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The Effect of a Pandemic on Computed Tomography Pulmonary Angiography Results and Utilize in the Emergency Department

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ABSTRACT

Objective: The incidence of pulmonary embolism (PE) increases with COVID-19. With the pandemic, changes occur in the utilization of computed tomography pulmonary angiography (CTPA), which we use in the diagnosis of PE. In our study, we investigated the impact of the pandemic on the utilized and result of CTPA.

Methods: Patients over the age of 18 who applied to the emergency department between 01.03.2019 and 28.02.2021 and underwent CTPA was included in this retrospective study. Patients were separated to two groups based on the date of the first case. CTPA result and Polymerase chain reaction (PCR) positivity status were recorded. Data were compared between groups.

Results: While 757(1.022%) out of 74,063 patients underwent CTPA in the pre-pandemic period, 649(1.430%) out of 45,397 patients underwent CTPA in the during-pandemic period. The PE rate in patients who underwent CTPA in the during-pandemic period was statistically significantly higher compared to the pre-pandemic period (pre-pandemic: 89(11.9%), during-pandemic: 122(19%), p<0.001). In the during-pandemic period, there was no statistically significant difference in the rate of PCR positivity in any time in patients with PE detected as a result of CTPA compared to patients without PE (PE: 14(11.5%), non-PE: 54(10.4%), p=0.725).

Conclusion: Higher rate of CTPA shoot was obtained in the during-pandemic period compared to the pre-pandemic period. Additionally, the rate of PE detection among patients who underwent CTPA was statistically significantly higher in the during-pandemic period compared to the pre-pandemic period.

Keywords: COVID-19, Pulmonary embolism, Computed Tomography Angiography, Emergency Department

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a viral disease that starts with symptoms of upper respiratory tract infection (1). Since spreading worldwide, it was considered in the pandemic (2). COVID-19 causes hypercoagulation by causing endothelial damage (3). It was found that the rate of pulmonary embolism (PE) is increased in hospitalized COVID-19 patients and critical care unit COVID-19 patients (4). Although prophylactic anticoagulant therapy, deaths due to PE occurred in COVID-19 patients (5).

Although methods such as D-dimer and echocardiography are used to diagnose PE in the emergency department (ED), the most commonly used diagnostic method is computed tomography pulmonary angiography (CTPA) (6). Along with the pandemic, changes have occurred in ED patient management and the utilization of computed tomography (CT) (7,8). To manage the diagnosis and prophylaxis of PE, which is a life-threatening disease, epidemiological researches are needed to appraise the change in the use of CTPA with the pandemic.

In this study, we analyzed the impact of the pandemic on the utilization and result of CTPA. For this purpose, we examined the utilization of CTPA and the results of CTPA in patients who applied to the ED in the 1-year periods before and after the onset of the pandemic.

2. METHODS

The study was designed as retrospective, observational and single-center. The local ethics committee granted approval for the study (Necmettin Erbakan University Meram Medical Faculty Pharmaceutical and Non-Medical Device Studies Ethical Committee, 2021/3310, 18.06.2021). Patients over the age of 18 who applied to the ED of a tertiary university hospital between 01.03.2019 and 28.02.2021 and underwent

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CTPA at any time until they left the hospital was included in the study. After the pandemic started, the hospital where the study was conducted served both COVİD 19 patients and non-COVID 19 patients. Data were obtained by scanning the CTPA code over the hospital information management system program. Patients whose CTPA not be interpreted due to shooting artifact and/or contrast deficiency were excluded from the study. Although the contrast and shooting method were not optimal, patients whose CTPA was interpreted were included in the study. Patients were separated to two groups based on the date of the first case in the country where the study was conducted (March 2020). The period from 01.03.2019 to 29.02.2020 was named as the pre-pandemic period, and the period from 01.03.2020 to 28.02.2021 was named as the during-pandemic period. Besides, the groups were named as pre-pandemic and during-pandemic.

Age, gender, hospital outcomes (discharge, exitus in-hospital, discharged against medical advice (AMA), and referral), in-hospital mortality status (survivior, non-survivor), length of hospital stay, optimality status of CTPA in terms of contrast and shooting, CTPA shooting status in ED, CTPA result (PE positive, PE negative), localization of the thrombus (if there is PE), bilateral thrombus status (if there is PE), and thrombolytic treatment status were recorded. Additionally, polymerase chain reaction (PCR) positivity in the last 2 weeks and PCR positivity at any time was recorded in the during-pandemic group. It was compared the collected data between groups. The primary aim of the study was to evaluate the frequency of CTPA scans and PE between the two periods. The secondary aim of the study was to compare the PCR positivity rates between those who were diagnosed with PE and those who were not.

SPSS program was used for statistical analysis. Normality analysis of the data was performed. Kolmogorov–Smirnov test was used for this. All quantitative data were not normally distributed. Quantitative and categorical data were expressed as median (Q1 –Q3) and frequency (percentage). The differences between the groups were investigated using the Mann–Whitney U test and chi-square test. For statistical significance, $p<0.05$ was accepted.

3. RESULTS

A total of 74,063 patients over the age of 18 in the pre-pandemic period and 45,397 patients over the age of 18 in the during-pandemic period were admitted to the ED of hospital where the study was conducted. While 757(1.022%) patients underwent CTPA in the pre-pandemic period, 649(1.430%) patients underwent CTPA in the during-pandemic period. In total, 1406 patients underwent CTPA. Fifteen of these 1406 patients were excluded because CTPA not be interpreted due to contrast deficiency and shooting artifact (8 pre-pandemic, 7 during-pandemic). Of the remaining 1349 patients, 749 (53.8%) were in the pre-pandemic group and 642 (46.2%) were in the during-pandemic group. PE was detected in 211 (15.2%) of the patients who underwent CTPA. Additionally, in 274(19.7%) patients, CTPA was not shot in the emergency room, but in the service where they were hospitalized. In-hospital mortality was observed in 230 (16.5%) of the

Table 1. *The Result of parameters according to the periods*

		All (1391)	Pre-pandemic (749)	During-pandemic (642)	p value
Age		67(55-78)	66(53-77)	70(57-79)	0.001
Length of Hospital Stay (Day)		4(0-12)	3(0-12)	6(1-13)	<0.001
Gender	**Male**	710(51%)	382(51%)	328(51.1%)	0.974
	Female	681(49%)	367(49%)	314(48.9%)	
CTPA shooting location	**ED**	1117(80.3%)	630(84.1%)	487(75.9%)	<0.001
	Admission clinic	274(19.7%)	119(15.9%)	155(24.1%)	
CTPA result	**PE positive**	211(15.2%)	89(11.9%)	122(19%)	<0.001
Contrast enhancement is optimal		1329(95.5%)	721(96.3%)	608(94.7%)	0.161
Hospital Outcome	**Discharged**	1026(73.8%)	580(77.4%)	446(69.5%)	0.001
	Exitus	230(16.5%)	98(13.1%)	132(20.6%)	
	Referral	50(3.6%)	22(2.9%)	28(4.4%)	
	Discharged against medical advice	85(6.1%)	49(6.5%)	36(5.6%)	
In-Hospital Mortality	**Survivor**	1161(83.5%)	651(86.9%)	510(79.4%)	<0.001
	Non-Survivor	230(16.5%)	98(13.1%)	132(20.6%)	

CTPA: Computed tomography pulmonary angiography; ED: Emergency department; PE: Pulmonary embolism.

patients included in the study. The data of the patients are given in Table 1 in detail.

The PE rate in patients who underwent CTPA in the during-pandemic period was statistically significantly higher compared to the pre-pandemic period (pre-pandemic: 89(11.9%), during-pandemic: 122(19%), p<0.001). In-hospital mortality rate of patients who underwent CTPA in the during-pandemic period was statistically significantly higher compared to the pre-pandemic period (pre-pandemic: 98 (13.1%), during-pandemic: 132 (20.6%), p<0.001). The comparison of the data according to the periods is given in Table 1 in detail.

There was no statistically significant difference between the pre-pandemic period and the during-pandemic period in parameters such as age, gender, hospitalization time, localization of thrombus, in-hospital mortality of patients with PE. However, the rate of bilateral PE detected in the pre-pandemic period was statistically significantly higher compared to during-pandemic period (pre-pandemic: 54(60.7%), during-pandemic: 57(46.7%), p=0.045) (Table 2).

Table 2. *Evaluation of PE patients according to periods*

		All(211)	Pre-pandemic(89)	During-pandemic(122)	p value
CTPA shooting location	ED	154(73%)	65(73%)	89(73%)	0.989
	Admission clinic	57(27%)	24(27%)	33(27%)	
Is embolism bilateral?	Yes	111(52.6%)	54(60.7%)	57(46.7%)	0.045
Localization of thrombus	Subsegmental artery	12(5.7%)	7(7.9%)	5(4.1%)	0.245
	Segmental artery	131(62.1%)	50(56.2%)	81(66.4%)	
	Main and lobar pulmonary artery	68(32.2%)	32(36%)	36(29.5%)	
Thrombolytic Therapy		17(8.1%)	6(6.7%)	11(9%)	0.549
Age		71(57-78)	66(55.5-74.5)	72(58-81)	0.019
Gender	Male	104(49.3%)	42(47.2%)	62(50.8%)	0.603
Length of Hospital Stay (Day)		9(4-17)	10(4.5-16.5)	9(4-17.25)	0.531
In-Hospital Mortality	Survivor	150(71.1%)	68(76.4%)	82(67.2%)	0.146
	Non-Survivor	61(28.9%)	21(23.6%)	40(32.8%)	

CTPA: Computed tomography pulmonary angiography; ED: Emergency department; PE: Pulmonary embolism.

Table 3. *Evaluation of parameters according to CTPA result*

		PE(211)	Non-PE(1180)	P value
Age		71(57-78)	67(55-78)	0.118
Length of Hospital Stay (Day)		9(4-17)	3(0-12)	<0.001
Gender	Male	104(49.3%)	606(51.4%)	0.580
	Female	107(50.7%)	574(48.6%)	
Hospital Outcome	Discharged	132(62.6%)	894(75.8%)	<0.001
	Exitus	61(28.9%)	169(14.3%)	
	Referral	20(4.7%)	40(3.4%)	
	Discharged against medical advice	8(3.8%)	77(6.5%)	
In-Hospital Mortality	Survivor	150(71.1%)	1011(85.7%)	<0.001
	Non-Survivor	61(28.9%)	169(14.3%)	
CT shooting location	ED	154(73%)	963(81.6%)	0.004
	Admission clinic	57(27%)	217(18.4%)	
Contrast enhancement is optimal		207(98.1%)	1122(95.1)	0.050

CTPA: Computed tomography pulmonary angiography; ED: Emergency department; PE: Pulmonary embolism.

The median length of hospital stay of patients with PE detected by CTPA was statistically significantly higher compared to patients without PE (PE: 9(4–17), non-PE: 3(0–12), p<0.001). In-hospital mortality rate of patients with PE detected as a result of CTPA was statistically significantly higher than patients without PE (PE: 61(28.9%), non-PE: 169(14.3%), p<0.001). A detailed comparison of the data according to the result of CTPA is given in Table 3.

In the during-pandemic period, there was no statistically significant difference in the rate of PCR positivity in the last 2 weeks in patients with PE detected as a result of CTPA compared to patients without PE (PE: 7(5.7%), non-PE: 32(6.2%), p=0.863) (table 4). In the during-pandemic period, there was no statistically significant difference in the rate of PCR positivity in any time in patients with PE detected as a result of CTPA compared to patients without PE (PE: 14(11.5%), non-PE: 54(10.4%), p=0.725) (Table 4).

Table 4. *Evaluation of CTPA result with PCR positivity in the during-pandemic period*

	All(642)	PE(122)	Non-PE(520)	P value
PCR positivity in any time	68(10.6%)	14(11.5%)	54(10.4%)	0.725
PCR positivity in the last 2 weeks	39(6.1%)	7(5.7%)	32(6.2%)	0.863

PCR: Polymerase chain reaction; CTPA: Computed tomography pulmonary angiography; PE: Pulmonary embolism.

4. DISCUSSION

In our study, patients who underwent CTPA in the 1-year periods before and after the onset of the pandemic were examined. The rate of CTPA shoot was found to be higher in the during-pandemic period. Additionally, in the during-pandemic period the number of patients with PE detected in patients who underwent CTPA was higher than in the pre-pandemic period.

PE is a life-threatening disease that requires rapid intervention. Due to the relationship between COVID-19 and hypercoagulation, it is thought that its frequency may increase with the pandemic. In this study, the rate of PE detection in the during-pandemic period was statistically significantly higher compared to the pre-pandemic period. In the study by Watchmaker et al., the number of patients with PE detected as a result of CTPA in March-April 2019 was 34, while the number of patients with PE as a result of CTPA in March-April 2020 was 87 (9). In the study by Finn et al., while Pulmonary Embolism Response Team (PERT) consultation was requested from 26 patients in March-April 2019, PERT consultation was requested from 74 patients in March-April 2020 (10). In the same study, PE was detected by CT in 24 patients in March-April 2019, whereas PE was detected by CT in 43 patients in March-April 2020 (10). There may be many reasons for the increase in the incidence of PE with the pandemic. The reason for this increase can be shown as the direct effect of the COVID-19. Because the rate of PE in patients hospitalized due to COVID-19 was found to be higher compared in patients hospitalized for other reasons (11). Additionally, in a meta-analysis of 22 studies by Suh et al., the rate of PE in 3342 COVID-19 patients was found to be 16.5% (12). Espallargas et al. determined that PE detected in COVID-19 patients was predominantly unilateral and on the right side (13). In our study, the rate of bilateral PE in the during-pandemic period was found to be statistically significantly low. This result may be an explanation that the cause of increased PE in the during-pandemic period can be attributed to COVID-19.

In our study, in the during-pandemic period, there was no statistically significant difference in the rate of PCR positivity between patients with and without PE according to the CTPA result. This result is in contrast to the conclusion that the increase in PE may be due to COVID-19. There are studies with similar results in the literature. Freund et al. found similar PCR positivity between 500 patients with PE and 2753 patients without PE in a retrospective multicenter study conducted with 3253 patients who underwent CTPA (14). The reason for this situation be that the pre-diagnosis of PE should be considered more in patients with PCR positivity. For this reason, it may have increased CTPA negativity for PE in patients with PCR positivity. Additionally, the inadequacy in the number of PCR tests during the pandemic process and the fact that a patient who does not have a PCR test even if he/she has COVID-19 can be included in the PCR-negative group also causes this increase. To eliminate this bias in a patient with PCR positivity, studies with prospective and CTPA shot criteria are needed. In the study by Agarwal et al., it was determined that the number of CTPA shoots decreased after the pandemic compared to the pre-pandemic period (8). They thought that the reason for this decrease might be the decrease in ED admission (8). Because we know from the literature that the number of ED admission has decreased during the pandemic period compared to the pre-pandemic period. In the study by Hartnett et al., they found that the number of ED visits decreased by 42% compared to the pre-pandemic period (15). In our study, while the number of CTPA shoots was found to be less in the during-pandemic period, the CTPA shoot rate was found to be higher. The increase in CTPA shoot rate increase the number of patients with PE. However, in our study, the rate of CTPA shoots increased by 39.92% in the during-pandemic period compared to the pre-pandemic period, while the rate of PE detection increased by 59.66% in patients who underwent CTPA (1.022%-1.430%=39.92%; 11.9%-19%=59.66%). In other words, we cannot attribute the increase in PE detection rate only to the increase in CTPA shoot rate.

In our study, statistically significant difference was not found in the in-hospital mortality rates and length of hospital stays of patients with PE in the during-pandemic period compared to the pre-pandemic period. Additionally, the rate of thrombolytic therapy and the localization of the thrombus (segmental, subsegmental) were found to be similar between the two groups in our study. In the study by Finn et al., the length of hospital stays, in-hospital mortality rates and severity of PE (massive-submassive) was found to be similar between the pre-pandemic group and the during-pandemic group (10). However, high mortality rates have been shown in the literature in COVID-19 patients with PE. In a meta-analysis of 8 studies by Liao et al., they found a mortality rate of 45.1% in COVID-19 patients with PE (16). We can explain this contradiction by the small number of patients in our study. Because, although there was no statistical difference in our study, the in-hospital mortality rate was 32.8% in patients with PE in the during-pandemic period, while it was 23.6% in the pre-pandemic period.

This study has some limitations. The fact that the study is retrospective and single-centered is a limitation. Failure to evaluate the CTPA shoot criteria creates a limitation. The inability to explain a clear reason for the difference between the two periods is a limitation. Not evaluating patients receiving anticoagulant prophylaxis is a limitation.

5. CONCLUSION

According to the results of the study, a higher rate of CTPA shoots was obtained in the during-pandemic period compared to the pre-pandemic period. Additionally, the rate of PE detection among patients who underwent CTPA was statistically significantly higher in the during-pandemic period compared to the pre-pandemic period.

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Clinical and Experimental Health Sciences

Psychological Morbidity, Fatigue and Burden of Disease in Patients With Connective Tissue Diseases

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ABSTRACT

Objective: Depression and anxiety disorders are significant health problems that can coexist with other diseases and exert adverse effects on these diseases' course and treatment response. Fatigue is a common and disabling symptom in chronic inflammatory diseases. The present study aimed to evaluate the fatigue, anxiety, depression and burden of disease (eg, disease activity, function, quality of life) in autoimmune connective tissue disease (CTD) patients.

Methods: 160 patients diagnosed with CTD and 50 healthy control patients were included in the present study. Disease activity scores were recorded. All patients were asked to fill the Short Form-36, Fatigue Severity Scale, Hospital Depression and Anxiety Scale (HADS).

Results: In all patients groups, anxiety, depression, and fatigue scores were significantly higher, and quality of life scores significantly lower than those of healthy controls. A significant positive correlation was found between rheumatoid arthritis activity, HADS anxiety and depression scores. It was also established that in scleroderma patients with diffuse skin involvement and pulmonary involvement, depression and anxiety scores were high.

Conclusion: It is clear that psychiatric comorbidity and fatigue may be present in CTD and adversely affects quality of life. It is important to assess them and they should be an important treatment target.

Keywords: Anxiety, connective tissue diseases, depression, fatigue, quality of life

1. INTRODUCTION

In patients with connective tissue diseases (CTD), comorbid depression and anxiety prevalence are 2 to 4-fold higher than the general population (1, 2). Although its exact etiology is unknown, it has been demonstrated that physical symptoms, physical limitations, pro-inflammatory cytokines, and other factors related to having the chronic disease are associated with depression (1). It is thought that fatigue may be more marked in these patient groups, and the inflammatory process, which becomes more intensive inactive disease periods, may further increase fatigue (3). Important points that are stressed in the pathophysiology of fatigue are autonomous dysfunction, sympathetic overactivation, decrease in brain volume, CRH release defect, cytokines and immune reactivation, and defects in central neurotransmitter release. Fatigue, anxiety, and depression may lead to a reduction in physical activity and compliance with treatment, which may result in aggravation of the disease and worse health outcomes. Adequate CTD treatment influencing well-being and quality of life in patients may decrease the risk of such comorbid conditions (4).

Although fatigue, anxiety, and depression have been studied separately in each CTD patient, few results examine this in all connective tissue diseases. Therefore, this study aimed to evaluate fatigue, anxiety, depression, and burden of disease (disease activity, function, quality of life) in CTD patients.

2. MATERIALS AND METHODS

2.1. Study Population and Design

The study included 160 consecutive patients with CTD, who were admitted to Rheumatology outpatient clinic, and 50 healthy volunteers admitted to routine health follow-up outpatient clinic between October 2020 and January 2021. The study group included 30 patients with systemic sclerosis

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(SSc), 30 patients with Sjögren's syndrome (SS), 30 patients with Systemic Lupus Erythematosus (SLE), 70 patients with rheumatoid arthritis. Those with a chronic neurological disease, history of head trauma, or the central nervous system, metabolic or endocrinological disease, known psychiatric disorder, alcohol and/or substance abuse, and those who are on drugs that can lead to depression were excluded from the study. All procedures complied with the 1964 Declaration of Helsinki ethical standards. The study was approved by the Ethics Committee with the decision dated 30.09.2020 and numbered 220/419.

2.2 Assessment of Laboratory Markers and Clinical Parameters in Patients with CTD

In all patients, demographic (age, sex, marital status, education level, work status) and clinical data (the type of disease, duration of disease, organ involvement, disease activity, drugs used, immunological evaluation, physical examination, and laboratory tests) were recorded. Erythrocyte sedimentation rate (ESH) was measured with the Westergren method (mm/h) and serum C-reactive protein (CRP) levels with nephelometer(mg/dl).

2.3. Definition and Assessment of Clinical Scores in Patients with CTD

In order to evaluate disease activity in each patient group, for SLE patients SLEDAI (Systemic Lupus Erythematosus Disease Activity İndex), for RA patients, DAS28 (Disease Activity Score) and for SS, EULAR SS Disease Activity Index (ESSDAI) were used. For SSc, skin and pulmonary involvement degree was determined. DAS28 was interpreted as follows: <2.6: remission, >2.6 and <=3.2: low disease activity, >3.2 and <=5.1: moderate disease activity, >5.1: High disease activity. SLEDAI score range was interpreted as follows: 0-1: no activity; 1-5, mild activity; 6-10, moderate activity; 11–19, high activity and ≥20 very high activity. Low moderate, and high activity was defined as respectively ESSDAI scores of <5, 5-13, and ≥ 14.

2.4. Assessment of Quality of Life (QoL) and Functional Status in Patients with CTD

To measure both physical and mental components of HRQoL, we utilized the Short Form-36 (SF-36), a robust and accurate questionnaire. The SF-36 comprises 36 elements that are organized into eight dimensions (5). These subscales are physical functioning (PP) role limitations due to physical problems (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE) and mental health (MH). Scores vary between 0-100 points, with 100 indicating the best quality of life and 0 the worst.

2.5. Assessment of Anxiety and Depression in Patients with CTD

The Hospital Anxiety and Depression Scale (HADS) is a self-reported questionnaire to assess and measure the risk of depression and/or anxiety. HADS is a reliable and validated psychological scale that consists of 14 questions; half of them evaluate anxiety and the others evaluate depression with four probable responses (score 0–3). A score over 8 points is accepted as the presence of anxiety and depression. In accordance with the HADS Turkish validation study, if scores ≥11 acknowledged as anxiety, and ≥8 accepted as depression in the present study (6).

2.6. Assessment of Fatigue in Patients with CTD

The Fatigue Security Scale (FSS), valid in Turkish, is a self-reported questionnaire consisting of 9 items, each ranging from 1 to 7 points in a Likert scale, was used to evaluate the severity of fatigue in various situations (motivation, exercise, physical functioning, carrying out duties, interference with work, family, or social life) during the past week (3). FSS final score 4 was interpreted as fatigue (7).

2.7. Statistical Analysis

Statistical analyses were conducted with R (version 3.6.0., The R Project for Statistical Computing, https://www.r-project.org). Shapiro-Wilk's test and Q-Q plots were used to assess the normality, and Levene's test was used to check the homogeneity of variance. Quantitative variables were presented as mean ± standard deviation or median (interquartile range), and qualitative variables were described as counts (n) and percentages (%). One-Way ANOVA (one-way analysis of variances) followed by Tukey HSD (honestly significant difference) post-hoc test, Kruskal Wallis test followed by Dunn post-hoc test using Bonferroni correction, and Chi-square test was used to compare the study groups according to the demographic characteristics, laboratory findings, disease activity scores, FSS, anxiety and depression scores, and SF36 scores. Moreover, Spearman's rho correlation test was used to define the relationship between RA, SLE, and SS diseases' activity scores and FSS score, HADS score and SF36, and also, student's t-test, Welch's t-test, and Mann-Whitney U test were run to analyze the differences between SSc and these scores. Also, univariate binary logistic regression analysis was carried out to identify the risk factors of anxiety and depression for each disease group. Fisher's exact test and Yates continuity correction were used to calculating the p-value of SSc in anxiety and depression, respectively, and the odds ratio was calculated with 2×2 crosstabs. The significance level was set at 5%.

3. RESULTS

210 participants were included in the present study. Of these, 169 were female and 41 males. Demographic characteristics of patients, laboratory results, disease activity scores, FSS, HAD

anxiety and depression scores, and SF36 scores are illustrated in Table 1. There was no significant difference between disease groups with respect to age, duration of disease, and smoking status, while in a control group, the rate of females and married patients was lower than disease groups. There was a significant relation between FSS, HADS-A, HADS-D, and SF36 scores. For each disease, factors that can influence the anxiety and depression levels of patients are showed in Table 3. The Depression and anxiety level of patients with each disease compared to healthy controls was shown in Table 3. In the comparison of HADS-A and HADS-D scores, anxiety and depression scores were found to be significantly higher in CTD patients than healthy controls (Figure 1). FFS score was higher in all patient groups than the control group. SF 36 subscale scores were markedly lower in all patient groups than healthy controls. RA patients had lower SF36 scores, with a pronounced decrease in RP and RE subscales.

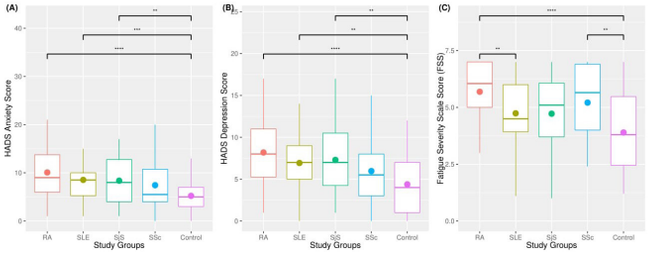

Figure 1. *The comparisons of HADS Anxiety Score, HADS Depression Score and Fatigue Severity Scale Score (FSS) in study groups.*

In RA patients, HADS-D was found to be 8 (5.25–11) and HADS-A was 9 (6 – 13.75). According to these results, it was established that in RA patients, anxiety risk was 5.646 (2.476–12.874) fold higher and depression risk 4.750 (2.121 – 10.636) fold higher than healthy subjects. There was a significant positive relation between DAS28 CRP, ESH and FSS, and HADS anxiety and depression scores, whilst a significant negative correlation was found with SF36 parameters. Besides, in RA patients, an increase in FSS score increased anxiety risk 1.516 (1.048–2.194) fold and depression risk 1.714 (1.156 – 2.542) fold, which was significant (p=.027 and p=.007, respectively). It was found that a rise in DAS28 – CRP, increased the risk of anxiety 1.653 (1.074 – 2.544) fold and risk of depression 2.963 (1.717–5.113) fold. (p=.022 and p<.001, respectively). Besides, in patients who have active disease according to DAS28 CRP, the risk of anxiety was 3.173 (1.165–8.641) fold and risk of depression 8.455 (2.821 – 25.339) fold higher than those who are in remission. (p=.024 and p<.001, respectively). It was also determined that in the SF36 scale, an increase in RP, RE, VT, MH, SF, and GH values significantly correlated with anxiety, whereas PF and BP had no impact on anxiety. However, a negative correlation was observed between all subscales of SF-36 and depression.

It was found that while in SLE patients, anxiety risk was 6.124 (2.254 – 16.639) times as high as healthy subjects, there was no difference in depression risk. (p=.074). No significant relation was found between SLEDAI scores and FSS, HADS, and SF36 parameters. It was determined that in SLE patients, a rise in FSS score significantly increased anxiety risk 2.961 (1.233 – 7.107) fold and depression risk 2.304 (1.131 – 4.697) fold (p=.015 and p=.022, respectively). SLEDAI scores were found to exert no effect on anxiety and depression levels. (p>.05). An increase in SF36 PF, RP, RE, and GH subscale values was found to decrease anxiety significantly, while VT, MH, SF, and BP had no effect on anxiety. As to depression, it was determined that an increase in SF36 RT, RE, and MH values decreased depression while other SF36 parameters exerted no significant effect on depression.

It was established that in SS patients, anxiety risk was 3.545 (1.331-9.444) times and depression risk 2.771 (1.053-7.290) times higher than healthy subjects. No significant relation was found between ESSDAI scores and FSS, HADS, and SF36 parameters. It was established that ESSDAI scores had no impact on anxiety and depression. (p>.05). Among SF36 parameters, an increase in VT, MH, SF, and GH scores was found to decrease anxiety significantly, and an increase in PF, RP, RE, SF, and GH scores reduced depression significantly. In addition, it was also established that in SS patients, the FSS score exerted no effect on anxiety while an increase in this score increased the risk of depression 2.304 (1.131 – 4.697) fold (p=.167 and p=.019, respectively).

SSc patients were found to be similar to healthy subjects in terms of anxiety and depression risk (p=.089 and p=.556, respectively). The relation between SSc and FSS, HADS, and SF36 parameters are demonstrated in Table 2. In SSc cases, patients with diffuse skin involvement (dcSSC) were found to have a significantly higher mean HADS anxiety score (9.44 ± 5.32) than those with limited skin involvement (lcSSC) (4.42 ± 2.23). (p=.002). However, it was established that patients with dcSSC and pulmonary involvement had significantly higher mean HADS scores (7.33 ± 4.58 and 7.24 ± 5.03, respectively) than those with lcSSC and without pulmonary involvement (3.92 ± 2.68 and 4.31 ± 2.06, respectively). (p=.027 and p=.041, respectively). Also, dcSSC were found to have significantly lower SF36 MH scores (56.44 ± 17.67) than those with lcSSC (72.67± 14.05) (p=.013). Besides, lcSSC were found to have a 95.8 % lower risk of anxiety than those with dcSSC (OR=0.058, 95% CI=0.006 – 0.552, p=.013). Nevertheless, the effect of skin involvement status on depression was not significant. (p=.114). Patients without pulmonary involvement were found to have a 96.7% lower risk of depression than those without pulmonary involvement. (OR=0.033, 95% CI=0.002-0.646, p=.003). An increase in FSS score in SSc patients was established to increase the risk of depression by 2.499 (1.118-5.587) fold (p=.026). Finally, an increase in SF36 parameters of VT, MH, and SF decreased the risk of anxiety, while an increase in PF, RP, MH, SF, BP, and GH decreased the risk of depression.

Table 1. *Demographics characteristics, laboratory findings, disease activity scores, FSS, HADS and SF36 scores in study groups*

	Control (*n*=50)	RA (*n*=70)	SLE (*n*=30)	SjS (*n*=30)	SSc (*n*=30)	*p*-value
Demographics						
Age (years)	46.52 ± 9.82	49.63 ± 10.28	43.37 ± 12.72	50.10 ± 8.52	47.23 ± 12.83	.057
Female gender	27 (54)	61 (87.1)	25 (83.3)	27 (90)	29 (96.7)	**<.001**
Disease duration (years)	NA	4 (2 – 8)	5.5 (4 – 10)	5 (2 – 7)	5 (2.25 – 7)	.333
Marital status (married)	15 (30)	66 (94.3)	22 (73.3)	28 (93.3)	26 (86.7)	**<.001**
Smoking	16 (32)	8 (11.4)	8 (26.7)	9 (30)	5 (16.7)	.051
Laboratory findings						
CRP	NA	8 (4 – 15.75)[a]	2.60 (1.90 – 3.30)[b]	4 (2.85 – 5)[b]	3.80 (2.05 – 6.45)[b]	**<.001**
ESH	NA	22 (12.25 – 38.75)	14 (6 – 21)	15 (8 – 27.25)	17.5 (11.5 – 29.25)	.088
Disease activity scores						
DAS28-CRP score		2.99 ± 1.23				
DAS28-CRP level						
Remission (<2.6)		32 (45.7)				
Low activity (>2.6 and ≤3.2)		7 (10)				
Moderate activity (>3.2 and ≤5.1)		28 (40)				
High activity (>5.1)		3 (4.3)				
DAS28-ESR score		3.79 ± 1.39				
DAS28-ESR level						
Remission (<2.6)		17 (24.3)				
Low activity (>2.6 and ≤3.2)		11 (15.7)				
Moderate activity (>3.2 and ≤5.1)		29 (41.4)				
High activity (>5.1)		13 (18.6)				
SLEDAI score			14.90 ± 8.86			
SLEDAI level						
No activity (0)			0 (0)			
Mild activity (1–5)			3 (10)			
Moderate activity (6–10)			6 (20)			
High activity (11–19)			14 (46.7)			
Very high activity (≥20)			7 (23.3)			
ESSDAI score				5.73 ± 4.01		
ESSDAI level						
Low activity (<5)				16 (53.3)		
Moderate activity (5–13)				11 (36.7)		
High activity (≥14)				3 (10)		
Kutanöz involvement						
dcSSc					18 (60)	
lcSSc					12 (40)	
Pulmoner involvement						
Absent					13 (43.3)	
Present					17 (56.7)	
FSS	3.80 (2.45 – 5.47)[a]	6.05 (5 – 7)[b]	4.50 (3.92 – 6)[ac]	5.10 (3.70 – 6.07)	5.65 (4 – 6.90)[bc]	**<.001**
Anxiety and depression scores						
HADS anxiety score	5 (3 – 7)[a]	9 (6 – 13.75)[b]	8.5 (5.25 – 10)[b]	8 (4 – 12.75)[b]	5.5 (4 – 10.75)	**<.001**
HADS anxiety level						**<.001**
Absent (<8)	39 (78)	27 (38.6)	11 (36.7)	15 (50)	18 (60)	
Possible (8–10)	8 (16)	16 (22.9)	13 (43.3)	2 (6.7)	4 (13.3)	
Probable (11–14)	3 (6)	13 (18.6)	2 (6.7)	10 (33.3)	4 (13.3)	
Extreme (15–21)	0 (0)	14 (20)	4 (13.3)	3 (10)	4 (13.3)	
HADS depression score	4 (1 – 7)[a]	8 (5.25 – 11)[b]	7 (5 – 9)[b]	7 (4.25 – 10.5)[b]	5.5 (3 – 8)	**<.001**
HADS depression level						**.013**
Absent (<8)	38 (76)	28 (40)	17 (56.7)	16 (53.3)	21 (70)	
Possible (8–10)	10 (20)	23 (32.9)	9 (30)	6 (20)	6 (20)	
Probable (11–14)	2 (4)	14 (20)	4 (13.3)	6 (20)	1 (3.3)	
Extreme (15–21)	0 (0)	5 (7.1)	0 (0)	2 (6.7)	2 (6.7)	
Short form (36) heath survey (SF36)						
SF36-PF	90 (80 – 100)[ac]	50 (21.25 – 75)[b]	80 (56.25 – 98.75)[c]	57.5 (42.5 – 98.75)[bc]	60 (45 – 85)[bc]	**<.001**
SF36-RP	100 (100 – 100)[a]	0 (0 – 100)[b]	100 (6.25 – 100)	75 (0 – 100)[b]	12.5 (0 – 75)[b]	**<.001**
SF36-RE	100 (100 – 100)[a]	0 (0 – 91.67)[b]	100 (0 – 100)	100 (0 – 100)	66.67 (0 – 100)	**<.001**
SF36-VT	59.10 ± 21.01[ac]	36.07 ± 18.37[b]	49.67 ± 20.21[c]	40.50 ± 17.14[bc]	45.33 ± 23.15[bc]	**<.001**
SF36-MH	70.48 ± 16.10[a]	55.20 ± 18.29[b]	63.47 ± 13.44	58.93 ± 17.89[b]	62.93 ± 17.98	**<.001**
SF36-SF	75 (50 – 100)[a]	50 (37.5 – 62.5)[b]	75 (50 – 87.5)[a]	62.5 (50 – 84.38)	62.5 (37.5 – 87.5)	**<.001**
SF36-BP	90 (77.5 – 100)[ac]	45 (22.5 – 67.5)[b]	77.5 (55 – 90)[c]	67.5 (37.5 – 77.5)[bc]	61.25 (45 – 77.5)[bc]	**<.001**
SF36-GH	71.06 ± 16.73[a]	36.79 ± 15.74[b]	47 ± 21.03[b]	45.30 ± 16.86[b]	46.50 ± 20.68[b]	**<.001**

Data were expressed as mean ± standard deviation or median (interquartile range).Bold values indicate that statistically significance (p<.05). [a,b,c] Each different superscript letters in each row indicate that statistically significant difference between groups.p-values calculated using One-Way ANOVA with Tukey HSD, Kruskal Wallis test with Dunn-Bonferroni post-hoc test, or Chi-square test, as appropriate..Abbreviations: RA: Rheumatoid Arthritis, SjS: Sjogren's Syndrome, SLE: Systemic Lupus Erythematosus, SSc: Systemic Sclerosis, FSS: Fatigue severity scale, HADS anxiety and HADS depression: Hospital anxiety and depression scale, SF36: Short form health survey, PF: Physical function, RP: Role limitations due to physical health, RE: Role limitations due to emotional problems, VT: Energy/fatigue, MH: Mental health, SF: Social function, BP: Bodily pain, GH: General health

Table 2. *Relationship between RA, SLE and SjS diseases' activity scores and FSS score, HADS score and SF36, and also differences between SSc and these scores.*

	RA (*n*=70)		SLE (*n*=30)	SjS (*n*=30)	SSc (*n*=30)			
		DAS28	SLEDAI	ESSDAI		Diffuse/Present	Limited/Absent	*p*-value
FSS			0.028 (.885)	0.289 (.122)				
	CRP	0.629 (<**.001**)			Kutanöz	5.29 ± 1.65	5.09 ± 1.47	.741[a]
	ESR	0.489 (<**.001**)			Pulmoner	5.42 ± 1.59	4.93 ± 1.52	.400[a]
HADS								
HADS anxiety			–0.154 (.418)	–0.008 (.967)				
	CRP	0.351 (**.003**)			Kutanöz	9.44 ± 5.32	4.42 ± 2.23	**.002**[b]
	ESR	0.255 (**.033**)			Pulmoner	8.76 ± 5.78	5.69 ± 3.07	.073[b]
HADS depression			0.042 (.826)	0.153 (.421)				
	CRP	0.547 (<**.001**)			Kutanöz	7.33 ± 4.58	3.92 ± 2.68	**.027**[a]
	ESR	0.513 (<**.001**)			Pulmoner	7.24 ± 5.03	4.31 ± 2.06	**.041**[b]
SF36								
SF36-PF			0.206 (.274)	0.045 (.813)				
	CRP	–0.576 (<**.001**)			Kutanöz	57.22 ± 26.58	66.67 ± 26.57	.348[a]
	ESR	–0.551 (<**.001**)			Pulmoner	57.35 ± 26.64	65.77 ± 26.68	.399[a]
SF36-RP			0.322 (.082)	0.011 (.955)				
	CRP	–0.556 (<**.001**)			Kutanöz	12.50 (0 – 75)	25 (0 – 75)	.964[c]
	ESR	–0.508 (<**.001**)			Pulmoner	0 (0 – 75)	50 (0 – 75)	.892[c]
SF36-RE			0.169 (.372)	–0.135 (.478)				
	CRP	–0.477 (<**.001**)			Kutanöz	83.34 (0 – 100)	50 (0 – 100)	.597[c]
	ESR	–0.403 (<**.001**)			Pulmoner	66.67 (0 – 100)	100 (0 – 100)	.555[c]
SF36-VT			–0.002 (.992)	–0.135 (.478)				
	CRP	–0.514 (<**.001**)			Kutanöz	40.28 ± 21.45	52.92 ± 24.44	.146[a]
	ESR	–0.482 (<**.001**)			Pulmoner	43.53 ± 24.61	47.69 ± 21.85	.634[a]
SF36-MH			–0.045 (.812)	–0.074 (.698)				
	CRP	–0.484 (<**.001**)			Kutanöz	56.44 ± 17.67	72.67 ± 14.05	**.013**[a]
	ESR	–0.369 (**.002**)			Pulmoner	59.29 ± 20.51	67.69 ± 13.31	.186[b]
SF36-SF			0.286 (.125)	–0.039 (.837)				
	CRP	–0.380 (<**.001**)			Kutanöz	59.03 ± 27.05	69.79 ± 27.93	.301[a]
	ESR	–0.330 (**.005**)			Pulmoner	61.03 ± 20.51	66.35 ± 25.71	.608[a]
SF36-BP			0.251 (.182)	0.195 (.301)				
	CRP	–0.533 (<**.001**)			Kutanöz	56.53 ± 21.46	69.17 ± 24.57	.147[a]
	ESR	–0.508 (<**.001**)			Pulmoner	59.26 ± 23.30	64.62 ± 23.67	.541[a]
SF36-GH			0.073 (.703)	–0.213 (.258)				
	CRP	–0.601 (<**.001**)			Kutanöz	32.50 (30 – 61.25)	60 (42.50 – 66.25)	.060[c]
	ESR	–0.536 (<**.001**)			Pulmoner	30 (30 – 50)	65 (35 – 65)	.073[c]

Data were expressed as mean ± standard deviation or median (interquartile range) for SSc, and presented as Spearman's rho (p-value) for RA, SjS and SLE. Bold values indicated that statistically significance (p<.05)

[a] *student's t test*

[b] *Welch's t test*

[c] *Mann-Whitney U test*

Abbreviations: RA: Rheumatoid Arthritis, SjS: Sjogren's Syndrome, SLE: Systemic Lupus Erythematosus, SSc: Systemic Sclerosis, FSS: Fatigue severity scale, HADS anxiety and HADS depression: Hospital anxiety and depression scale, SF36: Short form health survey, PF: Physical function, RP: Role limitations due to physical health, RE: Role limitations due to emotional problems, VT: Energy/fatigue, MH: Mental health, SF: Social function, BP: Bodily pain, GH: General health, DAS28-CRP: Disease activity score that uses c-reactive protein, DAS28-ERP: Disease activity score that uses erythrocyte sedimentation rate, ESSDAI: European league against rheumatism SS disease activity index, SLEDAI: Systemic lupus erythematosus disease activity index, dcSSc: Diffuse cutaneous scleroderma, lcSSc: Limited cutaneous scleroderma

Table 3. *Risk factors for the anxiety and depression in each disease group.*

	HADS Anxiety (≥8 vs <8)		HADS Depression (≥8 vs <8)	
	OR (%95 CI)	*p*-value	OR (%95 CI)	*p*-value
Rheumatoid Arthritis (vs control)	5.646 (2.476 – 12.874)	**<.001**	4.750 (2.121 – 10.636)	**<.001**
Age (years)	0.973 (0.926 – 1.022)	.272	0.953 (0.906 – 1.004)	.070
Disease duration (years)	0.990 (0.914 – 1.073)	.807	0.981 (0.905 – 1.062)	.635
CRP	1.029 (0.976 – 1.085)	.287	1.102 (1.023 – 1.187)	**.011**
ESH	1.006 (0.980 – 1.032)	.670	1.016 (0.989 – 1.044)	.243
FSS	1.516 (1.048 – 2.194)	**.027**	1.714 (1.156 – 2.542)	**.007**
SF36-PF	0.987 (0.972 – 1.002)	.088	0.983 (0.968 – 0.998)	**.031**
SF36-RP	0.984 (0.972 – 0.995)	**.005**	0.986 (0.975 – 0.997)	**.012**
SF36-RE	0.981 (0.970 – 0.993)	**.002**	0.975 (0.963 – 0.988)	**<.001**
SF36-VT	0.950 (0.920 – 0.982)	**.002**	0.932 (0.898 – 0.968)	**<.001**
SF36-MH	0.941 (0.908 – 0.976)	**<.001**	0.900 (0.854 – 0.948)	**<.001**
SF36-SF	0.954 (0.928 – 0.981)	**<.001**	0.952 (0.925 – 0.979)	**<.001**
SF36-BP	0.986 (0.968 – 1.004)	.129	0.976 (0.957 – 0.995)	**.015**
SF36-GH	0.955 (0.922 – 0.989)	**.010**	0.909 (0.867 – 0.952)	**<.001**
DAS28 CRP score	1.653 (1.074 – 2.544)	**.022**	2.963 (1.717 – 5.113)	**<.001**
DAS28 ESH score	1.362 (0.944 – 1.965)	.098	2.369 (1.487 – 3.772)	**<.001**
DAS28 CRP activity (vs remission)	3.173 (1.165 – 8.641)	**.024**	8.455 (2.821 – 25.339)	**<.001**
DAS28 ESH activity (vs remission)	3.025 (0.982 – 9.318)	.054	5.550 (1.677 – 18.368)	**.005**
Systemic Lupus Erythematosus (vs control)	6.124 (2.254 – 16.639)	**<.001**	2.422 (0.917 – 6.394)	.074
Age (years)	0.998 (0.941 – 1.059)	.952	0.981 (0.926 – 1.040)	.523
Disease duration (years)	0.874 (0.751 – 1.016)	.080	0.899 (0.772 – 1.047)	.171
CRP	1.059 (0.790 – 1.419)	.702	0.848 (0.594 – 1.210)	.364
ESH	1.006 (0.971 – 1.042)	.751	0.997 (0.965 – 1.031)	.868
FSS	2.961 (1.233 – 7.107)	**.015**	2.304 (1.131 – 4.697)	**.022**
SF36-PF	0.953 (0.913 – 0.995)	**.028**	0.956 (0.920 – 0.993)	**.019**
SF36-RP	0.976 (0.953 – 0.999)	**.040**	0.987 (0.970 – 1.004)	.139
SF36-RE	0.978 (0.958 – 0.998)	**.034**	0.983 (0.967 – 0.999)	**.046**
SF36-VT	0.958 (0.915 – 1.003)	.064	0.940 (0.892 – 0.990)	**.019**
SF36-MH	0.955 (0.897 – 1.015)	.140	0.955 (0.897 – 1.017)	.154
SF36-SF	0.967 (0.933 – 1.003)	.076	0.969 (0.937 – 1.003)	.076
SF36-BP	0.968 (0.928 – 1.011)	.141	0.980 (0.947 – 1.014)	.245
SF36-GH	0.929 (0.875 – 0.987)	**.016**	0.969 (0.932 – 1.008)	.120
SLEDAI score	0.971 (0.891 – 1.057)	.490	1.040 (0.954 – 1.133)	.379
SLEDAI High/Very high (vs mild/moderate)	0.138 (0.015 – 1.302)	.084	1.818 (0.357 – 9.272)	.472
Sjogren's Syndrome (vs control)	3.545 (1.331 – 9.444)	**.011**	2.771 (1.053 – 7.290)	**.039**
Age (years)	0.986 (0.905 – 1.074)	.744	1.005 (0.922 – 1.095)	.910
Disease duration (years)	1.219 (0.958 – 1.550)	.107	1.113 (0.931 – 1.331)	.240
CRP	1.127 (0.901 – 1.410)	.296	1.189 (0.930 – 1.518)	.167
ESH	0.984 (0.942 – 1.028)	.477	0.993 (0.951 – 1.037)	.761
FSS	1.373 (0.876 – 2.151)	.167	2.219 (1.139 – 4.322)	**.019**
SF36-PF	0.980 (0.955 – 1.007)	.139	0.959 (0.928 – 0.990)	**.011**
SF36-RP	0.990 (0.974 – 1.005)	.186	0.982 (0.965 – 0.998)	**.028**
SF36-RE	0.990 (0.975 – 1.005)	.177	0.981 (0.965 – 0.997)	**.018**
SF36-VT	0.937 (0.882 – 0.996)	**.035**	0.959 (0.911 – 1.010)	.113
SF36-MH	0.782 (0.636 – 0.960)	**.019**	0.951 (0.901 – 1.003)	.063
SF36-SF	0.960 (0.927 – 0.995)	**.024**	0.927 (0.880 – 0.977)	**.005**
SF36-BP	0.984 (0.955 – 1.014)	.297	0.971 (0.941 – 1.003)	.078
SF36-GH	0.921 (0.862 – 0.984)	**.015**	0.903 (0.836 – 0.975)	**.009**
ESSDAI score	1.009 (0.841 – 1.210)	.926	1.168 (0.951 – 1.434)	.138
ESSDAI moderate/high (vs low)	0.583 (0.137 – 2.481)	.466	3.960 (0.865 – 18.119)	.076
Systemic Sclerosis (vs control)	2.364 (0.878 – 6.365)	.089	1.357 (0.492 – 3.746)	.556
Age (years)	1.042 (0.978 – 1.109)	.201	1.027 (0.963 – 1.096)	.418
Disease duration (years)	1.089 (0.929 – 1.276)	.293	1.119 (0.943 – 1.328)	.196
CRP	1.051 (0.858 – 1.288)	.629	1.051 (0.851 – 1.299)	.644
ESH	1.015 (0.967 – 1.066)	.537	0.976 (0.921 – 1.035)	.419
FSS	1.371 (0.824 – 2.283)	.224	2.499 (1.118 – 5.587)	**.026**
SF36-PF	0.980 (0.951 – 1.009)	.176	0.942 (0.900 – 0.987)	**.012**
SF36-RP	0.989 (0.970 – 1.007)	.228	0.971 (0.944 – 0.998)	**.035**
SF36-RE	1.004 (0.988 – 1.020)	.664	0.986 (0.969 – 1.004)	.133
SF36-VT	0.962 (0.925 – 0.999)	**.048**	0.945 (0.901 – 0.992)	**.021**
SF36-MH	0.938 (0.888 – 0.991)	**.023**	0.927 (0.871 – 0.988)	**.019**
SF36-SF	0.960 (0.928 – 0.993)	**.019**	0.953 (0.917 – 0.991)	**.016**
SF36-BP	0.973 (0.939 – 1.008)	.131	0.953 (0.911 – 0.997)	**.035**
SF36-GH	0.960 (0.921 – 1.002)	.059	0.941 (0.891 – 0.994)	**.030**
lcSSc (vs dcSSc)	0.058 (0.006 – 0.552)	**.013**	0.114 (0.012 – 1.076)	.114
Pulmoner involvement absent (vs present)	0.267 (0.054 – 1.326)	.201≠	0.033 (0.002 – 0.646)	**.003**≠≠

≠ calculated using Yates continuity correction. ≠≠ calculated using Fisher's exact test. Bold values indicated that statistically significance (p<.05).Abbreviations: FSS: Fatigue severity scale, HADS anxiety and HADS depression: Hospital anxiety and depression scale, DAS28-CRP: Disease activity score that uses c-reactive protein, DAS28-ERP: Disease activity score that uses erythrocyte sedimentation rate, ESSDAI: European league against rheumatism SS disease activity index, SLEDAI: Systemic lupus erythematosus disease activity index, dcSSc: Diffuse cutaneous scleroderma, lcSSc: Limited cutaneous scleroderma

4. DISCUSSION

This study evaluated fatigue, anxiety and/or depression in all CTD with details. Fatigue anxiety and/or depression were more common in patients with CTD. Patients who were at higher risk for anxiety and / or depression had more fatigue severity and lower QoL.

In all CTD such as RA, SS, SLE, and SSc, high rates of depression have been established (1). The etiopathology of depression in RA is quite complex. It has been suggested that an uncertain and variable prognosis of the disease leads to dysfunction, intensive pain, and social isolation caused by the disease, which may set the stage for both anxiety and depression. A decrease in drug compliance attributed to impaired quality of life and social withdrawal has also been reported. In a study by Van Korff et al., a strong relationship was found between RA disease activity and depression. Although it has been reported in some studies that there is an association between the severity of arthritis and depression, a direct relation between disease activation markers and severity of depression could not be demonstrated (8-10). In the present study, a statistically significant relation was found between disease activity and depression and anxiety. Even though anxiety and depression are usually coexisting disorders, anxiety has not drawn as much attention as depression in RA. It has been stated by El-Miedany et al. (11) that anxiety is as common as depression in RA patients. Investigators have established that the rate of depression is 66.2% and that of anxiety is 70%. However, in these studies, depression and anxiety levels in patients were not compared with those in non-patients (12).

Depression was found to be associated with high levels of fatigue. Although the etiology of fatigue is not known in RA, it is thought that it is multifactorial, including pain, disability, inflammation, sleep disorders, and psychosocial factors. Especially, increased production of IL-6 was stated to be associated with fatigue, which is one of the main components contributing to RA-related disability and morbidity. Although there are variable results in the studies on the relation of fatigue with disease activity, it has mainly been shown to be related to disease activity and in some studies, it has been proposed that fatigue be considered a sign of disease activity. In patients with high levels of inflammatory markers, lower HRQoL, higher levels of pain and fatigue are observed. The study of Gök K et al. revealed that levels of fatigue which were assessed by VAS, SF-36-vitality, FSS, and MAF, were quite similar between patients with RA and SSc. Levels of fatigue, measured by different scales, were significantly correlated with physical functions and HRQoL measures and psychometric variables in both groups (13). In the present study, consistent with the literature, fatigue scores have been high and associated with disease activity and low HRQoL. In SLE, depression is a multi-factor condition caused by the interaction between behavioral and biological pathways. Previous studies have reported that anxiety disorders occur twice as commonly in SLE patients as controls. In individual studies, depression prevalence has been estimated to vary between 2% and 91.7% (14, 15). It has been stated that the severity of depression has a positive correlation with increased disease activity, which may reflect significant CNS damage in SLE (16). In another study, the prevalence of depression was found to be 16 %, without any relation with disease activity and organ damage, and it was established that merely 7% of patients were on antidepressant treatment (17). In the present study, no relation was found between disease activation and depression and anxiety, which may be explained by the lack of patients with CNS clinical involvement and few cases. Depression is associated with an increase in pro-inflammatory cytokines, and increased cardiovascular risk may be associated with the accumulated inflammatory burden. Therefore, improvement of depression management in SLE patients may directly or indirectly affect psychological health and physical health, including cardiovascular disease risk (16, 17).

Fatigue is a symptom that occurs in 80% of SLE patients and markedly reduces the quality of life (18, 19). Consistent with the literature, in the present study, fatigue was associated with depression and anxiety. The relation between fatigue and organ damage and disease activity varies between studies. Wysenbeek et al. observed arthritis more commonly in SLE patients with fatigue than those without fatigue. Similarly, Krupp et al. found a strong association between fatigue and clinicians' opinion regarding disease activity. However, no relation could be demonstrated between fatigue and disease activity in three studies, similar to the present study (18-21).

Fatigue, pain, and depression are factors that exert adverse effects on HRQoL. In the study of Mok et al., in comparing SLE patients in long-term remission with those in short-term remission, better HRQoL and MH were observed in the former in all SF36 components except PF. Besides, in the study of Tench et al., it was observed that although fatigued has a correlation with sleep quality, anxiety, depression, and all components of SF-36, even in early and mild periods of the disease, fatigue exerts a markedly adverse effect on morbidity (22, 23). Compatible with the literature, it was established that patients had higher levels of fatigue, depression, and anxiety and generally lower levels of HRQoL compared to healthy controls.

SS may also have some extra glandular features such as pain, fatigue, anxiety, and depression, which have the potential of impairing HRQoL and exert a negative impact on psychological, physical, and social functioning. The prevalence of depression was established to be high, and estimations range from 8.33% to 75.5 %. The exact etiopathogenesis of depression in SS remains uncertain (24). In SS, a relation has been observed between pain, depression, fatigue, and pro-inflammatory cytokines. It has been reported that the prevalence of fatigue is around 70 % among SS patients. In SS patients, psychological distress and constant fatigue were correlated with low HRQoL, loss of work productivity, increasing levels of disability, and medical costs. Therefore, depression and anxiety may be suitable targets for interventions to improve subjective health and

quality of life (25-28). In the present study, consistent with the literature, high rates of fatigue, anxiety, and depression and low levels of HRQoL were observed, but they were not found to be associated with disease activity. Despite the effect of fatigue enhancing depression, it was found to be effective in anxiety.

SSc patients report high levels of pain, fatigue, and physical dysfunction. In addition to physical impairment and symptoms, SSc patients have been stated to have higher rates of depression and anxiety (18%-65%) compared to the general population and other patient groups, and a significant correlation has been reported between depression and anxiety. In the present study, it was observed that the risk of depression and anxiety did not increase in SSc patients compared to controls, which is discrepant with the findings of previous studies. This difference may be explained by our small patient group and the divergence between measurement parameters used. In some studies, depressive symptoms, fatigue, and other physical symptoms were related to SSc severity (28, 29). Fatigue occurred more commonly in patients with pulmonary symptoms. In the present study, fatigue was observed to increase the risk of depression. In the study of Legendre et al., it was stated that in patients with SSc, depressive symptoms were associated with pulmonary involvement but not with skin involvement, organ involvement, and disability. In the study of Beretta et al. on SSc patients, depressive symptoms were found to be associated with diffuse skin involvement, pulmonary involvement, generalized pain, Reynaud phenomenon, severe disease, and digital ulcer. In many studies in patients diagnosed with SSc, diffuse skin involvement and visceral organ involvement were found to be associated with depressive symptoms.

Similarly, Danieli et al. established that in patients with diffuse skin involvement, quality of life was impaired at a higher degree. The study of Muller et al. stressed that quality of life and female sex are independent risk factors for depressive symptoms. Functional disability and anxiety were found to be associated with low HRQoL (30, 31). In keeping with previous studies, depression and anxiety were associated with functional status and quality of life in the present study. The present study may be considered significant in that it is a comprehensive study on depression, anxiety, fatigue, and HRQol in CTD patients. However, as it is a cross-sectional study, it is not possible to reach any definitive conclusion on the relation between disease activity and psychiatric impairment. Prospective studies with larger patient series may shed light on factors that effect the development of depression and anxiety in CTD patients.

Some limitations in our study should be addressed. The present study was performed at a single tertiary care center. Moreover, the research included a relatively small number of patients. The strengths of this study include our assessment of fatigue and psychological morbidity with a valid and trustworthy tool such as FSS and HADS, as well as extensively investigated laboratory and clinical markers.

5. CONCLUSION

It is obvious that irrespective of the causality relation, psychiatric morbidities and fatigue may be present in CTD patients. Early diagnosis and judicious intervention are essential in order that the adverse effect of anxiety and depression on quality of life and disease outcomes can be reduced to the minimum.

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Clinical and Experimental Health Sciences

Increased Systemic Inflammatory Response with Mast Cell Activation in Elder Children With Cerebral Palsy

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ABSTRACT

Objective: Increased systemic inflammatory response during intrauterine period or period before the age of 3 is associated with cerebral palsy (CP) pathogenesis; however, effects of inflammatory processes involving mast cell activation in elder children with CP remain unclear. We aimed to investigate the role of mast cells and proinflammatory cytokines in children with CP at 3-18 years of age.

Methods: In this cross-sectional study, venous blood samples were obtained from 30 volunteers with CP and 26 healthy volunteers at 3-18 years of age. Plasma levels of proinflammatory cytokines (IL-1β, IL-6 and IL-9) and mast cell biomarkers (histamine and tryptase beta-2) were determined using ELISA.

Results: IL-1β, IL-6 and histamine levels were higher in individuals with CP compared to healthy controls. Likewise, IL-1β, IL-6, IL-9 and histamine levels were higher in the female patients with CP compared to the male patients, and in the female patients in adolescence compared to the female patients in pre-adolescence.

Conclusion: Our findings indicate that the increased inflammatory response contributes to the pathogenesis of the disease in children with CP who are older than 2 years of age. Moreover the increased inflammatory response is more effective in female patients than in male patients, suggesting that there may be a gender difference in CP. Additionally mast cell activation contributes to the exacerbation of systemic inflammatory response in children with CP at 3-18 years of age.

Keywords: Cerebral palsy, Children, Inflammation, Mast cells, Cytokines, Gender.

1. INTRODUCTION

Cerebral palsy (CP) is one of the main disabling disorders, characterized by permanent brain damage that affects motor and cognitive functions with different clinical symptoms in children (1). Cerebral palsy has a complex etiology that involves intrauterine pathologies, intrapartum complications, and postpartum sequelae.

Central nervous system injury is multifactorial and involves the exposure of the fetus or the newborn to acute and chronic inflammation, or perinatal hypoxia-ischemia (2). Neuronal lesions related to inflammation lead to the initiation of a series of immune responses, including an increase in circulating cytokines, particularly interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α), which may be involved in the development of brain injury (3).

In the previous studies, it was demonstrated that there was a relationship between high levels of proinflammatory cytokines such as TNF-α, IL-6 and IL-8 in the amniotic fluid, plasma or umbilical cord blood and, the occurrence of cerebral palsy and periventricular leukomlacia, the common white matter damage of the brain (4-6). In the meantime, the clinical evidence indicating higher plasma IL-1, IL-8, IL-9 and TNF-α levels in children with cerebral palsy compared to the healthy controls also supports the role of proinflammatory cytokines in the pathophysiology of CP (3,7,8). Thus, the relationship between the inflammation triggered by proinflammatory cytokines in various developmental processes, mentioned above, and the CP pathogenesis has been well established. Yet, clinical studies revealing this relationship have focused on the postnatal period of children with cerebral palsy from

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the prenatal period to 3 years of age. On the other hand, the relationship between proinflammatory cytokines and the course of the disease is unknown in children with CP after 2 years of age. Moreover, it is not clear whether inflammation in the pathogenesis of CP varies by gender and developmental periods. Therefore, clarification of the pathophysiological processes and possible gender and developmental period differences in the course of the disease in childhood after the age of 2 is of vital importance.

Mast cells are the multifunctional immune cells that are one of the most important sources of proinflammatory cytokines. The preclinical studies reporting that the deficiency of mast cells or the stabilization of mast cells by cromolyn substantially reduced brain lesions in different experimental models (9,10) indicated that the mast cells may play an important role in inflammatory processes associated with the pathogenesis of CP. Nevertheless, studies investigating the role of mast cell activation in the pathogenesis of CP are limited to experimental animal studies; and clinical studies are needed on this subject.

In the present study, we aimed to compare the concentrations of plasma proinflammatory cytokines and, the markers of mast cell count and activation in children diagnosed with CP older than 2 years of age, with the healthy individuals of the same age, thereby investigating the relationship between proinflammatory cytokine levels and mast cell activation in elder children with CP. In addition, we aimed to investigate if there is a difference in terms of these cytokines and mast cell markers, whether this difference changes according to the gender and developmental periods.

2. METHODS

2.1. Subjects

The research protocol was approved by the Bolu Abant Izzet Baysal University Clinical Research Local Ethics Committee (approval number 2018/181). This cross-sectional study included children with clinically confirmed CP, who were diagnosed with CP at the Pediatric Neurology department in one of the hospitals in Western Black Sea region between November 2018 and June 2019 and were benefiting from the rehabilitation services at the *Izle Special Education and Rehabilitation Center* located in Duzce city. The study was participated by 30 volunteering individuals with CP between 3-18 years of age (15 male, 15 female), and 26 volunteering healthy individuals of the same age group in the control group (14 male, 12 female). Inclusion criteria for the group of patients with CP were, i) being clinically diagnosed with CP, ii) being between the ages of 3-18, iii) having stable health conditions; and the exclusion criteria were, i) having any chronic (neuro)inflammatory diseases (autoimmune or microbial), ii) having any type of infection, iii) having any type of chronic mast cell disease (mastocytosis etc. Healthy controls were term born and normal born individuals with no diseases. All of the healthy controls and the patients were from the the Western Black Sea Region of Turkey. The demographic and clinical characteristics of the patients and healthy controls are presented in Table 1 and Table 2.

2.2. Study design

In accordance with the Helsinki Declaration, signed and informed consent was obtained from all volunteering healthy controls, patients and their parents. Age, gender, type of CP, height, weight, body mass index and ambulation characteristics were recorded for the patients. Age, gender, height, weight, body mass index and findings of the normal physical and neurological examinations were recorded for the healthy individuals in the control group. From each volunteering patient and individual in the control group, a blood sample of 5 ml was taken from the median cubital vein for once, while resting in a sitting position between 09:00 and 12:00 in the morning. Blood samples were immediately transferred to ice-cold glass tubes containing anticoagulant EDTA. The blood was then centrifuged at 3000 rpm, 4°C for a period of 15 minutes. Supernatants were stored at – 80°C until their immunoreactivity was determined against proinflammatory cytokines IL-1β, IL-6 and IL-9 as well as the mast cell count and activation markers histamine and tryptase beta-2, using the ELISA method.

