patients included in the study were experiencing disability due to stroke. This situation showed a considerable rate among the disability status applications.

In the study of Türkel *et al*. [13], at the end of the 6-month follow-up of stroke patients under 65 years of age, a significant functional improvement was observed in most of the patients. We know that functional

Table 2. Disability rates and age

		Stroke-related disability scores	
		Correlation coefficient (r)	*p* value
Age			
Median	64.5	0,676	**< 0.001**
Min/Max	21.0-87.0		

Data are expressed as median (minimum-maximum).

Table 3. Disability rates and related factors

		Patients evaluated for stroke-related disability	Stroke-related disability scores	*p* value
Sex				
	Women	14 (30.4%)	86.0 (58.0-96.0)	**0.047**
	Men	32 (69.6%)	70.0 (20.0-98.0)	
Aphasia				
	Yes	19 (41.3%)	92.0 (28.0-98.0)	**< 0.001**
	No	27 (58.7%)	68.0 (20.0-92.0)	
Dementia				
	Yes	10 (21.7%)	92.0 (70.0-98.0)	**< 0.001**
	No	36 (78.3%)	73.0 (20.0-92.0)	
Stroke type				
	Hemorrhagic	12 (26.1%)	63.0 (20.0-92.0)	0.056
	Ischemic	34 (73.9%)	82.5 (20.0-98.0)	
Affected side				
	Right	23 (52.3%)	84.0 (20.0-98.0)	0.094
	Left	21 (47.7%)	67.0 (20.0-98.0)	

Data are expressed as n (%) and median (minimum-maximum).

recovery is more common especially in the early stages after stroke. From this point of view, the evaluation period of the patients will affect the level of disability. In our study, we did not evaluate stroke patients in terms of disability in the acute phase, where the condition of the patients can change rapidly and we cannot make a clear decision in terms of disability. The disability status assessment of the patients we evaluated was made at least 3 months after the event date.

In the study of Martin *et al*. [14], female gender was associated with increased disability in atrial fibrillation-related stroke. Kes *et al*. [15] found that advanced age and female gender were associated with increased disability at discharge in stroke patients. Because stroke pathology varies depending on sex [16], disability may vary after stroke. In our study, stroke-related disability scores were found to be statistically significantly higher in female patients than in male patients ($p < 0.05$). Since the patients we included in the study were selected only among the patients who applied to the disability board, they could not fully reflect the stroke patients in the community. Therefore, we cannot make a definitive judgment between sex and stroke-related disability.

In the study of Oyewole *et al*. [17], post-stroke disability status increased with advanced age. Lee *et al*. [18] found that recurrent stroke was associated with advanced age. Based on this, it was concluded that recurrent stroke is associated with increased disability in individuals, and disability due to stroke increases with advanced age [18]. Farzadfard *et al*. [19] found in their study that advanced age was associated with increased disability. In our study, stroke-related disability scores increased significantly with increasing age ($p < 0.001$). In the correlation analysis, a strong positive correlation was found between age and disability scores ($r = 0.676$). In this respect, we can say that our results are compatible with the literature.

In one study, no significant difference was found between the affected extremity in stroke and the quality of life of patients [20]. Oyewole *et al*. [17] found that right dominant extremity involvement was associated with worse disability status. In our study, although the disability scores were higher in the right-sided affected patients, no statistically significant relationship was found between the affected side and disability scores ($p > 0.05$). In fact, this situation is contradictory considering that the dominant extremity

is the right side in most of the population. Normally, patients affected by the dominant side are expected to score higher in the upper extremity according to disability legislation. Since the upper and lower extremity disability scores of most of the patients we evaluated were not written separately, whether the side involved between the upper extremities was effective or not could not be compared. Therefore, we cannot reach a clear conclusion on this issue.

In one study, the presence of aphasia was associated with poor functional outcomes in stroke patients [21]. Flowers *et al*. [22] found that patients with aphasia 28 days after stroke had more disability. In another study, it was stated that the coexistence of stroke and dementia was associated with an increase in disability status [23]. In our study, we thought that disability in stroke patients may be related to comorbidities. Of the stroke patients included in our study, 41.3% had aphasia and 21.7% had dementia. Stroke-related disability scores of patients with both aphasia and dementia were found to be statistically significantly higher ($p < 0.001$).

Rehabilitation is widely used to reduce disability and improve functional status in stroke patients [24]. In a systematic review, it was concluded that home-based rehabilitation program is effective in reducing disability in stroke and other diseases that cause physical disability [25]. In our retrospective analysis, we could not determine whether most of the patients received rehabilitation in the disability status reports. Therefore, we cannot comment on the relationship between disability and rehabilitation.

In a study, 56% of patients with ischemic stroke and 49% of patients with hemorrhagic stroke were found to be severely disabled at discharge [26]. In another study, patients with hemorrhagic stroke were found to have lower quality of life and mobility scale scores than patients with ischemic stroke [27]. Another study found a strong association between hemorrhagic stroke and worse lower extremity function [28]. In general, ischemic stroke is more common, but hemorrhagic stroke is thought to be responsible for more deaths and disability-adjusted life-year losses [29]. In our study, it was concluded that the type of stroke did not have a statistically significant relationship with the disability scores of the patients ($p > 0.05$). The reason why our study differs from the literature may be due to the fact that the patients who applied to the disability board did not show all stroke patients in the community homogeneously.

Limitations

There have been some limitations in our study. First of all, the study was a retrospective study. In addition, since the patients we evaluated were selected only from among the patients who applied to the disability board of our hospital, we do not reflect all the stroke patients in the society homogeneously, and therefore we cannot form a general judgment. Finally, the evaluation of the patients only in terms of the pathology constituting the disability rate prevented us from looking at the event from a broad perspective.

CONCLUSION

In conclusion, stroke patients constitute a significant portion of disability status assessment. When evaluating stroke patients in terms of disability, comorbidities and demographic characteristics of patients should also be taken into account.

Authors' Contribution

Study Conception: UE; Study Design: UE; Supervision: UE; Funding: UE; Materials: UE; Data Collection and/or Processing: UE; Statistical Analysis and/or Data Interpretation: UE; Literature Review: UE; Manuscript Preparation: UE and Critical Review: UE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Investigation of antioxidant and antimicrobial activities of walnut (*Juglans regia* L.) kernel septum

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ABSTRACT

Objectives: Walnut (*Juglans regia* L.) kernel septum (or septa) (WKS), a traditional nutraceutical material in China, has not been explored in detail. In this study, antimicrobial activity, total phenolic content (TPC) and antioxidant-oxidant status of WKS was investigated in case it may be clinically important in the management of various complications.

Methods: The WKS was extracted with ethanol in a Soxhlet device. TPC of WKS was analysed by using Folin-Ciocalteu's method. Antioxidant activity was obtained by using Rel Assay Diagnostics kits. The antimicrobial activity of WKS was evaluated against two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*), one Gram-negative bacteria (*Escherichia coli*) and one fungus (*Candida albicans*) strains using the agar diffusion method.

Results: The TPC of WKS was found to be 119.42 ± 2.39 mg GAE/gDW. It was determined that total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) values were 7.542 ± 0.389 mmol/L, 3.718 ± 0.287 µmol/L and 0.049 ± 0.001, respectively. WKS selectively inhibited the growth of Gram-positive bacteria and fungus, while *S. aureus* was the most susceptible one with 16 mm of inhibition zone. Gram-negative bacteria was resistant to the extract.

Conclusions: As far as we know, this paper is the first work that demonstrates the antioxidant-oxidant status of WKS by using the method described above, and moreover there are no scientific reports which have examined WKS in such a multidisciplinary experimental design. This study strongly supports the reported traditional use of WKS. Results indicated that WKS can be used as a pharmacological natural agent due to its high antioxidant and antimicrobial activities.

Keywords: *Juglans regia* L, walnut kernel septum, antioxidant, total phenolic content, antimicrobial activity

Herbal drugs are the oldest type of health service known by humankind. Medicinal plants have been used by all cultures throughout the history [1]. It is demonstrated in several studies that aromatic and medicinal plants are the source of many molecules with antioxidant and antimicrobial characteristics which can protect the body against both cellular oxidation reactions and pathogens. Thus, characterization of different parts of different types of plants is important in order to show their antioxidant and antimicrobial potentials [2].

In cases where reactive oxygen species (RES) are excessively produced or antioxidant mechanisms are insufficient, the failure of the balance between oxi-

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dant-antioxidant systems is defined as oxidative stress, and this condition has a significant role in the etiopathogenesis of many diseases [3]. Triggering factors caused by the modern lifestyle such as processed food, exposure to several chemicals, lack of exercise makes oxidative stress inevitable [4]. Weakening of antioxidant protection mechanisms that play an active role in decreasing the oxidative stress effects is a condition which increases tendency of body cells and tissues to develop diseases. Therefore, to continue having sufficient antioxidant levels without having excess dose are necessary to prevent many numbers of diseases and to take them under control [5].

It is known that phytochemicals such as phenolic compounds in plants decrease the risk of degenerative diseases by inhibiting the oxidation of macromolecules or decreasing the oxidative stress [6]. Bioactive materials including phenolic compounds have a large number of chemical functions and structures. It has been reported that bioactive components have a potential effect on health and particularly they have beneficial effects on prevention or delay of chronical diseases. However, complex, controlled, and long-term studies which would demonstrate these effects are still required [7].

On the other hand, increasing antibiotic resistance in bacteria is one of the main reasons of failure in fight with infectious diseases. Especially for an important number of immunosuppressed patients, infections are the most important reason of mortality and morbidity [8]. The rapid emergence of multidrug-resistant bacteria results in a constant requirement for novel antimicrobial components [9].

To date, walnut is accepted as a natural functional food due to its nutritious content and medical benefits [10]. Many previous pharmacological studies have shown that different parts of *Juglans regia* L. have nutritious, cardiovascular, antidepressant, antisedative, antimicrobial, antioxidant, anticancerous, antidiabetic, antiinflammatory, antiparasitic, immunological, analgesic, gastrointestinal, endocrinal, and several other pharmacological effects [1]. Use of walnuts and walnut trees has a long history. Therefore, green husk, kernel, shell, or other parts such as flowers or leaves of walnut have always drawn attention and not only found place in conventional medicinal systems but they are also used in pharmaceutical and beauty industries [11-13].

The woody tissue, that divides the walnut fruit into two parts from inside called as 'Walnut Kernel Septum' (WKS) is a by-product which has been included in traditional medical literature and has been used in diseases such as diabetes, insomnia, diarrhea, renal diseases and reproductive disorders. However, it has not gained the reputation it deserves as there are very few numbers of scientific studies about it [14].

This study focuses on this part of the walnut which is treated as a waste and has only limited usage area. The aim of this study is to evaluate the total phenolic content, antioxidant, and antimicrobial capacity of ethanolic WKS extract with a multidisciplinary experimental design.

METHODS

Chemicals

All chemicals/reagents used in this study were purchased from Sigma (Sigma Aldrich Chemie GmbH, Schnelldorf, Germany) and Merck (Darmstadt, Germany). All reagents and solvents used were of analytical grade. Water used in all analyses was ultra-pure produced by a Milli-Q system (Millipore, Bedford, MA, USA). Total oxidant status (TOS) and total antioxidant status (TAS levels) were determined by using Rel Assay Diagnostic kit RL009 and RL010, respectively.

Plant Material and Extraction Procedure

Walnuts (*Juglans regia* L.), which is naturally grown in Kaman district of Kırşehir province, were supplied commercially. The WKS of dried walnuts which separates the two kernel pieces were removed. The shadow-dried plant materials were chopped, just prior to pulverization with the help of a blender (Waring 8011 EB). WKSs (10 g) were extracted by a Soxhlet device (BUCHI Extraction System Model B811) with 100 mL ethanol (EtOH-%95, v/v, Merck) at 60°C for 8 hours. The extracts were filtered using Whatman filter paper (No:1) and then concentrated under vacuum at 40°C using a Rotary evaporator (Heidolph Hei-Vap Rotary Evaporator). Obtained ethanolic WKS extract was stored in a freezer at -20°C for further tests.

Biological Materials

Based on their pathogenic importance, the following test micro-organisms were selected for the antimicrobial activity assay; S*taphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633 as gram-positive bacteria; *Escherichia coli* ATCC 25922 as gram-negative bacteria and *Candida albicans* ATCC 90028 as fungus. All standard bacterial and fungal strains were obtained from Department of Medical Microbiology, Ahi Evran University, Kırşehir.

Quantitative Determination of TPC

Total phenolic content (TPC) of ethanolic WKS extract was determined by using the Folin-Ciocalteu (FC) method with minor modifications [15]. The principle of the method is based on the separation of a phenolic proton in alkaline medium and formation of a phenolate anion capable of reducing the FC reagent.

The prepared ethanolic WKS extract was diluted 100 times with Milli-Q water. 1 mL of the diluted sample was mixed with 1 mL of five times diluted FC reagent, prepared freshly before use. The reaction medium was kept at room temperature for 3 min. The mixture was then mixed with 3 mL of sodium carbonate (Na_2CO_3) solution (12.5%) and 15 mL of Milli-Q water. The solution was vortexed for 1 min and kept at 25 °C for 90 min. The absorbance value was obtained at 760 nm by using a spectrophotometer (Thermo Scienteilfic-Evolution 60S UV-Visible). Calibration curve used for quantification was obtained by using different concentrations of gallic acid. Gallic acid was used to obtain the standard curve (linear range 10-70 µg/mL with $R^2 = 0.9991$). The concentrations of phenolic compounds in all samples were expressed as micrograms of gallic acid equivalents per gram dry weight [µg of GAE/gDW].

Measurements of TOS

The measurement is based on the principle that the oxidants in the sample oxidize the ferrous ion-orthodianicidine complex to ferric ion and the ferric ion forms a blue-green complex with the chromogen in an acidic environment [16]. The colour intensity of the complex, which is proportional to the total amount of oxidant present in the sample, is measured spectrophotometrically at 530 nm wavelength. Hydrogen peroxide (H_2O_2) solution is used as a standard in the calibration of the method and the results are expressed as µmol H_2O_2 equiv/L. The assay was carried out as follows: 500 µl of Reagent 1 was mixed with 75 µl ethanolic WKE and OD1 (optical density) values were obtained at 530 nm 25 µL Reagent 2 was added to the mixture. OD2 values were obtained at 530 nm after 5 min incubation at 37°C. The TOS value was calculated by using the formula given in the kit prospectus.

Measurements of TAS

The principle of the measurement is based on the reduction of dark blue-green 2,2'-azinobis 3-ethyl benzothiazoline-6-sulphonate radical to colourless ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) form by the antioxidants in the sample [17]. The degree of ABTS reduction, which depends on the amount and capacity of antioxidants, is determined by the change in absorbance caused by the colour difference at 660 nm wavelength in the spectrophotometer. The method is calibrated with a stable antioxidant standard solution called "Trolox", which is widely used as a traditional standard for TAS measurement assays. The assay results are expressed in mmol Trolox equiv/L.

The assay was carried out as follows: 500 µl of Reagent 1 was mixed with 30 µl ethanolic WKE and OD1 values were obtained at 660 nm 75 µL Reagent 2 was added to the mixture. OD_2 values were obtained at 660 nm after 5 min incubation at 37°C. The TAS value was calculated using the formula given in the kit prospectus.

Measurements of OSI

The percentage ratio of TOS to TAS gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress. In order to perform the calculation, the unit of TAS, mmol Trolox equivalent/L, was changed to µmol Trolox equivalent/L. The OSI value was calculated according to the following formula [17]:

OSI = [(TOS, µmol H_2O_2 equivalent/L) / (TAS, µmol Trolox equivalent/L) × 100]

Determination of Antimicrobial Activity

Antimicrobial effects of ethanolic WKS extract were tested using the agar well method [18]. Bacteria was grown in Nutrient-Broth Agar (NBA) medium which was kept at 37°C overnight. Suspensions were prepared from these bacteria the next day according to 0.5 McFarland (a final inoculum of 1.5×10^8

CFU/mL) turbidity value. 100 µl of 0.5 McFarland bacterial suspension prepared in turbidity was added into 5 ml soft agar (0.5% agar) and poured onto the Müller-Hinton Agar (MHA) medium prepared in petri dishes. Wells were opened with the help of a 6 mm diameter glass pipette on the media that were left to dry for a while. Two wells were drilled in NBA medium where microorganisms were spread. 25 µl of ethanolic WKS extract was pipetted into one, while ethanol, the solvent of the extraction process, was pipetted into the other as a negative control. Ethanolic WKS extract and pure ethanol were sterilized by passing them through membrane filters with 0.22 µm pore diameter before use. On the other hand, Tetracycline (30 mg), Penicillin G (10 U), Sulbactam (10 mcg) + Ampicillin (10 mcg), Gentamicin (10 mcg), Rifampin (5 mcg), Teicoplanin (30 mcg), Ciprofloxacin (5 mcg), Chloromphenical Sterile discs containing (30 mg) were also carefully placed in MHA media and used as positive control. Antimicrobial activity was determined by measuring the zone diameters formed after 24 hours of incubation at 35 oC. After the incubation process, the inhibition zones were measured. The results were given as mean value of three independent measurements. The antibacterial activity was measured in terms of the diameter (mm) of clear zone of growth inhibition. The sensitivity was evaluated according to the National Committee for Clinical Laboratory Standards (NCCLS) [NCCLS, 1998] and the antimicrobial activity of the WKS was evaluated.

Statistical Analysis

All samples were analysed in triplicate (n = 3) and the results were expressed as mean ± SD (Standard Deviation).

RESULTS

TPC

In this study, phenolic concentration in ethanolic extract of WKS was measured and TPC of WKS was found as 119.42 ± 2.39 mg (GAE)/gDW. Experiments are made in 3 parallels.

Antioxidant Activity

In this study, antioxidant, and oxidant potentials of WKS has been determined and the oxidative stress index, which demonstrates how much the available antioxidant compounds can suppress the oxidant compounds, has been calculated. It was found that endogen antioxidant capacity produced by WKS was 7.542 ± 0.389 mmol/L, while oxidant compound level that is obtained due to environmental effects and metabolic activities was determined to be 3.718 ± 0.287 µmol/L. It was determined that the OSI, which shows the percentage of oxidant compounds that are tolerated by endogen antioxidant compounds was 0.049 ± 0.001 (Table 1). In the antibiogram test *E. coli* and *C. albicans* have been found to be resistant to the antibiotics; Ampicillin/Sulbactam, Rifampin, and Penicillin, while most effected by Ciprofloxacin and less effected by Chloramphenicol. *S. aereus*, has been effected by all antibiotics except Ampicillin/Sulbactam and has provided large inhibition diameters. *B. subtilis*; has shown resistance to the antibiotics; Rifampin, Penicillin and Teicoplanin, while most effected by Ciprofloxacin and least effected by Ampicillin/Sulbactam (Fig. 1). So, it has been demonstrated by the inhibition zone diameters formed that the antibiotics are efficient against many of the selected microorganisms.

Antimicrobial Activity

In this study, the antimicrobial potential of WKS has been investigated on two Gram-positive (*S. aureus, B. subtilis*), one Gram-negative (*E. coli*) bacteria, and *C. albicans*, an opportunistic yeast that can become pathogenic when our immune system is compromised. Ethanol, the extract solvent, was used as negative control, while Tetracycline (30 mg), Penicillin G (10 U), Sulbactam (10 mcg) + Ampicillin (10 mcg), Gentamicin (10 mcg), Rifampin (5 mcg), Teicoplanin (30 mcg), Ciprofloxacin (5 mcg), Chloram-

Table 1. TAS, TOS and OSI values

	TAS (mmol/L)	TOS (µmol/L)	OSI
Walnut kernel septum	8.407 ± 0.399	6.533 ± 0.366	0.078 ± 0.008

Values are presented as mean ± SD. Experiments are made in 3 parallels. TAS = Total antioxidant status, TOS = Total oxidant status, OSI = Oxidative stress index

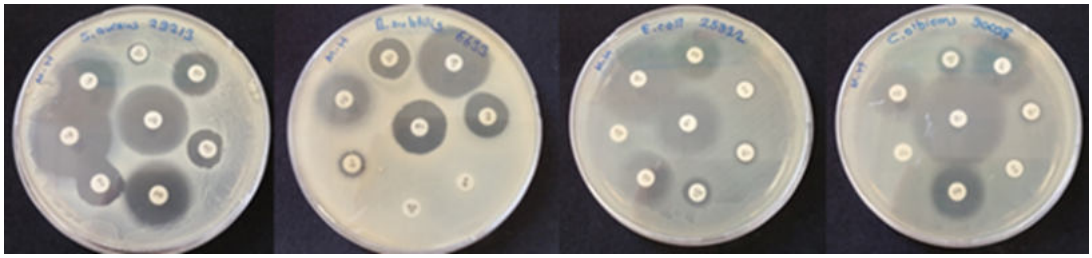

Fig. 1. **Antibiogram test.**

phenicol (30 mg) were used as positive control (Fig. 1).

In the antibiogram test *E. coli* and *C. albicans* have been found to be resistant to the antibiotics; Ampicillin/Sulbactam, Rifampin, and Penicillin, while most effected by Ciprofloxacin and less effected by Chloramphenicol. *S. aereus*, has been effected by all antibiotics except Ampicillin/Sulbactam and has provided large inhibition diameters. *B. subtilis*; has shown resistance to the antibiotics; Rifampin, Penicillin and Teicoplanin, while most effected by Ciprofloxacin and least effected by Ampicillin/Sulbactam (Fig. 1). So, it has been demonstrated by the inhibition zone diameters formed that the antibiotics are efficient against many of the selected microorganisms.

According to our findings, the results indicate that WKS ethanolic extract had antimicrobial effects against S. aereus (16 mm), *B. subtilis* (15 mm), and *C. albicans* (14 mm) with varying extent, but did not have any inhibitory effect on *E. coli* (12 mm) in terms of antimicrobial activity. The diameter of inhibition zone is shown in Table 2.

DISCUSSION

Due to their strong antioxidant characteristics and significant effects on preventing several diseases related with oxidative stress, herbal polyphenols are increasingly drawing attention. In the last few years, identification of phenolic compounds has been an important field in the researches about health and medicine. Phenolic compounds are the most found secondary metabolites of plants and have a wide distribution among plant tissues. Therefore, there are several studies in which different parts of plants are examined [19].

In a recent study by Hu *et al*. [20] in which phenolic composition and nutritious characteristics of WKS was investigated, TPC of WKS has been reported as 73.66 ± 0.73 g/100 gDW. In another study conducted by Liu *et al*. [21], polyphenol profile of WKS was aimed to be identified and it was determined that there were 75 individual phenolic compounds. Also, TPC of WKS has been expressed in terms of 122.78 ± 2.55 mg GAE/gDW. In an experimental study conducted by Ghiravani *et al*. [22] in 2016 on diabetic rats, polyphenol content of WKS ethanol extract has been found as 21.64 ± 1.44 mg GAE/gDW.

Also, Regueiro *et al*. [23] has reported mean total polyphenol content of different walnut extracts as 2.464 ± 22 mg GAE/100 g and highlighted that the results have varied between 1.576 and 2.499 mg GAE/100 g and this was consistent with other studies [24, 25]. In a comprehensive review article by Jahanban-Esfahalan *et al*. [26] , methanolic extract of walnut leaves were compared with aqueous extract (27.92 ± 1.40 mg); and it was stated that methanolic extract has demonstrated the maximum polyphenol content (94.39 ± 5.63 mg GAE/g extract). In another study conducted by Akbari *et al*. [27], phenolic compounds of different parts such as walnut hull, shell, pellicle (brown skin), and kernel have been investigated. TPC for hull, shell, skin and kernel were reported as follows, respectively: 24.68 ± 4.28mg GAE/g, 18.04 ± 4.20 mg GAE/g, 52.05 ± 1.27 mg GAE/g and 1.45 ± 0.12 mg GAE/g. In another study conducted by Popovici [28], ethanol extracts of walnut leaves, shell and WKS were investigated in respect of their total phenol content. In that article, total phenol content of WKS has been found significantly more than that of both leaves and shell extracts. Solvent extractions are the most convenient procedures to prepare extracts of plant materials due to their ease of use, efficiency, and large applicability. However, many methods such as

Table 2. Antibacterial and antifungal activity of WKS

Mean inhibition zone (mm)*	Microorganisms	Positive control								Negative control	
		SAM	C	RIF	PCN	TET	TEC	GM	CPFX	Ethanol	WKS
	B. subtilis 6633	11	22	R	R	18	R	16	34	12	15
	S. aereus 29213	R	23	27	15	20	14	17	25	12	16
	E. coli 25922	R	19	R	R	17	15	17	26	12	12
	C. albicans 90028	R	22	R	R	18	R	19	28	12	14

*Inhibition zone including well diameter (5 mm). SAM = Ampicillin/sulbactam, C = Chloramphenicol, RIF = Rifampin, PCN = Penicillin, TET = Tetracycline, TEC = Teicoplanin, GM = Gentamicin, CPFX = Ciprofloxacin R = Resistant

microwave, ultrasound supported extractions have been developed in recent years. Rusu *et al*. [29] have found total phenol content of WKS as 67.03 ± 9.76 mg GAE/gDW by Ultra-Turrax extraction method and 31.27 ± 5.24 mg GAE/gDW by maceration method. Taken together, different extraction methods, different types and parts of plants, use of different solvents have made it more difficult to compare the results of these studies; however, it can be easily distinguished that WKS is a rich source of phenolic compounds and yet, it has more phenolic content than other different parts of walnut.

It is reported that oxidative stress is related with development of many metabolic and chronical disorders. Within this context, antioxidants are referred as valuable molecules as they prevent the damage due to oxidative stress in defense mechanisms [30]. It is also reported that in case of antioxidant insufficiency, exogen antioxidant supplement (food/preparation) for the organism can also prevent formation of oxidative stress [31]. Therefore, it is especially important to evaluate antioxidants present in biological materials and food in respect of quantity and activity. Kusano and Ferrari [32] have not only highlighted that TAS measurement can be a reliable biomarker which can be used in diagnosis and prognosis of several pathophysiological conditions, but also have stated that to prevent initiation and development of diseases, to execute nutritional interventions including anti-aging strategies, it can also be used in determination of antioxidant-rich food.

The exploration of new antioxidant sources in nature can be possible by determining the antioxidant capacity of different parts of plants. Yet, these parts of plants might have significant medical potential even if they do not have nutritional features [33]. Currently, many total antioxidant capacity tests such as FRAP (Ferric Reducing-Antioxidant Power) [34], ORAC (Oxygen Radical Absorbance Capacity) [35], TEAC (Trolox Equivalent Antioxidant Capacity) [36] are widely used in the analysis of biological tissues and/or food materials [32]. In this study, a different automated method was used which directly measures the total antioxidant capacity colorimetrically, which is far less affected by the presence of uric acid, as reported by Erel *et al*. [17]. The most important advantage of this method is that it has not measured only a single compound's antioxidant capacity, but it has measured the total antioxidant capacity of all antioxidants in the biological sample [37]. So, known or unknown, any type of antioxidant interactions including possible additive effects have been evaluated as a whole.

No reference values could have been found as there were not found any study in literature which determines the antioxidant potential of WKS by the method we use. However, with the method reported by Erel, by different studies conducted on different plant types, TAS; TOS; OSI values of *Thymbra spicata* [38], *Gundelia tournefortii* [39], *Rumex crispus* [40] have been reported as 8.399, 6.831, 6.758; 6.530, 3.712, 5.802; 0.078, 0.054, and 0.086, respectively. When compared with these studies, TAS value of WKS was higher than that of *G. tournefortii* and *R. crispus*, and lower than that of *T. spicata*, which have been evaluated as having antioxidant properties. It is considered that these differences are due to the potential of the plants to produce compounds with antioxidant characteristics. In this regard, it can be said that WKS has a significant antioxidant potential.

In a review study where different extracts of dif-

ferent walnut parts such as kernel, skin, shell, husk have been examined comparatively in respect of antioxidant features, results obtained by DPPH (2.2.-Diphenyl-1-picrihydrazile), FRAP and ORAC methods have been reported [26]. In another study in which antioxidant activity of walnut male flowers was investigated, IC50 values were reported for DPPH and ABTS as 75.17 ± 4.43 µg/ml and 63.40 ± 5.73 µg/ml, respectively, while FRAP value was reported as 54.35 ± 3.12 µmol/L FeSO4/mg ethanolic extract [41]. It is understood that different parts of walnut might have different antioxidant potentials.

The only study that has been found in literature about the antioxidant activity of WKS has been the study of Rusu *et al*. in which ABTS, DPPH and FRAP methods were used. According to this study, antioxidant activity was reported as 174,28 ± 8,22 mg trolox equivalent (TE)/g DW WKS by ABTS method, while 255,89 and 400,97 mg TE/g WKS extract by DPPH and FRAP methods; WKS was evaluated as having good antiradical effects [29].

Wang *et al*. [42] have reported that WKS has formed anti-inflammatory activity by inhibiting nitric oxide production and claimed that this activity has been due to the presence of gallic acid, ethyl gallate and (+)-dehydrovomifoliol. Meng *et al*. [43] have also reported that semi-maximal inhibitor concentration of WKS extract (IC50) has been 1.06 mg/mL, and this was far less than that is for polysaccharide obtained from potato peel (11.57 mg/mL) and more than that of ascorbic acid (0.077 mg/mL).

Although it is not possible to compare these values as antioxidant activity is expressed in different ways, different methods are applied and results are given in different units, in general all of them has demonstrated that WKS has high antioxidant activity. It is claimed that highly phenolic compounds can be responsible from high antioxidant activity of WKS [44].

Pharmacological agents already in use modulate oxidative stress [45]. WKS is also considered to have high antioxidant potential and can be recommended as a natural antioxidant agent.

Nowadays, the emergence of antibiotic-resistant strains and the presence of various side effects are problems to be solved in the fight against infectious diseases. Limited options for preventing or treating bacterial/fungal infections have led medical science to nature, to discover new and different antimicrobial agents [46].

In this study the results indicate that fungal/bacterial species have different sensitivities towards WKS extract. It can be said that WKS is more effective on gram (+) bacteria compared to gram (-) bacteria. There are other studies which have reported that plant extracts are more effective on gram (+) bacteria [47]. It is claimed that this is caused by the lipopolysaccharide layer outside the cell wall of gram (-) bacteria which makes them more resistant [48].

Many numbers of herbal extracts with antimicrobial characteristics have been reported [49]. But, there are limited number of studies whose subject area is antimicrobial characteristics of WKS. Similar to our study, in a recent study, it was reported that WKS has shown varying degrees of antibacterial effects on Gram-positive bacteria (*S. aureus, Staphylococcus epidermidis, Enterecoccus faecalis* and *Enterecoccus faecium*) (with MIC (minimum inhibitory concentration) ranging from 8.59 to 275 µg/ml); while Gram-negative (*E. coli, Klepsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis*) strains have shown very low sensitivity (with MIC values of 275 µg/ml). In the same study in which antimicrobial activity of WKS has been determined by using Ciprofloxacin which is a wide-spectrum antibiotic, it was also reported that administration of WKS in highest doses (275 ug/ml) have affected the growth of Gram-positive and Gram-negative bacteria strains most of which are resistant to Ciprofloxacin [50]. In another study conducted by Rusu *et al*. [51], it was reported that WKS has shown MIC (minimum inhibition concentration) levels between 0.012-3.12 mg/mL; WKS antimicrobial activity was measured against Gram-positive (*S. aureus*) and Gram-negative (*E. coli, P. aeruginosa, Salmonella enteritidis*) bacteria and two fungi (*C. albicans* and *C. parapsilosis*), and the lowest effect was found against E. coli. Different than our study, in two experimental studies conducted by Meng *et al*. [43], the water soluble polysaccharide fraction isolated from WKS has been found to show significant antibacterial activity against two Gram-negative bacteria strains (*E. coli* and *P. aeruginosa*) and also against two Gram-positive strains (*S. aureus* and *Listeria monocytogenes*) depending on the dosage (0.2-1.2 mg/mL).