2.3. Measurement of plasma IL-1β, IL-6, IL-9, histamine and tryptase beta-2 concentrations

IL-1β, IL-6, IL-9, histamine and tryptase beta-2 contents in the samples were measured by enzyme-linked immunosorbent assay kits (BT Lab, Shanghai, China). The detection limit was 10.07 pg/L for IL-1β, 1.03 ng/L for IL-6, 9.83 pg/mL for IL-9, 0.51 ng/mL for histamine, and 36.15 ng/L for tryptase beta-2, respectively. Assay protocols were performed in duplicate according to the instructions of the manufacturer. In brief, 50 µL of IL-1β, IL-6, IL-9, histamine and tryptase beta-2 standard was added to the standard wells. Then, 40 µL of plasma samples, and 10 µL of antibodies of these markers were added to the sample wells. Then, 50 µL streptavidin-HRP was added to each well. Then, the 96-well plates were incubated at 37 °C for 60 min. After washing the 96-well plates 5 times with wash buffer, 50 µL substrate solution A and B were added to each well, and the plates were incubated at 37 °C for 10 min. After the incubation, 50 µL stop solution was added to each well. The optical density of the wells was measured at 450 nm using a microplate reader (Epoch BioTek Instruments, Inc. Highland Park, Winooski, VT, USA). Optical density curves were obtained using the standards with defined IL-1β, IL-6, IL-9, histamine and tryptase beta-2 concentrations.

2.4. Statistical analysis

Data were given as median values, and first and third quartiles. SPSS statistical package program was used for the statistical analysis of the data obtained from the study (IBM SPSS Statistics for Windows, Version 22.0, IBM Corp, Armonk, NY, USA). The data distribution was analyzed for normality using the Shapiro-Wilk-W test. For the comparison of the two independent groups, independent sample t-test

Table 1. *Demographic and clinical characteristics of subjects with cerebral palsy for each patient.*

Patient	Gender	Classification	Age	Height (cm)	Weight (kg)	BMI (kg/m2)	Ambulation
1	Male	Spastic – Hemiplegic	10	135	40	21,9	Yes
2	Female	Spastic – Kuadriplegic	4	90	12	14,8	No
3	Male	Spastic – Diplegic	15	160	52	20,3	Yes
4	Female	Spastic – Hemiplegic	4	110	20	16,5	Yes
5	Male	Spastic – Hemiplegic	9	125	30	19,2	Yes
6	Female	Spastic – Hemiplegic	11	148	36	16,4	Yes
7	Male	Spastic – Diplegic	9	150	45	20	Yes
8	Female	Spastic – Hemiplegic	4	112	18	14,3	Yes with orthosis
9	Male	Spastic – Hemiplegic	4	108	19	16,2	Yes
10	Male	Spastic – Diplegic	16	165	70	25,7	Yes
11	Female	Ataxic	14	155	55	22,8	Yes
12	Male	Ataxic	11	156	42	17,2	Yes
13	Female	Spastic – Kuadriplegic	8	117	18	13,1	No
14	Female	Spastic – Diplegic	5	102	15	14,4	Yes with orthosis and walker
15	Male	Spastic – Hemiplegic	10	140	28	14,2	Yes
16	Female	Spastic – Diplegic	11	152	53	22,9	Yes with orthosis and walker
17	Male	Spastic – Hemiplegic	3	90	18	22,2	Yes
18	Female	Spastic – Kuadriplegic	17	153	65	27,7	No
19	Female	Spastic – Diplegic	5	98	14	14,5	Yes with orthosis and walker
20	Male	Spastic – Diplegic	15	158	60	24	Yes with orthosis and walker
21	Male	Spastic – Hemiplegic	8	132	23	13,2	Yes
22	Male	Spastic – Diplegic	10	133	30	16,9	No
23	Female	Spastic – Hemiplegic	8	112	19	15,1	Yes
24	Male	Spastic – Diplegic	6	98	16	16,6	Yes with orthosis and walker
25	Female	Spastic – Diplegic	13	165	60	22	Yes with orthosis and walker
26	Female	Spastic – Hemiplegic	11	135	43	23,5	Yes
27	Female	Spastic – Kuadriplegic	15	145	56	26,6	No
28	Male	Spastic – Hemiplegic	8	122	30	20,1	Yes
29	Female	Spastic – Diplegic	14	150	65	28,8	Yes with orthosis and walker
30	Male	Spastic – Diplegic	16	168	49	17,3	Yes with orthosis and walker

Table 2. *Demographic and clinical characteristics of healthy control subjects.*

Subject	Gender	Age	Height (cm)	Weight (kg)	BMI (kg/m2)	Physical examination	Neurological examination
1	Female	16	155	58	24,1	Normal	Normal
2	Male	15	175	88	28,7	Normal	Normal
3	Male	14	170	75	25,9	Normal	Normal
4	Male	10	133	43	24,3	Normal	Normal
5	Female	8	130	32	18,9	Normal	Normal
6	Male	14	158	57	22,8	Normal	Normal
7	Female	11	140	45	22,9	Normal	Normal
8	Male	11	149	44	19,8	Normal	Normal
9	Female	9	125	28	17,9	Normal	Normal
10	Female	12	155	48	19,9	Normal	Normal
11	Female	5	102	16	15,3	Normal	Normal
12	Female	9	139	33	17	Normal	Normal
13	Female	7	118	20	14,3	Normal	Normal
14	Female	7	125	23	14,7	Normal	Normal
15	Female	6	120	20	13,8	Normal	Normal
16	Male	9	140	35	17,8	Normal	Normal
17	Male	17	169	70	24,5	Normal	Normal
18	Male	15	165	55	20,2	Normal	Normal
19	Male	12	160	49	19,1	Normal	Normal
20	Male	14	167	55	19,7	Normal	Normal
21	Female	9	135	35	19,2	Normal	Normal
22	Male	14	152	60	25,9	Normal	Normal
23	Male	13	173	57	19	Normal	Normal
24	Male	3	102	21	20,1	Normal	Normal
25	Male	9	125	27	17,2	Normal	Normal
26	Female	11	140	35	17,8	Normal	Normal

or Mann-Whitney U test was used. The level of $p<0.05$ was considered statistically significant.

3. RESULTS

Venous blood samples were obtained from 30 volunteers (15 male) with CP (mean age, 9.8±4.2 years) and 26 healthy controls (14 male) (mean age, 10.7±3.5) between the ages of 3-18. The mean age of individuals with CP was not statistically different compared to the healthy individuals in the control group (P=0.362, independent sample t-test). Body mass index value (BMI) of individuals with CP (19.2±4.5) was not statistically different from compared to the healthy controls (20.0±3.8) (P=0.513, independent sample t-test).

3.1. High plasma proinflammatory cytokine levels and mast cell activation in patients with cerebral palsy

Since plasma IL-1β, IL-6, IL-9, histamine and tryptase beta-2 values were not normally distributed in the control and CP groups, they were compared using the Mann-Whitney U test. The plasma levels of proinflammatory cytokines IL-1β (P=0.021, Fig 1A) and IL-6 (P=0.037, Fig 1B) and also the plasma level of histamine (P=0.002, Fig 1D), which indicates mast cell activation, were significantly higher in the CP group compared to the healthy controls. On the other hand, there was no significant difference between patients with CP and the healthy controls in terms of the plasma levels of proinflammatory cytokines IL-9 (P=0.411, Fig 1C) and tryptase beta-2 (P=0.593, Fig 1E), which is a marker of increased count of mast cells.

3.2. High plasma IL-1β, IL-6, IL-9 and histamine levels in female patients with cerebral palsy, but not in male patients, compared to healthy female controls

Since plasma IL-1β, IL-6, IL-9, histamine and tryptase beta-2 values were not normally distributed in the control and CP groups, the independent paired groups were compared using the Mann-Whitney U test. In the female gender, the plasma levels of IL-1β (P=0.006, Fig 2A), IL-6 (P=0.005, Fig 2B), IL-9 (P=0.003, Fig 2C), and histamine (P=0.017, Fig 2D) were found to be significantly higher in the CP group (n=15) compared to control group (n=12). Nonetheless, there was no significant difference in terms of tryptase beta-2 (P=0.205, Fig 2E). On the other hand, in the male gender, none of these values differed between control (n=14) and

CP (n=15) groups (P>0.05 for all unpaired comparisons, Fig 2A-E).

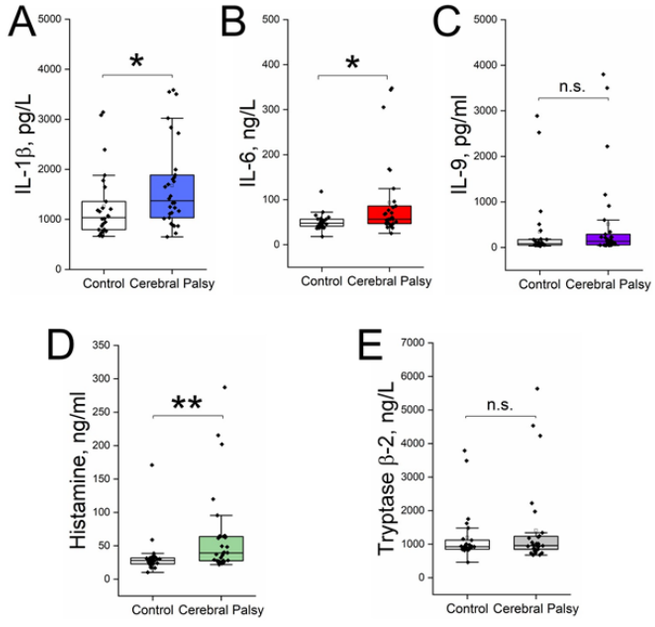

Figure 1. *High plasma IL-1β, IL-6 and histamine levels in subjects with cerebral palsy compared to healthy controls. Each box represents the median±SD, minimum and maximum values of the results. Plasma levels of these mediators between the cerebral palsy and control groups are compared by Mann-Whitney U test. n.s.: non-significance, *P < 0.05 and ** P < 0.01.*

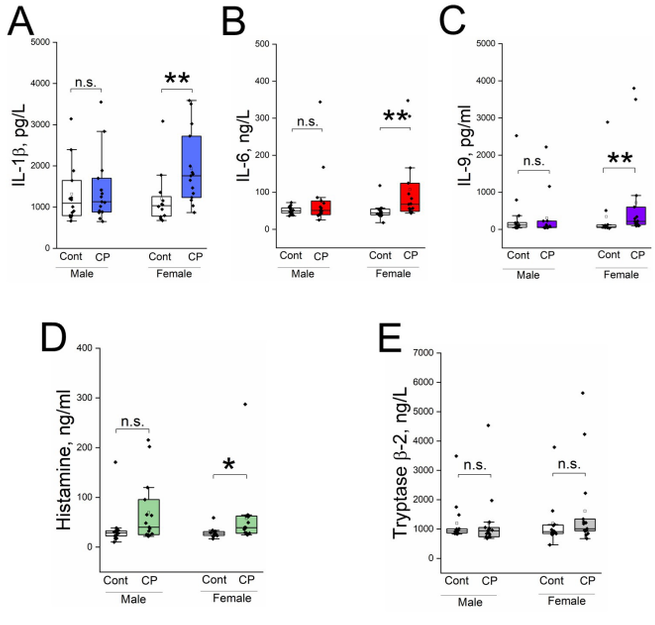

Figure 2. *High plasma IL-1β, IL-6, IL-9 and histamine levels in female subjects with cerebral palsy compared to healthy female controls. Each box represents the median±SD, minimum and maximum values of the results. Plasma levels of these mediators between the cerebral palsy and control groups are compared by Mann-Whitney U test. n.s.: non-significance, *P < 0.05 and ** P < 0.01.*

3.3. High plasma IL-1β and IL-9 levels in the females compared to the males in the group of patients with cerebral palsy.

Since plasma IL-1β, IL-6 and IL-9 levels mentioned above were found to be higher in the females with CP compared to the female healthy controls, we also compared these inflammation-related parameters of the males and females in the CP group to confirm that this difference stemmed from the females with CP. As above, Mann-Whitney U test was used for paired comparisons since the values did not exhibit a normal distribution.

Among the patients with CP, the plasma IL-1β (P=0.04, Fig 3A) and IL-9 (P=0.007, Fig 3C) levels were found to be significantly higher in the females (n= 15) compared to the males (n=15). But, there was no difference between male and female patients in terms of plasma IL-6 (P=0.165, Fig 3B), histamine (P=0.852, Fig 3D) and tryptase beta-2 (P=0.178, Fig 3E) levels.

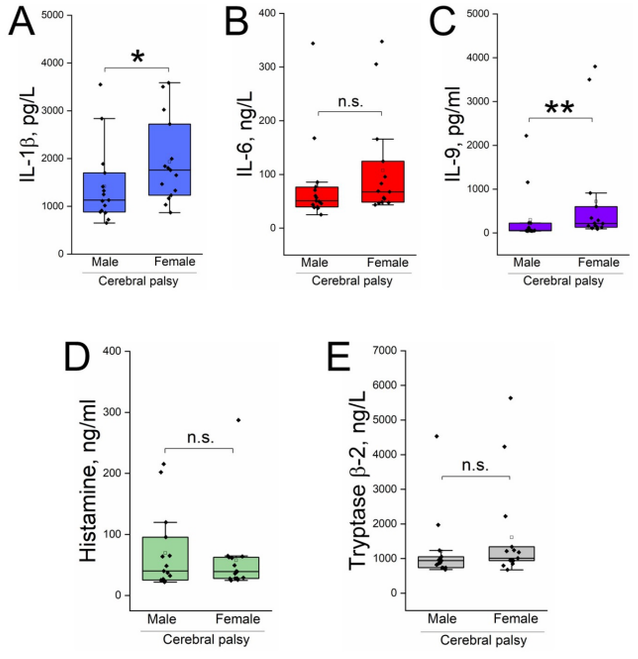

Figure 3. *High plasma IL-1β and IL-9 levels in females compared to males in the cerebral palsy group. Each box represents the median±SD, minimum and maximum values of the results. Plasma levels of these mediators between the cerebral palsy and control groups are compared by Mann-Whitney U test. n.s.: non-significance, *P < 0.05 and ** P < 0.01.*

3.4. Plasma proinflammatory cytokine levels and mast cell activation in patients with cerebral palsy by age groups (pre-adolescents and adolescents)

As a result of the analyses mentioned above, which indicated high levels of plasma proinflammatory cytokines and mast cell activation in females with CP, we asked the question of whether there was a difference according to pre-puberty and puberty periods. Accordingly, in order to find the answer to this question, we divided the male and female patients

in both groups into two subgroups as pre-adolescent (3-10 years of age, n=12 for control; n=17 for CP) and adolescent (11-17 years of age, n=14 for control; n=13 for CP) according to their developmental periods. Paired comparisons were compared using the Mann-Whitney U test since the data did not have normal distribution.

Among the female individuals, the plasma IL-6 (P=0.042, Fig 4B), IL-9 (P=0.043, Fig 4C) and histamine (P=0.041, Fig 4D) levels were found to be higher in patients with CP in the adolescence period compared to the healthy controls; however, no difference was found in terms of these parameters in the pre-adolescent period (P>0.05, Fig 4B-E). While plasma IL-1β levels were higher in patients with CP in pre-adolescence compared to the healthy controls (P=0.028, Fig 4A), no difference was found in terms of adolescent period (P>0.05, Fig 4A). Additionally, plasma tryptase beta-2 levels were not different in female patients with CP in neither pre-adolescence nor adolescent period compared to the healthy controls (P>0.05 for both comparisons, Fig 4E).

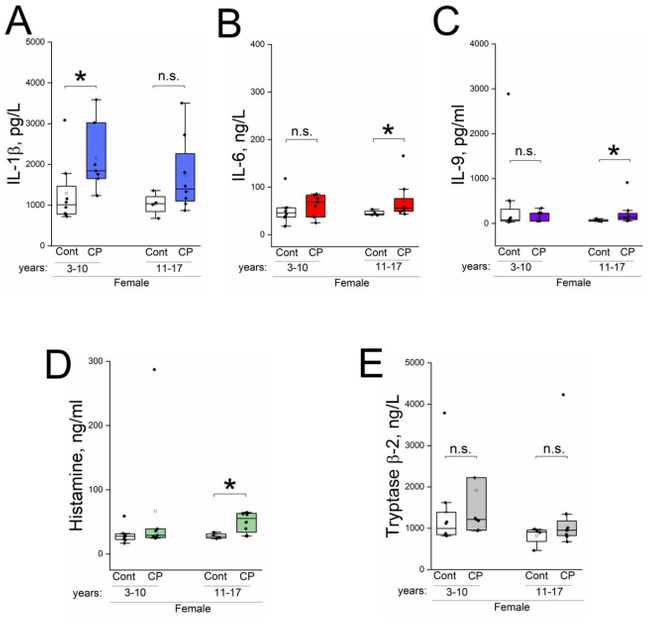

Figure 4. *High plasma IL-6, IL-9 and histamine levels in female subjects with cerebral palsy compared to healthy female controls in adolescence. Each box represents the median±SD, minimum and maximum values of the results. Plasma levels of these mediators between the cerebral palsy and control groups are compared by Mann-Whitney U test. n.s.: non-significance, *P < 0.05.*

On the other hand, unlike the females, no difference was found in male patients with CP in neither pre-adolescent nor adolescent period in terms of the plasma levels of IL-1β, IL-6, IL-9, histamine and tryptase beta-2 compared to the healthy controls (P>0.05 for all comparisons, Fig 5A-E).

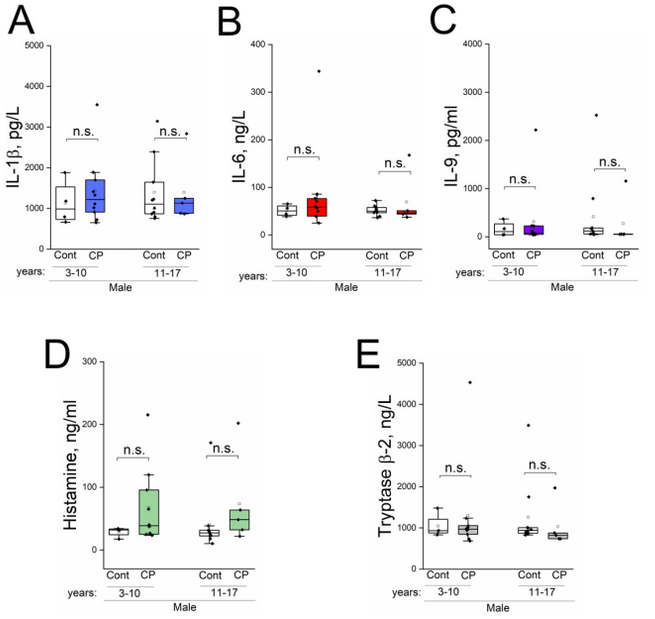

Figure 5. *Plasma levels of proinflammatory cytokines and mast cell mediators in male subjects with cerebral palsy by age periods. Each box represents the median±SD, minimum and maximum values of the results. Plasma levels of these mediators between the cerebral palsy and control groups are compared by Mann-Whitney U test. n.s.: non-significance.*

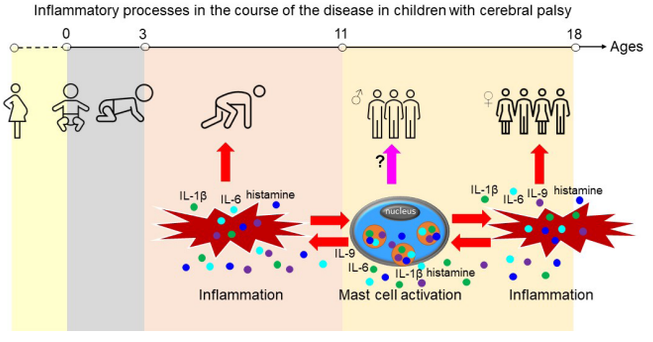

Figure 6. *Illustration of possible inflammatory processes in the course of the disease in children with cerebral palsy.*

4. DISCUSSION

The present study investigated the role of proinflammatory cytokines and immune mast cells in the course of the disease in children with CP between 3 and 18 years of age. The major findings of the study included higher plasma levels of proinflammatory cytokines IL-1β and IL-6, as well as histamine as a mast cell activation marker, in individuals with CP compared to the healthy controls. Moreover, it was found that these high inflammatory parameters in individuals with

CP stem from the females. The further analyses we made revealed that these high inflammatory values in female patients with CP were concentrated in the adolescent period, and they were rarely seen in the pre-adolescent period.

It is well known that abnormal expression of inflammatory cytokines can initiate perinatal brain injury in the pathogenesis of cerebral palsy (11-13). In addition to studies including the intrauterine period, it has been revealed that increased cytokine levels in neonatal blood at term delivery are strongly associated with the possibility of cerebral palsy (14,15).

Our findings revealed that the concentrations of proinflammatory cytokines IL-1β and IL-6, and the concentration of histamine as one of the mast cell activation markers, were higher in the plasma in patients with CP compared to the healthy controls. These findings are consistent with the results reported in the previous studies covering the intrauterine period or the period of early infancy before 2-3 years of age. In a previous clinical study, it was reported that the levels of proinflammatory cytokines IL-1β, IL-8, IL-9 and TNF-α in neonatal blood samples were higher in the group with CP compared to the controls (16). Similarly, in a clinical study conducted by taking blood samples of term infant with perinatal asphyxia, the levels of proinflammatory cytokines IL-1β, IL-6 and TNF-α were reported to be higher in children diagnosed with CP (17). In accordance with these studies, our findings have revealed that the increase in inflammatory cytokine levels plays an important role in both the pathogenesis and the course of the disease.

It is considered that proinflammatory cytokines such as IL-1β, which are released during the course of intrauterine infection, cause to the white matter damage in preterm infants developing CP (18). In the present study, the significantly higher plasma IL-1β, IL-6, and histamine concentrations of individuals with CP at 3-18 years of age compared to the healthy controls suggest that the increased inflammatory response still continues in childhood after 2 years of age, regardless of the initial trigger.

On the other hand, in our study, difference in the IL-9 levels was not statistically significant. This may be due to the fact that different proinflammatory cytokines may further come into prominence in the course of the disease in different age groups of children with CP. Moreover, gender difference may be another possible explanation of this. Because, in our study, plasma IL-9 concentration in the females with CP was found to be significantly higher compared to both the female healthy controls and the males with CP.

On the other hand, it is an important question whether there is a relationship between the gender difference and inflammatory processes that are effective in the course of CP. In the present study, plasma IL-1β and IL-9 levels were found to be higher in the females compared to the males among the patients with CP. Besides, interestingly, the plasma levels of IL-1β, IL-6, IL-9 and histamine were significantly higher in the females with CP compared to the female healthy controls; however, there was no difference between the males with CP and the males in the control group. These findings suggest that the increased inflammatory response, which plays a role in the course of CP in childhood after the age of 2, is more effective in female patients compared to the male patients; and in our findings, the major differences were due to mostly the female patients with CP.

Looking at the change of proinflammatory cytokine concentrations in female individuals by age groups, it was found that IL-6, IL-9 and histamine levels in females with CP were higher in the adolescents compared to the individuals of the same gender and age group in healthy controls. However, in females with CP, only IL-1β level in pre-adolescence period was higher compared to the healthy controls of the same gender and age group; and no difference was found in terms of other inflammatory parameters. Unlike females, no significant difference was found for any of these inflammatory parameters in males during the pre-adolescence or the adolescence period.

Estrogens whose levels begin to increase in adulthood may be responsible for the increase in inflammatory responses in the females with CP during adolescence. Because, estrogens are able to activate the mast cells (19). Activation of mast cells leads to induce inflammatory cascades. Mast cells can be activated by a large number of immunological and non-immunological agents (20,21). These include IgE, estrogen, and the inflammatory cytokines such as IL-1β, IL-3, IL-4, IL-9, IL-33 (19,21,22). It is well known that mast cell activation is increased in many inflammatory diseases (21,23). Consistent with this information, the high histamine levels found in patients with CP in our study indicated that the activation of mast cells were increased due to the increase of inflammatory cytokines (IL-1β, IL-6 and IL-9) in these patients.

On the other hand, in the present study, no difference was found between individuals with CP and the control group in terms of tryptase beta-2 levels, which are released from mast cells and accepted as an indicator of mast cell amounts. This finding suggests that mast cells play a key role in the course of the disease through their increased activation without changing in number.

In an experimental study mimicking hypoxic-ischemic brain injury in CP, IL-9 administration was reported to increase ibotenate-induced brain injury in normal mice, while no effect was observed on the mice genetically lacking mast cells (9). Moreover, it was demonstrated that a mast cell stabilizer cromolyn significantly reduced ibotenate-induced brain lesions (IL-9 treated) (10). In accordance with these studies, our study revealed that the mast cell activation plays an important role in the course of CP as in other brain injury models, and that this effect may continue in childhood.

5. CONCLUSION

Future clinical trials with mast cell stabilizers such as ketotifen and cromolyn may provide further information on whether

the mast cell stabilizers can be used to limit the inflammation in patients with CP.

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Conflicts of interest

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

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Clinical and Experimental Health Sciences

Compliance with Public Health Measures and Psychological Effects of COVID-19: Two-Group Cross-Sectional Research

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ABSTRACT

Objective: COVID-19 has spread since the day it emerged and was declared as a global pandemic. Determining the psychological effects of this situation and the compliance with public health measures will be a guide both in the fight against the COVID-19 pandemic and possible epidemics in the future. This two-group, cross-sectional descriptive study aimed to determine compliance with public health measures against COVID-19 and to evaluate the levels of fear, depression, anxiety, and stress by analysing individuals who experienced and did not experience COVID-19.

Method: The study sample consisted of a total of 636 participants. Of these participants, 328 had a positive Polymerase Chain Reaction test and 308 had a negative test. Data was collected using the Diagnostic Form, Questionnaire for the Compliance with Public Health Measures Against the COVID-19, Fear of COVID-19 Scale, and the Short Form Depression Anxiety Stress Scale in our online database.

Results: Although the participants' compliance with public health measures regarding the use of masks is high, their compliance with the measures related to physical distance is low. Moreover, fear of COVID-19, anxiety, stress, and depression levels are higher in individuals who experienced COVID-19 than those who did not experience COVID-19. Also, both groups have high levels of fear, anxiety, stress, and depression.

Conclusion: In conclusion, priority should be given to strategies aimed to increase compliance with physical distance and to detect and control the psychological effects of COVID-19 in the whole society, especially in individuals experiencing COVID-19.

Keywords: COVID-19, public health measures, compliance, psychological effects

1. INTRODUCTION

The novel Coronavirus (COVID-19), a virus detected for the first time in Wuhan, China, has spread around the world since the day it emerged and the spread was declared as a global pandemic by the World Health Organization (WHO) on March 11, 2020 (1,2). As of April 25, 2021, there have been 146,054,107 confirmed positive cases of COVID-19 resulting in 3,092,410 deaths worldwide (3).

The COVID-19 virus spreads when an infected person coughs, sneezes or talks. After dispersion, while smaller droplets suspend in the air, larger droplets settle on the surfaces and remain alive for a while (4). Transmission may occur through inhalation of these droplets by non-infected individuals or by taking hands to the mouth, nose, or eye mucosa after contact with infected surfaces (5). Public health measures are being implemented across the globe to limit transmission and reduce mortality and morbidity due to COVID-19 (6-9). These measures include wearing a mask, ensuring hand hygiene, avoiding potentially contaminated people or environments, and physical distancing (5,8). Immunization is considered the definitive solution for controlling the pandemic. Some of the vaccine studies conducted for this purpose have been completed, and as of 18 February 2021, at least seven different vaccines across three platforms have been rolled out in countries. The vaccination process is continuing, and as of 25 April 2021, a total of 899,936,102 doses of vaccine have been administered worldwide (3). However, despite vaccination, the pandemic continues in some countries without slowing down. As reported by WHO, "Being vaccinated does not mean that we can throw caution to the wind and put ourselves and others at risk." Because it is still not clear the degree to which the vaccines can protect not only against disease but also against infection and transmission. It is not yet known

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how long immunity from different COVID-19 vaccines will last. That is one reason why we still need to follow all public health measures even though COVID-19 vaccines are rolling out (10,11). Therefore, it is necessary to identify the society's compliance with the proposed public health measures and prepare plans according to the compliance status.

In addition to the fact that new cases and deaths have been continuing without pausing since the emergence of the pandemic, media broadcasts on the subject and the fear of getting unable to access healthcare services, of getting infected and dying have led to the development of various phobias (12). It has been suggested that as previous outbreaks caused fear among people, increased stress levels, and led to anxiety and depression, individuals' psychological status should be evaluated in cases of such events that affect large masses (13,14). Psychological outcomes of the COVID-19 pandemic are not yet fully known (13,15,16). There is a limited number of studies addressing psychological status, especially in individuals who experienced COVID-19 (17). For a healthy population, identifying the psychological effects of this pandemic is critical for planning appropriate psychiatric treatment and determining future strategies. Additionally determining the psychological effects and the compliance with public health measures will be a guide both in the fight against the COVID-19 pandemic and possible epidemics in the future.

This study aimed to determine compliance with public health measures against COVID-19 and to assess the fear, depression, anxiety, stress levels by analysing individuals who experienced and did not experience COVID-19.

2. METHOD

2.1. Research Question

The research question was as follows:

What is the level of compliance of the society with public health measures against COVID-19?

Does the compliance with public health measures against COVID-19 differ between individuals who experienced COVID-19 and those who did not?

Is there a difference between the individuals who experienced COVID-19 and those who did not regarding the fear, anxiety, depression, and stress levels?

Has COVID-19 affected the general psychological wellbeing of society?

Type of the study

This study was conducted as two-group cross-sectional research.

Inclusion criteria

- Having a positive PCR test and receiving only medical treatment for COVID-19 (Group Positive-GP);
- Having no positive PCR test and receiving no treatment for COVID-19 (Group Negative-GN);
- Being aged from 18 to 85 years;
- Being a non-healthcare professional;
- Giving consent to participate in the study.

2.2. Study sample

According to the study by Zhanga et al (17) the sample size was estimated to be 200 individuals in each group considering the effect size of 0.4, with a confidence level of 95% and a test power of 95%. The sample was kept larger considering the possibility that data loss would occur. Data of patients who presented to the COVID-19 outpatient clinic of a university hospital between 10 February 2021 and 20 April 2021 were accessed. There were 26,181 applications in total, and the number of patients who met the inclusion criteria and had contact details was 9,522. These patients were divided into two groups according to their PCR test result: there were 2,477 patients with positive PCR (GP) and 7,045 patients with negative PCR (GN). A stratified sampling method was used for sample selection. Three strata were formed according to the age ranges (18-24 years of age, 25-64 years of age, and 65-84 years of age) specified in WHO's COVID-19 Situation Report-198.[18] Using a simple random numbers table, a total of 1,200 people (600 people in GP; 600 people in GN) including 200 people from each stratum were sampled. In the end, data of 636 people were accessed: for GP, 328 individuals who could be reached by phone and agreed to participate in the research, and for GN, 308 individuals who could be reached by phone and agreed to participate in the research (Figure 1).

2.3. Data collection tools

Diagnostic form

The diagnostic form was created by the authors. This form consists of 9 items (rated on a 1-5 scale) designed to describe the participants› sociodemographic characteristics, medical history (6 items), reasons for the fear of COVID-19 (1 open-ended item).

Questionnaire for compliance with public health measures against the COVID-19

The questionnaire was prepared based on WHO's (COVID-19) advice list for public health measures (9). It is a 15-item questionnaire and all items are positively worded. Responders were asked to answer each item as "yes" (1 point) or "no" (0 points).

Fear of coronavirus (COVID-19) scale

The Fear of COVID-19 Scale is a one-dimensional scale consisting of 7 items, all of which are worded positively (19). It

has a Likert-type rating system. Scoring is as follows: 1=Strongly disagree, 2=Disagree, 3=Neither agree nor disagree, 4=Agree, 5=Strongly agree. The total score calculated by adding up each item score shows the level of the COVID-19 fear the individual is experiencing. The possible total score ranges from 7 to 35. Higher scores reflect greater fear. The reliability and validity study of the Turkish version of the scale was conducted by Bakioglu et al. (20) reporting the Cronbach's alpha coefficient as 0.88. In this study, the Cronbach's alpha coefficient of the scale was 0.85.

Short-form depression anxiety stress scale (DASS-21)

The Depression Anxiety Stress Scale (DASS) originally consisted of 42 items. Later, the scale was shortened to 21 items, and the short version (DASS 21) was reported to have the validity to perform the same measurement (21,22). DASS 21 compromises 7 items selected from each of the depression, stress, and anxiety subscales. It is a Likert-type scale and each item is scored on a 4-point scale: 0 "Did not apply to me at all", 1 "Applied to me to some degree", 2 "Applied to me to a considerable degree", and 3 "Applied to me very much". The validity study of the Turkish version of the short form was carried out by Yılmaz et al. (16). The omega value of the scale was found to be 0.82 for the depression sub-scale, 0.80 for the anxiety sub-scale, and 0.75 for the stress sub-scale. Depending on these results, the Turkish form was reported to be valid and reliable. In the present study, the Cronbach's alpha value was found to be 0.80 for the depression sub-scale, 0.78 for the anxiety sub-scale, and 0.75 for the stress sub-scale. Table 1 shows the evaluation of the scores from this scale.

Table 1. *Interpretation of the scores received from the short-form depression anxiety stress scale (DASS-21)*

	Depression	Anxiety	Stress
Normal	0-4.5	0-3.5	0-7
Mild	4.6-6.5	3.6-4.5	7.1-9
Moderate	6.6-10	4.6-7	9.1-12.5
Severe	10.1-13.5	7.6-9.5	12.6-16.5
Very Severe	14.6+	9.6+	16.6+

2.4. Data collection method

The data collection tools were converted to online tools keeping their original form. All participants were called and informed about the research. After the information, the data collection form was sent online (via e-mail, WhatsApp, SMS) to the individuals who agreed to take part in the study, and the participants were asked to complete the form. The first part of the form consisted of consent, participants who did not consent was not allowed to answer other parts of the form.

2.5. Statistical analysis

The responses to the open-end question "What is the reason that scares you the most about the COVID-19 pandemic?" were analysed by two separate independent researchers. A total of 258 different responses were examined, and the causes of fear were grouped under eight headings by combining different expressions containing the same reason for fear. Descriptive statistical methods (percentage, mean, standard deviation) were used for evaluating the data. The Pearson Chi-square test was used for testing categorical variables between the two groups. For the inter-group comparisons, the Independent Sample-T test was employed for normally distributed data and the Mann-Whitney U test for non-normally distributed data.

2.6. Ethical Dimensions of the Research

This study was performed only after obtaining the ethics committee approval (2020/514/181/3), agency approval, and other necessary permissions for the use of the data collection forms. The individuals who agreed to participate in the research were informed about the research, and their consent was obtained. The study started on February 2021 and was finished by April 2021. For the performance of the research, we adhered to the World Medical Association (WMA) – Ethical Principles for Medical Research Involving Human Subjects. In this study, data analysers were blinded.

2.7. Limitations of the study

1. The study sample is limited to individuals who applied to the COVID-19 outpatient clinic of a university hospital between 10 February 2021 and 20 April 2021.

2. The results are limited to the data collected via the Diagnostic form, the Questionnaire for compliance with public health measures against the COVID-19, the Fear of coronavirus (COVID-19) scale, and the Short-form depression anxiety stress scale (DASS-21).

3. RESULTS

The groups were similar in terms of the characteristics that would affect the outcomes of the study (p>0.005) (Table 2).

When the responses to the questions on public health measures against the COVID-19 were evaluated, it was found that 96.6% (n=614) of the participants said "yes" to the item "'I wear a mask when I go out", 96.2% (n: 612) of the participants said "yes" to "I wear a mask in the way covering my mouth and nose", and 95.6% (n: 608) of the participants said "yes" to "I wear a mask in crowded places".

When it came to the statements that got the highest rates of "no"; "I do not make home visits to my close relatives/ friends", "I do not leave the house unless necessary", and "I pay attention to keep the physical distance (minimum 1 meters)" were the top three with the rates of 25.8% (n=164), 18.9% (n=120), and 18% (n=103), respectively. When the groups were compared regarding the responses they gave to and the scores they got from the compliance questionnaire, no difference was found (p>0.05) (Table 3).

Table 2. *Descriptive characteristics of the groups*

Results			Group Positive (n=328)	Group Negative (n=308)	T	P
Age (Mean±SD)			44.29±16.04	37.83±12.08	4.03	0.052
			n (%)	**n (%)**	**χ2**	**P**
Age Ranges	18-24		113(35.5)	110(35.7)	09.12	0.985
	25-64		148(45.7)	139(45.2)		
	64-84		67(18.8)	59(19.1)		
Vaccinated individual	18-24		4(5.3)	3(4.5)	24.26	1.00
	25-64		26(34.6)	23(27.3)		
	64-84		49(65.1)	55(68.2)		
Gender		Female	168 (51.2)	186 (60.4)	2.70	0.114
		Male	160 (48.8)	122 (39.6)		
Employment		Employed	144 (43.9)	118 (38.3)	10.07	0.063
		Unemployed	184 (56.1)	190 (61.7)		
Systemic Disease		Yes	140 (42.7)	130 (42.2)	21.07	0.082
		No	188 (57.3)	178 (57.8)		
A Family Member Who Have Had COVID-19		Yes	169 (51.5)	93 (30.2)	29.38	0.001
		No	159 (48.5)	215 (69.8)		
A Family Member Who Died of COVID-19		Yes	2 (0.6)	1 (0.3)	0.27	1.00
		No	326 (99.4)	307 (99.7)		

t= independent samples T χ2 = Pearson Chi-Square

Table 3. *Scores obtained from the questionnaire for compliance with public health measures against COVID-19 and the state of compliance*

		Group Positive (328)		Group Negative (308)		Total (636)			
		Median (IQR)		Median (IQR)		Median (IQR)		U	p
Score of the Questionnaire for Compliance with Protection Measures		13 (3-15)		12.5 (7-15)		13(3-5)		11327	0.102
Prevention Measures against the Pandemic		**Yes**	**No**	**Yes**	**No**	**Yes**	**No**		
		n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**χ^2**	**p**
1	I avoid entering crowded places.	302(91.9)	26(8.1)	282(91.6)	26(8.4)	584(92.0)	52(8.0)	-0.00	0.947
2	I wear mask when I go out.	318(96.7)	10 (3.3)	296(96.1)	12(3.9)	614(96.6)	22(3.4)	-0.00	0.780
3	I wear mask in crowded places.	314(95.7)	14(4.3)	294(95.5)	14(4.5)	608(95.6)	18(4.4)	-0.00	0.986
4	I wear mask in the way covering my mouth and nose.	315(96.0)	13(4.0)	297(96.4)	11(3.6)	612(96.2)	24(3.8)	-0.31	0.872
5	I pay attention to keep the physical distance (minimum 1 meters).	276(84.1)	52(15.9)	246(79.8)	62(20.2)	522(82.0)	114(18.0)	-0.04	0.403
6	I do not make home visits to my close relatives/ friends.	260(79.2)	68(20.8)	212(68.8)	96(31.2)	472(74.2)	164(25.8)	-0.12	0.058
7	I ensure hand hygiene (washing/scrubbing hands) after touching surfaces open to contact.	320(97.5)	8(2.5)	297(96.4)	11(3.6)	617(97.0)	19(3.0)	0.12	0.727
8	For ensuring hand hygiene (washing/scrubbing hands), I scrub my hands at least for 20 minutes.	288(87.8)	40(12.2)	262(85.0)	46(15.0)	550(86.4)	86(13.6)	-0.33	0.561
9	I do not leave the house unless necessary.	270(82.3)	58(17.7)	246(78.9)	62(21.1)	516(81.1)	120(18.9)	-0.02	0.693
10	I change my clothes after coming from outside.	276(84.1)	52(15.9)	274(89.0)	34(11.0)	550(86.4)	86(13.6)	0.78	0.165
11	I ensure hand hygiene after coming from outside (handwashing or handrubbing with disinfectant).	320(97.5)	8(2.5)	297(96.4)	11(3.6)	617(97.0)	19(3.0)	0.20	0.727
12	I use disposable tissues when I cough or sneeze.	276(84.1)	52(15.9)	248(80.5)	60(19.5)	524(82.3)	112(17.7)	-0.39	0.489
13	I am careful to keep my hands away from my eyes, mouth and nose.	276(84.1)	52(15.9)	268(87.0)	40(13.0)	544(85.5)	92(14.5)	0.48	0.387
14	I frequently ventilate the indoor areas I am in.	312(95.1)	16(4.9)	296(96.1)	12(3.9)	608(95.6)	28(4.4)	-0.02	0.727
15	I do not make contact with other people like shaking hands or hugging.	300(91.4)	28(8.6)	298(96.8)	10(3.2)	598(94.0)	38(6)	0.83	0.137

U= Mann-Whitney U χ2 = Pearson Chi-Square IQR=interquartile range

Table 4. *Scores obtained from the fear of COVID-19 scale and reasons for fear*

		Group Positive (328)	Group Negative (308)		
		Median(IQR)	**Median(IQR)**	**U**	**P**
Fear of COVID-19 Scale Score (Lowest Possible Score 7, Highest Score 35)		29 (7-35)	28 (7-35)	1116	0.073
		n (%)	**n (%)**	**χ^2**	**P**
Does the COVID-19 pandemic scare you?	**Yes**	276 (84.1)	246 (79.9)	21.82	0.52
	No	52 (15.9)	62 (20.1)		
What is the reason that scares you most?	Dying	90 (32.7)	36 (14.6)	31.93	0.001
	Getting intubated	58 (21.0)	40 (16.3)		
	Losing a family member	56 (20.3)	82 (33.3)		
	Becoming permanently disabled	20 (7.2)	6 (2.4)		
	Getting ill	14 (5.1)	20 (8.1)		
	Obscurity	14 (5.1)	2 (0.8)		
	Being quarantined	12 (4.3)	18 (7.3)		
	Infect others	12 (4.3)	42 (17.1)		

χ2= Pearson Chi-Square IQR=interquartile range

Table 5. *Scores obtained from the DASS-21 scale and the stress, anxiety and depression level*

DASS-21		Group Positive (328)	Group Negative (308)		
		Median(IQR)	**Median (IQR)**	**U**	**p**
Stress Score		14 (7-21)	9 (5-21)	12316	0.002
Anxiety Score		9 (3-21)	6 (2-18)	10485	0.008
Depression Score		7 (4-21)	5 (3-21)	11705	0.011
		n (%)	**n (%)**	**χ^2**	**p**
Stress	Normal	72 (22)	74 (24.1)	4.05	0.013
	Mild	86 (26.2)	66 (21.4)		
	Moderate	55 (16.7)	161(52.4)		
	Severe	112 (34.1)	2 (0.5)		
	Very Severe	3 (1)	5 (1.6)		
Anxiety*	Normal	64 (19.6)	59 (19.1)	8.06	0.015
	Mild	76 (21.5)	96 (29.2)		
	Moderate	30 (9.2)	134 (43.2)		
	Severe	150(46.8)	11 (3.1)		
	Very Severe	8 (2.9)	18 (5.4)		
Depression	Normal	56 (18.2)	117 (35.5)	10.80	0.019
	Mild	87 (28.2)	155 (47.3)		
	Moderate	142 (46.1)	40 (12.2)		
	Severe	11 (3.6)	10 (3.1)		
	Very Severe	12 (3.9)	6 (1.9)		

U= Mann-Whitney U test χ2= Pearson Chi-Square

The median (interquartile range [IQR]) of the scores obtained by the groups on the COVID-19 scale was 29 (7-35) in GP, 28 (7-35) in GN. We observed that 84.1% (n=276) of the GP patients and 79.9% (n=246) of the GN patients said "yes" to the question, "Does the COVID-19 pandemic scare you?" It was found that the top three reasons for fear in GP were dying, getting intubated, and losing a family member with the rates of 32.7% (n=90), 21% (n=58), and 20.3% (n=56), respectively. In GN, on the other hand, the top three causes of fear were losing a family member, infecting other people, and getting intubated stated by 33.3% (n=82), 17.1 (n=42), and 16.3% (n=40) of the participants respectively (p=0.001) (Table 4).

In terms of median DASS-21 scale score (IQR) of the groups, the GP/GN ratio was as follows: stress 14 (7-21) (severe)/ 9

(5-21) (moderate) (p=0.002); anxiety 9 (3-21) (severe)/ 6 (2-18) (moderate) (p=0.008); depression 7 (4-21) (moderate)/ 5 (3-21) (mild) (p=0.011). It was determined that 34.1% (n=112) of the individuals in GP experienced severe stress and 52.4% (n=161) of the individuals in GN experienced moderate stress (p=0.013). Similarly, it was determined that 46.8% (n=150) of the individuals in GP experienced severe anxiety and 43.2% (n=134) of the individuals in GN experienced moderate anxiety (p=0.015). When depression levels were examined, it was found that 46.1% (n=142) of the individuals in GP experienced moderate depression and 47.3% (n=155) of the individuals in GN experienced mild depression (p=0.019) (Table 5).

4. DISCUSSION

In this study, we evaluated compliance with public health measures proposed for protection from COVID-19 and found the scores of the compliance survey to be similar in GN and GP. The responses to this questionnaire demonstrated that both groups had a high level of compliance with the recommendations for using masks, whereas the compliance with the recommendations for physical distancing was low. Whereas physical distancing is one of the critical measures. Maintaining physical distance has been reported as the most important public health measure in the COVID-19 pandemic and previous outbreaks. It is noteworthy that compliance with such a vital measure is low. It is known that no mask can provide 100% protection against the virus. It has been also reported that the surgical masks used by the public allow 20-30% of the droplets to pass through, and this rate increases, even more, when the mask is worn loosely, if it does not fit the face properly, and in closed environments (23). For this reason, transmission is possible even when a mask is worn unless social distancing is maintained. A modelling study investigating the data of more than 40,000 participants in the United Kingdom reported that without physical distancing, all other measures were insufficient (24). Unfortunately, it was revealed in this study that none of the participants were in full compliance with public health measures. It is thought to be a factor contributing to the rapid spread of the pandemic. Lessa et al. (25) reported that some personality traits and some sociodemographic characteristics were effective in compliance with public health measures. Conducting similar studies can be a guide in determining the reasons for the low level of compliance and which strategies may be beneficial for encouraging higher compliance. Additionally, measures should be taken especially for social distancing, and public awareness on the issue should be raised. It was thought that investigating the reasons for non-compliance with the physical distance and conducting studies to increase compliance will be an important strategy in facilitating the fight both with the COVID-19 epidemic and any epidemic that may arise later.

It was revealed when the data were examined to reveal the psychological effects of COVID-19 that the participants got high scores on the Fear of COVID-19 Scale. The causes of fear were asked and the top three causes stated by the individuals in GP were dying, getting intubated, and losing a family member. On the other hand, the top three causes mentioned by the individuals in GN were losing a family member, infecting others, and getting intubated, respectively. All answers involve the fear of disintegration of the family and deterioration in health status. Additionally, the individuals' use of the medical term "getting intubated" suggests the frightening effect of the news in the media on society. Mertens et al. (26) stated in their study that media increases the fear of COVID-19. Even though fear is motivating in some situations, excessive fear can also bring about hopelessness, concentration on negative thoughts, and psychological problems in society (20). Therefore, it is important to keep fear under control. Furthermore, encouraging individuals to obtain information only from reliable sources and preventing fearful expressions about COVID-19 in the news may contribute to the reduction of fear among people.

In our study, the groups were evaluated in terms of the scores they got from the DASS-21 sub-scales for anxiety, stress, and depression, which are the other psychological effects of the pandemic. It was found that the majority of the individuals in GP experienced severe stress and anxiety, and moderate depression; whereas the majority of individuals in GN experienced moderate stress and anxiety, and mild depression ($p < 0.05$). Besides, the stress, anxiety, and depression levels of the individuals in GP were found to be higher than that of the individuals in GN ($p<0.05$). Similar to our research, a study conducted in China reported higher anxiety and depression levels in patients who experienced COVID-19 as compared to the general population (17). It was thought that the higher level of anxiety in GP was due to unknown long-term effects of COVID-19 on health as well as the increased concerns for the future. Moreover, these consequences were thought to emerge due to the fear of death and feelings of helplessness, which are likely to be experienced at the time of illness and quarantine process.

Stress and anxiety at a mild level have a useful function in dealing with difficulties, reacting to physical ailments, and taking the necessary measures to prevent and alleviate the disease. However, excessive and prolonged stress and anxiety can cause physical and psychological health problems (27,28). It is known that advanced stress and anxiety are associated with depression. Moderate and severe depression requires medical treatment, while mild depression requires psychosocial treatment. If depression is not treated, it results in social isolation and a decrease in the quality of life. Especially when it is long-lasting and with moderate to severe intensity, depression may become a serious health condition. It can cause the affected person to suffer greatly and function poorly at work, at school, and in the family. At its worst, depression can lead to suicide (29,30) or increase the rate of death (31). For this reason, the results of this study are important as they reveal that psychological support strategies should be developed in Turkey and in other countries affected by the pandemic.

5. CONCLUSION

In conclusion, the compliance of individuals with public health measures against COVID-19 is not at the desired level. Especially the compliance with the measures for social distancing is low. The levels of fear, anxiety, stress, and depression related to COVID-19 are higher in the individuals who experienced COVID-19 compared with those who did not experience COVID-19, with both groups having high levels of fear, anxiety, stress, and depression. Priority should be given to strategies aimed to increase compliance with public health measures against COVID-19 and to identify and control the psychological effects of COVID-19 in the whole society, especially in individuals experiencing COVID-19. Based on these results, we recommend:

- Raising awareness of the individuals for the compliance with public health measures against COVID-19;
- Adopting more rigorous measures for the compliance with social distancing;
- Screening of the whole society, especially individuals experiencing COVID-19, in terms of the psychological effects of COVID-19;
- Planning initiatives for psychological support.

Disclaimer

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Declaration of competing interest

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Clinical and Experimental Health Sciences

In Vitro Evaluation of Root Surface Roughness in the Use of an Ultrasonic Device with Different Tips Having Different Mechanism of Action: A Profilometric Study

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ABSTRACT

Objective: The aim of this study was the profilometric evaluation of the changes in root surface roughness created by different types of ultrasonic tips and mechanism of action.

Methods: Thirty root dentine samples obtained from 15 maxillary premolars, extracted for orthodontic reasons, were included in the study. The sample surfaces were embedded into acrylic blocks, polished, and divided into 3 study groups as linear oscillating device (LOD) with straight tip (ST); LOD with perio-curette tip (PCT); conventional ultrasonic scaler tip (CUST). A calibrated clinician instrumented all surfaces in each group. The root surfaces were evaluated before and after instrumentations with a profilometer device.

Results: There were no statistical differences between the initial roughness values of the groups ($p<0.05$). Multiple comparisons of after-treatment values and differences before and after instrumentations revealed statistical significances ($p=0.041$; $p=0.016$, respectively). CUST group showed the highest surface roughness in comparison with the LOD groups. LOD with ST revealed the smoothest surface followed by LOD with PCT and CUST.

Conclusion: Within the limits of this study, it may be concluded that fine and delicate tips with linear oscillating movement may be considered as the choice of insert for subgingival instrumentation due to the gentler mechanism of action than the conventional ultrasonic scalers.

Keywords: in-vitro, profilometer, root debridement, surface roughness, ultrasonic device

1. INTRODUCTION

Periodontal diseases are multispecies microbial infections, characterized by continuous passage and thread of microorganisms from external environment into the periodontal tissues and the human body. Oral microorganisms are organised in the oral cavity as biofilms on desquamation free, non-shedding hard surfaces as the main etiological factor. The accumulation and attachment of microbial dental plaque biofilm are facilitated by retentive areas including rough root surfaces (1). The corner stone of anti-infective non-surgical approaches as the first phase of periodontal treatment protocol is the mechanical debridement for physical removal of biofilm and other disease mediating factors contaminating hard surfaces, namely the root surfaces (2, 3).

Sonic/ultrasonic devices, hand instruments (such as scalers and curettes), lasers and rotating burs are widely used to remove tooth-surface associated biofilm, calculus, and contaminated root cementum (4-6). Although hand instruments together with sufficient time/manual dexterity are accepted as the gold standard, ultrasonic devices with various tips are also considered as options during periodontal treatment (7,8). Power-driven ultrasonic scalers are mostly used in daily routine practice and have become increasingly popular for subgingival debridement due to less operator strain, similar effectiveness with hand tools, newly designed tips, and effective debridement (9, 10). Although periodontal therapy with power driven devices do indeed offer some clinical advantages to the clinician, there are still some conflicting results and issues to be solved. Different results have been shown in the literature regarding the physical effects of magnetostrictive and piezoelectric ultrasonic scaling devices on tooth surfaces (11,12).

The amount of removed root substance during subgingival instrumentation is as important as the removal of bacterial deposits (13), since the clinician may end up with excessive root surface roughness leading to increased sub-gingival plaque retention (13,14). Besides the aforementioned factors, the type, and the tips of the ultrasonic devices as well as the orientation, the distance, and the movement of the tip

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in relation to the root surface are of critical importance to avoid harmful effects.

This is an open research area to overcome the limitations and disadvantages (7,14-16). Novel tools are continuously being designed and developed. A power-driven ultrasonic device presenting linear oscillating movement has been introduced in the market as a gentle and effective alternative tool for mechanical periodontal therapy. The tips of this new linear oscillating device (LOD) move linearly parallel to the root surface during instrumentation (17). In a limited number of studies, this device, avoiding horizontal vibrations, revealed better patient perception with less pain compared to other power-driven instruments (18,19) and caused less hypersensitivity compared to hand instruments (20,21). The unique vertical vibrational energy of the instrument is transmitted to the tooth and root surface as well as to the periodontal tissues in conjunction with or without a hydroxyapatite (HA) particle containing fluid. The use of the device with water or polishing fluid directed to the instrument tip helps to soft and hard debris removal occurring through hydrodynamic forces rather than by the chipping action of the conventional ultrasonic tips (22-24).

Therefore, the aim of this study was to evaluate the effects of two subgingival tips of the LOD in comparison to a scaler tip of the conventional ultrasonic system on the roughness of the root surfaces following non-surgical periodontal instrumentation in vitro.

2. METHODS

The present study was approved by Yeditepe University, Dentistry Faculty Scientific committee and Ethical Board of Yeditepe University (number:37068.608.6100-15-2228/13.10.2021). The sample calculation was carried out with another vitro research (25): a:0.05, power (β) of 80%, d effect size of 1.365 for the mean surface roughness (Ra) parameter and SD of 1.5. The sample number was calculated to be minimum of 10 in each group.

Fifteen human maxillary premolar teeth, freshly extracted for orthodontic reasons, were used. Teeth with cracks, large carious lesions, or restorations were excluded. Care was taken to keep the root surfaces intact during extraction. Immediately after extraction of the teeth, they were rinsed in running tap water for approximately 20 seconds to remove the surface debris or blood. Afterwards stored in 4°C distilled water with thymol as a preservative to inhibit microbial growth until their use (26).

2.1. Sample Preparation

Dentine specimens obtained from 15 teeth were prepared as described in a previous study (27). After preparation procedures, 30 dentin samples with a thickness of 5 mm were randomly divided into 3 groups through a randomization table (randomlists.com). To avoid the inclusion of the two pieces of the same teeth in the same group, the halves were coded as a and b and then distributed. The prepared dentine specimens were mounted in a specially designed rectangular cast filled with acrylic resin keeping either of the two surfaces (buccal/palatal) exposed without any visible surface irregularities. The specimen surfaces were wet-polished with a sequence of silicone carbide papers 320.600.1200-and 2000 grit). All specimens were rinsed with sterile water solution and air dried.

2.2. Application Groups

Straight Tip Group (ST) (n=10).

Perio Curette Tip Group (PCT) (n=10).

Conventional Ultrasonic Scaler Tip Group (CUST) (n=10).

2.3. Application Procedures

In a previous study (27), an experienced periodontist (OLT) was educated and calibrated to operate with stable lateral forces for both power-driven and hand instruments according to calibration methodology (1,8,28,29). The pressure was aimed to 40 g for power-driven instruments.

All tips were used in connection with the same ultrasonic device (Vector® Paro Pro, Dürr Dental, Bietigheim-Bissingen, Germany), consisted of two different handpieces, one specially designed for linear oscillating movement (Vector Paro Handpiece, 25-35 kHz operating frequency) and the other with conventional scaler handpiece generating a spatial vibration (Vector Scaler Handpiece, 25-35 kHz operating frequency) (Figure 1).

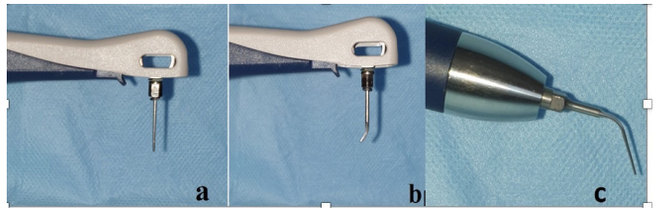

Figure 1. *Vector® Paro and Vector® Conventional Ultasonic tips used in treatment groups a) straight tip, b) perio-curette tip c) ultrasonic scaler tip*

ST, like a periodontal probe in shape and PCT, like a periodontal curette, were used only with water according to the setting instructions given by the manufacturer (70% power setting), while CUST (P1) was used through conventional piezoelectric ultrasonic handpiece and settings for the instrumentation of the specimen surfaces.

For Paro handpiece, HA particle containing fluid was intentionally not used during the application to avoid the possibility of jeopardizing the profilometer readings of roughness, since it provides a polished surface after usage. The water was not in spray aerosol form but held hydrodynamically on the instrument by the linear ultrasonic movement (21).

The tips were guided by a parallel position (0°) hold onto the specimen surfaces in contact mode and applied in an imbricate sweeping movement without pressure during surface scanning of 30 sec.

2.4. Profilometer Roughness Calculation

Surface roughness of all dentin specimens were evaluated before and after instrumentation procedures with a profilometer (Perthometer M1 Mahr, Göttingen, Germany) as described in a previous research (27). For each specimen, 5 measurements were recorded at different locations and in different directions (blinded, EÖK). Ra (µm) was taken as the average value of these five readings. The surface-roughness tester was used during the whole evaluation to periodically calibrate the profilometer (Mahr GmbH, Göttingen, Germany).

2.5. Statistical Analysis

Surface Ra was chosen as the primary outcome variable. IBM SPSS Statistics 22 Program (SPSS IBM, Turkey) was used for statistical analysis. Kolmogorov-Smirnov and Shapiro Wilks tests were used to evaluate the normal distribution of the data. Owing the fact that parameters did not show a normal distribution, Kruskal Wallis test was used for comparing the parameters between the groups, whereas Dunn's test was used for the paired evaluation when a significant difference was detected in multiple comparison. Wilcoxon sign test was used for intra-group comparisons. Significance was set at $p < 0.05$.

3. RESULTS

All groups showed Ra increases in intra-group comparisons (p=0.028). No significant difference was detected between the initial roughness of the groups before instrumentation (Table 1). Multiple comparisons of after-treatment values and differences before and after instrumentations revealed statistical significances (p=0.041; p=0.016, respectively). When after-treatment mean values and mean differences between the groups were evaluated in pairs, statistical significances were detected between the groups of (ST and CUST) (p= 0.031; after-treatment) (p=0.035; mean difference), (PCT and CUST) (p= 0.027; after-treatment) (p=0.017; mean difference) (Figure 2).

***Table1. Roughness** values of the groups*

	ST	PCT	CUST	
Roughness (µm)	**Mean±SD (median)**	**Ort Mean±SD (median)**	**Mean±SD (median)**	**[1]p**
Before	0.17±0.03 (0.17)	0.15±0.06 (0.13)	0.16±0.04 (0.14)	0.523
After	0.27±0.02 (0.26)	0.24±0.12 (0.22)	0.46±0.25 (0.38)	0.041*
[2]p	0.028*	0.028*	0.028*	
Difference	0.10±0.04 (0.1)	0.09±0.09 (0.05)	0.30±0.21 (0.23)	0.016*

*[1]Kruskal Wallis Test [2]Wilcoxon sign test [*a] significant difference (p<0.05). ST: Scaler tip, PCT: Perio-curette tip, CUST: Conventional ultrasonic scaler tip*

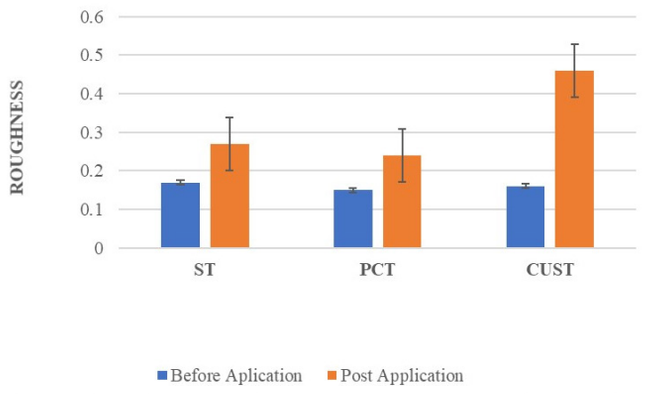

***Figure 2.** Diagram of roughness changes after surface instrumentations with straight, perio-curette and conventional ultrasonic scaler tip*

5. DISCUSSION

The main goal of nonsurgical periodontal therapy is to reduce or to eliminate the amount of tooth associated biofilms and their biological products, such as bacterial endotoxins, antigens, enzymes and other tissue-irritating substances on root surfaces (30). Nominately both primary etiologic factor as supra/subgingival plaque biofilm and other disease contributing factors on the root surfaces should be removed during nonsurgical periodontal therapy. These can be achieved through especially changing the subgingival environment by root debridement procedures. Instruments that are used for root debridement include hand instruments, ultrasonic devices, air-powder abrasive systems, and lasers etc.

The root surface roughness influence the supragingival and subgingival plaque biofilm formation (31). Conventional root instrumentation with curettes removes root irregularities that harbor plaque and calculus, and renders the diseased root surfaces free of detectable endotoxins (32-34). Therefore, there is a demand for smoothness on after-treatment surfaces in order to minimize plaque formation, thereby reducing periodontal or restorative needs. The roughness of the root surface after debridement is a factor to consider for maintenance, because it has been shown that bacterial plaque biofilm adheres easily onto rough root surfaces (35) and initial bacterial adhesion always occurs on surface irregularities (31).

According to the literature, ultrasonic devices are less time consuming but leave more rough surfaces after instrumentation compared to hand instruments (36). However, it has been found that new age ultrasonic systems are described as delicate but also effective in removing plaque and calculus (24). The results of our present study showed that the LOD application with oscillating vertical movements on root surfaces seemed to be gentler with regards to roughness parameters than the conventional ultrasonic scaler. It has been proposed that Ra value of 0.2 µm was the threshold of initial bacterial adhesion on root surfaces

(31). Ra for both the ST (0.27±0.02 µm) and PCT (0.24±0.12 µm) was close to this threshold value even without using the polishing liquid of the LOD system containing HA particles, expected to smoothen the surface. On the other hand, Ra value in the literature for various sonic or ultrasonic devices varies within a range from 0.6 to 1.8 µm (37,38). The CUST result of this present study (0.46±0.25 µm) was found below this range, but greater than ST and PCT Ra values. In this study LOD tips caused the least amount of roughness increase and the results are compatible with the findings in the literature as above.