The results presented above clearly prove that this part of the plant is a promising source of new antimicrobial agents.

Limitations

This study was carried out on crude WKS extract. The elucidation of metabolic pathways, metabolic regulations, or the biosynthesis and roles of macromolecules are still in obscurity. More in-depth studies are needed in the future to clarify the molecular mechanisms responsible for the antioxidant and antimicrobial effect of WKS.

CONCLUSION

In our study, it was aimed to get more information about WKS which is a by-product with a limited use at present. In sum, the results from our study showed that WKS has a high phenolic content, and remarkable antioxidant and antimicrobial activity. Nevertheless, clinical studies are also required in order to investigate other possible pharmacological activities, safety, and efficacy of WKS.

Authors' Contribution

Study Conception: EAÖD, EK; Study Design: EAÖD, EK; Supervision: EAÖD, EK; Funding: EAÖD, EK; Materials: EAÖD, EK; Data Collection and/or Processing: EAÖD; Statistical Analysis and/or Data Interpretation: EAÖD; Literature Review: EAÖD; Manuscript Preparation: EAÖD and Critical Review: EAÖD, EK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The most common persistent symptoms in patients with COVID-19 who were evaluated in the Internal Medicine polyclinic

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ABSTRACT

Objectives: To identify continuing symptoms of individuals in the post-COVID period. To begin with our study was planned to research the most common persistent symptoms in the post-COVID period, and additionally to research whether or not there were differences between the most frequent initial symptoms in the 1st, 2nd and 3rd waves of the pandemic.

Methods: Cases attending the internal medicine clinic infected with COVID-19 who were minimum 120 days past the infection were included in the study. The study was shaped by responses of cases to open-ended questions.

Results: From a total of 2,802 clinical attendances, 1,005 cases were included in the study. Of cases, 9.3% required clinical monitoring and 1.7% required intensive care during infection. The mean number of persistent symptoms was 1.38, with the most common persistent symptoms being 11.2% fatigue/tiredness, 6.1% shortness of breath, and 4.6% back and low back pain. Mean number of initial symptoms was 1.63, with the most common initial symptoms being 21.2% fatigue, 19.4% fever and 19.1% headache. Persistent chest pain was most common among those infected in the 1st wave, while there were no other significant differences observed between pandemic waves.

Conclusions: The most common persistent symptoms were consistent with the general literature data; however, our condition of minimum 120 days past infection allowed a range of symptoms to ameliorate. Our results are more realistic in this way. A range of rare persistent symptoms emerging in our results were not encountered in the literature, while our study is unique as there is no other study comparing pandemic periods.

Keywords: COVID-19, persistent symptom, post-COVID-19

The first case of the coronavirus disease 2019 (COVID-19) pandemic linked to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was recorded in Wuhan city in China in December 2019 and it spread rapidly around the world since this first case [1]. The mortality rate for SARS-CoV-2 is 2%, while this novel coronavirus generally is associated with mild and/or severe respiratory tract disease [2]. The term Long COVID or post-COVID describes the situation characterized by continuation of symptoms for at least 12 weeks after initial infection. This situation forms an observation problem and may last several months. Symptoms and clinical findings are very diverse and include cardiovascular system symptoms

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leading to consideration of multiple organ/system involvement. The most commonly observed symptoms are fatigue, shortness of breath, muscle pain, headache, memory loss and concentration disorders. Patients frequently report loss of psychological and physiological performance in this period [3]. Our study was planned to research the frequency of COVID-19 infection among patients attending the internal medicine clinic in our hospital, initial symptoms, persistent symptoms if any and whether there were differences between the 1st, 2nd and 3rd waves of the pandemic.

METHODS

This cohort study questioned whether patients attending the internal medicine clinic for any reason had been infected with COVID-19. Cases aged 18 and over at the time of infection were included in the study. Cases with minimum 120 days since infection and with SARS-CoV-2 infection confirmed by PCR who provided consent were included in the study. Cases were asked open-ended questions. Patients were asked about age, sex, body mass index, additional diseases and comorbidities, the wave of the pandemic when they were infected, clinical and/or intensive care unit admission and if so, number of days of hospitalization, features and duration of initial symptoms during the disease period, persistent symptoms in the post-COVID period in spite of 120 days passing since recovery from acute infection, vaccination status, whether they were infected with COVID-19 more than once, smoking habit and detection of any SARS-CoV-2 variant apart from the common strain during the infection period. Patients with clinical/intensive care monitoring were included in the study if they were monitored by the clinic/intensive care in our hospital. The wave of the pandemic when cases were infected was identified as 1st, 2nd or 3rd and we planned to research whether there were differences in initial complaints, unresolved complaints and descriptive findings between the pandemic waves. The 1st wave was defined as the period from March 2020 when the first case was identified in Turkey to June 2020, the 2nd wave was September 2020 to January 2021 and the 3rd wave was March 2021 to September 2021. Individuals under the age of 18 years, not infected with COVID-19, infected within the last 120 days and who did not provide consent were not included in the study. Ethics committee approval was obtained (decision no:2021/514/207/5)

Statistical Analysis

For statistical analyses, the Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) program was used. When assessing study data, descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used. Fit of quantitative data to normal distribution was tested with the Shapiro-Wilk test and graphical investigations. Comparison between more than two groups of quantitative data with normal distribution used the one-way analysis of variance. Comparison of more than two groups of quantitative data without normal distribution used the Kruskal-Wallis

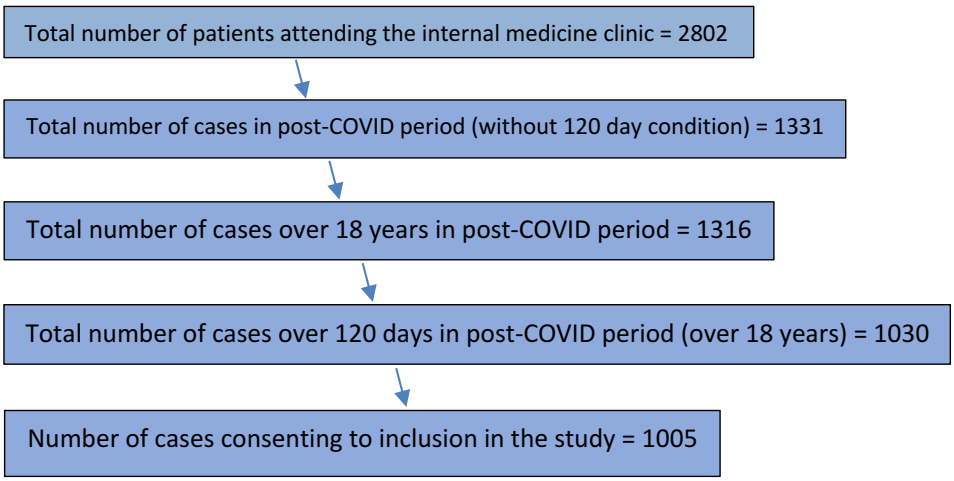

Fig. 1. Flow scheme for clinic attendance.

test. Comparison of qualitative data used the Fisher-Freeman-Halton exact test. Statistical significance was accepted as $p < 0.05$.

RESULTS

From July 2021 to October 2021, 2802 patients attending the internal medicine clinic were randomly asked whether they had been infected with COVID-19 and 1005 cases (35%) who abided by the criteria were included in the study (Fig. 1).

The total of 1005 cases, 60.2% (n = 605) were women and 39.8% (n = 400) were men. The ages of those participating in the research varied from 18 to 95 years, with mean age determined as 41.94 ± 14.78 years. Mean BMI of participants was identified as 27.64 ± 5.91 kg/m2. Of cases, 17.3% (n = 174) smoked. At least one comorbid disease was identified in 35.9% (n = 361) of cases. When comorbid diseases are investigated, 14.7% (n = 148) had hypertension, 12.3% (n = 124) had diabetes mellitus, 3.8% (n = 38) had coronary artery disease/chronic obstructive pulmonary disease and 0.9% (n = 9) had other diseases (Table 1).

Among the cases, 9.3% (n = 93) were admitted to hospital. Hospitalization varied from 1 to 22 days, with mean hospitalization duration determined to be 8.32 ± 4.58 days. Of the cases, 1.7% (17) required intensive care admission. It appeared that 39.8% (n = 400) of cases had received at least 1 vaccination. Of cases, 98.3% (n = 988) were infected with COVID-19 once, 1.6% (n = 16) were infected twice and 0.1% (n = 1) were infected three times. Among the cases, 2.6% (n=26) had different variants observed. Total disease duration varied from 0 to 36 days, with mean duration 10.55 ± 9.10 days (Table 2).

Of cases, 92.1% (n = 926) had at least one initial symptom. When initial symptoms are investigated, 21.2% (n = 213) of cases had fatigue, 19.4% (n = 195) had fever, 19.1% (n = 192) had headache, and 15.9% (n = 160) had cough. Among cases, 9.8% (n = 98) had joint pain, 9.7% (n = 97) had low back/back pain, 8.6% (n = 86) had sore throat, 8% (n = 80) had muscle pain, and 5.9% (n = 59) had loss of taste and smell. Of cases, 5% (n = 50) had shortness of breath, 3.6% (n =

Table 1. Distribution of descriptive characteristics

	Data
Sex	
Female	605 (60.2)
Male	400 (39.8)
Age	41.94 ± 14.78 42 (18-95)
BMI (kg/m^2)	27.64 ± 5.91 27.2 (0.0-62.3)
Smoker	
No	829 (82.7)
Yes	174 (17.3)
Comorbid disease	
No	644 (64.1)
Yes	361 (35.9)
DM	124 (12.3)
HT	148 (14.7)
COPD-CAD	38 (3.8)
Other	9 (0.9)

Data are mean ± standard deviation or median (minimum-maximum) or n (%)

Table 2. Distribution of findings related to disease

		Data
Hospitalization	No	912 (90.7)
	Yes	93 (9.3)
Admission duration (n = 81) (days)		8.32 ± 4.58 7 (1-22)
ICU admission	No	988 (98.3)
	Yes	17 (1.7)
Vaccination	No	605 (60.2)
	Yes	400 (39.8)
Number of infections	1	988 (98.3)
	2	16 (1.6)
	3	1 (0.1)
Different variants	No	979 (97.4)
	Yes	26 (2.6)
Total duration of disease (days)		10.55 ± 9.10 10 (0-36)

Data are shown as mean ± standard deviation or median (minimum-maximum) or n (%)

Table 3. Distribution of Initial Symptoms and Unresolved Complaints

Initial symptom	Data
No	79 (7.9)
Yes	926 (92.1)
Fatigue	213 (21.2)
Fever	195 (19.4)
Headache	192 (19.1)
Cough	160 (15.9)
Joint pain	98 (9.8)
Low back/back pain	97 (9.7)
Sore throat	86 (8.6)
Muscle pain	80 (8.0)
Loss of taste/smell	59 (5.9)
Shortness of breath	50 (5.0)
Nausea/vomiting	36 (3.6)
Shivering/shaking	36 (3.6)
Rhinitis/nasal blockage	32 (3.2)
Bone pain	26 (2.6)
Chest pain/	15 (1.5)
Leg pain	14 (1.4)
Diarrhea	14 (1.4)
Loss of appetite	14 (1.4)
Other	63 (6.3)
Number of initial symptoms	1.63 ± 0.75 1 (1-5)
Unresolved complaints	
No	589 (58.3)
Yes	419 (41.7)
Fatigue	113 (11.2)
Low back/back pain	46 (4.6)
Shortness of breath	61 (6.1)
Loss of taste/smell	29 (2.9)
No	29 (2.9)
Yes	29 (2.9)
Fatigue	28 (2.8)
Forgetfulness	26 (2.6)
Chest pain	17 (1.7)
Leg pain	16 (1.6)
Palpitations	11 (1.1)
Muscle pain	7 (0.7)
Other	97 (9.7)
Number of unresolved complaints	1.38 ± 0.65 1 (1-4)

Data are shown as mean ± standard deviation or median (minimum-maximum) or n (%)

36) had nausea/vomiting, 3.6% (n = 36) had shivering/shaking, 3.2% (n = 32) had nasal rhinitis/blockage, 2.6% (n = 26) had bone pain and 1.5% (n = 15) had chest pain or stabbing chest pain. Among cases, 1.4% (n = 14) had leg pain, 1.4% (n = 14) had diarrhea, 1.4% (n = 14) had loss of appetite and 6.3% (n = 63) had other complaints. The other 7.9% of cases were asymptomatic and were identified as positive due to contact and/or coincidental testing. While cases encountered 1 to 5 initial complaints, the mean number of symptoms was identified as 1.63 ± 0.75 (Table 3).

At least one persistent symptom was observed in 41.7% (n = 419) of cases. When unresolved complaints are investigated, 11.2% (n = 113) of cases had fatigue/tiredness, 4.6% (n = 46) had back/low back pain, 6.1% (n = 61) had shortness of breath, 2.9% (n = 29) had loss of taste and smell, 2.9% (n = 29) had cough, 2.9% (n = 29) had joint pain, 2.8% (n = 28) had headache, 2.6% (n = 26) had forgetfulness, 1.7% (n = 17) had chest pain, 1.6% (n = 16) had leg pain, 1.1% (n = 11) had palpitations, 0.7% (n = 7) had muscle pain and 9.7% (n = 97) had other complaints (Fig. 2 and 3).

Cases were identified to have between 1 and 4 unresolved complaints, with the mean number of persistent symptoms calculated as 1.38 ± 0.65. There were no statistically significant differences between the groups according to sex, age, height, weight, BMI, smoking status and presence of comorbid diseases ($p > 0.05$) (Table 4).

There was a statistically significant difference between groups according to vaccination status and COVID infection times ($p = 0.001$ and $p < 0.01$, respectively). Among those who were vaccinated, the rate with COVID infection during the 2nd wave was higher than the rate with COVID infection during the 3rd wave. There were no statistically significant differences between hospitalization and intensive care admission status, admission durations and total disease duration according to group ($p > 0.05$) (Table 5).

There were no statistically significant differences between initial symptoms of cases according to group ($p > 0.05$) (Table 6). According to the groups, there was a statistically significant difference between rates of chest pain among unresolved complaints of cases ($p = 0.015$ and $p < 0.05$, respectively). Those who were infected during the 1st wave had higher incidence of chest pain among unresolved complaints compared to those infected in the 2nd and 3rd waves. There was

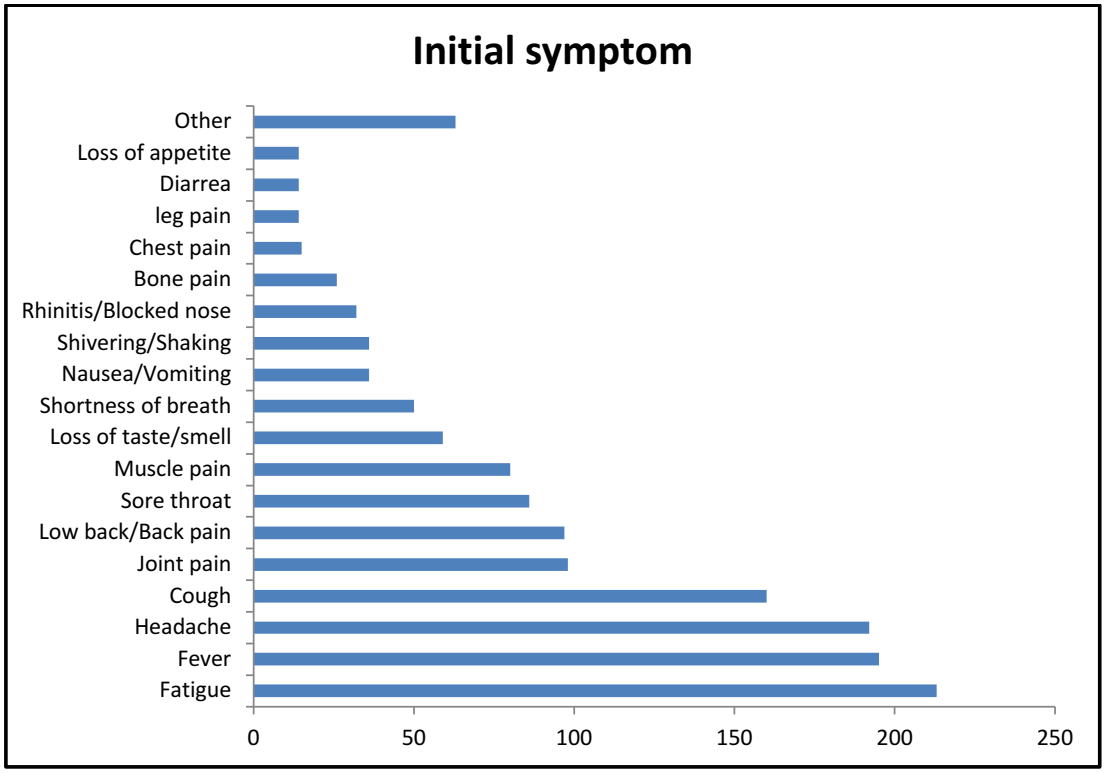

Fig. 2. Distribution of initial symptoms.

no statistically significant difference in incidence of unresolved complaints and other unresolved complaint types among cases according to groups ($p > 0.05$) (Table 7).

DISCUSSION

In the literature, the most frequent symptoms during the post-COVID-19 period are reported to be cough,

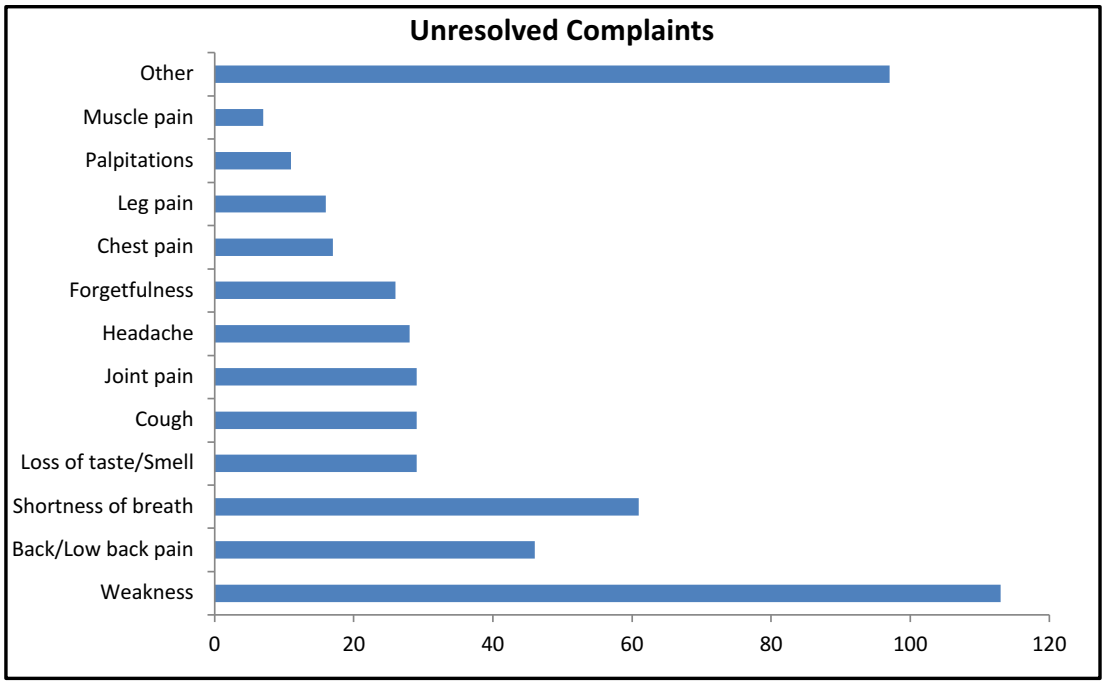

Fig. 3. Distribution of unresolved complaints.

Table 4. Assessment of descriptive characteristics according to group

	COVID infection time			
	1st wave (n = 40)	2nd wave (n = 520)	3rd wave (n = 445)	*p value*
Sex				
Female	22 (55.0)	304 (58.5)	279 (62.7)	[a]0.312
Male	18 (45.0)	216 (41.5)	166 (37.3)	
Age (years)	43.18 ± 13.05 42.5 (18-68)	42.22 ± 15.35 42 (17-95)	41.5 ± 14.26 42 (18-83)	[b]0.620
BMI (kg/m^2)	27.93 ± 5.81 27.2 (17.9-45)	27.77 ± 5.71 27.3 (0-51.4)	27.47 ± 6.14 27 (14.4-62.3)	[b]0.713
Smoker				
No	33 (82.5)	436 (84.2)	360 (80.9)	[a]0.406
Yes	7 (17.5)	82 (15.8)	85 (19.1)	
Comorbid disease				
No	26 (65.0)	337 (64.8)	281 (63.1)	[a]0.859
Yes	14 (35.0)	183 (35.2)	164 (36.9)	
DM	1 (2.5)	69 (13.3)	54 (12.1)	[a]0.117
HT	9 (22.5)	71 (13.7)	68 (15.3)	[a]0.262
COPD/CAD	0 (0)	18 (3.5)	20 (4.5)	[a]0.438
CRF	0 (0)	4 (0.8)	5 (1.1)	[a]0.817
Other	4 (10.0)	68 (13.1)	60 (13.5)	[a]0.904

Data are shown as mean ± standard deviation or median (minimum-maximum) or n (%). BMI = Body Mass Index, DM = Diabetes Mellitus, HT = Hypertension, COPD = Chronic obstructive pulmonary disease, CAD = Coronary artery disease, CRF = Chronic renal failure

[a]Fisher Freeman Halton Test, [b]One-way ANOVA Test

Table 5. Assessment of disease findings according to group

		COVID infection time			
		1st wave	2nd wave	3rd wave	*p value*
Hospitalization	No	33 (82.5)	480 (92.3)	399 (89.7)	[a]0.067
	Yes	7 (17.5)	40 (7.7)	46 (10.3)	
ICU admission	No	40 (100.0)	512 (98.5)	436 (98.0)	[a]0.820
	Yes	0 (0)	8 (1.5)	9 (2.0)	
Hospitalization duration (days)		6.83 ± 3.82 6 (3-14)	8.75 ± 5.38 7.5 (1-22)	8.15 ± 3.88 7 (1-18)	[b]0.588
Vaccination	No	9 (22.5)	108 (20.8)	154 (34.6)	**[a]0.001****
	Yes	31 (77.5)	412 (79.2)	291 (65.4)	
Total duration (days)		7.70 ± 4.01 7 (0-15)	10.40 ± 9.32 9.5 (0-120)	10.97 ± 9.12 10 (0-60)	[c]0.255

Data are shown as mean ± standard deviation or median (minimum-maximum) or n (%).

[a]Fisher Freeman Halton Test, [b]One-way ANOVA Test, [c]Kruskal Wallis Test, **$p < 0.01$

Table 6. Assessment of initial complaints according to group

	COVID infection time			
Initial symptom	**1st wave**	**2nd wave**	**3rd wave**	***p value***
No	5 (12.5)	41 (7.9)	33 (7.4)	[a]0.455
Yes	35 (87.5)	479 (92.1)	412 (92.6)	
Fatigue	7 (17.5)	107 (20.6)	99 (22.2)	[a]0.731
Fever	6 (15.0)	95 (18.3)	94 (21.1)	[a]0.445
Headache	8 (20.0)	106 (20.4)	78 (17.5)	[a]0.500
Cough	5 (12.5)	78 (15.0)	77 (17.3)	[a]0.544
Joint pain	2 (5.0)	55 (10.0)	41 (9.2)	[a]0.537
Low back/back pain	3 (7.5)	50 (9.6)	44 (9.9)	[a]0.967
Sore throat	3 (7.5)	40 (7.7)	43 (9.7)	[a]0.560
Muscle pain	5 (12.5)	39 (7.5)	36 (8.1)	[a]0.466
Loss of taste/smell	2 (5.0)	35 (6.7)	22 (4.9)	[a]0.514
Shortness of breath	1 (2.5)	24 (4.6)	25 (5.6)	[a]0.730
Nausea/vomiting	0 (0)	18 (3.5)	18 (4.0)	[a]0.566
Shivering/shaking	1 (2.5)	14 (2.7)	21 (4.7)	[a]0.214
Rhinitis/nasal blockage	1 (2.5)	19 (3.7)	12 (2.7)	[a]0.809
Bone pain	2 (5.0)	15 (2.9)	9 (2.0)	[a]0.281
Chest pain/	2 (5.0)	5 (1.0)	8 (1.8)	[a]0.060
Leg pain	1 (2.5)	8 (1.5)	5 (1.1)	[a]0.520
Diarrhea	1 (2.5)	7 (1.3)	6 (1.3)	[a]0.591
Loss of appetite	1 (2.5)	5 (1.0)	8 (1.8)	[a]0.255
Other	3 (7.5)	34 (6.5)	26 (5.8)	[a]0.800

Data are shown as n (%). [a]Fisher Freeman Halton Test

fever, dyspnea, musculo-skeletal system symptoms (myalgia, joint pain, fatigue), gastrointestinal symptoms and anosmia/dysgeusia [4]. A study of 143 patients in Italy by Carfi *et al*. [5] assessed cases for minimum 60 days after recovery from COVID-19 infection. Only 12.6% of cases did not have any symptom related to COVID-19, while 32% had 1 or 2 symptoms and 55% had 3 or more symptoms. No patient had any fever or acute disease finding or symptom. Cases described fatigue/weakness (53.1%), shortness of breath (43.4%), joint pain (27.3%) and chest pain (21.7%). After discharge, 87% of patients were identified to have minimum 1 symptom [5]. In our study assessing a total of 1005 cases, the most common persistent symptoms were parallel to those reported most frequently in studies by Carfi *et al*. [5], Mandal *et al*. [6], Halpin *et al*. [7], Huang *et al*. [10], Peterson *et al*. [11], and Karaaslan *et al*. [12], with 11.2% reporting fatigue/tiredness. A study by Mandal *et al*. [6] of 384 cases in the United Kingdom showed general improvement during early follow-up of symptom burden in subjects recovering from COVID-19 after hospitalization. During the post-COVID period, 53% had permanent shortness of breath, 34% had permanent cough, 69% reported permanent tiredness and 15% of patients reported depression [6]. Halpin *et al*. [7] assessed 100 cases in the post-COVID period with 68 followed by the clinic and 32 in intensive care and investigated the effect of symptoms continuing after discharge and on daily life in the post-COVID period. New fatigue linked to the disease was the most frequently reported symptom among 72% of participants in the ICU group and 60% of those in the ward group. The next most common symptoms were shortness of

Table 7. Assessment of unresolved complaints according to group

	COVID infection time			
Unresolved complaints	**1st wave**	**2nd wave**	**3rd wave**	***p value***
No	27 (67.5)	292 (56.2)	267 (60.0)	[a]0.253
Yes	13 (32.5)	228 (43.8)	178 (40.0)	
Fatigue	4 (10.0)	50 (9.6)	59 (13.3)	[a]0.228
Low back/back pain	3 (7.5)	27 (5.2)	16 (3.6)	[a]0.272
Shortness of breath	1 (2.5)	29 (5.6)	31 (7.0)	[a]0.529
Loss of taste/smell	0 (0)	18 (3.5)	11 (2.5)	[a]0.494
Cough	0 (0)	13 (2.5)	16 (3.6)	[a]0.377
Joint pain	0 (0)	19 (3.7)	10 (2.2)	[a]0.326
Headache	0 (0)	20 (3.8)	8 (1.8)	[a]0.115
Forgetfulness	0 (0)	13 (2.5)	13 (2.9)	[a]0.680
Chest pain	3 (7.5)	5 (1.0)	9 (2.0)	**[a]0.015***
Leg pain	1 (2.5)	8 (1.5)	7 (1.6)	[a]0.643
Palpitations	2 (5.0)	4 (0.8)	5 (1.1)	[a]0.092
Muscle pain	0 (0)	1 (0.2)	6 (1.3)	[a]0.097
Other	1 (2.5)	50 (9.6)	46 (10.3)	[a]0.295

Data are shown as n (%). [a]Fisher Freeman Halton Test

breath (65.6% ICU group, 42.6% ward group) and psychological problems (46.9% ICU group and 23.5% ward group) [7]. A prospective cohort study of 131 COVID-19 patients after discharge from a clinic in Wuhan by Wang *et al*. [8] showed that 86% of patients had no symptoms 3 to 4 weeks after discharge, while only 1.5% were identified to have dyspnea. Tenforde *et al*. [9] performed a telephone survey of symptomatic COVID-19 patients not admitted to hospital and identified 35% had not returned to their normal health level and had permanent symptoms like fatigue, cough and headache. A study by Huang *et al*. [10] investigating a total of 1655 post-COVID cases found the most frequent symptoms were fatigue or muscle weakness affecting 63% and sleep difficulties affecting 26%. A study by Peterson *et al*. [11] of 180 participants without clinical follow-up reported that 53.1% still had at least one symptom mean 125 days after the onset of symptoms. Of participants, 33.3% were identified to have one or two symptoms and 19.4% had three or more symptoms. The most common permanent symptoms were fatigue, loss of taste and smell and arthralgia [11]. During the post-COVID period, Karaaslan *et al*. [12] showed that 31% had fatigue, 18% had joint pain and 15% had myalgia, while other COVID-19 symptoms included 25.3% shortness of breath and 20% hair loss in their study. Kayaaslan *et al*. [13] showed fatigue/tiring easily, myalgia and weight loss were the most frequently seen permanent symptoms (generally 29.3%) while respiratory symptoms had 2nd-highest frequency (25.4%).

In our study, the most frequent persistent symptoms were fatigue, shortness of breath, back and low back pain, loss of taste/smell, cough, joint pain, headache, forgetfulness, chest pain, leg pain, palpitations and muscle pain. With varying rates and rankings, tiredness-fatigue, shortness of breath and cough were the most frequent post-COVID persistent symptoms as supported in studies by Mandal *et al*. [6], Halpin *et al*. [7], Wang *et al*. [8], Tenforde *et al*. [9], Huang *et al*. [10], and Peterson *et al*. [11]. The first of these studies by Carfi *et al*. [5] overlaps in general with the most frequent persistent symptoms in studies from all around the world. In Turkey, Karaaslan *et al*. [12] and Kayaaslan *et al*. [13] reported the most frequent symptom was fatigue. No study contradicting these studies was encountered. We identified that 58.3% of cases did not have any symptom, while

41.7% had at least one persistent symptom. As our study included adequate numbers of cases with at least 120 days since recovery from viremia, we identified lower rates of tiredness-fatigue, shortness of breath and cough symptoms compared to the literature. At the same time, we observed that more than half of cases had full symptomatic recovery by benchmarking this duration. Previous studies are generally observed to keep the post-COVID duration shorter. As our study data belong to a longer period, we believe they reflect objective outcomes related to the post-COVID process.

In our study, different to the general literature, the 3rd most common persistent symptom was back and low back pain. Similarly, forgetfulness (2.6%) was encountered with higher frequency in our study. Cases rarely mentioned (< 0.5%) diarrhea, sweating, aggression, nasal blockage, loss of appetite, pollacuria, insomnia, numbness of distal extremities, edema of the legs, non-healing oral aphthous, feeling of pressure in head and chest, epigastric pain, regurgitation, vertigo, effort dyspnea, hair loss, toothache, hallucinations, facial pain, dry throat, miliaria skin rash, itching, night sweats, breast pain, atopia, ear pain, muscle cramps, confusion, nasal rhinitis, burning eyes, hypoglycemia attacks, hoarseness, and oily skin among persistent symptoms.