The used new generation LOD instrument with two different fine tips comprises a ring-shaped resonant body vibrated by an ultrasonic drive (at 25kHz), which is attached to the working end at an angle of 90°. This configuration eliminates ellipsoid vibrations of the tips moving in a plane parallel to the tooth surface, contrary to the horizontal vibrations in conventional ultrasonic scalers (17). As a result, the tips move parallel along the axis of the special handpiece presenting an obvious difference mechanism of action. One of the limitation of this in vitro study maybe the performance of the tested instrument are expected to be higher due to lack of one-to-one stimulation of the oral conditions.

6. CONCLUSION

In conclusion, the root surface roughness with the investigated ultrasonic system significantly depends on the selection of handpieces and tips. Within the limits of this study, fine and delicate tips with linear oscillating movement may be considered as the choice of insert for subgingival instrumentation due to the gentler mechanism of action than the conventional ultrasonic scalers.

Disclosure Statement

The authors do not have any financial interest in the companies whose materials are included in this article.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

The association of factor V Leiden mutation (G1691A) with pregnancy complications (miscarriage) in the Iran, East Azerbaijan

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ABSTRACT

Objective: The pathogenesis of human spontaneous abortion involves a complex interaction of several genetic and environmental factors. Resistance to anticoagulant activity of activated protein C (APC) is often due to point mutations in the Factor V gene. Leiden mutation is definitely associated with pregnancy complications. This study investigated the association of factor V Leiden with recurrent miscarriage in Iranian patients.

Methods: 200 women included in this study: 100 women as patients' group with two or more consecutive unexplained miscarriage and 100 women as controls group with at least one childbirth and without any abortion or pregnancy complications. Genomic DNA is extracted from the must be delete from peripheral blood of each person. Presence or absence of mutations in the factor V Leiden gene performed by factor V Leiden coagulation test, and to determine homozygous and heterozygous of the factor V Leiden mutation, used HRM techniques by PCR Factor V Leiden Kit.

Results: In this study, Chi-square analysis was a significant relationship between mutant G1691A and recurrent pregnancy loss, and other environmental variables in the statistical analysis, gestational age, and family history, statistically significant association with recurrent pregnancy loss.

Conclusion: The results of this study showed that Factor V Leiden mutation has an effective role in the risk of pregnancy complications and recurrent miscarriage. Factor V Leiden mutation frequency in various countries due to genetic differences and different geographic can prone to recurrent pregnancy loss, better management of patients, and adoption of effective preventive methods.

Keywords: Recurrent pregnancy loss, miscarriage, Factor V Leiden, mutation, pregnancy

1. INTRODUCTION

Aborsement is denominated as frequent spontaneous abortions and losing the pregnancy product. Frequent spontaneous abortions are classically defined as thrice spontaneous abortions in 20 weeks of gestation or less or the birth of fetuses less than 500 grams (1). Genetic abnormalities in recurrent miscarriages in the first quarterly are significantly low. Karyotype was normal in half of all recurrent miscarriages; this was only a quarter of the sporadic abortions. Since the timing of abortion can lead us to the cause of the miscarriage, it is observed that autoimmune or anatomical abnormalities generally cause miscarriage in the second quarterly (2). Additionally, the prevalence of recurrent miscarriage is one in every 300 births. Epidemiological studies have shown that 1-2% of women have experienced recurrent miscarriages (3). Recurrent miscarriage as a clinical problem requires diagnostic tests and therapeutic interposed to reduce the risk of the disease and then find a treatable cause for the disorder. Mutation of FVL in people is according to each record and leads to pregnancy complications such as miscarriage.

Generally, women who are getting frequent abortions have some problems in their blood coagulation system. During blood circulation, some clots erupt in capillaries, which may be hemophiliac to the fetus (4). It seems clot creation in blood vessels or thrombosis is probably caused due to disorder in blood circulation between mother, fetus and fetus and will lastly cause fetus abortion (5). One of the main genetic factors that have a role in thrombosis creation thrombosis is Factor V Leiden. The clot demonstration reason is a mutation in the factor V gene. Factor V acts as a helper agent in enzyme reaction, creates fibrin in a clot (6). According to the lack of studies in genetic variants prevalence of this factor in the Azerbaijan region, this research aims to evaluate the different alleles abundance of factor V in the G1691A situation among women in East Azerbaijan.

Resistance to active protein C is characterized by plasma resistance to the anticoagulant effects of active protein (7). Thrombin (factor IIa) converts fibrinogen to fibrin and active platelets, and this starter protein initiates the responsibility for fibrin inhibition activities (8). When thrombin binds to

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existing thrombomedulin at the level of endothelial cells, it is activated and can convert protein C to the active form. Protein C is only active when it binds to its cofactor (the S protein) is connected. Protein S is active when it is not bound to C4b. At baseline, about 40% of S protein is unconnected. The active C / S protein complex decomposes the Va and VIIa factor, reducing fibrin formation and ultimately reducing blood clotting. The role of these factors during pregnancy is vital; a successful pregnancy requires proper growth and development of placental circulation (9&10).

Factor V Leiden mutation inhibits APC's effects on factor V and is inherited in form of dominant autosome (11). This missense mutation replaces glutamine (CAA) instead of arginine (CGA) at position 506 of polypeptide factor V and 1691 position of gene F5, Exon 10. This case is due to omitting one of definition position factor V by APC. Finally, factor V is resistant to decomposition by activated protein C and facilitates the occurrence of thrombosis (20). This gene is cytogenetically located on the long arm of chromosome 1 at situations 23-24. And molecularly includes 169481191 to 169555768 pair bases. Factor V gene has 25 exon and 24 intron, and the size of this fragment is a 75-kilo base. Heterozygote form of active factor V is the most common hereditary thrombophilia (8). Factor V Leiden mutation can be found in about half of non-pregnant who, suffering from thrombophilia diseases. In heterozygote form, risk of thrombosis emergence increases ten times in pregnancy (12). The resistance of active protein C was measured in the biometric method. It should be noted that, anti-phospholipid antibody syndrome can cause the resistance of active protein C. the early pregnancy naturally increases the resistance due to changes in other coagulation proteins. During pregnancy, DNA analysis is used to confirm the mutation in the V factor gene (9). Studies have shown that, mutation of factor V Leiden increases the risk of the first episode of intravenous thromboembolism during pregnancy about 4 to 8 times. In addition to the aforementioned mutations, other single-nucleotide polymorphisms have been reported in the factor V gene, including the following: SNP in the promoter region (99930G> A), exon 16 nucleotides (42855A> G), intron 19 (37833T> G). (13) Coagulation Factor V plays a key role in regulating the homeostasis of coagulation pathways. GT691A point mutation in the coagulation factor V gene is called the Leiden thrombophilia factor (FVL), and it eliminates a breakdown position in coagulation factor 5; therefore, by affecting the function of the thrombinase complex, it induces resistance of coagulation factor V to activated protein C (APCR), which is associated with recurrent miscarriage (14). FVL polymorphism is due to increased risk of fetus abortion in people who carrying this polymorphism more than three times (6%-16%) in comparison with to healthy people. Many investigations approved the increasing risk of frequent abortion in women with this polymorphism. Coagulation V factor acts as a co-factor for factor X. Factor X converts prothrombin to thrombin, which plays an important role in the coagulation cascade. This factor analysis by active protein C. However, the V-factor Leiden is not inactivated by the action of protein C, which causes clots in the individual. As mentioned, the Leiden variant was caused by a G1691A point mutation in Exon 10. Because of this missense mutation, arginine amino acid converted to glutamine. FVL mutation prevents FV digestion, accordingly cause to blood clotting (15). May be deep vessels thrombosis (DVT) arising as a painful and protuberant and achy area (usually in the foot). If thrombosis is displaced, it might cause an obstruction of pulmonary bloodstream alongside dyspnea, tachycardia, arrhythmia, or sudden death. Insufficiency of C or S Proteins and presenting the FVL mutation are dangerous factors for causing effects on DVT (16). In 20% to 40% of people with signs of venous thrombosis, FVL mutation is observable, which is the most common factor of causing the effects on DVT. Heterozygotes with a copy of FVL mutation have a 7-fold increase in the risk of infection, and homozygotes with two copies of Leiden FV mutation have an 80-fold increased risk of DVT. It is expected that positive homozygote people have at least a thrombotic event during their age, while heterozygote people, according to thrombotic problems expression, are different (16).

Due to the deep relationship between thrombophilia and recurrent miscarriage, these polymorphisms were studied in various non-Iranian and Iranian populations (for a limited time) for many years, and contradictory results were obtained from these studies. For this reason, the study of these polymorphisms is recommended once again. If it is widespread in this area, it can be used as a diagnostic marker in women who have recurrent miscarriages. Undoubtedly there is a correlation between factor V mutation and pregnancy complications and the occurrence of recurrent miscarriage; Therefore, it was hypothesized that this relationship could be present in people with recurrent miscarriages in the East Azarbaijan region Tabriz city, which persuaded me to do this research. The purpose of this study is to investigate the relationship between mutation FVL in causing pregnancy complications and recurrent miscarriage in this region, which can consequently help us in the treatment process, prevention of such complications, and having healthy children.

2. METHODS

The research was done in Doctor Arami medical laboratory by evaluations accomplished and essential data obtained with a questionnaire. 100 patients and 100 healthy subjects (as a control group) with a mean age of 45 ± 1.28 in the age group of 18-45 years were selected randomly. The Control group were pregnant women who had no pregnancy complications and were healthy. The patient group was pregnant women who had a background of abortion. All health and patients took written subscriptions then registered their information about the number of children, occupation, place, and age of the pregnancy. Patient sampling does not require special preparations. 5 ml of blood took from people, 3 ml of which were transferred to the EDTA test tubes for PCR tests and 2

ml to the sodium citrate test tubes for the coagulation panel tests. Blood samples were stored in the freezer at a +2 /+ 8 degrees temperature for three days. For long-term storage, samples stored between – 85 and – 10 degrees.

Enter criteria to the study were pregnant women who had previous abortion affection and each complications kind of pregnancy because of thrombosis and exit criteria were existing anatomical raucousness, hormone disorders, autoimmune diseases, approved infection of genital and person who used heparin, aspirin or enoxaparin. Information obtained by a questionnaire was adjusted after reviewing texts and scientific articles and collected according to study aims, and blood sampling was directly from women.

Data collection tools included questionnaires and sampling of healthy pregnant women and pregnant women with miscarriages who were referred to gynecologists. At the request of the medicine, the patient was referred to the relevant laboratory and the pregnant women with abortion whose disease previously confirmed by the treating medicine and performed tests and the control group who did not have any evidence of any personal or family history of abortion with full consent examined with complete satisfaction.

This research was accepted with the Ethical standard code: IR.IAU.TABRIZ.REC.1395.63 in 06/17/2018 in Faculty of Medical of Islamic Azad University of Tabriz branch.

2.1 Clotting Panel test

In the possible shortest time (maximal 2 hours), the sample was centrifuged at 2500-3000 rpm for 20 minutes and separated plasma. Therefore, kept in – 20 degrees centigrade. HEMOCLOT Quanti.

V-L Ref CK065K done by coagulation, fully automatic method. The test does in 37 degrees centigrade. The device itself automatically adjusts this temperature during the test. The rack stand for the samples has a barcode reader system to detect the sample entered into the device. Regent solutions also placed in their own racks and identified by the device's barcode reader system. After placing the samples and solutions, we set the device, 1. from the main menu, press the start key, wait until the sample loading station lights change to green. Slide the sample tray into the sample loading station. 2. Press the ID entry area. Write and confirm the ID for each sample. 3. Confirm the test order. 4. Press the corresponding number field in the "POS" column. After these steps, we started the device and waited for the answers. After a few minutes, the answers were prepared on the device monitor. Results greater than two were reported as normal, and results less than 1.8 were positive (with V factor mutations). After confirming the results of the coagulation test, the HRM test was done with a Real-Time PCR device to detect the heterozygote and homozygote of the samples. Purification of nucleic acid should do according to the desired kit GeneProof Factor V Leiden lot No. P4101-16097 prepared for specific clinical materials. The nucleic acid separation must adjust to prepared intended kit for special clinical materials. Whole blood kit DNA for purification of DNA used health and purified blood. Using method in the present study was an ex-filled cartridge including salt for cells lysis and breaking proteins. DNA is surrounded by cellulose cover, which has magnet grains. After washing pollutants, purified DNA was washed with low salt washing buffer. Purified DNA was obtained about 20-30 kb long in this level proper of PCR. This method replied for all existing samples in the test and due to doing the polymerase chain reaction test with real-time device moved to the freezer, after ramp rate setting with real-time device samples placed in the device as the following progress: 1. Determine the RT-PCR approach. In performing RT-PCR, one-step and two-step methods are the two common approaches, each with advantages and disadvantages. 2. Prepare sample. 3. Design primers. 4. Remove genomic DNA. 5. Perform one-step RT-PCR. 6. Determine the RT-PCR approach. 7. Prepare sample. 8. Design primers. After samples, embedment, and program set up, we started the device and waited for results. The PCR Steps Explained: Step 1 – Denaturation. The solution contained in the tube is heated to at least 94°C (201.2°F) using a thermal cycler. Step 2 – Annealing. Step 3 – Extension. Step 4 – Analysis with Electrophoresis. Results got ready after one and a quarter-hour. Obtained results had some graphs.

2.2 Evaluating results of HRM

Results of HRM were obtained into graphs, in which FAM and HEX graphs reflect the kind of person genotype (homozygote and or heterozygote) (Figure 4).

In figure 5, there were both positive and negative results. FAM and hex graphs are both ascending, which present to be heterozygote of the sample. Ascending FAM and anticlimactic hex graphs indicate to being homozygote of the sample. GG genotype indicates the sample to be homozygote. Demonstrate AG genotype, which is show sample, be heterozygote and positive Factor V mutation (Figure 6).

2.3 Method of data analysis

After experimental techniques and collecting the genetic data, this information merged with another data outcome from questionnaires and after editing in excel software entered to SPSS software. On the device monitor were appearances some graphs adjust to 1, 2 and 3 figures that show the kind of gene and with the help of obtained graphs can diagnose the heterozygote or homozygote of the sample (Figure. 1, 2, 3). For accounting allele and genotypes abundance, chi-square statistical and Hardy-Weinberg balance test used pop gin s1 software and studied the relation of genotype kind and presence or absence of abortion in women used chi-square test in probability level of 95% and SPSS software version 24.

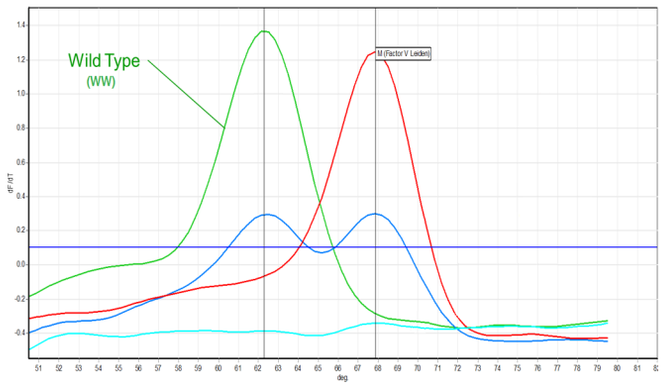

Figure 1. *Graph related to negative sample or WW genotype*

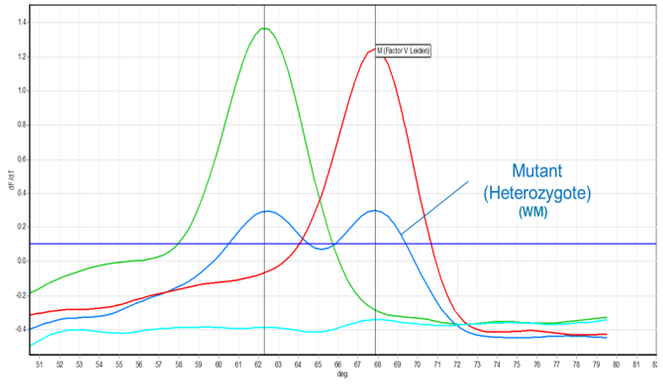

Figure 2. *Graph related to heterozygote positive sample or WM*

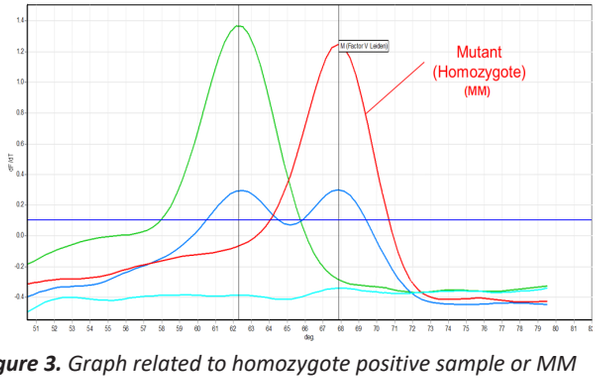

Figure 3. *Graph related to homozygote positive sample or MM*

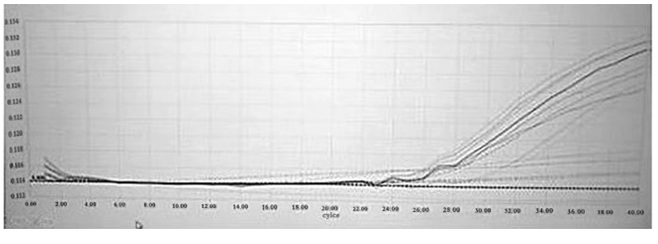

Figure 4. *Image of FAM and HEX graphs*

Figure 5. *GG genotype graph*

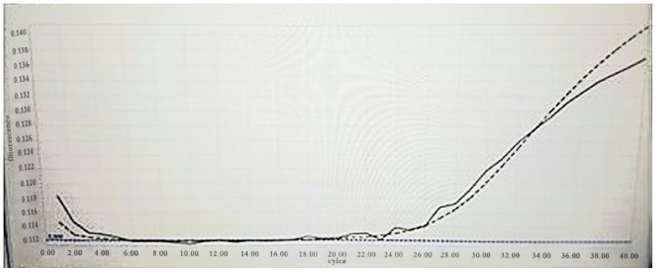

Figure 6. *Ag genotype graph*

3. RESULTS

3.1. Genotypic analysis of test groups

According to HRM obtained data, abundance genotypes from patient and control groups are like table 1 and table 2.

Table 1. *Abundance genotypic in-patient group*

Allele abundance	Number	Observed abundance	Expected abundance	Chi-square	Allele abundance
AA	0	25	0.029	3.95	A = 0.17 G = 0.83
AG	32	0.64	0.102		
GG	62	85	0.25		

Table 2. *Abundance genotypic in control group*

Control group	Number	Observed abundance	Expected abundance	Chi-square	Allele abundance
AA	0	0	0	0.003	A = 0 G = 1
AG	0	0	0.01		
GG	73	50	0.99		

3.2. Statistical analysis

Obtaining the expected frequency and using chi-square coefficient to obtain a value of p<0,05, it concluded that there is a significant relationship between the parameters like

pregnancy age (P=0.01), family history (P=0.01), and recurrent miscarriage with V-factor Leiden (P=0.02).

GG allele abundance is high in control and patient samples, which is reckon normal factor. Moreover, the AA allele was not observed in any of the samples. Also, AG allele was just observed in samples with high age and previous familial affected to frequent abortion. Generally, observed genotypes abundance in both groups is in table 3.

Table 3. *Genotypic abundance in both patient and control groups*

Total	Number	Observed abundance	Expected abundance	Chi-square	Allele abundance
AA	0	0	0.0092	1.87	A = 0.096 G = 0.904
AG	32	0.54	0.1733		
GG	135	0.12	0.8176		

3.3. Evaluating number of laborers with frequent abortion

Results of table 4 demonstrated the number of labors in studied samples that the most sample members are their first pregnancy. There is no significant relation between frequent abortion and numbers of labor.

Table 4. *Number of labors by tests*

Number of labors	Number	Percent
0	53	56.5
1	74	37
2	12	6
3	1	0.5

3.4. Studying relationship between previous familial with frequent abortion

Analyzing the last familial indicates the presence of significant relation between access abortion and previous familial diseases that 171 people of sample members had previous familial diseases and 29 people had no previous familial diseases (Table 5).

Table 5. *Previous familial diseases by tests*

Previous familial	Number	Percent
Had	158	85.5
Hadn't	29	14.5

3.5. Evaluating effective factors on results of coagulation test

Results of the correlation test indicate the effect of each three Leiden genotypes (P=0.02), previous familial (P=0.01), and age of a pregnancy (P=0.01). Factors are significant, and they significantly influence the coagulation test (Table 6).

Table 6. *Evaluating effective factors on result of coagulation test*

	MS	P value
Leiden genotype	1.03	0.027
Previous familial	3.91	0.01
Age of pregnancy	1.744	0.011
Error	0.262	-

3.6. Evaluating genotype effect

For surveying the effect of patient genotype on number of abortions, used Mann-Whitney test, which results indicate that genotype has a significant effect on abortion number (P=0.01) and heterozygote genotype is more than homozygote due to more abortion numbers (Table 7).

Table 7. *Evaluating effect of patient genotype on abortion numbers*

Genotype	Number	Average level	Mann-Whitney test	P value
AG	32	122.7	921.5	0.001>
GG	135	74.83		

3.7. Effect of the presence of previous familial

Also, due to indicating previous familial on frequent abortion, Mann-Whitney test and results of test demonstrated that previous familial has a significant effect on frequent fetus abortion (P=0.01) and samples with previous familial had more frequent fetus abortion than samples without previous familial (Table 8).

Table 8: *Studying effect of presence previous familial*

Previous familial	Number	Average level	Mann-Whitney test	P value
Presence	29	154.86	903.00	0.001>
Absence	171	91.28		

3.8. Compact people genotype

Due to compact health and patient people genotype, using chi-square test, which test results indicated genotype in health and patient people of samples are different with each other (P=0.01), and heterozygote genotype were more than homozygote genotype in patient samples, and health samples were more homozygote genotype (Table 9).

Table 9. *Compact genotype of health and patient samples*

	AG	G genotype	Chi-square
Patient	32	62	0.001>
Health	-	73	

4. DISCUSSION

According to the National Center for Health, pregnancies include 13 percent of births. Fertility under 19 and over 34 is considered high-risk pregnancies (17). Successful pregnancy is a pregnancy which the fetus grows and develops while maintaining mother's health, leading to the birth of a healthy baby (18). Bleeding, infection and bacterial shock (infection-induced shock) are complications of abortion. Patients who have had a previous miscarriage are about 20% more likely to have a miscarriage in their next pregnancy (19). In some women, fetal developmental defects in the fetus can lead to a bicornuate Uterus or bicornuate or various forms of uterine deformities, leading to miscarriage. Fibroma or uterine viscosity can also cause miscarriage in pregnant women (20). The risk of miscarriage is related to the mother's age, as studies show that abortion is more common in pregnant women under the age of 20 and over 40. Increasing abortions are observed in very young or old fathers. Abortion rates with women's pregnancy increase within three months of giving birth (20). How APS can cause recurrent miscarriage is not fully understood, but what is the evaluation of antibodies to anti-cardiolipin and lupus anticoagulants in these patients could be helpful. The recommended treatment for these people is a low dose of aspirin with heparin (21). Normal homeostasis requires balancing between pre-coagulation and anticoagulant factors. Factor V is one of the important pre-coagulation factors. Factor V glycoprotein acts as a prothrombinase complex and converts the protein to thrombin to form fibrinogen from the fibrin of the polymerized network and to form the primary clot (22). The A1691G variant is known as the FVL, which is the most common disorder of all three variants. It is associated with increased coagulation and increases the risk of thrombosis in the venous arteries of various tissues. FVL Polymorphism has a predominant autosomal recessive factor and occurs at the displacement of a nucleotide (23). The G>A mutation in nucleotide 1691 on Exon 10 of factor V altered arginine amino acid to glutamine, which eliminated the main site of failure at 506 and led to the resistance of factor V active to protein C function. In normal people, after the clot formation, the remnants of active factor V by protein C activated in arginine 506 site broke and inactive, but in people with factor V lead, factor V refracted to analysis, decompose, and remains active for a longer period. Increased risk of thrombosis associated. Fracture factor V was activated by protein C activation and protein S cofactor was performed. In these people, an increase in thrombin leads to the production of excess fibrin and more clots produced. 90 to 95% of mutations are heterozygous and other cases are homozygous (24). Pregnant women who have a FVL mutation are more likely to have recurrent miscarriages, preeclampsia, or even stillbirth. Thrombosis in placental capillaries disrupts the blood flow to the mother and fetus and eventually leads to miscarriage (25&26). FVL polymorphism is present in approximately 5% of the Caucasian population (whites), 3% – 8% of Europeans, and 4% – 7% of Americans, and is less common among Asian populations (27). The FVL allele of Europeans is 1 – 8.5 percent and is less common among Israelis, with large immigrant backgrounds, the allele frequency various, but, the highest rate reported among Turkish and Greek about 0.87.(13). In Iran, due to the existence of various ethnic groups and various indigenous groups, the Allele frequency for the FVL is somewhat different from the living area and ethnicity (27). In Tehran, the prevalence of FVL is 5.5% and the allele frequency is 2.7%. In southern Iran, the prevalence rate is 4.1% and the frequency of allylic for the V-factor varies is different from 0.207 to 0.209. The obtained allylic frequency in the present study in the northeast of the country is equal to 0.19% for the AG genotype and 0.808% for the GG genotype, and the prevalence of the V-factor is 1.8%. Which is more than the west and south of Iran and has a significant difference (1). The study of the Australian population in 2012, reported that heterozygosity related to intra-uterine death risk of the fetus, placenta separation, and clamps of preeclampsia for V Leiden factor mutations (28). A study in 2011, defined that hereditary thrombophilia is related to a high risk of thromboembolism and accompanied by undesirable pregnancy pages (29). In 2012 in Poland, defined Leiden Factor V mutation as a common disorder in patients with an abortion background, and clearly, it has a higher prevalence with frequent abortion and suggested that Leiden Factor V mutation was high in person with frequent abortion (27). A study in 2012 reported that Leiden Factor V mutation was observed in 4.5% of studied people who had frequent abortions (18). In a study in 2013, scientists reported that one of the effective reasons for increasing affliction risk to frequent abortion is Leiden Factor V mutation (29).

In our study, similar results were obtained and a significant correlation was found between heterozygous motility factor V Leiden and miscarriage risk. Chi-square analysis was a significant relationship between mutant G1691A and recurrent pregnancy loss, and other environmental variables in the statistical analysis, gestational age, and family history, statistically significant association with recurrent pregnancy loss.

5. CONCLUSION

In the present study, mutations in the factor V gene were investigated by using the HRM technique in 100 patients with recurrent miscarriage in northeastern Iran (Tabriz) whose disease was confirmed by relevant specialists and 100 healthy individuals treated as a control group. By examining and identifying mutations, genetic testing can diagnose recurrent miscarriages in people with the disease, and early detection of the disease can be a big step in treating these people and reducing fetal mortality from the disease. The frequency of allylic obtained in the present study is 0.19% for AG genotype

and 0.8% for GG genotype and the prevalence of factor V Leiden is 1.8%. In addition to studies, there is no relation to the situation of occupation, housekeeping and educational situation, and the number of labor and pregnancy with the rate of patient affect. The result of the correlation test demonstrates that in patient groups every three factors of Leiden genotype, previous familial, and age of pregnancy on coagulation test is more than to health group and has a significant effect on coagulation test. This means there is a significant and direct relationship between the age of the pregnancy, previous familial and frequent abortion.

In this research, we found a significant relation between heterozygosis of Leiden Factor V mutation and the risk of abortion. And it concluded that surveying Leiden Factor V as the main marker in patients affected by frequent abortion and pregnancy complications can avoid to next abortions and aggravation of pregnancy complications in the future. Some researchers suggested that a mutation in the factor V Leiden gene as a marker for recurrent miscarriage and thrombosis, due to its low frequency, could not affect the diagnosis, but in our study, it concluded that the factor V Leiden investigated. The title of an important marker in patients with recurrent miscarriages and pregnancy complications can prevent further miscarriages and exacerbation of future pregnancies. PCR testing is used only once to diagnose the type of mutation; therefore, patient control will only be possible by testing the low-cost V factor coagulation panel. In order to ensure the results, more studies proposed to confirm or reject the findings of this study. So that, investigate the relationship between recurrent miscarriage and other genetic factors, the causes of lack of factors in patients and better matching between the case group and the control in terms of other patients can examine.

The authors do not have conflict interests. We thank all people who participated in this research.

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Clinical and Experimental Health Sciences

The Effect of Gestational Diabetes on Depression and Breastfeeding Self-Efficacy in Pregnancy and Postpartum Period

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ABSTRACT

Objective: This study aims to explore the effect of gestational diabetes mellitus (GDM) on depression and breastfeeding self-efficacy during pregnancy and the postpartum period.

Methods: This descriptive study was conducted in Obstetrics and Perinatology clinics of two university hospitals in Turkey between July 2016 and June 2017. Women were followed up twice. The first follow-up was performed face-to-face in the gestational week 34th to 38th and the second one was performed by telephone in the 8th week of the postpartum period. In the first follow-up, 104 pregnant women with GDM and 133 pregnant with non-GDM women were interviewed. In the second follow up, 30 women could not be reached in both groups. Data were collected by the Individual Description Form, Edinburgh Postpartum Depression Scale, and Breastfeeding Self-Efficacy Scale-Short Form. Descriptive statistics, repeated measures analysis of variance, and correlation analysis were used in the analysis.

Results: The depression risk of mothers with GDM was found higher compared to non-GDM mothers. No significant difference was found between the depression and breastfeeding self-efficacy mean scores in the antenatal and postpartum period of women by the presence of GDM. There was no significant difference between the depression and breastfeeding self-efficacy mean scores in the antenatal and postpartum period by the presence of GDM and some confounding variables.As the breastfeeding self-efficacy level of mothers with GDM increased, the depression risk decreased.

Conclusion: It has been concluded that GDM does not have an impact on depression and breastfeeding self-efficacy during pregnancy and in the postpartum period.

Keywords: Breastfeeding self-efficacy, depression, gestational diabetes, nursing

1. INTRODUCTION

Gestational diabetes mellitus (GDM) first occurs in pregnancy especially in the second or third trimester and is defined as the hyperglycemia that continues during pregnancy (1). The prevalence of GDM in all pregnancies is approximately 7% (2). International Diabetes Federation stated that 21.3 million or 16.2% of live births in a year had hyperglycemia during pregnancy and 86.4% of these cases were affected by GDM (3). The prevalence of GDM in Turkey changes between 3.17% and 9.2% (4,5). There are four factors that may lead to lower breastfeeding rate in women with GDM: increased cesarean rate, delayed lactation and milk production, separation of newborn from the mother, and early feeding provided to newborn with formula (6–8). Previous studies (9–12) have reported that breastfeeding rates of the mothers with GDM are low, breastfeeding duration is short, and they have a negative perception about the inadequate lactation. These negative thoughts about breastfeeding are related to the breastfeeding self-efficacy perception of the mother which is one of the factor affecting breastfeeding (13). Breastfeeding self-efficacy perception is influenced by four main sources of information; namely the individual's previous breastfeeding experience, others' experiences, the presence of support for breastfeeding and such psychological answers as anxiety, stress, fatigue and depression (13–15).

The increase in insulin resistance during pregnancy may cause the development of GDM and also depression (16). Increase in the inflammatory response and in stress hormones such as cortisol during pregnancy (17), increase of insulin resistance and hyperglycemia combination may increase the probability of depression in pregnant women with GDM (16). Pregnant women with GDM were more likely to have a higher rate of depression compared to healthy pregnant women, but the

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difference was not significant (18,19). Huang et al. reported that hyperglycemia was correlated to antenatal depressive symptoms in pregnancy but it wasn't correlated to postpartum depression (20). In another research (21), it was reported that women with GDM were at risk for antenatal depression three to four times higher compared to non-GDM women when the age, income level, education level, and parity were adjusted.

Few studies focused on the relationship between depression and breastfeeding self-efficacy, being one of the psychological responses that may affect the self-efficacy perception of the mother about breastfeeding. It was reported in two previous studies that (22,23) the higher the depression scores of mothers were, the lower their breastfeeding self-efficacy was.

The negative mood of women with GDM, as a result of their higher inclination to depression, reduces the self-efficacy of the individual and may negatively affect the breastfeeding self-efficacy. In addition to the negative impact of existing insulin resistance on breastfeeding in women with GDM, the presence of depression reduces their breastfeeding self-efficacy and may increase the negative breastfeeding outcomes in postpartum (14,15,24). In this regard, the evaluation of breastfeeding self-efficacy in pregnant women with GDM in the antenatal period is of great importance. The purpose of this study was to explore the effect of GDM on depression and breastfeeding self-efficacy during pregnancy and the postpartum period.

2. METHODS

2.1. Design and Population

This descriptive study was conducted in Obstetrics and Perinatology clinics of two university hospitals in Izmir province between July 2016 and June 2017. The number of individuals to be included in the sample by using the calculation formula for sample size with an unknown population (25) was determined as 100 (GDM prevalence was accepted as 0.07 according to ADA (2). One hundred and four pregnant women with GDM and 133 non-GDM pregnant women who complied with the inclusion criteria were incorporated into the study through a convenience sampling method.

The inclusion criteria for both groups are as follows: (a) Elder than 18 years old, (b) reading and writing in Turkish, (c) being in the gestational age 34th to 38th, (d) not being diagnosed with chronic illnesses (Type 1 and Type 2 Diabetes Mellitus, renal failure, epilepsy, hypertension etc.), depression or any other psychiatric disease before, (e) voluntary participation in the research. In addition to these inclusion criteria, the special conditions were (f) not having any other pregnancy complication apart from GDM for GDM group and (g) not having any pregnancy complication for the non-GDM group.

2.2. Measurements

Data were collected by using "Individual Description Form", "Edinburgh Postpartum Depression Scale (EPDS)" and "Breastfeeding Self-Efficacy Scale (BSES) – Short Form".

"Individual Description Form": The form prepared by the researcher using the literature (5,9,10,14,22) which was composed of antenatal and postpartum period description form. This form contains 23 questions related to socio-demographic and obstetrics characteristics and breastfeeding conditions of the women.

"Edinburgh Postpartum Depression Scale (EPDS)": The scale developed by Cox et al. (26) is used to determine both the antenatal and postpartum depression risk (26,27). This is a self-evaluation Likert type scale composed of ten questions. Turkish reliability and validity of the scale was performed by Engindeniz (28) in 1996. The cut-off score of the scale was stated to be 12-13 and the individuals getting the score of 12 and above were evaluated as a risk group. Cronbach's alpha value was found as 0.79 in the reliability and validity study of Engindeniz (28) In this study, Cronbach alpha value was determined as 0.78 in the antenatal and postpartum period.

"Breastfeeding Self-Efficacy Scale (BSES) – Short Form": This five-point Likert type scale was developed by Dennis and Faux (15) and composed of 14 items that evaluates how competent the mother feel about breastfeeding. The lowest score that can be obtained from the scale is 14 while the highest score is 70 and an increase in scores denotes that breastfeeding self-efficacy is higher. The scale is suitable to use in the postpartum period but it can also be used in antenatal period by using the "future tense" in the items of the scale (15,29). Cronbach alpha value of the scale was found as 0.94 by Dennis and Faux and 0.86 by Aluş Tokat and Okumuş who performed its Turkish reliability and validity (30). In this study, Cronbach alpha's value was found as 0.84 for Antenatal Breastfeeding Self-Efficacy Scale and 0.74 for Postpartum Breastfeeding Self-Efficacy Scale.

2.3. Data Collection

Data were collected by the first researcher from pregnant women, who came for routine control, in the Non-Stress Test polyclinic in the hospital. The researcher regularly went to the hospital two days a week to collect data. Women in both groups were followed up two times. The first follow-up was performed face-to-face in the gestational age 34th to 38th and the second one was performed by telephone in the 8th week of the postpartum period. In the first follow-up, 104 women with GDM and 133 non-GDM women were asked to fill Antenatal Description Form, EPDS, and Antenatal BSES-Short Form. In the second follow-up, 74 women with GDM and 103 non-GDM women were reached by telephone and they were asked to fill Postpartum Description Form, EPDS, and Postpartum BSES-Short Form. In the second follow-up, 30 women could not be reached in both groups (Figure 1).

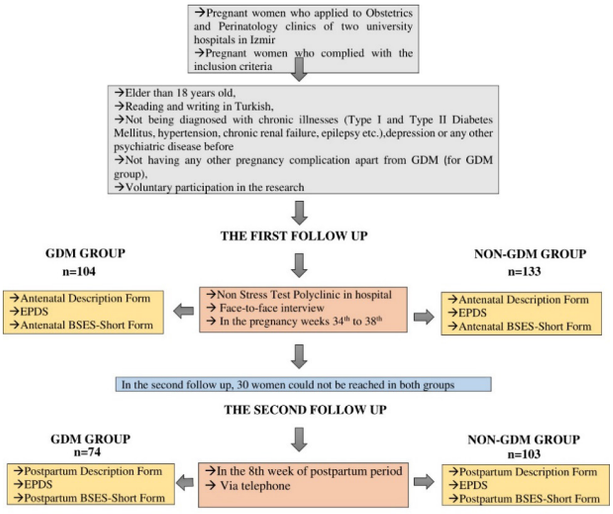

Figure 1. *Flow diagram of the research process*

2.4. Data Analysis

The data were analyzed by using SPSS 25 (IBM Corp. Released in 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). All values were presented as mean±standard deviation, percent, and frequencies. Repeated measures of analysis of variance were analyzed by Mauchly's sphericity test and Box's Test of Equality of Covariance Matrices. "Repeated Measurements Analysis of Variance" was used to compare the means of repeated measurements. If parametric tests (factorial design for repeated measures analysis) did not provide the preconditions, Greenhouse-Geisser (1959) correction or Huynh-Feldt (1976) correction was used for corrections to the "Degrees of Freedom" or "Friedman Test". "The Corrected Bonferroni Test" was used for multiple comparisons. Variables were evaluated after controlling for normality and homogeneity of variances with the "Shapiro Wilk and Levene Test". "Pearson Correlation Coefficient" was used for the relationship between continuous variables, and "Fischer's Exact Test" and "Chi-square Test" were used for categorical data. p values<0.05 were considered statistically significant.

2.5. Ethical Considerations

Ethical approval was obtained from the "Non-Interventional Clinical Research Ethics Committees" of both universities in which the study was performed (Date: 26.05.2016, Decision No: 129; Date: 26.01.2017, Decision No: 2). Written consent was provided by participants and hospitals where the study was conducted.

3. RESULTS

The descriptive characteristics of the women are shown in Table 1. The mean age (t=3.905; p=0.001), number of pregnancies (χ^2=6.684; p=0.035) and the rate of GDM history in previous pregnancies (χ^2=13.336; p=0.001) were higher for the mothers with GDM compared to non – GDM mothers. The mothers' with GDM education level (χ^2=9.963; p=0.019) and social supports (χ^2=5.090; p=0.024) were lower and breastfeeding in postpartum (χ^2=15.928; p=0.001) started later (Table 1).

The depression risk for the mothers with GDM was 39.4% in the antenatal period and 27% in the postpartum period while these rates were 30.8% and 19.4% respectively in the non-GDM mothers and the difference was not statistically significant (Table 2).

No significant difference was found between the mean scores of depression and breastfeeding self-efficacy in the antenatal and postpartum period in the women by the presence of GDM. Including the age and BMI as covariance, there was no significant difference between the mean scores of depression and breastfeeding self-efficacy in the antenatal and postpartum period by the presence of GDM (Table 3).

The mean scores of depression and breastfeeding self-efficacy in antenatal and postpartum period of women by the presence of GDM and some variables were given in Table 4. No statistically significant difference between antenatal and postpartum periods in the mean scores of depression and breastfeeding self-efficacy according to the presence of GDM and type of birth, GDM history in previous pregnancy, presence of social support and planned pregnancy. In the presence of GDM and parity model, there was a statistically significant difference between the mean scores of antenatal and postpartum depression while no significant difference was found between the mean scores of breastfeeding self-efficacy (Table4).

The higher the breastfeeding self-efficacy level of mothers with GDM both in the antenatal (r=-0.248; p=0.033) and postpartum period (r=-0.392; p=0.001), the lower their depression risk became. No correlation was found in non-GDM mothers in the antenatal period while the higher the breastfeeding self-efficacy in the postpartum period (r=-0.351; p=0.001) resulted in the lower the depression risk (Table 5).

Table 1. *Descriptive characteristics of mothers*

Variables	With-GDM (n=74)		Non-GDM (n=103)		Statistical Significance*
	Mean±SD		Mean±SD		
Mean age (year) (Min-max)	31.94±5.96 (19-46)		28.33±6.15 (18-43)		t=3.905 p=0.001
	N	**%**	**n**	**%**	
Education					
Literate	11	14.9	4	3.9	
Primary education	44	59.5	64	62.1	χ^2=9.963 p=0.019
Secondary education	11	14.9	28	27.2	
High education	8	10.8	7	6.8	
Income status**					
Low income	26	35.1	42	40.8	
Equal to income expense	47	63.5	54	52.4	
High income	1	1.4	7	6.8	
Having social support					
Yes	62	83.8	97	94.2	χ^2=5.090 p=0.024
No	12	16.2	6	5.8	
Number of pregnancy					
1	10	13.5	29	28.1	χ^2=6.684 p=0.035
2	20	27.0	30	29.2	
≥3	44	59.5	44	42.7	
Status of planned pregnancy					
Planned	41	55.4	55	53.4	χ^2=0.773 p=0.680
Unplanned but I am grateful	25	33.8	40	38.8	
Unplanned, I do not feel ready	8	10.8	8	7.8	
GDM history in previous pregnancy					
Primigravid/abortion	13	17.6	35	34.0	χ^2=13.336 p=0.001
Yes	12	16.2	3	2.9	
No	49	66.2	65	63.1	
Pre-pregnancy BMI classification**					
Underweight (<18.5)	-	-	11	10.7	
Normal weight (18.5-24.99)	24	32.4	48	46.6	
Pre-obesity (25.00-29.99)	20	27.0	30	29.1	
Obesity (>30)	30	40.5	14	13.6	
Type of birth					
Vaginal birth	18	24.3	33	32.0	χ^2=1.249 p=0.264
Caesarean birth	56	75.7	70	68.0	
Time to start breastfeeding after birth					
15-30 minute	9	12.2	19	18.4	χ^2=15.928 p=0.001
31-60 minute	5	6.7	24	23.3	
61-180 minute	31	41.9	42	40.8	
>180 minute	29	39.2	18	17.5	

*Note: *Chi-square test was used.*

***Cannot be statistically analyzed because the sample set is less than five*

Table 2. *The depression risks of mothers in antenatal and postpartum period*

Depression Risk (EPDS≥ 12)	With GDM		Non-GDM		Statistical Significance*
	n	%	n	%	
Antenatal Period					
Have risk	41	39.4	41	30.8	χ^2=1.906 p=0.167
Not have risk	63	60.6	92	69.2	
Postpartum Period					
Have risk	20	27.0	20	19.4	χ^2=1.426 p=0.232
Not have risk	54	73.0	83	80.6	

*Note: * Chi-square test was used.*

Table 3. *EPDS and BSES means of mothers according to the presence of Gestational Diabetes*

Variables	With GDM Mean±SD	Non-GDM Mean±SD
Antenatal EPDS	10.28±5.64	10.17±5.03
Postpartum EPDS	7.98±5.16	7.84±5.07
Depression*presence of GDM[a]	p=0.967	
Depression*presence of GDM[b]	p=0.779	
Antenatal BSES	60.06±8.71	58.37±9.65
Postpartum BSES	62.09±7.47	60.88±8.83
Self-efficacy*presence of GDM[c]	p=0.563	
Self-efficacy*presence of GDM[d]	p=0.661	

Note: Multivariate test was used for statistical analysis.
[a]Design: Intercept + presence of GDM, Within Subjects Design: Depression, [b]Design: Intercept + Age + BMI + presence of GDM, Within Subjects Design: Depression, [c]Design: Intercept + presence of GDM, Within Subjects Design: Self-efficacy, [d]Design: Intercept + Age + BMİ + presence of GDM, Within Subjects Design: Self-efficacy

Table 4. *EPDS and BSES Means of Mothers According to the Presence of Gestational Diabetes and Some Variables*

Variables	EPDS		BSES	
	Antenatal Mean±SE	Postpartum Mean ±SE	Antenatal Mean ±SE	Postpartum Mean ±SE
Parity				
Nulliparous	9.900±1.663	6.600±1.610	57.900±2.883	63.600±2.923
Primiparous	9.950 ±1.176	7.400±1.139	59.350 ±2.038	63.200±2.068
Multiparous	10.523 ±0.793	8.568±0.768	60.886 ±1.374	61.250±1.394
Statistical analysis	depression * presence of GDM * parity[a] **p=0.044**		self-efficacy * presence of GDM * parity[b] p=0.922	
Type of birth				
Vaginal birth	10.611±1.254	6.889±1.209	60.056±2.198	63.000±2.191
Caesarean birth	10.179 ±0.711	8.339±0.685	60.071 ±1.246	61.804±1.242
Statistical analysis	depression * presence of GDM * type of birth[c] p= 0.197		self-efficacy * presence of GDM * type of birth[d] p=0.898	
GDM history in previous pregnancy				
Yes	6.833±1.514	8.000±1.488	59.750±2.587	61.667±2.640
No	11.143 ±0.749	8.163±0.737	61.163 ±1.280	62.755±1.306
Statistical analysis	depression * presence of GDM * GDM history[e] p=0.217		self-efficacy * presence of GDM * GDM history[f] p=0.968	
Having social support				
Yes	9.500±0.646	7.661±0.650	59.790±1.182	62.371±1.183
No	14.333 ±1.468	9.667±1.477	61.500 ±2.687	60.667±2.688
Statistical analysis	depression * presence of GDM * social support[g] p=0.512		self-efficacy * presence of GDM * social support[h] p=0.967	
Status of planned pregnancy				
Planned	8.463±0.786	6.317±0.781	60.146±1.452	63.000±1.460
Unplanned but I am grateful	11.520 ±1.007	10.000±1.000	61.480 ±1.860	61.080±1.869
Unplanned, I do not feel ready	15.750 ±1.780	10.250±1.768	55.250 ±3.288	60.625±3.304
Statistical analysis	depression * presence of GDM * planned pregnancy[k] p=0.977		self-efficacy * presence of GDM * planned pregnancy[m] p=0.618	

Note: [a]Design: Intercept + presence of GDM + parity + presence of GDM * parity, Within Subjects Design: depression
[b]Design: Intercept + presence of GDM + parity+ presence of GDM * parity, Within Subjects Design: self-efficacy
[c]Design: Intercept + presence of GDM + type of birth + type of birth * type of birth, Within Subjects Design: depression
[d]Design: Intercept + presence of GDM + type of birth + type of birth * type of birth, Within Subjects Design: self-efficacy
[e]Design: Intercept + presence of GDM + GDM history + presence of GDM * GDM history, Within Subjects Design: depression
[f]Design: Intercept + presence of GDM + GDM history + presence of GDM * GDM history, Within Subjects Design: self-efficacy
[g]Design: Intercept + presence of GDM + social support + presence of GDM * social support, Within Subjects Design: depression
[h]Design: Intercept + presence of GDM + social support + presence of GDM * social support, Within Subjects Design: self-efficacy
[k]Design: Intercept + presence of GDM + planned pregnancy + presence of GDM * planned pregnancy, Within Subjects Design: depression
[m]Design: Intercept + presence of GDM + planned pregnancy + presence of GDM * planned pregnancy, Within Subjects Design: self-efficacy

Table 5: *The correlation between mothers' EPDS and BSES mean*

	Variables	Antenatal BSES	Postpartum BSES	Postpartum EPDS
With GDM	Antenatal EPDS	r=-0.248 p=0.033	r=-0.277 p=0.017	r=0.642 p=0.001
	Postpartum EPDS	r=-0.257 p=0.027	r=-0.392 p=0.001	
	Postpartum BSES	r=0.338 p=0.003		
Non-GDM	Antenatal EPDS	r=0.001 p=0.987	r=-0.261 p=0.008	r=0.425 p=0.001
	Postpartum EPDS	r=-0.021 p=0.834	r=-0.351 p=0.001	
	Postpartum BSES	r=0.258 p=0.008		

4. DISCUSSION

In this study, the mean age of women with GDM, the number of pregnancies and the rate of having GDM history in a previous pregnancy were found to be higher compared to non-GDM mothers. Similarly, in other studies, mean age (9,31,32) and the number of pregnancies (9,33,34) in mothers with GDM were found to be higher compared to non-GDM mothers and the difference was significant. As mentioned in the literature, having GDM history in a previous pregnancy was found to be one of the relevant factors that increase the risk of GDM development in future pregnancies (9,35,36). The high mean age of women with GDM can be related to the high prevalence of high-risk pregnancies in advanced maternal age (33,35–38). An increase in the number of pregnancies is also one of the factor that increase the GDM risk (38) and the difference between the mothers with and without GDM by the number of pregnancies is thought to be associated with this condition. Nurses should be aware of these risk factors for GDM and should provide antenatal care to women.

In our study, the depression risk of the mothers with GDM was found higher compared to mothers without GDM in the antenatal and postpartum period; however, the difference was not statistically significant. This result was consistent with some results obtained in the literature (39,40). Similarly, in the study of Beka et al. (41) it was reported that women with GDM were found not to be at higher risk for new-onset mental disorders in pregnancy compared to non-GDM women. In other studies, as different from the result of this research (20,21,42,43), the women with GDM were found to be at higher risk for antenatal and postpartum depression compared to non-GDM women. In the prospective cohort study of Silverman et al. (43), it was determined that individuals with GDM were at risk for 1.7 times more in terms of postpartum depression. In reviewing the literature, it is thought that obtaining different results for both antenatal and postpartum depression risk in women with GDM can be associated with the different cut off score of the scale used in indicating the depression risk, size of sample and use of different scales. Based on these results, nurses should consider the risk of developing depression in women with GDM.

In this study, in the depression and breastfeeding self-efficacy scores of women by the presence of GDM, no difference was found in terms of the antenatal and postpartum period. The statistical significance did not change when age and BMI values were adjusted. There are few studies in literature related to GDM and breastfeeding self-efficacy. Similar to our study, it was stated that GDM did not have a significant contribution to the development of postpartum depression in the studies (44,45). In other similar studies (41,46), no statistically significant difference was found between the women with and without GDM for all mental disorders during pregnancy and the postpartum period when such characteristics as age and BMI were adjusted and these values were stated not to be associated with depressive symptoms. Contrary to our research result, it was stated in a study (32) that there were significant differences between breastfeeding self-efficacy scores of women with and without GDM. In studies unlike our research result, the women with GDM were stated to be at higher risk for perinatal depression (45,47). It has been established that there is a significant relationship between GDM and depression when age and BMI are adjusted (48) and the women with GDM suffer from antenatal (21) and postpartum depression (49) more.

In the study, no time-dependent difference was ascertained in the depression and breastfeeding self-efficacy scores of the participant mothers by the presence of GDM, type of birth, GDM history in a previous pregnancy, and presence of social support and planned pregnancy. In the presence of the GDM and parity model, a significant difference was found between antenatal and postpartum periods in terms of depression scores. In studies conducted similar to the research result (21,46), low parity in women with GDM affects the frequency of depressive symptoms. In another study being different from the research result (41), a statistically significant difference was not established between the women with and without GDM in terms of all mental disorders during the antenatal and postpartum period when nulliparity was adjusted.

It was determined in the research that the higher the depression risk was both in the antenatal and postpartum period in mothers with GDM, the lower their postpartum breastfeeding self-efficacy was. In previous studies as parallel to the result of this research, a significant and negative relationship was indicated between postpartum breastfeeding self-efficacy and postpartum depression (22,23). In the study of Dennis and McQueen (50), breastfeeding self-efficacy scores of those with the scores BSES >12 are lower than those with the scores BSES<12 in the first week of postpartum. Informing the pregnant women regarding the complications of GDM may cause anxiety, concern, and despair and depression about the pregnancy, which may result in failure of mother to make future plans about her baby. It is thought in this study that depression

and breastfeeding self-efficacy relationship of the pregnant women with GDM in the antenatal period can be associated with these negative thoughts.

5. CONCLUSION

We found that GDM did not have a significant effect on depression and breastfeeding self-efficacy during the antenatal and postpartum period. In terms of some confounding factors, no significant difference was found between the mean scores of depression and self-efficacy in the antenatal and postpartum period by the presence of GDM. It was determined that an increase in the depression risk of mothers with GDM in the perinatal period, negatively affect breastfeeding self-efficacy in postpartum. Early diagnosis of pregnant women by nurses considering the possibility of a relationship between GDM and depression, prediction of the possible risks and their support given to the pregnant women in this regard may contribute to the nursing care. Nurses can reduce the negative breastfeeding outcomes by helping mothers, change their negative emotional inclinations and increasing breastfeeding self-efficacy.

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Clinical and Experimental Health Sciences

Corpus Callosum Volume in Patients with First-Episode Psychosis

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ABSTRACT

Objective: In first-episode psychosis, a relationship may exist between disruption communication between two brain hemispheres and psychosis symptomatology. We aimed to investigate the relationship between corpus callosum (CC) volume and psychosis symptomatology in patients with first-episode psychosis.

Methods: This is a retrospective case-control study wherein first-episode psychosis cases and healthy controls were included from inpatient unit archives of the Department of Psychiatry and Department of Radiology, Dokuz Eylül University School of Medicine. Psychosis symptoms were assessed using the positive and negative syndrome scale (PANSS). The CC, the chief connection between two brain hemispheres, was examined using magnetic resonance imaging (MRI); 27 patients with first-episode psychosis and 29 healthy volunteers were evaluated via 1.5-T MR. MRI findings of CC volumes of the two groups were compared. Correlations between PANSS scores and CC volume were also evaluated.

Results: The CC volume was lower in patients with first-episode psychosis than in healthy controls. Moreover, we observed a significant negative correlation between the CC volume and emotional withdrawal scores, and a significant positive correlation between the CC volume and hallucinations.

Conclusions: The CC is a vital structure that connects two frontal lobes of the brain. There may be CC abnormalities in first-episode psychosis. Emotional withdrawal is associated with decreased CC volume, whereas hallucinations are associated with increased CC volume. The development of these symptoms may be associated with changes in CC connections.

Keywords: First-episode psychosis, corpus callosum, emotional withdrawal, hallucinations

1. INTRODUCTION

The most frequently occurring signs associated with psychosis and detected in advanced MRI studies were, until recently, as follows: (a) temporolimbic changes caused by the enlargement of lateral and third ventricles, (b) cerebral volume loss (especially in the frontal lobe), and (c) the effacement of cortical sulci (1,2).

Abnormal constitutional connections between hemispheres in schizophrenia lead to variability in brain asymmetry. Numerous reports have revealed a lower CC volume or lower CC to brain ratio in patients with schizophrenia compared with subjects in control groups (3). Decreases in CC dimensions were noted more frequently in first-episode schizophrenia cases compared with psychotic patients and normal control groups. Smaller anterior genu of the CC and a decrease in the isthmus and anterior splenium are relevant in schizophrenia cases. This decrement is caused by cellular loss and a decrease in cell size. Together with this, a decrease in CC volume is observed in anterior genu, corpus (prefrontal cortex connections), isthmus (inferior parietal cortex connections), and anterior splenium (superior temporal cortex connections) (4,5,6,7).

Studies including treatment-naïve patients with first-episode psychosis are crucial with respect to differentiating between alterations due to the chronic nature of the disease and alterations induced by drug therapies. In cases of first-episode psychosis, corpus callosum changes were detected, which supports the disconnection hypothesis, which is thought to be due to the clinical pattern observed in the formation of psychotic attacks (8).

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Psychosis is associated with a significantly smaller CC area, as well as a smaller anterior splenic area (9). Neuroimaging studies describe that negative symptoms are linked with volume loss in the frontal cortex (10). Corpus callosum volume and the association of first-episode psychosis symptomatology have not yet been investigated. In this study, we aimed to investigate the association between CC volume and psychosis symptomatology in patients with first-episode psychosis.

2. METHODS

2.1. Study Design and Participants

This investigation was designed as a case-control study in which patients with first-episode psychosis admitted to the Inpatient Unit of the Psychiatry Department of Dokuz Eylül University School of Medicine between 2009 and 2013 were included. Archived case records were utilized in this retrospective study. This study was approved by the ethical board of Dokuz Eylul University Faculty of Medicine (No: 2015/02-36). The cases diagnosed as first-episode of acute psychosis according to DSM-IV criteria composed the case group of the study (n=27), while the control group was composed of records of healthy volunteer subjects who attended the radiology department and underwent MRI imaging (n=28).

Inclusion criteria for first attack acute psychosis patients; Satisfying DSM-IV diagnostic criteria for acute psychosis and being literate. The control group has identified healthy individuals with similar age, gender, and educational status. This group consisted of persons who had never had any psychiatric treatment and were not present with any psychiatric illnesses. Volumetric evaluation of 28 individuals aged 18-55 years who volunteered and met certain criteria (especially those with no neurological, psychiatric, and chronic illnesses, no problems with hearing and vision functions, and no continuous use of drugs) Exclusion criteria; The history of any psychiatric illness in the control group and the presence of neurological disease in the control and patient group.

Power of study calculated in G power software. According to minimum r=0,37 correlation, Type I error=0.05, 1-β=0,80 total sample size was 55. We have a total of 55 participants in the study.

2.2. Data Collection

Sociodemographic data and positive and negative syndrome scale (PANSS) scores of the patients were obtained from their medical records, PANNS scores are calculated routinely inpatient clinic. In patients with first-episode psychosis, the standard procedure was to undertake MRI scans to establish that the psychosis was not associated with other neurological disorders. Volumetric measurements of the CC were evaluated using patients' MRI scans taken in the first week of diagnosis.

Measurement of the Size of the Corpus Callosum

MRI images used in the study were obtained using standard head coil Achieva and Intera (Philips, Holland) 1.5 Tesla strength MRI units available in the Radiology Department of Dokuz Eylül University Medical Faculty.

Volumetric measurements were carried out on sagittal plane SE T1-weighted images (parameters; 25 consecutive sections, 5 mm section thickness, TR 600 msec, TE 15 msec; FOV 24 cm; matrix 256 × 256).

MRI images stored in Digital Imaging and Communications in Medicine format were analyzed using Easy Vision 4.4 workstation (Philips, Holland). Segmentation of the CC (volume of interest) and volumetry was carried out using a region-growing algorithm assisted by using a mouse-guided cursor. A midline section of the CC and sections on both sides of this section (three sections in total) were used for volumetry measurement.

For measurements, sections were selected from T1-weighted images in which CC was best shown in the midsagittal plane. The area contained within a closed line drawn around the circumference of CC was calculated. Area measurements were automatically calculated by the tool, which had a sensitivity of 1% per square millimeter. Measurements were conducted retrospectively using the TSE/T1 sequence, which is routinely used in brain MRI protocols. All the measurements were carried out by the same investigator.

2.3. Data Analysis

Categoric variables were defined as numbers and percentages. Chi-Square test was used for the comparison of categorical variables. Continuous variables were defined by mean, standard deviation, and quartile; continuous variables to the normal distribution were examined by Kolmogorov–Smirnov test. CC volume for patients with psychosis and control group subjects was measured, and t-test or Mann–Whitney U test were used to compare groups according to the distribution of data. The correlations between PANSS points and CC measurements were analyzed using Pearson's correlation coefficient; if the normal distribution was not determined, analysis was conducted using Spearman's rank correlation coefficient (Spearman Rho).

CC callosum values were compared between case and healthy control groups with age and gender adjustment in multiple linear regression. Significant subscales of PANSS associated with CC were analyzed with multiple linear regression, and age – and gender-adjusted subscales were analyzed using multiple linear regression analysis; B coefficients were expressed together with a 95% confidence interval (CI).

3. RESULTS

A total of 27 first-episode psychosis cases were examined with regard to sociodemographic characteristics, disease characteristics (PANSS score), and CC volume.

3.1. Descriptive Statistics

Mean age of first-episode psychosis cases was 30.2±12.6 (median=26.0 25p–75p=21.0–35.0 minimum–maximum=18.0–57.0); 63% of those included in the study were male (n=17), 70.4% (n=19) single, 25.9% (n=7) married, 3.7% (n=1) widowed. When looking at the educational status, 18.5% (n=5) of subjects had graduated from primary school, 25.9% (n=7) from high school, and 55.6% (n=15) from university. None of the participants had graduated from secondary school (Table 1).

Table 1. *In first episode psychosis cases and healthy controls, evaluation of the association between gender and volume of corpus callosum.*

	Control (n=28)	First Episode psychosis (n=27)	Test values	p
Gender				
Female	60,0% (15)	40,0% (10)	Chi-Square =1,5	0,218
Male	43,3% (13)	56,7% (17)		
Age	30,2±12,6	35,7±7,3	Mann-Whitney-U=224,0	0,009
Volume of corpus callosum	24,0±3,0	22,0±2,3	t=-2,8	0,008

The frequency of alcohol use was low in first-episode psychosis cases (user 3.7% n=1). The frequency of smoking was 22.2% (n=6). Only 11.1% (n=3) of first-episode psychosis cases were cannabis users. The frequency of first-episode psychosis cases in which there was a family history of the psychiatric disease was 40.7% (n=11) (Table 2).

Table 2. *Results of volume of corpus callosum in first attack psychosis and control group adjusted by age and gender.*

Model*	Coefficient		P	95% Confidence Interval for B coefficient	
	B	Std. Error		Minimum	Maximum
Age	0,019	0,036	0,598	-0,054	0,092
Gender	-0,479	0,742	0,521	-1,969	1,010
Healthy control (r)	-1,791	0,764	0,023	-3,324	-0,258

**Association between age, gender, first episode psychosis case or healthy control, and volume of corpus callosum was tested.*

When examining emotional characteristics of patients with first-episode psychosis, it was observed that 40.7% (n=11) of them were blunted, 15.5% (n=5) irritable, 14.8% (n=4) inappropriate, 11.1% (n=3) depressive, and 11.1% (n=3) anxious.

A total of 10 patients (37%) with first-episode psychosis cases had visual-auditory hallucinations. No hallucinations were observed in 33.3% (n=9) of cases. A total of 11% (n=3) cases were auditory, 7% (n=2) were separately existing visual hallucinations, and in 3% (n=1) olfactory–visual, olfactory–visual–auditory, and auditory–tactile–visual hallucinations were present.

In 70.4% (n=19) of first-episode psychosis cases, insight was not present; in 25.9% of cases (n=7) it was restricted; and in 3.7% of cases (n=1) insight was present.

The mean age of first-episode psychosis cases was 30.2±12.6, mean age in healthy controls was 35.7±7.3; there was a statistically significant difference between groups concerning mean age (p=0.009). In total, 40% (n=10) of females and 56.7% (n=17) of males were included in the study. There was no significant difference between the case and control groups regarding gender (p=0.218).