In our study, one COVID-19 case with rheumatoid arthritis diagnosis reported an acute attack after viremia, while a case with restless leg syndrome reported increased severity and frequency of symptoms after viremia. There is no literature data related to these two clinical situations.

Additionally, there were patients with newly-diagnosed DM, HT, pulmonary embolism, panic attack, asthma and Hashimoto disease in the post-COVID period. There are studies related to development of panic disorder [14], Hashimoto thyroiditis [15] and pulmonary embolism [16] in the post-COVID period, but there is inadequate literature data related to DM, HT and asthma newly developing in this period. We think patients with new DM diagnosis in the post-COVID period may be the effect of steroids administered during the disease period.

Of our cases, 35.9% had at least one comorbid disease. The most frequent chronic diseases were HT (14.7%), DM (12.3%) and COPD-coronary artery disease (3.8%). There are studies reporting that the presence of chronic disease and multiple morbidities reduces quality of life [17]. The most common comorbidities in the literature were observed to be hypertension (15.8%), cardiovascular and cerebrovascular situations (11.7%) and diabetes mellitus (9.4%) [18-20]. A meta-analysis including 61 studies emphasized that there was strong epidemiological evidence for the association of comorbidities with COVID-19 severity and prognosis [21].

While 4% of cases were infected with SARS-CoV-2 during the 1st wave, 44.3% during the 2nd wave and 51.7% during the 3rd wave, statistical differences were not observed between the sex, age, height, weight, BMI values, smoking habits and presence of comorbidities between the pandemic periods for our cases. The rates of those vaccinated who were infected with COVID in the 2nd wave was higher than during the 3rd wave ($p < 0.01$). We believe this situation is due to the increase in awareness about the pandemic over time and the better adoption of general hygiene rules related to COVID-19 in society.

There were no statistically significant differences between the hospitalization and intensive care admission status, admission durations, total disease duration, initial complaints and persistent complaints of cases according to pandemic period.

Only the incidence of chest pain was observed to be statistically high for those infected with SARS-CoV-2 during the first wave. The different treatment protocols in the period of the first wave, the lack of vaccines, progression of the process and accumulation of literature data changed the treatment protocols over time. Linked to all these factors, cases infected during the 1st wave are thought to have developed pneumonia more. However, there is no literature data found to support our hypothesis.

The most common initial symptoms stated in the literature [22] were fever and cough at 71.5%, while in our study, fatigue (21.2%), fever (19.4%) and headache (19.1%) were observed to be proportionally different from the general literature.

Limitations

Our study assessed the post-COVID period and could not access sufficient data about strains in the COVID-19 period. We could have gained information about variants apart from the common strain in line with patient declarations. When assessing strains,

cases with PCR kit use, severe clinical findings and assessed in health organizations may have been examined. At the same time, cases with chest pain, shortness of breath and respiratory distress were not assessed with radiological images/ECHO in line with the design and scope of our study. Both situations comprise limitations in terms of our study.

CONCLUSION

In the literature there are articles in parallel to our study, though our study differs from others both in terms of the emerging results and the post-COVID findings that were not reported in other studies. For the first time, our study included persistent symptoms and findings. Strong aspects of our study are that participants were not asked closed and directive questions about persistent findings and that cases were not directed toward certain answers. The minimum 120 days since disease ensured a more objective assessment of the post-COVID process. In the literature, there is no article comparing the COVID-19 period and the 1st, 2nd and 3rd waves. This situation is the strongest aspect of our study. Our study included all patients infected with COVID-19 without regard to follow-up in intensive care/ward or without being admitted. Strong aspects of our study are that patients with clinical follow-up were monitored with very similar treatment protocols, access to results and disease progression for all cases in the information-communication system in our hospital, and assessment during the post-COVID period by the same pandemic experts in our hospital's clinics. The clinician group in our hospital worked in the internal medicine specialist ward, intensive care, clinic and emergency service since the start of the pandemic, employed primarily during the COVID-19 pandemic. The internal medicine clinics are the health branch most frequently attended due to symptoms linked to COVID in the post-COVID period. The results of our study are important in terms of reflecting clinical practices in internal medicine.

Authors' Contribution

Study Conception: ZK; Study Design: ZK; Supervision: SA; Funding: ZK; Materials: SA; Data Collection and/or Processing: ZK; Statistical Analysis and/or Data Interpretation: SA; Literature Review: ZK; Manuscript Preparation: ZK and Critical Review: ZK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The role of frailty score in early surgical treatment of elderly cholecystitis patients

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ABSTRACT

Objectives: The average life expectancy is increasing all over the world, and as a result, the number of elderly patients is increasing. Acute cholecystitis is one of the most common diseases requiring emergency surgical treatment in the elderly. In the literature, it has been reported that in the treatment of elderly patients with acute cholecystitis, physicians do not fully comply with the current literature recommendations due to some concerns and do not apply surgical treatment in the early period. The concept of frailty, which has come to the fore in geriatric assessment today, provides objective information about the general health status of the patient. In our study, the role of frailty assessment in the decision made regarding the early surgery in elderly patients with acute cholecystitis treated in our hospital was investigated.

Methods: In our study, the clinical features, comorbidities, American Society of Anesthesiologists (ASA) scores, Canadian Study of Health and Aging (CSHA) frailty scale, treatment modalities and prognosis of patients over the age of 65 who were treated with the diagnosis of acute cholecystitis between January 2018 and January 2021 were evaluated retrospectively.

Results: Of the 182 patients included in the study, 24 (13.2%) were found to be frail. It was observed that the mean age and multi-morbidity were higher in the frail group ($p < 0.001$). It was observed that the mean C-reactive protein and leukocyte values, which are inflammatory mediators, increased in both groups, both fragile and non-fragile, and complicated cholecystitis accompanied by cholangitis or pancreatitis was observed in a total of 64 (35.16%) patients. There were 57 (31.3%) patients using anticoagulant or antiaggregant agents. The patients were most frequently treated with medical treatment (n = 108; 59.3%), the other treatment methods were early surgery ((n = 46; 25.3%), endoscopic retrograde cholangiopancreatography (n = 22; 12.1%) and percutaneous cholecystostomy (n = 11; 6.0%). Surgical treatment was more common in ASA I and II patients, and percutaneous cholecystostomy was more common in frail patients ($p < 0.001$). There were 20 (14.70%) patients who were re-admitted to the hospital and 6 (3.29%) patients ended up with mortality. No statistical relationship could be demonstrated between these conditions and frailty ($p > 0.05$).

Conclusions: In the treatment of the elderly cholecystitis patients, early surgical treatment is the most favorable treatment method in order to reduce re-admission and prevent possible complications. However, we think that a detailed geriatric evaluation should be made in a multidisciplinary manner for the decision making regarding the of surgical treatment of elderly patients, and frailty evaluation should also be made in this context.

Keywords: Acute cholecystitis, early surgical treatment, frailty, elderly

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The risk of developing gallstones increases with age, and its prevalence increases to around 20% over the age of 65 [1]. Acute cholecystitis due to cholelithiasis is one of the most common surgical emergencies in the elderly population. As in young patients, the recommended treatment for acute cholecystitis in the elderly patients is laparoscopic cholecystectomy in the early period [1, 2]. However, in clinical practice, it has been observed that the decision to operate on elderly patients is more conservative than younger patients [3]. In the surgical decision regarding elderly patients with acute cholecystitis, presence of comorbidities, anticoagulants drug usage and a high American Society of Anesthesiologists (ASA) score bring up alternative treatment options [4].

The chronological age limit of 65 used for the evaluation of elderly patients does not reflect the biological status of each patient. One of the commonly used variables to evaluate the general health status of the geriatric patient group is frailty [5]. Frailty refers to a state in which the person is generally vulnerable, accompanied by a decrease in the general health status, physical activity energy and cognitive skill reserves [6].

In our study, the usability of the Canadian Study of Health and Aging (CSHA) frailty scale was investigated in the surgical treatment of elderly patients with acute calculous cholecystitis who were hospitalized in our hospital.

METHODS

In our study, patients over the age of 65 who were hospitalized in the general surgery service for acute calculous cholecystitis between January 2018 and January 2021 were evaluated. The diagnosis of acute cholecystitis was made according to the ultrasound and computed tomography imaging results of the patients, as well as the laboratory and physical examination findings. Patients with acalculous cholecystitis and chronic cholecystitis were not included in the study.

Patients' age, gender, presence of comorbid disease, use of anticoagulants, C-reactive protein (CRP) and leukocyte values, ASA classification value, frailty score value, concurrent cholangitis or pancreatitis, type of cholecystitis treatment, presence of re-admission to the hospital within 30 days with similar complaints and mortality were evaluated retrospectively by accessing the patients' file data.

CSHA frailty scale was used to assess the frailty of the patients. In this scoring system, patients are classified from 1 to 7. Patients with CSHA class 1 are very fit, class 2 are well, class 3 are well, with treated comorbid disease, class 4 are apparently vulnerable, class 5 are mildly frail, class 6 are moderately frail, and class 7 are severely frail (Table 1). In our study, patients were evaluated in two groups; patients in CSHA category 1-4 as non-fragile and those in category 5-7 as fragile based on the guidelines.

Anesthesia preoperative evaluation forms are used for ASA classification. Patient anamnesis form and nutritional risk screening (NRS) form, patient fall risk assesment form, decubitus wound screening form file data records were evaluated for CSHA score evaluation.

Consent was obtained with the decision numbered

Table 1. Frailty scale

1	Very Fit	Robust, active, energetic, well-motivated and fit; these people commonly exercise regularly and are in the most fit group for their age
2	Well	Without active disease, but less fit than people in category 1
3	Well, with treated comorbid disease	Disease symptoms are well controlled compared with those in category 4
4	Apparently vulnerable	Although not frankly dependent, these people commonly complain of being "slowed up" or have disease symptoms.
5	Mildly frail	With limited dependence on others for instrumental activities of daily living
6	Moderately frail	Help is needed with both instrumental and non-instrumentalactivities of daily living.
7	Severely frail	Completely dependent on other for the activities of daily living, or terminally ill.

2021/72 of the scientific research ethics committee of Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital.

Statistical Analysis

Study data were analyzed with SPSS 21.0. Skewness and Kurtosis (normal distribution of data) values, normal distribution curve and Levene (equality of variances) test results were examined whether the data met the prerequisites of parametric tests. T-test and deviation of data from normal distribution in independent groups based on comparison of mean and variance between low and high-risk groups according to ASA scores and surgical treatment, percutaneous transluminal coronary angioplasty (PTCA), medical treatment variables, and also frail and non-frail groups according to frailty scale results and surgical treatment, PTCA, medical treatment variables. In case the data deviated from the normal distribution, comparisons were made with non-parametric Mann Whitney U and Kruskal Wallis tests. $P < 0.05$ was accepted for statistical significance.

RESULTS

A total of 182 patients over 65 with acute calculous cholecystitis, whose file data could be accessed retrospectively within the specified period of time, were included in the study. The number of female patients was 110 (60.4%) and the number of male patients was 72 (39.6%). The mean age of female and male patients was similar (74.53 ± 7.08 years and 74.93 ± 6.21 years, respectively).

Table 2. The Canadian Study of Health and Aging (CSHA) frailty scale frequencies of patients

Frailty	Number of patient/percent
1	33/18.1%
2	65/35.7%
3	37/20.3%
4	23/12.6%
5	16/8.8%
6	6/3.3%
7	2/1.1%

When the distribution of the patients according to the CSHA frailty scores was evaluated, it was seen that the patients were most frequently (n = 135, 74.17%) in CSHA classes 1, 2 and 3, which were not considered as frail, and a total of 24 (13.2%) patients were in the frail group (Table 2).

When patients were sub-grouped as frail and non-frail, the mean age was 84.71 ± 5.09 years in the group considered frail, while this value was 73.16 ± 5.55 years in the non-fragile group ($p < 0.001$). The number of patients with more than two additional diseases in the frail group was 9 (4.9%) and 12 (6.6%) in the non-frail group. Similar to the mean age distribution, multimorbidity was statistically higher in the frail patient group ($p < 0.001$) (Table 3).

The mean CRP value was 16.18 ± 7.53 mg/dLin the frail group, and 12.10 ± 10.96 mg/dL in the non-

Table 3. The comparison of means in independent groups according to frailty

	Non-frail (CSHA 1, 2, 3, 4)	Frail (CSHA 5, 6, 7)	Total	*p* value
Age (years) (mean ± SD)	73.16 ± 5.55	84.71 ± 5.09		**< 0.001**
Male/Female, n (%)	63/95 (34.6/52.2)	9/15 (4.9/8.2)	72/110 (39.6/60.4)	0.825
Multimorbidity > 2, n (%)	12 (6.6)	9 (4.9)	21(11.5)	**< 0.001**
Use of anticoagulant agent, n (%)	45 (24.7)	12(6.6)	57(31.3)	**0.034**
CRP (mg/dL)	12.10 ± 10.96	16.18 ± 7.53		0.081
Leucocyte (cells/mm^3) (mean ± SD)	11465.11 ± 5227.04	14054.58 ± 6523.78		**0.030**

CSH = Canadian Study of Health and Aging, CRP = C-reactive protein, SD = standard deviation

Table 4. The concomitant cholangitis or pancreatitis prevalence according to age groups

	65-80 years	> 80 years	Total	*p* value
Patients with concomitant cholangitis or pancreatitis, n (%)	44 (24.2)	20 (11.0)	64 (35.2)	**0.017**
Total, n (%)	143 (78.6)	39 (21.4)	182 (100)	

Table 5. Treatment modalities according to ASA scores

	ASA I, II	ASA III, IV	Total	*p* value
Surgery, n (%)	36 (19.8)	10 (5.5)	46 (25.3)	**< 0.001**
Percutaneous cholecystostomy, n (%)	1 (0.5)	10 (5.5)	11 (6.0)	**0.004**
Medical treatment, n (%)	47 (25.8)	61 (33.5)	108 (59.3)	**0.008**
ERCP, n (%)	12 (6.6)	10 (5.5)	22 (12.1)	0.772

ASA = American Society of Anesthesiologists, ERCP = endoscopic retrograde cholangiopancreatography

frail group. The mean leukocyte value was 14054.58 ± 6523.78 × cells/mm^3 in the frail group, and 11465.11 ± 5227.04 × cells/mm^3 in the non-frail group. There was no significant difference between the groups in terms of mean CRP and leukocyte values (p = 0.081 and p = 0.030, respectively) (Table 3).

In total, 57 (31.3%) patients were using anticoagulant or antiaggregant agents. There was no significant difference between the frail and non-frail groups in terms of gender distribution and use of anticoagulants (p = 0.825 and p = 0.034, respectively) (Table 3).

It was observed that there were 39 patients in the octogenarian (over the age of 80) group. 20 patients had concomitant cholangitis or pancreatitis diagnoses and regarded as complicated cholecystitis. There were 44 patients with complicated cholecystitis in the 65-80 age group (p = 0.017) (Table 4).

It was observed that medical treatment was the most common (n = 108; 59.3%) treatment option and surgery was planned in elective conditions for this group. The number of patients who underwent emergency surgical treatment in the early period was 46

Table 6. Treatment modalities according to The Canadian Study of Health and Aging (CSHA) frailty scale

	Non Frail	Frail	Total	*p* value
Surgery, n (%)	46 (25.3)	0	46 (25.3)	**0.002**
Percutaneous cholecystostomy, n (%)	3 (1.6)	8 (4.4)	11 (6.0)	**< 0.001**
Medical treatment, n (%)	94 (51.6)	14 (7.7)	108 (59.3)	0.914
ERCP, n (%)	19 (10.4)	3 (1.6)	22 (12.1)	0.957

ERCP = endoscopic retrograde cholangiopancreatography

Table 7. The readmission and mortality frequencies according to ASA scores

	ASA I, II	ASA III, IV	Total	*p* value
Readmission, n (%)	10 (5.5)	10 (5.5)	20 (11)	0.876
Mortality, n (%)	2 (1.1)	4 (2.2)	6 (3.3)	0.361

ASA = American Society of Anesthesiologists

Table 8. The readmission and mortality frequencies according to The Canadian Study of Health and Aging (CSHA) frailty scale

	Non Frail	Frail	Total	*p* value
Readmission, n (%)	19 (10.4)	1 (0.5)	20 (11)	0.251
Mortality, n (%)	4 (2.2)	2 (1.1)	6 (3.3)	0.180

(25.3%). The number of patients who underwent percutaneous cholecystostomy was 11 (6.0%) and the number of patients who underwent endoscopic retrograde cholangiopancreatography (ERCP) was 22 (12.1%) (Tables 5 and 6).

When the treatment modalities applied to the patients were divided into subgroups according to their ASA scores and state of frailty, emergency surgical treatment was applied more frequently for patients with ASA I-II compared to ASA III-IV patients ($p < 0.001$) and percutaneous cholecystostomy was performed more frequently for frail patients compared to non-frail patients ($p < 0.001$) (Tables 5 and 6).

It was observed that 20 (14.70%) patients who were not operated, were admitted to the hospital again within 30 days due to similar complaints. It was observed that re-admissions to 125 the hospital were not associated with the ASA scores and frailty score ($p = 0.876$ and $p = 0.251$, respectively) (Tables 7 and 8). A total of 6 (3.29%) patients included in the study ended up with mortality. When the relationships between mortality and ASA and frailty scores were evaluated, no significant difference was found ($p = 0.361$ and $p = 0.180$, respectively) (Tables 7 and 8).

DISCUSSION

Cholelithiasis is a very common disease in the society. As the prevalence of cholelithiasis increases with age, gallbladder stones are detected in 20% of men and 35% of women by the seventh decade [7]. In our patient group, acute cholecystitis was more common in female patients. Complications related to cholelithiasis are the most common cause in the elderly patient group referring to the emergency services due to acute abdomen [8].

It has been reported that the risk of morbidity and mortality is higher in the elderly patients compared to the young adult patients. The incidence of patients with complicated cholecystitis accompanied by gangrenous cholecystitis, cholangitis or pancreatitis is higher the in elderly [9-11].

In our study, when evaluated numerically, complicated cholecystitis was frequently encountered in the elderly patients. It has been observed that this risk may be higher in older ages. Today, early surgical treatment is recommended for both young and elderly patients who are admitted to the hospital due to biliary pancreatitis and cholecystitis attacks [1]. It has been shown that there is an increase in the frequency of re-admissions to the hospital with similar complaints and morbidity in patients who did not undergo surgical treatment [12, 13]. Laparoscopic cholecystectomy usually does not cause severe endocrine, metabolic, and inflammatory responses in patients because it is a minimally invasive surgery. However, studies have shown that there is a significant increase in inflammatory markers in the postoperative period in the elderly patients after cholecystectomy [9]. When the cholecystitis patients in our study group are evaluated, it is seen that the mean CRP and leukocyte values, which help to show the inflammation in the body, are higher at the time of admission, more higher in frail patients. Due to comorbidities and general health problems in the elderly patients, the patients in this group become more sensitive to acute stresses and traumas [14, 15]. It has been reported that the risk of mortality increases significantly in the perioperative period in the elderly patients, even in low-risk surgical procedures, when compared with the younger patient group. It has been reported that the risk of mortality increases dramatically in every decade over the age of 50, and can reach values as high as 40-50%, although it varies according to the diagnosis and surgical procedure for those over 80 [14].

When the literature data is evaluated, it is seen that the recommendations specified in the Tokyo guideline are not followed in the surgical treatment of the elderly cholecystitis patients even in the developed countries

[3]. It is thought that cholecystectomy rates are low in the elderly patients due to difficulties in diagnosis, comorbidities, and increased perioperative morbidity and mortality risks in the elderly patients. In a study conducted even in the developed European countries such as the United Kingdom, Scotland and Sweden, the rates of patients who underwent surgical treatment in the early period ranged from 20% to 62% [3].

In the study of Mclsaac *et al*. [16], it was reported that 15% of the 65-69 age group patients who underwent cholecystectomy in emergency conditions and up to 40% of the patients over the age of 80 had a decrease in their ability to do their daily activities and an increased risk of being dependent on other people in the postoperative period.

In the study of Lupinacci *et al*. [13] on 81 cholecystitis patients over 80, they compared the surgical treatment on emergency conditions with semi-elective conditions. As a result, they reported that need for intensive care, days of hospitalization and complication rates were higher in the group that underwent emergency surgery. They showed that length of hospital stay of the patients was prolonged, and the mortality risk increased up to 32% in the emergency surgery group. At the end of the study, they recommended that more studies should be conducted for the decision making and timing of surgery in patients with acute cholecystitis [13].

In our study, medical treatment in acute phase and surgery in elective conditions was the most frequently preferred treatment method in the elderly patients with cholecystitis.

Trust *et al*. [3] suggested to apply non-surgical treatment methods in the acute period for patients who use anticoagulant or antiaggregants such as aspirin and clopidogrel, or who have uncontrolled comorbidities and later on surgical treatment in elective conditions [3]. Similarly, Elixhauster *et al*. [17] reported in their study that profit and loss should be evaluated for patients who use anticoagulant or antiaggregant agents and have comorbidities when making a surgical decision.

The high number of patients using anticoagulants in our study was one of the factors that distracted us from the decision to apply surgical treatment in the early period.

In the WSES and SICG 2017 guidelines, in which many studies have been rewieved, it has been reported that it is more difficult to decide between surgery, medical treatment or percutaneous cholecystostomy for the treatment of elderly patients with acute cholecystitis compared to younger patients. In elderly patients with acute cholecystitis, it has been recommended to use scores that determine surgical risk and frailty in making the decision for laparoscopic cholecystectomy in the early period [1].

The effect of the ASA score, which determines the surgical risk of the patient, was evaluated for the surgical treatment method and timing of surgery in elderly cholecystitis patients. Accordingly, it has been shown that there is an increased risk of complications and mortality after cholecystectomy in patients with a high number of additional diseases and a high ASA score. It was emphasized that the post-operative intensive care unit need of these patients were increased [18, 19].

It has been reported that percutaneous cholecystostomy is an effective temporary treatment that can be applied before the final surgical treatment for cholecystitis patients with ASA 3 and above [20]. However, percutaneous cholecystostomy was not recommended as the gold standard treatment due to high mortality rates, prolonged hospitalization, and high rate of re-admissions to the hospital [21].

Frailty refers to the clinical sensitivity of the person as a result of the loss of physiological systems with aging [6]. Geriatric studies have shown that frailty is superior to chronological age in assessing the patient. In fact, it has been reported that frailty of the patient is more effective in predicting perioperative morbidity and mortality than the assessment made with the ASA score alone [22]. Some scoring systems have been developed to objectively define frailty. However, some of the defined scoring systems are difficult to use in daily clinical practice. CSHA scoring system is a widely used method due to its ease of use and high accuracy in determining mortality risk [6].

Goeteyn *et al*. [23], reported that mortality risks of frail patients after emergency surgery were significantly increased compared to the non-frail patient group. For the frail patient group, mortality rates increase up to one third within 1 year after the emergency surgical procedure. The fact that most of the deaths occur in the early postoperative period draws attention to the stress factor that surgery creates on the patient [23]. Similarly, Khan *et al*. [5], in their study

on 326 elderly surgical patients, reported that frailty has an effect on the prognosis of patients and that frailty should be taken into consideration when making an emergency surgical procedure. Studies have shown that frailty score is an independent marker for postoperative complications, length of hospital stay, and mortality risk in the elderly patients after surgical procedures [24, 25].

In our patient group, it is seen that the ASA score and CSHA frailty score are effective in planning the treatment choice of the elderly cholecystitis patients. In our study, it has been shown that these evaluation methods are especially effective in making the decision on performing surgery and percutaneous cholecystostomy in the early period.

When all patients are evaluated, our mortality rate is not high. However, the high rate of re-admission of patients to the hospital within 30 days is high. It can be attributed to the low rate of patients who underwent surgery in the early period.

Limitations

One of the limiting factors of our study is that, the time interval of our study coincides with Covid 19 pandemic. The increase in the occupancy rate of intensive care beds due to Covid disease has been one of the factors that distracted us from the decision on early surgical treatment of the elderly patients who may need intensive care after surgery. When the treatment option applied to our patients is evaluated, our early surgical treatment rate is less than the literature recommendations. As another limitation of our study, we can say that the number of our patients is low in the frail and octogenarian patient groups. Therefore, we could not show statistically significant difference when evaluating the distribution of patients with comorbid diseases and complicated cholecystitis. We think that a prospective multicenter study conducted on more patients under favorable conditions after the pandemic, will yield clearer results.

CONCLUSION

Today, with the increase in the number of elderly patients, the number of emergency surgical treatment is also increasing for elderly patients. In the treatment of acute cholecystitis, early surgical treatment is much recommended for elderly patients as young patients. However, conditions such as additional diseases, use of anticoagulant agents, poor general condition and the need for intensive care unit in the elderly patients affect the surgical decision of surgeons. We think that multidisciplinary geriatric evaluation is necessary when deciding surgical intervention in the elderly patients and it is beneficial to include frailty in the evaluation.

Authors' Contribution

Study Conception: NT, CD, DE; Study Design: NT, CD, DE; Supervision: NT, CD, DE; Funding: NT, CD, DE; Materials: NT, CD, DE; Data Collection and/or Processing: NT, CD, DE, ÜBB; Statistical Analysis and/or Data Interpretation: NT, CD, DE, ÜBB; Literature Review: NT, CD, DE, ÜBB; Manuscript Preparation: NT, CD, DE, ÜBB and Critical Review: NT, CD, DE, ÜBB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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A window of opportunity against diabetes: frequency of microvascular and macrovascular complications in prediabetes

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ABSTRACT

Objectives: To determine the chronic complications of diabetes mellitus (DM) in patients with prediabetes, and to compare prediabetics with normoglycemic group participants in terms of the presence of the complications of DM.

Methods: An observational study was conducted between December 2018 to April 2019. The patients aged 18-65 years were recruited from an internal medicine outpatient clinic of a tertiary care hospital. A total of 106 prediabetic patients and 54 normoglycemic subjects were included to the study. OGTT-0th, OGTT-2nd and HbA1c levels, lipid parameters, blood pressure, the homeostasis model assessment of insulin resistan (HOMA-IR), body mass index (BMI) were estimated. Nephropathy (urine protein/urine creatinine ratio, serum creatinine [sCre], Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation-- creatinine clearance), retinopathy by dilated fundus examination and neuropathy (10-g monofilament testing and electroneuromyography [ENMG]) were assessed.

Results: Age, gender, BMI, HOMA-IR, smoking status, lipid parameters, systolic blood pressure were similar in both groups. The values of oral glucose tolerance test (OGTT)-0th, OGTT-2nd and glycated hemoglobin (HbA1c) were higher in prediabetics. Although not statistically significant, proteinuria was slightly more occurred in the prediabetics than the controls. sCre was significantly higher, and CKD-EPI equation was significantly lower in prediabetics than in controls (p = 0.012, p = 0.001, respectively). We did not detected diabetic retinopathy in any participants. Neuropathy was slightly more occured in prediabetics, but it was not significantly different (p = 0.309). There were no correlation between sCre, CKD-EPI, proteinuria and age, BMI, HOMA-IR, OGTT-0th, OGTT-2nd, and HbA1c.

Conclusions: Managing the prediabetes by early diagnosis is very meaningful in terms of prevention from DM and its complications. So, prediabetes may be a window of opportunity for diabetes associated morbidity.

Keywords: Complications, nephropathy, neuropathy, prediabetes, retinopathy

Prediabetes (PD) is explained as an intermediate condition with plasma glucose levels ranging between normoglycemia and diabetes mellitus (DM) [1]. PD is classified as isolated impaired fasting glucose

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(IFG) (a fasting plasma glucose value of 100 to 125 mg/dL) or impaired glucose tolerance (IGT) (a 2-hour plasma glucose value of 140 to 199 mg/dL) in the 75-gram oral glucose tolerance test (OGTT). Glycated hemoglobin (HbA1c) level of 5.7% to 6.4% is also considered to be PD [1, 2].

The complications of PD and DM, which has rapidly increasing prevalence in many countries such as USA [2] and Turkey [3], are a major public health problem. These complications contain microvasculary (retinopathy, nephropathy and neuropathy) and macrovasculary complications (cardiovascular events, cerebrovascular disease and peripheral artery disease). As a result of complications of DM; hemodialysis, cardiac interventions, eye exams, various interventional procedures and surgical operations are being performed, that leads to an increase in economic costs, hospitalizations and mortality [4, 5]. In addition, quality of life and labor conditions are also severely affected [6]. Although these complications are relatively less in PD than DM, they can be seen even in the prediabetic stage of hyperglycemia [7, 8]. It is shown in many studies that PD was associated with cardiovascular events and mortality, but there are only a few studies that determining microvasculary complications in prediabetic patients compared to the normal population [9-11].

In our study, we aimed to determine the chronic complications of DM in prediabetic patients, and to compare prediabetics with normoglycemic control group participants in terms of the presence of the diabetic complications.

METHODS

Participants

A cross-sectional study was conducted between December 2018 - April 2019. Fasting plasma glucose (FPG) and HbA1c levels were determined for all participants who admitted to recruit voluntary healthy subjects in the tertiary hospital's internal medicine outpatient clinic for routine health control. Glucose values of OGTT-0th and OGTT-2nd were conducted for all participants without diagnosed diabetes. Then, people whom had blood glucose levels in prediabetic range, or normal range were included to the study, consecutively. PD was defined as 0-hour plasma glucose value (OGTT-0th) of 100-125 mg/dL (IFG) and/or 2-hour plasma glucose value (OGTT-2nd) of 140 mg/dL to 199 mg/dL (IGT) (1). HbA1c value of 5.7% to 6.4% was also considered to be PD [1].

A total of 160 participants (106 prediabetic and 54 control group participant), 18-65 years old, were enrolled to the study. Exclusion criteria were as follows: renal failure, proteinuria, recent urinary tract infection (UTI), corticosteroids use, and endocrinological disorders (diabetes mellitus, thyroid function disorders, cushing disease, acromegaly). Also, the patients did not have any announcement or educational programs including diet restriction or regular exercise.

Health Indicators

Height and weight were measured and body mass index (BMI) calculated as weight in kilograms divided by height in meters squared. BMI was categorized as normal (BMI < 30 kg/m^2), and obese (BMI: 30 kg/m^2 and above) [12].

Measurement of Laboratory Parameters

A fasting venous blood sample was collected after an overnight fast of at least 12-h for biochemical investigations and samples were processed at the hospital laboratory on the same day. Fasting plasma insulin (FPI) and glucose, serum blood urea nitrogen (BUN), serum creatinine (sCre), plasma and urine protein were estimated using a Roche Cobas 8000 immunoassay analyzer (Roche Diagnostics, USA). Plasma glucose values at 0th and 2nd hours were conducted by OGTT, and HbA1c levels were measured for all participants. HbA1c were estimated using an Adams A1c HA-8180V automatic analyzer (Arkray Diagnostics, USA). All assays were performed with specific kits and calibrators supplied by the manufacturers. While evaluating the status of complications, laboratory analyzes of the participants were also made on the same day.

Insulin Resistance (IR)

Twelve-hour fasting blood samples were obtained for FPI and FPG determinations in order to calculate the homeostasis model assessment of insulin resistance (HOMA-IR). It was defined by the formula [13]: HOMA-IR = FPI (mU/L) × FPG (mmol/L)/22.5. If the result is ≥ 2.5, it indicates the presence of insulin resistance. The higher the score, the greater the insulin

resistance is measured.

Nephropathy Assessment

A random spot urine sample was collected as part of each routine clinical assessment. Proteinuria is measured by "urine protein/urine creatinine ratio (PCR)". Creatinine clearance was evaluated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [14].

Ophthalmic Assessment

All patients with hyperglycemia received a comprehensive ophthalmic assessment consisting of autorefractometer measurement, visual acuity assessment with Snellen, slit-lamp biomicroscopy, stereoscopic fundus examination measurements. Mydriatic eye drops to dilate the pupils are administered before retinal examinations. A combination of 0.5% tropicamide and 2.5% phenylephrine was used to dilate the pupil. Patients with microaneurysms, retinal hemorrhage, macular edema, hard exudates, soft exudates, intraretinal microvascular abnormalities, neovascularization, vitreous hemorrhage who have one or more of findings were classified as diabetic retinopathy after dilated fundus examination by an ophthalmologist (ES) who was blinded to the paticipants' clinical data. The screening protocol was performed in accordance with the guideline recommendation [15].

Neuropathy Assessment

Symptoms and signs of neuropathy were assessed in all patients by a neurologist (MFG) who were blinded to the participants' clinical data. All patients were performed 10-g monofilament testing to identify feet at risk for neuropathy. Assessment for distal symmetric polyneuropathy were included a careful history and assessment of either temperature or pinprick sensation (small-fiber function). The screening protocol was performed in accordance with the guideline recommendation [15]. Patients who had suspicion of neuropathy referred to neurology department for electroneuromyography (ENMG). In all patients, neurophysiological studies were done using standard procedures by a neurologist (MFG) by using Nihon Kohden Neuropack MEB-9200 (4-channel amplifier). Measurements were performed at temperatures of 33-34 °C. The criteria suggested by the American Academy of Neurology and the American Academy of Physical Medicine and Rehabilitation were used in order to entrapment neuropathy and polyneuropathy [16]. Participants who had clinically neuropathy, and verified with electrodianostical test, determinated as neuropathy.

Macrovasulary Complications

Information about the diseases of the patients was obtained by anamnesis. Cardiovascular disease (CVD) was asked to the participants. It was accepted that there was no cardiovascular disease complication in patients with normal electrocardiogram and coronary angiography findings.

Ethical Issues

The patient's written informed constent to publish the clinical informations and materials was obtained. Ethical approval for the study is received from Erciyes University Ethical Committee. This trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice.

Statistical Analysis

A power analysis program, G*Power version 3.0.10 software (Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany), The values of CKD-EPI were taken into consideration for the post-hoc power analysis. The effect size of CKD-EPI values was 0.492. The study power was calculated as 0.90 for $\alpha = 0.05$ with a sample size of 54 in the control group and of 106 in the study group. Statistical analyses were performed using the SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Number of cases and percentages were used for categorical variables. Categorical data were analyzed by Chi-square or Fisher's exact test, where appropriate. Shapiro-Wilks test and histograms were used to determine whether continuous variables were normally distributed. Normally distributed variables were presented as means and standard deviations (SD), non-normally distributed variables were presented as medians and interquartile ranges (IQR). Two independent groups of parametric variables were compared using Student t test. For non-parametric variables Mann-Whitney U test was administered. Relationship between non-parametric variables were analyzed by Spearman correlation tests and relationship between parametric variables were analyzed by Pearson correlation tests. A p value of <

0.05 was considered to indicate statistically significant differences.

RESULTS

A total of 106 prediabetic patients and 54 control group participants, 18-65 years aged, were recruited to the study, consecutively. In prediabetic group, 54 (50.9 %) patients had IFG, 15 (14.2%) patients had IGT, 32 (30.2%) patients had both IFG and IGT and 5 (4.7%) patients had only isolated elevated HbA1c.

Age, gender, BMI and the presence of obesity, HOMA-IR and the presence of insulin resistance, systolic blood pressure, presence of hypertension and ACE-i/ARB user, lipid profile were similar in both

Table 1. Comparison of clinical data between prediabetics with the control group

	PD (n = 106)	Control (n = 54)	*p* value
Age (year) (mean ± SD)	49.07 (9.77)	47.00 (11.21)	0.253
Gender (F/M), n (%)	80/26 (75.5/24.5)	38/16 (70.4/29.6)	0.488
Smoking, n (%)			0.075
Never	79 (74.5)	38 (70.4)	
Quit	12 (11.3)	2 (3.7)	
Smoker	15 (14.2)	14 (25.9)	
Obesity (+), n (%)	72 (67.9)	30 (57.7)	0.206
BMI (kg/m^2) (mean±SD)	33.80 (7.50)	32.66 (8.17)	0.400
Hypertension (+), n (%)	22 (21)	8 (15.1)	0.375
ACE-I/ARB user, n (%)	18 (17.3)	4 (7.5)	0.096
Blood Pressure (S/D), median (IQR)	120 (20)/80 (10)	120 (17.5)/70 (10)	0.401/**0.019**
Lipid Profile			
Total cholesterol (mean ± SD)	204.4 (37.69)	200.6 (39.54)	0.353
LDL (mean ± SD)	126.7 (37.76)	120.3 (33.21)	0.234
HDL, median (IQR)	45 (13)	46.5 (12.5)	0.141
Triglyserides, median (IQR)	144 (91)	127 (92.25)	0.252
OGTT-0 (mean ± SD)	105.28 (8.03)	91.59 (5.54)	**< 0.001**
OGTT-2 (mean ± SD)	131.71 (32.36)	108.48 (16.28)	**< 0.001**
HbA1c, median (IQR)	5.90 (0.50)	5.50 (0.30)	**< 0.001**
HOMA-IR, median (IQR)	2.41 (2.20)	2.11 (2.08)	0.318
sCre (mean ± SD)	0.76 (0.11)	0.70 (0.13)	**0.012**
CKD-EPI equation--creatinine clearance (mean ± SD)	96.21 (12.35)	103.28 (11.50)	**0.001**
Proteinuria (mg/24 h), median (IQR)	70.30 (37.86)	64.07 (30.86)	0.298
Neuropathy, n (%)	20 (19.40)	8 (15.70)	0.309
Retinopathy, n (%)	0	0	-
CVD, n (%)	3 (2.83)	0	-

ACE-I = angiotensine converting enzyme inhibitör, ARB = angiotensine receptor blocker, BMI = body mass index, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration, CVD = cardiovasculary disease, HbA1c = glycated hemoglobin, F/M = female/male, OGTT = oral glucose tolerance test, HOMA-IR = the homeostasis model assessment of insulin resistance, sCre = serum creatinine, S/D = systolic/diastolic, SD = standard deviation. $p < 0.05$ considered statistically significant.

groups. OGTT-0th, OGTT-2nd and HbA1c were significantly higher in the prediabetics than in the normoglycemic participants.

Although proteinuria levels were similar (p = 0.298), sCre was significantly higher, and CKD-EPI-creatinine clearance was significantly lower in prediabetic group than in the control group (respectively; p = 0.012, p = 0.001).

Neuropathy was more occurred in PD group, but it was not significantly important (p = 0.309). Twenty (19.4%) prediabetic patients, and 8 (15.7%) control group participants had neuropathy. All neuropathic participants had entrapment neuropathy (median nerve), but in PD group two patients had also polyneuropathy (PNP). One of them had axonal PNP in lower extremities and the other one had sensory-motor mixt type PNP. Nobody had retinopathy in both groups.

In prediabetic group, three patients had CVD. In control group, participants had not any macrovascular complications. The comparison of prediabetic and control groups' data were summarized in Table 1.

Correlation analyses between sCre, CKD-EPI, proteinuria and age, BMI, HOMA-IR, OGTT-0th, OGTT 2nd, HbA1c were performed (Table 2). There were no significant relationship between parameters (r or rho < 0.250), except CKD-EPI and age, that was a negative good correlation (r: -0.511, p < 0.001).

DISCUSSION

In this study, the frequency of microvascular and macrovascular complications in prediabetic patients were determined, and when compared to the control group participants who had the similar age, gender, BMI and IR, similar frequency of microvascular complications were found.

Impaired glucose metabolism has a significant role in atherosclerosis. Previous studies show that increased plasma glucose level is a risk factor for CVD (cardiovascular death, myocardial infarction, stroke and peripheral artery disease) regardless of the presence of diabetes [9, 10, 17]. In our study, we determined three prediabetic patients with CVD. There was no CAD in the normoglycemics. Although our data in terms of macrovascular complications seemed to be incompatible with the literature [1, 3, 15], all of the prediabetic patients whom recruited to the study were newly diagnosed patients because of the study design. It is a new data for the literature that the lower frequency of macrovasculary complications in newly diagnosed prediabetic patients. This outcome also suggests that the earlier the prediabetes is diagnosed, the less complication and the economic burden can be prevented.

Diabetic nephropathy is the leading cause of renal failure and is responsible for morbidity and mortality in diabetes. Proteinuria is a marker of kidney injury, serving as a screening test as well as a means of assessing the degree of nephropathy and risk for cardiovascular events and death in both the diabetic and the non-diabetic population [18, 19]. Lots of studies have shown that the prevalence of microalbuminuria in patients with prediabetes was higher than normoglycemics [20, 21]. It was also reported that prediabetes is a significant risk factor for proteinuria compared to people with completely normal glucose levels in a population-based study conducted with 228778 subjects [22]. Proteinuria is associated with the presence of hypertension, and it is known that proteinuria can be prevented by using angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) [23]. Whereas there were twenty-two patients (21%) with hypertension in prediabetic subjects, in control group eight participants (15.1%) had hypertension in our study, and that difference between groups were not statistically significant. While there were eighteen (17.3%) participants

Table 2. Correlations of kidney function tests and glucose metabolism associated factors

	Age		BMI		HOMA-IR		OGTT-0		OGTT-2		HbA1c	
	r	*p* value	r	*p* value	rho	*p* value	r	*p* value	r	*p* value	rho	*p* value
sCre	-0.005	0.959	-0.237	0.014	-0.085	0.402	-0.016	0.869	-0.125	0.203	-0.064	0.515
CKD-EPI	**-0.511**	**< 0.001**	-0.047	0.632	0.171	0.090	-0.086	0.385	-0.018	0.857	-0.085	0.389
Proteinuria	0.103	0.300	0.171	0.085	0.170	0.094	0.050	0.615	0.066	0.507	-0.128	0.197

BMI = body mass index, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration, HbA1c = glycated hemoglobin, HOMA-IR = the homeostasis model assessment of insulin resistance, OGTT = oral glucose tolerance test, sCre = serum creatinine

using ACEi or ARB in prediabetics, there were four (7.5%) participants using ACEi or ARB in control group. Again the difference between groups were not statistically significant. Thus, direct effect of prediabetes on proteinuria could observed. Although it was not statistically significant, proteinuria was a little more occured in the prediabetic patients than in the control subjects in our study. Insulin resistance has the main role in the pathophysiology of PD [24]. IR may also be one of the pathological links between prediabetes and renal dysfunction, as reflected by proteinuria [25, 26]. In our study, similar frequency of IR and similar values of HOMA-IR may be the reason of similar proteinuria levels in the prediabetics and control subjects.

Although PD was known to be a risk factor for development of proteinuria, it was shown to have no effect on CKD-EPI in a cross-sectional study with 228.778 Japanese whom aged ≥ 20 years [22]. Several previous population-based studies with 4 to 8 years of follow-up reported that PD did not decrease or increase CKD-EPI [27-29]. More recently, Kawata *et al*. [30] and Melsom *et al*. [31] were obtained unusual results in their studies. CKD-EPI values of prediabetic patients were higher than control group, so they interpreted it as a risk for development of glomerular hyperfiltration related to PD. But in our study, PD has a statistically significant worsening effect on the value of CKD-EPI ($p = 0.001$). To clarify the impact of PD on CKD-EPI, further studies with more patients are needed. In correlation analyses, there were no significant relationship between sCre, CKD-EPI, proteinuria and BMI, HOMA-IR, OGTT-0th, OGTT 2nd, HbA1c. These outcomes may be associated to study protocol. Because all the patients were newly diagnosed. As expected, only age and CKD-EPI had a negative good correlation.

Some authors described narrowing of arterioles lumen, reducing of blood flow in retinal vessels, venulary dilatation and chronic inflammation in PD and early DM without signs of retinopathy [32, 33]. But we did not detected diabetic retinopathy findings in any participants. This result of our study might depend on our evaluation of retinopathy by screening instead of advanced technological methods (laser dopler, adaptive optics, optical coherence tomography, etc.).

Though the epidemiological link between neuropathy and PD is controversial, common thought is that the frequency of neuropathy increases in patients with PD. One case-control study has been shown that a neuropathy incidence is 2% in both prediabetics and normoglycemics, but small fiber neuropathy was not evaluated in that study [34]. In a study, an age-adjusted prevalence of neuropathy of 11.2% in patients with PD and 3.9% in normoglycemic subjects was found [33]. The MONICA/KORA study demonstrated that neuropathy was more common in PD compared to normoglycemics [35]. Although not statistically significant, in our study, neuropathy was slightly more occured in the prediabetic group than the control one. Two third of the patients were obese. As the control group received a similar ratio of obese patients as the prediabetic group, the median nerve entrapment neuropathy in the control group was higher than the incidence in the normal population [36, 37]. Further studies evaluating CTS are needed in more prediabetic patients without obesity.

Limitations

One of the limitations of our study is that we did not measure the excretion of albumin. Although the proteinuria evaluation in the spot urine sample is more accurate than the use of a dipstick test, a timed 24-hour urine collection or/and albumin:creatinine ratio might be more precise in measuring proteinuria and diabetic nephropathy. Although the number of patients included in the study is more than the number of studies in the literature, another limitation is the small number of patients. Because PD is a common condition in the community. And, there is a need for longitudinal studies with a very large population to obtain clear data. Also, the nature of this study is a cross-sectional observational study. Prospective studies are needed for better detection of complications in patients with prediabetes.

CONCLUSION

Managing the PD by early diagnosis is very meaningful in terms of prevention from DM and its complications. Prediabetes may be a window of opportunity for diabetes associated morbidity and mortality. Further analysis on large cohort of patients would be helpful to understand the potential of PD.

Authors' Contribution

Study Conception: UST, MFG; Study Design UST, FT; Supervision: ES, FT; Funding: UST, MFG; Materials: UST, MFG; Data Collection and/or Processing: UST, ES; Statistical Analysis and/or Data Interpretation: UST, FT; Literature Review: UST, FT; Manuscript Preparation: UST and Critical Review: FT.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Ethics Committee Approval

Ethics committee aproval was received from Erciyes University Ethical Committee (Approval Date: Fabruary 6, 2019; Approval Number: 2019/100).

Informed Consent

Written informed consent was obtained from the individuals who participated in this study.

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The differences between tattooed and non-tattooed individuals in body image coping strategies and attitudes toward cosmetic surgery: a cross sectional study

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ABSTRACT

Objectives: This study aims to compare tattooed and non-tattooed individuals in terms of their attitude toward cosmetic surgery in multiple motivational sources. Also, the differences across groups are examined by use of three different body image coping strategies

Methods: Participants were 108 tattooed and 202 non tattooed individuals. Respondents were 267 females and 43 males from Turkey in a private university. Their age ranged between 18 to 29, and the mean age is 21.

Results: The analysis indicated that tattooed individuals had a more positive attitude toward any cosmetic surgery application. Also, there is a difference in appearance fixation coping strategy between two groups. Tattooed individuals preferred to use appearance fixation to cope with their body image. However, the effect size of this difference was negligible to small.

Conclusions: These results suggest that tattooed and non- tattooed individuals differ remarkably in cosmetic surgery attitude. Having tattoos has an effect on cosmetic surgery motivational sources. Tattooed individuals are more motivated and look positive toward cosmetic surgery interventions than non-tattooed individuals. Also, tattooed individuals use more appearance fixation coping strategy.

Keywords: Body image, coping strategies, cosmetic surgery, cosmetic surgery acceptance, tattoos

Body image is a concept that expresses individuals' perceptions and attitudes towards their own bodies and an important domain of interpersonal functionality [1]. Individuals' satisfaction with their body manifests a sign of positive body image whereas negative body image relies on dissatisfaction from their body image [2]. Individuals with a positive body image value, appreciate and love their body and therefore avoid potentially harmful and permanent procedures [3]. Therefore, positive body image is expected to decrease the interest in aesthetic surgery.

Body-oriented interventions and body modifications are one of the most common ways for individuals to express themselves, create identity and the self. Body modifications; tattoos, piercings, stamping the body, cutting the body, is a term used to cover a wide variety of practices such as implant placement, and even actions such as dieting are included in this con-

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cept [4]. Transforming or modifying the body in the desired way is a common practice in all cultures. One of the most common interventions to make changes on the body is to get a tattoo.

Tattoos, which are one of the body ornaments and modifications, differ from temporary body ornaments [5] with their permanence and become a part of the individual's personality that needs to be understood. Historically, getting a tattoo has been associated with people experiencing difficulties, traumas, or great victories, but the way tattoos are perceived has changed over time [6]. While tattoos were once more common among outgroups, tattoos are now preferred by all levels of socioeconomic or different cultural groups [5, 7]. Although tattooing has become widespread in a wide range of groups, academic interest in the psychosocial aspects of tattooing has remained rather limited [8].

According to Le Breton [9], a tattoo is like a screen on which an imagined identity is projected. With symbols, shapes, forms, letters embroidered on the body people's predisposition to a certain type of music, literature, and life culture is revealed. Atkinson stated that tattoos are "indicating a contextual and negotiated identity" [10]. Tattoos are thought to represent both a powerful form of nonverbal communication [11] and symbolic communication [12]. Tattoos are also defined as individuals' attempts to establish control over their own bodies [13].

Although studies on tattoos and their psychological effects have increased rapidly in recent years, [14] discovered the lack of this literature on the relationship between body image and tattoos. The study found that both women and men reported significant reductions in body dissatisfaction and concerns about their appearance immediately after getting a tattoo [15]. In addition, participants in this study reported significant improvements in body appreciation three weeks after getting a tattoo. These results have been interpreted as tattoos can create a more positive effect in the short term to correct the body perception of individuals. The positive change in body perception can be thought of as individuals using tattoos as a way of coping with their body images. Additionally, It is thought that making permanent changes to the body may increase their willingness to accept cosmetic surgery. Based on the studies supporting that tattooing positively affects body image, it is thought that getting a tattoo may be related to a strategy that individuals prefer to use in coping with body dissatisfaction. In this line, the current study compares tattooed and non-tattooed individuals in terms of their attitude toward cosmetic surgery in multiple motivational sources and preferred body image coping strategies.

Individuals develop various cognitive and behavioral strategies to adapt to or cope with thoughts, feelings and situations that cause stress in body image [16]. When individuals are exposed to stressful stimuli or events related to body image, individuals use three coping strategies [17]. Firstly, the avoidance coping strategy is defined as an attempt to avoid threats to one's body image, thoughts and feelings [18]. For example, individuals who use this strategy avoid looking at themselves in the mirror or confronting themselves. Secondly, appearance fixing coping strategy can be seen as an effort to change the person's appearance by hiding or correcting a perceived physical defect. People who use this strategy can spend a lot of time changing and correcting something about their appearance. Third, and finally, a positive rational acceptance strategy involves mental and behavioral activities that emphasize accepting one's bodily experience. For example, someone might change their focus by reminding themselves that they have other important qualities as well.

Cosmetic surgery describes procedures applied to change the visible shape of the body without any disease, injury, deformation or hereditary condition that can be a factor in improving quality of life [19]. Since the main purpose of aesthetic interventions is to correct a patient's defects, it is optional and includes interventions that are not medically necessary [20]. There are a wide variety of applications and techniques for this purpose. One of the most frequently researched and correlated psychological variables in cosmetic surgery is low body satisfaction [21]. Individuals who have low levels of body satisfaction, more likely show interest in cosmetic surgery operations [19]. Similarly, getting a tattoo is one of the processes in which individuals make permanent changes in their bodies without any necessity. Therefore, there could be e several different motivators behind getting a tattoo. People getting a tattoo and cosmetic surgery may have similar tendencies to make lasting changes and interventions to the body.

Body image coping strategies might affect the peo-

ple's view of aesthetic interventions. The study showed that fixation appearance coping strategy positively correlated with surgical aesthetic intervention [22]. It would be valuable to understand the individuals' motivation and body image coping strategy behind the decisions before making permanent changes in their body. In this study, it was aimed to compare attitudes toward cosmetic surgery and body coping strategies between the tattooed and non-tattooed individuals.

METHODS

Personal Information Questionnaire

This form includes demographic questions such as gender, age, education, as well as participants were asked to indicate whether or not they were tattooed.

Acceptance of Cosmetic Surgery Scale (ACSS)

Henderson-King's [23] 15 item scale was used to measure individuals' acceptance of cosmetic surgery in general, and also underlying motivational reasons for having it done. It consists of three factors; intrapersonal, social and consider. The personal factor includes the personal evaluations of individuals about their appearance; social factor interests the opinions affirming aesthetic surgery regarding the individuals' feeling better in their social relationships and social environments are evaluated. Participants responded to each item on a scale from 1 (disagree strongly) to 7 (agree strongly). High scores indicate a more positive attitude towards cosmetic surgery. In the original study, it was reported that Cronbach Alpha coefficients ranged between .84 and .92. The Turkish validity and reliability study of this scale was conducted by Karaca *et al*. [24] and the total internal consistency coefficient was found to be Cronbach's alpha .92. Also, for interpersonal is .81 for, for social .86 , and for thoughts .90 Cronbach's alpha coefficient was reported. The Cronbach's alpha coefficient for this study is .93 for total score, .91 for intrapersonal; .84 for social; .90 for consider factor.

Body Image Coping Strategies Inventory (BICSI)

Body Image Coping Strategies Scale was developed to measure how individuals manage their body image threats or difficulties by Cash *et al*. [18]. It consists of 29 items and three body-image coping factors as positive rational acceptance, appearance fixing, and avoidance. Cronbach's alpha coefficient ranged between.74 and .91.of the factors. In the Turkish version validity and reliability study was carried out by Doğan *et al*. [25]. The three factor structure confirmed and reliability of the factors ranged .81 and .84. Cronbach's alpha coefficient for positive logical acceptance factor is .86, appearance fixation is .90, and avoidance is .83.

Participants

Respondents (n = 310) were 267 females (86.1%) and 43 males (13.9%) studying in the associate, undergraduate and master programs of the faculty of humanities and social sciences and arts and sciences of Doğuş University in Istanbul. Their age ranged from 18 to 29, and the mean age in this sample was 21. Participants were reached by a convenience sampling method. The questionnaire form was sent to 500 people in total, but 310 of these people participated in the study. The rate of participation in the research is 62%. The majority of participants were undergraduate students (73%), the rest of associate degree (24%), and master students (3%). The tattooed and non-tattooed groups compared in the sample of the study are equivalent in demographic terms such as age and educational status. Inclusion criteria for the study was being willing and motivated to participate in the study, being between 18-25 ages, continuing education in the educational institutions where the study was conducted. Exclusion criteria were determined for this study as having a p congenital physical anomaly, having undergone plastic surgery due to trauma or illness, having undergone or are considering cosmetic surgery simultaneously for functional and health purposes, having severe medical illness (e.g., cerebral palsy), persons with psychiatric disorders that may affect their ability to make decisions (e.g., psychotic disorders, bipolar disorder, body dysmorphic disorder, eating disorders). Exclusion criteria were chosen among common causes and related factors known to increase the tendency to cosmetic surgery. Demographic characteristics of the participants were presented in Table 1.

Procedure

Participants were informed of the purpose of the research and voluntary nature of their participation via online form. The study was complied with the decla-

Table 1. Demographic characteristics

	Frequency	%
Gender		
Male	43	14
Female	267	86
Marital status		
Single	306	98,7
Married	4	1,3
Education level		
Associate degree	9	23,9
Undergraduate	74	73,2
Master	227	2,9
Department		
Psychology	144	46,5
Child development	62	20
Interior architecture	52	16.7
Engineering	20	6.5
Banking and finance	15	4.8
Physiotherapy	10	3.2
Nutrition and dietetics	7	2.3

ration of Helsinki. Answering all the questions in the study on average 20 minutes lasted. Ethical permission to conduct the study was granted by the Doğuş University Ethics Committee (Decision No: E-42435178-050.06.04). Data collection process lasted two months, from March to April 2021.

Statistical Analysis

Data were analyzed by using SPSS 22.0 statistics programme, an independent t-test and multivariate analysis of variance analyses was conducted to test hypotheses.

RESULTS

Tattoo status

Overall, 108 (34.8 %) respondents reported having tattoos and 202 (65.3 %) people reported that they had no tattoos in the total sample of 310.

Preliminary between-group comparisons

We first examined differences in key demographics between tattooed and non-tattooed individuals. An independent t-test showed that there is no significant differences in age $t(308) = 1.62$, $p = .107$, sex $t(308) = -.68$, $p = 0.50$, between tattooed and non-tattooed individuals.

Between-group comparison

Multivariate analysis of variance (MANOVA) test was conducted to compare body image coping strategies and attitudes toward cosmetic surgery in tattooed and non-tattooed individuals. The three subdimensions of body image coping strategy and three motivational factors of cosmetic surgery acceptance of were entered as dependent variables, being tattooed versus non-tattooed was entered as the independent variable. First of all, the assumptions of MANOVA analysis, normal distribution, collinearity and homogeneity of variances were examined [26]. The normality analysis showed that skewness and kurtosis values ranged between -2 +2 values, so data were distributed normally [27]. The result of Levene's test revealed that dependent variables are equal between tattoo and non-tattoo groups, p-values greater than 0.05. Also, results of Box's M test showed covariance matrices are equal, $p > 0.05$. Thus, data has m*et al*l of the assumptions.

The results of analysis showed that the omnibus MANOVA effect was significant, $F(7,302) = 4.02$, $p < 0.0001$; Wilk's $\lambda = 0.96$, , $\eta p\ 2 = 0.09$. Descriptive statistics, along with the results of the univariate ANOVA are reported in Table 2.

As seen, results of the analysis indicated that tattooed individuals (mean: 35.25 ± 7.53) compared to non-tattooed individuals (mean: 33.36 ± 7.91), $t(308) = 2.03$, $p = 0.04$) showed high scores on appearance fixing body coping strategy. Although the difference between the group means was relatively small, these results suggest that having tattoos increases usage of appearance fixing body coping strategy. As predicted, the result of the analysis showed that there are no significant differences in positive rational acceptance, $t(308) = -.03$, $p = 0.97$. and avoid strategies, $t(308) = 1.79$, $p = 0.09$ between tattooed and non-tattooed individuals.

Cosmetic surgery acceptance scores showed a significant difference between two groups. Specifically, tattooed individuals (mean: 28.64 ± 9.69) have significantly higher scores in intrapersonal sub-dimen-

Table 2. Descriptive statistics for tattooed and non-tattooed participants and the results of multivariate analysis of variance

Variable	Tattoo status				Main effect of tattoo status		
	Tattooed (n = 108)		Non- tattooed (n = 202)		*F*	*p value*	η_p^2
	Mean	Standart deviation	Mean	Standart deviation			
Positive rational acceptance	22.417	0.468	22.436	0.342	0.001	0.974	0.000
Appearance fixing	35.250	0.749	33.366	0.547	4.125	**0.043***	0.013
Avoidance	15.917	0.475	14.866	0.347	3.185	0.075	0.010
Intrapersonal	28.639	0.926	24.144	0.677	15.353	**< 0.001****	0.047
Social	12.435	0.660	10.777	0.482	4.117	**0.043***	0.013
Consider	22.528	0.859	17.733	0.628	20.320	**< 0.001****	0.062

M = mean, SD = , *$p < 0.05$, **$p < 0.01$

sion than non tattooed individuals (mean: 24.14 ± 9.59), t (308) = 3.92, $p < 0.01$. Similarly, for consider sub-dimension of cosmetic surgery acceptance showed significantly higher scores tattooed individuals (mean: 22.53 ± 8.39) than on non-tattooed individuals (mean: 17.73 ± 9.20), t (308) = 4.51, $p < 0.01$. Tattooed individuals (mean: 12.44 ± 6.49) have significantly higher scores on social sub-dimension than non-tattooed individuals (mean: 10.78 ± 7,05), t (308) = 2.03, $p = 0.04$. These findings revealed that having tattoos has an effect on cosmetic surgery motivational sources. Tattooed individuals are more motivated and look positive toward cosmetic surgery interventions than non-tattooed individuals.

DISCUSSION

The purpose of the study was to compare tattooed and non-tattooed individuals in terms of their attitude toward cosmetic surgery in multiple motivational sources. Also, the differences across groups are examined by use of three different body image coping strategies which are appearance fixing, positive rational acceptance, and avoidance. The results showed that tattooed individuals use significantly higher appearance fixing body coping strategy than with non-tattooed. Appearance fixing body coping style reduces intrusive or unwanted ideas toward the body through aesthetic surgery [28]. Tattooing is classified as a type of appearance-related risky behaviors that contain potential health risks that people do to improve their appearance [29]. Earlier findings were consistent with these results, in a study of conducted by Italian adolescent girls showed that interest in tattoos and piercings are associated with greater interest in cosmetic surgery [30]. These findings may indicate that tattooed individuals are more prone to body-oriented changes to create desired self with permanent body changes like tattoos. Similarly, Armstrong *et al*. [28] found that getting tattoos is a way to make an impression on others and draw attention to specific areas of their body. The positive rational acceptance strategy was found to be associated with a more positive body image, a higher quality of life and self-esteem, and it was found that individuals using these strategies were less likely to define themselves according to their physical appearance [31]. Also, Pajor *et al*. [32] found no differences in self-esteem and life satisfaction between people with body modifications and those without such modifications. In line with all, these current findings support nonsignificant relationships between two groups in terms of positive rational acceptance strategy. On the contrary, earlier studies revealed the positive correlation between the number of body modifications and negative attitudes towards one's

own body [33], in this study two groups were not differentiated. Discrepancy in previous findings can be explained by methodological differences. As a last, the difference between two groups based on avoidance strategy is not significant. It can be thought that people with tattoos do not experience stress to cause avoidance in the current situation. Furthermore, another study showed that tattooed individuals have a positive effect on their body perceptions in the medium and long term [15], it supports our findings. The motivational sources to cosmetic surgery differs across two groups. People with tattoos are affected more by intrapersonal and consider reasons to accept permanent changes in their body. These motivators can be directly related to self-oriented benefits and improve self regard, general well being by making those lasting changes. Current analysis revealed that social factor which measures whether an individual would prefer cosmetic surgery for social reasons is relatively small effect size. This result may be interpreted as self-oriented evaluation is more important for tattooed individuals rather than others' evaluations and thoughts. The sample of this study consists of young adults. It is defined as a period in which individuals in this age period focus on themselves and self-oriented beliefs [34]. These findings can be explained by the characteristics of young adulthood.

Limitations

The fact that the sample of the study consisted of university students and a female-dominated sample limits the generalizability of the results. It may be recommended for future studies to test the same study in different age groups and in samples with equal gender distribution. Also, the effect sizes of between-group differences were small in some cases. Although these results are consistent with previous studies, it may also stem from inequality of numbers of people with tattoos and non-tattos. For future studies, examination of both quantity and quality of tattoos are also suggested. Another limitation of the study is that the participants were evaluated with self-report scales whether they had any psychiatric diagnosis or not. The fact that the psychometric characteristics of the participants before or after tattooing were not measured is also one of the limitations of the study due to the nature of this study. Despite the stated limitations, the current study provides a significant contribution to our understanding of the psychological dimensions that explain the motivation to get tattoos, and its relation to body image coping strategies, and preference for aesthetic interventions.

CONCLUSION

The findings of the study showed that tattooed individuals use more appearance fixing style to cope with their body image than non tattooed individuals. Also, tattooed and non- tattooed individuals differ significantly in attitudes toward cosmetic surgery. Tattooed individuals are more accepting of cosmetic surgery and look more positively to making permanent changes in their body than non-tattooed individuals.