3.2. Corpus Callosum Volumes

The mean volume of CC in first-episode psychosis cases was 22.0cm3±2.3cm3; in healthy controls, it was 24.0cm3±3.0cm3 (Table 1).

The volume of CC in first-episode psychosis cases was significantly smaller than in healthy controls (p=0.008) (Table 1). When age and gender were adjusted, CC volume was 1.7 cm lower in first-episode psychosis cases than in healthy controls (R square=0,137, B=−1.7, p=0.023, 95% CI=from −3.3 to −0.3) (Table 2).

First episode psychosis symptomatology and CC volume

We observed a significant positive correlation between CC volume and hallucinations subitem of the positive symptoms subscale of PANSS. As CC volume increased, hallucination subitem scores increased (42.7%, p=0.026) (Table 3). No significant associations were determined between CC volume and other items of the positive symptom subscale of PANSS (Table 3). When adjusting by age and gender effect, the volume of CC increased by 0.781 units with each one-point increase in hallucination score (R square change=0,395, p=0.002, 95% CI=0.308–1.251) (Table 4).

We observed a significant negative correlation between the emotional withdrawal of negative symptom subscales of the PANSS scores and CC volume. Emotional withdrawal subscale scores increased with decreasing CC volume (−%46.8, p=0.014) (Table 3). There were no significant associations between CC volume and the scores of other components of the negative symptom subscale of PANSS. When age and gender effects were controlled, CC volume decreased by −0.752 units with each point increase in emotional withdrawal score (R square change=0,264, p=0.028, 95% CI= from −1.416 to −0.088) (Table 4).

Table 3. *Correlation coefficient (Rho) and significance levels of association between volume of Corpus Callosum (CC) and PANSS scores*

	Spearman rho	p		Spearman rho	p
P1. Delusions	-0,122	0,546	N1. Affective blunting	-0,342	0,081
P2. Thought dispersion	-0,233	0,242	N2. Emotional withdrawal	-0,468*	0,014
P3. Hallucinations	0,427*	0,026	N3. Difficulty in communicating	-0,214	0,284
P4. Expansiveness	0,009	0,965	N4. Social withdrawal	-0,225	0,258
P5. Grandiose sensations	0,051	0,799	N5. Difficulty in abstract thinking	-0,258	0,194
P6. Scepticism/ persecution	0,023	0,910	N6. Impairment of speech	-0,170	0,396
P7. Hostility	0,167	0,404	N7. Stereotypical thinking	-0,247	0,214

Table 4. *Age and Gender Adjusted Results of Corpus Callosum Volume and Hallucination and Emotional Withdrawal Scores.*

		Coefficient		p	95% Confidence Interval for B coefficient	
		B	Standard Deviation		Minimum	Maximum
Model I	Age	0,106	0,035	0,005	0,035	0,178
	Gender	0,804	0,832	0,344	-0,917	2,525
	Hallucinations	0,781	0,228	0,002	0,308	1,253
Model II	Age	0,033	0,036	0,366	-0,041	0,107
	Gender	-0,111	0,882	0,901	-1,934	1,713
	Emotional withdrawal	-0,752	0,321	0,028	-1,416	-0,088

4. DISCUSSION

Numerous studies have reported the decrease in the size of the CC in schizophrenia; however, in some studies, no differences in size or larger CC were determined (11,12). These inconsistencies could be based on the differences in case of characteristics (gender, experiencing different psychotic symptoms, use of dominant hand, age at disease onset), and the measurement method of CC volume could also be effective as the others (4). Thus, as antipsychotic therapy is another confounding factor, it has been reported that such therapy causes alterations in white matter volume (13). For example, olanzapine therapy in schizophrenia led to an increase in white matter (14). Similarly, treatment with risperidone increased myelinization in white matter (15). Antipsychotic therapy also probably results in a potentially increased size of the CC. In addition, few studies have investigated the CC of patients who did not use antipsychotic agents (11).

Age at disease onset is an important factor with respect to neurodevelopmental differentiations and to effects of CC measurements beside the others (16,17). The CC is the last part of the brain to mature, and its development continues until late adolescence/early adulthood (18).

In a large number of neuroimaging studies investigating schizophrenia, a substantial amount of evidence regarding morphological brain pathology was gathered (19). The major interhemispheric commissure, which is part of the CC, is one of the affected parts of the brain (20). The CC constitutes the transition of axons emanating from the neocortex and fibrils of white matter from both brain hemispheres. Abnormal interhemispheric connections have been identified on the basis of the variability of brain asymmetry in schizophrenia (21,22,23). A large number of studies have shown that in psychosis cases, a lesser volume of the CC or lower CC to brain ratio is present in comparison with control groups (24). Two groups of investigators examined first-episode psychosis cases, and one group determined gender differences in case and control groups (25), whereas the other group determined the deformation of shape (26).

Even though considerable data point to disturbed communication between the two hemispheres of the brain in schizophrenia, communication disorder cannot be anatomically shown. Diffusion tensor imaging studies can reveal that connection has been focally disturbed in commissural relations (27).

In patients with schizophrenia who had not yet been treated, smaller corpus callosa were identified compared with healthy controls; together with this, the existence of larger corpus callosa was greater in females compared with males. Evidence regarding gender was consistent with the findings for the brain of normal subjects (7). In our study, there were no statistically significant associations between CC volume, age, and gender. Consistent with previous studies, the volume of CC in first-episode psychosis cases was significantly smaller than in control cases in our study.

In the present study, a decrease in CC volume was found to be associated with emotional withdrawal, which is one of PANSS negative scores. It was shown that hallucinations that were part of the positive signs were positively associated with CC volume. Hence, it led us to think that variabilities of CC volume could be separated from symptomatology. Furthermore, it was thought that the calculation of the volume of subsections of the CC could elucidate this situation.

The association between negative symptoms and loss of gray matter in the schizophrenic brain has been investigated. Alterations were most prominently observed in prefrontal, temporal, limbic, and subcortical regions. In schizophrenia cases where negative symptomatology was dominant, decreases in the bilateral limbic and prefrontal gray matter had been determined. These results explained cases where social functioning deteriorated, a situation created

by beginning and ongoing negative symptoms. These insufficiency symptoms have shown consistency with PANSS titles such as "emotional withdrawal" or "blunted affect," which consisted of a high factor burden consistent with core negative symptom content of negative signs. These PANSS titles reflected disorders of social and emotional functioning (28).

In Walterfrang's study, no associations between positive symptoms determined by PANSS and CC measurements were detected in patients with first-episode psychosis diagnosed with schizophrenia and similar spectrum disorders.

On the contrary, negative symptoms were negatively correlated with the tangle of twist of CC ($r=-0.215$, $p=0.013$). While there were no associations between the variables of CC and relevant variables in the groups of the same study, a positive correlation was observed between negative symptoms, and callosal area ($r=0.307$, $p=0.018$), and thickness ($r=0.288$, $p=0.027$). These associations could not be shown in regression analysis. In addition to this, CC was found to be significantly different in patients with schizophrenia compared with healthy controls, and some changes were determined even in first-episode cases. A decrease in volume may be associated with a decrease in axon number of axon diameter or myelin sheath loss. It may be mentioned about the decrease in genu and isthmus as fibers of the connection cortex (29).

Correlation analysis carried out between symptom severity scores achieved from PANSS and CC measurements in schizophrenia cases revealed that increased superior genu volume was associated with increased severity of positive symptoms (30,31). Similarly, in the study by Whitford et al., a positive correlation was noted between the sum of hallucination and delusion severity scores of the positive signs' subscale and the existence of fractional anisotropy in the region of the CC that frontal fibrils passed through. In the same study, a negative correlation was noted between the severity of difficulty in abstract thinking (N5) and the existence of fractional anisotropy in the same region (32).

In studies including schizophrenia cases under therapy, healthy controls, and healthy relatives, a negative association was found between scores of the hallucination severity subscale of the PANSS scoring system and CC volume. A negative correlation was also determined between fractional anisotropy values of the hallucination severity subscale of PANSS and the volume of the posterior genu region of the CC. In our study, a statistically significant positive correlation was determined between CC volume and hallucination severity scores achieved from the PANSS subtitle. This finding could be specific to first-episode psychosis cases in which therapy has not yet been started and evaluated with imaging modalities during onset. Volume increase in white matter may be related to increased efficiency of pathways in connection with the CC. However, the relationship between emotional withdrawal and decreased CC volume may be interrelated with decreased efficiency of these pathways. For detailed information, CC requires investigation at a regional level.

There are some limitations, and generalizations should be made with caution. First, the study sample was small. In some details, we relied on the information given by our healthy controls and patients. Since we acquired retrospective data, our findings can only support the hypothesis of first-episode psychosis symptoms as hallucinations and emotional withdrawal. However, because first-episode psychosis cases were examined, the response of the CC with respect to chronic alterations could have been excluded. In addition, the dominant hand could not have been determined. Control group and first-episode psychosis cases could not have been matched case by case according to age and gender. The results have been analyzed by statistically adjusting to age and gender.

5. CONCLUSIONS

In this study, we found that hallucinations, one of the positive signs observed in first-episode psychosis, were associated with increased CC volume. Decreased CC volume may be associated with emotional withdrawal. Volumetric changes in the CC are associated with axonal structure, cellular changes, and the number of fibrils in this region. First-episode psychosis cases are those in which pathology has not yet been treated, and as such, it is known that the pathology has not been altered using drug therapy. In this group of patients, volumetric changes in the CC suggest that the neurodevelopmental hypothesis and the hypothesis of disrupted connection are in play. It was observed that different cognitive functions implicated by the fibers emanating from the frontal lobe were altered as suggested by correlation with CC volume in first-episode psychosis cases.

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Clinical and Experimental Health Sciences

Reducing the Pain of Infants due to Vaccine Injection: A Randomized Controlled Trial

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ABSTRACT

Objective: This study was conducted as a randomized controlled, and experimental to compare the effect of breastfeeding and distraction methods on vital signs, pain level, and the duration of crying due to vaccine injection in healthy infants.

Methods: The population of the study consisted of 120 infants between 1 and 12 months of age who had met the inclusion criteria. The sample group was randomized and divided into two groups. The control group was breastfed according to the clinical procedure, including 58 infants, and the distraction grincludedlude 62 infants. For both groups, the oxygen saturation (SpO_2), heart rate (HR), crying duration, and pain scores were compared both before and following vaccination sessions.

Results: The vaccination pain scores of the infants from the distraction group (4.39±2.18) were significantly lower than those of the breastfeeding group (7.05±1.55; p=0.001). The post-vaccination SpO_2 was higher in infants in the distraction group; whereas HR was lower in this group compared to the breastfeeding group. The post-vaccination crying durations of those in the distraction group were also shorter than those in the breastfeeding group.

Conclusion: The distraction method adopted by the use of a toy has been found to be effective in decreasing acute pain during vaccine administrations.

Keywords: Pain, infant, breastfeeding, distraction, vaccine injection

1. INTRODUCTION

Painful interventions such as the pricking of one's heel, venous blood sampling, and vaccination are frequently administered to infants (1). The pain experienced by infants during these interventions has a negative impact on their behaviour, their interaction with parents, their cerebral & sensory development, their growth development, and their diet, thus leading to eventual physiological and behavioural problems (2,3). Behavioural symptoms such as crying, impaired sleep patterns, exhaustion, changes in facial expression, irregularities in breastfeeding, and leg movement are among the symptoms (2-5). Physiological changes include symptoms such as an increased peak heart rate (HR) and decreased oxygen saturation (SpO_2) during pain (1,6).

Nonpharmacological methods are used alone or in accompaniment with pharmacological methods in order to reduce the pain that infants experience (4,6-8). Since pain during routine vaccination in infants is not a sign of disease, it is more appropriate to use non-pharmacological methods to reduce pain (3,5). Nonpharmacological methods include; position change, oral glucose/sucrose administration, pacifier giving, breastfeeding, reducing environmental stimuli, kangaroo care, massage, and games can be counted. While these methods used to reduce pain reduce the baby's pain, they also reduce the parent's stress (5, 8, 9).

Determining the method to be used in order to reduce the perception of pain in infants should be suitable for the age, development level, and area of interest of the baby and should stimulate major senses such as hearing, sight, touc,h, and movement. These are musical games with rhythm, blowing balloons, kaleidoscope, et,c. according to the age group (4, 6-10). Nurses play an important role when it comes to identifying pain, thus adversely affecting the quality of life of infants in particula and thus initiating the appropriate intervention for pain management and alleviation (3,6). In a study by Asadi-Noghabi et al. (2014), in which they examined what nurses know in the management of newborn pain, it

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was stated that nurses should initiate appropriate strategies for the evaluation, recognition, and treatment of infants in order to alleviate their pain (9).

In accordance with the literature (2-3,8, 10-12), this study was designed as a randomized controlled trial with the aim of comparing the effect of the methods of breastfeeding and distraction on the vital signs, pain level, and the duration of crying induced by vaccination.

2. METHODS

2.1. Design

The study was conducted as a randomized controlled experimental trial. In this study, two methods, breastfeeding, and distraction, were implemented and compared by examining how each reduced the acute pain level felt by infants during vaccination.

2.2. Sample

The population of the study consisted of infants of mothers who volunteered to participate in the study and met the inclusion criteria in the Vaccination Room of Child Health Clinic at the Istanbul University Cerrahpaşa Medical Faculty Hospital between June and December of 2015. The inclusion criteria for infants were determined as follows: being between 1-12 months of age, being terma newborn (>37+6 GW) according to the new Ballard method (14), having a birth weight of 2500-3500 g a,andd being breastfed, being deemed healthy by a physician. The exclusion critea for infanwasere determined as follows; mother's unwillingness to participate in the study; having any congenital abnormality, displaying any symptom of disease, has a health problem, having a developmental problem according to the Ankara Developmental Screening Inventory (15), taking analgesic medication within four hours prior the procedure (1,6).

In accordance with the Pain diagnostic scale (Face, Legs, Activity, Cry, Consolability: FLACC) used for pain assessment in the sample group, it was considered that the difference of "1" unit in pain level would refer to a clinical/medical significance, whereupon the power analysis was then performed using G*Power (v3.1.7) software in order to determine the sample size. When the probability of Type 1 error (α) was accepted as 0.05 (at confidence level of 95%) and the probability of Type 2 error (β) was accepted as 0.10 (at confidence level of 90%), the value of delta was determined to be 2.86. By using the mean and standard deviations obtained from the study entitled as "The Effect of Foot Reflexology on Acute Pain in Infants: A Randomized Controlled Trial" (7), the effect size (d) was calculated as 0.666. Accordingly, the minimum sample size was calculated as a total of 74 infants, including 37 infants per group. When the data reliability and possible case losses were considered, 120 infant/mother couples, who had agreed to participate in the study, were contacted for data collection.

2.3. Randomization

In this study, the Urn method, which is a method of randomization that corresponds to full randomization, was used. In this method, there are two parameters, α, and β. These parameters refer to two balls of two different colours (16,17). The white ball was determined as being the "breastfeeding" g,oup; whereas the green ball was determined the as "distraction" group. The researcere placed the balls into a black bag, and the nurse administering the vaccination selected a ball from that bag. According to the colour of the selected ball, the infant was assigned to either the breastfeeding group or the distraction group (16,17) (Figure 2).

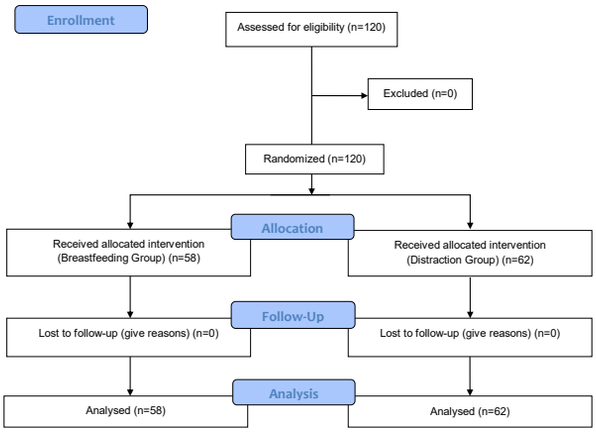

Figure 2. *Flow diagram of participant enrollment (CONSORT 2010).*

2.4. Measures and Equipments

2.4.1. Data collection form

The form is included the study application steps and descriptive characteristics of infants. The researcher had then filled out the form through the face-to-face interview method.

2.4.2. Ankara Developmental Screening Inventory (ADSI)

The inventory was developed by Savaşır et al. in 1994. The inventory is an assessment tool that provides in-depth and systematic information on the current development of infants and children. The reliability and validity study of the inventory adapted to Turkish society was conducted on 860 infants and children. The present skills and development of both (between 0-6 years) were assessed based uponhe the information provided by their mothers. The inventory also allows for the early detection and identification of those wh risk of developmental delay and irregularity. The inventory consists of 154 items target is obtained by asking their mothers, who respond with either "yes", "no, or "I don't know.".

The Cronbach's alpha coefficients of the inventory, which were calculated overa total score for 0-12 months, were declared as 0.98 for General Development(GD), 0.93 for Language/Cognitive(L-C), 0.92 for Fine Motor(FM), 0.91 for Gross Motor(GM) (0.92), and 0.92 for Social Skill/Self-Care(SS-SC) (15).

2.4.3. Pain diagnostic scale (Face, Legs, Activity, Cry, Consolability:FLACC)

The FLACC scale developed by Merkel et al. (18), includes the evaluation of five basic behavioural categories. The validity and reliability of the Turkish version of the Pain Diagnostic Scale (FLACC) was conducted by Şenaylı et al. (19). Each of facial expressions, leg movements, activity, crying, and consolability parameters also consists of three sub-items (19). The items are scored as 0, 1, and 2 point(s) respectively, with total score ranging between 0 to 10 point(s). The "0" point shows that there is no pain, 1-3 points refer to mild pain, 4-6 points refer to moderate pain, and 7-10 points refer to severe pain in infants (18,19).

2.4.4. Pulse oximeter device

The plus MED plus-50DL model Fingertip calibrated pulse oximeter (serial no:12.06.2012-70235-Made in P.R.C/China) was used to measure the oxygen saturation and HR of the infants.

2.4.5. Weighing instrument

The calibrated infant scale (SECA Mark-Production number:07.02.00700, made in Germany) was used to measure the weight of the infants in both groups.

2.4.6. Height measuring tape

A plastic, inflexible measuring tape was used to measure height of the infants in both groups.

2.4.7. Head circumference measuring tape

A plastic, inflexible measuring tape was used to measure the head circumference of the infants in both groups.

2.4.8. Stopwatch

Infant's crying duration was evaluated using Samsung Note II smart phone.

2.4.9. Toy

For distraction, a CE-approved single-unit multi-coloured toy with music and mirror, which is suitable for the characteristics of the infants, was used (8,10). (Photographs 1).

2.5. Procedures

2.5.1. Pilot study

The pilot study was conducted to a total of 10 infants. Following the pilot study, data collection form and process were reorganized.

2.5.2. Application phase

Infant-mother couples from both groups were acquainted in the vaccine room. The groups (breastfeeding or distraction) of mother-infant couples, who volunteered to participate in the study and met the inclusion criteria, were determined using randomization. All of the infants in the study were vaccinated by the same nurse, with the injector having the same sized needle tip (23G), sent by the Turkish Ministry of Health in a ready-to-use form. The pain score (both before and after vaccination) of the infants in both groups in accordance with the FLACC was assessed by both the researcher and the observing nurse. The infants in both groups were respectively subjected to the procedures shown in Figure 1.

Figure 1. *Toy used in the study*

Infants in the Breastfeeding Group (Control): In the hospital procedure, before the vaccination, the infants were left on the mothers' chest, whereupon they breastfed for 1 minute. The control group was breastfeed according to hospital procedure. The vaccination took place during that time slice. Given that the breastfeeding is a pain relief method that currently is used during vaccinations at the clinic in question, the breastfeeding group was determined as the control group.

Infants in the Distraction Group: The infants were given to lay upon their mothers' chests before the vaccination, whereupon the mothers were asked to shake the toy in front of her infant (in 25-30 cm distance). The infant was vaccinated while the mother distracted it with the toy.

During the vaccination, the stopwatch was started as soon as the infant first started to cry as from insertion in both groups. The stopwatch was stopped when the infant ceased crying. Immediately after the needle was removed from the

injection site, the subjects' physiological measurements (SpO_2, HR) were read from the pulse oximetry and FLACC and the pain scores were evaluated by both the researcher and the observer.

2.6. Data Assessment

The data obtained in the study were analyzed using the IBM SPSS Statistics 22 (IBM SPSS, Turkey) software. In the study, the compatibility of the parameters to normal distribution was evaluated using the Shapiro-Wilk test. The descriptive statistical methods (Mean, Standard deviation, Frequency) were used in order to assess the data of the study. The student t-test was employed in order to compare normally distributed quantitative data between the two groups; whereas, Mann Whitney U test was used to compare the data not showing normal distribution between two groups. The Kruskal Wallis test was used in the comparison of the data not showing normal distribution among more than two groups, whilst the Mann Whitney U test was re-used in order to determine the group causing the difference. In the evaluation of the pre – and post-vaccination data, the Paired Samples t-test was used for normally distributed data; whereas, the Wilcoxon Signed-Rank test was used from those who did not show normal distribution. The Chi-square, the Yates' correction for Continuity, and Fisher's Exact Chi-Square tests were used with the aim of comparing the qualitative data. The results were evaluated at confidence interval of 95% and significance level of $p<0.05$.

2.7. Ethical Considerations

In order to conduct the study, the permission was obtained via e-mail from Yesim Şenayli, who had translated Pain Diagnosis Scale into Turkish. Both written approval from Ethics Committee of Istanbul Universiy Cerrahapaşa Medical Faculty (IRB no: 83045809/604.01/02-69828) as well as permission from the institution where the data would be collected were obtained. The mothers of all of the infants were informed both about the purpose, plan, and period of the study, as well as about how the data would be used via "Informed Consent Form" before starting the study, alongside their consent asked both in written and verbal form.

3. RESULTS

It was determined that there was no statistically significant difference between the infants in the breastfeeding and distraction groups in terms of gender, diet, age of gestation, age, body weight, height, and head circumference in this study ($p>0.05$;Table 1).

Table 1. *Comparison of descriptive and vaccination characteristics of infants (N=120)*

Descriptive Characteristics		Breastfeeding Group (n=58)	Distraction Group (n=62)	t	[a]p
		Mean±SD	Mean±SD		
Age	Gestational age (week)	39.07±0.81	38.87±0.78	1.362	0.176
	Current age (month)	6.17±4.31	6.61±4.05	-0.573	0.567
Weight (g)	Birth	3405.34±436.10	3328.39±359.70	1.057	0.293
	Current	7284.07±2032.80	7526.94±1993.94	-0.661	0.510
Height (cm)	Birth	50.78±2.40	50.63±1.64	0.394	0.695
	Current	66.84±7.97	67.29±7.77	-0.316	0.753
Head circumference (cm)	Birth	35.60±0.78	35.41±0.68	1.373	0.172
	Current	42.55±5.38	42.11±3.18	0.542	0.589
Descriptive Characteristics		**n (%)**	**n (%)**	χ^2	[b]p
Gender	Girl	28 (%48.3)	30 (%48.4)	0.001	[b]0.990
	Boy	30 (%51.7)	32 (%51.6)		
Nutrition type	Only breast milk	27 (%46.6)	24 (%38.7)	1.879	[c]0.598
	Breast milk+baby food	5 (%8.6)	7 (%11.3)		
	Breast milk +supp. food	26 (%44.8)	31 (%50)		
Vaccine Application Characteristics					
Vaccine type	Hepatitis B	12 (%20.7)	12 (%19.4)	0.667	0.881
	KPA	22 (%37.9)	22 (%35.5)		
	DaBT-IPA-Hib	16 (%27.6)	16 (%25.8)		
	Measles	8 (%13.8)	12 (%19.4)		
Vaccine area	Vastus Lateralis	50 (%86.2)	50 (%80.6)	0.327	0.567
	Deltoid	8 (%13.8)	12 (%19.4)		
Practice method	IM	50 (%86.2)	50 (%80.6)	0.327	0.567
	SC	8 (%13.8)	12 (%19.4)		

[a]*Student t Test;* [b]*Chi-square TestYates' Continuity Correction;*[c]*Fisher's exact Chi-square Test*

Moreover, no statistically significant difference between the groups in terms of vaccine type, the injection site, or the method of vaccine administration (p>0.05;Table 1) was found.

When the physiological symptoms of the infants in both groups were evaluated before the vaccination, no statistically significant difference was found between the groups in terms of SpO_2 (t=-1.941; p=0.055) and HR (t=1.762; p=0.081). It was determined that after the vaccination, the mean SpO_2 levels of the infants decreased less (t=-13.499; p=0.001), and HR increased less in the distraction group than the breastfeeding group, which hence was statistically significant (t= 9.203; p=0.001; Table 2).

When pain total mean scores of the infants were compared within the group in terms of the age, the pain score was determined to be statistically significantly higher in the breastfeeding group (Table 3). The crying duration of the breastfeeding group was significantly longer than that of the distraction group in terms of the age (p<0.05; Table 2).

When the pain total mean scores of the infants were compared within the group in terms of vaccine type, it was determined that pain mean scores in both breastfeeding (χ^2=28.434; p=0.001) and distraction (χ^2=-36.457; p=0.001) groups showed a statistically significant difference according to the vaccine type. It was also found that while the vaccine causing the highest level of pain in the breastfeeding (8.23±0.87) and distraction (6.23±1.15) groups was the CPV vaccine, the least painful vaccine was measles vaccination for both groups. The pain scores in the infants of breastfeeding group were higher than those of the infants in distraction group, which in turn was statistically significant (p<0.01; Table 4).

When pain total mean scores of the infants were compared within the group in terms of the method of vaccine administration, the pain score of the infants vaccinated with the IM method in both breastfeeding (Z=-2.462; p=0.014) and distraction (Z=-3.204; p=0.001) groups was found to be higher (Table 5). The crying durations of the infants, moreover, confirmed this result. The crying duration in both groups after the IM method was significantly longer than that of the SC administration (p<0.05; Table 5).

Table 2. *Distribution and comparison of SpO2. HR and crying duration of infants before and after vaccination (N=120)*

Physiological Features		Breastfeeding Group (n=58)	Distraction Group (n=62)	t	[a]p
		Mean±SD	Mean±SD		
Oxygen Saturation (%)	Before vaccination	96.90±1.52	97.44±1.52	-1.941	0.055
	After vaccination	90.72±2.02	95.31±2.34	**-11.446**	**0.001****
	Difference	**-6.17±1.77**	**-2.13±1.51**	**-13.499**	**0.001****
	t	**27.279**	**-19.142**		
	[d]p	**0.001****	**0.001****		
Heart Rate (HR)	Before vaccination	126.67±9.56	123.71±8.85	1.762	0.081
	After vaccination	148.29±11.74	132.85±10.37	**7.649**	**0.001****
	Difference	**21.62±8.60**	**9.15±5.90**	**9.203**	**0.001****
	t	**11.107**	**-12.198**		
	[d]p	**0.001****	**0.001****		
Crying Duration (seconds)	After vaccination	59.22±26.72	24.69±18.53	**8.174**	**0.001****

[a]*Student t Test;* [d]*Paired Sample t Test* *p<0.05;**p<0.01

Table 3. *Comparison of pain scores of infants before and after vaccination (N=120)*

FLACC		Breastfeeding Group (n=58)	Distraction Group (n=62)	Z	[e]p
		Mean±SD	Mean±SD		
Face	Before vaccination	0.09±0.28	0.08±0.27	-0.110	0.913
	After vaccination	1.52±0.50	1.11±0.55	**-3.871**	**0.001****
	Difference	**1.43±0.57**	**1.03±0.57**	**-3.653**	**0.001****
	Z	**-6.718**	**-6.764**		
	[f]p	**0.001****	**0.001****		
Legs	Before vaccination	0.03±0.18	0.03±0.18	-0.068	0.946
	After vaccination	1.67±0.47	0.98±0.78	**-4.972**	**0.001****
	Difference	**1.64±0.48**	**0.95±0.76**	**-5.039**	**0.001****
	Z	**-6.888**	**-5.929**		
	[f]p	**0.001****	**0.001****		
Activity	Before vaccination	0.00±0.00	0.00±0.00	0.001	1.000
	After vaccination	1.12±0.50	0.55±0.50	**-5.430**	**0.001****
	Difference	**1.12±0.50**	**0.55±0.50**	**-5.430**	**0.001****
	Z	**-6.834**	**-5.831**		
	[f]p	**0.001****	**0.001****		
Cry	Before vaccination	0.00±0.00	0.03±0.18	-1.374	0.170
	After vaccination	1.57±0.50	0.89±0.52	**-6.157**	**0.001****
	Difference	**1.57±0.50**	**0.85±0.51**	**-6.397**	**0.001****
	Z	**-6.847**	**-6.761**		
	[f]p	**0.001****	**0.001****		
Consolability	Before vaccination	0.02±0.13	0.03±0.18	-0.524	0.600
	After vaccination	1.16±0.37	0.85±0.40	**-4.010**	**0.001****
	Difference	**1.14±0.40**	**0.82±0.43**	**-3.933**	**0.001****
	Z	**-7.107**	**-7.005**		
	[f]p	**0.001****	**0.001****		
Total pain score	Before vaccination	0.12±0.42	0.18±0.69	-0.060	0.952
	After vaccination	7.05±1.55	4.39±2.18	**-6.300**	**0.001****
	Difference	**6.93±1.58**	**4.21±2.13**	**-6.373**	**0.001****
	Z	**-6.652**	**-6.753**		
	[f]p	**0.001****	**0.001****		

[e]*Mann Whitney U Test;* [f]*Wilcoxon Signed Ranks Test* ***p<0.01*

Table 4. *Comparison of pain scores and crying duration of infants according to vaccine types (N=120)*

Total pain score	Applied Vaccine Types				χ^2	[g]p
	Hepatitis B	CPV	DaBT-IPA-Hib	Measles		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Breastfeeding Group	7.33±1.37	8.23±0.87	5.88±1.15	5.75±1.39	**28.434**	**0.001****
Distraction Group	5.08±1.73	6.23±1.15	2.69±1.40	2.58±1.73	**36.457**	**0.001****
Z	**-2.970**	**-4.799**	**-4.546**	**-3.153**		
[e]p	**0.003****	**0.001****	**0.001****	**0.001****		
Crying Duration (sec)						
Breastfeeding Group	60.75±26.06	78.41±22.27	41.38±19.04	39.88±12.83	**23.602**	**0.001****
Distraction Group	22.42±13.28	43.77±11.95	9.31±7.16	12.50±12.02	**39.386**	**0.001****
Z	**-3.669**	**-4.803**	**-4.640**	**-3.286**		
[e]p	**0.001****	**0.001****	**0.001****	**0.001****		

[g]*Kruskall Wallis Test;* [e]*Mann Whitney U Test* ***p<0.01*

Table 5. *Comparison of pain score and crying duration of infants according to vaccine application method (N=120)*

Total Pain Score		Vaccine Application Method		Z	[e]p
		IM	SC		
		Mean±SD	Mean±SD		
Breastfeeding Group		7.26±1.48	5.75±1.39	**-2.462**	**0.014***
Distraction Group		4.82±2.06	2.58±1.73	**-3.204**	**0.001****
	Z	**-5.670**	**-3.153**		
	[e]p	**0.001****	**0.002****		
Crying Time (seconds)					
Breastfeeding Group		62.32±27.13	39.88±12.83	**-2.087**	**0.037***
Distraction Group		27.62±18.70	12.50±12.02	**-2.612**	**0.009****
	Z	**-6.007**	**-3.286**		
	[e]p	**0.001****	**0.001****		

[e]*Mann Whitney U Test *p<0.05;**p<0.01*

4. DISCUSSION

This study set out to compare the effects of breastfeeding and distraction using a toy on the pain level experienced by infants during vaccinations both of which are two effective methods in accordance with the studies examining the efficiency of the pain relief intervention.

In a study conducted by Karimi et al. (20), including 4-6 months-old infants, the infants were divided into breastfeeding, sensorial saturation, and control groups. It was reported that peripheral mean SpO_2 of the infants in the breastfeeding group (95.3+2.1 in 4 month-old infants; 95.5+1.7 in 6 month-old infants) receiving pentavalent vaccination was lower than those in the sensorial saturation group (96.9+1.2 in 4 month-old infants; 96.3+1.8 in 6 month-old infants), who received a stimulus in all of their five senses ($p<0.001$). When comparing with results of the present study, SpO_2 was found to be 90.72±2.02 in infants belonging to the breastfeeding group and 95.31±2.34 in those of the distraction group after vaccination. The SpO_2 level of those in the breastfeeding group in the study by Karimi et al., was higher than those of breastfeeding group in the present study. In addition, the SpO_2 level of the infants in the distraction group was similar to that of participants in Karimi et al.,'s study. In the present study, a stimulus was given for the distraction group infants' senses of hearing (a toy with sound and music), sight (a colorful and moved toy) and touch (given that the procedure was performed on the mother's chest). In addition, in Karimi's study, the stimuli were also given to stimulate those in the sensorial saturation group's senses of taste and smell. Similar SpO_2 levels suggested that when the sensory stimuli were used effectively, they relieved the infants. In the present study, a stimulus was given for the distraction group infants' senses of hearing (a toy with sound and music), sight (a colorful and moved toy) and touch (given that the procedure was performed on the mother's chest). In addition, in Karimi's study, the stimuli were also given to stimulate those in the sensorial saturation group's senses of taste and smell. Similar SpO_2 levels suggested that when the sensory stimuli were used effectively, they relieved the infants.

In study conducted by Thomas et al. (21), involving 40 infants aged 5-15 weeks, the pain scores of infants after the DBT vaccination were evaluated using the NIPS (Neonatal Infant Pain Scale). Here, it was reported that pain total score of the infants was 4.7±1.3 in the breastfeeding group and 6.6±0.5 in the control group. When the pain score (5.88±1.15) of the breastfeeding group after the DaBT-IPA-Hib vaccination was assessed over 10 points in the present study, this score was observed to be closer to Thomas et al.,'s results (21). Furthermore, both the pain score (Table 3) and crying duration of the distraction group were statistically lower than the breastfeeding group (Table 4). These results suggested that auditory and visual sensory stimuli such as color, motion, and sound were more effective in relieving pain the infants in this age group.

Gedam et al. (10), compared the effectiveness of three methods on reducing the pain experienced by infants and children during vaccination and divided 350 infants into three groups: a distraction with a sound and light toy group, a cartoon group, and a control group. When the methods were compared, it was reported that while the FLACC pain score of the infants in the distraction with toy group was 2.30 during the vaccination, this score increased to 4.62 after the vaccination. In the same study, while the pain score of the infants in the control group was 5.3 during the vaccination, it increased to 6.20 after the vaccination. It was determined that post-vaccination pain score of the group, to which the sound&light toy was applied, was lower than those in the breastfeeding group, thus entailing a statistically significant difference ($t=11.29$; $p<0.05$). When compared with the results of the study, it was found that while the FLACC pain total score of the infants in the distraction group was close to the results of Gedam et al. (10). It was also revealed in the present study that the infants in the breastfeeding group had a similar level of pain (Table 3) with those in control group in Gedam et al's study (10).

Özdemir and Tüfekçi (11) conducted a study involving 120 two month-old infants by holding a multi-coloured toy phone that played music 20-25 cm away from their faces in order to reduce the acute pain felt during the DaPT-IPV-Hib vaccination, and thereupon their FLACC pain scores were evaluated. When the methods applied in the study were compared, the FLACC scores were found to be 5.13±2.11 among those in the experimental group, and 6.65±2.69 among those in the control group during the vaccination. When both groups were compared based on the results of the study, the pain scores during the vaccination were reported to be lower in the toy group at a statistically significant level (t=3.66; p=0.001). The FLACC pain total score was determined as 2.69±1.40 in the distraction group vaccinated with DaPT-IPV-Hib (Table 4). When the results of the present study were compared with those of Özdemir and Tüfekçi's study (11), it was observed that the post-vaccination pain score of those in the distraction group was lower in the present study. This difference was thought to be associated with the toy used, the sample size, and measurement time. In fact, unlike the present study, only one vaccination was applied in Özdemir and Tüfekçi's (11) study, and the pain scores both during and after the vaccination were evaluated. It was reported that the pain score of the infants, for whom toy method was used, decreased to 1.26±2.01 after the vaccination. This value was similar with the present study given that it was very close to the pain score (2.69±1.40) of the infants in the distraction group vaccinated with DaPT-IPV-Hib (Table 4).

When the groups were compared in terms of the administration of vaccination method, the pain scores of the infants in the distraction group were found to be lower than the breastfeeding group in both IM and SC vaccines (Table 5). In both groups, the pain scores in IM injection were higher than those in SC injection (Table 5).

In the systematic review, there was only low quality evidence suggesting that touch/massage, non-nutritive sucking, water; holding, and toy distraction were effective on the pain regulation of older infants. They also emphasized that key reasons for the lack of reliable evidence for the findings stemmed from the low quality of randomised controlled trials in the field, as well as from the limited number of studies within the same intervention (6). The results of the current study are important for the quality of evidence involving a larger sample group.

5. CONCLUSION

It was determined in accordance with the results of the study that breastfeeding was effective in reducing pain felt by infants, but when pain scores and crying durations were compared according to the vaccine type and vaccination method, distraction was more effective in reducing pain experienced by infants older than one month. This result has suggested that infants beyond one month of age are more extroverted and they are more susceptible to the stimuli from their surrounding environment. In fact, this result confirmed higher HR and lower SpO_2 level in the breastfed infants, as well. Breastfeeding is preferable prior to one month of age to reduce interventional pain, whereas distraction appears to be preferable for those above one month of age.

Implications for clinical pratice

Distraction method is more effective than breastfeeding when it comes to reducing acute interventional pain experienced by infants older than one month of age since those who fall within these age groups are very much affected by environmental stimuli. Breastfeeding is preferable prior to one month of age, whereas distraction appears to be preferable for those above one month of age. Practice such as distraction, should be used as a nursing intervention additionally breastfeeding to reduce the pain after vaccination among infants. Its use should be expanded and encouraged through in-clinic trainings for nurses.

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Clinical and Experimental Health Sciences

Prevalence of Orthorexia Nervosa in Academicians and the Influencing Factors

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ABSTRACT

Objective: Orthorexia Nervosa/Healthy Eating Obsession is defined as a pathological obsession for consuming appropriate and healthy food. The aim of this study is to determine the prevalence of orthorexia nervosa in academicians working in a public university and the influencing factors.

Methods: This study was concluded between 01 January– 01 March 2020 to determine the prevalence of Orthorexia Nervosa and its correlation with influencing factors. The data of the study were collected using the Eating Attitude Test-40 (EAT-40) and the ORTO-11 along with the information form.

Results: In this study, it was observed that an advanced age caused a higher risk of eating disorder. In addition, the variables of being in the age range of 34-41 years, being female, and receiving undergraduate education were significant in terms of reducing the risk of Orthorexia Nervosa ($p>0.05$).

Conclusion: Factors such as industrialization, developments in the socio-economic structure, and mass media cause changes and new tendencies in dietary habits and food consumption. As it is believed that these conditions will become even more widespread in the future and affect all sections of society, including academicians who are accepted to be a risk group due to their perfectionist structure; it is of prime importance to raise awareness of society and inform individuals from a multidisciplinary approach including dieticians.

Keywords: Academician, Healthy Eating Obsession, Influencing Factors, Orthorexia Nervosa, Prevalence

1. INTRODUCTION

Orthorexia Nervosa (ON), also known as “healthy eating obsession”, was defined by Steven Bratman in 1997 for the first time to diversify Anorexia Nervosa (AN). Because “ortho” means “straight” and “truth”, Bratman used the term ON to define a pathological obsession related to consuming appropriate and healthy food (1). ON has not been included in the DSM-V (Diagnostic and Statistical Manual of Mental Disorders-V, 2013) of the American Psychiatric Association, yet (2).

ON has been included in the research objectives of clinicians in the world recently. The increasing prevalence of obesity and especially the increasing number of studies aiming to prevent obesity in the United States of America have attracted attention to the necessity of leading a healthy life from all aspects and have led individuals to suffer from healthy eating obsession (1). Healthy eating habits are not actually pathological. However, ON can be evaluated as a personality and behavioral disorder when it becomes excessive, lasts too long and causes negativities in daily life (3).

ON is a new category in which the researchers focus intensely and deliberately on whether it can be defined as a disorder or not (4). It also requires discussions regarding whether ON will be examined as a subset of Anorexia Nervosa or Obsessive Compulsive Disorder (OCD) or as a different disorder. Similar properties of ON and AN are perfectionism, constant high anxiety, intense effort for keeping the control, and significant weight loss. Orthorexic and anorexic individuals are success-oriented, consider a commitment to their diet as an indicator of self-discipline, and evaluate diversion from the diet as a failure of self-control (5). While eating disorders like AN and bulimia nervosa are expressed in a quantitative context in orthorexia (for example, the amount of food consumed), they are expressed in a qualitative context in ON (quality of food consumed) (6).

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It is indicated that patients with healthy eating obsession spend most of their time engaging in strict rules just like OCD patients and consequently their social functions may get damaged in the course of time (4). Extreme orthorexia cases state that they prefer making themselves starve instead of consuming "impure" food that harms their health (7). The obsessive situation drives individuals to follow a strict diet or exclude essential nutrients from their diet and thus, individuals with these properties suffer from poor nutrition and malnourishment (4). In other words, orthorexic individuals can be evaluated in the group with risk of malnutrition as they have a tendency to gradually restrict their diet due to their effort of reaching perfectionism (8).

The purpose of this study is to determine the prevalence of orthorexia nervosa in the academicians working in different departments of Bingol University and the influencing factors.

2. METHODS

This descriptive study was conducted with the academicians working in different departments of Bingol University between 01 January and 01 March 2020 to determine prevalence and correlation of orthorexia nervosa. The population of the study consisted of 606 academicians working in different departments. No sample selection was performed. The entire population was included in the study (560 people, Responsivity rate; 92.40%).

The data of the study were collected using the Eating Attitude Test-40 (EAT-40) and the ORHO-11, along with information form. The data were collected by a research associate using the face-to-face interview method in order to prevent any bias possibility.

2.1. Information Form

It was prepared to determine some characteristics (age range, gender, marital status, educational level, title, perception of appearance, weight and height, state of having any health problem, the desire of eating according to mood, receiving any hormonal therapy, and doing physical activities regularly) of the participants. Body Mass Index (BMI) was calculated for the weight and height values reported based on personal statements of the participants (with the formula BMI: weight (kg)/ height (m^2)). The BMI classification was based on the criteria established by the World Health Organization and the weight of < 18.5 is classified as slim, the weight of 18.5-24.9 as normal, the weight of 25.0-29.9 as overweight and the weight of >30.0 as obese.

Waist and hip measurements of the individuals were measured with tape measure and recorded in the surveys. Waist circumference was measured with tape measure passing through the middle point by finding the region between lower rib and crista iliaca and the measurements were recorded by taking the circumference measurement from the highest point for the hip circumference.

2.2. Eating Attitude Test-40 (EAT-40)

The test was developed by Garner and Garfinkel (1979) to measure eating disorder symptoms. The Turkish validity and reliability study of the EAT-40 was conducted by Savaşır and Erol (1989) and its test-retest reliability was found to be 0.65 (9). The responses of this Likert-type scale consists of six stages range from "always" to "never". For the scale items 1, 18, 19, 23, 27, and 39; the option "sometimes" is evaluated as 1 point, "seldom" as 2 points, "never" as 3 points, and other options as 0 point. Total score of the scale ranges between 0 and 120. Higher scores signify that eating disorder pathology increased. A score of ≥30 points imposes a risk for an eating disorder (9).

2.3. Orthorexia-11 (ORTO-11) Scale

The scale was developed by Steven Bretmen in 2000 to determine orthorexia nervosa in individuals. It is a self-report scale. It was adapted into Turkish by Arusoglu in 2008 as ORTO-11. The scale is a 4-point Likert scale. Individuals rate the frequency of feeling the situations in the items with the options "always", "often", "sometimes" and "never". The cut-off point of the scale is 27 points and individuals receiving any value less than cut-off point are accepted as orthorexic. The Cronbach's Alpha value of the scale was reported to be 0.62. Higher scores signify the decreased risk of ON (4).

The limitation of the study is that only the academicians working in a public university in one geographical region in 2019 were included in the study and only cross-sectional design was used. In addition, it is an important limitation that the study was based only on the self-report of the participants.

Before starting the study, ethical approval from Bingol University Scientific Research and Publishing Ethics Committee and verbal consents from the participants were obtained (approval number: 2017-28, date: 07.11.2017). They were informed about the criteria of the Declaration of Helsinki in written and through an informed consent form.

2.4. Data Analysis

In the study, the data were evaluated using the SPSS-21 software package. Responses given by the participants to the questions in the information form were given as numbers and percentages in tables and they were accepted as the independent variables. In the distributions of EAT-40 and ORTO-11 scores of the participants; mean, standard deviation, standard error, minimum value, and maximum value were utilized and these scales were accepted as the dependent variables. The difference between the independent variables and dependent variables was evaluated with analyses of parametric conditions and also proper tests (T/U test, F/KW test) were used. Post-hoc analyses (Tukey HSD) were performed in case of difference

in multiple variables. Correlation analyses were performed to determine the correlation between the scale scores. In the study, the significance level was accepted as 0.05.

3. RESULTS

A great majority of the participants (45.7%) were in the age range of 34-41 years. 82.5% were male. 58.4% of the participants were married. The number of academics and research associates was more than the number of academic members (Table 1).

Mean and standard deviation in total scores of the quantitative data obtained in the study were 25.09±2.68 (15.67-34.72) in BMI, 142.67±9.43 (112-167) in EAT-40, and 25.51±4.06 (11-37) in ORTO-11.

As seen in Table 2, BMI mean rank was higher in those who were male, were at an advanced age, specified their marital status as other, had a higher level of education, had a higher title and did not do physical activities regularly. A statistical difference was observed in these variables ($p<0.05$). According to the table, it was also seen that the mean rank of the EAT-40 score was higher in those at an advanced age ($p<0.05$). In addition, the variables of being in the age range of 34-41 years, being female and having undergraduate education were significant in terms of reducing the risk of ON ($p>0.05$).

As seen in Table 3, it was determined that there was no correlation between the participants in terms of EAT-40, ORTO-11, BMI and waist circumference values.

Table 1. *Distribution of the Participants According to Some Characteristics (N=560)*

Variable	Characteristics	Number (n)	Percentage (%)
Age	18-25 years	14	2.5
	26-33 years	182	32.5
	34-41 years	256	45.7
	42-50 years	80	14.3
	51-60 years	28	5.0
Gender	Female	98	17.5
	Male	462	82.5
Marital status	Married	327	58.4
	Single	221	39.5
	Other	12	2.1
Education level	Undergraduate	15	2.7
	Postgraduate	234	41.8
	Doctorate	311	55.5
Present title	Professor	31	5.5
	Assoc. Prof. Dr.	26	4.6
	Asst. Prof.	173	30.9
	Research Assoc.	192	34.3
	Instructor	138	24.6
Health problem	Available	125	22.3
	N/A	435	77.7
The emotional condition causing to eat more	Excitement	135	24.1
	Sadness	160	28.6
	Stress	126	22.5
	Happiness	139	24.8
Have received any hormonal therapy?	Yes	108	19.3
	No	452	80.7
Doing physical activities regularly	Yes	255	45.5
	No	305	54.5
BMI (n=532)	Slim	2	0.4
	Normal	294	55.3
	Overweight	206	38.7
	Obese	30	5.6

Table 2. *Distribution of the BMI, EAT-40 and ORTO-11 Scale Scores of the Participants According to Some of Their Characteristics (N=560)*

		n	BMI	Test value	EAT-40	Test value	ORTO-11	Test value
			Mean Rank		Mean Rank		Mean Rank	
Age	18-25 years	14	175.67		143.79		208.79	
	26-33 years	182	249.94	KW=17.794	291.94	KW=11.25	288.85	KW=19.27
	34-41 years	256	274.53	**p=0.001**	278.94	**p=0.024**	296.98	**p=0.001**
	42-50 years	80	299.74		287.60		257.90	
	51-60 years	28	354.50		268.50		176.00	
Gender	Female	98	254.98	U=19822.0	272.70	U=21874.0	325.52	U=18226.0
	Male	462	275.06	p=0.254	282.15	p=0.599	270.95	p=**0.002**
Marital status	Married	327	291.92		293.33		278.04	
	Single	221	236.95	KW=20.04	261..58	KW=5.08	281.31	KW=1.338
	Other	12	363.67	**p=0.001**	279.17	p=0.079	332.67	p=0.512
Education level	Undergraduate	15	182.97		228.83		379.57	
	Postgraduate	234	247.11	KW=16.616	287.98	KW=2.149	293.98	KW=9.964
	Doctorate	311	294.22	**p=0.001**	277.36	p=0.341	265.58	**p=0.007**
Present title	Professor	31	280.40		294.37		206.76	
	Assoc. Prof. Dr.	26	271.21	KW=24.302	260.12	KW=2.750	259.58	KW=7.545
	Asst. Prof.	173	319.15	**p=0.001**	274.96	p=0.601	286.34	p=0.110
	Research Assoc.	192	239.40		273.66		285.35	
	Instructor	138	256.88		297.70		286.94	
Health problem	Available	125	284.15	U=24481.0	303.64	U=24295.5	282.84	U=26895.5
	N/A	435	267.71	p=0.303	273.85	p=0.069	279.83	p=0.854
The emotional condition causing to eat more	Excitement	135	252.95		291.89		282.78	
	Sadness	160	268.73	KW=3.217	264.29	KW=6.564	278.54	KW=1.131
	Stress	126	282.63	p=0.359	305.67	p=0.087	291.33	p=0.770
	Happiness	139	282.82		265.28		270.72	
Have received any hormonal therapy?	Yes	108	276.26	KW=2263.0	302.47	KW=22035.0	291.44	KW=23226.0
	No	452	270.34	p=0.727	275.25	p=0.116	277.88	p=0.432
Doing physical activities regularly	Yes	255	255.69	U=32446.5	269.29	U=36029.5	272.87	U=36942.5
	No	305	284.06	**p=0.036**	289.87	p=0.134	286.88	p=0.306

Table 3. *Correlation between the Body Mass Indices, Waist Circumferences, Eating Attitudes and Orthorexic Scores of the Participants* (N=560)*

		EAT-40	ORTO-11	BMI	Waist Circumference
EAT-40	Rho	1			
	p	-			
ORTO-11	Rho	-0.040	1		
	p	0.343	-		
BMI	Rho	-0.023	0.059	1	
	p	0.590	0.172	-	
Waist Circumference	Rho	0.035	-0.071	-0.009	1
	p	0.409	0.094	0.843	-

**Spearman's correlation analysis*

4. DISCUSSION

ON is accepted as important because of pathological efforts made by individuals. It is believed that these individuals experience emotional displeasures and social isolations (10). In the literature, it is stressed that ON can turn a personality and behavioral disorder after a certain time (11). This study was conducted to determine the prevalence of ON (orthorexia nervosa) in the academicians working in different departments of Bingol University and the influencing factors.

Mean and standard deviation values in EAT-40 and ORTO-11 scores of the participants were 142.67±9.43 (112-167) and 25.51±4.06 (11-37), respectively; whereas, the mean and standard deviation in BMI was 25.09±2.68 (15.67-34.72). In the study by Yeşil, it was determined that the ORTO-11 mean score was 26.3±3.61 in women and 26.9±3.85 in men (10). It is known that although orthorexic individual aims to protect her/his health, his/her self-limiting behavior will show differences in terms of many variables. In this study, the BMI mean rank was higher in those who were male, were at an advanced age, specified their marital status as other, had a

higher level of education, had a higher title and did not do physical activities regularly. These conditions are important in terms of statistical difference. In the study, it was observed that being at an advanced age led to a higher risk of eating disorder. In addition, the variables of being in the age range of 34-41 years, being female and having undergraduate education were significant in terms of reducing the risk of ON ($p>0.05$). In the study by Atmaca and Durat, the EAT-40 mean score was found to be 21.0000±20.00 in women and 21.0000±16.00 in men (12).

In the study conducted by Öztürk and Yabancı Ayhan with individuals in the age range of 18-25 years, they determined that the participants' age did not create a difference in terms of the ORTO-11 scores (13). The study was conducted in a narrow age range like 18-25 years; therefore, it is expected that this result was different from the result of this study. Hence the fact that the ORTO-11 score was remarkable at an advanced age in this study was significant.

Likewise, the literature emphasizes that eating disorders are encountered in women more frequently (1). In the study, being female was found to be an important variable in terms of the risk of ON. There are studies indicating that orthorexic tendency is higher in women (10,14,15). Other studies also indicate that it is higher in men (16-18). These differences between the studies are associated with cultural differences of study groups and study designs.

In this study, it was found that the variable of marital status caused no difference in terms of being orthorexic, which shows parallelism with other studies in the literature (10, 19). In a study conducted on adults in Italy, orthorexia nervosa tendencies of singles were found to be higher than the others (20).

In the study, it was observed that individuals with undergraduate education were more orthorexic than those with higher education in terms of educational level. The result in the study by Arusoglu et al., is compatible with the result of the present study (4). In the study of Oktay (21) on university students, it was stated that 19.40% of those whose fathers were literate and primary school graduates, 16.30% of those who graduated from high school, and 12.50% of those who graduated from university showed orthorexic tendency but reported no statistically significant difference.

BMI is an important variable in terms of eating disorders (10). No correlation was determined between the participants in terms of the EAT-40, ORTO-11, BMI and waist circumference values in this study. Likewise, Öztürk and Yabancı Ayhan found no correlation between the BMI and ORTO-11 scores in their study (13). However, some studies obtained different results. For example, in their study, Fidan et al., determined that there was a reverse correlation between the BMI and orthorexic scores (17). In another study conducted in 2017, it was determined that there was a negatively weak correlation between being orthorexic and eating attitude scores, which shows contradistinction to this study (12). In a study, it was stressed that orthorexic individuals faced problems such as malnutrition, hypoproteinemia, B12 vitamin deficiency and emphysema more frequently and they even become bedridden as a result of routinized nutrition (22). In this study, it was concluded that the participants' ORTO-11 scores were not correlated with their eating attitude scores. In the literature, it is pointed out that eating disorders are common among models, dancers, yogi, sportsmen, and medical personnel (23-25). In this study, it was determined that there was a significant difference between occupations, eating attitudes, and orthorexia scores of the participants working as academicians.

5. CONCLUSION

In the study, it was determined that the BMI mean rank average was higher in those who were male, were at an advanced age, specified their marital status as other, had a higher level of education, had a higher title and did not do physical activities regularly. Being at an advanced age led to a higher risk of eating disorder. In addition, the variables of being in the age range of 34-41 years, being female and having undergraduate education were significant in terms of reducing the risk of ON ($p>0.05$).

Consequently, factors such as industrialization, changes and developments in the socio-economic structure, opening to the western culture, the desire of living a long healthy life, mass media and advertisements today cause changes and new tendencies in dietary habits and food consumption. As it is believed that these conditions will become even more widespread in the future and affect all sections of society, including academicians who are accepted to be a risk group due to their perfectionist structure, it is of prime importance to raise awareness in society and inform individuals from a multidisciplinary approach including dieticians.

Informed Consent: Written informed consents were obtained from all participants.

Peer-review: Externally peer-reviewed.

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Clinical and Experimental Health Sciences

Development of a Parental Attitude Scale for Rational Drug Use

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ABSTRACT

Objective: The aim of this study is to develop a valid and reliable measurement tool to identify parental attitudes towards rational drug use.

Methods: The sample of the methodological study included 517 parents. "The Parent Information Form" and the "Parental Attitude Scale for Rational Drug Use (PASRDU)" were used to collect data. In the assessment of the data, validity and reliability analyses were applied.

Results: In the study, CVI was calculated as 0.71. For the exploratory factor analysis, KMO score was 0.86, and Bartlett's test was x^2=7.559.22 in the study. For the confirmatory factor analysis, X^2/Sd was measured at 3.47, GFI at 0.94, AGFI at 0.93, CFI at 0.92, RMSEA at 0.0,6 and SRMR at 0.06, and the scale structure was approved according to these findings. Consequently, the scale was formed of 40 items and two sub-scales. The Cronbach's alpha value of the scale was 0.88. Item-total correlation values were 0.32-0.61, and test-retest value was r = 0.85.

Conclusion: Validity and reliability analyses conducted during the process of scale development showed that PASRDU is a valid and reliable scale that finds out parents' attitudes towards rational drug use. It is also useful for nurses to use within the safety criteria of drugs. This scale enables the nurse to determine the lack of rational drug use and provide training and consultancy on this subject. This scale can be used in primary and preventive health services.

Keywords: Attitude, nurse, parents, rational drug use, scale.

1. INTRODUCTION

Rational drug use (RDU) has become an important issue in primary and preventive health services today. Great importance has been attached to rational drug use in Turkey and the world, and many institutions, especially the World Health Organisation (WHO), work on this issue.

Rational drug use is defined as "a set of rules the patients need to obey to receive medications by their clinical needs, in doses to meet their own individual requirements, for an adequate period, and at the lowest cost for them and the society" (1). This definition highlights four basic principles; appropriate medication, appropriate dose, adequate period, and appropriate cost (2). RDU aims to decrease the social and financial burdens that incorrect use of drugs imposes on society and to pent related physiological, biological, and psychological damages (3).

A report published by WHO significantly emphasises that more than half of all drugs are not administered correctly. Its results indicate that this is also true for Turkey (4). The rate of irrational drug use among parents has also increased in developed countries (5). The studies have reported that drugs are not used correctly in paediatric patients (6-8).

Rational drug use includes the planning, conducting, and monitoring process for the prescribed drugs, administered in a safe, economical, and effective manner and requires behaving rationally based on the country, the drug industry, healthcare professionals, and the society (9,10). RDU knowledge/skills and sensitivity of all the physicians, pharmacists, nurses, and patients are effective in the prevention of current and future problems. It is important for healthcare professionals to be knowledgeable about RDU within the context of their jobs and to raise awareness by training society (11). One of the most important responsibilities of nurses is to enable patients to use drugs within the criteria of safety (12).

Drugs are used commonly for treating diseases and providing vitamin/mineral support in paediatric patients. Basic differences between adults and children in terms of drug

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administration are age, weight, and physiological differences (13,14). Absorption, distribution, and metabolism of drugs, certain processes in their excretion, and deficiencies in specific drugs in children make them one of the high-risk populations in drug use. There are several factors that make the paediatric population more susceptible to complications associated with drug therapy. These factors are the administration of the same drug at different doses, wrong dose adjustment, lack of standard dose regimen, and immature organ system (15-22). The most important RDU problems in the paediatric population are seen in the use of antipyretantibioticsoti,c and cough medicine groups (23). For this reason, RDU is important for all individuals but much more important for children. Parents' attitudes and behaviours become much more important in the rational use of drugs in their children.

Parents administer drugs to their children. During non-hospital treatment stages, parents should pay attention to the effect, method of use, time, dose, and side effects of the drugs. The role of the nurse in providing correct guidance and training to parents concerning RDU is crucial. However, it is also important to identify the attitudes of the parents towards rational drug use and the factors effective in planning the counselling and training. The responsibilities of the nurse in RDU are to provide training on possible side effects of the drugs to parents. The nurses should first inquire about the parents' knowledge of the indications, use, time, dose, and side effects of the drugs. Then, they should show indications of the drugs, the dose amount, the right time, and the points to consider (24).

Nurses can positively affect parents' attitudes towards RDU and contribute to the benefit of society by replacing false information with correct ones. They can contribute to the promotion of child health by providing training and counselling to their parents, especially mothers, about the rational use of drugs in their children (24).

There is no national or international standard measurement tool assessing RDU in parents in the literature. In most studies assessing RDU, the data were collected using different questionnaires. However, there is a need for a valid and reliable measurement tool that will measure more objectively the results in order to obtain data based on scientific information. The aim of this study is to develop a valid and reliable measurement tool to identify parental attitudes towards rational drug use.

Research Questions

Is the Parental Attitude Scale for Rational Drug Use valid and reliable?

2. METHODS

2.1. Design

This methodological study was conducted to develop a valid and reliable Parental Attitude Scale for Rational Drug Use to identify parental attitudes towards rational drug use.

2.2. Setting and Sample

The study included parents with children aged between 0 and 12 years who applied to the outpatient clinics and the Family Health Centre (FHC) in the area where the study was conducted. As is stated in the literature about scale development and adaptation studies, it is necessary to reach 300–500 people or 5 to 10 times the number of items ionthe scale (25,26). Based on this information, the sample of the study included 63 items and 517 parents. As a result, it was calculated as 517, which is approximately eight times larger than the item number for the 63-item scale. These parents were literate, had no hearing and vision loss, had a 0-12-year-old child, had previously administered medicine to their child, and were voluntary to participate in the study.

2.3. Data Collection

The data were collected in the paediatrics outpatient clinics of a university hospital, a training-research hospital, and a FHC in Erzurum between May and December 2017. "The Parent Information Form" and the "Parental Attitude Scale for Rational Drug Use (PASRDU)" were used to collect data. The researcher collected the data using the face-to-face interview method. Parents filled out the questionnaire themselves. Each interview lasted for 20-30 minutes.

2.4. Item Selection

Upon the literature review conducted by using the "rational drug use, child, parent, nursing" keywords in order to form an item tool for the draft PASRDU, the related studies and scales were reviewed. The researcher prepared a total of 77 items about positive and negative attitudes. Later, the draft scale was sent to the experts for content validity.

2.5. Content Validity and Pilot Application

In the study, Lawshe's Technique was used for the content validity of the draft scale. The draft PASRDU was presented to 16 experts. These experts were academicians experienced in rational drug use, paediatric nursing and scal,e development. 14 items out of 77 items having a Content Validity Ratio (CVR) of 0, negative (-,) and less than 0.49 at the significance level of α:0.05 were removed from the study. As a result of the content validity study, the draft scale included 63 ite,ms anthe d Content Validity Index (CVI) was calculated as 0.71 (>0.67).

Later, in a pilot application, the draft scale was applied to 50 parents from different educational levels who agreed to participate in the study through the face-to-face interview method. The parents were asked to fill out the draft scale and then evaluate each item in terms of understanding and relevance. It took 30-40 minutes per each parent to complete the data collection process. After the pilot application, five items were revised in terms of spelling and punctuation based on their comments. Thus, the draft scale was prepared for advanced analysis with 63 items.

2.6. Measurement

2.6.1. Parent Information Form

This form, which was prepared by the researcher based on literature (7,10,12), includes a total of 13 questions about parents' age, gender, educational status, where they go to most frequently for medication when their child gets ill, and the drugs they give most frequently without a prescription.

2.6.2. Parental Attitude Scale For Rational Drug Use (PASRDU)

The scale with 40 items and two subscales was put into final form. Subscales of the scale are *Correct and Conscious Use* (29 items) and *Effective and Safe Use* (11 items). 12 of the 40 itemonin the scale consist of negative expressions. Negative items are reversely coded. Every item in the Likert-type scale is rated from 1 to 5. High scores signify that parenteral positive attitudes for rational drug use increase.