Authors' Contribution

Study Conception: YK, ET; Study Design: ET, YK; Supervision: YK, ET; Funding: ET, YK; Materials: YK, ET; Data Collection and/or Processing: ET; Statistical Analysis and/or Data Interpretation: YK, Literature Review: ET, YK; Manuscript Preparation: ET, YK and Critical Review: ET, YK.

Informed consent

Informed consent was presented to each individual before participating in the study. All of them declared voluntary participation.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The effects of gold nanoparticles with different surface coatings and sizes on biochemical parameters in mice

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ABSTRACT

Objectives: Gold nanoparticles are very popular metallic nanomaterials and they have a wide spectrum of biomedical applications. This study was aimed to the production of stable and monodisperse polyethyleneimine (PEI) and polyethylene glycol (PEG) coated gold nanoparticles (AuNP20 and AuNP50), investigation of their in vivo biochemical effects in the BALB/c mice.

Methods: Gold nanoparticles were synthesized and their surfaces were modified by PEI and PEG. All the necessary physicochemical characterizations were performed. After the single high dose i.v. injection (5 mg Au/kg animal weight) of the AuNP groups, their in vivo biochemical effects were evaluated multiparametrically in the mice on day 14.

Results: Highly monodisperse and stable AuNPs were synthesized successfully. Significant changes in the biochemical hemogram parameters were observed depending on the surface coatings of the AuNPs. PEI and PEG surface coatings increased biocompatibility. No excessive oxidative stress response was observed in all the gold nanoparticle groups.

Conclusions: It has been concluded that the surface chemistry of the particles is a more decisive parameter than the size in terms of in vivo biochemical toxicity. The surface functionalization, stability and biocompatibility of the AuNPs are important parameters for the potential biomedical applications of gold nanoparticles in future studies.

Keywords: Gold nanoparticle, hemogram, nanotoxicity, oxidative stress, PEI/PEG surface coatings

Due to their unique chemical and physical properties, gold nanoparticles (AuNPs) have a wide range of applications in the biomedical field [1]. With the development of more innovative and effective nanomaterial designs, AuNPs have been widely preferred in biomedical research recently, with applications that include antibacterial studies [2], biosensors [3], detection systems [4], imaging [5], DNA/RNA delivery [6, 7], drug delivery [8], photothermal therapy [9] and targeted therapy [10]. The synthesis method, surface coating, size of the nanomaterials, duration and concentration of the exposure play a decisive role on the toxicity of the nanoparticles [11].

In some *in vitro* studies in the literature, it has been shown that gold nanoparticles without surface functionalization were clustered in larger endosomes in the cells by forming aggregates. It has also been reported that generally smaller particles cause more cellular

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toxic effects. It has been reported that PEG-coated gold nanoparticles including small ones, do not cause cellular toxic effects. In addition, it has been shown that particles are localized to smaller endosomes, reduced aggregation and increased cellular particle uptake, thanks to the PEG coating. In the literature, it has been evaluated that PEI surface coating improves cellular nanoparticle uptake, stability and biocompatibility. Under controlled synthesis conditions, a thin layer of low molecular weight PEI surface coating increases the stability of the particles and provides a positive charge [12-19].

Previous in vivo studies have been conducted to evaluate the toxicity and biochemical effects of different gold nanoparticles. It has been demonstrated that the PEG coated various gold nanoparticles increase the stabilization and circulation time in the blood and reduce toxicity [20]. Researchers have shown that smaller nanoparticles lead to more toxic effects and excessive oxidative stress production [21]. Further in vivo studies are needed to determine the biochemical effects of the various gold nanoparticles and their role in nanotoxicity.

This study was aimed to the production of stable and monodisperse polyethyleneimine (PEI, positively charged) and polyethylene glycol (PEG, slightly negatively charged) coated gold nanoparticles (AuNP20 and AuNP50), investigation of their in vivo biochemical effects in the BALB/c mice. Thus, in addition to conventionally synthesized citrate stabilized gold nanoparticles, AuNP variations with different surface chemistries and charges were obtained. In addition, considering the two different AuNP sizes, biochemical effects of various physicochemical parameters of the particles were evaluated apart from the literature.

METHODS

Gold Nanoparticles Synthesis, Surface Functionalization and Characterization

As a first stage, gold seed nanoparticles ($AuNP_{20}$) were synthesized and used in the synthesis of gold nanoparticles with average size ($AuNP_{50}$) in the following stages. Seed AuNPs were produced by modifying the Turkevich synthesis method [22]. Briefly, after 100 ml, 0.25 mM chloroauric acid ($H[AuCl_4]$) solution (Sigma-Aldrich) was prepared, the trisodium citrate dihydrate (reducing agent, Sigma-Aldrich) solution was added at a concentration of 0.033% by increasing the rotational speed of the boiling solution. After the prepared seed AuNP solution was cooled, it was centrifuged (Thermo-Scientific, MicroCL 21R) at 7,000 g for 30 min. Then, it was dispersed in deionized water. AuNPs of 50 nm size were synthesized by the seeding-growth method [23]. 100 ml of 0.25 mM chloroauric acid solution was prepared again, then 2.4 ml of seed AuNPs were added to the solution and mixed at medium rotational speed. Trisodium citrate was added at a concentration of 0.15 mM, and 1 ml of 25 mM hydroquinone (Sigma-Aldrich) solution was added and mixed for 10 min. In the next step, the synthesized AuNPs of 50 nm size were centrifuged at 7000 g for 30 min and then dispersed in deionized water.

For the purpose of PEI coating of all the AuNPs, 2% PEI (Mw: 10000-25000, Sigma-Aldrich) stock solution was prepared and it was added to the AuNPs solution at 0.005% final concentration and mixed for 1 h [17]. After the PEI coating, all the AuNPs solutions were centrifuged at 7000 g for 30 min and then dispersed in deionized water. For the purpose of PEG coating of all the AuNPs, 0.15 mM PEG-SH (Mw: 5000, Nanocs) solution was prepared and 100 µl of the solution was added to each ml of the synthesized AuNPs solution and mixed for 1 h. After the PEG coating, all the AuNPs solutions were centrifuged at 7000 g for 30 min and then dispersed in deionized water [18].

Inductively coupled plasma mass spectrometry (ICP-MS) measurements were applied (Perkin Elmer ICP-MS, Nexion 300X) to precisely quantify the amount of the gold (Au) in all the synthesized AuNPs solutions. For subsequent *in vivo* studies, all the AuNPs solutions were standardized to contain gold (Au) at 500 µg ml^{-1} concentrations. All the synthesized AuNPs were characterized regarding size and zeta potential using a zeta-sizer (Zetasizer Ultra-Malvern). Scanning electron microscope (SEM) images were taken (SEM-Zeiss Geminisem 500). The shifts in surface plasmon resonance (SPR) spectrum was determined by UV-visible spectrophotometer (Shimadzu UV-1800).

Fourier Transform Infrared Spectra analysis were carried out with Pelkin Elmer Spectrum Two equipped with ATR apparatus within the spectral region of

4000-400 cm^{-1} wave number at room temperature. Samples were scanned 4 times at a resolution of 2 cm^{-1} to get average spectra. To obtain FTIR spectra of samples, two drops of solution located on the crystal and the solvent were evaporated to eliminate solvent peaks from the spectra of the samples. FTIR spectra of $AuNP_{20}$, $AuNP_{23}$-PEI, $AuNP_{24}$-PEG, $AuNP_{50}$, $AuNP_{56}$-PEI and $AuNP_{57}$-PEG were obtained and analyzed.

Animals

A total of 21 male adult BALB/c mice were used for all the in vivo biochemical analysis. All the animal procedures were performed by virtue of Istanbul Medipol University Institutional Animal Care and Use Committee (IMU-HADYEK, Approval date/number: May 18, 2017 / 21). For the biochemical analysis, all the AuNP groups were dissolved in sterile PBS (250 µl) and injected as a single high dose (5 mg Au/kg animal weight) from the tail vein of the mice. All the mice were allowed free access to drinking water and diet. The cages were located in temperature-controlled normal room conditions and a light:dark period of 12 h.

Biochemical Analysis

The effects of various AuNPs groups on the changes in selected biochemical parameters (including; hemogram values, creatinine, alanine aminotransferase-ALT, aspartate aminotransferase-AST, total antioxidant capacity-TAC, total oxidant status-TOS) in mice were investigated. For this purpose, blood samples were collected via cardiac puncture which is considered a euthanasia procedure and should be performed only after ensuring that the animal was under deep anesthesia, from the mice 14 days post injection. Serum and plasma samples were obtained quickly by centrifugation at 3000 rpm for 10 min. Creatinin levels were measured with Jaffe method, and also ALT/AST parametres were measured with colorimetric method by the fully automatic analyzer (ROCHE module Cobas 6000), and the kits were procured by ROCHE. Hemogram values were analyzed with flow cytometry method by using hematology analyzer system (SYSMEX XN 1000).

The TAC and TOS values were measured colorimetrically in the mice serums using the methods developed by Erel [24, 25]. The principle of TAC measurement is briefly as follows: ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (Sigma-Aldrich) reagent is made radical with hydrogen peroxide by keeping the pH of the environment constant in the presence of acetate buffer (Sigma-Aldrich) solution. Following the addition of serum, antioxidants in the serum neutralize the existing ABTS radicals. As a result, the solution's color becomes lighter and the absorbance is measured at 658 nm. The principle of TOS measurement is briefly as follows: Fe_2SO_4 (Sigma-Aldrich) dissolves in water and releases Fe^{2+} ions. The oxidants in the serum enable Fe^{2+} to be oxidized to Fe^{3+}. The X-orange reagent (Sigma-Aldrich) used gives a colored complex with Fe^{3+}. The absorbance is measured at 658 nm. After TAC and TOS values are obtained, oxidative stress index (OSI) is calculated using the formula (TOS) / (TAC × 10).

Statistical Analysis

All the measurement and analysis results were given in tables. The numerical results represented the average values of three replicate measurements. Only the measurement results in the confidence interval of the device were used. No additional statistical approaches were used for differences between groups. Due to the large number of measured parameters, differences between the groups were discussed in the text.

RESULTS

Characterization of the AuNPs

The main characteristic properties of the synthesized gold nanoparticle groups are shown in Table 1. Seed gold nanoparticles ($AuNP_{20}$) were synthesized by the modified Turkevich method and were used in the synthesis of nanoparticles with the medium size ($AuNP_{50}$).

The gold nanoparticle size increased depending on the PEI and PEG surface functionalizations. Additionally, the polydispersity index (PDI) values remained below 0.2, showing highly monodisperse level. The changes in UV-visible peaks for seed and medium sized AuNRs occurred depending on the surface coatings. After the PEI coating, there was a significant positive increase in the zeta potentials. On the other hand, after the PEG coating, the zeta potential values re-

Table 1. Main characteristic properties of synthesized gold nanoparticle groups

AuNP Groups	Hydrodynamic diameter (nm)	Polydispersity index	UV-visible peak (nm)	Zeta potential (mV)
$AuNP_{20}$	20.04 ± 0.17	0.11	520	-48.53
$AuNP_{23}$-PEI	23.53 ± 0.20	0.15	522	+32.25
$AuNP_{24}$-PEG	24.92 ± 0.30	0.17	523	-22.76
$AuNP_{50}$	50.74 ± 0.14	0.12	535	-38.15
$AuNP_{56}$-PEI	56.21 ± 0.20	0.14	538	+30.16
$AuNP_{57}$-PEG	57.35 ± 0.30	0.16	540	-32.17

AuNP = gold nanoparticles, PEI = polyethyleneimine, PEG = polyethylene glycol

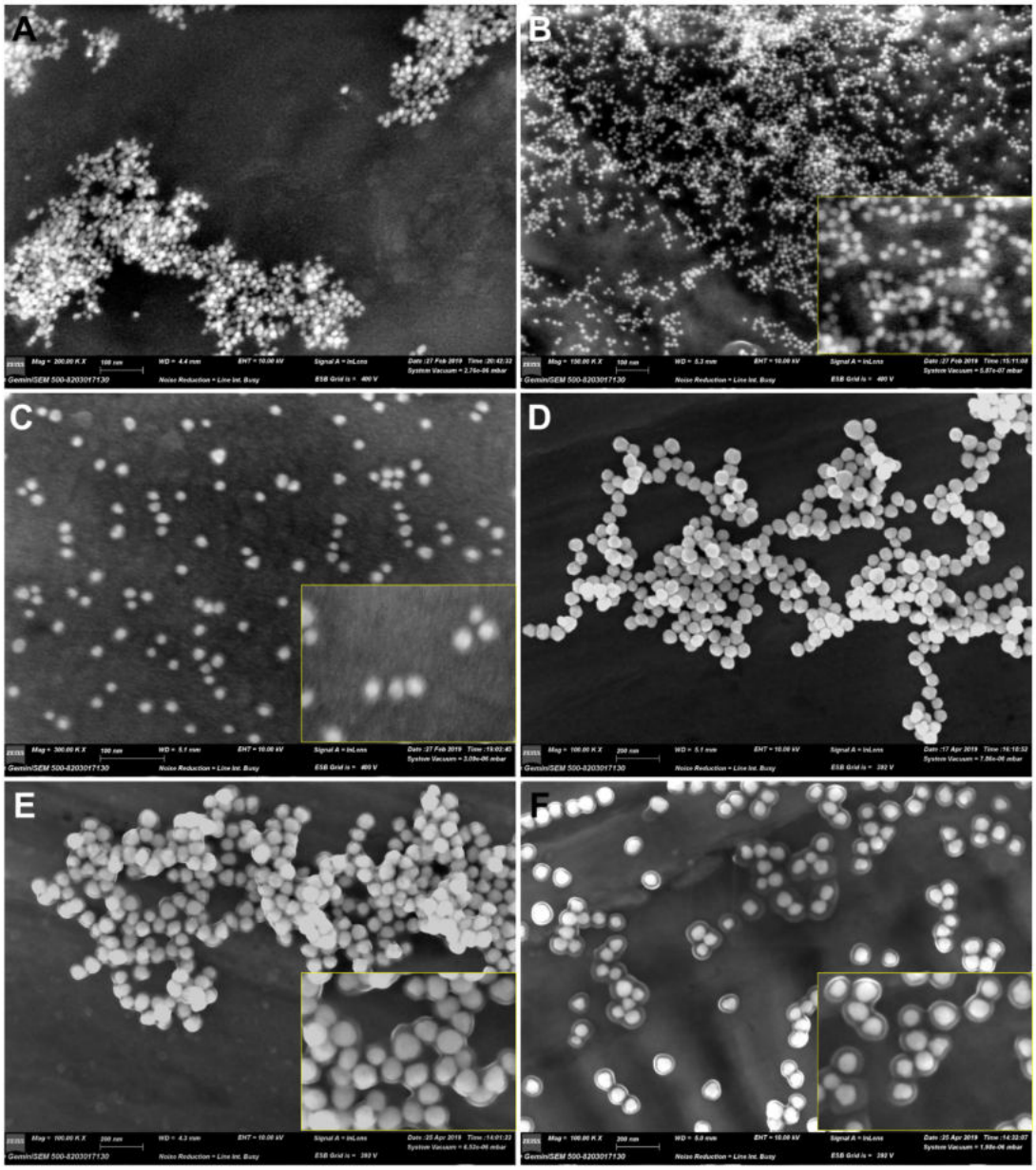

Fig. 1. SEM images of synthesized gold nanoparticles. (A) AuNP20. (B) $AuNP_{23}$-PEI. (C) $AuNP_{24}$-PEG. (D) $AuNP_{50}$. (E) $AuNP_{56}$-PEI. and (F) $AuNP_{57}$-PEG. AuNP = gold nanoparticles, PEI = polyethyleneimine, PEG = polyethylene glycol.

mained as negative (Table 1). According to the SEM images shown in Fig. 1, it is seen that highly monodisperse and stable gold nanoparticles have been synthesized. Again, the size measurements were also verified based on the SEM images. The PEI and PEG surface coatings are clearly visible, especially for the medium sized AuNPs (Figs. 1E and 1F). As shown in Figs. 1B and 1E, when AuNP was covered with PEI molecules, FTIR spectra of $AuNP_{23}$-PEI and $AuNP_{56}$-PEI yielded similar bands as PEI. The C-H stretching bands and N-H bending bands were observed at 1116 cm^{-1} and at 1574 cm^{-1} respectively which are similar to the positions observed with PEI. The peak at 3276 cm-1 and the shoulder at 3344 cm^{-1} are N-H stretching bands. The characteristic C-H stretching band is located at 2813 cm-1 [26]. The similar bands as PEG were shown in Figs. 1C and 1F. PEG, $AuNP_{24}$-PEG and $AuNP_{57}$-PEG spectrum peaks observed at 2883 cm^{-1} and 2971 cm^{-1} represent -CH_2 and -CH_3 asymmetrical vibrations respectively, whereas the peaks at 1115 cm-1 show -OH asymmetrical vibrations (Fig. 2) [27].

The Effects of the AuNPs on the Various Biochemical Parameters

The effects of different AuNP groups on the changes in the selected biochemical parameters in the mice were investigated on day 14 after a single i.v. injection (5 mg Au/kg animal weight) of the gold nanoparticle groups (Table 2). All the biochemical investigations were carried out using AuNP groups at a considerably higher dose than the literature. Significant changes in the biochemical parameters were observed depending on the surface coatings of the gold nanoparticles. Up to two-fold increases in the leukocyte percentages were observed compared to the control group with no AuNPs exposure. Additionally, PEI and PEG surface coatings slightly reduced this excessive increase. When the hemogram data were evaluated in terms of other standart parameters, generally the values were observed closer to the control group in the AuNP-PEI and AuNP-PEG groups.

According to the results of creatinine analysis applied to investigate kidney function, it was observed that the values increased up to two times compared to the control group. Nevertheless, it was evaluated that the values especially in PEI coated AuNP groups were closer to the control group. The liver, which is the organ where nanomaterial accumulates the most, is also very important in terms of nanotoxicity evaluations. The measured ALT and AST values were similar to the results of the cretain analysis. Additional surface coatings of the AuNPs (especially in the PEI groups) significantly reduced the excessive rise of these values. The ALT and AST levels excessively increased in direct relation to liver dysfunctions in the $AuNP_{20}$ and $AuNP_{50}$ groups. The TAC, TOS and OSI values measured as a result of these two values were generally considered to be close to each other. No excessive oxidative stress response was observed in all the gold nanoparticle groups. Again, TAC and TOS values in the surface functionalized AuNP groups were closer to the control group. Generally, in terms of all the bio-

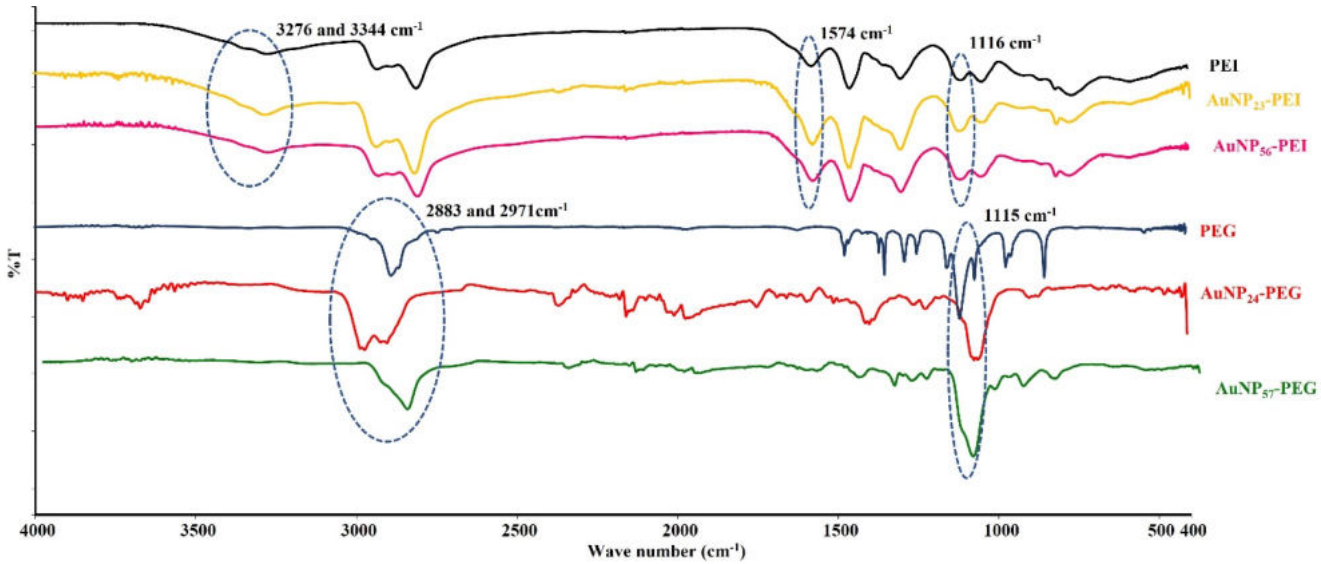

Fig. 2. FTIR-ATR analysis. FTIR = Fourier Transform Infrared, ATR = Attenuated Total Reflection.

Table 2. Effects of the AuNPs over the different biochemical parameters of the mice on day 14 after a single i.v. injection (5 mg Au/kg animal weight) of the gold nanoparticle groups

Biochemical parameters	Control	$AuNP_{20}$	$AuNP_{23}$ PEI	$AuNP_{24}$ PEG	$AuNP_{50}$	$AuNP_{56}$PEI	$AuNP_{57}$PEG
Leukocyte (%)	5.49	7.34	6.72	6.90	10.71	8.28	9.01
Erythrocyte (%)	4.82	4.43	4.83	4.77	3.22	4.98	4.99
Hemoglobin (g/dl)	13.49	10.20	14.60	14.40	9.80	13.40	13.60
Hematocrit (%)	40.00	34.00	43.60	41.60	29.20	42.30	42.10
MCV (μm^3)	86.00	76.70	87.20	90.30	84.40	84.90	90.70
PLC ($10^3/mm^3$)	270.00	207.00	264.00	239.00	201.00	282.00	251.00
RCDW (%)	14.11	13.20	17.10	13.10	14.50	14.20	13.00
PDW (%)	11.90	11.70	14.70	11.40	14.10	11.80	10.40
MPW (%)	11.40	9.90	11.50	10.00	9.40	11.40	10.00
Creatinin (mg/dl)	0.92	1.22	1.17	1.18	1.95	1.42	1.84
ALT (U/L)	36.15	71.40	44.80	64.40	76.00	68.10	75.00
AST (U/L)	159.25	272.20	184.90	243.70	352.40	257.40	298.00
TAC (mmol Trolox Equiv.)	0.43	0.53	0.43	0.46	0.62	0.56	0.60
TOS (µmol H_2O_2 Equiv.)	18.40	23.20	22.40	21.60	27.20	26.40	24.40
OSI	4.26	4.36	5.19	4.73	4.39	4.72	4.07

AuNP = gold nanoparticles, PEI = polyethyleneimine, PEG = polyethylene glycol, MCV = Mean cell volume, PLC = Platelet count, RCDW = Red cell distribution width, PDW = Platelet distribution width, MPV = Mean platelet volume, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, TAC = Total antioxidant capacity, TOS = Total oxidant status, OSI = Oxidative stress index.

chemical data, the PEI coating was evaluated to be more advantageous than the PEG coating.

DISCUSSION

Two different sizes of gold nanoparticles ($AuNP_{20}$ and $AuNP_{50}$) were successfully synthesized by the modified Turkevich method and seeding-growth method. Highly stable and monodisperse AuNPs were obtained. PEI and PEG surface coatings of the AuNPs made the nanostructures more stable, additionally prevented the aggregation of the particles. The particle sizes increased depending on the PEI and PEG surface modifications. Especially, after the PEI coating, there was a significant positive increase in the zeta potentials. Gold nanoparticles have a strong binding affinity to chemical groups such as; amine, thiol and disulfide. In this approach, nanoparticles can be easily physically modified with various polymers or biomolecules without the need for covalent bonds [28, 29]. In the protocol we followed, the PEI coating was carried out as a thin layer after synthesis under highly controlled conditions. In the PEG coating, polymer containing thiol group was used and an increased chemical stability was successfully achieved again. Positively charged nanoparticles could easily interact electrostatically with negatively charged molecules such as DNA and RNA directly. As this situation may facilitate DNA or RNA transfer, this is a great advantage for potential nanotherapeutic areas such as gene delivery, gene silencing and other molecular therapies [30-33].

Foreign molecules in the bloodstream are recognized by reticuloendothelial system (RES) elements through the opsonization process. The foreign substances are then transported to the liver and spleen by coating with antibodies for inactivation process [34]. After the intestinal nanomaterial absorption, significant accumulation occurs in the liver and spleen, followed by the kidneys and circulatory system [35]. As a result of i.v. injection of the AuNPs to mice with a single high dose (5 mg Au/kg animal weight) through

the tail vein, the various biochemical effects of particle groups were investigated on day 14. When the biochemical blood analysis data were evaluated, significant changes were observed depending on the surface coatings of the gold nanoparticles. Generally, in the AuNP-PEI and AuNP-PEG groups, the biochemical values were observed closer to the control group. PEI and PEG surface coatings increased biocompatibility. No excessive oxidative stress response was observed in all the AuNP groups. It has been evaluated that the surface chemistry of the particles is a more decisive parameter than the size over the biochemical toxicity in our study. Unlike our results, Lopez-Chaves *et al*. demonstrated that smaller particles lead to more toxic effects after the I.P. injection of the AuNPs of different diameters (10, 30 and 60 nm in sizes) to Wistar rats [36].

In terms of all the biochemical data in our study, the PEI functionalization was considered to be more advantageous than the PEG coating. In a study using glutathione coated AuNPs of 1.2 nm in diameter as an alternative to PEG coating, the researchers have demonstrated a low toxicity and immune response over the 4-weeks after the subcutaneous administration to the BALBc/cAnNHsd mice [37].

In the literature, it is seen that similar results were obtained in some studies using gold nanorods (AuNRs). After intratumoral injection of PEG-functionalized gold nanorods in cats and dogs, the researchers showed that the surface-modified nanostructures did not adversely affect biochemical blood parameters, kidney and liver functions within one-month period [38]. In another study evaluating the in vivo effects of PEG coated AuNRs functionalized with RGD and GLF, it was interestingly shown that PEG coated nanostructures increased ALT levels more than CTAB stabilized ones [39].

In a gold nanoparticle study, the *in vivo* toxic effects of CALNN pentapeptide coated AuNPs of 20 nm in diameter have been evaluated in a long period of 28 days. As a result of administration of a single dose (0.7 mg Au/kg of body weight) intravenously of the AuNPs to the rats, Fraga *et al*. showed that the ratio of red blood cells and hemoglobin/hematocrit levels decreased significantly. They evaluated the other hemogram values as normal [40]. Based on all these results; the surface coating, size, stability and biocompatibility of the AuNPs are important parameters for the potential biomedical applications of gold nanoparticles in future studies.

Limitations

The limitation of this study was that the biochemical analyses were carried out for only one day (day 14) due to the insufficient number of animals. If the measurement days could be determined for short and long time period, time dependent effects of the nanoparticles could be investigated as well.

CONCLUSION

In summary, we have performed a multiparametric study to understand the biochemical effects of the gold nanoparticles with different surface coatings and sizes in the mice. It has been concluded that the surface chemistry of the particles is more decisive parameter than the size in terms of in vivo toxicity. PEI and PEG surface coatings increased biocompatibility. No excessive oxidative stress response was observed in all the AuNP groups. Generally, it has been evaluated that PEI surface functionalization is more advantageous than PEG coating for potential in vivo applications. The surface modification, stability and biocompatibility of the AuNPs are important parameters for the biomedical applications of gold nanoparticles in future studies.

Authors' Contribution

Study Conception: İÖ; Study Design: İÖ, ÇÇ; Supervision: İÖ; Funding: İÖ; Materials: İÖ, ÜCE; Data Collection and/or Processing: İÖ, ÇÇ, NA, ÜCE; Statistical Analysis and/or Data Interpretation: İÖ, ÇÇ, NA; Literature Review: İÖ, ÜCE; Manuscript Preparation: İÖ and Critical Review: İÖ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The impact of the COVID-19 pandemic on domestic abuse against Turkish immigrant women in Germany

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ABSTRACT

Objectives: This study aimed to investigate the relationship between psychological violence, psychological maltreatment, and depression, anxiety among Turkish immigrant women living in Germany during the COVID-19 pandemic.

Methods: The Profile of Psychological Abuse of Women, Psychological Maltreatment of Women Inventory, Generalized Anxiety Disorder-7 (GAD-7) Scale, and The Patient Health Questionnaire-9 (PHQ-9) were delivered to participants.

Results: Our results showed that participants who had been exposed to psychological abuse and domestic violence reported having higher depression and anxiety scores. Furthermore, participants with low income and married to spouses with no formal education or only primary school graduates were reported to have higher depression, anxiety, domestic abuse, and psychological maltreatment scores during the COVID-19 outbreak.

Conclusions: Our findings demonstrated that Turkish immigrant women who had experienced psychological abuse and domestic violence by their partners during the pandemic were reported to have higher depression and anxiety.

Keywords: COVID-19, psychological violence, depression, anxiety

COVID-19, which emerged at the end of 2019 in the city of Wuhan, quickly spread to many countries and gained a global dimension. Thus, the World Health Organization declared this epidemic as a pandemic on March 11, 2020. As a result, all living areas such as social and working life, education, as well as our daily life habits were affected all over the world. While the pandemic caused a global crisis in the macro plan due to its threatening nature, problems occurred on the family and individual basis in the micro plan as well [1]. The pandemic continues to affect individuals' not only leisure activities and hobbies but also their core problem solving skills in the dynamics of marriage, family, friendship, and work. Tension among couples increased and started to have a devastating effect. In this process, factors such as education level, income status, and unemployment pose a risk to relationships. As it was reported in a study which was aimed to investigate the quality of life (QoL) among married people during the COVID-19 pandemic, employed participants stated higher QoL scores than unemployed; higher educated participants stated higher QoL than those with lower education; respondents with higher income reported higher QoL than those with lower income [2]. External factors caused by COVID-19, such as changes in daily routines, de-

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crease in social relations, finding a balance between intertwined roles, reduce the quality of couples' relationships.

Studies have shown that increased domestic violence and psychological abuse against women during COVID-19 have become a risk that threatens family relationships. The most common but hidden form of violence against women is domestic violence. As a result of the social isolation measures and quarantine practices brought by the COVID-19 pandemic, reports of domestic violence against women have increased [3]. Women have been subjected to greater surveillance and coercive control, especially by their partners during the pandemic. The cause of domestic violence and psychological abuse is related to various factors such as the uncertain nature of the pandemic, economic stress, and increased exposure to abuse [4].
According to a study prepared by the United Nations, Germany, the United Kingdom, France, Cyprus, the United States, Canada, and Singapore, it was stated that there was an increase in reports of domestic violence [5]. In addition, a study conducted by Prof. Steinert from the Technical University of Munich and Dr. Ebert from the RWI – Leibniz Institute for Economic Research scientifically proved an increase in domestic violence and psychological abuse in German society. The research was conducted through an online survey, in which 3,800 women aged 16-65 reported violent crimes they encountered in their homes. The findings illustrated that approximately 2.2% of participants could not go out without their husband's permission, 3% were subjected to physical violence and 3.6% to sexual violence and 3.8% were threatened by their partners. Prof. Steinert and Dr. Ebert's report showed that victims of domestic violence experienced not only physical violence but also sexual and emotional violence during the pandemic [6].

According to the findings of a literature review of longitudinal studies conducted in Western European countries, unemployment, and low socioeconomic status were associated with symptoms of depression and anxiety [7]. As a result of the recent outbreak the economic, mental, and emotional difficulties caused increased stress, depression, and anxiety disorders, which adversely affected social relationships and marriages at a high level. Depression, somatic complaints, and anxiety were more common in women even before the pandemic [8]. Immigrant women were mentally more vulnerable to the COVID-19 pandemic as they stay at home for a long time due to the recent regulations and try to meet the care needs of family members. Research showed that women suffered more emotional and life distress than men during COVID-19 and according to a report published by German Institute for Economic Research, immigrant women laborers were more likely to be affected by socio-economic difficulties [9, 10]. It was reported that depression was the most prevalent negative mental health consequence of domestic violence as well [11].

Studies highlighted that domestic violence and psychological abuse were serious issues against not only the native German female population but also immigrant women during the COVID-19 pandemic in Germany. An increase in workloads at home, traditional gender roles and time spent at home with a partner due to social isolation during the pandemic might be considered possible reasons for domestic violence and psychological abuse against immigrant women. The findings of another study carried out by the Federal Ministry for Family, Senior Citizens, Women and Young People in Germany, which sampled native German women and immigrant women from Turkey, showed that 9% of women living in Germany reported that they experienced several forms of abuse and misuse; 45% was in the domestic setting [12]. Therefore, it is necessary to address the factors that may increase domestic violence against immigrant women during the pandemic process. It was highlighted by previous research that the COVID-19 pandemic had an unprecedented impact on Turkish immigrants in Germany. Unemployed respondents and participants with low education levels were reported to worry more during the pandemic [13]. In addition, social support plays a significant role in the mental conditions of Turkish immigrants. Individuals, who received more family and friend support, showed better coping strategies for different problems [14]. Employees' working hours were cut by some employers as a response to the current pandemic. 17% of the population, particularly immigrants, in Germany had reduced working hours as of April 2020 and people with low education levels and income had a higher percentage of "short-time work" [15].

Considering the previous studies, our research aimed to investigate the relationship between domestic abuse, psychological maltreatment and depression,

and anxiety among Turkish immigrant women living in Germany during the COVID-19 pandemic. Furthermore, their mental health conditions and the sociodemographic data of the participants and their impact were analyzed.

The study was designed based on the following hypothesis:

H1: There is a positive relationship between domestic abuse, psychological maltreatment, and anxiety scores of the participants.

H2: There is a positive relationship between domestic abuse, psychological maltreatment, and depression scores of participants.

H3: There is a positive relationship between domestic abuse, anxiety, depression scores, and the partners' education level.

METHODS

Study Design and Population

One hundred ninety-two female participants were recruited among patients who were first- and second-generation immigrants treated at the Neuro Psychiatrisches Zentrum Riem (NPZR) living in Munich, Germany. The data was collected between December 2021 and March 2022. The NPZR is specialized in transcultural psychotherapy and offers neurological, psychiatric, and psychotherapeutic therapies as well as the treatment of psychosomatic diseases in ten different languages. Applied culture-specific therapies consist of mother tongue therapies for Turkish patients, and the knowledge about several migration-specific topics such as discrimination, fear of cultural assimilation, different concepts of sickness and healing, and different ways to express the disease, e.g., psychosomatic reactions to mental problems.

Procedure and Ethics

The Ethics Committee of the Bavarian State Medical Association has confirmed that no additional ethical approval is required. An informed consent form was signed by all participants of the study. The participants had the opportunity to ask further questions and seek clarification about any aspect of the research before taking part in it.

Data Collection Tools

"Profile of Psychological Abuse of Women", "Psychological Maltreatment of Women Inventory (PMWI)-Short Form", "Generalized Anxiety Disorder-7 (GAD-7) Scale" and "Patient Health Questionnaire-9 (PHQ-9)" were distributed to collect the data.

Sociodemographic Form

The sociodemographic form consists of sociodemographic questions including age, income, marital status, education status of both participants and their partners, and employment status of participants and their partners.

Profile of Psychological Abuse of Women Scale

The Profile of Psychological Abuse of Women was developed by Sackett and Saunders [16], to measure psychological abuse and show the relationship of different forms of abuse to self-esteem and depression. The scale contains 21 items and responses are rated on a Likert scale. Four types of abuse were derived from factor analysis: ridiculing of traits (e.g., "He mocks the traits you like or value most in yourself"), criticizing behavior (e.g., "He mocks the traits you like or value most in yourself"), ignoring (e.g., "He ignores that you need help when you are sick, tired or overworked"), and jealous control (e.g., "He gets angry or upset when you want to be with others instead of with him"). Each subscale had sufficient internal reliability. Scales were related to outcome variables in expected ways, supporting their validity. The validity and reliability of the Turkish version of the scale was performed and approved by Boyacioglu [17]. In the current research, Cronbach's α for the Profile of Psychological Abuse of Women Scale was 0.94, demonstrating very good internal reliability.

Psychological Maltreatment of Women Inventory

Psychological Maltreatment of Women Inventory was developed by Tolman and measures the psychological violence of women by their male partners [18]. The scale includes 18 questions. The assessment was performed between 1-5 points Scale from 1 (never) to 5 (very often). Furthermore, not applicable option with a score value of 0 was added for some relationships and participants such as items related to childcare. Cronbach's alpha of the Psychological Maltreatment

of Women Inventory was between 0.93. In the current study, Cronbach's α for the scale was 0.94, demonstrating good internal reliability. The validity and reliability of the Turkish version of the scale was performed and approved by Cem-Ersoy [19].

Generalized Anxiety Disorder-7 (GAD-7) Scale

GAD-7 Scale was developed by Spitzer to determine anxiety levels of participants [20]. The scale is not only used for screening generalized anxiety but also, panic disorder and social anxiety disorder. The high scores obtained from the individuals show the severity of the anxiety. The following cut-off scores determine the level of anxiety: 5, 10, and 15 were determined for mild, moderate, and severe anxiety respectively. The scale includes 7 items and is a measure of 4 Likert types between 0–3. The scale was adapted to the Turkish language by Konkan [21]. The internal consistency of the Turkish version of the scale was 0.85. In the current study, Cronbach's α for the Generalized Anxiety Disorder-7 (GAD-7) Scale was 0.88, demonstrating very good internal reliability.

The Patient Health Questionnaire-9 (PHQ-9)

PHQ-9 was designed to diagnose the severity of depression by asking for 9 diagnosis criteria included in DSM-IV. It was developed by Kroenke [22] and the Turkish reliability study was performed by Sari [23]. The scale contains 9 questions, and each item was assessed between 0 (not at all) and 3 (nearly every day). Scores between 1-4 are evaluated as minimal, 5-9 mild, 10-14 moderate, 15-19 moderately severe, and 20-27 severe depression. The Turkish version of the scale was found to be reliable (Cronbach's α 0.842). In the current study, Cronbach's α for the PHQ-9 Scale was 0.90, demonstrating very good internal reliability.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) was performed for data analysis. First of all, the kurtosis and skewness coefficients were analyzed to determine the conformity of the scores to the normal distribution. The data showed a normal distribution according to the Kolmogorov-Smirnov (K-S) test. Therefore, the correlation between the scale scores was conducted with the Pearson correlation test. Parametric test techniques were used in the study due to the normal distribution of the scores. The t−test and analysis of variance (ANOVA) were used to analyze the differences in scale scores according to the variables. While the t−test was used in the analysis of demographic variables with two groups, the ANOVA was used in the analysis of the variables with k (k > 2) groups.

RESULTS

Description of the Sample

Sociodemographic of participants were demonstrated in Table 1. Among the sample of participants, 138 (71.9%) were married, 24 (12.15%) were divorced, 11 (5.7%) were widowed, 12 (6.3%) were living with a partner and 7 (3.6%) were single. Most participants (n = 59; 30.7%) were primary school graduates, followed by high school graduates (n = 38; 19.8%), secondary school (n = 34;17.7%), and university graduates (n = 39; 16.6%). Only 10.4% of the participants (n = 20) had a master's or PhD degree and 5.7% (n = 11) were illiterate. While the rate of employed participants was 49.0% (n = 94), the percentage of unemployed participants was 51.0% (n = 98). 66.1% (n = 127) had average income status.

Investigating the Relationship Between Scale Scores

Correlation between scale scores is represented in Table 2. To investigate the relationship between the scale scores, the Pearson correlation test was carried out. The findings demonstrated that there was a positive and moderate correlation between Profile of Psychological Abuse of Women Scale scores and the Generalized Anxiety Disorder Scale (r = 0.533) as well as Patient Health Questionnaire scores (r = 0.532). According to the results participants who had encountered psychological abuse were reported to have higher depression and anxiety scores. In addition, there was a moderate and positive correlation between the Psychological Maltreatment of Women Inventory and the Generalized Anxiety Disorder Scale (r = 0.519) as well as Patient Health Questionnaire scores (r = 0.487). The findings showed that participants who had experienced psychological violence by their partners were reported to have higher depression and anxiety scores. Furthermore, there was a strong and positive correlation (r = 0.852) between Profile of Psychological Abuse of Women Scale scores and the Psy-

chological Maltreatment of Women Inventory. Generalized Anxiety Disorder Scale scores and The Patient Health Questionnaire were positively correlated as well (r = 0.803).

Analysis of Scale Scores in terms of Monthly Income

Table 3 shows the analysis of scale scores in terms of monthly income. The ANOVA test was conducted to investigate the relationship between scale scores and the income levels of participants. The findings showed that there was a significant relationship between psychological resilience scale scores and monthly income of participants ($p < 0.05$). According to the findings, anxiety, and depression scores were higher among participants with low-income levels. There was also a statistically significant difference between different monthly income groups and Profile of Psychological Abuse of Women Scale scores ($p < 0.05$). The findings show that psychological abuse was higher among participants with low income. ($p < 0.05$).

Analysis of Scale Scores in terms of Partner's Education Level

Analysis of scale scores in terms of partner's education level in Table 4. There was a significant relationship between Profile of Psychological Abuse of Women Scale, Generalized Anxiety Disorder Scale,

Table 1. Demographic characteristics (n = 192)

		n	%
Marital Status	Married	138	71.9
	Single	7	3.6
	Divorced	24	12.5
	Partner	12	6.3
	Widowed	11	5.7
Employment Status	Employed	94	49.0
	Unemployed	98	51.0
Education Level	Uneducated	11	5.7
	Primary School	59	30.7
	Secondary School	34	17.7
	High School	38	19.8
	University	30	15.6
	Master's / PhD	20	10.4
Which income group do you think you belong to according to your monthly income?	Low	39	20.3
	Average	127	66.1
	High	26	13.5
What is your partner's education level?	Uneducated/Primary School Secondary School	99	51.5
	High School	47	24.5
	University	23	12.0
	Master's / PhD	23	12.0
Did you lose your job during the pandemic?	Yes	23	12.0
	No	169	88.0
Did your partner lose his job during the pandemic?	Yes		
	No		

Table 2. Investigating the relationship between scale scores

		Profile of Psychological Abuse of Women Scale	Psychological Maltreatment of Women Inventory	Generalized Anxiety Disorder	The Patient Health Questionnaire
Profile of Psychological Abuse of Women Scale	r	1	.852	.533	.532
	p		**< 0.001**	**< 0.001**	**< 0.001**
Psychological Maltreatment of Women Inventory	r		1	.519	.487
	p			**< 0.001**	**< 0.001**
Generalized Anxiety Disorder	r			1	.803
	p				**< 0.001**
The Patient Health Questionnaire	r				1
	p				

Patient Health Questionnaire, Psychological Maltreatment of Women Inventory, and education level of the participants' partners ($p < 0.05$). The findings illustrated that depression, anxiety, domestic abuse, and psychological violence scores were higher among participants whose partner was either illiterate or had a primary school degree.

Analysis of Scale Scores in terms of Partner's Job Loss During the Pandemic

Table 3. Analysis of scale scores in terms of monthly income

Which income group do you think you belong to according to your monthly income?		n	Mean	SD	F	*p* value
Profile of Psychological Abuse of Women Scale	High	39	1.32	1.86	6.032	**0.003**
	Average	127	1.78	1.69		
	Low	26	2.84	1.84		
Psychological Maltreatment of Women Inventory	High	39	2.02	1.19	2.740	0.067
	Average	127	2.21	1.14		
	Low	26	2.70	1.27		
Generalized Anxiety Disorder Scale	High	39	.96	.88	17.394	**< 0.001**
	Average	127	1.79	.92		
	Low	26	2.21	.82		
The Patient Health Questionnaire	High	39	.98	.81	22.006	**< 0.001**
	Average	127	1.73	.75		
	Low	26	2.16	.67		

SD = standard deviation

Table 4. Analysis of scale scores in terms of partner's education level

What is your partner's education level?		n	Mean	SD	F	*p* value
Profile of Psychological Abuse of Women Scale	Uneducated/Primary School	99	2.29	1.71	8.300	**< 0.001**
	High School	47	1.59	1.79		
	University	46	1.08	1.68		
Psychological Maltreatment of Women Inventory	High	39	2.02	1.14	4.799	**< 0.001**
	Average	127	2.21	1.20		
	Low	26	2.70	1.14		
Generalized Anxiety Disorder Scale	High	39	.96	.85	19.375	**< 0.001**
	Average	127	1.79	1.05		
	Low	26	2.21	.81		
The Patient Health Questionnaire	High	39	.98	.68	20.525	**< 0.001**
	Average	127	1.73	.89		
	Low	26	2.16	.75		

SD = standard deviation

The findings showed that there was a statistically significant relationship between Profile of Psychological Abuse of Women Scale, Generalized Anxiety Disorder Scale, Patient Health Questionnaire, Psychological Maltreatment of Women Inventory, and job loss status during the pandemic ($p < 0.05$). According to the results, depression, anxiety, domestic abuse, and psychological violence scores were higher among participants whose partner had lost his job during the pandemic. Table 5 illustrates the analysis of scale scores in terms of partner's job loss during the pandemic.

Table 5. Analysis of scale scores in terms of partner's job loss during the pandemic

Did your partner lose his/her job during the pandemic?		n	Mean	SD	F	*p* value
Profile of Psychological Abuse of Women Scale	Yes	99	2.29	1.71	8.300	**< 0.001**
	No	46	1.08	1.68		
Psychological Maltreatment of Women Inventory	Yes	39	2.02	1.14	4.799	**< 0.001**
	No	26	2.70	1.14		
Generalized Anxiety Disorder Scale	Yes	39	.96	.85	19.375	**< 0.001**
	No	26	2.21	.81		
The Patient Health Questionnaire	Yes	39	.98	.68	20.525	**< 0.001**
	No	26	2.16	.75		

SD = standard deviation

DISCUSSION

According to the latest reports and studies domestic violence against women has increased in Germany during the pandemic outbreak. However, there is not much research conducted about the problems of immigrant women, especially of Turkish origin in Germany. We aimed to investigate whether Turkish immigrant women suffered from psychological abuse and domestic violence as well. Furthermore, the purpose of the current study was to investigate the relationship between psychological abuse, psychological maltreatment, and depression, anxiety among Turkish immigrant women living in Munich, Germany during the COVID-19 pandemic. Furthermore, the relationship between socio-demographics of the participants and mental health conditions was analyzed.

According to the results of previous research, participants stated incidents of physical conflict and emotional abuse during the COVID-19 outbreak [6]. In addition, another study conducted even before the pandemic revealed that Turkish immigrant women living in Germany reported that almost half of the participants experienced physical or psychological violence from their husbands [24]. Psychological violence can turn into physical violence over time. Accordingly, the increase in the time spent at home with a partner during the pandemic may show many negative outcomes. In accordance with previous studies, participants in our study reported psychological abuse and domestic violence as well.

When the types of violence against women are discussed, even though physical violence is considered first, it is also very important to mention the psychological and emotional violence against women. It was reported in several previous studies that women who encountered psychological violence more frequently experienced mental health problems as well. For instance, several mental disorders including depression, anxiety, and panic attacks occurred among those who were affected by psychological abuse or psychological maltreatment [25, 26]. Furthermore, in another study conducted among a Turkish population by Kıvrak *et al*. [27] with women who had been exposed to psychological violence and women who had not been exposed to violence, it was observed that the depression scores of the group exposed to psychological violence were much higher than the other group. Our findings were in line with the previous findings reporting that Turkish immigrant women who had experienced psychological abuse or domestic violence by their partners during the pandemic were reported to have higher depression. In addition to previous studies, women's anxiety levels are higher than men. It was stated that anxiety is mostly low in perceived social support from the family and higher in those who experience psychological violence [28]. Our study conducted in Germany showed similar results with researchers in Turkey. Our participants who suffered from psychological abuse and domestic violence reported higher anxiety scores.

There are some common factors affecting women being exposed to economic, psychological, and physical violence including the education and income level of both women and their spouses, employment of the spouses [29-30]. Our findings demonstrated that there was a statistically significant difference between the scales used in our study and variables such as the education level of the spouses and income level of participants who encountered psychological violence or abuse. Participants married to spouses with no formal education or primary school graduates were reported to have higher depression, anxiety, psychological abuse, and domestic violence during the COVID-19 outbreak in Germany. In addition, Turkish women, whose spouses lost their jobs during the COVID-19 outbreak, reported higher depression, anxiety, psychological abuse, and domestic violence scores as well.

Limitations

Our research has some limitations that should be mentioned. First, the sample is restricted to Munich and the surrounding area in Germany. This characteristic of the sample clearly limits generalization of our findings. Second, another research is needed for the special conditions of domestic violence in immigrant communities such as discrimination, insufficient proficiency of German language skills to ask for help in precarious situations. Third, the reality of women with a migration background in Germany in terms of gender roles might be still different from the situation of German women. Without falling into the trap of trying to explain everything with different cultures and religions and customs and virtues we should investigate the migration status of women. Their economic and social status were addressed in this study and can be

compared with their level of integration, discrimination, and legal status.

CONCLUSION

Our findings demonstrated that Turkish immigrant women who had experienced psychological abuse and domestic violence by their partners during the pandemic were reported to have higher depression and anxiety. Participants married to spouses with no formal education or primary school graduates were reported to have higher depression, anxiety, psychological abuse, and domestic violence scores during the COVID-19 outbreak.

Authors' Contribution

Study Conception: EDC-H; Study Design: EDC-H; Supervision: EDC-H; Funding: EDC-H; Materials: EDC-H; Data Collection and/or Processing: EDC-H, RD; Statistical Analysis and/or Data Interpretation: EDC-H, RD; Literature Review: EDC-H, RD; Manuscript Preparation: EDC-H and Critical Review: EDC-H.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(1):150-154 Original Article

DOI: 10.18621/eurj.1193758 *Plastic, Reconstructive and Aesthetic Surgery*

Interpolation sural flaps in acute traumatic defects

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ABSTRACT

Objectives: Distal lower leg wounds, regardless of cause, and acute trauma wounds, regardless of site, are difficult. Reconstruction of both situations are much more challenging. Interpolation sural flap is a good alternative for this type of reconstruction.

Methods: Fourteen interpolation sural flap were harvested to reconstruct for distal leg region wounds due to acute trauma. Ten male and four female patients aged from 10 to 59 years old were included this study.

Results: All flaps survived. No venous congestion and total flap necrosis was seen. There were 4 complications, and all of them were corrected with short interventions. After second stage, no complication was seen.

Conclusions: Interpolation sural flap modification has many advantages including reliability, no venous congestion, immediate reconstruction without a surgical team. However, it is a-two staged reconstruction and its donor site scarring can be serious.

Keywords: Acute trauma, distal lower leg defects, interpolation, sural flap, venous congestion

Distal lower leg wounds are difficult to reconstruct with local flaps due to the small amount of soft tissue being available. If the wound cause is acute trauma the tissue around the zone of injury has uncertain fate, whether it is viable or not. Edema is another serious problem associated with the acute trauma zone. The application of free flaps is a good choice for this area; however, teamwork and operational preparation are needed. Sural flaps are a good and frequently used approach for heel reconstruction, around the ankle and the distal lower leg. Sural flaps have a good axial blood supply, but partial or total necrosis related to venous congestion have complicated their use. Unfortunately, the distal tip of the flap, which is the area that is needed the most, is often the part that fails [1].

In this study we investigated the use of interpolation sural flaps for acute trauma patients, and we analyzed the outcomes and complications associated with this flap in the selected injury type.

METHODS

Between 2019 and 2022 ten males and four females patients underwent interpolation sural flap surgery to reconstruct the ankle and foot region with orthopedic surgeons immediately or within a week following trauma.

Surgical procedures were performed under spinal or general anesthesia. The patients were placed in the supine, prone, or lateral decubitus positions. All flaps

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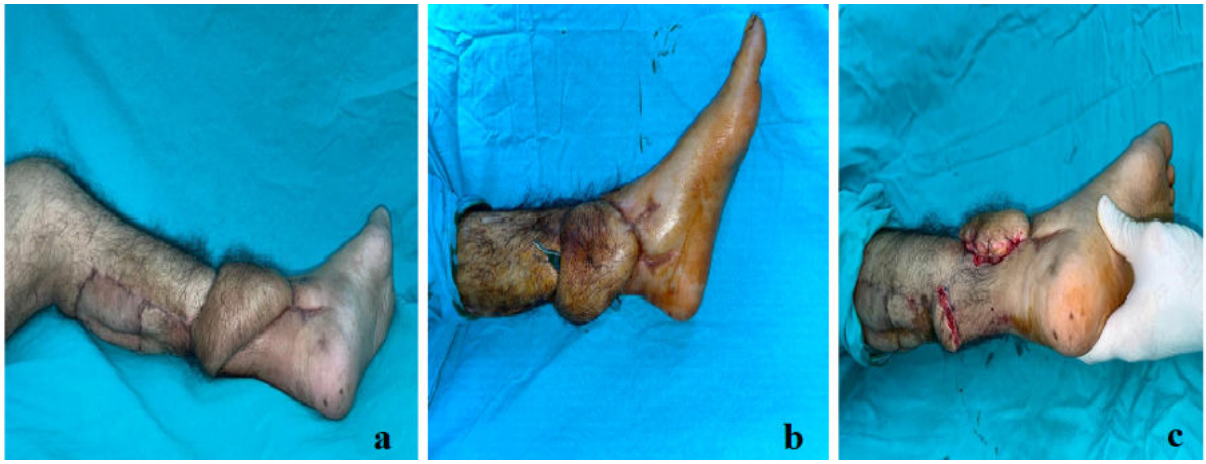

Fig. 1. a) Interpolation sural flap b) At the second stage, the flap can be divided with the help of a forceps. c) Raw surface was sutured.

were raised sub-facially and included the skin, soft tissue, sural nerve, sural artery, and lesser saphenous vein. We did not ligate small saphenous vein. The sural nerve was located at the center of the flap course. The flap width was designed to be at least 4 to 8 cm. The main peroneal perforator located about 5 cm above the lateral malleolus was checked with handheld Doppler ultrasound. However, the perforator was not dissected and the base of the flap with an intact skin was at the perforator level. Sutures were placed few and far between each other, as the flap would have had a natural edema process. The donor site of the flap was primarily sutured or skin-grafted. At the second stage, we found the skin bridge between the donor site and the reconstructed area with the help of a forceps (Fig. 1). Division was made and the sural nerve was sutured proximally and distally at the flap margins. The raw surfaces of the flap margins were then sutured loosely on the skin.

RESULTS

Mean age was 47 years. Nine patients had medial malleolus defect. Mean interval period between first and second stages was 7 weeks. All flaps survived and no total flap necrosis were observed; no venous congestion was observed either (Fig. 2). Only four of the patients had complications. Three of them needed an additional short time revisional operation under local anesthesia. These operations were flap readvancement and scar revision. The remaining patient had exposure

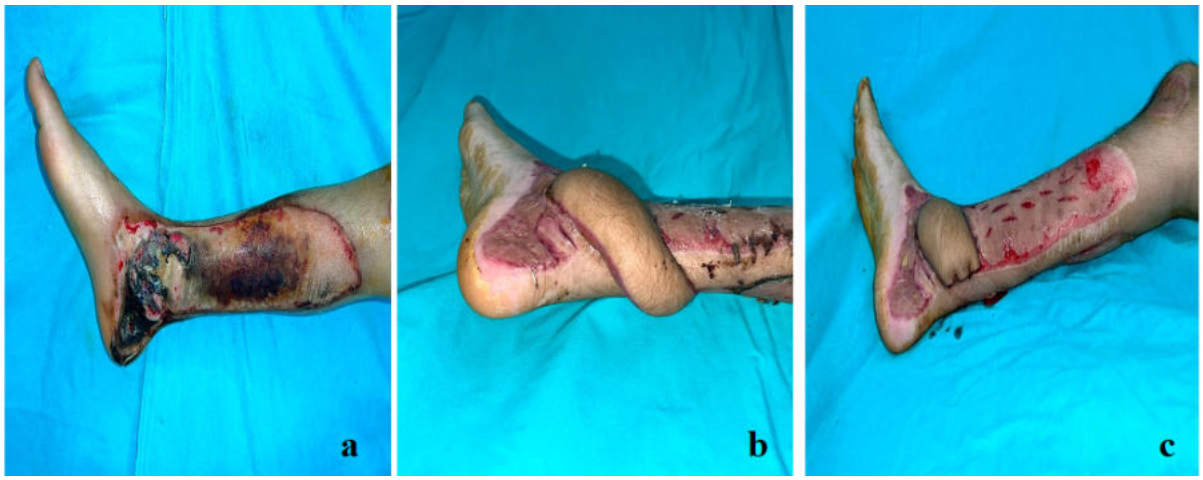

Fig. 2. a) The demarcation of the injury zone was clear at the end of a week b) An interpolation sural flap was inset on the exposed bone c) After 3 weeks, the interpolation sural flap was divided.

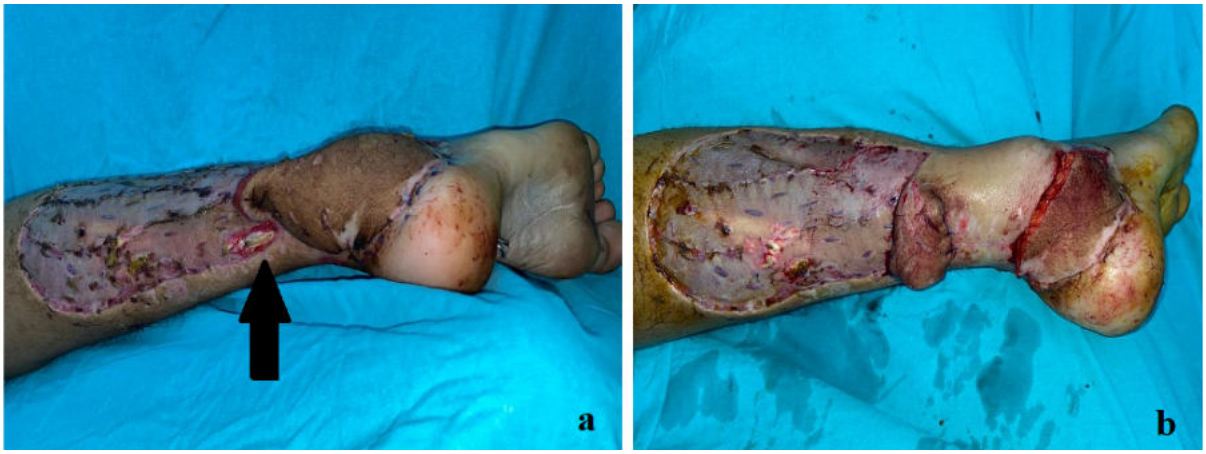

Fig. 3. a) The black arrow is indicated the exposed Achilles tendon b) The remnant of the interpolation sural flap was transposed to the iatrogenic defect when the flap was divided at the second stage.

of the Achilles tendon in donor site, and was reconstructed during division, the second stage of the interpolation flap procedure (Fig. 3). A summary of the patients is shown in Table 1.

For the first stage of the operation, all patients were advised to limit their mobility for a 7-day period. Postoperatively, the extremity remained elevated for a 7-day period as well. The planned second stage was at 3 weeks postoperatively, unless there was a complication or orthopedic surgery or the patient's compliance. For the second stage of the operation there were no limitations regarding mobilization and extremity elevation. No patient needed any custom shoes or flap debulking surgeries, and we did not see any complications after this stage.

Table 1. Patients' summary

Patient Number	Age	Sex	Defect Location	Complications after Immediate Reconstruction	Interval Period between First and Second Stages of Interpolation Flap	Follow up
1	47	F	Heel	-	3 weeks	14 months
2	59	F	Medial malleolus	-	4 weeks	12 months
3	10	M	Medial malleolus	-	3 weeks	12 months
4	49	M	Heel	-	4 weeks	10 months
5	43	M	Medial malleolus	-	7 weeks	9 months
6	42	M	Medial malleolus	Exposion of Achilles tendon	4 weeks	15 months
7	42	M	Heel	-	9 weeks	13 months
8	56	M	Medial malleolus	-	6 weeks	12 months
9	38	M	Medial mallelus	-	8 weeks	10 months
10	59	M	Lateral malleolus	Partial necrosis	10 weeks	16 months
11	54	F	Medial malleolus	-	6 weeks	8 months
12	59	M	Anterior side of tibia	Partial necrosis	12 weeks	15 months
13	56	F	Medial malleolus	-	8 weeks	12 months
14	48	M	Medial malleolus	Wound dehissance	16 weeks	9 months

DISCUSSION

Since 1981 there have been many studies on the anatomical basis of the sural flap [2-7]. Several modifications of this flap have also been reported in the literature, including interpolation, propeller, free, and turnover types [1, 8-11]. The main purpose of these modifications is the viability of the flap itself, aesthetic improvements in appearance, and the ability of the patient to wear their own shoes.

According to a systematic review, propeller flaps in lower extremities have 1.1% total flap necrosis, 11.3% partial flap necrosis, and 25.8% total complications [12]. On the other hand, interpolation sural flaps are reported in the literature to have fewer complications [1, 13]. In our study we also had very low complication rate, and total flap necrosis did not occur. All the complications we saw were related with hematomas, postoperative edema of the flap, and incompliance with the suggested positioning of the leg. Thus, in our experience, if tension and edema were minimized the flap survived.

Although sural flaps as a reverse flow have axial pattern and reliable perfusion, impaired venous outflow can be the most significant difficulty associated with this flap [14, 15]. Veins are compressible structures. We therefore designed the flap with supplying dermal circulation without cutting the skin base. Following this approach we did not face complications associated with venous congestion.

In one patient, we saw exposure of the Achilles tendon in the donor site ten days after the first stage of the interpolation sural flap procedure, which should had been covered by the flap. In that case we waited for the second stage, and we transposed the remnant of the interpolation flap after division.

Although a 2-stage reconstruction procedure is a limitation associated with this flap, secondary debulking surgeries are usually needed after free flap reconstruction of the lower extremities. [16, 17]. In the second stage of the interpolation sural flap procedure we also made the debulking of the flap, and reshaped the flap as well as possible.

The interval period between the first and second stage of the interpolation sural flap procedure varied in our study. Many studies have suggested that this time ranges between 3 weeks to 20 months [18-20]. In our study we preferred the interval between the first and second stage operation to be at least 3 weeks only in healthy, non-complicated patients. Generally, this interval depended on complications, orthopedic surgery, and on the patient's compliance with the treatment.

Selecting the right donor site for lower extremity reconstruction is associated with many uncertainties. Although the treatment of choice is often a free flap harvesting, there are some questions including the laterality of the lower extremity and duplex ultrasound result [21, 22]. On the other hand, sural flaps have several advantages compared to free flaps: constant vascular anatomy, and the same skin features with the defect. However, the donor site of the interpolation sural flaps is usually grafted skin and the aesthetic appearance of the leg may not be as good. For this reason, the patients' concerns about the donor site should be considered preoperatively.

CONCLUSION

Interpolation sural flap is a reliable, versatile, and quick solution for patients with acute trauma. Although this technique requires a 2-stage procedure, the second stage is simple and not associated with complications. Finally, the patients' expectation and concerns about the donor site should be reviewed preoperatively.