2.7. Data Analysis

The data were analysed using SPSS for Windows 22.0 and LISREL programme. Face and content validity, construct validity, and reliability analyses were used in the data assessment. Content Validity Index was performed for content validity of the scal,e and exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) were performed to determine its construct validity. Kaiser-Meyer Olkin (KMO) test, Bartlett's test, and Varimax Rotation test for EFA as well as X^2/Sd, Root Mean Square Error of Approximation (RMSEA), Standardised Root Mean Square Residual (SRMR), Goodness of Fit Index (GFI), Adjusted Goodness of Fit Index (AGFI), Comparative Fit Index (CFI), fit tests and PATH diagram for CFA were used. Item-total correlation tests, Cronbach's Alpha internal consistency coefficient,t and correlation analysis related to test-retest reliability were used in order to determine the reliability analysis. In addition, the demographic characteristics of the parents were analysed using descriptive statistics.

2.8. Ethical Considerations

Ethics committee permission was obtained from the Faculty of Health Sciences Ethics Committee (dated 06/02/2017 numbered 2017/01/06). Written permissions from the related outpatient clinics and FHC were obtained. After the parents were informed about the purpose and method of the study, their verbal and written consents were taken. Ethical principles were met in the study.

3. RESULTS

3.1. The Demographic Characteristics of the Parents

The average age of the parents was 31.72±5.59, 80.1% were mothers, 35.2% were university graduates, and 54% were unemployed. The place people most commonly went to when their children fell ill was the state hospital (40.2%), 83.2% of the parents most commonly administered antipyretics without a prescription. The total mean score of the parents was 157.13±16.25 (Table 1).

Table 1. *Distribution of Parents' Score from the Attitude Scale towards Rational Drug Use*

Subscales and scale	n	Min-max	Min.	Max.	Mean	SD
Accurate and Conscious Use	517	29-145	40.00	140.00	120.62	11.55
Effective and SafeUse	517	11-55	17.00	52.00	36.51	7.68
PASRDU	517	40-200	85.00	190.00	157.13	16.25

SD: Standard Deviation
PASRDU: Parental Attitude Scale for Rational Drug Use

3.2. Construct Validity

Exploratory and confirmatory factor analyses were carried out for construct validity.

3.3. Exploratory Factor Analysis (EFA)

The KMO was used for sampling adequacy, and Bartlett's test of sphericity was used to determine the correlation between the items for factor analysis. While the KMO value of the scale was 0.863, its Bartlett's test values were x^2=7.559.228 and p<0.001, which indicated that the data were correlated and suitable for factor analysis.

Principal component analysis and the varimax rotation method were used to perform EFA. In the principal component analysis, a 2-factor structure was determined. After the rotation was appltwoed 2 times, 18 items out of 63 items with factor loadings of less than 0.30 were removed from the scale. The factor loading values of the scale items ranged from 0.332 to 0.701 (Table 2). In additiontwo 2 subscales of the scale accounted for 28.356% of the total variance. As a result, PASRDU was put into final form wtwoth 2 subscales and 45 items.

3.4. Confirmatory Factor Analysis (CFA)

Confirmatory factor analysis was used to determine whether or not the items represented the subscales and whether or not the subscales accounted for the scale structure. X^2/Sd, SRMR, RMSEA, CFI, AGFI, and GFI compliance tests were used in confirmatory factor analysis. X^2/Sd was measured as 3.47, GFI as 0.94, AGFI as 0.93, CFI as 0.92, RMSEA as 0.069, and SRMR as 0.066 (Table 3). The fit indices showed that the model was acceptable as it was.

According to PATH diagram results, two items with factor loadings of lower than 0.30 and 3 items with a t-value less than 1.96 were removed from the scale. The 40-item version of the model was accepted without further modifications

instead of its 45-item version. The factor loadings of the model ranged from 0.32 to 0.,69 and the t values of all items were higher than 1.96 (Figure 1).

Table 2. *Items and Factor Loads of Parental Attitude Scale for Rational Drug Use (45 Item)*

Item	Subscales	
	Correct and Conscious Use	*Effective and Safe Use*
s 1	**0.386**	0.105
s 2	**0.538**	0.128
s 4	**0.476**	0.314
s 6	**0.572**	0.199
s 7	**0.445**	0.130
s 9	**0.499**	-0.157
s 10	**0.630**	0.048
s 11	**0.554**	-0.082
s 12	**0.398**	0.028
s 16	**0.522**	0.085
s 17	**0.602**	0.109
s 18	**0.576**	-0.009
s 19	**0.475**	-0.139
s 23	**0.351**	0.001
s 29	**0.594**	-0.002
s 33	**0.347**	0.271
s 35	**0.597**	0.142
s 36	**0.617**	-0.008
s 41	**0.439**	-0.199
s 42	**0.701**	0.038
s 44	**0.454**	0.201
s 48	**0.490**	0.197
s 51	**0.481**	0.078
s 52	**0.488**	0.001
s 53	**0.465**	0.090
s 55	**0.340**	-0.305
s 58	**0.400**	0.338
s 59	**0.564**	0.348
s 60	**0.594**	0.207
s 63	**0.475**	0.074
s 3	0.097	**0.359**
s 8	-0.173	**0.603**
s 15	0.019	**0.542**
s 20	0.115	**0.579**
s 30	0.150	**0.612**
s 31	-0.132	**0.442**
s 32	0.163	**0.390**
s 34	0.104	**0.332**
s 37	0.264	**0.384**
s 45	0.211	**0.512**
s 47	0.209	**0.401**
s 49	0.114	**0.535**
s 50	0.181	**0.623**
s 54	0.091	**0.607**
s 62	0.102	**0.404**

Table 3. *Compliance Index Values Determined for the Scale*

Index	Normal Value	Acceptable Value	Found Value
X^2/Sd	<2	<5	**3.47**
GFI	>0.95	>0.90	**0.94**
AGFI	>0.95	>0.90	**0.93**
CFI	>0.95	>0.90	**0.92**
RMSEA	<0.05	<0.08	**0.069**
SRMR	<0.05	<0.08	**0.066**

GFI: Goodness of Fit Index
AGFI: Adjusted Goodness of Fit Index
CFI: Comparative Fit Index
RMSEA: Root Mean Square Error of Approximation
SRMR: Standardised Root Mean Square Residual

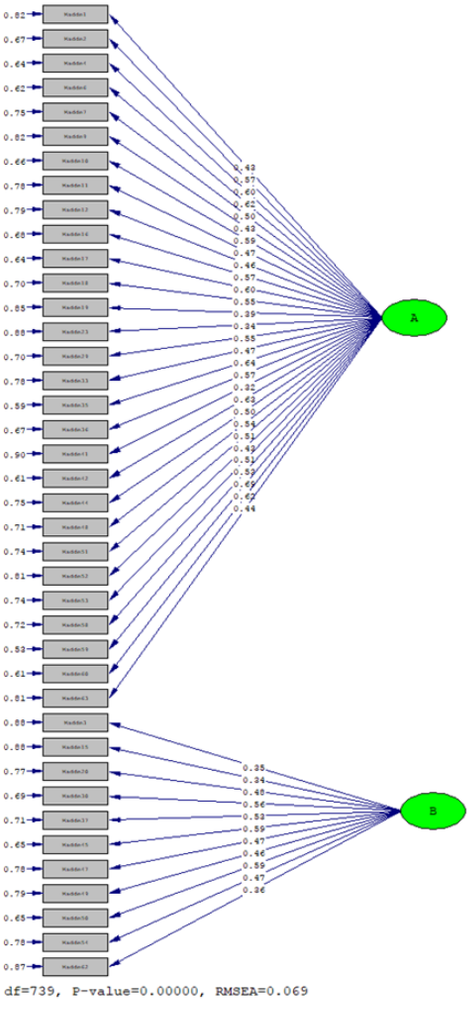

Figure 1. *PATH Diagram of PASRDU*

3.5. Reliability

A test-retest reliability analysis was conducted using Cronbach's α calculation and item-total correlation tests in order to determine the reliability of the PASRDU. The Cronbach's α coefficient of the scale was found to be 0.887 for the overall scale, 0.894 for the *Correct and Conscious Use* subscale, and 0.771 for the *Effective and Safe Use* subscale.

The item-total score correlations varied between 0.22 and 0.61 (Table 4).

To determine the time dependent invariance of PASRDU, the scale was applied to the same individuals (n=50) again 15 days later by using the test-retest method. A statistically positive correlation was found between both measurement scores (r: 0.85; p< 0.001).

Table 4. *Scale Items, Mean Values, Item-Total Correlation, and Cronbach's α Values if Item is Deleted*

Item	n	Mean	SD	Item-Total Correlation	Cronbach's α Values if Item is Deleted
1. I learn about my child's medicines from healthcare professionals.	517	4.44	0.56	0.348	0.885
2. I know what the medicine I give my child will be used for.	517	4.36	0.74	0.466	0.883
4. I check the prescription written to my child.	517	4.06	0.93	0.517	0.882
6. I give my child her/his medication as recommended.	517	4.46	0.64	0.532	0.883
7. I prepare my child's medicines in suspension as indicated in the instructions for use.	517	4.39	0.80	0.392	0.884
9. I know the side effects of the medicines I will give to my child.	517	3.69	1.16	0.302	0.886
10. I look at the expiration date of the medicines I will give to my child.	517	4.41	0.73	0.518	0.882
11. I keep my child's medication with instructions for use.	517	4.32	0.92	0.385	0.884
12. If I think the medicines I will give to my child are spoiled, I will throw them away.	517	4.43	0.85	0.311	0.885
16. I do not use the medicine recommended by others for my child.	517	4.08	1.02	0.447	0.883
17. I read the instructions for the medicines I will give to my child.	517	4.35	0.76	0.52	0.882
18. I keep the medicines out of the reach of my child.	517	4.51	0.74	0.433	0.883
19. If the medicine I give to my child causes side effects, I will stop using the medicine.	517	4.38	0.75	0.283	0.885
23. I do not give my child medicine without a prescription.	517	4.07	0.96	0.266	0.886
29. I give my child her/his medication for the recommended time.	517	4.25	0.80	0.454	0.883
33. If I need to give my child more than one medicine, I will mix the medicines together.	517	4.00	1.18	0.396	0.884
35. Before I give my child the suspension medications, I shake them.	517	4.36	0.76	0.524	0.882
36. When storing my child's medicines, I pay attention to the storage conditions written in the instructions for use.	517	4.43	0.72	0.456	0.883
41. I keep my child's medicine with its box to protect from light.	517	4.34	0.84	0.222	0.886
42. I give my child their medication at the recommended time intervals.	517	4.52	0.55	0.555	0.883
44. Unnecessary use of drugs is harmful to health.	517	4.43	0.86	0.447	0.883
48. If I do not see the benefit of the medicine I use for my child, I consult the doctor.	517	4.36	0.78	0.448	0.883
51. I pay attention to the hunger-satiety condition before giving the medicine to my child.	517	4.38	0.74	0.389	0.884
52. I give my child the medicine with the scale that came out of the box.	517	4.42	0.70	0.359	0.885
53. I give my child their medication in the recommended dose / amount.	517	4.48	0.73	0.374	0.884
58. If my child's medicines in suspension form are not finished within 10 days, I discard the remaining part.	517	4.10	0.95	0.611	0.880
59. I use boiled and cooled water while preparing my child's medicine in suspension form.	517	4.50	0.63	0.555	0.882
60. If the medicine I give to my child causes side effects, I consult the doctor.	517	4.38	0.74	0.389	0.884
63. I do not give my child antibiotics without a prescription.	517	4.66	0.51	0.393	0.885
3. When my child is sick, I give the medicines that are at home before applying to the health institution.	517	3.37	1.27	0.238	0.887
15. When my child is sick, I use more than one medicine alternately that has the same effect.	517	3.17	1.34	0.260	0.887
20. I give my child's medicine in capsule form by opening the capsule.	517	3.11	1.26	0.355	0.885
30. I recommend the drug, which I think is effective for my child, to someone else.	517	3.26	1.39	0.407	0.884
37. If the medicine I give to my child causes side effects, I will look for a solution myself.	517	3.08	1.45	0.393	0.885
45. I learn about my child's medicines from my environment.	517	3.95	1.14	0.402	0.884
47. I give my child the medicine with milk.	517	3.78	1.17	0.359	0.885
49. I give my child's medicine in tablet form by crushing.	517	2.84	1.20	0.345	0.885
50. When my child has similar complaints as before, I give home remedies without consulting anyone.	517	3.59	1.25	0.441	0.883
54. After my child's medication is over, I keep the remainder of her/his medication at home.	517	2.62	1.31	0.354	0.885
62. I give my child the medicine with the juice.	517	3.75	1.10	0.345	0.885
Total Cronbach's α	**0.887**				
Correct and Conscious Use	**0.894**				
Effective and Safe Use	**0.771**				

SD: Standard Deviation

4. DISCUSSIONION

No national or international standard measurement tool was found in the literature to assess RDU in parents. In most dies assessing RDU, the data were collected using different questionnaires (5-8,17,18).

A newly developed measurement tool should identify two characteristics: validity and reliability (27-30). The item pool was formed for face validity of the draft PASRDU; then the peer assessment was made by reassessing the scale according to the expert views before the pilot study was conducted. For content validity, the CVI value is expected to be greater than 0.67 (31). PASRDU's CVI value was calculated as 0.71, confirming PASRDU's content validity and demonstrating that the draft PASRDU is a sufficient scale of rational drug use.

Afterward, the construct validity of the draft scale was assessed. In this study, exploratory and confirmatory factor analyses were carried out to determine the construct validity of the PASRDU. The construct validity of the scale is evaluated to determine how accurately a measuring tool measures the abstract concept or behaviour to be measured. Factor analysis is one of athe nalyses commonly used to evaluate construct validity and to test whether items gather under different factors (27). Prior to factor analysis, KMO was performed to evaluate whether or not the sample was sufficient and suitable for factor analy is. Moreover, Bartlett's test of sphericity was conducted to determine whether or not the data were suitable for factor analysis. For factor analysis, KMO values should be greater than 0.5 (32). A KMO criterion between 0.90-1.00 is assumed to be perfect, while it is assumed to be very good between 0.80-0.89, good between 0.70-0.79, mild between 0.60-0.69, low between 0.50-0.59 and inadmissible when lower than 0.50 (33). Results of Bartlett's test indicate significance at $p<0.05$ (34). Seçer (25) stated that this value should be at least 0.70 and, values of 0.80 or higher would be most appropriate for factor analysis. As the KMO value of the 45-item PASRDU was greater than 0.80, the sample adequacy can be considered very good for factor analysis. The Bartlett's test was also found to be significant at a very good level, thus determining that there was a correlation between the variables and factors of PASRDU.

Within the scope of EFA of the draft PASRDU, a varimax rotation method was conducted, maintaining the structure of the factors. Vertical rotation methods are frequently preferred in scale development since they are commented easier (34). The draft PASRDU was rotated twice using varimax rotation and principal component analysis, revealing 18 items out of 63 items with factor loadings lower than 0.30. These items were subsequently removed from the scale. A factor loading of 0.30 and higher is considered as a good value for the scales (35). The factor loading values of the draft PASRDU were found to range between 0.332 and 0.70. When these values were compared with analysis coefficients, they were found to be at acceptable levels. Therefore, after the EFA, PASRDU had 45 items and two subscales (*Correct and Conscious Use* and *Effective and Safe Use).*

In scale studies, confirmatory factor analysis is used to test the accuracy of exploratory factor analysis results (27). In the nursing literature, the structures of scales determined after EFA are accepted as models and their accuracy is tested with CFA (36). PASRDU's structure was analysed with X^2/Sd, GFI, AGFI, CFI, RMSEA and SRMR consistency tests within the context of CFA. An X^2/Sd value of 5 or lower indicates a tested model with a good level of fit (27). This study's analysis found an X^2/Sd value of 3.47, indicating that PASRDU is an acceptable model. PASRDU's GFI value was 0.94, its AGFI value was 0.93, and its CFI value was 0.92. For these indices, values of 0.90 or higher are considered acceptable. The remaining fit indices (RMSEA and RMR) produced values of 0.06 for PASRDU. An RMSEA value of < 0.08 shows a good fit.AnA SRMR value of 0.05 < RMR < 0.10 shows that a model has a rational fit (37).

After the validity of PASRDU was tested, reliability analyses were conducted. Within the context of PASRDU's reliability analyses, internal consistency was tested first. Internal consistency indicates that all subgroups address and measure the same construct. Cronbach's alpha is widely used to assess the fit between items to determine internal consistency (38). Scales with a coefficient between 1.00-0.80 have high reliability, those with a coefficient between 0.60-0.79 are quite reliable, and those with a coefficient between 0.40-0.59 have low reliability (26). While PASRDU's internal consistency coefficient was found as 0.88 for the whole test, it was found to be 0.89 for the Correct and Conscious Use subscale and 0.77 the for Effective and Safe Use subscale. Cronbach's Alpha values of > 0.80 showed that PASRDU is a highly reliable scale. The fact that items in the scale are consistent with each other and the scale consists of items testing the items of the same feature depends on the high Cronbach's α reliability coefficient of the scale. (39). Accordingly, items in the PASRDU are consistent with each other and the PASRDU consists of items that test the elements of the same feature.

Item-total score correlation is also used to determine internal consistency. It is stated that the items which have an item-total correlation of 0.20 and higher, which is another criterion for reliability, can be included in the scale and differentiate between individuals well in terms of the related characteristics (34). The item-total correlations of the scale were assessed and were found to be 0.20 and higher. This result shows that the participants understood the expressions and answered objectively, and the scale had high item discrimination.

Following item-total correlation, another reliability method, test-retest, was applied to find out the time-dependent fit of PASRDU. High correlation coefficient between the two measurements shows that the test gives consistent time-dependent measurements. The correlation coefficient of 0.80 and above is interpreted as high correlation, 0.60-0.80 as strong correlation, 0.40-0.59 as mild correlation, 0.20-0.39 as low correlation, and below 0.20 as weak correlation (31). The correlation coefficient of PASRDU was 0.85, and the test-retest results showed that the reliability of the scale was

hig,h and it made consistent measurements independent of time.

One of the limitations of the study is that Explanatory and confirmatory factor analysis was conducted on the same sample. Another limitation is that mothers constituted the majority of the sample.

5. CONCLUSION

Validity and reliability analyses conducted during the process of scale development showed that PASRDU is a valid and reliable scale that finds out parents' attitudes towards rational drug use. It is also useful for nurses to use within the safety criteria of drugs. This scale enables the nurse to determine the lack of rational drug use and provide training and consultancy on this subject. This scale can be used in primary and preventive health services.

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Clinical and Experimental Health Sciences

The Relationship Between Health-Promoting Behaviors and Socio-demographic and Clinical Characteristics of Patients with Diabetes Mellitus

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ABSTRACT

Objective: In this study, it was aimed to investigate the relationship between health-promoting attitudes and socio-demographic and the clinical characteristics of patients with diabetes.

Methods: The study sample comprised a total of 267 patients with diabetes mellitus aged 18 years and older, who had previously been diagnosed as Type 1 or Type 2 diabetes mellitus for at least 6 months, who had no gestational diabetes mellitus and who had presented to the Internal Diseases Outpatient Clinic in Kocaeli between June-September 2015. Collection of the data were realized by the researchers through face-to-face interviews using the "Patient Information Form" and the "Health-Promoting Lifestyle Profile-II". The validity-reliability study of the scale for the Turkish population was carried out by Bahar et al. The scale comprises six factors including health responsibility, physical activity, nutrition, spiritual growth, interpersonal relations and stress management.

Results: The patients scored highest in the interpersonal relations and scored lowest in the physical activity factors. Patients who were aged between 18-44 years, those who were high school graduates, those who were retired, those who had a well-balanced income and those with no additional chronic disease had higher scores in Health-Promoting Lifestyle Profile-II compared to others. We found that the health-promoting attitudes were not affected by the duration of diabetes mellitus, body mass index or the presence of diabetes in the family.

Conclusion: We determined that healthy lifestyle attitudes were demonstrated moderately by the patients with diabetes mellitus, and these attitudes were found to be associated with socio-demographic and clinical variables such as patient's age, education status, diabetes type and presence of diabetes complications.

Keywords: Demographic factors, disease attributes, diabetes mellitus, health promotion, nurse.

1. INTRODUCTION

Diabetes mellitus (DM) is a common chronic disorder which deteriorates the glycemic control, affects the life activities, life span and the quality of life of the individual, leads to acute and chronic complications and requires continuous medical care (1-3).

DM is treated with oral antidiabetics, insulin, diet and exercise (4-6). Glycemic control is achieved with medical treatment, educating the patients and changing their lifestyles (7). Patients with DM should adopt a healthy lifestyle to reduce the complications of disease in physical, psychological and social terms. A number of factors including environmental factors, economic status and social support affect the patients with DM health-promoting attitudes, adaptation effort and concomitant control of blood glucose levels (8). Previous studies have reported that demographic characteristics such as cognitive and social factors, age and gender affect the quality of life and glycemic control in patients with diabetes (9,10).

A number of models have been developed to explain the relationship between health and illness. The "Health Promotion Model (HPM)" is one of these, which was developed by Pender. It has been used in practice to determine the cognitive and affective factors affecting the health-promoting attitudes. According to this model, demographic and biological characteristics, interpersonal interactions, situational and behavioral factors affect the development of health (11). The "Health Promoting Lifestyle Profile" was developed based on the Health Promotion Model. This scale measures the individual's health-promoting behaviors and it is used to assess the healthy lifestyle attitudes in daily practice (12).

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Health-Promoting Attitudes that play a significant role in disease prevention and chronic disease management are classified as health responsibility, spiritual growth, physical activity, nutrition, interpersonal relations and stress management. The health responsibility expresses that an individual care about his/her health and he/she can take responsibility and decide on his/her health. Spiritual growth is a positive approach towards events in order to enhance the inner peace of the individual, the desire to achieve life goals and the state of well-being. Physical activity is the planning of mild, moderate and severe activities by the individual throughout the day. Nutrition is the individual's conscious choice of food and proper nutrition according to the meal order. Interpersonal relations include having a verbal or non-verbal communication with the social environment, establishing meaningful relationships and sharing own feelings. Stress management is an individual's ability to reduce and control tension arising from physiological and psychological causes (13). A patient with DM should be able to take health responsibility, deal with the disease, perform physical activity according to the treatment plan, adapt to the diet, express emotions and thoughts and reduce the stress burden of the disease by learning to cope with stress (4, 8,14).

Previous studies in the literature have reported that healthy lifestyle attitudes contribute positively to the health status in chronic diseases. Furthermore, social and medical factors including age, gender, education level, marital status and the duration of DM affect the health-promoting behaviors and the quality of life in patients with DM. Moreover, 'Type 2 DM prevention programs' were found to be effective in promoting healthy lifestyle behaviors (15-20). In this context, we aimed to investigate the association between socio-demographic and clinical characteristics of patients with diabetes and their health-promoting attitudes in order to contribute to nursing practice and the current literature.

Study Questions

Q1: Do healthy lifestyle attitudes show alterations in patients with Type 1 and Type 2 DM based on socio-demographic characteristics?

Q2: Do healthy lifestyle attitudes show alterations in patients with Type 1 and Type 2 DM based on clinical characteristics?

2. METHODS

2.1. Study sample and design

This study, which was descriptive and cross-sectional, was conducted with patients who had presented to the Internal Diseases Outpatient Clinic of a public hospital in Kocaeli, a city located in western Turkey, between June and September 2015. Considering that the number of patients with DM presenting to the outpatient clinic during the same period of the previous year was 872, the sample size representing the universe was calculated as 267 patients at a confidence interval of 95%, an error rate of 5% and a statistical significance level of $p<0.05$. 290 patients were interviewed to reach the sample. Participants who did not fulfill the inclusion criteria during the study and refused to participate in the study (n = 23) were not included in the sample.

The study population comprised 267 patients aged 18 years and older, who had been diagnosed as Type 1 or Type 2 DM for at least 6 months, who had no gestational DM, who were cognitively competent to respond to the scale and the questionnaire and who gave consent to participate in the study.

2.2. Data collection

Patients with DM eligible for the inclusion criteria were first informed about the procedure of the study and written informed consent forms were signed by those who agreed to participate. The data were collected by the researchers in the Internal Diseases Outpatient Clinic through the face-to-face interview method and each interview lasted for nearly 30 minutes. The data were collected in the waiting room of the clinic after the examination during the non-busy hours (afternoon) of the outpatient clinic. The patients filled in the "Patient Information Form" and then, the "Health Promoting Lifestyle Profile-II (HPLP-II)" questionnaire was administered to collect data about the healthy lifestyle attitudes. Responses were recorded assuming that the patient statements were correct. The data were collected by the researchers through the face-to-face interview method and there were no missing data.

2.3. Instruments

2.3.1. The patient information form: This form consisted of 19 questions, which were generated by the researchers. The questions aimed to gather information about the socio-demographic and clinical characteristics of the patients.

2.3.2. Health-Promoting Lifestyle Profile-II (HPLP-II): The original "Health Promoting Lifestyle Profile" (HPLP) scale, developed by Walker, Sechrist and Pender (1987), consists of 48 items and 6 subscales (self-actualization, health responsibility, exercise, nutrition, interpersonal support, and stress management). The scale was revised in 1995 and named as "Health Promoting Lifestyle Profile-II" (HPLP-II). The revised scale consists of 52 items and 6 subscales (health responsibility, spiritual growth, physical activity, nutrition, interpersonal relations, stress management). This is a 4-point likert-type scale, and it is scored as never (1), sometimes (2), frequently (3) and regularly (4). The lowest score for the scale is 52 and the highest score is 208. The lowest score for physical activity and stress management subscales is 8 and the highest score is 32. The lowest score for the other subscales is 9 and the highest score is 36. The overall score of the scale gives the score of health-promoting lifestyle and all items are positive. A high score indicates that the health-promoting lifestyle attitudes are displayed at a high level, while low scores indicate that these behaviors are displayed

less frequently (13). The validity-reliability study of the scale for the Turkish population was conducted by Bahar et al. in 2008, and the Cronbach's Alpha reliability coefficient was determined as 0.92. The Alpha reliability coefficients of the subscales were between 0.79 and 0.87 (21). In this study, the Cronbach's Alpha reliability coefficient of the total scale was determined as 0.94, while the Cronbach's Alpha reliability coefficients of the subscales were determined as 0.87 for interpersonal relations, 0.85 for spiritual growth, 0.65 for nutrition, 0.76 for stress management, 0.83 for physical activity and as 0.81 for health responsibility.

2.4. Ethical Aspect

Before commencing the study, permission was obtained from Bahar and his colleagues who conducted the Turkish validity-reliability study of HPLP-II. Ethics approval was obtained from the Ethics Committee of the Institute (22/06/2015-1) and the institution where the study was conducted (09/07/2015-1307). Oral and written informed consents were obtained from patients with DM who agreed to participate in the study. The study was conducted based on ethical principles. The study was carried out in accordance with the principles of the Helsinki Declaration.

2.5. Data Analysis

The data obtained in the study were analyzed using the SPSS package program. The Mann-Whitney U test was used to compare the non-parametric continuous data between two independent groups, and the Kruskal Wallis H-Test was used for comparison of the non-parametric continuous data between three and more independent groups. The Spearman's correlation analysis was used to determine the relationship between two continuous variables. The Mann Whitney U test was used as a post-hoc test to perform pair-wise analyses of the differences between each of the two groups following the Kruskal Wallis H test. The results were evaluated at a confidence interval of 95% and a significance level of 5%.

3. RESULTS

The socio-demographic and clinical characteristics of patients with DM (n=267) have been demonstrated in Table 1. In addition to the results shown in the table, we found that mean age of the patients was 57.52±13.77 years old; the mean body mass index (BMI) was 30.19 ± 6.73 kg/m2 and the mean duration of DM was 9.61 ± 7.86 years. All of the patients had health insurance. The most common complication of diabetes was nephropathy, 15.0% had diabetic foot and 10.1% had coronary heart disease furthermore, 21% of the patients had concomitant hypertension. We found that 36% of the patients used insulin, 45.3% of the patients used oral antidiabetic and 50.2% were on a diabetic diet.

The patients had a mean score of 122.83±24.154 from the whole scale. On analysis of the mean scores of patients from the subscales, we found that the highest score was obtained from the interpersonal relations subscale (24.70±5.99) and the lowest score was obtained from the physical activity subscale (12.74±4.39) (Table 2).

Table 1. *Socio-demographic and clinical characteristics (n=267)*

Characteristics	n	%
Age (57,52±13,77 [18-89])		
18-44	44	16,5
45-64	133	49,8
65 and above	90	33,7
Gender		
Female	177	66,3
Male	90	33,7
Marital status		
Married	221	82,8
Single	46	17,2
Educational status		
Illiterate	35	13,1
Literate	43	16,1
Primary school	147	55,1
High school	30	11,2
University	12	4,5
Occupation/job		
Officer	11	4,1
Worker	20	7,5
Self-employment	4	1,5
Housewife	169	63,3
Retired	56	21,0
Unemployed	7	2,6
Economic status		
Income< expenditure	44	16,5
Income> expenditure	13	4,9
Income= expenditure	210	78,7
BMI (30.19±6.73 [17,30-49,94])		
Normal (18.5-24.9 kg/m2)	67	25,1
Overweight (25-29.9 kg/m2)	79	29,6
Obese (≥30 kg/m2)	121	45,3
Type of DM		
Type 1	41	15,4
Type 2	226	84,6
Family history of DM		
Present	171	64,0
Absent	96	36,0
Diabetes education		
Received	143	53,6
Not received	124	46,4
Complication of DM		
Present	215	80,5
Absent	52	19,5
Other chronic disease		
Present	59	22,1
Absent	208	77,9
Duration of DM (year) (9,61±7.86 [1-40])		

Min: Minimum, Max: Maximum, Sd: Standard deviation

Table 2. *The mean HPLP-II and subscale scores (n=267)*

Subscales	Min.	Max.	Mean±Sd
Health responsibility	10	36	21,99±5,29
Physical activity	8	30	12,74±4,39
Nutrition	9	29	20,28±3,99
Spiritual growth	11	35	23,77±5,70
Interpersonal relations	9	36	24,70±5,99
Stress management	10	32	19,34±4,62
HPLP-II total	61	178	122,83±24,15

Min: Minimum, Max: Maximum, Sd: Standard deviation

Table 3. *Comparison of HPLP-II and subscale scores according to socio-demographic characteristics (n=267)*

Characteristics	Health Responsibility Mean±Sd	Physical Activity Mean±Sd	Nutrition Mean±Sd	Spiritual Growth Mean±Sd	Interpersonal Relations Mean±Sd	Stress management Mean±Sd	HPLP-II (Total) Mean±Sd
Age							
	r=-0,109	r=-0,266[a]	r=-0,064	r=-0,189[a]	r=-0,211[a]	r=-0,116	r=-0,202[a]
	p=0,074	**p<0,001**	p=0,299	**p=0,002**	**p=0,001**	p=0,057	**p=0,001**
18-44[1]	21,61±5,09	14,80±4,96	20,41±3,35	24,30±5,00	25,82±4,90	9,77±3,50	126,71±18,72
45-64[2]	22,74±5,08	12,72±4,03	20,59±4,04	24,72±5,72	25,28±5,93	19,76±4,55	125,81±23,73
65 and over[3]	21,08±5,58	11,77±4,31	19,78±4,21	22,10±5,67	23,32±6,37	18,51±5,12	116,56±26,05
	p=0,037[b]	**p<0,001**[b]	p=0,346	**p=0,004**[b]	**p=0,032**[b]	p=0,080	**p=0,009**[b]
	KW=6,574	KW=15,641	KW=2,122	KW=11,118	KW= 6,874	KW= 5,042	KW= 9,420
	2>3	1>2>3		1>3, 2>3	1>3, 2>3		1>3, 2>3
Gender							
Female	22,10±5,31	12,02±3,80	20,25±3,85	23,31±5,66	24,55±6,08	19,07±4,51	121,31±23,03
Male	21,78±5,29	14,16±5,10	20,34±4,29	24,68±5,70	25,01±5,84	19,88±4,83	125,84±26,10
	p=0,766	**p=0,002**[c]	p=0,616	**p=0,038**[c]	p=0,509	p=0,249	p=0,176
	MW=7 788,0	MW=6 095,5	MW=7 667,0	MW=6 731,5	MW=7 572,0	MW=7 279,5	MW=7 158,0
Marital status							
Married	22,25±5,48	12,91±4,43	20,55±3,90	23,97±5,68	24,93±5,99	19,42±4,68	124,05±24,52
Single	20,76±4,11	11,91±4,17	19,02±4,25	22,72±5,75	23,63±5,95	18,93±4,38	116,98±21,60
	p=0,077	p=0,109	**p=0,018**[c]	p=0,177	p=0,238	p=0,477	p=0,084
	MW=4 243,0	MW=4 326,0	MW=3 960,0	MW=4 441,0	MW=4 521,5	MW=4 745,0	MW=4 260,0
Education							
Illiterate[1]	19,97±4,48	10,00±2,69	19,23±3,71	21,02±4,48	22,23±4,83	18,00±4,28	110,46±18,37
Literate[2]	20,26±5,30	11,28±3,62	19,23±4,37	22,00±6,57	23,19±7,04	18,18±5,13	114,14±27,24
Primary school[3]	22,78±5,32	13,30±4,53	20,56±3,87	24,35±5,33	25,23±5,90	19,72±4,47	125,95±23,20
High school[4]	23,00±5,42	14,10±4,23	20,97±4,13	25,70±6,34	26,97±5,65	20,83±4,66	131,57±24,23
University[5]	21,92±4,25	15,75±4,73	22,00±3,67	26,08±4,25	25,33±3,80	19,00±4,20	130,08±18,99
	p=0,003[b]	**p<0,001**[b]	p=0,061	**p=0,001**[b]	**p=0,003**[b]	**p=0,037**[b]	**p<0,001**[b]
	KW=15,995	KW=33,789	KW=9,006	KW=19,416	KW=15,724	KW=10,237	KW=22,635
	3,4>1,2	3,4,5>1,2		3,4,5>1,2	3,5>1; 4>1,2	3,4>1,2	3,4>1,2; 5>1
Occupation/job							
Officer[1]	22,00±3,85	15,55±4,76	21,09±2,95	24,09±3,86	24,82±3,63	19,90±3,24	127,45±16,10
Worker[2]	21,40±4,99	14,50±4,73	19,95±2,78	24,45±5,49	25,35±4,91	18,10±3,54	123,75±20,06
Self-employment[3]	22,00±1,41	12,50±3,11	23,50±3,32	26,00±2,71	23,25±3,09	21,00±2,58	128,25±4,87
Housewife[4]	21,86±5,38	11,83±3,73	20,11±3,88	23,13±5,57	24,32±6,10	18,98±4,50	120,24±23,39
Retired[5]	22,89±5,66	14,45±5,15	20,95±4,71	25,68±6,24	26,08±6,42	20,86±5,41	130,91±28,44
Unemployed[6]	19,71±3,99	11,86±5,79	17,00±3,42	20,14±4,84	21,71±5,82	17,57±3,99	108,00±19,41
	p=0,498	**p=0,001**[b]	**p=0,048**[b]	**p=0,024**[b]	p=0,245	p=0,108	**p=0,043**[b]
	KW=4,364	KW=21,373	KW=11,197	KW=12,976	KW=6,682	KW=9,023	KW=11,439
		1,2,5>4	1,2,3>6; 4,5>6	3,5>6; 5>4			5>4,6
Income							
Income<expenditures[1]	20,32±4,90	11,95±3,33	18,70±4,01	20,98±5,79	22,05±5,95	17,68±4,38	111,68±23,26
Income>expenditures[2]	21,92±4,99	14,54±5,78	20,00±3,02	25,07±6,20	25,15±5,27	19,69±5,02	126,38±22,44
Income=expenditures[3]	22,35±5,34	12,79±4,47	20,63±3,93	24,27±5,50	25,24±5,92	19,66±4,95	124,95±23,89
	p=0,112	p=0,462	**p=0,019**[b]	**p=0,003**[b]	**p=0,011**[b]	**p=0,031**[b]	**p=0,008**[b]
	KW=4,373	KW=1,544	KW=7,896	KW=11,581	KW=9,006	KW=6,977	KW=9,734
			3>1	3>1	3>1	3>1	3>1

Sd: Standard deviation, aSpearman's correlation analysis, bKruskal Wallis H-Test (KW), cMann Whitney-U Test (MW).

Table 3 shows the comparison of HPLP-II and its subscale scores of the patients based on the socio-demographic characteristics (Table 3). There were differences in some subscales or total scores of HPLP-II based on age, gender, marital status, educational status, occupation, and the economic status of the patients. Table 4 demonstrates the comparison of HPLP-II and its subscale scores of the patients based on clinical characteristics (Table 4). Those with Type 1 DM, those who had been educated on diabetes care and those without complication of diabetes were found to have higher physical activity scores compared to the others ($p<0.05$, for all). The mean scores of the patients who had chronic diseases other than DM were lower in both the physical activity subscale and the overall scale compared to those without other chronic diseases ($p<0.05$, for both). In addition, patients using oral antidiabetics together with insulin therapy (n = 52, 19.5%) had a lower physical activity score than the others ($p <0.05$).

Table 4. *Comparison of HPLP-II and subscale scores according to clinical characteristics (n=267)*

Charecteristics	Health Responsibility Mean±Sd	Physical Activity Mean±Sd	Nutrition Mean±Sd	Spiritual Growth Mean±Sd	Interpersonal Relations Mean±Sd	Stress management Mean±Sd	HPLP-II (Total) Mean±Sd
Type of DM							
Type 1	21,24±5,02	14,95±4,79	19,98±3,62	23,83±5,39	25,12±5,47	19,46±4,02	124,58±22,56
Type 2	22,12±5,33	12,34±4,20	20,34±4,07	23,76±5,77	24,63±6,09	19,32±4,73	122,52±24,46
	p=0,387	**p=0,001**[a]	p=0,503	p=0,784	p=0,486	p=0,795	p=0,535
	MW=4 240,0	MW=3 094,0	MW=4 329,0	MW=4 508,5	MW=4 316,5	MW=4 515,0	MW= 4 351,0
Diabetes education							
Received	22,55±5,53	13,24±4,47	20,28±3,97	24,13±5,30	25,32±5,65	19,59±4,54	125,10±23,37
Non-received	21,35±4,95	12,16±4,24	20,29±4,04	23,35±6,12	24,00±6,32	19,05±4,72	120,22±24,87
	p=0,083	**p=0,023**[a]	p=0,919	p=0,257	p=0,088	p=0,328	p=0,121
	MW=7 778,0	MW=7 448,5	MW=8 802,5	MW=8 154,5	MW=7 794,5	MW=8 252,0	MW=7 891,5
Complication of DM							
Present	22,06±5,42	12,32±4,25	20,20±4,11	23,53±5,74	24,58±6,18	19,26±4,60	121,96±24,57
Absent	21,73±4,76	14,50±4,55	20,59±3,50	24,75±5,49	25,23±5,18	19,65±4,75	126,46±22,22
	p=0,791	**p=0,001**[a]	p=0,739	p=0,221	p=0,652	p=0,748	p=0,322
	MW=5 458,0	MW=3 889,0	MW=5 424,0	MW=4 979,5	MW=5 365,0	MW=5 429,5	MW=5 095,5
Other chronic disease							
Present	20,93±5,36	11,39±4,03	19,47±4,66	22,54±5,95	23,76±5,78	18,91±4,91	117,01±25,56
Absent	22,29±5,24	13,12±4,42	20,51±3,77	24,11±5,59	24,98±6,04	19,46±4,54	124,49±23,54
	p=0,086	**p=0,002**[a]	p=0,167	p=0,056	p=0,180	p=0,487	**p=0,042**[a]
	MW=5 238,5	MW=4 529,0	MW=5 414,5	MW=5 137,0	MW=5 434,5	MW=5 773,0	MW=5 070,5
BMI							
Normal	21,49±5,42	13,97±5,23	20,51±4,51	23,67±5,78	24,88±6,02	19,12±4,42	123,64±25,41
Overweight	22,30±4,97	12,59±4,39	19,82±3,85	23,81±5,85	24,52±5,50	19,25±5,04	122,30±24,07
Obese	22,06±5,44	12,16±3,73	20,46±3,79	23,79±5,60	24,74±6,32	19,52±4,48	122,74±23,68
	p=0,528	p=0,121	p=0,465	p=0,998	p=0,853	p= 0,784	p=0,875
	KW=1,278	KW=4,219	KW=1,530	KW=0,005	KW=0,318	KW=0,486	KW=0,266
Family history of DM							
Present	22,28±5,23	12,92±4,32	20,29±4,09	24,05±5,92	24,96±6,28	19,60±4,61	124,12±24,42
Absent	21,48±5,39	12,43±4,52	20,26±3,83	23,26±5,27	24,26±5,46	18,86±4,63	120,55±23,62
	p=0,213	p=0,192	p=0,997	p=0,177	p=0,245	p=0,165	p=0,151
	MW=7 456,0	MW=7 423,5	MW=8 205,5	MW=7 391,0	MW=7 504,5	MW=7 369,5	MW=7 338,0
Duration of DM (year)							
	r=0,077	r=0,029	r=0,102	r=-0,108	r=-0,079	r=0,013	r=-0,004
	p=0,209	p=0,638	p=0,095	p=0,077	p=0,199	p=0,837	p=0,953

Sd: standard deviation, r: Spearman's correlation analysis, KW: Kruskal Wallis H-Test, aMann Whitney-U Test (MW).

4. DISCUSSION

In the present study, we found that patients with DM moderately practiced healthy lifestyle attitudes, and that the socio-demographic and clinical variables affected the health-promoting attitudes (Table 2). Patients with DM should acquire health-promoting lifestyle attitudes in order to protect their own health and maintain their well-being.

When the mean scores of the patients with DM from healthy lifestyle attitudes were examined based on the socio-demographic characteristics, the age, educational status, occupation groups and the economic status were observed to affect the healthy lifestyle attitudes (Table 3). With regard to clinical characteristics, the presence of a chronic disease other than DM was found to affect the healthy lifestyle attitudes. Furthermore, variables such as BMI, duration of diabetes and a history of diabetes in the family were not found to affect healthy lifestyle attitudes (Table 4).

There are a number of studies in the literature assessing the health-promoting lifestyle attitudes in various groups. Vahedi reported that patients with DM "moderately" practiced health-promoting lifestyle attitudes. The highest scores were noted in the health responsibility subscale (33.68±7.14) and the lowest score was found in the physical activity subscale (10.08±3.57) in patients with DM. (16). In their study with young black women at risk of Type 2 DM, Jefferson et al. found that these women practiced health attitudes at a "moderate" level. Young black women at risk of Type 2 DM were found to have the highest score from the spiritual growth subscale and the lowest score from the physical activity subscale (22). In another study by Sutherland et al. evaluating health-promoting lifestyle attitudes based on the risk status for DM, patients with DM were found to obtain the lowest score from the physical activity subscale (18).

In their study comparing the healthy lifestyle behaviors of people with and without diabetes, Vahedi et al. observed that patients with DM practiced healthy attitudes at a moderate level and that both the diabetic and non-diabetic groups received the lowest score from the physical activity subscale, which is consistent with our results (16). Thus, we may conclude that patients with DM practice health-promoting lifestyle attitudes moderately.

When the healthy lifestyle attitudes of patients with DM were examined based on age groups, significant differences were found between the subscales of health responsibility, physical activity, spiritual growth, interpersonal relations and the total mean score of HPLP-II. The total HPLP-II scores of the 18-44 and 45-64 years age groups were found to be higher than the score of the 65 years and older age group (Table 3). It was observed that the level of practicing healthy lifestyle attitudes decreased as age increased in patients with DM. Age is a demographic feature affecting the health-promoting attitudes. The decrease in the functional competence with aging prevents the free actualization of health behaviors (23). A decrease in the level of practicing health-promoting lifestyle attitudes is expected with aging. Tol et al. found that the "age" variable affected the health-promoting lifestyle attitudes in their study with Type 2 DM patients and reported that these attitudes were better in patients under the age of 50 years (15).

When the health-promoting lifestyle attitudes were evaluated according to gender, the mean scores in the physical activity and spiritual growth subscales of male patients were found to be higher than those of female patients. The HPLP-II total score of male patients also tended to be higher than female patients, although not statistically significant (Table 3). In their study on patients with heart disease, Kucukberber et al. found that psychological development, the physical activity subscale scores and the total HPLP-II scores of male patients were higher than female patients (24). Moreover, Sutherland et al. determined that the overall HPLP-II score of males having a higher risk of DM was higher than the score of females (18). Our results were also consistent with the previous studies mentioned above. Considering the facts that educational level of men is higher than women, that women are further away from working life and that many women do not have the freedom of making decisions on their own health, it may be stated that women are away from health-promoting activities and programs, and they practice healthy lifestyle attitudes to a lower extent than men.

In Turkish society, marriage brings along a regular lifestyle. For this reason, it is thought that married patients with DM may adapt to disease more easily and they can practice healthy lifestyle attitudes more than single individuals. In our study, we found that the health-promoting lifestyle attitude scores of married patients were higher than those of single patients (Table 3). Chen and Lin reported that marital status did not affect the health-promoting lifestyle attitudes in their study on pre-diabetic adults (25). This difference may arise from the difference in cultural characteristics of the study samples.

Patients with a primary school, high school and higher education degree were found to have higher scores from health-promoting lifestyle attitudes compared to patients with DM who were illiterate or only literate (Table 3). Tol et al. found that patients with higher education level had a higher total score of health-promoting lifestyle attitudes (15). In another study, it was determined that the level of diabetes knowledge increased as the level of education increased (26). These results show that as the educational level increases, the healthy lifestyle attitudes also increase in a positive manner. Accordingly, we may suggest that education increases the awareness on healthy lifestyle and provides an increase in practicing health attitudes.

When the health-promoting lifestyle attitudes were examined based on job/occupation status, there was a statistically significant difference between the occupational groups in terms of the physical activity, nutrition, spiritual growth subscales and the mean total HPLP-II scores (Table 3). In a study by Kuru and Piyal, a significant difference was determined in the total score of HPLP-II scores between occupational groups in patients with coronary artery disease

(17). Tol et al. found that patients with DM who worked as an officer had a higher total HPLP-II score compared to other occupational groups (15). Working individuals can organize their meals and social lives according to working hours. We may suggest that the regular lifestyle rendered by this process has a positive influence on healthy lifestyle attitudes, and that working individuals place a higher emphasis on healthy lifestyle attitudes, and they are physically more active.

According to our results, the economic status was found to affect the health-promoting lifestyle attitudes (Table 3). Softa et al. found in their study that elderly individuals with low economic status had lower scores from health-promoting lifestyle behaviors, consistent with our results (27). Individuals with a good economic status can meet their health expenses more comfortably. The results suggest that improvement in the economic status of patients will affect their healthy lifestyle attitudes in a positive way.

Chilton et al. conducted a study to investigate the relationship between the demographic characteristics and the health-promoting lifestyle attitudes and diabetes awareness, and a high-income level was found to positively affect the health attitudes in the physical activity subscale (28). In our study, there was no significant difference in the physical activity subscale based on the income level; however, the mean score of the patients having "a higher income than expenses" was found to be higher (Table 3). This result suggests that societies in developed countries pay more attention to physical activity compared to Turkish society.

We found that BMI and the duration of diabetes did not affect the health-promoting lifestyle attitudes in our study (Table 4). Chen and Lin also reported that BMI did not affect the health-promoting lifestyle attitudes in pre-diabetic adults (25). In a study by Tol et al., patients with a duration of disease of more than 10 years had the highest score in the health responsibility subscale, and this result suggests that long time ago diagnosed patients pay more attention to their health (15).

Patients with a family history of diabetes were expected to practice health-promoting lifestyle attitudes at a higher level than those without a family history of diabetes. We hypothesized that if the individual witnessed a family member suffering from a complication of diabetes due to poor diabetes management, it would encourage the individual to practice a higher level of positive health attitudes. However, no significant relationship was determined between the family history of diabetes and health-promoting lifestyle attitudes (Table 4).

In our study, the mean physical activity subscale score of patients with Type 1 DM was determined to be higher than those of patients with Type 2 DM (Table 4). Compared to Type 2 DM, Type 1 DM starts at an early age when patients can be physically more easily active. In this respect, it is thought that patients with Type 1 DM practice more physical activity attitudes.

The mean physical activity subscale scores of patients who received diabetes education were found to be higher than those without education (Table 4). According to the study by Kucukberber et al. on patients with heart disease, patients who received education about the disease were found to have higher physical activity, health responsibility, stress management and HPLP-II total scores than those who were not educated about the disease (24). In their study with patients with pre-diabetes, Chen and Lin found that knowledge on pre-diabetes affected the health-promoting lifestyle attitudes in all subscales and that the scores from the scales increased as the level of knowledge increased (25). Our results were in parallel with the previous studies mentioned above in terms of the higher physical activity subscale scores in educated patients.

Patients with DM who had complications had lower physical activity subscale scores than those who were free of complications (Table 4). This finding reflects an expected situation. It is considered that inclusion of more physical activities in the education of patients with DM will affect their healthy lifestyle attitudes more positively.

Patients who did not have any additional chronic disease scored higher in the scale, and the difference was found to be statistically significant in the physical activity subscale and the total score of the HPLP-II (Table 4). The low score of health-promoting lifestyle attitudes of patients with an additional chronic illness suggests that they are challenged in diabetes management. It may be useful to keep the existing additional chronic disease under control with health attitudes and treatment methods.

According to the Turkey Diabetes Epidemiology Study (TURDEP-I), which was carried out during 1997-1998, the rate of diabetes was 7.2% and the rate of impaired glucose tolerance was 6.7% (29). The rate of diabetes was found to have increased up to 13.7% in Turkey Diabetes, Hypertension, Obesity and Endocrinological Diseases Prevalence Study (TURDEP-II) in 2010 (30). The studies performed in our country show that the incidence of diabetes is increasing steadily despite some regional variations. This study was conducted in one centre and its outcomes should be evaluated in line with this limitation.

Limitations

The most important limitation of our study was its cross-sectional design and the fact that it was conducted at a single center. It is recommended to carry out similar studies with larger sample groups.

5. CONCLUSION

Our findings show that healthy lifestyle attitudes are affected by various variables and that physical activity is the healthy lifestyle attitude that is practiced at the lowest level. In this context, it is important to determine the socio-demographic and clinical characteristics that affect the attainment of

health-promoting lifestyle attitudes in patients with DM in order to ensure effective diabetes management in clinical practice.

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Clinical and Experimental Health Sciences

Effectiveness of the Bicinchoninic Acid Method in Patient Unit Cleaning in Intensive Care

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ABSTRACT

Objective: This study was conducted to identify the effectiveness of the Bicinchoninic Acid/(BCA) method applied for evaluate the cleaning the beds of the patients with infections or colonization requiring strict contact isolation after discharge.

Methods: This is an experimental study and it was used the ORION checklist. In this study, 480 BCA and 480 microbiological samples were taken from 40 patient units before and after cleaning and the results were compared. The cleaning procedure was evaluated by examining whether there was post-cleaning gel residue in the areas stained with fluorescent gel before the cleaning.

Results: When post-cleaning BCA and post-cleaning microbiological sampling data were compared, no statistical difference was found. When the data of the areas stained with fluorescent gel before and after the cleaning were compared, it was observed that there was a statistical difference. It was revealed that the bedside and the bed controller were mostly contaminated in both methods.

Conclusions: It was concluded that BCA was an effective method that could be used to evaluate the cleaning applied to the infected patient unit. It is thought that cleaning only areas that are considered to be contaminated after evaluating the cleaning with an effective method will prevent contamination due to cleaning and will provide more positive results in terms of time, labor, and cost. The control of cleanliness using objective methods can help maintain a safe environment.

This study is registered to ClinicalTrials.gov with the number ID:NCT04212130.

Keywords: Intensive care, patient unit cleaning, bca method, microbiological sampling, fluorescent gel

1. INTRODUCTION

Florence Nightingale attached the highest priority to the control of environmental factors and hygiene in the nursing care process, which gained a professional identity with her(1). She emphasized that sanitation was an indispensable basic factor in the protection and improvement of health(2). Environmental cleaning is one of the vital components of infection control(3). In Intensive care the cleaning of frequently touched environmental surfaces and the monitoring and verification of cleaning results are important for patient safety(4–6). There are many studies indicating that environmental cleaning in hospitals is not always sufficient(7–11).

The evaluation of the effectiveness of environmental cleaning and disinfection procedures has received great interest in recent years(12,13). The effectiveness of environmental cleaning can be evaluated using different methods. Visual evaluation, ATP (Adenosine Triphosphate) measurement with fluorescent gel, protein tests or microbiological sampling methods can be used to measure/evaluate the effectiveness of the cleaning and disinfection procedures(11).

Despite the increase in the number of intensive care beds in recent years, the length of stay of the patients in the intensive care unit has been prolonged due to the increasing service quality and improving health services, so the need for intensive care beds is increasing day by day. Therefore, the effective use of intensive care beds is very important(14). Due to high demands for intensive care beds(15) and it is very important to perform effective cleaning so that patients can be admitted as soon as possible and to evaluate the cleaning with a fast and safe method(7–9,11).

Nurses, who have always been at the forefront and the largest group of healthcare workers from past to present, have attempted to develop strategies to identify and reduce preventable sources of contamination in hospitals and other healthcare settings(1,16,17).

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This study was conducted to identify the effectiveness of the BCA method applied to evaluate the cleaning the beds and medical equipment of the patients with infection or colonization requiring strict contact isolation after discharge.

Hypotheses of the Study

H^0 = The BCA method has no effect on the evaluation of patient unit cleaning performed in intensive care.

H^1 = The BCA method has an effect on the evaluation of patient unit cleaning performed in intensive care.

2. METHODS

2.1. Type and Sample of the Study

This is an experimental study. In this study was used the "Guidelines for transparent reporting of outbreak reports and intervention studies of nosocomial infection checklist". The patient units of patients with infections or colonization requiring strict contact isolation after discharge constituted the sample of the study. In this study, when the values of alpha 0.05, β= 0.10, 1-B 0.90 were taken, it was decided to include 32 patient units in the study, and the power of the test was found to be p=0.9657. Data collection was maintained until the BCA protein pen and fluorescent gel were exhausted, and a total of 43 patient units were included in the study. During the data collection stage, while two patient units were excluded from the study since the patients were admitted before the data collection process was completed, one patient unit was excluded from the study due to the disruption of blinding. Consequently, the study was conducted with the data of 40 patient units. 480 BCA and 480 microbiological samples were taken before and after cleaning from a of 40 patient units

2.2. Data Collection Tools

In the study, while the data on the effectiveness of cleaning performed in the patient unit were collected by the fluorescent gel marking method, the data on the presence of biological agents on the surface after cleaning were collected by the BCA and microbiological sampling methods.

2.3. Data Collection

The data of the study were collected between 01.12.2019 and 31.03.2020. The number of patient units included in the study is shown in the ORION flow diagram (Figure 1)

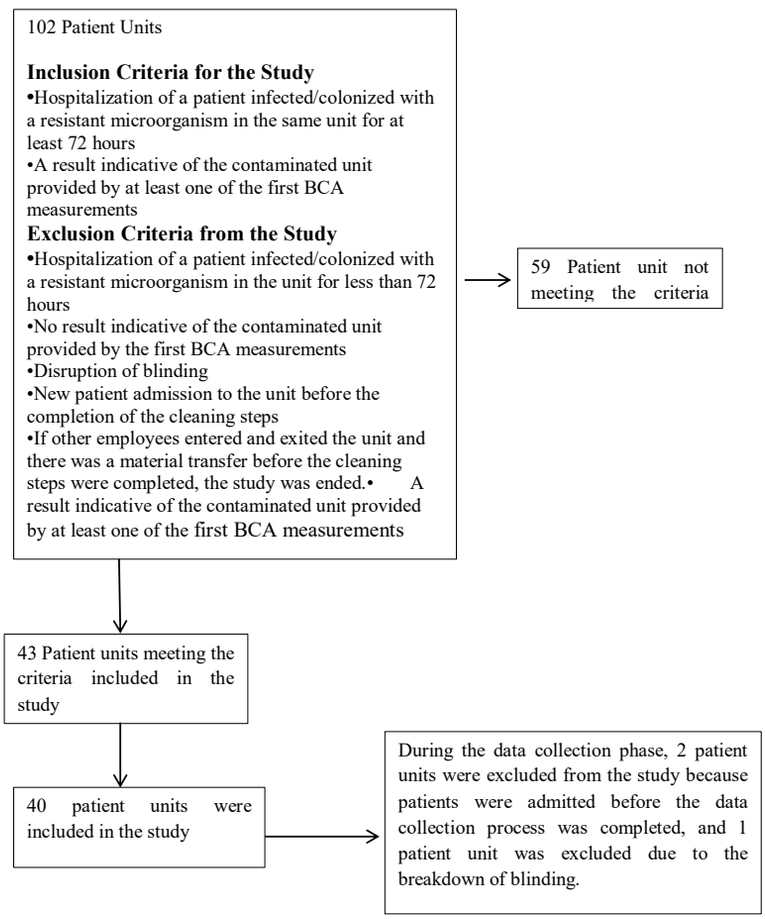

Figure 1. *ORION flow diagram*

2.4. Implementation of the Study

The cleaning staff who volunteered to participate in the study were trained, and the cleaning instruction of the institution was used as the training material. As a result of the preliminary application carried out before the study, dining tables and shelving units were excluded from the sample since both the BCA and microbiological examination tests gave clean results before and after the cleaning. As it was indicated in the study conducted by Adams et al. (2017)(18), the fact that contamination was less on surfaces that were frequently touched by healthcare workers but away from the patient is similar to the results of our preliminary application. Therefore, samples were taken from (1).bedsides, (2).patient beds, (3).monitors, (4).mechanical ventilators, (5).infusion pumps, and (6).bed controllers from infected patient units in intensive care while obtaining the data of the study. The implementation process of the study is presented in below.

Implementation Stages of the Study	
1	When the infected patient unit was emptied, the first BCA swab samples were collected from the planned areas. The compliance of the unit with the study inclusion criteria was evaluated when at least one of the BCA swabs was found to be contaminated. The units that were found to be contaminated were included in the study
2	Swabs were collected for microbiological sampling and sent to the microbiology laboratory
3	The areas to be cleaned with a fluorescent gel were stained and marked, and it was waited for the gel to dry for 3-5 minutes
4	After the measurements, the whole unit was cleaned in accordance with the cleaning standards of the institution
5	After the cleaning, it was waited for the setting to dry for 30 minutes, and within this period, the doors of the unit were closed, and a warning note was written to prevent entrance and exit to the unit
6	After the waiting period, the cleanliness of the unit was checked visually. After the visual evaluation, the gel applied areas were checked with a UV lamp. The cleaning of the areas with gel residues was repeated
7	The swab samples were collected from the same areas again for post-cleaning BCA and microbiological sampling. The study was ended in the units that were considered to be clean according to the BCA measurement results. In a case when the BCA measurement results were found to be contaminated after the first cleaning, the second cleaning was performed.
During the application process, the 30-minute waiting period for the area to dry between two cleaning procedures was determined in parallel with the study carried out by Donskeys, 2013(9) to reduce the possibility of contamination of the cleaned surfaces	

2.5. Data Evaluation Criteria

The effectiveness of cleaning was evaluated with BCA swab samples in accordance with the instructions and reference parameters of the device used for BCA measurement, and the results below 5 micrograms were considered clean. The absence of gel residues in the setting as a result of checking the cleaned areas with a UV lamp was considered as an indicator of effective cleaning. The failure to produce bacteria in microbiological samples or the bacteria produced below 2 colonies per cm^2 was considered to be an indicator that the unit was clean.

2.6. Objectivity of the Study and Ensuring Blinding

The cleaning of the infected units was performed by the same people so that it would not be affected by the individual differences and sensitivities of the cleaning staff. Since the control stage with a UV lamp was performed with the cleaning staff, the staff were prevented from guessing the areas by changing the stained areas in each unit to eliminate the possibility of guessing the stained areas. The study was conducted in a single-blind manner.

2.7. Inclusion Criteria for the Study

- Hospitalization of a patient infected/colonized with a resistant microorganism in the same unit for at least 72 hours
- A result indicative of the contaminated unit provided by at least one of the first BCA measurements

2.8. Exclusion Criteria from the Study

- Hospitalization of a patient infected/colonized with a resistant microorganism in the unit for less than 72 hours
- No result indicative of the contaminated unit provided by the first BCA measurements
- Disruption of blinding
- New patient admission to the unit before the completion of the cleaning steps
- If other employees entered and exited the unit and there was a material transfer before the cleaning steps were completed, the study was ended.

2.9. BCA Test Measurements

There are many types of protein tests, which are one of the cleaning evaluation methods, and the Bicinchoninic Acid (BCA) method is one of these tests(19). The BCA test, which was first defined by Smith et al. (1985), is based on the biuret test. This test is based on the conversion of Cu (II) to Cu (I) under alkaline conditions. Cu (I) is then reacted with BCA. As a result of this reaction, a dark purple color is formed and changes the color of the solution in which the reaction takes place(19). The test gives results in 1-5 minutes.

In the study, surface protein residues were detected by the BCA method (Terragene, Chemdye®P RO1 MICRO, Bionova MiniPRO, Santa Fe, Argentina). The PRO1 MICRO measuring device can measure between 0.3 micrograms and 10 micrograms. This system can detect a low amount of protein residues(20). The measuring range of the PRO1 MICRO hygiene system complies with international standards such as ISO 15883-1, DGSV (Germany), HTM 01-01 and HTM 01-05 (England)(20).

2.10. Evaluation of Data

The data obtained from the study were loaded into the SPSS v.22 package program and statistically analyzed. In the evaluation of the data, the McNemar test was used when the data obtained from the same regions were compared with dependent groups at different times, and the chi-square test in multiple cells was used when the groups were compared in terms of a variable obtained by counting. The level of significance was accepted to be 0.05.

2.11. Ethical Aspect of the Study

Each stage of the study was conducted in accordance with the ethical principles. Before starting the application, written permissions were obtained from the ethics committee of Cumhuriyet University (dated 04.07.2019, decision number 2019-17/15) and the institution where the study would be conducted (dated 05.07.2019, numbered 692).

3. RESULTS

When pre-cleaning BCA and pre-cleaning microbiological sampling results were compared, while 78.75% (n=189) of the samples collected from the patient unit by BCA were found to be contaminated, 46.66% (n=112) of the samples collected from the patient unit by microbiological sampling were contaminated (Table 1). When the initial BCA and microbiological sampling contamination rates were compared, it was found that the difference was not statistically significant (p=0.129).

When the BCA data obtained from the same areas before and after cleaning were compared, it was determined that 230 of a total of 240 samples, namely 95.83% of them, were cleaned after the first cleaning, there was a difference between BCA levels before and after cleaning, and this difference was statistically significant (p=0.001) (Table 1).

When the microbiological sampling results obtained from the same areas before and after cleaning were compared, it was determined that the patient units were cleaned after the first cleaning by 96.7%. It was determined that there was a difference between the microbiological sampling results obtained from the same areas before and after cleaning and that this difference was statistically significant (p=0.019) (Table 1).

When post-cleaning BCA and post-cleaning microbiological sampling data were compared, no statistical difference was found (p=0.230). While 230, namely 95.84% of the samples obtained from the patient units by the BCA method, were found to be clean, 232, namely 96.67% of the samples obtained by microbiological sampling, were clean. No statistically significant difference was found between the methods in terms of cleaning values (p=0.292) (Table 1).