Authors' Contribution

Study Conception: MT; Study Design: MT; Supervision: YEŞ, TCZ; Funding: MT; Materials: MT, YEŞ, TCZ, AEK; Data Collection and/or Processing: MT, AEK; Statistical Analysis and/or Data Interpretation: MT, AEK; Literature Review: MT; Manuscript Preparation: MT and Critical Review: ÖÖ, ÖÖ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(1):155-163 Original Article

DOI: 10.18621/eurj.1213036 *Orthopaedics and Traumatology*

Clinical and radiological results of posterior ankle endoscopy treatment for the flexor hallusis longus tenosynovitis and os trigonum syndrome

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ABSTRACT

Objectives: This study investigated the effect of two portal posterior ankle arthroscopy (PAA) procedures using American Orthopaedic Foot and Ankle Society (AOFAS) and Visual Analog Scale (VAS) scores for the treatment of patients with ankle pain associated with Os trigonum (OT) and Flexor hallusis longus (FHL) tenosynovitis. The effect of PAA treatment on the degree and localization of effusion around the FHL tendon was also investigated.

Methods: Between March 2016 and August 2021, 41 patients who underwent PAA with the diagnosis of OT and stenosing FHL tenosynovitis, whose arthroscopy video records could be reviewed retrospectively, and who had at least 1 year of follow-up results were included in the study. Patients in the pediatric age group, diabetes patients, patients with inflammatory disease, and those with subtalar and tibiotalar osteoarthritis were excluded from the study. Preoperative and postoperative physical examinations, lateral radiography of the pressing foot, MRI, and the VAS and AOFAS scores were evaluated. In the statistical analysis, data were statistically analyzed using SPSS 19.0 (SPSS, Chicago, Illinois, USA). $p < 0.05$ was accepted as statistically significant.

Results: The mean age was 35.6 years (range: 19-55), among which the mean age of the women was 36.2 years (range: 24-48), and the mean age of the men was 35.2 years (range: 19-55). The mean follow-up was 34 months (range: 14-62). The AOFAS value increased from 38.61 ± 7.176 preoperatively to 89.83 ± 6.34 at the postoperative follow-up, and the difference was statistically significant ($p < 0.001$). Five patients fully regained their normal function (AOFAS score = 100 points). The VAS value increased from 90 ± 5.916 preoperatively to 18.682 ± 7.688 at the last postoperative follow-up, and the difference was statistically significant ($p < 0.001$). Pre-PAA FHL tenosynovitis was seen only in zone 1 in 26 patients, zones 1 and 2 in 14 patients, and in zones 1, 2, and 3 in two patients. There was no significant decrease in effusion in the magnetic resonance imaging (MRI) at 1 month after the PAA ($p = 0.117$). A significant decrease in effusion was observed in the MRI taken at the last control ($p < 0.001$).

Conclusions: In the treatment of patients with ankle pain associated with OT and FHL tenosynovitis, the two-portal PAA treatment was observed to be an effective method that resulted in significant improvement in the AOFAS and VAS scores.

Keywords: Ankle, arthroscopy, flexor hallusis longus, os trigonum

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The two-portal endoscopic approach to the back of the foot was first described by van Dijk *et al*. [1]. It has been reported that posterior ankle arthroscopy (PAA) can be used in the treatment of os trigonum syndrome (OTS), flexor hallucis longus (FHL) tenosynovitis, bone and soft tissue impingements, free osteochondritis, and talus osteochondral lesions (TOL). In addition, PAA is an important option for the treatment of synovitis, tenosynovitis, subtalar joint pathologies, hypertrophic posterior talar process, Haglund deformity, and Achilles tendon pathologies [2, 3].

At the level of the ankle, the FHL tendon passes through the flexor retinaculum adjacent to the medial aspect of the talar process posteromedial to the talus and inferior to the sustentaculum tali via a fibrous tunnel. In the presence of OT, the FHL tendon sheath is compressed and narrowed, and stenosing tenosynovitis develops [1]. The effusion seen in the magnetic resonance imaging (MRI) around the FHL often develops due to stenosing tenosynovitis developing in the fibro-osseous tunnel region [4].

The OT is the most common accessory bone of the foot. It is posterolateral to the talus, triangular or oval, usually single-parted, and approximately 1 cm in size. While it is often asymptomatic, symptoms may be seen in persistent posterior ankle pain and posterior impingement syndrome known as OTS [5, 6].

OTS with stenosing FHL tenosynovitis is one reason for posterior ankle impingement syndrome. A radiological diagnosis of OT in the absence of any complaint has no clinical significance. However, in patients with OT compression and FHL tenosynovitis, PAA is required in the presence of such symptoms as pain, swelling, joint stiffness, locking, instability, and a feeling of insecurity in the posterior and inner part of the ankle, despite conservative treatment for more than 3 months [7].

This study investigated the effect of two portal PAA procedures on the American Orthopaedic Foot and Ankle Society (AOFAS) and Visual Analog Scale (VAS) scores in the treatment of patients with ankle pain associated with OT and FHL tenosynovitis. The effect of PAA treatment on the degree and localization of effusion around the FHL tendon was also investigated.

METHODS

A retrospective evaluation was conducted on 62 patients who received PAA for effusion due to OT and stenosing FHL tenosynovitis between March 2016 and August 2021 and whose arthroscopy video records could be retrospectively reviewed. Forty-one of these patients who had at least 1 year of follow-ups and complete records were included in the study. Diabetic patients, patients with systemic inflammatory diseases, and those with talotibial and subtalar joint osteoarthritis were excluded. No neurovascular deficits were observed. Consent was obtained from the patients included in the study.

OT was diagnosed with a lateral radiograph of the foot taken in a standing position (Fig. 1A). In addition to the standing lateral radiograph, a lateral radiograph taken with the ankle in plantar flexion (Fig. 1B)

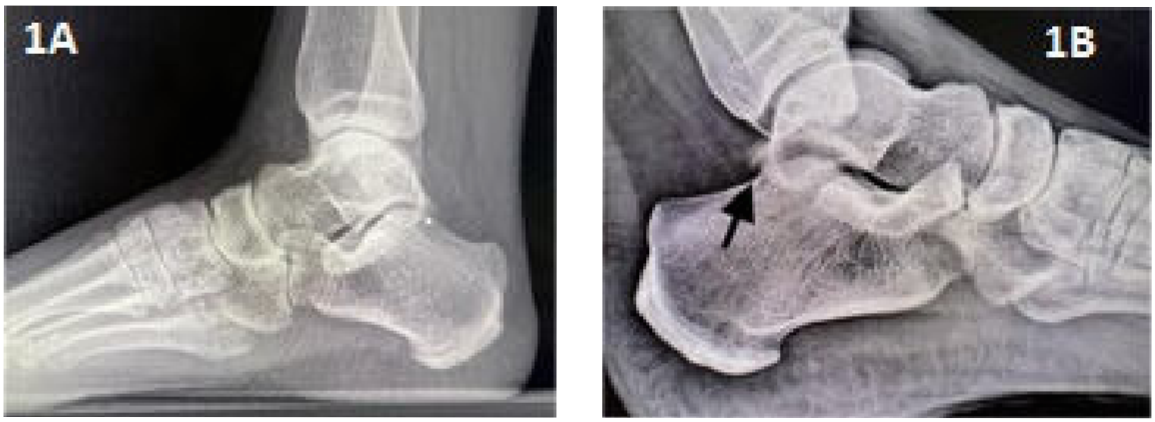

Fig. 1. (A) OT (white arrow) image on the lateral radiograph of the pressing foot. (B) Posterior compression (black arrow image) image on the lateral radiograph taken while the foot is in plantar flexion.

showed OT posterior impingement syndrome.
A physical examination, lateral radiography, computed tomography (CT), and MRI (Figs. 2A, 2B, 2C) were used for the diagnosis of OT and FHL tenosynovitis. Pre-operative and post-operative evaluations were performed by a physical examination, lateral radiography, MRI, VAS, and AOFAS. Clinical and radiologic results at the pre-operative and final follow-ups were compared.

Pain posterolateral to the ankle and a positive impingement test on the physical examination were typical findings for OT. Pain and swelling in the medial aspect of the ankle were seen in all patients with FHL tenosynovitis. There was local tenderness, crackling on palpation, and a mobile nodule in the medial aspect of the ankle in the localization corresponding to the fibro-osseous tunnel entrance of the FHL. Frequent ankle sprains and a feeling of emptiness in OT were the most common complaints of the patients. In FHL tenosynovitis, the pain increased with thumb dorsiflexion.

If accessory OT was suspected, a further evaluation was carried out with a 3D ankle CT or MRI. The degree and localization of FHL tenosynovitis before PAA were determined using an MRI. The three-zone differentiation described by Lui *et al*. [8] for FHL tenosynovitis was used. Zone 1 is located between the tendon–muscle belly junctional zone and sustentaculum tali, zone 2 is between the sustentaculum tali and the Henry nodule of the foot sole, and zone 3 is at the distal end of the Henry nodule until the endpoint of the phalanx of FHL. The localization and level of FHL tenosynovitis were evaluated through an MRI after PAA. The presence of effusion was divided into three groups (Moderate-Mild-None).

Conservative treatment included soft heel support, ice application, nonsteroidal anti-inflammatory drugs, restriction of hyperflexion, and physiotherapy. PAA was performed in patients who did not respond to conservative treatment for more than four months.

The clinical status of the ankle-hindfoot was evaluated using the AOFAS Ankle-Hindfoot Assessment System, first published in Foot and Ankle International in 1994. According to this scale, pain contributes 40 points (none, mild-occasional, moderate-daily, severe-almost always), function (activity limitations, need for support, maximum walking distance-blocks, walking surfaces, gait abnormality, loss of flexion-extension motion, hindfoot inversion-eversion motion) contributes 50 points, and degree of ankle and hindfoot varus-valgus and foot–ankle alignment contributes 10 points. Lower point scores indicate a poor result.
The VAS score was divided into three groups according to the pain scale of the World Health Organization. A score of less than three was considered mild pain, three to six was mild-moderate pain, and more than six constituted moderate-severe pain.

Surgical Technique

All PAAs in this study were performed in a single center by the same surgeon who was experienced in ankle arthroscopy. General or spinal anesthesia was used. A tourniquet was applied to the proximal thigh while the patient was in the supine position, and then the patient was moved to the prone position. The foot was moved outward from the operating table to perform plantar flexion and dorsiflexion, and the ankle was elevated with the support of a green sterile drape.

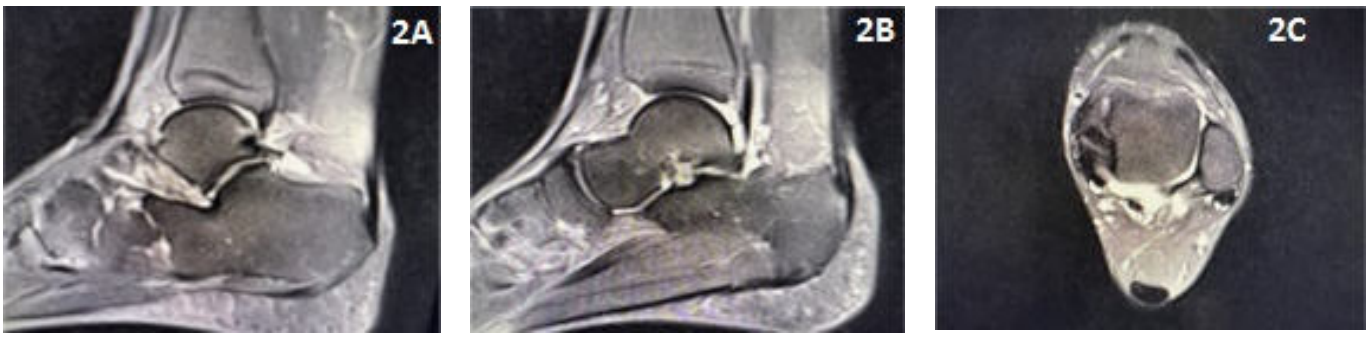

Fig. 2. (A) OT (white arrow) image on ankle sagittal MR section. (B) Degree and localization of effusion (white arrow) due to FHL tenosynovitis on sagittal MR section of the foot. (C) Degree and localization of effusion (white arrow) due to FHL tenosynovitis on axial MR section of the foot.

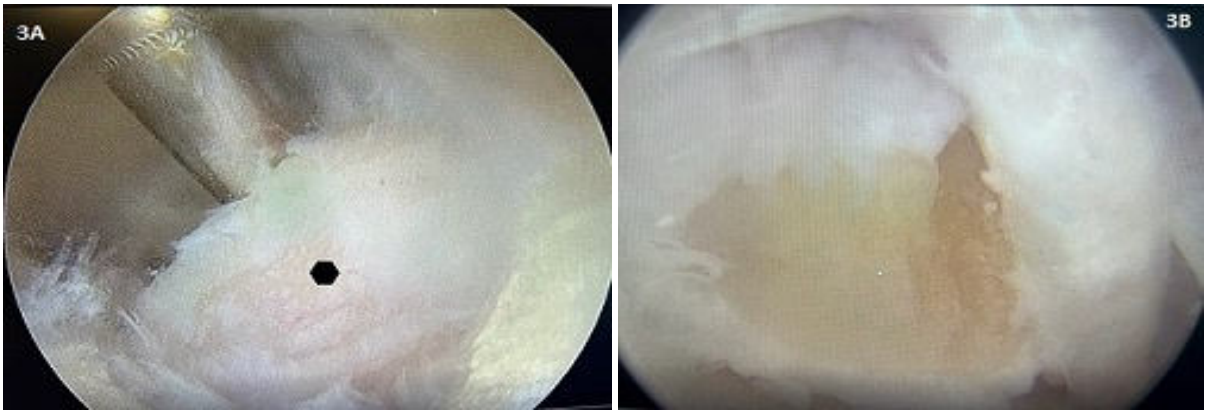

Fig. 3. (A) Separation of the OT (polygon) from the posterior talofibular ligament, flexor retinaculum, posterior talocalcaneal ligament by Sheaver. (B) Excision of OT with bone sheaver and position with FHL after synovectomy.

The two portal techniques described by Van Dijk [1] were applied. The lateral portal was opened lateral to the Achilles tendon at the malleolar junction line. Great care was taken to avoid damage to the small saphenous vein and nerve. The medial portal was opened at the same level as the lateral portal and medial to the Achilles tendon. The lateral portal served as the visualization portal, while the medial portal served as the process portal. A synovectomy was performed through these portals in the safe area between the posterior region of the tibiotalar and subtalar joint and the Achilles tendon without damaging the neurovascular structures. The subtalar joint was imaged first. By moving slightly upward and medially from the joint, the OT and FHL tendons were visible behind the talus. Since this vascular nerve bundle passes in front of the FHL tendon, crossing in front of it was avoided. The OT was loosened from the surrounding tissues (posterior talofibular ligament, flexor retinaculum, posterior talocalcaneal ligament) with a shaver and the released OT (Fig. 3A) was removed from the joint with a grasping punch. The OT was removed as a single 12-20 mm piece. If the OT was whole with the talus, it was excised using a bone bur (Fig. 3B). Stiffness and impingement in the tendon sheath, similar to de Quervain's tenosynovitis, which developed in FHL, was resolved by separating the flexor retinaculum from the posterior talar process using arthroscopic cutting scissors and a shaver. Surrounding adhesions were also loosened with a shaver. Aspiration was performed along the FHL tendon. The hallux was flexed and dorsiflexed, and it was observed that the FHL tendon moved easily in the sheath. The arthroscopic intervention was terminated by placing a skin suture on the portals. A compressive bandage was applied around the foot and ankle. On the first evening post-surgery, the area was compressed with support to the maximum pain threshold the patient could tolerate. After the second week, controlled compression without support was applied. After the first month, patients were permitted to resume light daily activities. The second month allowed for a return to active life, and the third month allowed for light sports. There were no intraoperative complications. Post-operatively, pa-

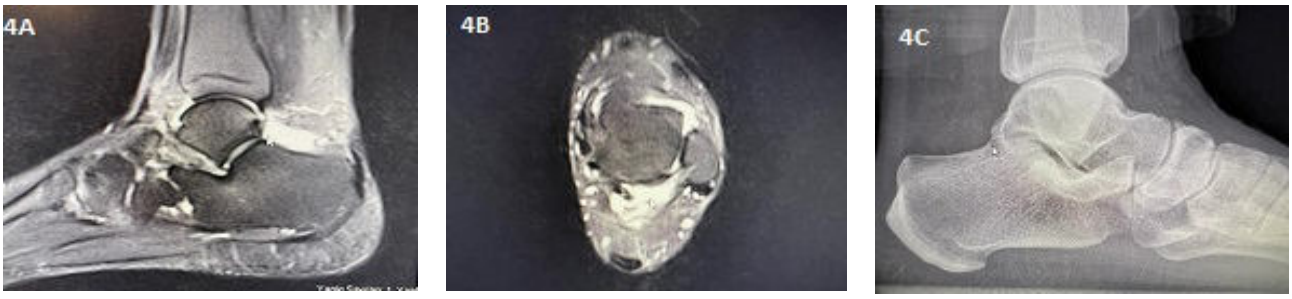

Fig. 4. (A) FHL effusion grade and localization (white arrow) in the ankle sagittal MR section after PAA. (B) FHL effusion degree and localization (white arrow) on ankle axial MR section after PAA. (C) Excision of the OT on the lateral radiograph of the pressing foot at the last follow-up after PAA (white arrow).

tients were evaluated with sagittal-axial MRI (Figs. 4A and 4B) and lateral radiograph (Fig. 4C)

Statistical Analysis

Data were statistically analyzed using the SPSS 19.0 (SPSS, Chicago, Illinois, USA) program. Pre- and post-operative AOFAS midfoot scores and VAS scores were analyzed by paired t-test. Pearson correlation coefficient was used to analyze whether significant correlation exists between the parameters. Two-tailed hypothesis was considered in the analyses, and the significant differences were accepted if p value was < 0.05.

RESULTS

A retrospective evaluation was conducted on 41 patients who underwent PAA for OT and FHL tenosynovitis between March 2016 and August 2021. The mean patient age was 35.6 years (range: 19-55), with the mean female age being 36.2 years (range: 24-48) and the mean male age being 35.2 years (range: 19-55). The mean follow-up period was 34 months (range: 14-62). A table indicating the scores of the demographic characteristics of the patients was made (Table 1).

The AOFAS value pre-PAA increased from 61.5 ± 11.7 to 90.4 ± 9.35 at the final follow-up, with this difference being statistically significant ($p < 0.001$). Five patients completely regained normal function (an AOFAS score of 100 points).

The pre-operative VAS value of 88.5 ± 7.3 increased to 43.5 ± 5.2 at the final follow-up, with this difference also being statistically significant ($p < 0.001$).

There was a significant difference between preoperative VAS and postoperative VAS ($p < 0.001$), and there was a significant difference between preoperative AOFAS and postoperative AOFAS ($p < 0.001$).

Pearson correlation test was performed which showed the relationship of all parameters with each other (Table 2). There is no significant difference between age and scores, but the degree of FHL involvement ($p = 0.019$), and the number of traumas ($p = 0.031$) increase significantly as age increase.

The degree of preoperative FHL involvement has no efffect on the results, but as the degree of postoperative FHL involvement increases, the postoperative VAS score alsıo increases ($p = 0.017$).

The higher the preoperative AOFAS score, the lower the preoperative VAS ($p = 0.011$), and the higher the postoperative AOFAS score, the lower the postoperative VAS score ($p = 0.000$).

It was obserwed that the preoperative AOFAS score did not affect the postoperative AOFAS score ($p = 0.091$), and the preoperative VAS score did not affect the postoperative VAS score ($p = 0.787$) as well.

Prior to PAA, FHL tenosynovitis was observed in zone 1 in 26 patients, in zones 1 and 2 in 14 patients, and in zones 1, 2, and 3 in two patients. In the final follow-up MRI, there was a significant decrease in effusion ($p < 0.001$).

Post-PAA complications included superficial infections treated with oral anti-biotherapy in two patients, stiffness requiring physiotherapy in three patients, mild plantar paresthesia in one patient, and sural nerve paresthesia in two patients. There was no evidence of deep infection, persistent pain, dysesthesia, or major complications requiring re-operation. No

Table 1. The patients demograpfic characteristics scores

	Males (n = 27)	Females (n = 14)	Total (n = 41)
Age (mean)	35.2	36.2	35.6
Preop AOFAS Score	38.4	38.9	38.6
Postop. AOFAS Score	89.7	90.1	89.8
Preop. VAS Score	8.9	9.3	9.0
Postop. VAS Score	1.8	1.7	1.8

Preop = Preoperative, Postop = Postoperative, AOFAS = American Orthopaedic Foot and Ankle Society, VAS = Visual Analog Scale

patients were subjected to revision.

There were seven patients with pes planovalgus and three with plantar fasciitis. For plantar fasciitis, a local lidocaine and corticosteroid injection was administered intraoperatively; no surgery was performed. Patients with pes planovalgus were fitted with special insoles.

DISCUSSION

In this study, FHL tenosynovectomy and OT resection provided significant improvement in the AOFAS and VAS values of PAA for patients with OT and FHL tenosynovitis and ankle pain.

Plantar flexion of the first metatarsophalangeal joint and the first finger interphalangeal joint is the primary function of the FHL tendon. Its secondary function is to support the subtalar joint and thumb joints and restrict passive dorsiflexion of the first metatarsophalangeal joint [4, 9]. When the ankle is excessively plantar flexed, angular incompatibility occurs between the FHL and fibro-osseous tunnel. The FHL tendon can be subjected to abnormal stress during an ankle sprain in excessive plantar flexion, and tenosynovitis may develop [4]. The fact that all patients except

Table 2. Pearson correlation test was performed which showed the relationship of all parameters with each other

		Age (n= 41)	Preop AOFAS (n = 41)	Postop AOFAS (n = 41)	Preop VAS (n = 41)	Postop VAS (n = 41)	Preop FHL (n = 41)	Postop FHL (n = 41)	Ankle Trauma (n = 41)
Age	Pearson Correlation	1	-.090	.006	.083	-.070	.365*	-.088	.337*
	Sig. (2-tailed)		.577	.969	.604	.662	**.019**	.585	**.031**
Preop AOFAS	Pearson Correlation	-.090	1	.268	-.395*	-.083	.272	-.126	.289
	Sig. (2-tailed)	.577		.091	**.011**	.604	.086	.433	.067
Postop AOFAS	Pearson Correlation	.006	.268	1	.207	-.621**	.299	-.253	.180
	Sig. (2-tailed)	.969	.091		.195	**< .001**	.058	.111	.261
Preop VAS	Pearson Correlation	.083	-.395*	.207	1	.044	.088	.032	.238
	Sig. (2-tailed)	.604	**.011**	.195		.787	.584	.841	.134
Postop VAS	Pearson Correlation	-.070	-.083	-.621**	.044	1	-.121	.370*	.006
	Sig. (2-tailed)	.662	.604	**< .001**	.787		.450	**.017**	.972
Preop FHL	Pearson Correlation	.365*	.272	.299	.088	-.121	1	-.083	.306
	Sig. (2-tailed)	**.019**	.086	.058	.584	.450		.604	.052
Postop FHL	Pearson Correlation	-.088	-.126	-.253	.032	.370*	-.083	1	-.098
	Sig. (2-tailed)	.585	.433	.111	.841	**.017**	.604		.543
Ankle Trauma	Pearson Correlation	.337*	.289	.180	.238	.006	.306	-.098	1
	Sig. (2-tailed)	**.031**	.067	.261	.134	.972	.052	.543	

Preop = Preoperative, Postop = Postoperative, AOFAS = American Orthopaedic Foot and Ankle Society, VAS = Visual Analog Scale, FHL = Flexor Hallusis Longus

for eight had a history of ankle sprain in our study supports this result.

Effusion due to FHL tenosynovitis can be seen along the tendon trace, but tenosynovitis was most reported at the level of the fibro-osseous tunnel located posterior to the medial malleolus [10]. In the present study, effusion was most seen in zone 1 between the proximal FHL tendon (behind the medial malleolus) and the sustentaculum tali.

OTS can be diagnosed clinically and radiologically. The initial evaluation method to observe OT is a lateral weight-bearing foot radiography. Furthermore, in a sagittal proton density MRI, OT bone marrow edema and signal changes can be observed, and bone scintigraphy of the involved area will demonstrate an increase in activity [11]. In addition to a lateral weight-bearing foot radiography, an impingement between the posterior malleolus and Os trigonum may be observed in a lateral plantar flexion foot radiography [12]. For OT diagnosis in the present research, lateral weight-bearing foot radiography, lateral foot radiography in flexion, and MRI methods were utilized.

The most common cause of posterior ankle impingement is OT syndrome, accompanied by stenosing FHL tenosynovitis. Conservative therapy is recommended as the primary method for this syndrome. However, patients should be informed that conservative therapy is time-intensive and that symptoms may not be fully alleviated [13, 14]. Conservative therapy has been shown to produce better results in patients who do not actively exercise [15], with another study reporting that conservative therapy methods can produce successful results in approximately 60% of OT syndrome patients [16]. In the present study, without making a distinction between those who exercised and those who did not, PAA was applied to patients for whom successful results could not be achieved despite receiving conservative therapy. Conservative therapy was chosen as the initial treatment option. No results regarding the number of patients receiving conservative therapy who did not require surgery are available from the present research, as this was outside of the scope of this study.

Previous research has reported that for some patients who did not respond to conservative therapy, open or arthroscopic FHL tenolysis and OT excision can be used [15, 17]. These options have come to the forefront as arthroscopic approaches have become favored and because they result in less scar formation, less postoperative pain, a decrease in general morbidity, and allow for the early return to daily activities. Treatment with PAA has been reported to be the gold standard in the treatment of posterior impingement syndrome, OT, and FHL tenosynovitis due to benefits such as detailed imaging of the ankle posterior, faster recovery, return to sports, low morbidity, and less postoperative pain [18, 19]. However, difficulties related to the application of FHL tenolysis and OT excision include the length of learning required for ankle arthroscopy, particularly PAA, and the proximity of the portals to neurovascular structures.

In treatments of massive effusion occurring due to FHL tenosynovitis in patients undergoing extensive synovectomy via open surgery, there have been reports that effusion relapsed in both the proximal and distal of the fibro-osseous tunnel [20]. Patient satisfaction was 80% for PAA and 85%–92% for open surgery in a study comparing PAA and open tenosynovectomy treatments for FHL tenosynovitis. While the recovery time to return to average activities for open surgery was reported to be 12–25 weeks, the recovery time for PAA was 6–8 weeks. However, no statistical significance was reported between these results [21]. Since the present study had limited experience treating FHL tenosynovitis and OT with open surgery, no results related to this difference could be reported.

Previous research has shown that after PAA was used to treat stenosing FHL tenosynovitis, good or excellent results were reported in 70% of patients, and 81% returned to their pre-surgery activity levels [10]. Other research has reported that AOFAS scores increased to 83.2 from 48.7 in patients who underwent tenolysis and a synovectomy for stenosing FHL tenosynovitis [18]. In the present study, the AOFAS scores for 22 patients (55%) were 90 or higher after PAA.

One study observed OT in 50 of 59 patients who underwent PAA for posterior ankle impingement syndrome, a large posterior talar eminence in 14 patients, and FHL tenosynovitis in eight patients. The mid-phase results of the PAA treatment were reported to be good, and the rate of return to sports was high [22]. In another study, in which PAA was commmonly used to treat OT syndrome, there was a significant improvement after arthroscopy [23]. In their study, Morelli *et*

al. [24] reported that after a mean 38.9-month follow-up in PAA and OT excision, the mean AOFAS score increased from 67.8 pre-surgery to 96 post-surgery. Again, in a similar study, it was reported that the mean AOFAS score increased from 43 to 87 in the last follow-up [25]. In the present study, the outcomes of patients who received PAA for both OT and stenosing FHL tenosynovitis were evaluated.

Successful outcomes were reported in studies where OT and FHL tenosynovitis occurred concurrently and were treated with PAA. PAA has been reported to be an effective and safe method in the surgical treatment of both OT and stenosing FHL tenosynovitis [10, 26]. In another study, it was reported that PAA should be the accepted standard in the treatment of pathologies related to the feet due to the low complication rate and faster recovery time [27]. In one study, in the 1-year follow-up of patients whose OT excision and massive effusion around FHL were treated with synovectomy and whose tendon sheath excision was treated with PAA, massive effusion did not reoccur [4]. In the present study, the amount of effusion detected through the MRI before PAA was reduced by at least one degree, and in six patients, it completely disappeared.

Ribbans *et al*. [28] reported 3.7% nerve damage and 0.96% wound site complications after PAA. The present research observed a superficial infection in two patients, stiffness requiring physiotherapy in three patients, mild plantar paresthesia in one patient, and sural nerve paresthesia in two patients.

Limitations

The main advantage of this study is the fact that it is one of the few that combined OT and stenosing FHL tenosynovitis, along with the outcomes of treating both pathologies with PAA. However, this study did have some limitations. Since the study was retrospective, there were some natural deficiencies. Furthermore, there were no results comparing conservative therapy and surgery results. A final limitation is that the pediatric age group was not included, and thus, there were no results for this age group.

CONCLUSION

In the treatment of patients with ankle pain associated with OT and FHL tenosynovitis, two-portal PAA treatment was observed to be an effective method that resulted in significant improvement in the AOFAS and VAS scores. No significant relationship was observed between effusion localization and degree and VAS and AOFAS scores after PAA. The treatment of OT and stenosing FHL tenosynovitis with PAA is considered a safe method due to the low complication rates.

Authors' Contribution

Study Conception: MS; Study Design: MS; Supervision: MS; Funding: MS; Materials: MS; Data Collection and/or Processing: MS; Statistical Analysis and/or Data Interpretation: MS; Literature Review: MS; Manuscript Preparation: MS and Critical Review: MS.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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Investigation of knowledge, attitude and behaviors of university students on testicular cancer: results from two different cities

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ABSTRACT

Objectives: Testicular tumors are the most common malign tumor in men aged 15-35 years. Early diagnosis and treatment is crucial in testicular cancer because if detected at an early stage, testicular cancer can be completely cured. Because of this reason increase in awareness and regular testicular self-examination (TSE) is recommended in the early diagnosis of testicular cancer. This study aimed to investigate the knowledge, attitude and behaviors of students about testicular cancer and testicular self-examination.

Methods: This descriptive and cross-sectional study was conducted in Health care vocational schools of 2 different universities between November 2018 and January 2019. Data were obtained by a self-applied questionnaire comprised of four sections.

Results: One hundred and six (37.7%) participants could not answer any question correctly. While 65.8% (n = 185) of the participants stated that they had heard of testicular cancer, the rate of those who heard about TSE was 17.8% (n = 50). Only 5.7% (n = 16) of the participants reported performing TSE. Binary logistic regression analysis was shown that the following factors increase men's intention to perform TSE: Students' academic unit [OR = 4.36, 95% CI: 1.37-13.88], age [OR = 0.2; 95% CI: 0.008-0.72], city [OR = 0.64; 95% CI: 1.15-1.49], those who have heard of TC before [OR = 0.71, 95% CI: 0.016-0.917], received information about TC [OR = 0.001, 95% CI: 0.015-0.309], and those who have heard of TSE before [OR = 0.01; 95% CI: 0.001-0.079].

Conclusions: One-third of the university students had never heard of testicular cancer, and TSE was not sufficiently practiced. There is a lack of information on this issue. It was thought that organizing training programs on the subject would raise awareness and save lives by early diagnosis.