Table 1. *Comparison of BCA and microbiological sampling results*

Variables	Clean: n (%)	Contaminated: n (%)	Total: n (%)	Statistical Result
Pre-Cleaning BCA	51 (21.25)	189 (78.75)	240 (100)	
Pre-Cleaning Microbiological Sampling	128 (53.3)	112 (46.7)	240 (100)	p=0.129
Pre-Cleaning BCA	51 (21.25)	189 (78.75)	240 (100)	
BCA After the First Cleaning	230 (95.8)	10 (4.2)	240 (100)	p=0.001
Pre-Cleaning Microbiological Sampling	128 (53.33)	112 (46.67)	240 (100)	
Microbiological Sampling After the First Cleaning	232 (96.7)	8 (3.3)	240 (100)	p=0.019
BCA After the First Cleaning	230 (95.83)	10 (4.17)	240 (100)	
Microbiological Sampling After the First Cleaning	232 (96.7)	8 (3.3)	240 (100)	p=0.292

Mc Nemar test used

The contamination rates of the areas where the samples were collected before and after cleaning are presented in Figure 2. When the table was examined, according to the pre-cleaning BCA method, mostly contaminated areas were bedsides by 100%, pumps by 95%, beds by 90%, mechanical ventilators (MV) by 80%, and bed controllers by 67.5%, and it was determined that the monitor was least contaminated by 40%. When the cleaned area and pre-cleaning microbiological sampling results were examined, it was observed that the bedside (72.5%) and the bed controller (62.5%) were mostly contaminated. When the contamination rates of the areas where BCA swab samples were collected after the first cleaning were examined, it was determined that all of the MV, bed controllers, and monitors were cleaned after the first cleaning ($p<0.05$), and the lowest cleaning rate was 17.5% for pumps.

It was observed that while fluorescent gel residues remained mostly on the bedsides by 60%, the least fluorescent gel residue was on the bed controller by 2.5% (Table 2).

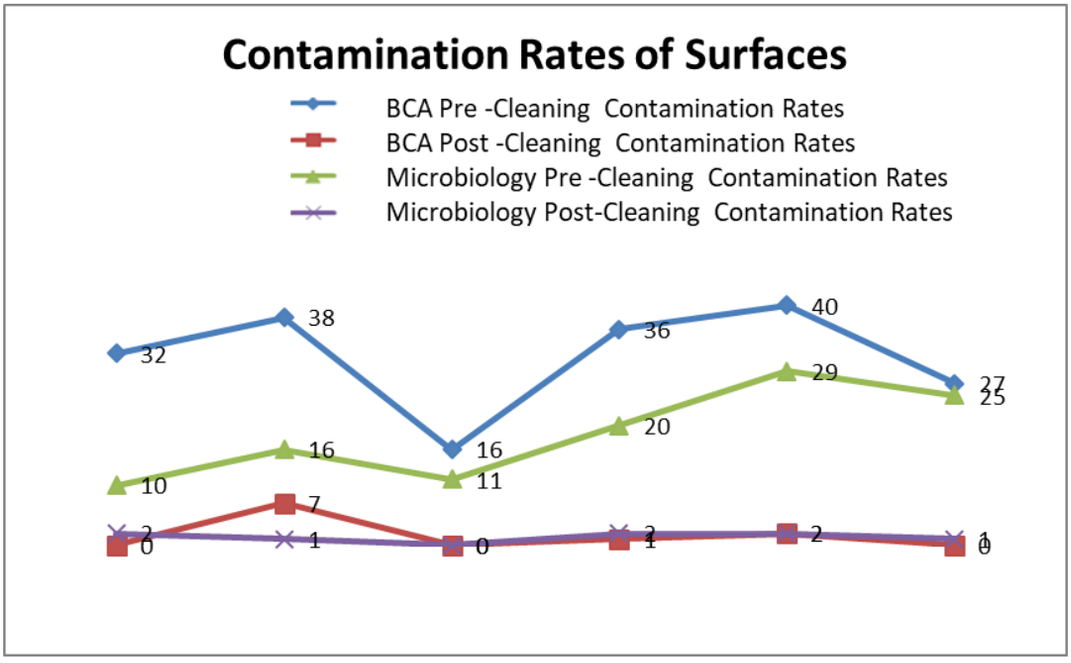

Figure 2. *Contamination rates of surfaces*

Table 2. *Comparison of the cleaned area and the fluorescent gel results*

		Fluorescent Gel Results		Total n(%)	Statistical Result
		Clean n(%)	Contaminated n(%)		
Cleaned Area	**MV**	33(82.5)	7(17.5)	40(100.0)	
	Pump	36(90.0)	4(10.0)	40(100.0)	
	Monitor	38(95.0)	2(5.0)	40(100.0)	
	Bed	23(57.5)	17(42.5)	40(100.0)	
	Bedside	16(40.0)	24(60.0)	40(100.0)	
	Bed controller	39(97.5)	1(2.5)	40(100.0)	
Total		185(77.1)	55(22.9)	240(100.0)	X^2=60,97* p =0,001

*Chi-square test used and * when the overall results are compared*

4. DISCUSSION

When the data obtained as a result of our study were examined, it was observed that while fluorescent gel residues remained mostly on the bedsides, the least fluorescent gel residue was on the bed controller (Table 2). In the study conducted by Choi et al. (2010) in which they performed microbiological sampling from the patient's environment in intensive care during the fight against the Acinetobacter outbreak, bedsides were among the most contaminated areas(21), as in our study. When the fluorescent gel residue rates of the surfaces after cleaning were examined, it was observed that the bedside was the area that was the most difficult to clean (Table 2). The low rate of cleaning at the bedside was associated with the cleaning staff having problems reaching narrow and irregular surfaces such as the joint of the bed and the bedside during cleaning works. It was

observed that the surface with the highest cleaning rate was the bed controller, which can be explained by the fact that the bed controller has a flat surface and is easily accessible. In general, when the surfaces stained with fluorescent gel after the cleaning and their cleaning rates were examined, it was concluded that cleaning staff tended to less frequently wipe the areas that were difficult to reach and the surfaces that were far from the patient. In a study conducted to evaluate the cleaning as a result of the increase in infection rates in the ICU, when the samples collected from the bedsides were evaluated with ATP, it was determined that the bedsides were not completely cleaned although there was a statistically significant difference between the ATP values after cleaning(10). This study also supports our results.

In some studies, it was emphasized that the bedsides were mostly contaminated surfaces(22–24). In our study, when contamination rates in the units were evaluated according to the criteria of the proximity of surfaces to the patient and the staff contact, it was observed that contamination rates were the highest on the bedsides and bed controllers that were closest to patients and mostly touched by healthcare personnel. However, the level of contamination was low in areas such as monitors that were far from patients but touched by healthcare personnel (Figure 2). Based on these data, it can be concluded that the surfaces that were closest to the patient and mostly touched by healthcare personnel were more contaminated than other surfaces. In their study conducted in the intensive care unit, Adams et al. (2017) found that the dining table was the area that was most frequently touched but had the lowest bio-load(18). These results support the data we obtained in our preliminary study.

When pre-cleaning BCA and pre-cleaning microbiological sampling results were compared with post-cleaning BCA and post-cleaning microbiological sampling data, no statistically significant difference was found between the methods (Table 1). The results of the experimental study conducted by Perçin and Renders (2018) revealed that the BCA method was a sensitive method with regard to presenting quantitative and qualitative data by detecting the protein residues in the setting(20). When BCA and microbiology data were compared both before and after the first cleaning, the fact that no difference was found between them supports that the BCA method can be used to evaluate environmental contamination in patient units. Although there was no statistically significant difference between BCA and microbiology data before cleaning, when the data were evaluated numerically, it was observed that 189 (78.75%) and 112 (46.66%) of the surfaces were contaminated according to pre-cleaning BCA data and microbiological data, respectively, and it is remarkable that there was a statistically significant difference between numerical data (Table 1). In line with these data, it is observed that the BCA method revealed the contamination rate more than the microbiology method. The fact that higher contamination rates were found in the BCA data can be attributed to the fact that the BCA method revealed not only the bacteriological load but also the entire biological load on the surface. The fact that the BCA method detects the presence of blood residues or body fluids in the environment and the conditions that are not infected but may become pathogenic over time if they are not cleaned can be considered as another indicator that BCA can be used to evaluate the effectiveness of cleaning. These data support our H^1 hypothesis. Furthermore, the cleaning of surfaces can be evaluated within 2-3 days by the microbiology method. Patients cannot be admitted to the unit during this long evaluation period, which causes the unit not to be used effectively. The failure to use the unit effectively leads to patient victimization and financial losses in institutions. The fact that the BCA method provides results in a short period of 15 minutes suggests that the BCA method is a method that can be used primarily in the evaluation of environmental cleaning in the clinic since it can prevent the specified problems.

When the data obtained from our study were examined, although the microbiology samples were clean before cleaning, the growth of microbiology samples after the first cleaning on the same surface suggests that the cleaning cloth used may have been contaminated and may not have been cleaned effectively in transitions between surfaces. After the second cleaning, it was determined that the same surface was clean by both the BCA and microbiology methods. In their study on the control of the cleaning of the patient environment with different cleaning agents, Bergen et al. (2009) indicated that there might be environmental contamination with cleaning cloths(25). In the study conducted by Gavalda et al. (2015) to determine the surface contamination that occurs within the first hour after cleaning in the intensive care unit, the reason for environmental contamination was associated with the contamination of cleaning cloths(7). This result supports the data in our study. In the study conducted by Gan et al. (2017), it was concluded that the use of three cleaning cloths instead of one in the cleaning of the same patient unit increased the effectiveness of the cleaning(8).

5. CONCLUSIONS

In this study, the following results were obtained. It was concluded that:

1. No difference was found between the first cleaning and the second cleaning applied to the patient unit. Although repeated cleaning is not required, cleaning should be repeated only in areas that are found to be contaminated as a result of controlling cleaning with objective methods.
2. Similar results were obtained with the BCA and microbiology methods in the evaluation of terminal cleaning, and cleaning can be controlled effectively and in a shorter time with the BCA method compared to the microbiology method.
3. When the surfaces stained with fluorescent gel and the cleaning rates are examined, cleaning staff tend to less

frequently wipe the rough areas that are difficult to reach and far from the patient.

4. In patient units, the surfaces that were the closest to patients and mostly touched by the staff were more contaminated than other surfaces.
5. The failure to comply with cleaning protocols during unit cleaning and to clean cleaning cloths effectively after surface passages may lead to environmental contamination originating from the cleaning cloth.

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Conflict of Interest

There is no conflict of interest between the authors.

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Clinical and Experimental Health Sciences

Use of Care Bundles to Prevent Healthcare-Associated Infections in Intensive Care Units: Nurses' Views

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ABSTRACT

Objective: The purpose of this study was to determine the views of nurses working in intensive care units regarding the use of care bundles in preventing healthcare-associated infections.

Methods: This study used the focus-group interview method, which is one of the qualitative methods. Two focus-group interviews, each lasting about half an hour, were conducted with 14 intensive care unit nurses. Qualitative data obtained from the interviews were recorded on tape and in note form. The interviews were then transcribed and analyzed. The transcribed data from the focus-group discussions were grouped by theme and concept, and the statements of the participants were coded numerically according to these groupings. Three themes and six subthemes emerged in analyzing the qualitative data.

Results: The nurses defined care bundles as "materials that provide integrated care for patients". They also stated that their benefits included providing a tool for self-monitoring, support and guidance for both patients and nurses. When whether they had experienced any difficulties while using care bundles, they stated that they had not experienced any. Furthermore, nurses stated that care bundles improved their perspectives, and that they were must-have items in intensive care units providing reminders rather than a waste of time.

Conclusion: It is that the participation of nurses is important so that care bundles are used more widespread in order to prevent healthcare-associated infections in intensive care units.

Keywords: Care bundle, healthcare-associated infections, intensive care units, nurses' views

1. INTRODUCTION

Healthcare-associated infections (HAIs) are a global health problem for both patients and healthcare professionals (1-3). HAIs are the most important indicator of care quality in hospitals, threaten patient safety, cause prolonged hospital stays, and increase morbidity, mortality and treatment costs (3-8). It has been reported that mortality rates due to HAIs vary between 8.2 and 29%, an additional hospitalization period of between 4.3-29.5 days and an additional cost of between $11,000 and $18 million (3-6,8,9). Turkey has reported that HAIs prolong hospital stays by an average of 10 days, cause an increase in mortality of 16% and an additional cost of $1500 (10). According to the World Health Organization (WHO), 15% of hospitalized patients catch HAIs worldwide. This rate is 7% in developed countries (general infection frequency is between 3.6% and 12%), while in developing countries it is 10% (general infection frequency is between 5.4% and 19.1%) (3). Often HAIs appear as catheter-related urinary tract infections, ventilator-associated pneumonias and catheter-related bloodstream infections (9).

While the incidence of infections associated with healthcare varies depending on the country, hospital and patient characteristics, the average is 3-17% across hospitals (9). While this rate rises to 30% in intensive care units (ICUs) in developed countries, it is 2-3 times more in the ICUs of developing countries than in those of developed countries (3). According to 2017 data from the Ministry of Health in Turkey, HAI rates in intensive care units vary between 1.08 and 4.86% (11). It is extremely important to prevent infections before they appear in order to provide a quality healthcare service and to protect against the negative effects of possible infections. These precautions should be implemented through initiatives that can be accepted by both the institution and the patients, and whose results have been proven by scientific studies (12). Studies on the prevention of HAIs, especially in the last 20 years, show that initiatives that have been proven

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to prevent a specific hospital infection make a rate of zero hospital infections possible if they are implemented as part of a whole bundle (13-15). One study using a healthcare bundle shows that ventilator-associated pneumonia decreased from 2.50 to 1.60cases per 1000 ventilator days; catheter-related bloodstream infections from 2.38 to 0.73cases per 1000 catheter days (16). Another study conducted to prevent ventilator-associated pneumonia showed that infection rates of ventilator-associated pneumonia decreased from 4.08 to 1.16 cases per 1000 ventilator days (17). In cases where compliance is at a high level the effectiveness of care bundles increases, and their use causes a significant decrease in mortality and morbidity (18). The fact that HAI rates decrease as a result of higher compliance with care bundles has increased demand for them and led to the development of many different types of care bundles (19,20).

A care bundle has been defined as a set of implementations used to standardize care and treatment in hospitals (21). Another definition has suggested that a care bundle involves the co-administration of several evidence-based practices (usually three to five) that positively affect the patient's healing process and improve the quality of care (20-23). The philosophy of the care bundle is to focus on how to provide the best care, not on what the care should be. The parameters that make the care bundle important are that "all its implementations have been tested with randomized controlled trials, level of evidence I, [and] it combines all these implementations and can be applied to every patient" (21,22,24). The key principle in evaluating packages is the logic of "all or nothing". Non-compliance with one of the parameters is accepted to be non-compliance with all the other parameters as well (21).

Intensive care nurses who look after a patient for 24 hours a day have an important responsibility in terms of using care bundles. Studies have stated that the presence of nurses during the implementation and monitoring of these bundles increases compliance by 100% (13,25). A study examining the use of care bundles to prevent infection found that if the responsible nurse was present in the ICU for the first three months of applying the care bundle the other nurses complied with it and infection rates decreased. In the second three-month period of the study, the responsible nurse was not present in the ICU for a long period and the compliance of the other nurses decreased while infection rates increased. This shows that the responsible nurse plays an important role, particularly in maintaining compliance (26). ICU nurses implement the procedures included in these bundles and maintain patient care, but they are also expected to be aware of the fact that HAIs are preventable, to keep up with the latest measures accepted worldwide for the prevention and control of infections, and to provide the best care for patients by using their knowledge to supplement their practice (27).

The literature includes studies on the effectiveness of care bundles with regard to infection rates. However, there is no study of the opinions of nurses on this subject. This study thus aimed to collect the opinions of the nurses who use care bundles. Knowing these nurse's opinions about the implementation of care bundles will be especially helpful in increasing their compliance with them.

Purpose of the Study

The study aimed to determine the views of nurses working in the ICU regarding the use of care bundles in preventing HAIs.

2. METHODS

Study design

The study employed a qualitative research design with the use of focus groups.

Setting and sample

Fourteen nurses working in the thoracic diseases ICU of a university hospital constituted the population and sample of this qualitative study. The study used the purposive sampling method in order to include intensive care nurses who had previously worked with the care bundles. Nurses use care bundles to prevent ventilator-associated pneumonia, catheter-related bloodstream infections, catheter-related urinary system infections in the intensive care unit.

In the study, 21.4% (n: 3) of the nurses were male and 78.6 (n: 11) were female. The average age of the nurses was 29.64±5.82. Total ratio of nurses who were graduates from vocational high school are 7.1% (n:1) and 92.9% (n: 13) of nurses had bachelor's degrees. While the average number of years of employment of the nurses was 7±5.09, the average number of years of employment in the ICU was 6±3.46. All of the nurses participating in the study had previously received training on HAIs and the use of care bundles and had worked with care bundles for six months.

Measurements/instruments

The study collected the data using a semi-structured questionnaire about the characteristics of the nurses (age, gender, educational status, total years employed, years employed in the intensive care unit) and their opinions regarding care bundles (Box 1).

Box 1: Questions for focus-group interviews
What is a care bundle?
Are care bundles useful in the ICU?
What difficulties have you experienced when implementing care bundles?

Data collection and analysis

Qualitative data collection and analysis

The data were collected in September 2019 using a semi-structured questionnaire. The study included two half-hour long focus-group interviews with seven nurses in each group,

for a total of 14 ICU nurses. The researchers conducted the focus-group interviews in the meeting room of the ICU. Before starting the interview, the researchers explained the purpose of the research to all the nurses participating in the study, and informed them that the interviews would be recorded. Their written permission and verbal consent were obtained.

The researchers conducted the focus-group interviews using three open-ended questions intended to open up a discussion. No additional questions were needed. The researchers ensured the nurses understood the questions and expanded them if necessary. The interviews were recorded on tape and in the form of written notes. The moderator of the focus group interviews was the first author, who had previously worked with care bundles, while the other researcher took notes during the interviews. The recorded interviews were then transcribed. The researchers then evaluated the data.

Analysis

The main researcher transcribed the focus-group interviews verbatim. The transcripts were later supplemented with the field notes which also contained information about the nonverbal responses that were observed during the interviews. The data transcribed from the focus-group interviews were grouped by theme and concept, and the statements of the participants were coded numerically according to these groupings.

Credibility

In order for the study to be credible to the nurses, the sessions were held in the meeting room of the ICU so that group members were in a familiar environment. The interviews started with casual conversation to create a relaxed atmosphere so that group members would feel free to express their opinions and speak openly. The researcher who had previously worked with care bundles was also previously acquainted with the nurses.

Dependability

In qualitative studies, dependability means that different researchers should come to almost the same conclusions when analyzing the data. Therefore, findings should be verified by more than one researcher (28). Both members of the research team analyzed the data in this study. They then compared the results of their analyses.

Ethical considerations

The study was conducted in accordance with the Principles of the Declaration of Helsinki. The study obtained approval from the Ankara Yıldırım Beyazıt University Ethics Committee (number 2019-351) in order to maintain the ethical standards of the research. In addition, permission was obtained from the institution where the study would be implemented after obtaining the permission of the Ethics Committee. The nurses participating in the study gave their written permission and verbal consent.

3. RESULTS

In the qualitative data analysis three themes and six subthemes emerged. These themes which are presented in Table 1.

Table 1: *Themes and subthemes*

Themes	Definition	Benefits	Difficulties
Subthemes	• Self monitoring – reminder • A preventive tool	• Helpful • A guide • Care	No problems

First theme: definition

Most of the participants defined a care bundle as "*a materials and reminder tool that provide integrated care for patients.*"

Below are some sample responses:

Self monitoring-reminder

"***Self-monitoring*** (informing ourselves)" (Nurse 4); "I can see my own deficiencies better by looking at the care bundle, ***it helps me make up what I lack***" (Nurse 6); "*Preventing errors, suggestions*, ***reminders*** to prevent infections" (Nurse 7).

A preventive tool

"***All-inclusive material*** that will prevent infection in the patient" (Nurse 12); "***A preventive tool*** for infection which allows action to be taken before infection occurs" (Nurse 13); "*Advantageous for the patient*, ***a tool that allows us to be careful***" (Nurse 14); "Material to prevent or minimize the risk of infection in patients" (Nurse 2).

Second theme: benefits

All of the nurses stated that a care bundle was beneficial, that it should be used in the ICU and that it was effective in reducing infection rates.

Below are some sample statements:

Helpful

"Helpful. ***It's like self-monitoring***. It helps us to check up on ourselves" (Nurse 2); "It's very important. It's especially effective for beginning nurses. Because nurses may miss something during implementations, a ***care bundle helps us to go back and carry out procedures we've missed***" (Nurse 3); "I saw many benefits, especially when I first started nursing. Since I can't remember everything, I look at the list and ***check everything*** (because I may have missed something)" (Nurse 5).

A guide

"You see the ***whole picture*** after a while, but something may be overlooked in the beginning because of information

overload. It functioned as ***a guide for me*** when I first started" (Nurse 6); "It allows us to follow up the patient in a better way and ***go back if there are any points that have been missed in the care***" (Nurse 8);

Care

"It's important for ***better care*** and to prevent infections" (Nurse 13); "It provides advantages for patient care and nurses, and ***reduces cost***" (Nurse 14); "***Makes nurses aware*** how to fight infections in intensive care units, ***if done regularly and properly***" (Nurse 12).

Third theme: difficulties

The nurses stated that they had no problems using care bundles and they were easy to apply once they had adapted to them.

Some of the nurses' statements are given below:

No problems

"I had ***no difficulty***" (Nurses 1, 2, 7, 12, 13, and 14); "***If you adapt to it***, it's ***not difficult*** and doesn't take any time. On the contrary, it's beneficial; it helps us to overcome any deficiencies" (Nurse 6) (Other nurses confirmed by nodding); "I didn't have any difficulty. I liked it because ***it improved my perspective***" (Nurse 8); "It is important that we use it ***as a stimulus***" (Nurse 5); "I've just started. It's very convenient for me. I see it as being ***a reminder, not a waste of time***" (Nurse 10).

4. DISCUSSION

Healthcare-associated infections are an important problem as they increase the length of hospital stay and costs, and cause mortality and morbidity (3-8). Nipping infections in the bud is extremely important in order to provide a quality healthcare service and to protect patients against the negative effects of possible infections. Hence, initiatives that are easy to implement for both the institutions and the patients, and that have evidentiary value should be developed (12). The literature suggests that care bundles are materials which fulfill this need. Studies on the prevention of HAIs show that implementing initiatives that are proven to prevent a specific hospital infection reduces the infection rate rapidly or even cancels it out when they are used as part of a whole bundle (13,14,16-18,26,29).

HAIs are seen most frequently in ICUs (3,9,30). These common infections need to be prevented because they increase mortality and morbidity rates and negatively affect the patients' quality of life.

ICU nurses are the most common users of the care bundles designed to prevent HAIs (13,16,17,26,29). Moreover, it has been found that the views of stakeholders are especially important in increasing compliance with care bundles (31). This study thus collected the opinions of the nurses who most frequently employ care bundles. Obtaining the opinions of these nurses about the implementation of the care bundles will be particularly helpful in increasing compliance.

The main purpose of care bundles is to provide guidance to their users. They are designed to be helpful tools which remind nurses of the required procedures in a step by step fashion. One study that included opinions of the nurses about care bundles stated that the nurses described the care bundle as a "good visual reminder" (32). Similarly, in our study, nurses defined care bundles as " ***a materials and reminder tool that provide integrated care for patients***". Nurses also identified cares bundle as "preventing errors", and as consisting of "all-inclusive material" or "reminder material. Furthermore, nurses in our study stated that their care bundles were a tool that allowed them to be more cautious in preventing infections. In fact, implementing specific steps through care bundles helps to eliminate errors by increasing nurses' attention (33,34). Care bundles are a strategy for increasing the incorporation of research evidence into clinical practice and assisting healthcare providers in providing optimal patient care in busy environments with limited resources.

The literature shows that care bundles are used in various fields (13,32,35-37). The objectives of care bundles include providing direct benefits to patients, shortening the duration of hospitalization in intensive care, reducing the economic burden and improving resource use (18). Care bundles, as mentioned earlier, provide reminders to nurses. The nurses in this study stated that using care bundles benefits them. They also stated that a care bundle is a tool that provides them with guidance and helps them to monitor themselves, as well as including very effective material for beginner nurses.

Also, They thought that care bundles lead to better quality care for patients and that they prevent infection when applied regularly and properly. Care bundles list all the specific steps that should applied in the correct order. Failure to carry out any of the steps included in a bundle thus negates its usefulness. In the study by Chaboyer and Gillespie (2014), nurses stated that it would be effective for them to use care bundles in their daily practices. In another study by Dampliang et al. (2015), in which opinions on the use of care bundles in emergency services were asked, the participants stated that using the care bundle helped them improve the quality of care and increase the knowledge, skills and confidence of nurses. Another study examining the results of using a care bundle in preventing ventilator-associated pneumonia concluded that using a care bundle provides a structured approach to the nursing care of a ventilated patient and that using a care bundle at the bedside is beneficial in an intensive practice environment (34). The introduction of care bundles to prevent/control infections creates an important opportunity for providing evidence-based, safe healthcare to patients.

There are no studies indicating difficulties in using/implementing care bundles. However, Chaboyer and Gillespie's study (2014) stated that nurses were worried that the use of care bundles would increase their workload. In the current study, they also stated that they had not experienced

any difficulties when asked about problems they had had while using care bundles. Rather, they talked about their benefits and how essential they were. Nurses stated: "I had no difficulties, I liked it very much because it improved my perspective"; "It is important that we use it as a stimulus", "I see it as a reminder, not a waste of time". It may have been only natural for the nurses participating in our study to think in this way as they had worked with care bundles before.

The literature has emphasized that compliance with care bundles is very important for them to be effective (18,25,26). There are some factors that affect adherence to care bundles. In a study that sought health professionals' opinions on the barriers to the implementation of evidence-based practices in the prevention of ventilator-related events, "inadequate use of evidence to underpin practice" was among the emerging ones (38). This study demonstrates that there is a need for care bundles that combine evidence-based practices, as stated by nurses in our study (38). Ladbrook et al. (2019), on the other hand, stated that the obstacle to the use of the care bundle is that the unit culture does not give priority to preventive care. In our study, a nurse stated the importance of complying with the use of the bundle as follows: "If you adapt, it's not difficult, doesn't take time. On the contrary, it's beneficial, it helps us to overcome any deficiencies". A care bundle consists of several interventions or approaches, all of which positively affect the patient's healing process and results when applied separately, but which provide a better result when applied together. The key principle of care bundles is high compliance with all their components (18,21,39).

The literature shows that the education and responsibility given to nurses play an important role in compliance with the care bundle (26,39,40). For this reason, effective communication should be established before implementing care bundles in a clinic, and nurses should be provided with adequate education and feedback, as well as the chance to express their own opinions about the effectiveness of care bundles (24,31,41).

Limitations

This study was conducted in the ICU of a university hospital; the data thus represent a single sample. Since this is a qualitative study, its results cannot be generalized to the population of nurses. Further studies should be conducted with larger samples and mixed methodologies.

5. CONCLUSIONS

Control of healthcare-associated infections is an important issue related to patient safety. Recent studies have shown that it is possible to prevent most HAIs through the introduction of care bundles that combine various evidence-based practices. Our study is the first study gathering the opinions of the nurses about the use of care bundles. The nurses participating in the research stated that the care bundles provide them with reminders and are useful, that they should be applied properly and regularly, and that they are easy to use once they have been adapted to. We know that effective communication, leadership, education, recommendations, supervision and feedback are required for proper use of nursing care bundles (24,31,41). In this regard, the participation of nurses in line with these guidelines is important for the widespread use of care bundles to prevent HAIs in ICUs.

In line with the results of the study, the care bundles used by nurses to prevent infection are determined to be beneficial. Contrary to popular belief, among the activities carried out to improve the quality of care in addition to routine work in ICUs, it has emerged that care bundles are not viewed as a workload by nurses, but rather as a guide for them, and they are especially important for nurses who have just started their profession. In this direction, managers of health institutions are recommended to expand the use of care bundles for HAIs, which is one of the quality indicators and one of the most important indicators of the quality of care.

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Clinical and Experimental Health Sciences

Evaluation of Complication Development in General Surgery Patients Admitted to the Post Anesthesia Care Unit

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ABSTRACT

Objective: This study was conducted to analyze the development of complications and risk factors in general surgery patients admitted to the Post-Anesthesia Care Unit (PACU).

Methods: This prospective and cross-sectional study was performed with 230 patients admitted to the PACU of a university hospital in Istanbul. The data were collected pre – and post-operatively in the PACU using the "Patient Monitoring Form", which was created by the researcher to track the patient's descriptive characteristics and development of complication. Percentage, mean, chi-square, Student's t, and logistic regression tests were used for data analysis.

Results: It was found that most of the patients were female aged between 50-65, with a chronic disease included in the ASA II class, who had undergone a laparoscopic cholecystectomy surgical operation, and had a profile of minimal obesity. The most common complications in the PACU were pain (75.7%), hypothermia (58.7%), nausea-vomiting (30.7%), and hypoxemia (20.0%) respectively. Length or duration of operation (OR:1.05; p=0.001) and age (OR: 1.08; p=0.027) were effective risk factors for development of complication; however, no correlation was found between descriptive characteristics of the patients and development of pain (p=0.023). A positive relationship was found between hypothermia and surgical operations with a duration of more than 120.3 minutes (p=0.001). Additionally, age of 57.8 (p=0.002), BMI of 30.8 (kg/m2; p=0.003), and inclusion in the ASA III/IV group (p=0.001) were significant in relation to hypoxemia.

Conclusions: It was found that pain, hypothermia, nausea-vomiting, and hypoxemia remained the most common complications in the PACU, and age and duration of operation were effective risk factors in the development of complications. Based on these results, it is recommended to identify high risk factors specific to the patient in advance and to increase nursing practices to prevent/reduce complications.

Keywords: Complication, post-anesthesia care unit, general surgery, nursing care.

1. INTRODUCTION

Patients require post-operative nursing care for a certain period of time in the post-anesthesia care unit (PACU). The aim of post-anesthesia care is to provide care for patients who have just undergone surgery with anesthesia in the PACU until vital signs are stable. Surgery and anesthesia always carry potential for the development of complications. In this critical period, when post-operative complications and mortality rates are the highest, factors such as having a limited patient follow-up period, complex patient care, emergency administrations of medications, and monitoring of the patient by multiple teams affect patient safety and increase the risk of complications (1-3).

Despite the advanced levels of science and technology available today, morbidity and mortality continue to be observed due to complications in the PACU, which in turn cause material and morale losses in patients and/or their families (3-5). When the reasons for the post-anesthesia death of 306 patients were reviewed in a study conducted in 1947, it was seen that approximately half the deaths could have been prevented by the presence of a PACU and one-third by post-operative professional nursing care (6). Half of the complications that occur in the PACU happen intra-operatively or in the first 15 minutes of post-anesthesia care (7-8). As well, 7.1% of post-operative malpractice cases in the United States of America (USA) result in anesthesia-associated death or neurological complications in the PACU (9). Studies have shown that complications increase the nursing care burden by 52.2% (10) and the cost of care by 89% (11).

While surgical operations generally vary based on the complexity of the procedure to be performed, the general condition of the patient, the available technology, and financial resources, all patients must stay in the PACU for a certain period of time. This period is critical as meeting the

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patient's basic needs and aiding in their recovery depend on nursing care. In this context, the effectiveness of the care given to the patient is based on the nurse's professional knowledge as well as the ability to identify and meet these needs (12-13). According to the literature review, it was observed that most of the research was retrospective and carried out with different types of surgical operations and anesthesia applications, but that prospective studies examining the development of specific complications in general surgery patients were very limited (9,14-19). Based on this need, the present study was conducted in order to determine the complications and analyze the risk factors in the complications by monitoring PACU patients who had undergone elective surgery in the general surgery department.

Research Questions

- Which complications are most common in the PACU?
- Do demographic and descriptive characteristics have an effect on the development of complications?

2. METHODS

2.1. Design and Sample

The study was designed and carried out as a cross-sectional and prospective study between October 2009 to August 2010 in Turkey. 230 patients were included in the study according to the medium effect size (effect size=0.2) and 80% power analysis in the calculation of the sample with a known population (n=422). The most frequently performed surgical interventions in general surgery were determined. Data were collected according to the surgical grade classification of the Turkish Society of Anesthesiology and Reanimation (TARD) from surgical interventions in 2 and 3 with a simple random method. Complication development of high surgical grade 4 (eg whipple operation) was not considered (20). Patients who had undergone laparoscopic surgery, thyroid surgery, colon and rectum surgery, hernia surgery and bariatric surgery, over 18 years of age, without communication problems, and with oral and written consent were included in the study.

2.2. Instruments

The data were collected using the *"Patient Follow-up Form"* prepared by the researcher, according to relevant literature (12,14) and considering expert opinions.

The patient follow-up form consists of three parts:

Part 1 consists of *"personal information"* [age, gender, body weight (kg), height (cm), BMI (kg/m^2), educational status, diagnosis, surgery performed, smoking and alcohol use, surgery history]; *"patient risk factors"* [Chronic Obstructive Pulmonary disease, heart failure, diabetes, drug use, ASA (American Society of Anesthesiologists) class]; and *"surgical operation information"* [pre-medication status, operation time, anesthetics and drugs used, complications during surgery].

The second part includes the *"Patient Monitoring and Evaluation Form in the Post-Anesthesia Care Unit"* (respiratory rate, partial oxygen saturation, heart rate, arterial blood pressure, body temperature, pain score, nausea and vomiting score, state of consciousness, recovery score, duration of stay in the PACU, color and condition of the skin, condition of the dressings and follow-up of fluid in-out].

The final section contains the *"Form for Monitoring Complications in the Post-Anesthesia Care Unit"* [airway obstruction, hypoxemia (oxygen saturation <90%), hypoventilation (respiratory rate <10, prolonged sleepiness), hypertension (30% increase compared to basal value), hypotension (30% reduction compared to basal value), tachycardia (heart rate 120/min), bradycardia (heart rate ≤50/min), bleeding, oliguria, agitation, delayed awakening (awakening ≥60 min), cognitive dysfunction, nausea, vomiting, hypothermia (parameters including body temperature <36°C (96.8°F), tremor, and pain (pain ≥4 according to the Visual Analogue Scale (VAS)].

The "Visual Analogue Scale" (VAS), created by Cline (1992), was used for evaluation of pain and nausea (21). The literature states that VAS is sensitive enough to detect the severity of a symptom and is a one-dimensional scale with proven validity and reliability, which is frequently used in the measurement of subjective parameters such as nausea, pain intensity, and patient satisfaction. In this study, 0-2 cm obtained from the scale was considered "mild", 2.1-4 cm was "uncomfortable", 4.1-6.0 cm was "moderate", 6.1-8.0 cm was "severe", and 8.1-10.0 cm. was "intolerable". It is reported that in the VAS, horizontal drawing tends to mark higher scores than vertical, and the scale drawn vertically gives more reliable results. Considering these data, the vertical VAS was used in the present study (22).

In the PACU, the "Modified Aldrete Scoring System" was used to evaluate recovery. In this system, if the score evaluation result is ≤7 in total, the patient remains in the post-anesthesia care unit or is transferred to the intensive care unit until general condition improvement. If the total score is ≥8, the patient can be sent to an inpatient department or home (23).

2.3. Data Collection

Patients who consented to remain in the study after the research objectives were explained were interviewed in the pre-operative period and the scales used to evaluate pain, nausea, and vomiting in the PACU were completed with patients who met the inclusion criteria. Patients were then followed-up and evaluated by the researcher.

In order to evaluate the clarity and usability of the data collection forms, a pilot study was performed in which 10 patients were asked to complete the forms on a voluntary basis. The questions were then rearranged based on feedback received from these volunteers. After the pilot study, follow-up was done on all patients included in the sampling and data were collected.

Vital signs, pain, and nausea-vomiting evaluations of the patients were measured every 15 minutes. A digital sphygmomanometer to measure blood pressure, a tympanic thermometer to measure body temperature, and a pulse oximeter (finger) to measure oxygen saturation were used.

2.4. Ethical Considerations

The study commenced after obtaining the required permission of the Ethics Committee of Marmara University (B.30.2.MAR.0.01.02/AEK/531) and of the University Hospital (B.30.2.MAR.0.H1.00.00/4064). In accordance with the Helsinki Declaration, patients were informed about the research via the Voluntary Information Form. Patients who volunteered to participate in the study were included in the study after their verbal consent was obtained.

2.5. Statistical Analysis

The SPSS (Statistical Package for the Social Sciences) 16.0 IBM statistics program was used for analysis of the data. Student's t test was used for parameters showing percentage, ratio, mean, and normal distribution for descriptive data, while the chi-square (x^2) test was used for categorical variables. In addition, a multivariate model was used to evaluate multiple factors. Significant variables were determined using logistic regression and the relationship was expressed as an Odds ratio. Parameters that may be effective in the development of complications were included in the model. A 95% confidence interval and $p<0.05$ error level were taken into consideration for the evaluation of the obtained results.

3. RESULTS

The descriptive characteristics of the patients in the PACU are given in Table 1. Review of these characteristics indicates that 62.5% of the included patients (n=143) were female, 37.3% (n=63) were aged 50 to 65, 42.7% (n=98) were overweight, 53.5% (n=123) had a chronic disease, 57.8% (n=133) took or had taken medicine, and 52.7% (n=121) were in the ASA II class. The average age of the patients was 53.49±14.57 and average BMI was 28.81±6.94 (Table 1).

Table 1. *Descriptive Characteristics of the Patients in the PACU (N=230)*

Demographics		N	%
Gender	Female	143	62.5
	Male	87	37.5
Age (53.49±14.57)	18 – 33	27	11.8
	34 – 49	63	27.5
	50 – 65	86	37.3
	66 – 71	30	13.0
	72 and↑	24	10.4
Education status	Elementary	130	56.6
	Secondary education	27	11.7
	High School	27	11.7
	Higher education	46	20.0
Body Mass Index (kg/m²) (28.81±6.94)	Normal (20-24.9)	59	26.7
	Minimal overweight (25-29.9)	98	42.7
	Moderate overweight (30-34.9)	34	14.7
	Maximal overweight (35-39.9)	24	10.4
	Morbid (40 and ↑)	15	6.5
Chronic disease	Yes	123	53.5
	COPD	26	20.9
	Diabetes	21	16.9
	Heart failure	39	31.5
	Other*	37	30.7
Use of medicine	Yes	133	57.8
	Antihypertensive	70	52.3
	Antithyroid, chemotherapy	45	33.9
	Steroid, tranquilizer	18	13.5
Previous surgery	Yes	155	67.3
	No	75	32.6
Smoking	Yes	75	32.6
	No	155	67.4
ASA score	ASA I	35	15.2
	ASA II	121	52.7
	ASA III / IV	74	32.1

**Other: patients with liver and kidney failure and cancer.*

COPD: Chronic Obstructive Pulmonary Disease

Multivariate analysis of the factors determining the development of complications is given in Table 2. In the logistic regression model for multivariate analysis in the development of complications, a significant difference was found between surgical operation time (Odds ratio: 1.05, p=0.001, confidence interval: 0.02-1.09) and age (Odds ratio: 1.08, p=0.027, confidence interval: 1.00-1.15). In addition, it was found that there was a statistically significant difference in females (Odds ratio: 6.58, p=0.052, confidence interval: 0.98-0.44) (see Table 2).

Table 2. *Multivariate Analysis of the Factors Determining the Development of Complications (N=230)*

Risk Factors:	Total *n=230*	Complication emerged *n=220*	Complication non-emerged *n=10*	Odds ratio (95% CI) *p*	*p*
Age (years), mean (SD)	53.4 (14.5)	54.0 (14.3)	41.5 (14.2)	1.08 (1.00 – 1.15) **0·027***	0.008†
Gender, n (%)					
Female	143 (62.5)	139 (63.2)	4 (40.0)	6.58 (0.98-0.44)	0.139‡
Male	87 (37.5)	81 (36.8)	6 (60.0)	**0·052***	
BMI (kg/m^2), mean (SD)	28.8 (6.9)	28.9 (7.0)	26.7 (34)	0.94 (0.81-1.09)	0.328†
ASA classification score, n (%)					
ASA I	35 (15.2)	34 (15.5)	1 (10.0)		1.000 ‡
ASA II	121 (52.7)	115 (52.3)	6 (60.0)		
ASA III / IV	74 (32.1)	71 (32.2)	3 (30.0)	0.79 (0.04 – 13.79) 0820*	
Duration of surgery (minutes) mean (SD)	117.4 (54.6)	120.3 (53.9)	54.5 (22.9)	1.05 (0.02-1.15) **0.001***	0.000†

ASA, American Society of Anesthesiologists; BMI, Body Mass Index
**Multi-factor logistic regression test; ‡ Based on chi-square test; † Based on Student' t test*

The distribution of complications developed in the PACU can be seen in Graphic 1. Of the included patients, pain in 75.7% (n=174), hypothermia in 58.7% (n=135), nausea and vomiting in 41.3% (n=95), hypoxemia in 30.7% (n=71), hypertension in 20.0% (n=46), tremor in 16% (n=37), agitation in 12.1% (n=28), and oliguria in 4.8% (n=11) emerged.

The comparison of risk factors that may cause pain, hypothermia, nausea-vomiting, and hypoxemia are shown in Table 3. It was determined that the descriptive characteristics of the patients were not effective in the development of pain (p>0.05). As the average age (p=0.002) and BMI (kg/m^2) (p=0.003) increased, the rate of hypoxemia was found to be statistically significant in patients in the ASA III/IV (p=0.001) group.

Factors that may cause nausea and vomiting were observed more frequently in female patients (p=0.023). A statistically significant (p=0.001) association with an increase in hyperthermia was found between surgical operation duration (120.3 minutes or more) and type of surgery (thyroid surgery).

Table 3. *The Comparison of Risk Factors That May Cause Pain, Hypothermia, Nausea-Vomiting and Hypoxemia (n=230)*

Risk factors	Pain*		Hypothermia		Nausea-Vomiting		Hypoxemia	
	Pain ≥ 4 *n=174*	Pain < 4 *n=56*	emerged *n=135*	non-emerged *n=95*	emerged *n=95*	non-emerged *n=135*	emerged *n=71*	non-emerged *n=159*
Age (years), mean (SD)	59.8 (15.0)	52.3 (13.1)	54.7 (14.4)	51.7 (14.6)	51.5 (14.8)	54.7 (14.3)	57.8 (13.4)	51.6 (14.6)
	-0.65 / 0.511†		-1.53 / 0.126†		1.63 / 0.103†		-3.09 / **0.002**†	
Female, n (%)	111 (63.8)	32 (57.2)	91 (67.4)	52 (54.8)	67 (70.5)	76 (56.2)	50 (70.4)	93 (58.5)
Male, n (%)	63 (36.2)	24 (42.8)	44 (32.6)	43 (45.2)	28 (29.5)	59 (43.8)	21 (29.6)	66 (41.5)
	0.79 / 0.372‡		3.80 / 0.051‡		5.13 / **0.023**‡		9.11 / 0.085‡	
BMI (kg/ m^2), mean (SD)			28.6 (6.9)	29.6 (7.2)	29.7 (8·2)	28.1 (5.7)	30.8 (8.2)	27.8 (6.0)
			-0.71 / 0.47†		1.74 / 0.082†		-3.04 / **0.003**†	
ASA I	28 (16.1)	7 (12.5)	21 (15.5)	14 (14.7)	14 (14.7)	21 (15.6)	5 (7.0)	30 (18.8)
ASA II	92 (52.9)	29 (51.8)	71 (52.6)	50 (52.7)	46 (48.4)	77 (57.0)	29 (40.9)	92 (57.9)
ASA III / IV	54 (31.0)	20 (35.7)	43 (31.9)	31 (32.6)	35 (36.9)	37 (27.4)	37 (52.1)	37 (23.3)
	0.44 / 0.931‡		6.12 / 0.442‡		6.06 / 0.067‡		19.90 / **0.001**‡	
Duration of surgery (minutes), mean (SD)	121.1 (65.6)	106.2 (50.2)	120.3 (53.9)	54.5 (22.9)	123.1 (59.8)	113.6 (50.6)	127.8 (51.3)	112.8 (55.5)
	-1.77 / 0.076‡		-3.60 / **0.000**‡		-1.28 / 0.200‡		-1.92 / 0.055‡	
Type of surgical operation								
Hernia surgery	18	11	13	16	9	20	4	25
Laparoscopic surgery	54	22	38	38	36	40	27	49
Thyroid surgery	22	6	21	7	10	18	5	23
Breast surgery	31	9	24	16	16	24	15	25
Colon/ rectum surgery	21	5	19	7	8	18	12	14
Bariatric surgery	15	3	11	7	10	8	5	13
Gastric surgery	13	-	9	4	4	9	3	10
	9.11 / 0.167‡		**10.62 /0.010**‡		6.06 / 0.416‡		11,077 / 0.086‡	

‡ Based on chi-square test (X^2/ p); † Based on Student t test t/ p)

4. DISCUSSION

One of the most important criteria in patient care and safety is the prevention or minimization of complications. Therefore, it is critical in this period to know the risk factors for complications, to prevent their occurrence, and to react effectively and quickly

when they are present (1,9,24). This study found that most of the patients displaying post-operative complications were female aged 50 to 65, with a chronic disease included in the ASA II class, had undergone a laparoscopic cholecystectomy surgical operation, had a profile of minimal obesity, and an average age of 53.4 (Table 1).

Distribution of Complications Emerged in the PACU

This study also found that the most common complications in the post-operative PACU were pain, hypothermia, nausea-vomiting, hypoxemia, and hypertension, respectively (see Graphic 1). In studies conducted at different times between 1986 and 2017, it was observed that the complication rate ranged from 4.5 to 36.2% depending on the type of surgery and anesthesia applications. Moderate-severe pain (7.2-54%), hypothermia (11-52%), hypoxemia (4-69%), nausea and vomiting (9-36%), hypotension (2-20%), and hypertension (3-20%) were observed as the most common complications. Bleeding, oliguria, delayed awakening, and airway obstruction were among the fewer common complications (9, 14-19, 25-27). Belcher et al. conducted a retrospective study including approximately 93 thousand patients in 2017 to evaluate complications emerged in the PACU and found most of the complications were hypertension, nausea, and hypoxemia without including pain and hypothermia (9). In this study, it is noteworthy that nausea-vomiting was included in the ranking after hypothermia, although this is similar to other studies when the profile of patients and types of complications and their incidence are compared. In some studies, it has been observed that feeling “chilly” can in fact be more disturbing than surgical pain. However, among the complications that occur in the PACU, hypothermia is the simplest complication as it is easily prevented and treated when it occurs (28,29). The complication of hypothermia ranking after pain in the present study can be explained by the fact that patients were transferred to inpatient departments earlier due to the physical conditions and lack of equipment (i.e. heaters were used alternately between patients) at the PACU where the study was performed.

Risk Factors That May Cause Complications in the PACU

This study identified 54-year-old patients and surgical operations 120 minutes or more in length as important risk factors for the occurrence of complications (Table 2). Studies have reported that age, without disease, before, during and after surgery is an independent risk factor for the emergence of complications and death. Post-operative complications have been reported to increase, especially in patients aged 60 and over (27, 30, 31). Tuchinda et al. (2010) stated that 47.9% of post-operative complications are caused by age-related problems and that 39.3% of deaths within 24 hours occur in patients aged 65 and over. In line with the literature, age in the present study was determined to be an important risk factor for the development of complications (32).

The duration of the surgical operation is another important risk factor for complications. Hines et al. (1992) determined that 120-240 minutes was significant for complications, and Yavaşcaoğlu, Kaya, and Özcan (2009) reported that surgical operations lasting more than 150.9 minutes lead to complications (14,19). Tarrac (2006) determined that emergence of complications increases in surgical procedures lasting 132 minutes or more. Again, data obtained within the present study are supported by the literature (17).

For the last 30 years, pain has continued to be an important factor affecting the comfort of patients as well as one of the complications emerging in the PACU (25,33). This research determined that the type and duration of the surgical operation and individual characteristics were not effective risk factors for the emergence of pain. Aubrun et al. (2008) and Fecho et al. (2009) reported that individual characteristics are not effective in the emergence of pain in patients with severe pain (34,35). Ip et al. (2009), in 48 studies including 23.037 patients, identified preoperative pain, anxiety, age, and type of surgery as the most important factors determining post-operative pain and analgesia consumption (36). A systematic review carried out by Aslan et al. (2018) determined that non-drug care practices of nurses for post-operative pain management were insufficient (37). In this study, care and treatment approaches for pain are considered to be more important than risk factors for pain complication.

This study also found that gender plays an important role in the development of nausea and vomiting, and that it is more often seen in women. Smith and Ruth-Sahd (2016), in a meta-analysis of 37 evidence-based studies, also identified gender and age as important risk factors for the occurrence of this complication (38). In all studies investigating the risk factors for nausea and vomiting, it is considered that these complications are more common in females and, although the cause is not clearly explained, serum gonadotropins or other hormones play a role in nausea and vomiting (39, 40).

It is stated that life-threatening respiratory complications occur within the first 15 minutes in patients transferred to the PACU and mostly hypoxemia is diagnosed (24), with this being the most common complication in malpractice cases (3). Another study states that patients stay in the hospital for about 17 days more due to respiratory complications and the cost of health care is $ 9,500 (41). An average age of 57.8 years, moderate obesity, being in the ASA III/IV class, and hypothermia were determined in this study as risk factors for the occurrence of hypoxemia. Although age is an independent risk factor in the emergence of hypoxemia, according to the literature, total lung capacity, vital capacity, residual volume, and lung elasticity decrease by 10% every decade and stiffness of the chest wall increases. These changes make it difficult for elderly patients to tolerate stress factors against anesthesia and surgery, and respiratory complications that occur in the post-operative period have an effect that is almost life-threatening (31). While Uakritdathikarn et al. (2008) stated that the age of 55 creates increased risk of hypoxemia, Murphy et al. (2008) indicated that 64 years of age or older increases its risk (24, 42). However, Aust et al. (2015) stated that in patients with an average age of 59, a BMI of 31, and a surgical operation duration of 144 minutes

or more, the emergence of hypoxemia is greater (43). The findings of the present study thus support the literature.

5. CONCLUSION

This study determined that pain, hypothermia, hypoxemia, and nausea-vomiting were the most common complications in the PACU. Age and duration of surgery are high risk factors that cause complications. Despite scientific and technological developments, similar complications were developed as in 30 years ago. Based on these results, it is thought that reviewing patient care for interventions, identifying patients with high risk of complications, taking precautions, training employees at regular intervals and increasing compliance with care protocols will reduce the rate of complication development.

Authors'contributions

All the authors took part in the formulation of the concept, data collection, data analysis and interpretation of results. All the authors reviewed and edited the manuscript and approved the final version of the manuscript.

DS, SKS: Conceptualization, Methodology; Data curation; Writing – Original draft preparation; Visualization, Investigation; Supervision; Software, Validation; Writing – Reviewing and Editing

Informed consent

Applicable. We have ethic form.

Conflict of interest

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Clinical and Experimental Health Sciences

Lactobacillus Species in Breast Milk: Do They Get Affected by Birth Style?

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ABSTRACT

Objective: Breast milk has an important function in the formation of the intestinal flora. Cesarean section bypasses the vertical transition of vaginal flora to the baby also usually causes the late start of lactation. The difference is in birth style and lactation period may affect the microbiota of breast milk. In this study, it was aimed to investigate how Lactobacillus species found in breast milk differ by the birth style and stages of milk.

Methods: Milk samples were taken from 72 mothers who had a vaginal birth (n:36) and cesarean (n:36) were divided into three groups as colostrum (n:12), early milk (n:12), and mature milk (n:12). *Lactobacillus* species were investigated from milk samples by real-time PCR.

Results: While Lactobacillus was detected in 70 (97.2%) of the samples, it was not detected in 2 (2.8%) of the samples taken from women with cesarean delivery. *L. acidophilus* and *L. rhamnosus* were detected simultaneously in all transitional milk samples of women who had a vaginal birth, and 82 *Lactobacillus* species were detected. The species identified were 33(39.3%) *L. rhamnosus*, 25(29.8%) Lactobacillus spp., and 24(28.6%) L. acidophilus. The rate of detection of *L. acidophilus* in milk samples taken from women who gave birth was found to be significantly higher than that found in milk samples taken from women who gave birth by cesarean section ($p < 0.05$).

Conclusion: Breast milk is not only a nutritional source but an important source of probiotics. *Lactobacilli* were found to be concentrated in breast milk. Also, *Lactobacillus* species detected in breast milk may differ according to the mode of delivery.

Keywords: Cesarean, *Lactobacillus,* microbiota, colostrum

1. INTRODUCTION

The microorganism assemblage, found in a certain ecological place or environment is called 'microbiota'. The microorganisms in the human body which are commensal, symbiotic, and pathogenic form the microbiota. The 'microbiome' usually used as the same meaning as microbiota, represents the gene pool of microbiota in a certain area and its relationship with the environment (1,2). In recent years, with the developments in molecular methods detection and identification of microbiota and microbiome of the human body becomes easier and more rapid (3). At present, developments in bacterial detection techniques especially those which are unrelated to culture methods and microbiome approach showed much more bacterial diversity in breast milk than predicted (4).

Human breast milk consists of high amounts of carbohydrates, essential fatty acids, proteins, vitamins, and minerals which are very important for feeding a baby, therefore it is recognized as the gold standard in baby nutrition (5-7). Breast milk plays a crucial role in the survival of the baby and baby development, not only with the nutrient source but also with the transfer of microflora (8). Breast milk is shown to be a consistent commensal, mutualistic, and probiotic bacterial source to baby intestines including *Staphylococcus, Streptococcus, Bifidobacteria*, and lactic acid bacteria (8-10).

Lactobacillus, Pediococcus, and *Lactococcus* species are belong to lactic acid bacteria (LAB) and strains of these bacteria often used in the production and preservation of many foods or used as probiotics for humans and animals (11,12). LAB like *L. gasseri, L. salivarius, L. rhamnosus, L. plantarum*, and *L. fermentum* which are found in breast milk and are considered to be probiotic species become more attractive targets (13). In this study, detection, and identification of *Lactobacillus* species in colostrum, transition, and mature breast milk from randomly selected mothers who are in the lactation period, also the determination of

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effects of factors like delivery method (vaginal or cesarean) and lactation duration was aimed.

2. METHODS

Between August 2019 and March 2020, women aged between 18 to 45 years old and in the lactation period who had full-time pregnancy and have 0-240 days lactation duration was randomly selected for this study. This research was reviewed and approved by the Gaziantep University Clinical Research Ethics Committee (protocol code: 2019/240, date: 19.06.2019), and participation involved informed consent.

2.1. Research Sample

Two groups were formed based on the delivery method. Two groups were formed based on vaginal and cesarean delivery and the minimum number for each group was defined as 36 based on statistical analysis, according to the literature, with 3% of expected prevalence, 5% precision, and %95 confidence interval.

Breast milk samples were classified into three different groups; breast milk between the 0-5 days after the birth was classified as colostrum, breast milk between the 6-15 days after the birth was classified as early milk, breast milk between the 15-240 days after the birth was classified as mature milk (14). Twelve colostrums, 12 early milk samples, and 12 mature milk samples, 36 in total, were collected from both groups consisting of women who gave birth by vaginal delivery and Cesarean section.

2.2. Exclusion Criteria

– Women who took antibiotics for any reason before 15 days

– Women with gestational diabetes, hypertension, heart disease, acute infectious diseases, and postpartum depression

– Women with breast-related diseases

– Breastfeeding women who received hormonal therapy during the three months prior to starting the study or who had inadequate skills in understanding study questionnaires were not included in the study

– Women using commercial probiotic supplements

2.3. Data and Sample Collection

Verbal instructions for standard sampling were given to mothers before sampling. Milk collection was taken by manually expressing the woman's breast. After the areola and its surroundings were cleaned with soap and sterile water and soaked in chlorhexidine and hand asepsis of the woman was achieved, the first few drops (0.5-1 mL) of milk were wiped off with a dry sterile sponge and 2-3 mL of milk was extracted from the subsequent milk sample and collected with sterile gloves in a sterile container.

2.4. Determination of demographic characteristics

The mother's level of education, working status, family type (core & large family), smoking/alcohol use, number of children, birth style, weight gained at birth, whether or not she received breastfeeding education during pregnancy, profession, where the family lives (city & rural), monthly income, sex of the baby, birth weight, birth height, weight and height of the baby during sample intake, sleep patterns of baby, gastrointestinal complaints (colic, vomiting) of baby, defecation count of the baby were questioned with a questionnaire form. While families consisting of a mother, father and children were accepted as core families, families living with adults in addition to these individuals were accepted as large families. No amount was questioned about alcohol and cigarette use, but the presence of habit was questioned. Babies with a birth weight of less than 2500 grams were considered low birth weight, while babies between 2500-4000 grams were considered normal birth weight, and those with a birth weight over 4000 grams were considered high birth weight. Male babies with a birth length of 48 cm or less were classified as short birth length, while those 48-58 cms were considered normal birth length and those larger than 58 cm were considered longer than normal birth lengths. Female babies with a birth length of 45 cm or less were classified as short birth length, while those with a normal birth length of 45-55 cm and those larger than 55 cm were considered as longer than normal birth lengths. Infants with bouts of restlessness, spasms, and crying for at least 3 days a week and lasting approximately 3 hours during the day were considered colic.

2.5. Species Identification with Real-Time PCR

All samples were kept frozen in the laboratory until work was carried out at −20 °C. DNA isolation was carried out using the Qiagen Stool Fast Kit (Qiagen, Hilden) protocol. Post-isolation DNA concentration was measured with the NanoDrop device (Thermo, USA). Using primary arrays (Primer Design Genesig (UK)) available from literature (15) according to brand species identification kits; *L. casei, L. acidophilus, L. delbrueckii, L. gasseri, L. reuteri, L. plantarum, L. rhamnosus,* species were defined, the species that are left out of these species were defined as Lactobacillus spp, negative and positive controls used according to producers recommendations. Amplification was run as 40 cycles on the Rotor-gene (Qiagen, Germany) PCR device.

2.6. Statistical Methods

Data suitability for normal distribution was tested by the Shapiro-Wilk test. Student t or Mann-Whitney U tests were used to compare numerical data in 2 independent groups. Correlation analysis of the relationships between numerical variables and relationships between categorical variables was tested with Chi-Square. SPSS 22 Windows version was used in the analysis and it was considered statistically significant that the P-value was smaller than 0.05.

3. RESULTS

The mean age of the women included in the research, in Group I (vaginal delivery) and Group II (cesarean section) was similar and was found to be 30.02±3.60 and 30.97±4.10, respectively. Statistical evaluation of the demographic characteristics of study groups is given in Table 1. Statistical analysis revealed a significant difference between vaginal and cesarean delivery based on family type only (p:.028). The rate at which mothers who gave birth by Caesarean section had a core family structure was significantly higher than those who gave vaginal birth (Table 1).

Lactobacillus was detected in 70 (97.2%) of the samples in the study, while 2 (2.8%) of the samples taken from women with cesarean birth Lactobacillus was not detected. *L. acidophilus and L. rhamnosus* were detected simultaneously in all early milk samples of women who gave vaginal birth and 82 Lactobacillus species were identified (Table 2).

As a result of statistical analysis to determine whether there was a difference between PCR results of women who gave vaginal birth (Group I) and cesarean (Group II), it was found that the rate of detection of *L. acidophilus* in vaginal birth (Group I) was significantly higher than in cesarean (Group II) (p:.000), with no significant difference in other types of bacteria (p>0.05) (Table 2). When we investigated the relationship between Lactobacillus species detected in breast milk and the birth weight of babies, no statistically significant correlation was found (p:.0809).

Table 1. *Comparison of demographic characteristics of vaginal and cesarean delivery groups*

	Vaginal Delivery	Cesarean Section	P
Age (Av ± sd)	30.02 ± 3.60	30.97 ± 4.10	0.303
Number of children n(%)			
1 child	9 (25.0)	7 (19.4)	0.571
2 and more children	27 (75.0)	29 (80.6)	
Smoking or alcohol use n (%)			
Yes	5 (13.9)	2 (5.6)	0.233
No	31 (86.1)	34 (84.4)	
Mother's Working Status n (%)			
Housewife	36 (100.0)	34 (84.4)	0.151
Working	0 (0.0)	2 (5.6)	
Family Type n (%)			
Large family	18 (50.0)	9 (25.0)	0.028
Core family	18 (50.0)	27 (75.0)	
Breastfeeding Training in Pregnancy n (%)			
Yes	8 (22.2)	8 (22.2)	1.000
No	28 (77.8)	28 (77.8)	
Weight Gain during Pregnancy n (%)			
<10 kg	11 (30.6)	11 (30.6)	1.000
10-15 kg	16 (44.4)	16 (44.4)	
>15 kg	9 (25.0)	9 (25.0)	
The sex of the baby n (%)			
Female	20 (55.6)	19 (52.8)	0.813
Male	16 (44.4)	17 (47.2)	
Baby's Birth Weight n (%)			
Low	2 (5.6)	0 (0.0)	0.068
Normal	31 (86.1)	36 (100.0)	
High	3 (8.3)	0 (0.0)	
Baby's Birth Height n (%)			
Low	5 (13.9)	3 (8.3)	0.753
Normal	29 (80.6)	31 (86.1)	
High	2 (5.6)	3 (8.3)	
Colic Complaint n (%)			
Have	17 (47.2)	23 (63.9)	0.155
No	19 (52.8)	13 (36.1)	

Table 2. *Distribution of Lactobacillus species among different milk types*

	Lactobacillus spp.	*L. casei-group d*	*L. acidophilus*	*L. delbrueckii*	*L. gasseri*	*L. reuteri*	*L. plantarum*	*L. rhamnosus*
Vaginal Delivery								
Colostrum n(%)	8 (88.9)	0 (0.0)	4 (20)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (19.1)
Early Milk n(%)	0 (0.0)	0 (0.0)	12 (60)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (47.6)
Mature Milk n(%)	1(11.1)	0 (0.0)	4 (20)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (33.3)
Cesarean Section								
Colostrum n(%)	10 (62.5)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
Early Milk n(%)	1 (6.3)	0 (0.0)	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (73.3)
Mature Milk n(%)	5 (31.2)	0 (0.0)	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (26.7)
Total n (%)	25 (30.5)	0 (0.0)	21 (25.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	36 (43.9)
p	0.083	1.000	0.000	1.000	1.000	1.000	1.000	0.157

4. DISCUSSION

Breast milk has a protein content that has a special structure and that is easy to digest and protects against infections. Recent studies on breast milk, which is known to be a living and biological mixture, show that microbial content varies between individuals in terms of both species and numbers. There are many different microorganisms in breast milk microbiota (16,17).

Soto et al. (18) examine the lactobacilli population in the breast milk of healthy women and investigate the effects of various factors (antibiotherapy during pregnancy and breastfeeding, country and date of birth, type of birth, or infant age), including 160 healthy women from Germany and Austria. The three most common Lactobacillus species in milk are *L. salivarius* (35%), *L. fermentum* (25%), and *L.gasseri* (21.88%), as well as *L. reuteri* in 11.88% and *L. plantarum* in 10.63%, *L. ramnosus* in 8.13%, and *L. casei* in 4.38% of the milk samples were detected. Pregnancy, breastfeeding period and antibiotherapy have been reported as the main factors affecting the detection rates of lactobacilli.