Keywords: Testicular neoplasms, testicular cancer, cancer screening, testiculer self-examination, knowledge, behaviours

Testicular tumors are the most common malignant tumor in men aged 15-35 years [1]. The probability of developing testicular tumors is 0.2% throughout the entire life [2]. Testicular cancer (TC) comprises 1-1.5% of male neoplasms, constitutes 13-23% of male urogenital system tumors, and 3-6 cases develop each year for every 100,000 men in Western societies [3]. In Turkey, 4.7% of all cancers originate from the re-

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productive system, while TC makes 1.3% of male neoplasms [4, 5].

A history of cryptorchidism or undescended testis, Klinefelter's syndrome, the presence of testicular tumors among first-degree relatives (sibling, father) in the family history, the presence of testicular tumors or testicular intraepithelial neoplasia (TIN) in the contralateral testicle and infertility were reported as the epidemiological risk factors of in the development of testicular tumors [6, 7].

Although testicular cancer is a relatively rare tumor, it has a very important place in urological oncology because it forms a curable cancer model [8]. Because of this feature, early diagnosis and treatment is crucial in testicular cancer. When detected at an early stage, testicular cancer can be completely cured. The 5-year survival rate with early diagnosis is 99% [9, 10].

In the literature, it was emphasized that young men should increase their knowledge and awareness of testicular cancer for early diagnosis [11]. Despite the increase in its incidence, many studies have confirmed that young men do not know about the prevalence of this tumor in their age group, and seldom perform testicular self-examination (TSE) [12-14]. In a study conducted with 7,304 university students across Europe, only 3% of the respondents reported having regular monthly TSE [15]. Routine TSE should be conducted by the person at least once a month and regularly in the shower or after the shower in front of a mirror. It has advantages such as being easy to learn and apply, safe and economical, not requiring special equipment, not being invasive, and not taking much time. Additionally, when the TSE is performed regularly every month, the person gets familiar with the testis tissue, and thus, can promptly recognize any changes [16]. Since testicular cancer is treatable if detected at an early stage, and TSE is applicable, easy, and effective for early diagnosis, testicular cancer should be explained to all males, especially the group with risk factors, and testicular self-examination should be performed by all males.

Because testicular cancers threaten males, especially in the 15-35 age groups, researches targeted especially this group, mentioning that further information is required in this area. Based on the lack of sufficient research on this topic in Turkey, we aimed to determine the knowledge, attitudes, and behaviors of the university students about TC and TSE, to determine the frequency of performing TSE, and to investigate the affecting factors.

METHODS

Participants

This descriptive and cross-sectional study was conducted in Elazığ and Diyarbakır cities between November 2018 and January 2019. The population of our study consisted of male students attending the vocational school of health services at Fırat University in Elazığ and Dicle University in Diyarbakır. In the 2018-2019 academic years, all 1st and 2nd -year male students enrolled in the Vocational Schools of Health Services (VSHS) were included in this study. We aimed to include the whole population without sampling. The target population attending both universities was 330 (160 from Fırat University, 170 from Dicle University). A total of 281 (85%) students volunteered to fill out the study questionnaires and gave written consent. Criteria for inclusion were "being a student in one of the mentioned VSHS's," "male sex," and "age above 18," while participants with a history of testicular cancer were excluded.

Ethical Clearance and Permissions

This study was approved by the local ethics committee of Fırat University Non-Invasive Research Ethics Committee (Date: 17.05.2018, IRB number: 09/17). Also, permissions were obtained from the directorates of Fırat University and Dicle University Vocational Schools of Health Services.

Data Collection

The questionnaires used in the research were applied to the students in the two universities by researchers. Questionnaire forms were distributed to students and they were asked to fill out. A researcher was kept ready to answer the questions of the participants. In order to reach the whole universe, we went to both universities 3 times.

A 10-item questionnaire was prepared to determine the sociodemographic characteristics of the participants. To measure the knowledge about testicular cancer, a multiple-choice testicular cancer knowledge test about the most common symptom of testicular

cancer, the most common age group affected, the probability of recovery, and risk factors were prepared. Information about testicular cancer and TSE characteristics were collected through a 10-item questionnaire, which was prepared after a literature search. Participants' awareness and attitudes about TSE were examined with a seven-item questionnaire of the yes/no response type. In the awareness and attitudes survey of the participants about TSE, 'yes' responses reflected positive attitudes and behaviors, while the 'no' answers were evaluated as negative attitudes and behaviors. The attitude questionnaire crohbach alpha score was 0.735. Four or more positive responses were categorized as 'sufficient', while three or fewer scores were regarded as "inadequate."

Statistical Analysis

Data were analyzed using the SPSS 22.0 SPSS (SPSS Inc., Chicago, IL, USA,) package program. Descriptive data were presented as percentages, means, and standard deviations. Awareness and attitudes survey reliability was calculated with the Kuder-Richardson 20 formula. The reliability coefficient calculated in this way was also stated as a measure of the internal consistency of the test. The Chi-square test was used to examine the relationships between categorical variables. Statistical significance was accepted as $p < 0.05$. The relationship between the number of correct answers given to the testis cancer knowledge test and the number of positive behaviors was evaluated by the Spearmen correlation analysis. Multivariate logistic regression analysis was applied to statistically significant variables ($P < 0.05$). TSE application status (Performing TSE: 0, Not performing TSE: 1) was compared with risk factors. Odds ratio (OR) and 95% confidence intervals (CI) were calculated for each categorical variable.

RESULTS

Mean age of the 281 participants was 22 (3.7) years old. Of the students, 59.4% (n = 167) were from Fırat University in Elazığ and 40.6% (n = 114) from Dicle University in Diyarbakır. The proportion of first and second grade students was 57.3% (n = 161) and 42.7% (n = 120), respectively. 6.4% of the participants were married. 76.9% (n = 216) of the students attended formal education, while 23.1% (n = 65) were receiving evening education. The smoking rate was 44.5%, and no participant had a known history of cancer.

The level of knowledge about testicular cancer was measured with four multiple-choice questions. One hundred and six (37.7%) participants could not answer any question correctly. The number of correct answers in the knowledge test is presented in Table 1.

While 65.8% (n = 185) of the participants stated that they had heard of testicular cancer, the rate of those who heard about TSE was 17.8% (n = 50). Only 5.7% (n = 16) of the participants reported performing TSE. Characteristics of the participants on testicular cancer and TSE are given in Table 2.

Of the participants, 59.1% (n = 166) were aware of the importance of TSE for their health. Only 20.3% (n = 57) of the respondents stated that they knew how to perform TSE. Fifty-two percent (n = 146) of the participants said that they would do regular TSE if reminded by somebody (Table 3).

The rate of hearing TC was higher among the participants who declared that they had been informed about TC before ($p < 0.001$) and were informed by health personnel ($p < 0.001$) . The rate of hearing TSE ($p = 0.003$) and the rate of getting information about TSE ($p = 0.013$) were higher in those who had heard of TC before. TSE practice rate was higher in those who heard TC before ($p = 0.021$). TSE awareness and positive attitude scores ($p < 0.001$) were found to be higher in those who had heard of TC before (Table 4). The final binary logistic regression model demonstrated that the following factors have an independent influence on whether students undertake TSE. Students aged 22 years and above (OR = 0.24; 95% CI: 0.08-0.72; $p = 0.006$), second-year students (OR =

Table 1. Distribution of the correct answers in the testicular cancer knowledge test

Number of correct responses	Frequency (n)	Percent (%)
0	106	37.7
1	105	37.4
2	57	20.3
3	11	3.9
4	2	0.7
Total	281	100.0

Table 2. Participant features on testicular cancer and TSE

Feature (n = 281)	Frequency (n)	Percent (%)
Having any health issues concerning the testicles		
Yes	2	0.7
No	279	99.3
Having heard of testicular cancer		
Yes	185	65.8
No	96	34.2
Information about testicular cancer		
Yes	100	35.6
No	181	64.4
The information source of testicular cancer knowledge		
Didn't get any information	181	64.4
Internet	30	10.7
School	25	8.9
Health personnel	30	10.7
Friend	15	5.3
Having heard of testicular self-examination		
Yes	50	17.8
No	231	82.2
Information about TSE		
Yes	41	14.6
No	240	85.4
TSE information source		
Did not get any information	240	85.4
Internet	8	2.8
School	9	3.2
Health personnel	21	7.5
Friend	3	1.1
TSE practice		
Yes	16	5.7
No	265	94.3
TSE frequency		
Does not perform	265	94.3
Once a month	9	3.2
Once every three months	1	0.4
Once every six months	2	0.7
Once a year	4	1.4
Reasons for not practicing TSE (n = 265)**		
I do not know how to do a TSE myself.	186	70,2
I feel guilty due to examination	9	3.4
I think this examination is a sin	12	4.5
I am negligent on this issue	59	22.3
Afraid of finding something bad	19	7.2

** more than one answer given

1.37; 95% CI: 1.37-13.88; $p = 0.007$), students of Elazig Firat University (OR = 0.64; 95% CI: 1.15-1.49; $p < 0.001$), those who had heard about TC (OR = 0.71; 95% CI:0.02-0.92; $p = 0.015$), those who received information about TC (OR = 0.001; 95% CI: 0.02-0.31; $p < 0.001$), and those who had heard and got information on TSE (OR = 0.01; 95% CI: 0.001-0.079; $p < 0.001$) higher rate of TSE compared to the other students (Table 5).

DISCUSSION

The majority (65.8%) of the participants stated that they had previously heard of testicular cancer. As to other studies from Turkey, proportions of 80% and 44% of students from the vocational school of health services had heard about testicular cancer [17, 18]. In a school of education from Turkey, the same proportion was reported as 57.6% [19]. In a study from Ethiopia, 66.8% of the respondents in a vocational school had heard about TC [20]. We found comparable results with studies using similar populations. On the other hand, studies conducted in risk groups but in the general population, have reported knowing TC as 10.4% in France and 26.4% in Nigeria [21, 22]. Although the rate of hearing about TC among the studied students was higher compared to the general population, it is parallel to the individuals in a similar population. Even though the rate of hearing is higher than the normal population, the students were considered not at the desired level.

The sociodemographic factors had no statistically significant relationship with testicular cancer awareness. In a similar study, Ward *et al*. [23] studied the effects of sociodemographic characteristics of the participants on hearing testicular cancer and reported that except college graduation, the sociodemographic factors were not related to the hearing of TC. In a study conducted on vocational high school students, Pour *et al*. [17] found that education on testicular cancer was the most important factor in raising awareness. Higher rates of awareness among participants who received information about TC suggest that education is a crucial factor in this issue. It has been thought that research about testicular cancer awareness and the effects of education on testicular cancer awareness will unveil the effects of education.

When we look at the situation in the early diagnosis of testicular cancer and the state of TSE awareness, only 17.8% of the participants reported that they had heard of TSE; 14.6% of them had previously received information about TSE, and as a result, only 5.7% reported that they performed TSE. The study of Pour *et al*. [17] reported the STE hearing proportion as 27.6%, education on TSE as 9.4%, and TSE performance as 10.6% among Turkish vocational school of health services' students. Ramim *et al*. [24] from Iran found the rate of getting information about TSE as 5% and TSE practice rate as 7.9%. As to studies conducted among the general public, the proportion of practicing TSE was reported as 1.0% in Nigeria [22], 17.4% in Poland [25], and 3.3% in Turkey [18]. Our findings are similar to the literature. Besides, the results are similar in both the health-related populations and the general population, suggesting insufficient TSE awareness and practice.

When we examined factors affecting the TSE

Table 3. Students' awareness and attitudes of TSE

Variables	Yes		No	
	n	%	n	%
I know TSE is important for my health	166	59.1	115	40.9
I know TSE is effective if done at the same time each month	125	44.5	156	55.5
It is easy for me to remember that I will examine my testicles every month	109	38.8	172	61.2
I know how to perform TSE	57	20.3	224	79.7
I think I can practice TSE correctly	135	48.0	146	52.0
If someone reminds me, I can examine my testicles every month	146	52.0	135	48.0
I know where to seek help if I have pain, swelling, or size differences in my testicles	182	64.8	99	35.2

Table 4. Comparison of the TC knowledge status of the students with some features

	Hearing about testicular cancer before							
Variables	**Yes, heard**		**No, did not hear**		**Total**		**Statistics**	
	n	**%**	**n**	**%**	**n**	**%**	**χ^2**	***p* value**
Age							2.61	0.114
≤ 21	111	62.4	67	37.6	178	100.0		
≥ 22	74	71.8	29	28.2	103	100.0		
Marital status							0.35	0.563
Married	13	72.2	5	27.8	18	100.0		
Single	172	65.4	91	34.6	263	100.0		
Class							3.17	0.079
1st	99	61.5	62	38.5	161	100.0		
2nd	86	71.7	34	28.3	120	100.0		
Type of instruction							0.29	0.593
Formal	144	66.7	72	33.3	216	100.0		
Evening	41	63.1	24	36.9	65	100.0		
City							1.08	0.304
Elazığ	114	68.3	53	31.7	167	100.0		
Diyarbakır	71	62.3	43	37.7	114	100.0		
Father's educational status							1.03	0.314
Below high school	102	63.4	59	36.6	161	100.0		
High school and above	83	69.2	37	30.8	120	100.0		
Income perception							0.31	0.856
Good	34	68.0	16	32.0	50	100.0		
Medium	117	66.1	60	33.9	177	100.0		
Bad	34	63.0	20	37.0	54	100.0		
Mostly resided place							3.66	0.159
City	134	67.0	66	33.0	200	100.0		
District	34	70.8	14	29.2	48	100.0		
Village	17	51.5	16	48.5	33	100.0		
Tobacco use							0.01	0.943
No	103	66.0	53	34.0	156	100.0		
Yes	82	65.6	43	34.4	125	100.0		
Received information on testicular cancer							20.33	**< 0.001**
Yes	83	83.0	17	17.0	100	100.0		
No	102	56.4	79	43.6	181	100.0		
Source of TC information (n = 83)							22.34	**< 0.001**
Internet	26	86.7	4	13.3	30	100.0		
School	19	76.0	6	24.0	25	100.0		
Health personnel	27	90.0	3	10.0	30	100.0		
Friend	11	73.3	4	26.7	15	100.0		
Have you ever heard of TSE?							8.92	**0.003**
Yes	42	84.0	8	16.0	50	100.0		
No	143	61.9	88	38.1	231	100.0		
Have you ever been informed about TSE?							6.23	**0.013**
Yes	34	82.9	7	17.1	41	100.0		
No	151	62.9	89	37.1	240	100.0		
Can you doTSE practice?							5.88	**0.021**
Yes	15	93.8	1	6.2	16	100.0		
No	170	64.2	95	35.8	265	100.0		
TSE awareness and attitude							15.82	**< 0.001**
Insufficient awareness and attitude	79	54.9	65	45.1	144	100.0		
Sufficient awareness and attitude	106	77.4	31	22.6	137	100.0		

Table 5. Multiple logistic regression model of predictors of TSE status

Variables	Odds Ratio	Confidence Interval (95.0%)	p value
Age			**0.006**
≤ 21	1		
≥ 22	0.24	0.08-0.72	
Class			**0.007**
1st	1		
2nd	4.36	1.37-13.88	
City			**< 0.001**
Diyarbakir	1		
Elazig	0.64	1.15-1.49	
I've heard of TC before			**0.015**
No	1		
Yes	0.71	0.016-0.917	
I was informed about TC			**< 0.001**
No	1		
Yes	0.001	0.015-0.309	
I've heard of TSE before			**< 0.001**
No	1		
Yes	0.01	0.001-0.079	
I was informed about TSE			**< 0.001**
No	1		
Yes	0.001	0.000	

TSE = Testicular self-exam, TC = Testicular cancer

practice, it was seen that being aged 22 years and above, studying in the second class, being at Elazığ vocational school of health services, having previously heard of TC, and having received some information on TC were statistically significant factors affecting TSE practice. In the literature, factors affecting the TSE practice were examined in two studies, which reported that father's education, family history of cancer, TC and TSE awareness, having a high level of knowledge, and being under TC risk were more likely to practice TSE [23, 25]. TSE practices of the vocational school of health services students were investigated in Iran, which revealed that the rates of practicing TSE were increasing with the school year and knowledge and experience on TSE [24]. Two studies from Turkey have indicated a lack of knowledge on TSE and negligence as the reasons for not practicing TSE [17, 18], while Farrow's study identified lack of knowledge as the primary reason for not practicing TSE [26]. In our study, the participants stated that they did not know how to perform TSE as the most common reason for not applying TSE. Our findings were similar to the literature. When considering factors affecting TSE practice as well as barriers to perform TSE, lack of knowledge and awareness emerged as the two fundamental reasons.

Based on the data we have obtained, and hypothesizing that the most important factor affecting TSE was knowledge, a multivariate logistic regression analysis was applied to the factors that significantly affected TSE practice. As a result of the logistic regression analysis, the ratio of applying TSE was 4.3 times higher in the second-class students compared to the first class. It is possible that the knowledge level of the participants was increasing with advancing school class, thus, leading to higher TSE practice. In a similar study conducted in Iran, the acquaintance and knowledge with TC were increasing as the study years

progressed [24]. Also, in a study conducted among medical students in Nigeria, a comparison was made before and after training on TC and TSE, and it was revealed that the knowledge levels and TSE practices increased [27]. Our results are conforming to the literature. To support our hypothesis, a correlation analysis was done between the TC knowledge and the positive attitude scores, which showed a significant positive relationship. Supported by the literature, we considered that the level of TC and TSE knowledge was one of the most crucial factors affecting TSE practice and early diagnosis.

Limitations

Although our research has been conducted in two different centers, it is not representative of the general population. Besides, the study data relied on self-report. The information on TC education could be retrieved by reviewing the school curricula. Thus, the difference between the schools could not be elaborated. Future studies should investigate the effects of educational status and educational content on TC and TSE.

CONCLUSION

As a conclusion, our study revealed that the knowledge and awareness of TC and TSE are insufficient even in the health team of the future, and the rates of practicing TSE are much lower. The aim of management of testicular cancer is to prevent delayed diagnosis and to make an early diagnosis. The aim of testicular cancer management is to prevent delayed diagnosis and to make an early diagnosis; this goal can be achieved with education programs that will increase cancer awareness among young people. This study demonstrated that the implementation of TSE is still not sufficient by men. The obtained data suggest that the main reasons for not practicing TSE are lack of knowledge and awareness. Since half of the participants were ready to apply TSE if reminded to do so, and students with higher knowledge and awareness were practicing considerably more TSE, we considered that programs targeting to increase knowledge and awareness of men would attract the attention of the people and contribute to increasing the early diagnosis of TC. As men aged 15-35 can be easily reached at the school and during their military services, these two activities can be an opportunity to implement educational activities. Another suggestion is that family physicians provide counseling on health education and testicular cancer to their patients at risk during periodic examinations.

Authors' Contribution

Study Conception: BY, EP; Study Design: BY, EP; Supervision: BY, EP; Funding: MAŞ, EY; Materials: MAŞ, EY; Data Collection and/or Processing: MAŞ, EY; Statistical Analysis and/or Data Interpretation: BY, EP; Literature Review: BY, EP, MAŞ, EY; Manuscript Preparation: MAŞ, EY and Critical Review: BY, EP, MAŞ, EY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Paraganglioma admitting with stage 4 hypertensive retinopathy

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ABSTRACT

Objectives: Paragangliomas and pheochromocytomas are rare tumors originating in chromaffin cells which are predominantly located in adrenal glands. This tumor is generally bilateral and much more rarely seen in pediatric patients. Sustained or paroxysmal hypertension is the most frequent sign of paragangliomas /pheochromocytoma. Here, we present a 15-year-old patient diagnosed with the complaint of blurred vision.

Keywords: Extraadrenal gland neoplasms, paraganglioma, pheochromocytoma, hypertension, childhood

Paragangliomas and pheochromocytomas (PGL/PHEO) are rare neuroendocrine tumors producing catecholamines and other neuropeptides [1]. Pheochromocytomas originate mainly from the adrenal medulla; however, they usually secrete catecholamines. Most of those that are called PGLs, which originate from the extra-adrenal gland, are nonfunctional [2]. The incidence of PGL/PHEO is estimated to be 0.3 cases per million on an annual scale, and about 20 percent of cases are diagnosed during childhood [3]. Contrary to adults, pediatric patients may present with atypical findings such as psychiatric disorders and orthostatic hypotension, aside from the classical triad (e.g episodic headaches, sweating, and tachycardia), and are therefore likely to be misleading [4]. Here, we present a 15-year-old patient diagnosed with PGL who presented with the complaint of blurred vision.

CASE PRESENTATION

A 15-year-old male patient was admitted with short-term palpitations after effort for the last year and blurred vision in the left eye for one month. In his physical examination, blood pressure was 170/100 mmHg, heart rate was 140 per min, and other examination findings were normal. Except for the high renin levels (4.75 ng/mL/h) in laboratory tests, other results were normal. There were signs of stage 4 hypertensive retinopathy in the eye examination. Abdominal ultrasonography revealed a 47×46×58 mm hypo-isoechoic solid mass in the para-aortic area, adjacent to the left kidney lower pole (Fig. 1). The normetanephrine level that was measured in the 24-hour urine for the diagnosis of the mass was as high as 15322.43 µg/day. In the echocardiography of the patient that was evaluated by pediatric cardiology, it was found that he had left

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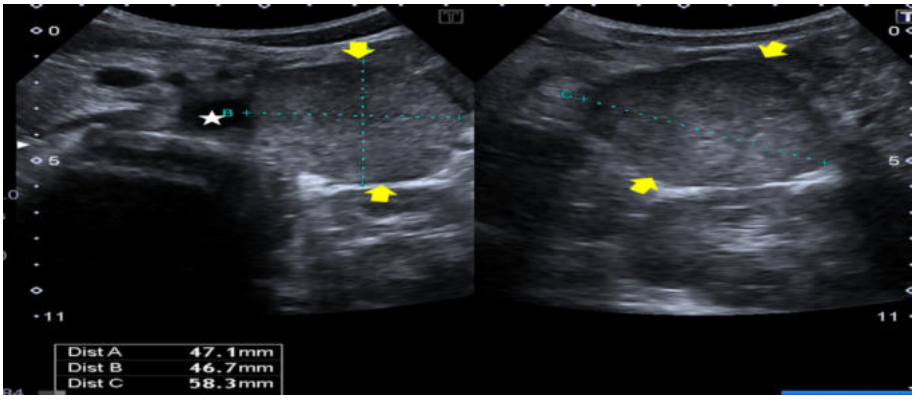

Fig. 1. Hypo-isoechoic solid mass image of 47×46×58 mm in the paraaortic area, adjacent to the left kidney lower pole in abdominal ultrasonography.

ventricular hypertrophy. The patient was initiated with nifedipine and enalapril for hypertension control.

Contrast-enhanced abdominal magnetic resonance imaging (MRI) revealed a mass lesion with homogeneous enhancement and smooth borders, adjacent to the lower pole of the left kidney, and the widest anterior-posterior diameter was 40 mm, the widest mediolateral diameter was 50 mm, and the widest craniocaudal length was 60 mm (Fig. 2). Oncological positron emission tomography (PET) scan (whole-body scan) showed a mass lesion that had dotatate in the left lower quadrant of the abdomen, adjacent to the kidney inferior area. The antihypertensive treatment was adjusted before the surgery as doxazosin and carvedilol. The patient, whose blood pressure measurements were examined, was operated on by the pediatric surgeon, after which blood pressure measurements were normal and antihypertensive treatment was not needed. Pathological examination of the mass revealed that it was compatible with PGL (Fig. 3).

DISCUSSION

Among hypertensive children, the incidence of surgically-confirmed catecholamine-secreting PGL/PHEO ranges between 0.8% and 1.7% [5]. However, it was reported in the case series that persistent or paroxysmal hypertension is the most common manifestation of PGL/ PHEO in children at a rate of 60-90% [6].

Our patient presented with hypertension that caused end-organ damage. Urinary metanephrine excretion, which was examined for the etiology, was elevated. Currently, the diagnostic test for PGL/ PHEO should reveal 87.5% and 99.7% specificity in a 24-hour urinary metanephrine and catecholamine excretion four-fold higher than the reference range [6, 7]. The tumor should be located with radiographic and nuclear imaging such as abdominal computerized tomography (CT) or MRI and 'iodine 131-metaiodobenzylguanidine scintigraphy after the biochemical diagnosis is made. Due to the predominant intra-abdominal location of PGL/PHEO, abdominal and

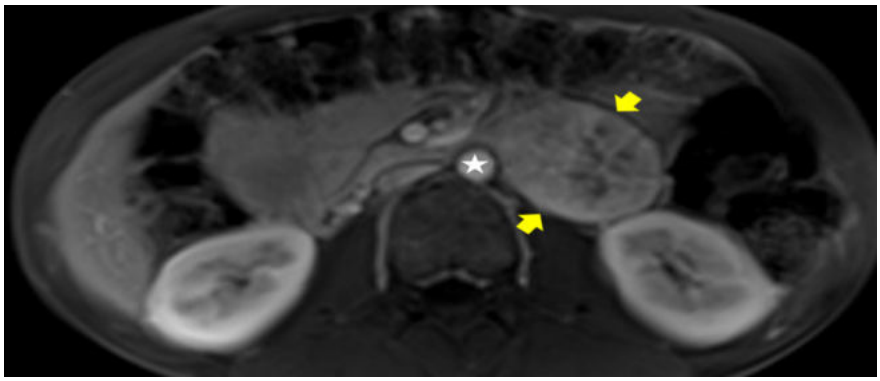

Fig. 2. In contrast-enhanced abdominal magnetic resonance imaging, a well-circumscribed mass lesion with homogeneous contrast, adjacent to the left kidney lower pole, has the widest anterior-posterior diameter 40 mm, the widest mediolateral diameter 50 mm, and the widest craniocaudal length 60 mm.

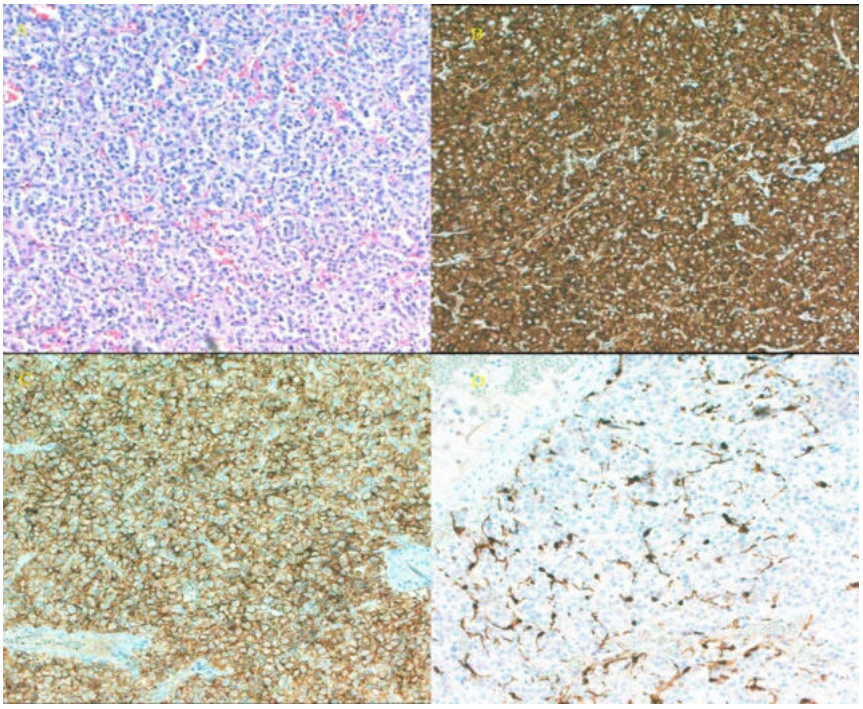

Fig. 3. Pathological examination. (A) Nests formed by tumor cells with oval, round, uniform nuclei and granular cytoplasm (HE×200), (B) Immunohistochemical synaptophysin positivity in tumor cells (×200), (C) Immunohistochemical CD56 positivity in tumor cells (×200), (D) Immunohistochemical S100 positivity in sustentacular cells surrounding tumor cells (×200).

pelvic CT/MRI are the first-choice imaging modalities [8]. Magnetic resonance imaging is recommended for (pregnant women, aged below 25 with metastatic PGL) with a contraindication of radiation exposure. Especially in patients with PGL, anatomical imaging is insufficient and further evaluation is required. Further assessment of localization may require functional investigation with scintigraphy (e.g.i. nuclear medicine modalities) or PET canning [9]. Functional imaging is recommended for the baseline evaluation of patients with large-size PHEOs or PGLs because it is associated with the risk of metastatic disease. Therefore, PET CT imaging was performed because the mass of our patient was located in the extra-adrenal gland.

Paraganglioma /pheochromocytoma is symptomatic in almost half of patients. These symptoms are typically paroxysmal [10]. We considered that our patient had longer hypertensive periods as he was admitted with stage 4 hypertensive retinopathy. However, echocardiography of our patient also revealed left ventricular hypertrophy that was secondary to hypertension. However, in the literature, patients with myocardiopathy secondary to catecholamine discharge were described in patients with pheochromocytoma, as was reported in the cases of Molaei *et al*. [11].

The mean age of diagnosis in children is 11-13 years. It is known that most of the cases are detected in males. In this age group, PGL/ PHEO tend to be bilateral, extra-adrenal, and benign [12]. The findings of our patient were compatible with the literature data since the tumor was in a 15-year-old male and was localized in the extra-adrenal area.

Surgery is the gold treatment for the treatment of PGL/ PHEO. However, as benign and non-functional tumors < 4-6 cm may rarely transform into malignant or become hormonally active, such tumors must be followed up with appropriate radiological imaging and hormonal evaluation. The purpose of surgery is complete resection. Pham *et al*. showed that survival was significantly increased in patients with radical resections that had macro and microscopic negative margins [13]. Medical treatment must be initiated 7-10 days before the surgery in PGL/PHEO cases to check hypertension as well as correct catecholamine-induced extracellular fluid volume contraction. Basically, alpha-adrenoreceptor blockers are used to control blood pressure; and phenoxybenzamine, which is a long-acting, irreversible, non-specific alpha-adrener-

gic antagonist, is the usual choice. Selective alpha-1-antagonists (e.g. prazosin, doxazocin or terazosin) can be used instead of phenoxybenzamine. Both phenoxybenzamine and doxazosin can effectively control perioperative blood pressure and prevent hemodynamic instability in patients of PGL. However, more often, beta adrenergic antagonist (e.g. metaprolol) or combined alpha and beta adrenergic antagonist (e.g. carvedilol, labetalol) are added to the treatment to control reflex tachycardia [14]. We applied premedication to our patient by using doxazosin and carvedilol. It is necessary during the surgery to avoid stimuli and drugs which may cause catecholamine release and to provide hemodynamic stabilization in anesthesia. Our patient, who had normal blood pressure after the surgery, did not require medication.

The surgical approach was adequate because our patient presented with a unilateral and single mass, and it was found that the pathology result was benign. There are controversial data on the malignant potential of PGL/PHEO in children between 3% and 47%. Despite this, overall 5-year survival is better in children than in adults in reported malignancy cases [11]. Long-term clinical and biochemical follow-up should be performed in all PGL/PHEO cases regarding increased recurrence and metastasis risk.

CONCLUSION

Paragangliomas and pheochromocytomas is one of the rare and important causes of secondary hypertension in pediatric patients. As the symptoms may have atypical progression in children, it may appear for the first time with end-organ damage. Even if there is no family history, it must be considered in hypertension cases that have an aggressive progression.

Authors' Contribution

Study Conception: İY; Study Design: ŞA; Supervision: FB; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ZEÇ; Statistical Analysis and/or Data Interpretation: MÖ; Literature Review: FÖS; Manuscript Preparation: İY and Critical Review: ŞA.

Informed consent

Informed consent form was not obtained as this was a patient followed up in the past.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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