Regardless of the geographical location and the analysis method used, it has been reported that there are most *Staphylococci* and *Streptococci* in breast milk. The limited type of microorganism, which will be named as the main microbiota of breast milk, constitutes 50% of the entire microbiota, while the remaining 50% is specific to the mother and varies according to environmental conditions (19). Factors such as the mother's health status, the living environment, diet, obesity, immunological status, gestational age, mode of delivery, antibiotic use, and lactation stage are the main factors affecting breast milk microbiota (20-22). Healthy microbiota development in infants is associated with a healthy maternal microbiota (23). In studies on the subject, microorganisms in breast milk have been shown to be effective in the development of the baby's microbiota (24).

Martin et al. (25) compared the *Lactobacillus* groups in five breast milk, vaginal swabs, and feces of babies in order to examine the effect of breast milk and vagina on intestinal colonization of the newborn. As a result of the study, none of the bacterial species detected in the vaginal samples were detected in breast milk samples, while a few species in the feces were detected in breast milk. Murphy et al. (20) compared breast milk with infant feces in the first three months and 10 months in their study. As a result of the study, it was seen that there were common bacterial species in both. The results obtained from the studies can be said that there is a vertical bacterial transmission from mother to baby through breastfeeding, and this affects the development of the baby's intestinal microbiota.

The transition of breast milk microbiota to the baby occurs as a result of extremely complex and developed processes (26). Especially in the first 3-4 months period is extremely important in terms of microbiota development (27). Bacteria that pass through the mother's vaginal, fecal, and skin microbiota are the first microorganisms to colonize in the neonatal intestine (3,28). The microbiota of the baby born with vaginal birth consists of facultative anaerobic bacteria such as *Staphylococcus, Streptococcus*, and *E. coli*, which are first transferred to the baby during passage through the birth canal. These bacteria multiply and form an anaerobic environment after a few days, which allows for the colonization and becoming dominance of *Bacteroides* and *Bifidobacteria* that pass from breast milk to the baby and can only reproduce in an anaerobic environment (27,29). Studies have reported that a variety of bacteria in the intestines of newborns who do not receive breast milk is higher and the amount of *Bifidobacteria* is less (27,30).

The first dietary factor affecting the microbiota is breastfeeding. Because it is accepted that breast milk is one of the most important factors affecting the formation of intestinal microbiota in babies. Breast milk is a symbiotic food that contains prebiotics (breast milk oligosaccharides) and probiotics (*Lactobacillus, Bifidobacterium*) together (31).

Studies have also shown that the milk microbiome can be affected by the mode of delivery (32). Also, maternal health can affect the breast milk microbiome. Bifidobacterium levels in the milk of mothers with problems such as obesity and allergies have been found to be lower than healthy mothers (33). The nutritional status of the mother affects microorganisms and immunomodulatory factors in milk (34).

After the presence of bacteria in breast milk was understood, the question of where these bacteria came from came to mind. In the first studies on this subject, it was suggested that the microorganisms in the breast skin are caused by the passage of the microorganisms into the milk during sucking, while some researchers have suggested that the bacteria in the infant oral mucosa pass into the milk (25). However, the detection of anaerobic bacterial cells or DNA in breast milk, which are mostly related to the intestinal environment and cannot live in aerobic regions, has led to the emergence of different opinions about the origin of milk-related bacteria. Because, despite the oxygen stress of the anaerobic *Bifidobacteria*, it is unlikely to be transferred from the mouth of the baby to the breast skin of the mother (4). Martin et al. (25) found that the bacteria in breast milk were not the bacteria found in the breast skin, but the fecal microbiota of the mother and baby. In this study, the researchers examined the mouth swabs and feces of eight babies who were breastfed, and samples were taken from their mother's milk, breast areola, and skin. They reported that lactic acid bacteria were isolated in all samples as a result of the examination. However, the RAPD (Random Amplified Polymorphic DNA) profile of the lactic acid bacteria found in the breast skin was found to be different from the other samples. As a result, it has been suggested that the presence of lactic acid in breast milk may not be due to the contamination of the milk with the breast skin environment and may have an endogenous origin. In the mechanism known as the Enteromammary Pathway, dendritic cells are advanced to have important functions. According to this view, dendritic cells can spread directly from the lumen to the intestinal epithelium to take a sample of bacteria. In addition, it can open tight junction points between the intestinal epithelium cells, extend beyond the epithelium through dendrites and directly sample bacteria without disrupting the epithelium cluster. After the bacteria bind to dendritic cells, they can travel to other regions including the milk glands via the monocyte circulation in the lymphocyte system associated with the mucosa (19). According to this knowledge, we formed the hypothesis that states mode of delivery can affect the bacteria species in breast milk because cesarean delivery may result in a late-onset of breastfeeding in the first hour of life and a reduction in the maintenance of breastfeeding in the first year of life (35). Failure to intake bacteria such as *Lactobacilli* and *Bifidobacteria* in breast milk, which are physiological stimulators of the physiological intestinal microbiota of babies born by cesarean section, may lead to a lower colonization level in the baby's intestinal flora (36, 37).

As limitations of our study, although we instructed the participants to collect milk samples under aseptic conditions, we would like to point out that we could not evaluate the contamination of the areola mammae and question the use of prebiotics, which is another factor that may affect the microbiota, and can be acquired from a very large group of nutrients.

5. CONCLUSION

As a result, the results of this study confirm that *Lactobacilli* are common members of the human milk microbiota in women who did not take antibiotics during pregnancy or breastfeeding. Therefore, the presence of such bacteria may be a marker of a healthy human milk microbiota without antibiotics, and this should be taken into account when defining a criteria standard for breast milk. Consequently, the administration of selected human milk lactobacilli to pregnant or breastfeeding women or their babies on antibiotics could create an attractive approach to restore the natural bacterial ecosystem found in breast milk. We think that long-lasting comprehensive studies should be conducted on the vital importance of the existing species, the development of the baby, and its various positive and negative effects.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Clinical and Experimental Health Sciences

3rd and 4th Degree Perineal Tears that Occurs During Vaginal Delivery

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ABSTRACT

Objective: Perineal injuries are common in vaginal delivery. In this study, we aimed to investigate the factors affecting the degree of perineal injury and the effect of injury degree on incontinence.

Methods: Fifteen patients, underwent sphincter repair by the general surgery unit, who had perineal tear during normal vaginal delivery between January 2018 and March 2019 in our hospital and were retrospectively evaluated. Those with grade 3a and 3b perineal tears were divided into 2 groups as group-1, and those with grade 3c and grade 4 perineal tears as group-2. Episiotomy type, fetal characteristics [head circumference and birth weight], early postoperative continence findings were compared in between groups.

Results: The average age of the patients was 30 ± 8.7 years. When the groups were compared, there was no significant superiority of episiotomy in terms of perineal injury (p=0.07). 4 patients had 3a, 3 patients had 3b, 6 patients had 3c and 2 patients had fourth-degree perineal injuries. The average birth weight of the newborns was 3438 ± 492 g, and the head circumference was 34.33 ± 1.23 cm. There was no significant difference in incontinence between the groups (p=0.55).

Conclusion: The treatment of anorectal injuries is surgery. The method of treatment varies according to the time elapsed between injury and intervention, fecal contamination, degree of injury, general condition of the patient, presence of accompanying injury, experience and preference of surgeon. We think that sphincter damage during delivery can be looked after successfully with early diagnosis and intervention before tissue edema develops.

Keywords: Perineal Tears, Fecal incontinence, Sugical treatment

1. INTRODUCTION

Perineal injury is one of the major complications during vaginal delivery. Perineal injury can develop during urological, gynecological and coloproctological interventions. [1,2]. Classification of perineal injury is used for grading perineal injury. Obstetric anal sphincter injury, including third and fourth degree perineal tears, occurs in approximately 3% of women after their first birth and 0.8% of women who have had at least one previous birth [3]. It has also been reported that the true incidence can be as high as 11% in some series [4]. There are publications in England and Norway showing that the incidence has increased in recent years [5,6]. It is unclear whether these changes are due to an actual uplift or greater awareness.

A perineal tear during delivery is an important cause of short and long term maternal morbidity. Urinary incontinence, anal incontinence, perineal pain, sexual dysfunction may cause many problems after perineal injury. Anal incontinence has been reported by 4.3% of women aged 15 to 60 years with or without injury to the perineum. [7]. Intestinal symptoms in women with perineal injuries vary between 7.6% and 61% depending on parity and injury type. [8]. Risk factors for perineal injuries involving anal sphincter include gestational age, nulliparity, duration of delivery, occipitoparietal diameter and birth weight. Episiotomy is controversial some studies have shown that the risk is reduced with a mediolateral incision, while in others it is increased or unchanged [9]. In this study, we aimed to investigate the factors affecting the degree of perineal injury and the effect of injury degree on incontinence.

2. METHODS

We retrospectively evaluated patients who have perineal injuries during normal vaginal delivery and underwent sphincter repair by general surgery unit in our hospital between January 2018 and March 2019. The study was conducted according to the Declaration of Helsinki. The patients identified

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for this series were retrieved from the surgical department records. Ethics committee approval was received for this study (E2-22-1623). During this period, the total number of 35.614 deliveries takes place in our hospital, including 23.816 spontaneous vaginal delivery and 11.798 cesarean delivery. Patients with first degree and second-degree perineal injury were not included in the study. Only perineal injury of 3rd degree and above was included [Table-1] [10]. The perineal injury classification was used to determine treatment and the degree of rectal injury. The degree of injury was determined by intraoperative physical examination.

Table 1. *Classification of perineal injury*

Grade 1	Laceration of the vaginal mucosa or perineal skin only
Grade 2	Laceration involving the perineal muscles
Grade 3	Laceration involving the anal sphincter muscles, being further subdivided into 3A, 3B, 3C:
3A	Where <50% of the external anal sphincter is torn
3B	Where >50% of the external anal sphincter is torn
3C	Where the external and internal anal sphincters are torn
Grade 4	Laceration extending through the anal epithelium (resulting with a communication of the vagina epithelium and anal epithelium)

Those with grade 3a and 3b perineal tears were divided into 2 groups as group-1, and those with grade 3c and grade 4 perineal tears as group-2. Age, parity, episiotomy type, fetal characteristics [head circumference and birth weight], anesthesia type, first intervention time, instrumentation during delivery, perineal injury classification, surgical intervention, and early postoperative continence findings were compared in between groups. Data of 18 patients were collected. Fifteen patients with complete data were included. Written consent was obtained from all patients for surgical procedures.

2.1. Surgical Technique

End-to-end repair was performed in all our cases. The ends of the torn muscle were grasped with Allis forceps and end-to-end repair was performed with 3/0 or 2/0 polyglactin sutures. Because overlap can be technically difficult, a torn internal sphincter mattress or single interrupted sutures should be approximated.

In addition to preoperative antibiotic prophylaxis, all patients were administered with post-operative broad-spectrum antibiotics. Medical constipation was achieved with the drug containing diphenoxylate + atropine sulfate active ingredients, preventing the wound from contacting with stool. Medical constipation was achieved for three days postoperatively. Oral food intake was started on the third postoperative day. Perineal examination and digital rectal examination was done at the postoperative follow-up and regular policlinic examination. They were advised to avoid vaginal delivery. In all patients, treatment with laxatives was started to promote bowel.

We used the Cleveland Clinic Florida Fecal Incontinence Score (CCFIS) system [Table-2] [11]. Scoring was done according to the follow-up data in the system. Patients whose follow-up did not come were called for control. There was no anorectal manometry in our hospital; Therefore, patients were referred to comprehensive centers.

Table 2. *Cleveland Clinic Florida Fecal Incontinence Score (CCFIS)*

	Frequency				
	Never	Rarely	Sometimes	Usually	Always
Solid stool leakege	0	1	2	3	4
Liquid stool leakege	0	1	2	3	4
Gas leakege	0	1	2	3	4
Pad use (for stool)	0	1	2	3	4
Lifestyle restriction	0	1	2	3	4

Never = 0; Rarely = <1/month; Sometimes = <1/week but >1/month; Usually = <1/day but >1/week; Always = >1/day.

2.2. Statistical Analysis

Social Science Statistical Package 22.0 (SPSS Inc., Chicago, IL, USA) software was used for biostatistical analysis. Kolmogorov-Simirmov and Shapiro-Wilk tests were used to check the distribution of parameters. Mann Whitney U test was used for comparison of independent groups and Fisher's exact chi-square tests in cross tables were used for categorical data. In the interpretation of the statistical hypothesis tests, a type 1 error was accepted as 0.05.

3. RESULTS

The average age of the patients was 30 ± 8.7 years. The mean number of delivery was 3 (min-max: 1-7). 14 patients underwent spinal anesthesia and 1 received general anesthesia during normal vaginal delivery and underwent surgery due to third and fourth-grade perineal tears. The mean duration of the first intervention was 70 minutes (min-max: 30-90 minutes). Episiotomy was performed in 11 patients (73.3%). When the groups were compared, there was no significant superiority of episiotomy in terms of perineal injury (p = 0.07). 4 patients had 3a, 3 patients had 3b, 6 patients had 3c and 2 patients had fourth-degree perineal injuries [Figure 1]. In 3 of the cases, birth weight was over 4000 g. The average birth weight of the newborns was 3438 ± 492 g, and the head circumference was 34.33 ± 1.23 cm . On the postoperative first day, rectal examination of the patients it was showed that sphincter tone decreased. On the 7th day, rectal examination showed that sphincter tones were more active than the first day examination but decreased compared to normal. Colostomy was not performed in any patient. 2 patients (13.4%) had less than one gas incontinence per month, 1 patient (6.7%) had less than one gas and stool incontinence per month. There was no significant difference in incontinence between the groups (p=0.50) [Table-3]. The average follow-up period was 9.8 ± 4 months. None of the patients developed surgical complications.

Table 3. *Properties of the groups and comparison results*

		Group-1 (Grade 3a-3b)	Group-2 (Grade 3c-4)	P
Incontinens	Yes	1 (14.2%)	2(25%)	**0.55**
	No	6(85.8%)	6(75%)	
Episiotomy	Done	7(100%)	4(50 %)	**0.07**
	No	0(0%)	4(50%)	
Head circumference(cm) (mean±SD)		34.1 ± 1.4	34.5 ± 1	**0.69**
Birth weight (g) (mean±SD)		3335 ± 424	3527 ± 558	**0.53**

SD: Standart Deviation

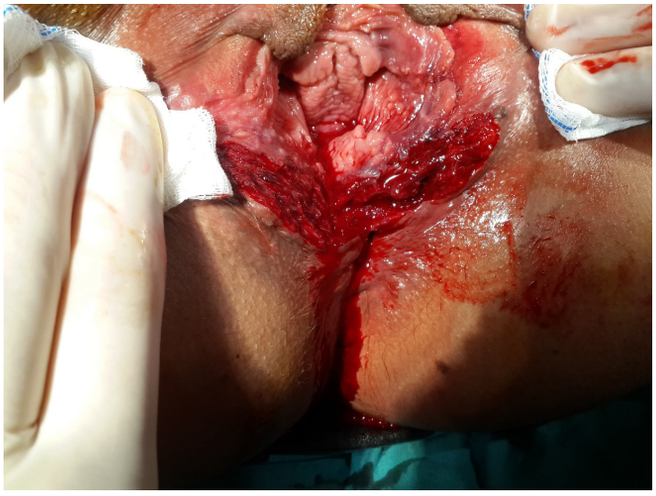

Figure 1. *A patient with type 3b injury*

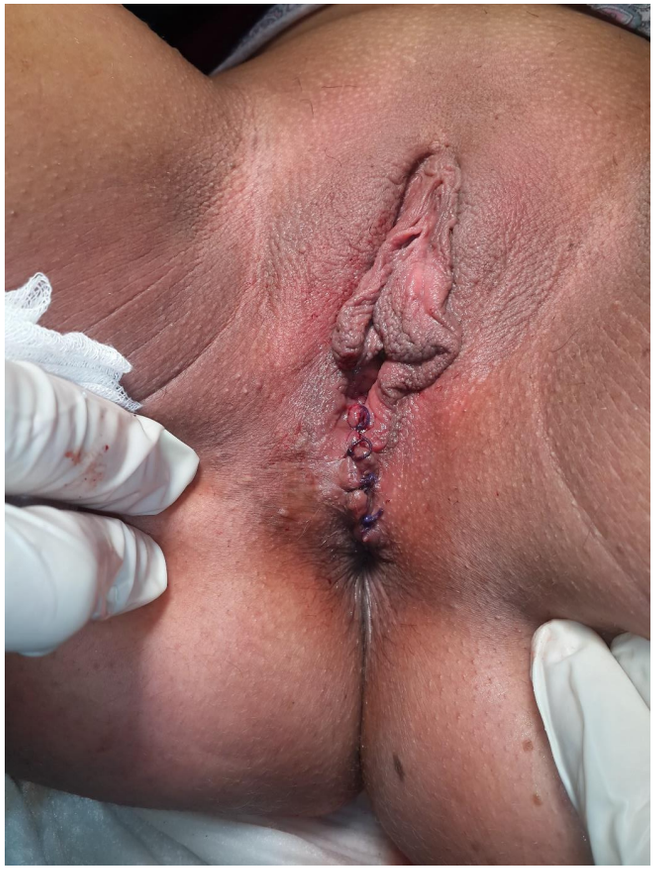

Figure 2. *Post-repair image*

Table 4. *Demographic characteristics of patients (n=15)*

	n	%	Mean±SD
Age (Years)			30±8.7
Number of delivery			3(1-7)
Anesthesia type			
General	1		
Spinal	14		
Time to first intervention (Minute)			70±24
Grade of injury			
Grade 3A	5	33.3%	
Grade 3B	3	20%	
Grade 3C	5	33.3%	
Grade 4	2	13.4%	
Episiotomy			
Done	11	73.3%	
None	4	26.7%	
Birth weight of the newborns (gr)			3438±492
Head circumference (cm)			34.33 ± 1.23
İncontinens			
Gas	2	13.4%	
Gas+stool	1	6.7%	
None	12	80%	
Average follow-up (month)			9.8 ± 4

4. DISCUSSION

The morbidity rate is high in perineal tears during delivery. Fecal incontinence is one of the most important of these. Obstetric trauma or sphincter defects after perianal field surgery are the main causes of stool incontinence. During vaginal delivery, 53% to 79% of patients have varying degrees of perineal injury. [8,12]. Rectal examination should be performed in vaginal deliveries so that perineal tears are not overlooked. Maternal or fetal features may cause perineal injuries. Fetal factors include abnormal presentation, birth weight, and head circumference, while maternal factors include rapid presentation, pelvic length, and tissue characteristics. [13]. A large volume study reported an association between shoulder dystocia and perineal injury [8]. Every 100 grams increase in birth weight caused a 10% increase in the rate of perineal injury. There were 3 children with shoulder dystocia. Their weight was over 4000 grams. One of the patients had a fetus over 4000 g, the other had a fetus transverse position and althought the last one had a low fetus weight, a perineum injury occurred for the pelvic incompatibility groove [Table-4].

The rate of anal sphincter injury in vaginal deliveries is approximately 18% [14]. In patients with sphincter injury at birth, the rate of fecal incontinence is 7.7% [15]. The incontinence rate is 6.3% in nulliparous women, 8.8% in

uniparous women and 8.4% in secundiparous women and 11.5% in triparous women and women with more than 3 deliveries [16]. In a study of patients with normal vaginal delivery, it was reported that complaints about incontinence ranged from 13% to 25% [17]. Two patients (13.4%) had less than 1 gas incontinence per month, 1 patient (6.7%) had less than 1 gas and stool incontinence per month. 3 patients (20%) were nulliparous and 12 patients (80%) were multipara.

It is reported in the literature that early intervention is a key factor in recovery [13]. The mean duration of the first intervention was 70 minutes (min-max: 30-90 minutes).

There are publications stating that episiotomy increases or decreases the risk of perineal injury [8]. In our study, 73.3% of our patients underwent episiotomy. When the groups were compared, there was no significant superiority of episiotomy in terms of perineal injury ($p = 0.07$). We found that episiotomy did not affect the risk of 3rd or 4th degree perineal injury. The low number of patients is also a factor in this result.

There is insufficient data to determine whether the next delivery of a patient with perineal injury after vaginal delivery is vaginal or caesarean section delivery. A vaginal delivery performed in a patient with perineal injury has been reported to have a 3% risk of re-anal sphincter injury [8]. We do not recommend vaginal delivery to prevent possible injury.

The first option in anal sphincter defect is sphincter repair. Perioperative second-generation cephalosporin antibiotic prophylaxis has been shown to reduce the rate of postoperative infection [19]. In sphincter repair performed after vaginal delivery injury, the infection rate can reach up to 20% [20]. None of our patients had surgical site infections. We use metronidazole and third generation cephalosporin for prophylaxis in our patients and we complete this treatment for 5 days. We think that the susceptibility to infection increases in the surgical area if vaginal discharge is high after delivery. For this reason, we pay attention to vaginal dryness.

All layers must be repaired separately. The internal and external sphincter should be repaired separately. The posterior anal canal mucosa and the anterior vaginal wall should be repaired separately. In the literature, there was no difference between overlapping and end-to – end suturing as a repair technique [21]. We performed end-to-end repairs in all our cases [Figure 2].

Although there is no significant difference between pelvic floor muscle training and biofeedback treatment, studies are suggesting early postnatal biofeedback treatment in symptomatic women with perineal injuries [18]. During follow-up up to 1 year, one of our cases had stool and two had gas incontinence. We have directed our patients with gas or stool incontinence to the centers where biofeedback treatment is applied. However, because of the low socioeconomic and sociocultural levels of our patients, none of our patients received this treatment. All of our results consisted only of patients who had performed pelvic floor exercises at home. This is one of the limitations of our study.

5. CONCLUSION

Sphincter repair should be done in experienced centers to obtain better results. Surgical repairs should be made in the early stages of injuries. It is known that the sphincter ends torn in the late intervention will separate more with time and repair in the late period may be more difficult. We think that sphincter damage during delivery can be looked after successfully with early diagnosis and intervention before tissue edema develops.

Authors' contributions *(Authors initials)*

Design of the study: MS

Acquisition of data for the study: YY

Analysis of data for the study: MS, YY

Interpretation of data for the study:

Drafting the manuscript: MS

Revising it critically for important intellectual content: MS, YY

Conflicts of interest

All authors have disclosed no conflicts of interest.

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Clinical and Experimental Health Sciences

Factors Affecting Perioperative Patient Satisfaction with Regional Anesthesia: A Patient-Centered Survey Study

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ABSTRACT

Objective: To determine the demographic and clinical characteristics that affect patient satisfaction with regional anesthesia.

Methods: This study was conducted at Kahramanmaras Sutcu Imam University Hospital between June-July 2019. The patients were included on a voluntary basis and all had undergone obstetrics, urology, orthopedics or general surgery, and met the following inclusion criteria: (1) age >18 years, (2) received regional anesthesia, (3) ASA-PS score of ≤ 3, and (4) no cognitive problem that would prevent self-expression. A Personal Information Form and the Evaluation of the Experience of Regional Anesthesia Questionnaire were applied to 402 patients at 48 hours after surgery performed under regional anesthesia in a university hospital in Turkey.

Results: The EVAN-LR total scores were 71.2±15.6 in obstetrics patients, followed by 54.9±24.9 in orthopedic patients, 26.6±24.4 in urology patients and 15.9±7.2 in general surgery patients ($p<0.001$). In the comparisons of the subscale points of the EVAN-LR points of attention (58.2±34.5), information (57.6±31.8), discomfort (41.1±31.8), waiting (45.4±36.4) and pain (36.5±32.7), the lowest mean scores of EVAN-LR were seen to be in the subscale of pain. The total mean scores of males were determined to be higher than those of females ($p<0.05$). The EVAN-LR total scores of the patients administered with premedication were statistically significantly higher than those of the patients who did not receive premedication ($p<0.001$). According to the multiple linear regression model, the best predictive variables for patient satisfaction with regional anesthesia were gender, history of anesthesia, and premedication.

Conclusions: The results of this study showed that the satisfaction with the regional anesthesia service of the participants was at a moderate level. This indicates the need to educate the anesthesia team to increase the patient satisfaction with regional anesthesia, especially in respect of postoperative pain management.

Keywords: Patient-centred surgery, patient satisfaction, perioperative satisfaction, regional anesthesia

1. INTRODUCTION

Traditionally, patient satisfaction, which is a complex and multi-dimensional concept, has been defined as the relationship between the patient's expectations and the perceived success of the treatment (1,2). The American Society of Anesthesiologists (ASA) recommend that it is important to evaluate patient satisfaction when evaluating clinical care quality and postoperative success, and the evaluation should be made with various measurement tools (2-5).

One of the functions of anesthetists is to determine the best anesthesia method for the patient, but the application of the method perceived to be best by the physician may not always provide satisfactory results from the patient's perspective (4). If it is considered that regional anesthesia increases patient comfort by providing the opportunity for operation with a conscious sedation strategy, it is necessary to make a detailed investigation of patient satisfaction with regional anesthesia (6). The success of the regional anesthesia technique affects patient satisfaction (2).

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The satisfaction of patients administered with regional anesthesia has been reported to be affected by several variables, such as previous experience of anesthesia, the technique used, the duration of the procedure, and communication (4-7,9,10). The technique used and the success of the regional anesthesia performance are known to affect patient satisfaction (2). It has been reported that when a block is applied and sedation is sufficient in the intraoperative period, patient anxiety is reduced and acceptance of the regional anesthesia is increased (8).

As patients are anxious and tense in the preoperative period in particular, the surgical team need to provide emotional support in this period (4,11). Mui et al. (12) reported that patients needed more emotional support and communication when regional anesthesia is applied. Respect for privacy of the patient has also been reported to be an important element in perioperative patient satisfaction (13). In busy surgical units, time pressure may present a challenge to the anesthesia team in respect of preserving and not violating the dignity of the patient during clinical applications. Accordingly, safeguarding patient dignity should be a paramount concern for all healthcare professionals. If the dignity and privacy of the patient are ignored in the application of regional anesthesia, he/she can feel weak and defenceless (11,13). Specifically informing the patient about potential postoperative complications such as surgical site infection, bleeding, paresthesia, back pain, headache and giving care recommendations not only increase patient satisfaction but are an important responsibility of the surgery team and other healthcare professionals (14,15).

The aim of this study was to determine the demographic and clinical characteristics that affect the patient satisfaction with regional anesthesia.

2. METHODS

2.1. Sample / Setting

This study was designed as a single-center, prospective, cross-sectional survey study. The study was conducted on 402 patients who were administered with regional anesthesia in a large public University Training and Research Hospital between July-September 2019. Using G*Power 3.19.4, the planned sample size was calculated as n=435 patients (0.85 power level, 0.12 effect size and 0.05 type I error). Therefore, it was decided to include 110 patients for each surgical branch evaluated. Due to incomplete data, 33 questionnaires had to be excluded, so the data of 402 surgery patients were analyzed. Quota sampling was used, which is defined as a non-probability sampling method in which the researchers create a sample including individuals that represent a population. The patients were selected according to the inclusion criteria and four surgical procedures in which regional anesthesia is most used. A self-report survey-based data collection procedure was applied in the patient's hospital room two days after surgery using the paper-and-pencil method face-to-face. The patients were included on a voluntary basis and had undergone surgery in obstetrics, urology, orthopedics or general surgery, had been applied with spinal anesthesia (for obstetrics, urology, and general surgery) or peripheral nerve block (for orthopedic surgery), were aged >18 years, had an ASA-PS score of ≤ 3, and had no cognitive problem that would prevent self-expression. All the participants in this study were recipients of publicly-supported healthcare. They were insured by the Social Insurance Institution of the Republic of Turkey and they were able to access standard clinical services. Patients were excluded from the study if surgery was performed under general anesthesia, or local infiltration anesthesia was applied for minor surgeries, if additional anesthesia was required for pain control, or if regional block was performed for chronic pain treatment.

2.2. Standard of Regional Anesthesia Care

In our clinic, regional anesthesia is administered to patients by experienced physicians specialized in anesthesiology. An experienced team (including anesthesia nurses, anesthesia residents) in regional anesthesia is involved in pre – and post-anesthesia care. In our hospital, there is adherence to the Basic Standards for Pre-anesthesia Care guidelines, which were developed by the American Society Anaesthesiologists for the administration of regional anaesthesia (https://www.ra-uk.org/index.php/guidelines-standards).

To improve a patient's overall perioperative experience, anesthesiology teams frequently administer preoperative anxiolytic medications to calm patients before they enter the operating room (16). However, it is not known how well the anxiety is treated by these medications or how they influence the overall perioperative experience (6,16). In the present study, the premedication selected according to the patient's preoperative assessment was applied for central antiemetic effect, sedation, anxiolysis and H2 receptor antagonism. Drug selection in premedication was made according to the patient's other concomitant diseases and medical conditions. With the exception of pregnant women, all the patients in the current study received diazepam to cope with surgery anxiety. Frequently, 10 mg Benzodiazepine premedication IM is administered half an hour before surgery to reduce anxiety in our clinic, but this is known to possibly cause amnesia, drowsiness, and cognitive impairment, which may be deleterious to some surgical patients (17). Therefore, the premedication was administered according to the patient's clinical condition and features. Apart from in an obstetric anesthesia setting, patients in Turkey typically receive Midazolam sedation, when undergoing regional anesthesia.

2.3. Instrumenets

Personal informational form

With the benefit of the literature, the researchers prepared an 11-item Personal Information Form to determine age, gender, the branch performing the operation, anesthesia history, and the development of anesthesia-related complications.

Evaluation of the Experience of Regional Anesthesia (EVAN-LR)

The 19-item "Evaluation of the Experience of Regional Anesthesia" (EVAN-LR) (Evaluation du Vécu de l'Anesthésie LocoRégionale) was applied. The EVAN-LR was administered up to 48 hours after surgery, as recommended by Maurice-Szamburski et al. (6) By restricting the questionnaire period to 48 hours, it was intended to weigh perceptions related to anesthesia over perceptions related to surgery, but with a risk of recall bias. Data were collected from patients in face-to-face interviews. The EVAN-LR scale has been proven to be a valid and reliable tool for the measurement of perioperative patient satisfaction with regional anesthesia in the Turkish population. The Cronbach alpha reliability coefficient was calculated as 0.95. A five-factor structure was confirmed, comprising the subscales of attention, information, discomfort, waiting, and pain (17).

The EVAN-LR consists of 19 items related to different procedures in the perioperative period. Negative items (numbers 7, 8, 9, 10, 11, 12, 13, 14, 18, 19) are reverse scored. The subscales of the scale are attention (items 6, 15, 16, 17), information (items 1, 2, 3, 4, 5), discomfort (items 7, 8, 9, 12), waiting (items 18, 19), and pain (items 10, 11, 13, 14) (for items see Appendix 1) (6). The subscale scores are totaled to give a raw score, then this is calculated as a standard score ranging from 0-100 (standard score = [raw score – minimum score] x 100 / possible score range). The mean standard scores obtained from the subscales are accepted as the total score. A higher total score and subscale scores indicate a higher level of patient satisfaction (6,18,19). There is no cutoff point for the scores.

The attention sub-dimension evaluates patient perception in respect of nursing and medical staff in the operating room, recovery room and patient's room. The information sub-dimension evaluates the information given by the anesthesiologist and surgeon about the operation. The discomfort sub-dimension evaluates unpleasant feelings such as thirst, hunger, nausea, or headache during and after the surgery. The waiting sub-dimension evaluates waiting times to obtain an appointment with the anesthetist or surgeon and waiting during the preoperative visits. The pain sub-dimension evaluates the patient's perception of pain after the surgery (17).

Clinical Forms

Intraoperative Anesthesia Assessment Form: This form is used by the anesthesia provider and is an integral part of the everyday anaesthesia practice in our clinic. It is used to document the preoperative anaesthetic assessment, the actions and interventions of the anaesthetist, the patient's vital signs throughout surgery, and important events or complications and provides information about the anaesthesia record. It contains the following sections about peri-anesthesia (time-based record of events): (A) Immediate review prior to initiation of anesthetic procedures: (1) Patient re-evaluation. (2) Check of equipment, drugs and gas supply. (B) Monitoring the patient, which states that during all anesthesia the patient's oxygenation, ventilation, circulation and temperature shall be continually evaluated. (C) Amounts of all drugs and agents used, and times given. (D) The type and amounts of all intravenous fluids used including blood and blood products, and times given. (E) The technique(s) used. (F) Unusual events during the anesthesia period. (G) The status of the patient at the conclusion of the anesthesia.

Perioperative Nursing Assessment Form; contains (A) Patient interview to review medical, anesthesia and medication history. (B) Appropriate physical examination. (C) Review of objective data (e.g., laboratory, electrocardiogram, x-ray). (D) Assignment of the American Society of Anaesthesiologists (ASA) physical status. (E) Formulation of an anesthesia plan with the patient and/or responsible adult. In this study, respiratory function including respiratory rate, airway patency, and oxygen saturation, cardiovascular function including pulse rate and blood pressure, mental status, temperature, pain, spinal cord damage, headache, infective complications such as epidural abscess, and postoperative hydration, nausea and vomiting were recorded with the Perioperative Nursing Assessment Form.

Postanesthesia Assessment Form; is completed and documented by a physician qualified to administer anesthesia no later than 48 hours after surgery or a procedure requiring anesthesia services. The form contains information about respiratory function including respiratory rate, airway patency, and oxygen saturation; cardiovascular function including pulse rate and blood pressure; mental status; temperature; pain; nausea and vomiting; and postoperative hydration. This form was used to follow up postoperative anesthesia complications such as cardiovascular function, respiratory function, mental status, pain, nausea and vomiting.

2.4. Statistical Analyses

The data were analyzed with SPSS v 22 software (IBM Corp., 2013). To determine the perioperative satisfaction level according to the demographic and clinical characteristics of the patients, conformity of the data to normal distribution was assessed with the Kolmogorov-Smirnov test. As the scale scores were not normally distributed, the Mann Whitney U-test and Kruskal-Wallis variance analysis (post-hoc Siegel-Castellan test) were used in the comparison of the scale scores of various groups. To evaluate the correlations between age and the scale scores and subscale scores, Spearman's rho correlation analysis was applied. Quantitative data were stated as mean ± standard deviation and median (minimum – maximum) values, and categorical data as number (n) and percentage (%). A value of $p<0.05$ was accepted as statistically significant.

To predict the patients' scores for satisfaction with regional anesthesia, multiple linear regression analysis was used

based on the independent sociodemographic and health-related variables considered. The collinearity between the factors was analyzed to avoid including correlated variables in the model. The model was constructed using backward stepwise regression, finally including the variables that were shown to be significantly associated in the bivariate analysis. Estimates of the model parameters and standard errors for these estimates were calculated. The independent associations of prespecified factors with patient satisfaction were examined with proportional odds multivariable regression analysis. To determine whether patient satisfaction with regional anesthesia was associated with personal and clinical features, the odds ratio (OR) with 95% confidence interval (95% CI) of patient satisfaction was calculated for each variable with univariate statistics (unadjusted OR), followed by multivariate logistic regression using backward variable selection to control for gender, education level, surgical branch, anesthesia history, premedication, intraoperative complications, complications in recovery room, and ASA-PS score (adjusted OR). Parameter estimates were exponentiated to obtain ORs for higher satisfaction scores together with the corresponding 95% CI. Statistical significance was concluded when the 95% CI did not include unity ($p<0.01$).

2.5. Ethical Approval

Approval for the study was granted by the Clinical Research Ethics Committee of Kahramanmaraş Sütçü Imam University (decision no:2019/10). Clinical Trials ID (NCT 04009018) was obtained for the study. Informed consent was obtained from all the study participants, who were assured about the confidentiality, protection, and anonymity of data. The research was conducted in accordance with the ethical criteria of the Helsinki Declaration.

3. RESULTS

3.1. Demographic characteristics of the participants

The study participants comprised 50.4% males and 49.6% females with a mean age of 44.1±18.4 years. Regional anesthesia was applied for an obstetrics operation in 24.1% of cases, urological procedures in 24.3%, an orthopedic operation in 26.4%, and a general surgery operation in 25.2%. Of the total patients, 56.7% had no history of anesthesia, and premedication was administered to 68.6%. No intraoperative complications were observed in 66.1% of the patients, and the most common intraoperative complication was pain in 16.9%. There were no complications in the recovery room in 77.6% of the patients. ASA-PS scores were determined as ASA-PS 1 in 35.5%, ASA-PS 2 in 50.3% and ASA-PS 3 in 14.2% of the patients (Table 1).

Table 1. *Demographic and clinical characteristics of the patients*

Characteristics	Mean±SD		
	Groups	**n**	**%**
Age (years)	18-30	**128**	31.8
	31-44	**94**	23.4
	45-54	**49**	12.2
	55-64	**59**	14.7
	65 and above	**72**	17.9
Gender	Male	203	50.4
	Female	199	49.6
Employment status	Employed	142	35.3
	Unemployed	260	64.7
Level of Education	Did not finish primary school	77	19.1
	Primary school	141	35.0
	High school	122	30.4
	University and above	62	15.5
Surgical Branch	Obstetrics	97	24.1
	Urology	98	24.3
	Orthopedics	106	26.4
	General Surgery	101	25.2
Anesthesia History	Yes	174	43.2
	No	228	56.8
Premedication	Yes	276	68.6
	No	126	31.4
Intraop Complications	None	266	66.2
	Hypotension	45	11.1
	Pain	68	17.0
	Nausea and Vomiting	16	3.9
	Dyspnea	7	1.8
Recovery Room Complications	None	312	77.6
	Hypotension	9	2.3
	Pain	39	9.7
	Nausea and Vomiting	38	9.5
	Shaking	4	0.9
ASA-PS score	1	143	35.6
	2	202	50.2
	3	57	14.2

Intraop: Intaraoperative; ASA-PS: The American Society of Anesthesiologists Physical Status Classification Score

3.2. The level of perioperative patient satisfaction with regional anesthesia

The level of perioperative patient satisfaction with regional anesthesia was evaluated in this study. In addition, the item total scores of the subscales of attention, information, discomfort, waiting and pain of the EVAN-LR scale were evaluated according to demographic and clinical characteristics. The total perioperative EVAN-LR mean score was determined to be 47.7±28.6. When the item total scores of the EVAN-LR were examined according to education level, the lowest level of satisfaction was determined in those with a high school level of education ($p<0.001$). The perioperative satisfaction level of males administered with regional anesthetic (57.1±25.5) was determined to be statistically significantly higher than that of females (38.2±28.5)

(p<0.001). The EVAN-LR item total scores were 71.2±15.6 in obstetrics patients, followed by 54.9±24.9 in orthopedic patients, 26.6±24.4 in urology patients, and 15.9±7.2 in general surgery patients (p<0.001). The EVAN-LR item total scores of the patients administered with premedication (73.4±13.9) were statistically significantly higher than those of the patients who did not receive premedication (30.0±25.8) (p<0.001).

When the development of complications in the intraoperative period was examined via the Intraoperative Anesthesia Assessment Form, the EVAN-LR item total scores were determined as 46.2±29.9 in patients who did not develop any complications, 34.3±22.6 in those who developed hypotension, 60.0±23.8 in those who experienced pain, 57.5±21.8 in those with nausea and vomiting, and 50.7±25.3 in those who developed dyspnea (p<0.01). In the evaluation of the satisfaction levels of the patients according to ASA-PS scores, the highest levels were seen in patients with ASA-PS 3 (53.3±27.0) (p<0.05). When perioperative patient satisfaction with regional anesthesia was evaluated according to the development of complications in the recovery room, the highest satisfaction levels were determined in patients who experienced pain (p<0.001) (Table 3).

Multiple linear regression analysis was performed in the study to determine which of the independent variables affect the dependent variable and to determine the value of the dependent variable using the data affecting the dependent variable. According to the multiple linear regression model, the best predictive variables for patient satisfaction with regional anesthesia were male gender, having a history of anesthesia, and not receiving premedication (Table 4). It was seen that the independent variables in the model explained 41.7% of the dependent variable (patient satisfaction).

3.3. The total mean scores of sub-scales of the EVAN-LR

The mean scores were 58.2±34.5 for attention, 57.6±31.8 for information, 41.1±31.8 for discomfort, 45.4±36.4 for waiting, and 36.5±32.7 for pain (Table 2). In the subscale total scores, the mean scores for attention were higher for employed patients compared to unemployed patients (59.2±34.9 vs. 57.6±34.4), the information subscale scores were the same, and the scores for the subscales of discomfort, waiting and pain were lower (Table 3).

Table 2. *Total scores of EVAN-LR and subscales*

Scale (Number of Items)	Mean ± SD	Median
EVAN-LR (19)	47.7±28.6	49.3
Subscales (Number of Items)	**Mean ± SD**	**Median**
Attention (4)	58.2±34.5	62.4
Information (5)	57.6±31.8	68.6
Discomfort (4)	41.1±31.8	55.0
Waiting (2)	45.4±36.4	58.4
Pain (4)	36.5±32.7	45.6

The scores of the subscales were seen to be higher for males than for females (p<0.001). The lowest level of patient satisfaction with regional anesthesia in all the subscales was determined in the general surgery patients (p<0.001). The satisfaction level of patients with a history of anesthesia (53.3±27.2) was found to be higher than that of patients with no anesthesia history (p<0.001). A statistically significant difference was determined between those with and without a history of anesthesia in respect of attention, information and pain subscales (p<0.05) (Tables 3).

There was a similar 40-point difference between those who received or did not receive premedication in the subscale scores of attention, information, discomfort, waiting and pain, and the differences were statistically significant (p<0.001) (Table 3). When the mean subscale scores of the EVAN-LR were examined according to the development of intraoperative complications, the highest levels of patient satisfaction with regional anesthesia were seen to be in the subscales of attention, discomfort, waiting and pain in those who developed intraoperative pain, and the highest satisfaction levels were seen in the subscale of information in those who experienced nausea and vomiting (Tables 3). When the subscales of the EVAN-LR were examined, the highest satisfaction level in the subscale of attention was determined in ASA-PS 1 patients (59.9±31.2), and the highest satisfaction levels in the subscales of information, discomfort, waiting and pain were seen in ASA-PS 3 patients (Table 3).

4. DISCUSSION

In today's highly competitive medical environment, traditional health care involves one or more elements (e.g. quality of health care, health care system costs, price, convenience, new technology and innovation and superior products or services). Patient satisfaction has become the core element in the competitiveness of medical and health institutions (19, 20). Evaluating patient satisfaction for the entire surgical process (nurses, anesthesiologist, surgeon) from the perioperative period to the postoperative period makes the EVAN-LR an important measurement tool (18). The scale consists of information, attention, waiting, discomfort and pain sub-dimensions that are determined as a guide for evaluating intraoperative patient satisfaction (6). The results of the current study showed a moderate level of perioperative patient satisfaction with regional anesthesia (47.7±28.6). The mean perioperative total EVAN-LR score was found to be 84.6±9.9 by Courtot et al. (19), and 78.83±15.61 by Maurice-Szamburski et al. (6). The lower level of patient satisfaction with regional anesthesia in the current study may be a result of the current research having been conducted in the region's largest university hospital. Since the number of daily operations (approximately150) and the number of patients per physician is high, there is less time to take care of the patients. Patient satisfaction rates improve as visit length increases (21). This finding is important to increase our awareness of our weaknesses regarding patient satisfaction with regional anesthesia and to review our practice.

Table 3. *The total points of the EVAN-LR subscales of attention, information, and discomfort, according to the demographic and clinical characteristics of the patients*

Participants' characteristics (n)	Attention			Information			Discomfort			Waiting			Pain			EVAN-LR		
	M ± SD	z/KW (p)	*Median (Min–Max)*	*M* ± SD	z/KW (p)	*Median (Min–Max)*	*M* ± SD	z/KW (p)	*Median (Min–Max)*	*M* ± SD	z/KW (p)	*Median (Min–Max)*	*M* ± SD	z/KW (p)	*Median (Min–Max)*	*M* ± SD	z/KW (p)	*Median (Min–Max)*
Gender																		
Female (203)	46.9±34.5	4.76	37.5 (0.0–100.0)	48.0±31.4	5.93	45.0 (0.0–100.0)	31.5±32.3	6.66	25.0 (0.0–100.0)	36.8±35.6	4.76	50.0 (0.0–100.0)	27.6±32.2	5.94	50.0 (0.0–100.0)	38.2±28.5	6.69	59.8 (0.0–100.0)
Male (199)	69.2±30.9	**<0.001**	81.3 (0.0–100.0)	66.9±29.3	**<0.001**	75.0 (0.0–100.0)	50.5±28.4	**<0.001**	50.0 (0.0–100.0)	53.8±35.3	**<0.001**	25.0 (0.0–100.0)	45.3±30.8	**<0.001**	12.5 (0.0–100.0)	57.1±25.5	**<0.001**	27.5 (0.0–100.0)
Employment Status																		
Employed (142)	59.2±34.9	2.67	62.5 (0.0–100.0)	57.9±32.8	0.21	60.0 (0.0–100.0)	35.9±32.9	2.78	25.0 (0.0–100.0)	39.5±38.5	2.67	25.0 (0.0–100.0)	33.9±33.0	1.19	25.0 (0.0–100.0)	45.3±29.4	1.41	42.6 (0.0–100.0)
Unemployed (260)	57.6±34.4	**0.008**	62.5 (0.0–100.0)	57.4±31.2	0.837	55.0 (0.0–100.0)	43.9±30.0	**0.005**	37.5 (0.0–100.0)	48.6±34.9	**0.008**	50.0 (0.0–100.0)	38.0±32.5	0.233	37.5 (0.0–100.0)	49.1±28.2	(0.159)	50.5 (0.0–100.0)
Level of Education																		
Literate (77)	64.2±31.6	11.24	68.8 (0.0–100.0)[a]	64.4±26.4	19.35	65.0 (0.0–100.0)[a]	47.2±25.8	33.21	43.8 (0.0–100.0)[a]	46.6±32.1	11.24	50.0 (0.0–100.0)[a,b]	38.9±27.9	30.16	31.3 (0.0–100.0)[a]	52.3±26.3	27.69	50.0 (6.5–100.0)[a]
Primary school (141)	65.6±32.5	**<0.001**	75.0 (0.0–100.0)[a]	63.7±29.0	**<0.001**	65.0 (0.0–100.0)[a]	49.4±31.3	**<0.001**	50.0 (0.0–100.0)[a]	51.8±35.5	**<0.001**	50.0 (0.0–100.0)[a]	46.5±32.7	**<0.001**	50.0 (0.0–100.0)[a]	55.4±29.1	**<0.001**	58.0 (0.0–100.0)[a]
High school (122)	48.3±34.6		31.3 (0.0–100.0)[b]	48.0±32.6		40.0 (0.0–100.0)[b]	30.7±31.1		21.9 (0.0–100.0)[b]	37.3±35.7		25.0 (0.0–100.0)[b]	26.1±31.6		12.5 (0.0–100.0)[b]	38.1±29.1		23.9 (0.0–100.0)[b]
University and above (66)	53.2±37.4		50.0 (0.0–100.0)[a,b]	54.0±37.2		55.0 (0.0–100.0)[a,b]	35.0±34.5		25.0 (0.0–100.0)[b]	45.2±42.3		25.0 (0.0–100.0)[a,b]	31.5±33.7		15.7 (0.0–93.8)[b]	43.8±33.5		27.0 (3.0–98.0)[b]
Surgical Branch																		
Obstetrics (97)	82.5±19.7	71.77	87.5 (18.8–100.0)[a]	78.5±21.8	160.16	80.0 (0.0–100.0)[a]	64.7±23.7	147.32	62.5 (0.0–100.0)[a]	67.5±32.8	71.77	75.0 (0.0–100.0)[a]	62.9±24.0	169.43	62.5 (0.0–100.0)[a]	71.2±15.6	182.85	72.3 (22.3–99.0)[a]
Urology (98)	29.5±28.1	**<0.001**	25.0 (0.0–100.0)[b]	30.4±26.7	**<0.001**	27.5 (0.0–100.0)[b]	24.2±30.9	**<0.001**	12.5 (0.0–100.0)[c]	32.4±31.0	**<0.001**	25.0 (0.0–100.0)[c]	16.7±29.1	**<0.001**	0.0 (0.0–100.0)[c]	26.6±24.4	**<0.001**	20.0 (0.0–93.8)[c]
Orthopedics (106)	70.0±29.2		75.0 (0.0–100.0)[a]	67.7±27.3		75.0 (0.0–100.0)[a]	46.4±28.8		43.8 (0.0–100.0)[b]	48.3±36.6		50.0 (0.0–100.0)[b]	42.4±29.3		37.5 (0.0–100.0)[b]	54.9±24.9		51.5 (0.0–100.0)[b]
General surgery (101)	19.6±13.0		25.0 (0.0–56.3)[b]	27.1±15.1		25.0 (0.0–60.0)[b]	10.1±11.3		6.3 (0.0–43.8)[c]	19.5±22.8		25.0 (0.0–100.0)[c]	3.7±5.3		0.0 (0.0–18.8)[c]	15.9±7.2		16.0 (0.0–33.8)[c]
Anesthesia history																		
Yes (174)	66.7±33.4	0.91	81.25 (0.0–100.0)	64.7±30.0	3.85	75.0 (0.0–100.0)	45.3±30.6	2.56	40.6 (0.0–100.0)	47.9±37.4	0.91	43.8 (0.0–100.0)	42.2±31.3	3.39	37.5 (0.0–100.0)	53.3±27.2	3.38	53.5 (0.0–100.0)
No (228)	51.7±34.1	0.363	50.0 (0.0–100.0)	52.1±32.1	**<0.001**	50.0 (0.0–100.0)	37.9±32.3	**0.010**	31.3 (0.0–100.0)	43.8±35.7	0.363	37.5 (0.0–100.0)	32.2±33.1	**0.001**	18.8 (0.0–100.0)	43.5±29.1	**0.001**	40.6 (0.0–100.0)
Premedication																		
Yes (276)	46.5±33.7	9.33	31.3 (0.0–100.0)	47.0±30.4	9.88	40.0 (0.0–100.0)	29.1±27.7	11.24	25.0 (0.0–100.0)	33.7±32.5	9.33	25.0 (0.0–100.0)	23.8±28.5	11.46	12.5 (0.0–100.0)	30.0±25.8	11.99	28.4 (0.0–100.0)
No (126)	83.7±19.1	**<0.001**	87.5 (6.3–100)	80.7±20.5	**<0.001**	85.0 (0.0–100.0)	67.4±23.1	**<0.001**	68.8 (0.0–100.0)	70.9±31.3	**<0.001**	75.0 (0.0–100.0)	64.4±22.4	**<0.001**	62.5 (0.0–100.0)	73.4±13.9	**<0.001**	75.0 (38.8–100.0)
Intraop complications																		
None (266)	56.2±35.4	1.19	62.5 (0.0–100.0)[b]	53.8±33.6	41.32	55.0 (0.0–100.0)[b]	39.4±33.0	11.18	37.5 (0.0–100.0)[a,b]	46.1±36.3	1.19	50.0 (0.0–100.0)	35.7±32.9	24.81	31.3 (0.0–100.0)[a,b]	46.2±29.9	23.39	48.9 (0.0–100.0)[a]
Hypotension (45)	34.2±26.6	0.880	25.0 (0.0–100.0)[a]	43.2±22.1	**<0.001**	35.0 (0.0–100.0)[a]	33.3±19.2	**0.025**	31.3 (6.3–87.5)[a,b]	40.8±27.4	0.880	37.5 (0.0–100.0)	20.1±25.8	**<0.001**	6.3 (0.0–100.0)[b]	34.3±22.6	**<0.001**	27.5 (3.8–93.8)[b]
Pain (68)	76.2±25.6		81.3 (6.3–100.0)[c]	75.3±21.9		75.0 (25.0–100.0)[c]	55.0±31.8		50.0 (0.0–100.0)[a]	47.6±41.5		37.5 (0.0–100.0)	48.7±30.5		50.0 (0.0–100.0)[a]	60.0±23.8		52.3 (22.0–97.8)[a]
Nausea and vomiting (16)	75.8±25.0		78.1 (18.8–100.0)[c]	79.1±17.9		85.0 (25.0–100.0)[c]	52.0±31.8		46.9 (6.3–100.0)[a,b]	42.2±40.0		43.8 (0.0–100.0)	45.3±29.6		34.4 (0.0–93.8)[a,b]	57.5±21.8		57.3 (11.3– 90.5) [a]
Dyspnea (7)	73.2±30.1		81.3 (31.3–100.0)[c]	70.4±27.2		75.0 (30.0–100.0)[c]	45.3±31.1		25.0 (0.0–81.3)[b]	33.9±35.9		25.0 (0.0–100.0)	34.8±38.5		12.5 (0.0–100.0)[a,b]	50.7±25.3		60.0 (14.8–83.8)[a]
Complications in Recovery room	56.3±34.8	4.67	62.5 (0.0–100.0)[b]	54.8±33.0	18.65	55.0 (0.0–100.0)[b]	40.2±33.0	7.61	34.4 (0.0–100.0)	45.9±29.6	4.67	37.5 (0.0–100.0)	36.5±33.5	11.57	31.3 (0.0–100.0)[b]	46.7±29.8	12.90	49.1 (0.0–100.0)[b]
None (312)	38.2±31.0	3.323	25.0 (6.3–100.0)[a]	53.9±26.5	**0.001**	50.0 (20.0–100.0)[b]	45.1±19.0	0.107	43.8 (25.0–75.0)	51.4±42.6	3.323	50.0 (25.0–100.0)	29.2±27.8	**0.021**	18.8 (0.0–81.3)[b]	43.6±25.6	**0.012**	33.8 (22.8–91.3)[b]
Hypotension (9)	80.8±23.0		93.8 (18.8–100.0)[c]	77.4±19.2		87.5 (40.0–100.0)[a]	53.5±31.2		50.0 (0.0–100.0)	49.0±42.6		37.5 (0.0–100.0)	49.8±28.9		50.0 6.3 (–100.0)[a]	62.1±21.8		59.5 (28.5–97.8)[a]
Pain (39)	53.1±34.4		53.1 (0.0–100.0)[b]	58.6±25.5		34.4 (0.0–100.0)[b]	34.7±19.3		31.3 (6.3–100.0)	34.2±31.8		37.5 (0.0–100.0)	24.5±24.8		18.8 (0.0–93.8)[b]	41.0±20.6		36.6 (11.3–90.5)[b]
Nausea and vomiting (38)	76.6±35.5		90.0 (25.0–100.0)[b]	78.8±28.4		80.0 (30.0–100.0)[a]	42.2±41.9		43.8 (0.0–81.3)	62.5±43.3		62.5 (25.0–100.0)	39.1±40.3		34.4 (0.0–87.5)[a]	59.8±28.9		61.3 (23.0–93.8)[a]
Shaking (4)																		
ASA-PS																		
1 (143)	59.9±31.2	23.58	62.5 (0.0–100.0)	57.2±30.8	0.19	60.0 (0.0–100.0)	34.9±30.3	13.38	25.0 (0.0–100.0)[a]	35.1±35.6	23.58	25.0 (0.0–100.0)[a]	32.5±30.1	3.10	25.0 (0.0–100.0)	43.9±27.3	6.25	42.5 (0.0–100.0)[a]
2 (202)	57.2±36.1	**<0.001**	62.5 (0.0–100.0)	57.3±33.4	0.912	60.0 (0.0–100.0)	42.5±33.3	**0.001**	37.5 (0.0–100.0)[a,b]	49.3±38.1	**<0.001**	50.0 (0.0–100.0)[b]	38.1±34.0	0.212	37.5 (0.0–100.0)	48.9±29.8	**0.044**	52.5 (0.0–100.0)[a,b]
3 (57)	57.1±37.3		62.5 (0.0–100.0)	59.5±28.6		50.0 (0.0–100.0)	51.6±27.1		43.8 (6.3–100.0)[b]	57.2±24.3		50.0 (25.0–100.0)[b]	41.2±33.5		37.5 (0.0–100.0)	53.3±27.0		63.8 (0.0–100.0)[a]

**The difference between groups with the same letter for each variable was significant (p<0.05)*

According to results of the original study by Maurice-Szamburski A et al. (6), female sex was associated with a significantly lower Information score, patients with ASA-PS score II had a significantly lower Attention score, and patients older than 55 years showed higher satisfaction scores for all dimensions except Attention. Similarly, in the current study, male sex was associated with a significantly higher Information score and patients with ASA-PS score I had a significantly higher Attention score (Table 3). Furthermore, according to the multiple linear regression model, the best predictive variables for patient satisfaction with regional anesthesia were male gender, having a history of anesthesia, and not receiving premedication (Table 4). As Maurice-Szamburski et al. (6) did not give regression analysis results in the original study, the best predictor variables in patient satisfaction could not be compared.

Table 4. *Results of the multivariate logistic regression (adjusted) with patient satistaction (n=402) as the dependent variable (OR and 95% CI)*

Variable	Coef	Std Error	Beta	t	Sig	95% Confidence Intervel
Constant	1.154	0.247		4.674	0.00	0.70-1.64
Male gender	0.265	.097	-0.113	-1.946	0.01	1.15-116
Having a history of anesthesia	0.301	.051	0.227	5.937	0.00	0.20-0.40
Not receiving premedication	1.361	.105	0.539	12.909	0.00	-0.45-0.73

In this study, it was aimed to explore the link between demographics or clinical status and patient satisfaction with regional anesthesia related to the information, attention, waiting, discomfort, and pain subscales of the EVAN-LR. When the subscales were examined in the current study, the subscale with the highest level of satisfaction was attention (58.2±34.5). In a study by Courtot et al. (19) of orthopedic patients, the subscale with the highest mean item total scores was also found to be attention (92.7±10.4), while in the study by Maurice-Szamburski et al. (6), it was discomfort (86.65±17.78). The lower level of patient satisfaction with regional anesthesia in the current study compared to other studies that have used the EVAN-LR can be attributed to the effect of the social, cultural, political and economic structure of the country where the patients live. In our hospital, the anesthesia care of patients who are to undergo surgery is applied in the anesthesia polyclinic, in the clinic at the preoperative visit, in the operating theatre immediately before surgery, and at the postoperative visit in the clinic. However, as this is a university hospital, there may be different anesthetists undertaking these steps in the anesthesia care process, because of training requirements. This can be thought to have a negative effect on patient satisfaction with regional anesthesia. The subscale score for pain (36.5 ± 32.7) in the present study was particularly lower than in other studies by Maurice-Szamburski et al. (6) (79.16 ± 26.15) and Courtot et al. (19) (75.2 ± 19.2). Insufficient analgesia might induce a lower subscale score for pain, which leads to a lower total satisfaction score (22, 23). At our hospital, when the condition of patients stabilize after the operation, the patients are transferred to the postoperative clinics of the relevant branch, and analgesia management is not performed by anesthesiologists. Pain management is performed by the specialist doctor of the branch performing surgery. Low patient satisfaction with regional anesthesia related to pain can arise from different approaches of specialist physicians in pain control.

In addition to gender and education, the levels of patient satisfaction were found to be higher in those undergoing obstetric surgery, those with previous experience of anesthesia and those who were not administered premedication. The reason for the highest level of patient satisfaction with regional anesthesia in obstetrics surgery can be considered to be that the majority of the sample were caesarean section delivery patients and they were excited about the birth of the infant. The duration of the operation was also thought to have an effect on the variable of surgical branch. In another study, which was conducted with gynaecology, urology, general surgery, and orthopedic surgery patients by Akpinar et al. (24) in Turkey, patients who underwent gynaecological and obstetric surgeries and the patients in the age group between 26 and 35 years were mostly satisfied with regional anesthesia. Furthermore, it was reported that patients mostly felt comfortable during urological surgeries, and mostly felt anxious during general surgical procedures. However, in the current study, orthopedic surgery patients had a higher satisfaction score than urology and general surgery patients. The reason for the high level of satisfaction in orthopedic patients may be the application of peripheral nerve block in surgery. There are known to be several benefits of peripheral nerve block compared with general anesthesia that directly affect patients, including reductions in postoperative pain, analgesic use, and postoperative nausea and vomiting (25). In addition, peripheral nerve block provides superior postoperative pain control compared with systemic opioids, with a corresponding reduction in opioid-related adverse events (22).

A statistically significant relationship was determined in the current study between perioperative satisfaction and the development of complications intraoperatively or in the recovery room ($p<0.05$). In contrast, Benwu and Gebremedhin (23) determined no relationship between patient satisfaction and the type of anesthesia applied or the development of complications. Although it has been stated in literature that the physical symptoms leading to substantial morbidity are associated with decreased satisfaction with care (1,6), patients in the current study who experienced pain intraoperatively or in the recovery room were found to have higher perioperative satisfaction levels than those who did not develop any complications (Table 3). As patients experience pain differently, it is difficult to develop a routine procedure to improve patient satisfaction with regional anesthesia (15,22,26). It has been previously reported that empathy with the patients and successful pain management have a positive effect on patient satisfaction (26,27). In the current study, the reason for the higher patient satisfaction with regional

anesthesia in patients experiencing intraoperative pain may have been due to the efforts of the anesthesia team to reduce the patient's pain during surgery. An interesting finding of this study is that patient satisfaction with regional anesthesia increases as the ASA score increases. The reason for this may be that patients with more chronic diseases are more likely to have more anxiety related to surgery (14,28) and that patients experience psychological relief when they come to the second postoperative day and they might be thankful.

Soltner et al. (28) stated that good medical communication, for example by changing anesthesiologists' attitude to increase empathy, has been reported to improve patient satisfaction and brings other benefits such as increasing adherence to medical advice. However, patient satisfaction is not only related to anesthesiologists' individual behavior (6), but it is a multi-dimensional healthcare construct affected by many variables, such as expectations, communication, connection with the patient and healthcare team, shared decision-making, and positive attitude and behaviour of the healthcare professionals (2,19,21). Ironfield et al. (22) emphasized that providing information, pain, and interaction with an anesthesiologist are three important areas in patient satisfaction with regional anesthesia. In the current study, a significant association was determined between patient satisfaction with regional anesthesia and gender, level of education, surgical branch, anesthesia history, premedication, intraoperative complications, complications in the recovery room and ASA-PS score. The multivariate regression model showed that the best predictor variables for EVAN-LR scores in the sample were gender, history of anesthesia, and premedication. Evaluation was made of preoperative, intraoperative, and postoperative processes with the EVAN-LR, but in another study conducted by Ironfield et al. (22) with 154 orthopedic and trauma surgery patients, regression analysis showed the complaint of needle puncture to be the greatest negative factor in patient satisfaction with regional anesthesia.

There were some limitations to this study, primarily that the patient group comprised only patients who presented at a single university hospital and underwent surgery in obstetrics, urology, orthopedics or general surgery, which are the branches where regional anesthesia is most applied. The heterogenous nature of the procedures included in the study made it difficult to compare the relevant covariates. Furthermore, excluding patients who required additional anesthesia for pain control was necessary because the different types of anesthesia would have affected the patient's consciousness and introduced a bias. Although the importance of using a standardized scale for patient satisfaction with regional anesthesia and anesthesia services is emphasized by ASA (30), there is no valid and reliable measurement tool used for this in Turkey. The insufficient number of valid and reliable measurement tools for the determination of patient satisfaction and the lack of a standardized scale are another limitation of the study. Therefore, there is a need for further larger studies to confirm the results of this study.

5. CONCLUSION

Patient satisfaction is emerging as an important indicator of the quality of health care, and identifying deficiencies in discrete aspects of satisfaction may allow targeted interventions to improve quality (22). Factors determined to have an effect on perioperative patient satisfaction were found to be male gender, a higher level of education, previous experience of anesthesia, obstetric surgery, an ASA-PS score of 3, complication development and the provision of premedication.

Conflict of Interest

There are no conflicts of interest in connection with this paper.

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Appendix 1. 19-Item Experience of Regional Anesthesia Questionnaire

The Evaluation du Vécu de l'Anesthésie LocoRégionale (EVAN-LR)*

During the preoperative visits with the anesthetist:	
1	I received information about what was going to happen
2	I was able to ask the questions I wanted
3	I felt reassured, relaxed, and confident
During the preoperative visits with the surgeon:	
4	I received information about what was going to happen
5	I felt reassured, relaxed, and confident
At operating room entrance:	
6	My privacy was respected
During the surgery:	
7	I had unpleasant feelings such as thirst, hunger, nausea, or headache…
8	I felt uncomfortable hearing and/or seeing what was happening
After the surgery:	
9	I had unpleasant feelings such as thirst, hunger, nausea, or headache…
10	I felt uncomfortable: cold, warm, badly positioned on the bed…
11	I had pain
Since I came back to my bedroom or home	
12	I had unpleasant feelings such as thirst, hunger, nausea, or headache…
13	I felt uncomfortable: cold, warm, badly positioned on the bed…
14	I had pain
Overall, about the nursing and medical staff:	
15	Upon OR admission, medical staff were attentive
16	In the recovery room, nursing and medical staff were attentive
17	Since I came back to my bedroom, nursing staff were attentive
Waiting times in the hospital seemed too long:	
18	To obtain an appointment with the anesthetist or surgeon
19	During the preoperative visits

Items of the sub-scales:

Attention: Items 6, 15, 16, 17; Information: Items 1, 2, 3, 4, 5; Discomfort: Items 7, 8, 9, 12; Waiting: Items 18, 19; Pain: Items 10, 11, 13, 14

**To use the English version of the EVAN-LR please contact Axel Maurice-Szamburski, to use the Turkish version of the Turkish version of the EVAN-LR please contact Omer Faruk BORAN*

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Clinical and Experimental Health Sciences

Surgical Teams' Attitudes and Views Concerning the Surgical Safety Checklist^{TR}

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ABSTRACT

Objective: This study was conducted to determine the surgical team members' views and attitudes concerning the Surgical Safety Checklist^{Turkey} (SSC^{TR}).

Methods: This descriptive-cross-sectional study was conducted in nine education and research hospitals in Istanbul with 561 surgical clinic and operating room nurses, surgeons, anesthetists, and anesthetic technicians. Data were collected using an information form to determine the demographic and professional characteristics of surgical team members, their attitudes and views concerning SSC^{TR}.

Results: According to the participants' responses, the use of SSC^{TR} was compulsory in the institution of 98% of the participants, 95.2% believed that this checklist should be used for each patient because it was effective in preventing complications, and 78.4% considered that it increased patient safety, but only 57.8% reported that SSC^{TR} was routinely used in daily practice. Although the participants who had received training on SSC^{TR} comprised 69% of the sample, 50.8% thought that training meetings were not beneficial. The rate of compliance was the lowest for SSC^{TR} item "Team members' introducing themselves" (32.7%) and the highest for "Confirmation of identity, surgery type, and surgical site of the patient" (95.3%).

Conclusion: The study findings showed that SSC^{TR} was not used regularly in half of the patients. It is recommended that regular trainings with the active participation of all team members to ensure the effective use of the checklist.

Keywords: Surgical safety checklist, patient safety, safe surgery.

1. INTRODUCTION

Safety errors can result in damage and injuries in patients during surgery and even lead to death (1). According to the Lancet Commission on Global Surgery, 313 million surgical procedures are performed globally every year, with at least 4.2 million people dying within 30 days of surgery, and approximately half of these deaths occur in low – and middle-income countries. It is also stated that post-operative deaths account for 7.7% of all deaths worldwide and rank third among all-cause mortality after ischemic heart disease and stroke (2). In industrialized countries, it is estimated that the rate of complications due to surgery is 3-17% (3,4), and the mortality rate is 0.4-0.8% (3-5). Therefore, safety errors due to surgery are seen as a global public health problem (6).

Studies have shown that the most common safety errors are wrong patient, site or surgery, anesthesia equipment problems, lack of necessary equipment, unpredictable blood loss, inadequately sterilized instruments, and retained surgical bodies (3,4,7). In addition to the negative effects of safety errors, it has been determined that they increase length of hospital stay and costs (3,8,9), approximately half of these errors can be prevented and the surgical team plays an important role in this process (3).

The "Safe Surgery Saves Lives" project initiated by World Health Organization (WHO) in 2008 is aimed at preventing adverse events related to surgical processes. This initiative prioritizes the administrative and managerial aspects of healthcare services, as well as the patient safety attitudes of the surgical team (1,10). The Surgical Safety Checklist (SSC) created within the scope of the project also aims to standardize practices to prevent possible surgical errors and improve teamwork and communication in the operating room in order to increase patient safety as a whole (1,11,12). The SSC consists of three sections with 19 items, and the pilot study conducted in eight different countries, four low-income and four high-income, showed significant reductions

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in the rate of mortality (from 1.5 to 0.8%) and complications (from 11 to 7%) (13). This provided that the list being widely used in many hospitals around the world (1,14,15). In 2009, the Turkish Ministry of Health included this list in the Health Quality Standards (16) by adding a fourth section called "Before patient leaves clinic", and since then, SSC[TR] has been utilized in hospitals throughout the country (17).

In previous studies about SSC usage, it has been shown to reduce surgical errors, postoperative complications (8,13,18-21) and mortality (19,20,22), improve the communication between surgical team members (3,8,22,23) and offers promising results in increasing patient safety (4,10,18,22). However, SSC usage is not sufficient alone to improve patient outcomes. Improvement in surgical outcomes depends on the adoption of SSC by surgical team and compliance with items (24). Despite the reported benefits, compliance with SSC is low (3,25,26). Although there are studies investigating compliance with the use of SSC in international literature (1,3,5,8,10,13,14,23,24,27), only limited research has been undertaken in Turkey (26,28-31). The current study aimed to determine the surgical team's views and attitudes concerning the use of SSC[TR].

2. METHODS

2.1. Study Design and Setting

This descriptive-cross-sectional study was carried out in the surgical clinics and operating rooms of nine training and research hospitals located in the Anatolian side of Istanbul between February-April 2019. Prior to the study, approval was obtained from the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date: 03.11.2018, number: 09.2017.647) and institutional permissions (date: 15.02.2019, number: 16867222-604.01.01) were taken. Informed verbal and written consent of all participants was obtained.

2.2. Participants

The study population consisted of 561 healthcare professionals (clinic and operating room nurses, surgeons, anesthetists and technicians) working in surgical units of hospitals. The inclusion criteria were having been regularly working in a surgical unit and/or operating room for at least six months and providing consent to participate in the study. The exclusion criteria were being on leave of absence (maternity, annual or bereavement leave) and not attending work for other reasons during the specified period. Further excluded were individuals that did not fully respond to the items included in the information form.

2.3. Data Collection

Data were obtained by interviewing healthcare professionals in hospital and using an information form consisting of three sections, prepared based on the literature (29,30,32,33). The first section contained questions related to the demographic and professional characteristics of the participants, the second section aimed to elicit the participants' views on SSC[TR], and the third section inquired about the participants' attitudes concerning the use of SSC[TR]. In the third section of the form, all the items in SSC[TR] were presented to the participants in a five-point Likert type (never, rarely, sometimes, often, and always). The items in the "Before patient leaves clinic" section of SSC[TR] were completed by clinical nurses, and those in the following three sections by operating room nurses, surgeons, and anesthetists/anesthetic technicians.

2.4. Data Analysis

Data were analyzed by using SAS (Statistical Analysis Software, North Carolina State University, SAS Institute, North Carolina, USA) package program. The participants' demographic and professional characteristics, views, and attitudes concerning SSC[TR] were presented with descriptive statistical methods (number, percentage, mean, standard deviation [SD], minimum and maximum values). Normal distribution of data were tested by Shapiro-Wilk and Chi-square test was used to compare categorical variables with normal distribution. Statistical significance was accepted as $p<0.05$.

3. RESULTS

Of the participants, 233 were clinic nurses, 187 were operating room nurses, 81 were anesthetic technicians, 34 were surgeons, and 26 were anesthetists. The mean time for practicing the profession was 7.3 (SD = 7.0) (range, 1-35) years and the mean time working in the current unit was 3.8 (SD = 4.7) (range, 1-25) years. The mean age of the participants was 29.8 (SD = 6.8) (range, 19-54) years. Of all the participants, 80.4% were women, 49% were working shifts (08-16/16-08/day), and 60.4% worked 40-49 hours a week.

Ninety-seven percent of the participants stated that they knew about SSC[TR], but only 69% had received training on SSC[TR]. While 98% of the participants stated that it was mandatory to use SSC[TR] in their institutions, 57.8% stated that SSC[TR] was used regularly in every patient. Of all the participants, 78.4% thought that the use of the checklist increased patient safety, and 92.2% of them stated that they would like SSC[TR] to be used if they were to undergo surgery themselves. The participants' views on the use of SSC[TR] are presented in Table 1.

Table 1. *Participants' views on the use of SSC[TR] (n = 561)*

Variables	n (%)
Knowledge of SSC[TR]	
Present	544 (97)
Absent	17 (3)
History of SSC[TR] training	
Present	387 (69)
Absent	174 (31)
Source of SSC[TR] training*	
In-service training	363 (64.7)
Scientific gatherings, such as congresses and symposiums	49 (8.7)
Courses	18 (3.2)
SSC[TR] training meeting in the unit	
Not organized	273 (48.7)

Organized once before the adoption of the checklist	187 (33.3)
Regularly organized both before and after the adoption of the checklist	101 (18)
Are SSC[TR] training meetings held in the unit beneficial?	
Yes	276 (49.2)
No	285 (50.8)
Is the use of SSC[TR] mandatory in the unit?	
Yes	550 (98)
No	11 (2)
Is SSC[TR] used regularly in every patient?	
Yes	324 (57.8)
No	237 (42.2)
Reasons for not using SSC[TR] regularly*	
Not believing it is important	129 (23)
Hierarchical structure of the unit	56 (10)
Unfamiliarity	75 (13.4)
Lack of motivation during day	69 (12.3)
Lack of time due to workload	167 (29.8)
No one assigned to provide leadership	41 (7.3)
Time-consuming	77 (13.7)
No support from hospital management	25 (4.5)
Responsibilities not distributed across surgical team members	76 (13.5)
Benefits of using SSC[TR] regularly in every patient*	
Increased team communication	283 (50.4)
Increased procedural awareness (right patient/procedure/site surgery)	412 (73.4)
Identification/increased awareness of patient risks	394 (70.2)
Increased patient-centered information	302 (53.8)
Increased compliance with patient safety precautions	386 (68.8)
Increased patient safety	440 (78.4)
Reduced adverse events/errors due to surgery	412 (73.4)
Should SSC[TR] be used in every patient?	
Yes	534 (95.2)
No	27 (4.8)
Would you like SSC[TR] to be used if you were undergoing surgery?	
Yes	517 (92.2)
No	44 (7.8)
Availability of a coordinator for the implementation of SSC[TR] in the unit	
Available	248 (44.2)
Not available	313 (55.8)
Profession of the coordinator, if available*	
Surgeon	68 (12.1)
Operating room nurse	205 (36.5)
Anesthetist/anesthetic technicians	116 (20.7)
Recommendation for the use of SSC[TR]*	
Regular training meetings	131 (63.6)
Leadership for the effective use of *SSC[TR]*	218 (38.3)
Ensuring the active participation of all employees in the implementation of *SSC[TR]*	390 (69.5)
Real-time feedback	289 (51.5)
Motivating all employees for the use of SSC[TR]	292 (52)

**Participants were allowed to select more than one option.*
SSC[TR]: Surgical Safety Checklist(Turkey)

The rate of compliance was the highest for SSC[TR] item "Confirmation of identity, surgery type, and surgical site of the patient" (95.3%) and the lowest for "Team members' introducing themselves" (32.7%). The rates of following the items included in SSC[TR] ranged from 75.1 to 95.3% (mean, 90.3%) for the section "Before patient leaves clinic", 66.8 to 85.1% (mean, 79.3%) for "Before induction of anesthesia", 54.9 to 87.5% (mean, 71.1%) for "Before skin incision", and 78 to 91.2% (mean, 84.8%) for "Before patient leaves operating room". It was determined that 54.9 to 95.3% (mean, 81.1%) of the participants had a general attitude toward the regular use of SSC[TR] (Table 2).

When the attitudes of the surgical team members toward the use of SSC[TR] are examined according to their professions, in the "Before patient leaves clinic" section of SSC[TR], the clinic nurses mostly (95.3%) complied with the item related to the confirmation of the patient identity, type of procedure, and surgery site, while they least complied with the item concerning the confirmation of whether the surgery site has been shaved. In relation to the "Before induction anesthesia" section of SSC[TR], the highest rates of compliance were observed in the confirmation of patient identity, type of procedure, surgery site, and patient consent for the surgeons (91.2%) and operating room nurses (86.1%) and checking whether the patient had any allergy for the anesthetists/technicians (88.8%). In the same section, the lowest rates of compliance were observed in the operating room nurses and anesthesiologists/technicians confirming the marking of surgery site (71.7% and 54.2%, respectively), and surgeons (70.6%) confirming the availability of necessary imaging devices. In the "Before skin incision" section, the item that was most confirmed was the adequacy of the sterility of materials (always confirmed by 93% of the operating room nurses, 82.4 of the surgeons, and 79.4% of the anesthesiologists/anesthetic technicians) while the least confirmed item was the confirmation of whether all team members have introduced themselves (66.8, 58.8 and 32.7%, respectively). In the "Before patient leaves operating room" section, the highest and lowest complied items were determined as always confirming the labeling of all samples taken from the patient (94.7% for the operating room nurses) and completion of instrument, sponge and needle counts (87.9% for the anesthetists/anesthetic technicians and 85.3% for surgeons). For the same section, the least confirmed item was postoperative critical requirements (79.7 and 76.5% for the nurses and surgeons, respectively) and confirmation of patient identity, type of surgery, and surgery site for the anesthetists/anesthetic technicians (72%) (Table 2).

The highest compliance with the SSC[TR] items was observed among clinical nurses at a rate of 75.1-95.3% (mean, 90.3%), followed by the operating room nurses at 68.8-94.7% (mean, 79.6%), surgeons at 58.8-91.2% (mean, 75.3%), and anesthetists/anesthetic technicians at 32.7-88.8% (mean, 72.7%) (Table 2). The participants' views and attitudes concerning the use of SSC[TR] did not significantly differ according to their individual and professional characteristics ($p>0.05$).

Table 2. *Participants' attitudes toward the SSC[TR] items*

SSC[TR] Items	Never	Rarely	Sometimes	Often	Always
	n (%)	n (%)	n (%)	n (%)	n (%)
Before Patient Leaves Clinic (n = 233)*					
Confirming the accuracy of patient identity, procedure, and surgery site and whether the surgery site has been marked	1 (0.4)	-	2 (0.9)	8 (3.4)	222 (95.3)
Confirming that the patient provided consent for the surgery	-	4 (1.7)	-	9 (3.9)	220 (94.4)
Checking whether the patient fasted	2 (0.9)	1 (0.4)	3 (1.3)	6 (2.6)	221 (94.8)
Checking whether the surgery site has been shaved	13 (5.6)	6 (2.6)	19 (8.2)	20 (8.6)	175 (75.1)
Checking whether there is any foreign substance on the patient's body (make-up, nail polish, prosthesis, etc.)	3 (1.3)	3 (1.3)	1 (0.4)	10 (4.3)	216 (92.7)
Confirming that all clothing has been removed and the surgical gown and bonnet have been put on the patient	3 (1.3)	-	3 (1.3)	7 (3)	220 (94.4)
Confirming the necessity of a special procedure requirement (enema, compression stockings, bladder catheterization, special treatment protocol, etc.) before surgery	5 (2.1)	5 (2.1)	3 (1.3)	11 (4.1)	209 (89.7)
Checking the preparation of required material, implant, blood or blood product	2 (0.9)	1 (0.4)	10 (4.3)	15 (6.4)	205 (88)
Checking the availability of the results of all examination findings (laboratory, radiological, etc.)	4 (1.7)	3 (1.3)	3 (1.3)	17 (7.3)	206 (88.4)
Before Induction of Anesthesia (n = 328)*					
Confirming patient identity, procedure, surgery site, and patient consent	6 (1.8)	5 (1.5)	8 (2.4)	30 (9.1)	279 (85.1)
Check whether the surgery site has been marked	14 (4.3)	15 (4.6)	28 (8.5)	52 (15)	219 (66.8)
Confirming that the Anesthesia Safety Checklist (anesthesia equipment and drugs) has been completed	5 (1.5)	4 (1.2)	15 (4.6)	37(11.3)	267 (81.4)
Confirming that the pulse oximeter is on the patient and functional	8 (2.4)	7 (2.1)	17 (5.2)	34 (10.4)	262 (79.9)
Checking whether the patient has any allergy	3 (0.9)	2 (0.6)	8 (2.4)	34 (10.4)	281 (85.7)
Checking the availability of necessary imaging devices	4 (1.2)	7 (2.1)	18 (5.5)	41 (12.5)	258 (78.7)
Checking the requirement of blood transfusion	5 (1.5)	4 (1.2)	20 (6.1)	44 (13.4)	255 (77.7)
Before Skin Incision (n = 328)*					
Confirming that all team members have introduced themselves by name and roles	32 (9.8)	31 (9.5)	42 (12.8)	43 (13.1)	180 (54.9)
One team member verbally confirming patient identity, type of surgery, and surgery site	20 (6.1)	27 (8.2)	29 (8.8)	55 (16.8)	197 (60.1)
Review of critical events (estimated operative time, expected blood loss, unexpected events during surgery, possible anesthesia risks, and patient position)	9 (2.7)	6 (1.8)	19 (5.8)	58 (17.7)	236 (72)
Confirming that antibiotic prophylaxis has been given within the last 60 minutes	14 (4.3)	14 (4.3)	18 (5.5)	56 (17.1)	226 (68.9)
Checking the preparation of materials to be used	3 (0.9)	3 (0.9)	5 (1.5)	44 (13.4)	273 (83.2)
Confirming the adequacy of the sterility of materials to be used	3 (0.9)	3 (0.9)	6 (1.8)	29 (8.8)	287 (87.5)
Checking the necessity of blood sugar monitoring throughout surgery	8 (2.4)	11 (3.4)	21 (6.4)	52 (15)	236 (72)
Checking the necessity of anticoagulant use	10 (3)	11 (3.4)	18 (5.5)	51 (15.5)	238 (72.6)
Checking the necessity of deep vein thrombosis prophylaxis	13 (4)	12 (3.7)	31 (9.5)	47 (14.3)	225 (68.6)
Before Patient Leaves Operating Room (n = 328)*					
Confirming patient, type of surgery, and surgery site	9 (2.7)	7 (2.1)	18 (5.5)	32 (9.8)	262 (79.9)
Completion of instrument, sponge/compress and needle counts	4 (1.2)	3 (0.9)	5 (1.5)	17 (5.2)	299 (91.2)
Labelling of all surgical specimens	4 (1.2)	4 (1.2)	5 (1.5)	22 (6.7)	293 (89.3)
Review of the patient's critical post-operative requirements by the surgeon and anesthetist	6 (1.8)	4 (1.2)	13 (4)	49 (14.9)	256 (78)
Confirming the hospital unit to which the patient will be transferred	2 (0.6)	3 (0.9)	7 (2.1)	35 (10.7)	281 (85.7)

**Marked by surgical clinic nurses*

***Marked by operating room nurses, surgeons, anesthetists and anesthetic technicians*

SSC[TR]: Surgical Safety Checklist(Turkey)

4. DISCUSSION

In Turkey, concerning the safety of surgical care in healthcare services, the national regulation (34) and safe surgical practice guidelines have been published (17). These practices contribute to the awareness of the surgical team and teamwork with higher participation in order to prevent possible surgery-related errors (1). In addition, studies have reported that the use of SSC[TR] is considered as a tool that can improve teamwork and patient safety (28,30,35).

This study findings showed that almost all the healthcare professionals working in surgical units knew about SSC[TR]. Although this result is consistent with the findings of previous studies (30,31,33,36,37), there are also researchers reporting otherwise. For example, 81.3% of 96 healthcare professionals in a study by Karayurt et al. (26) and 23.6% of 208 healthcare professionals in a study by Keskin et al. (28) reported that they did not have knowledge of SSC[TR] (26,28). In studies conducted with surgeons and anesthetists, the rate of SSC knowledge was very low (38,39). These results may be due to the SSC usage not being mandatory in the institutions where the studies were conducted and the employees not having received any training about SSC. In contrast, in the current study, almost all the participants stated that the use of SSC[TR] was mandatory in their units, and more than half had received training on the use of SSC[TR]. However, a very low rate of participants reported that regular training meetings were held before and after SSC[TR] was introduced. This can also be the reason why most of the participants did not find the training useful. Contradicting this finding, it has been shown that regular training organized for the surgical team for the use of SSC lead to significant changes in their awareness, attitudes, and behaviors and significantly increase their compliance with SSC usage (3,23,35,40). In addition, it has been reported that in addition to regular informative meetings (39,41,42), SSC usage improves team communication, information sharing, planning and decision-making processes (3,24). In this context, unit managers are required to hold continuous training meetings at regular intervals with the support of senior management to increase the compliance of the surgical team with SSC[TR].

A striking finding of our study is that almost all the participants stated that the use of SSC[TR] in their units was mandatory and it must be used in every patient, but approximately half of them stated that this checklist was not regularly applied to every patient. Similar to our findings, previous studies shows that SSC is not routinely used with every patient (26,28,41). Although it is recommended by many professional organizations to use of SSC, this internationally accepted checklist is not followed in every patient. This may be due to a number of reasons, including the unwillingness of healthcare professionals to change, the need for comprehension and their lack of awareness of the positive effects of SSC (43). In order to ensure the use of SSC[TR] in every patient, it is a priority to inform all the surgical team members about both the existence of it and the reasons for and importance of its implementation.

In the literature, it has been reported that the most common obstacle to the use of SSC is its inappropriate use; i.e., item duplication from existing checks (19). In a multicenter study, the participants considered the completion of SSC as time-consuming due to their intense workload, did not fully understand its importance, and marked some of the items on SSC without any evaluation just to comply with hospital directives due to lack of time (14). These findings were confirmed by the study of Vogts et al. (44). Levy et al. (45), on the other hand, stated that although there was 100% compliance in terms of including SSC in the medical records of patients, some of the items on SSC were not completed. Sivathasan et al. (43) also reported that only some of the items on SSC were used while others were overlooked due to time constraints in some operating rooms. Similar to the findings of previous studies (10,19,24,28-30,46,47), in this study, the most important reasons for not using SSC[TR] regularly in every patient were lack of time due to heavy workload, not believing in the importance, and perceiving its use as time consuming. However, it is known that the implementation of SSC is not a time-consuming process (38,47) since it only takes approximately two minutes to complete (32).

For the successful implementation of SSC[TR], training should be organized to provide convincing explanations for healthcare professionals concerning why and how SSC[TR] will be used in order to contribute to the understanding of its importance. In addition, the heavy workload of surgical units and the shortage of staff, as well as the lack of a trained coordinator responsible for ensuring the use of SSC[TR] can be considered as important obstacles to a successful implementation. Supporting this, both previous research conducted in Turkey (28,31) and our study revealed that a coordinator responsible for the implementation of SSC[TR] was not present in most institutions. However, WHO and the Turkish Ministry of Health underline the importance of assigning one person from the surgical team as a coordinator for the implementation of SSC to achieve complete and error-free surgical procedures (17,48). Previous studies have reported that the responsibility for the implementation of the checklist is generally undertaken by operating room nurses, which is in agreement with the results of our study (30,31,33,37,42). Considering various factors, such as assigning the responsibility of checklist implementation to surgical nurses in addition to their existing duties and the time that should be allocated to the necessary preparations for the next surgery to be performed in the same operating room, it is important for institutions to determine a coordinator for using SSC at all stages for an effective implementation. In this context, a nurse may be appointed as checklist-coordinator, as also recommended by WHO because our data showed that nurses had the highest compliance with the items of SSC[TR] and previous studies (4,29,31,35,43,49) also suggested that compared to the other members of the surgical team, nurses have higher awareness of the benefits of checklist, exhibit a more positive attitude toward its use, and tend to take more responsibilities.

Consistent with previous studies (3,24,27,30,32), we observed the highest compliance with SSC[TR] in relation to the item concerning the confirmation of patient identity, surgery site, and surgical procedure while the item with the lowest compliance was the team members introducing themselves. It is important to confirm the identity of the patient, surgery site, and type of procedure to prevent wrong patient, side and surgery; therefore, the high compliance with this item is a favorable finding. On the other hand, the low compliance in surgical team members introducing themselves by name and roles may be the result of the participants considered this item to be unimportant or unnecessary because they already know each other. However, there are also studies reporting that compliance with this item is very high (41,46,50).

Varying results are reported in the literature regarding the adaptation of surgical team members to the sections of SSC. For example, the highest compliance with SSC[TR] was seen in the "Before skin incision" section (mean, 91%) in one systematic review (25) while it was observed in "Before induction of anesthesia" (mean, 99%) in another systematic review (19). McGinley et al. (24) also determined that the most completed section of SSC was "Before induction of anesthesia" and the least completed section was "Before patient leaves operating room". Consistent with a similar study conducted in Turkey (35), our findings revealed that the highest compliance with SSC[TR] was for the "Before patient leaves clinic" section (mean, 90%) while the least completed section of the checklist was "Before skin incision" (mean, 71%), in contrast to the literature (24,25,35). Differences in results can be due to various reasons, including human, cultural and institutional factors, as well as the health systems of countries.

In our study, the general compliance with SSC[TR] varied between 54.9 and 95.3% (mean, 81.1%). Although this rate was higher than reported in the study of Borchard et al. (25) (mean, 75%), it does not indicate full compliance. In a systematic review, it was reported that adherence to the use of SSC was not 100% in any of the 22 studies examined (3). As the demand for medical resources increases worldwide, any security error can lead to more serious consequences and increased costs for both the healthcare institution and patient, adversely affecting the healthcare process. For example, increased length of hospital stay due to complications has a cascading effect, such as delayed discharge, prolonged waiting lists, and delays in other individuals' access to healthcare (43). Therefore, to achieve the effective implementation, there is a clear need to organize regular training sessions for healthcare professionals, ensure the active participation of surgical team members concerning the use of SSC[TR], and assigning a coordinator to lead all these processes with a multidisciplinary approach. In addition, in order to improve the use of SSC[TR], real-time feedback or group meetings should be offered, the team members' sense of belonging should be promoted by distributing the responsibilities across the team, checklist should be integrated into surgical culture, and the support of management should be sought (3,4,19,23).

The limitations of this study may be that data were obtained using a self-reported form over a certain period of time.

5. CONCLUSION

Study findings showed that although SSC[TR] was known by almost all of the surgical team, it was not used regularly and there was not sufficient training for its use. Despite each healthcare professional's compliance with the items related to their own profession, the surgical team did not have full compliance with SSC[TR]. Training, effective leadership, and communication are essential for the successful implementation of SSC[TR]. Considering the findings of the study, the following recommendations are made: Regular training and informative meetings should be held by hospital/unit managements to improve the use of SSC[TR], coordinators should be assigned and trained to supervise the implementation of SSC[TR], responsibilities should be allocated to surgical team members to ensure that all items are followed, real-time feedback or group meetings should be provided at the end of each case, and regular inspections should be undertaken.

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Clinical and Experimental Health Sciences

Preparation of Chitosan-Polyvinyl Prolidone (PVP) Hydrogels with Fibroblast Growth Factor (FGF) and Investigation of in Vitro Characteristics

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ABSTRACT

Objective: In this study, it was aimed to make in vitro characterization of the formulations by preparing hydrogel formulations using chitosan, a biocompatible and natural polymer, and PVP, a synthetic polymer. In addition, the effects of hydrogels containing FGF on the proliferation of keratinocyte cells were investigated.

Methods: Within the scope of the study, hydrogels with different properties were prepared and their water absorption capacity, and viscosities were examined. In addition, the hardness, adhesiveness, cohesiveness, and elasticity properties of hydrogels were investigated. The 3 – (4,5-dimethyl-2-thiazolyl) – 2,5-diphenyl tetrazolium bromide (MTT) test was applied to evaluate the toxicity of hydrogels on keratinocyte cell lines.

Results: It was observed that hydrogel formulations have high water absorption capacity and suitable viscosity values. In addition, the mechanical characterization results showed that the hydrogels have suitable mechanical properties. According to the results of in vitro cell culture studies, it has been observed that hydrogels stimulate the proliferation of keratinocyte cells.

Conclusion: Results showed that the mechanical properties of hydrogels containing FGF are suitable for application and according to the results of in vitro cell culture studies, hydrogels can be used in wound healing studies because they increase keratinocyte cell proliferation.

Keywords: Chitosan, In vitro characterization, Hydrogel, Cell proliferation.

1. INTRODUCTION

Wound is defined as the deterioration of tissue integrity due to physical factors, chemical factors, heat, surgery or spontaneously. Disruption of tissue integrity causes increased fluid loss, infection, hypothermia, immunity and changes in body image (1). Growth factors and cytokines are widely used in wound healing. Transforming growth factor beta (TGF-β), epidermal growth factor (EGF) and fibroblast growth factor (FGF) are growth factors used in wound treatment (2). FGF used in this study is produced by mast cell, lymphocytes, endothelial cells and keratinocyte cells. FGF is effective on keratinocyte, endothelial and fibroblast cells (2). The mechanism of action of FGF is that it creates mitogenic and chemotactic effects on keratinocytes and fibroblasts, and stimulates angiogenesis, granulation and epithelialization (3). When FGF is used only, it cannot maintain its stability due to immune system cells, natural barriers and various enzymes. Therefore, it shows low efficiency and requires continuous application. It is important to give FGF in a formulation to eliminate the stability problem and to work towards this purpose. Hydrogels are widely used for wound and burn treatment. Hydrogels have a three-dimensional network structure formed by the combination of hydrophilic molecules cross-linked by covalent bonds or held together by intramolecular physical interactions (4, 5). Hydrogels can absorb large amounts of water or biological fluids. The high hydrophilicity of hydrogels is due to the presence of hydrophilic moieties such as amino, carboxyl, amide and hydroxyl groups along the backbone of the polymer (6). In the swollen state, hydrogels have a soft and rubbery structure and closely resemble living tissues (7). Chitosan is widely used in the preparation of hydrogel formulations due to its superior properties (8). The absorption-enhancing feature of chitosan provides its use in mucosal administration, drug delivery systems, and formulation of hydrophilic macromolecular drugs (9). PVP, which is a synthetic polymer, can be used alone, or by adding it to another polymer or forming a copolymer with polymers, and it is used in various fields. PVP is a non-toxic, biodegradable, biocompatible, hydrophilic polymer with high gelling capacity as well as good complexing ability. It has a widespread use in the pharmaceutical industry (10, 11).

2. METHODS

2.1. Preparation of Chitosan-PVP Hydrogel Formulations

The ionic gelation method was used in the preparation of hydrogel formulations (12). Medium molecular weight

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(400 kD) chitosan was used while preparing the hydrogel formulations. The amounts of FGF, chitosan and PVP used in the formulations are indicated in Table 1. Hydrogels prepared according to these concentrations have important effects on physicochemical parameters such as adhesion to the application site, flexibility and cohesiveness. Chitosan was weighed on a precision balance and 1% concentration of glacial acetic acid was added to the chitosan in the beaker into sterile bi-distilled water and mixed with a magnetic stirrer. In a separate beaker, the determined amount of PVP was dissolved in a magnetic stirrer. The two solutions were then mixed in such a way that the volumetric association ratio was 3:1 (Chitosan: PVP). The mixing process was continued for two hours to form a homogeneous structure. Hydrogels were maintained in an ultrasonic water bath for 15 minutes to remove air bubbles. Hydrogels were maintained under UV light for 10 minutes in order for crosslinking and polymerization reactions to take place. The prepared hydrogels were stored in amber colored glass bottles at +4 °C until used in in vitro studies.

Table 1. *Hydrogel formulations and components*

Samples	FGF amount (mg)	Chitosan (% w/v)	PVP (% w/v)
A1	10	3	5
A2	20	3	10
A3	30	3	20
A4	10	2	5
A5	20	2	10
A6	30	2	20

2.2. Measurement of Viscosity and Water Absorption Capacity of Hydrogels

After the air bubbles were removed in the ultrasonic bath, the viscosity of the hydrogels at room temperature was measured using the Brookfield model viscometer. The tests were repeated 3 times for each formulation. A gram sample of the hydrogel, whose water absorbing capacity was to be measured, was weighed and lyophilized. Lyophilized hydrogels were placed in appropriate petri dishes. Then, 1 ml of PBS (pH 7.4) was added to the tared petri dish with the hydrogel at intervals of 15 minutes, and when the gel reached saturation, the excess liquid was removed from the hydrogel, and the tared petri dish was weighed again. The experiment was continued until the hydrogel reached a constant weight.

2.3. Investigation of Mechanical Properties of Hydrogels

Mechanical characterization studies were performed to determine the suitability of the hydrogel for physiological conditions in its application. The mechanical properties of the hydrogels were determined with the TA.XT Plus texture analyzer (12, 13). Before the test, the hydrogels in 50 ml standard bottles were maintained at room temperature for about two hours to reach room temperature. After the hydrogels were brought to room temperature, the weight and height calibrations of the device were made and the measurement was started. The bottle containing the hydrogel is fixed to the device to be measured. The probe of the device was immersed in the hydrogel at a speed of 2mm/s for 15 mm and, after being pulled back to the surface of the hydrogel for 2 seconds, the probe was dipped into the hydrogel a second time and pulled back to complete the test.

2.4. MTT Cell Viability Assay

MTT cell viability assay was performed on the HaCaT cell line. Before starting the MTT test, cells were seeded in 96-well plates in a sterile cabinet (14, 15). In this process, fetal bovine serum (FBS) was used as DMEM medium containing L-glutamine-streptomycin. Before starting the experiment, 5 ml of medium was added to a 25 ml flask, then keratinocyte cells were seeded and kept in an incubator (37 °C) overnight. Then, the medium in the flask was poured and washed 2 times with PBS and 1 ml of Trypsin EDTA was added to the flask and pipetted several times. The cells were then removed from the incubator. Cells were plated with 96 wells and 100 µl of medium was added to each well. Adhesion of cells to the wells occurred in approximately 24 hours. Then, our pre-prepared samples were applied to the wells. 50 µg hydrogel containing 5 µg FGF was added to each well by pipetting in 25 µl medium in a 500 µl eppendorf tube. Then, the 96-well plate was placed in the incubator and incubated for 24 hours. After 24 hours, the samples were taken into a sterile cabinet and 10 µl of MTT solution (room temperature) was added to each well. After the plate was kept in the incubator overnight, 50 µl of the SDS solution was added to each well and the plate was removed from the oven. After six or eight hours, the crystals on the plate were homogenized and the absorbance values were read at 550 nm and 690 nm wavelengths on the microplate ELISA reader. Cell viability rates were calculated using these results compared to the control group.

Statistical Analysis

The results of these studies were statistically evaluated using GraphPad Prism 8 ANOVA followed by Newman–Keuls multiple comparisons test. A value of $p<0.05$ was considered statistically significant. The results were expressed as the mean and ± standard deviation (SD) values.

3. RESULTS

3.1. Results of Mechanical Characterization Study of Hydrogels

The water absorption capacity, viscosity and mechanical properties of Chitosan-PVP hydrogels were given in Table 2. The water absorbing capacities of the hydrogel formulations are between 0.474±0.010 and 0.986±0.012 g. A3 formulation containing chitosan (3%) and PVP (20%) showed the highest water absorption capacity. The viscosity values of the hydrogel formulations varied between 22.800±4424 and 43200±3574 cPs. A3 formulation containing chitosan (3%) and PVP (20%) showed the highest viscosity value. A4 hydrogel formulation containing chitosan (2%) and PVP (5%) showed the lowest viscosity value.

Table 2. *Mechanical characterization results of hydrogels*

Samples	Water absorption capacity (g±SD)	Viscosity (cPs ±SD)	Adhesiveness (N.mm)	Cohesiveness	Elasticity (N.mm)
A1	0,772±0,008	30800±2100	0,086±0,003	0,840±0,009	0,842±0,009
A2	0,864±0,009	39400±3200	0,094±0,002	0,820±0,007	0,810±0,014
A3	0,986±0,012	43200±3574	0,099±0,002	0,850±0,011	0,795±0,006
A4	0,474±0,010	22.800±4424	0,067±0,002	0,800±0,013	0,973±0,009
A5	0,534±0,007	24600±1426	0,071±0,001	0,790±0,011	0,946±0,013
A6	0,598±0,013	26300±1590	0,074±0,002	0,810±0,010	0,910±0,016

Hydrogels are widely used due to the unique properties of its. In order for hydrogels to be applied, they should have significant properties such as high adhesive and cohesive properties, water absorption capacity and viscosity within certain values, and high elasticity and bio-adhesive properties (16, 17). Hydrogels should have high adhesiveness and water absorbing capacity in order to adhere to the wound area for the desired time and to keep that area moist. When these features are provided, a more effective treatment can be provided (18, 19). According to the mechanical characterization results of the hydrogel formulations, the A3 formulation had the highest adhesiveness (0.099±0.002 N.mm) and cohesiveness values (0.850±0.011, Table 2). In addition, the A4 formulation showed the lowest adhesiveness (0.067±0.002), while the A5 formulation had the lowest cohesiveness values (0.790±0.011). The elasticity values of the hydrogels range from (0.795±0.006) to (0.973±0.009) N.mm. According to the results, it was observed that the elasticity values of the hydrogels decreased depending on the concentration of chitosan and PVP used. By looking at the adhesive and cohesiveness results of the hydrogels, it was observed that the viscosity and water absorption capacity of the hydrogels increased depending on the concentration of chitosan and PVP used ($p<0.05$). According to the results obtained, it was observed that the changes in the amount of FGF did not have a significant effect on the mechanical properties, water absorption capacity and viscosity values of the hydrogels ($p>0.05$).

3.2. In vitro Cell Viability (MTT) Results of Hydrogels

In in vitro studies on wound and burn treatment, human keratinocyte and fibroblast cell lines are generally used to calculate and evaluate cell viability and proliferation (14, 20). In this study, keratinocyte cell lines were used to observe and evaluate the efficacy of hydrogel samples in the treatment of wounds or burns and their potential to increase cell proliferation. Cell viability results of hydrogels are given in Figure 1. According to the results obtained, it can be said that hydrogels containing FGF create significant differences on cell viability. It was observed that hydrogels containing 30 mg of FGF (A6: 108.600±1.417 % and A3: 105.800±1.651 %) showed the two highest cell viability. A1 (91.920±1.457 %) and A4 (94.120±2.647 %) formulations showed the lowest cell viability. According to the results, it was observed that the concentration of chitosan and PVP did not make a significant difference on cell viability ($p>0.005$). It was observed that hydrogels containing high amount FGF had higher cell viability than the control group and other formulations. It was observed that cell viability rates of hydrogels containing FGF were significantly different from the control group ($p<0.05$).

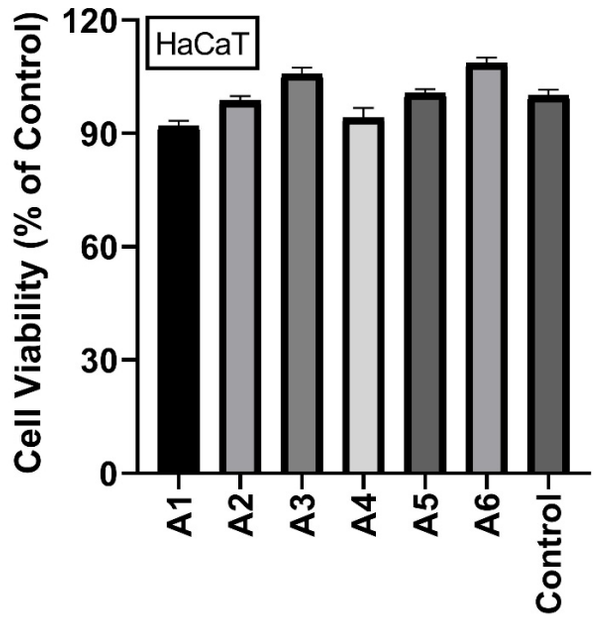

Figure 1. *Keratinocyte (HaCaT) cell viability result of hydrogel samples. The cell viability of the control group was determined as 100 %.*

4. DISCUSSION

Wounds and burns are the most common health problems in daily life. Treatment processes are also very arduous for both those who apply the treatment and the patient (21, 22). There are many drugs and medical support products for treatment (23, 24). In this study, as an alternative to the existing treatment options, a growth factor with known efficacy was planned to be formulated and applied via a suitable carrier system. It can be said that hydrogels are suitable for application in terms of their mechanical properties. In particular, the A3 formulation containing 30 mg of FGF has high water retention and viscosity, which shows that it is more suitable for application compared to others. In addition, it was observed that the A3 and A6 formulations increased the viability of keratinocyte cells more than the control group and other hydrogels. In this case, considering all

the features of the A3 formulation, it can be said that it is the most suitable hydrogel sample in terms of use and in order to shed light on future studies. have two important properties. First, the hydrogel must have suitable physicochemical properties, to be biocompatible and non-toxic. In order to meet these conditions, the polymers to be selected should have appropriate properties. Secondly, the active substance in the hydrogel is required to have bioactive properties suitable for its desired use and to maintain its effectiveness after being placed in the delivery system. In this study, it can be said that hydrogels are suitable for application in terms of their mechanical properties. In particular, the A3 formulation containing 30 mg of FGF has high water retention and viscosity, which shows that it is more suitable for application compared to others. In addition, it was observed that the A3 and A6 formulations increased the viability of keratinocyte cells more than the control group and other hydrogels. In this case, considering all the features of the A3 formulation, it can be said that it is the most suitable hydrogel sample in terms of use and in order to be beneficial for future studies.

5. CONCLUSION

According to the results of the characterization and cell culture studies of the hydrogels, it has been observed that the hydrogels containing FGF increased keratinocyte cell viability. In addition, it can be said that hydrogels have suitable viscosity and water retention values. Considering the adhesive strength, which is very important in terms of adhesion to the application area, it can be said that hydrogels prepared using high concentrations of chitosan and PVP have higher adhesive strength and are therefore more suitable for application. The meaningful results obtained in this study will be beneficial to the scientific literature by shedding light on future scientific studies.

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Conflict of Interests

The author declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

Analyzing Content and Quality of YouTube™ Videos on Removal of Amalgam Fillings

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ABSTRACT

Objective: The objective of this study was to analyze the information quality and content of operational videos available on YouTube™ regarding removal of amalgam fillings.

Methods: The videos were determined using the words "Removal of amalgam fillings" and "Replacement of amalgam fillings" in the YouTube™ search section. 85 videos were included for analysis. Demographics of videos, viewers' interactions, and viewing rates were evaluated. The videos were analyzed in two parameters in terms of audio-visual quality and the SMART (Safe Mercury Amalgam Removal Technique) protocol steps.

Results: It was determined that dentist accounts ranked first (62%) in the distribution of video sources. While only 19% of the videos were of "Excellent" audio-visual quality, 49% were rated as "Moderate" and 33% were rated as "Poor". In the SMART evaluation, while only 10% of the videos got the "Maximal Useful" score, the majority of the videos got the "Slightly Useful" score (58%). There was no statistical relationship between the "View Rate" and "Interaction Index" variables of the videos *(p> 0.05).*

Conclusions: Operational videos about removal of dental amalgam fillings should be uploaded to YouTube™ after approval by the experts of the subject. Students should be warned about videos which contain insufficient information. Videos should be prepared in line with current information in the literature.

Keywords: Dental amalgam; E-learning; Mercury; Social media; Toxicity.

1. INTRODUCTION

Nowadays, medical documents, seminars and YouTube™ videos available on the internet are more popular than traditional learning methods (researching through books, journals, and conferences) (1). Over 80% of web-based search activities are for seeking medical information and support (2). At the same time, the internet is an area where professionals and laypersons share their experiences and knowledge (3). While specialist physicians continue to be the most important source of information in guiding patients' decisions, the effect of internet-based information on patients is also clearly visible (4).

YouTube™, an online video sharing platform, is the second most popular website in the world after Google. Almost 5 billion videos are watched per day on YouTube™, and the average user spends an average of 18:35 minutes per day on YouTube™ (5). There are academic studies which analyze the nature and quality of the information on YouTube™ videos, which include topics from the fields of medicine and dentistry to the treatments and prevention methods of various diseases (2). YouTube™ videos are not reviewed by a controller due to the nature of this platform, and videos can be of low or high quality from different sources and/or often non-standard (6). This means that videos on YouTube™ could potentially contain incorrect or incomplete information. Most studies agree that YouTube™ contains scientifically incorrect and sometimes misleading details which can harm patients' health (7,8). Information disseminated through videos can be published and shared on the internet without any institutional or quality control; therefore, it is critical to determine whether the information shared is correct, incorrect or incomplete (9). It has been stated that audio-visual methods improve patients' knowledge more than traditional written and oral information (10). However, it has been shown that the information gathered from patient-sourced videos is often potentially misleading (11).

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Ease of access to social media is countered by studies investigating the content and quality of YouTube™ videos and focusing on the relationship between social media and dental treatments such as root canal treatments, orthognathic surgery, dental implants, teeth whitening, and botulinum toxin for bruxism (7,11–14).

With the increasing number of patients requesting replacement of amalgam fillings, it has come to the fore that some protective measures should be taken against mercury toxicity during restoration removals. In a study conducted in 2019, mercury vapor released in particles formed during the removal of amalgam restorations was evaluated. While it was assumed that amalgam particle surfaces could oxidize over time, which may reduce evaporation, it was noted that dental particles produced from amalgam fillings during filling removal produced mercury vapor above threshold levels for a significant period of time (15). In another study, a significant localized source of mercury vapor was identified on amalgam that may be present for hours after preparation with the bur. It was shown that micron amounts of amalgam particles produced from dental high-speed drilling process generated measurable amounts of mercury vapor that often exceeded occupational safety thresholds (15). In addition, the World Dental Federation (FDI) recommends avoiding direct skin contact and sources of mercury vapor, including mercury, freshly mixed dental amalgam, and particles formed during the extraction of dental amalgam (16).

In today's world, in which access to information has rapidly increased, significant concerns have been raised about the potential adverse health effects of mercury exposure released from dental amalgam restorations in patients (17). Besides, in the World Health Organization (WHO) report, the statement "Recent research suggests that mercury may not have a threshold at which some adverse effects occur" is included (18). A protocol has been established that protects patients, physicians and clinical staff from mercury vapor that may be released during amalgam removal (19). There is no study in the literature analyzing the existence of protective protocols to be followed during amalgam removal in YouTube™ videos. The objective of this study was to analyze the information quality and content of operational videos available on the YouTube™ platform regarding the removal of dental amalgam restorations.

2. METHODS

The research was designed as a cross-sectional study. The study videos consisted of YouTube™ (https://www.YouTube.com) videos containing operational videos on removing dental amalgam material from the tooth. The screening took place between 09.00-18.00 on January 22, 2021. A new account was created before the search, and the historical data and cookies of the computer used were deleted. The search filter used was the default filter "Sort by relevance".

The videos were determined using the words "Removal of amalgam fillings" and "Replacement of amalgam fillings" in the YouTube™ search section. It was shown that most YouTube™ users searched the first 60–200 videos and only scanned the top 30 videos (20). Based on the percentage measurement values of the methods to be studied in the literature review, the total sample size was n=84, with an effect size of 0.4, a power of 90%, and a margin of error of 0.05, using the G-POWER program. In this study, the first 200 videos were determined for each search term. Initially, non-English videos, duplicate videos, and irrelevant videos, such as other medical field advertisements and financial advice videos, were excluded from the study. In the second evaluation, non-operational videos (Conference, Lecture, Animation etc.) were excluded, depending on the purpose of the study. The number of videos excluded from the study is as indicated in Figure 1.

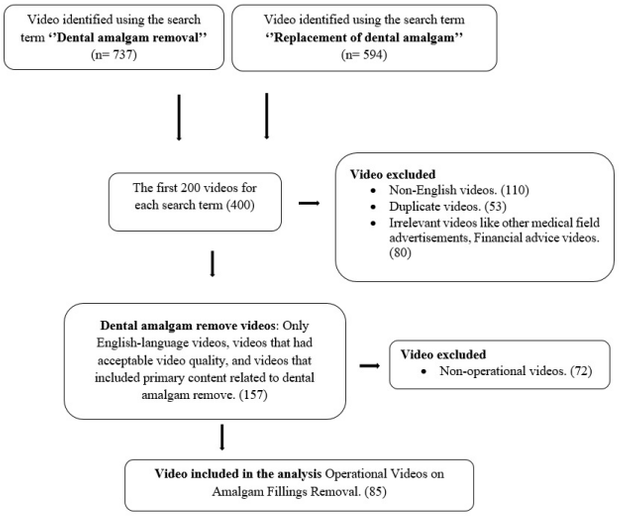

Figure 1. *Video Selection Workflow*

Evaluation of videos were made by two observers (M.B. and F.A.B) who were experienced in restorative dentistry. The few differences between observers in the evaluation criteria in the study were overcome by using two methods. The Safe Mercury Amalgam Removal Technique (SMART) protocol is a "yes-no" assessment without subjective data. To find out the reason for the difference between them, the observers watched the video together and gave a common score.

Prior to the audio-visual assessments, the researchers evaluated 10 videos (on different video subjects but on the same evaluation criteria) representing each score for each criterion. The correlation coefficient between the measurements was found to be 0.969, and a statistically significant, positive and very high-level relationship was obtained (*p=.000*). Since the audio-visual evaluation is a subjective evaluation and includes subjective comments, the final score was given by taking the average of the observers in the score differences in this criterion. Since the research was conducted on public internet data, it was decided that ethics committee approval was not required.

The data regarding view numbers, durations, comments, 'likes' and 'dislikes', and upload date were calculated for each video in our research. Viewers' interactions were calculated based on the interaction index ([likes − dislikes]/total views × 100%) and view rate (views/days after upload × 100%).

The account that uploaded the videos reviewed in this study to YouTube™ were classified under the following headings: Dentist/specialist, clinic/hospital/university, layperson, and other. According to their content, the videos were classified under three titles:

1. Only removal of dental amalgam fillings,
2. Removal and replacement of new restorations,
3. Safe removal of dental amalgam restoration procedures.

The videos were analyzed in two different parameters in terms of audio-visual quality and the presence of the Safe Mercury Amalgam Removal Technique (SMART) protocol steps (19). Audio-visual quality was graded by the researchers as "Excellent", "Moderate", or "Poor". The parameters considered in the assessment of audio-visual quality consisted of the following data: flow of video, information accuracy, quality of images or animations, video subtitles, and the extent to which the title included the projected video content (21).

Videos are graded between 0-20 according to the scores they get from the criteria specified in the SMART protocol (19), (Table 1). In this context, while they got 1 point for each criterion shown or mentioned in the video, they could not get any points from the wrong application or criteria not in the video.

Table 1. *SMART (Safe Mercury Amalgam Removal Technique) Protocols*

1	An amalgam separator must be properly installed and regularly maintained to ensure that mercury amalgam wastes are not released into the effluent leaving the practice.
2	Every room where mercury-containing fillings are removed must have a high-volume air filtration system (such as an at source oral aerosol vacuum and an adequate filtration system capable of absorbing the formed mercury vapor and amalgam particles).
3	Windows should be opened to reduce the mercury concentration in the environment. (If it is possible)
4	Before the procedure, the patient should be given a slurry of charcoal, chlorella or similar adsorbent to rinse and swallow.
5	Protective gowns and drapes should be used for the dentist, staff and patient.
6	The dentist and staff in the room should wear non-latex nitrile gloves.
7	The dentist and staff in the room should wear face shields and a hair/head cover.
8	The dentist and all dental staff in the room should wear a properly sealed, respirator-grade mask for mercury filtration.
9	A head/face/neck barrier should be used to protect the patient's skin and clothing.
10	External air or oxygen supplied to the patient through a nasal mask should also be used to ensure that the patient does not inhale any mercury vapor or amalgam particles during the procedure.
11	A rubber dam made of non-latex nitrile material should be used.
12	A saliva ejector should be placed under the rubber dam to reduce patient exposure to mercury.
13	During removal of amalgam filling, an oral aerosol vacuum should be used close to the operation area.
14	High-speed evacuation provides better capture when equipped with a non-essential but preferred cleaning device.
15	It requires copious amounts of water to reduce ambient mercury levels, and a conventional high-speed drain device to capture mercury discharges.
16	Amalgam needs to be cut into pieces and removed in as large pieces as possible.
17	After the removal process is completed, the patient's mouth should be rinsed with a slurry of charcoal, chlorella or similar adsorbent.
18	Dentists should follow local regulations regarding the proper handling, cleaning and/or disposal of mercury-contaminated components, clothing, equipment, room surfaces, and flooring in the office.
19	During the opening and maintenance of suction traps in operating rooms or the main suction unit, dental staff should use the appropriate personal protection equipment described above.
20	It should be noted that as a safety precaution, the IAOMT does not recommend removal of amalgam fillings for pregnant or breastfeeding women.
	Total: 20 Points: Not Useful (0p), Slightly Useful (1-5p), Moderately Useful (6-10p), Very Useful (11-15p), Maximal Useful (16-20)

In this study, the descriptive statistics of the data are given as number, percentage, mean and standard deviation. As the first step in analyzing the data, the assumption of normality was checked with the Shapiro Wilk test. The Kruskal Wallis test was used to examine the difference between the averages of three or more independent and non-normally distributed groups. The Bonferroni Post Hoc test was performed to identify the group or groups that made the difference. In order to examine the relationship between continuous variables, the Spearman correlation was used when the normal distribution assumption was not met. The Fisher's Exact Test was applied in the cases

where the sample size assumption was not provided in the analysis of categorical variables. The Kendall's Tau correlation analysis was conducted to examine the relationship between categorical and ordered variables and continuous variables. Analyzes were carried out using the IBM SPSS Statistics 25 program. The statistical significance level was set as *p<0.05.*

3. RESULTS

Descriptive analyzes are as stated in Table 2**.** In the classification made according to the video content, the videos containing the highest number of safe amalgam removal protocols (42% – n= 36) were included [Only removal of dental amalgam fillings (25% – n=21), Removal and replacement new restorations (33% – n=28)]. The distribution of videos in different video sources and the number of videos in these sub-groups were determined according to the evaluations received from the SMART score, and audio-visual quality (Fig.2). The Fisher's Exact test was used to examine the relationships between the video distributions based on audio-visual quality/SMART scores/Video sources variables. There was no statistically significant relationship between the variables in terms of video distributions *(p>0.05).*

The Kendall's Tau correlation analysis was performed to evaluate the relationship between the "Interaction Index" and "Viewing Rate" values with audio-visual quality and SMART score (Table 3). As a result of the correlation analysis, "Interaction Index" had a positive correlation with audio-visual quality and SMART score *(p <0.05).* In addition, while there was a positive correlation between "Viewing Rate" and "Audio-visual quality" *(p< 0.05),* there was no statistically significant correlation between "Viewing Rate" and SMART scores *(p> 0.05)*. In addition, Spearman correlation was calculated to examine the relationships between the "Interaction Index" and "Viewing Rate" variables. There was no statistically significant correlation between the "Viewing Rate" and "Interaction Index" variables *(p>0.05)* (Table 3).

There is no statistically significant difference between the groups formed according to the SMART assessment in terms of "Interaction Index" and "Viewing Rate" mean values *(p>0.05)* (Table 4). None of the videos scored zero points. There are two videos with the lowest SMART score (1p) and seven videos with the highest score (20p). The number of views of one of the videos with the lowest SMART score (1p) is 674.827. This number is the 2nd highest number of views in the study. The SMART score of the video with the highest number of views is 4p, and the lowest one is 3p. The SMART score of the video with the highest ''Interaction Index'' value is 3p, while the SMART score of the video with the highest ''Viewing Rate'' is 4p. Among the seven videos that received full SMART score (20p), the most viewed video was the 4th most watched video with 129.564 times.

As a result of the analysis, a statistically significant difference was found between the mean value of the "Interaction Index" and "Viewing Rate" variables according to the audio-visual quality score groups *(p<0.05)* (Table 4). In the "Interaction Index" variable, a statistically significant difference was found between the mean value of the "Poor" and "Excellent" groups *(p=,001)*. In the "Viewing Rate" variable, a statistically significant difference was found between the mean value of the "Poor" group and the mean value of the "Moderate" and "Excellent" groups *(p=.028* and *p=.037)*.

Table 2. *Descriptive statistics of videos*

	Minimum	Maximum	Mean	Standard Deviation
Number of views	13	1267533	37863.6	156.180.13624
Duration in minutes	0.16	30	4.6642	5.62949
Number of likes	0	9568	195.5765	1045.55743
Number of dislikes	0	425	13.4706	51.03677
Days since upload	26	4351	1491.8941	980.97557
Number of comments	0	417	30.4937	76.89485
Interaction index	-4.17	38.46	1.2055	4.52222
Viewing rate	2.45	130270.61	2854.2152	14477.03127

Table 3. *Correlation analysis evaluating the relationship between "Interaction Index" and "Viewing Rate" values, Audio-visual quality, and SMART score*

	Audio-Visual Quality		SMART Scores		Viewing Rate	
	Rho	p	Rho	p	Rho	*p*
Interaction Index	.307	.000*	.217	.011*	.122	.266
Viewing Rate	.240	.005*	.159	.059		

Spearman correlation: (Interaction index, Viewing Rate) Significance level (p>0.05)

*Kendall's Tau correlation analysis (Interaction index , Audio Visual Quality and SMART Scores / Viewing Rate, Audio Visual Quality) *(p<0.05)*

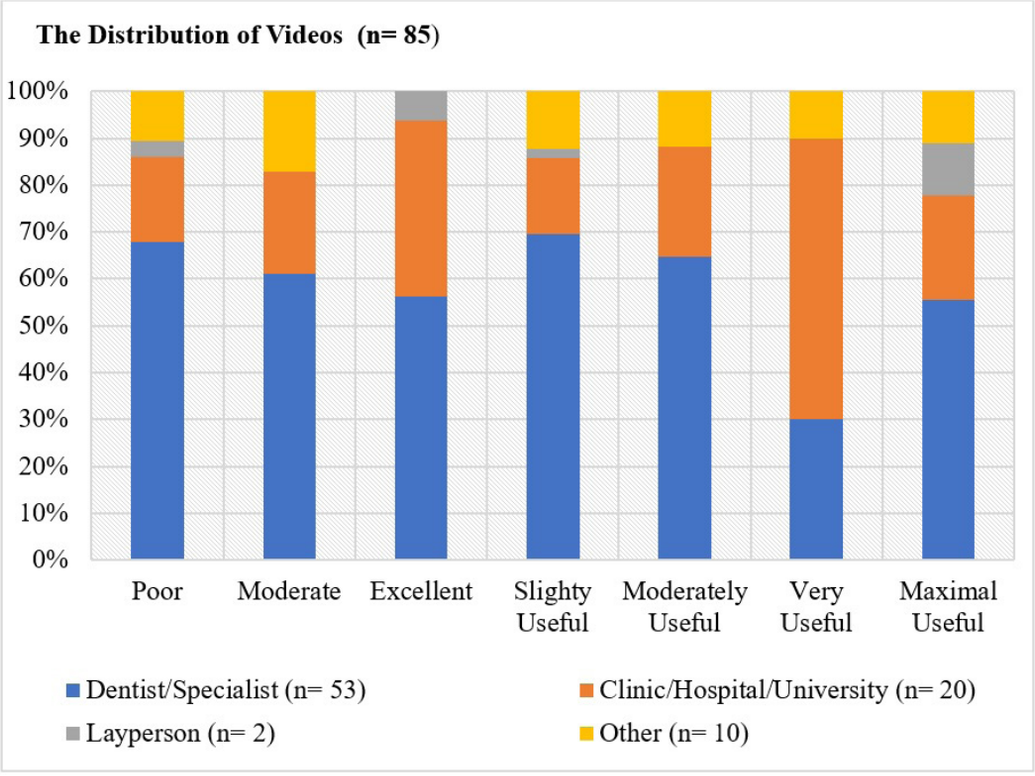

Figure 2. *The distribution of videos in different video sources and the number of videos in these sub-groups were determined according to the evaluations they received from SMART Score and Audio-visual quality. (Fisher's Exact test: (p>0.05)).*

Table 4. *The relationship between groups in the evaluation made according to SMART scores and Audio-visual quality rating*

SMART Scores										Audio-Visual Quality						
	Slightly Useful (n= 49)		Moderately Useful (n= 17)		Very Useful (n= 10)		Max Useful (n= 9)		*p*	Poor (n= 28)		Moderate (n= 41)		Excellent (n= 16)		*p*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	
Interaction Index	1.509	5.933	0.806	0.836	0.521	0.184	1.0623	0.722	.058	1.69	7.21	0.92	2.69	1.078	0.849	.002*
Viewing Rate	4138.37	18977.86	741.63	1181.76	2061.86	2992.73	733.50	1176.75	.164	804.69	2025.61	4650.84	20589.48	1837.029	4248.32	.012*

SMART scores; Kruskal–Wallis test= Significance level (p > 0.05)

*Audio-Visual Quality; Kruskal–Wallis test= Significant difference between groups *(p < 0.05)*

Bonferroni correction= (Interaction index/p=.001) ''Poor'' higher than ''Excellent''

= (Viewing Rate/p=.028 ve p=.037) ''Moderate'' and ''Excellent'' higher than ''Poor''

SD, standard deviation

4. DISCUSSION

The information provided by YouTube™ videos is not subject to peer review and is not pre-evaluated by the authors in the relevant field. This situation revealed the necessity of analyzing the videos in the related field. There are many studies evaluating Youtube™ videos on topics related to dentistry and general health (1,2,7–9,12–14,22–25). Our study is the first to analyze YouTube™ videos on removing dental amalgam fillings.

Dental amalgam is an alloy mixture of metallic mercury and mainly silver, tin, copper, and zinc, and its potential risk for chronic mercury toxicity is one of its major drawbacks (26). The use of amalgam fillings has been banned or restricted in Sweden, Norway, Denmark and Germany since 2008. In addition, as of 2018, the European Parliament has accepted a ban on the use of amalgam in clinical practice for children under 15 years of age and pregnant or breastfeeding women (27). Although the use of dental amalgam has decreased in favor of resin-polymer-based restorative materials in the last decade, many people will have amalgam fillings in their teeth for decades because well-placed dental amalgam restorations usually remain in the mouth for many years (26,28).

When the results of the recently published studies evaluating Youtube™ videos on dentistry are examined, it is seen that the "Viewing Rate" and "Interaction Index" variables are in a wide range. Although there are studies with similar results to the "Interaction Index" rate of the study (24, 25), it was determined that the closest one to our study was a study on cleft lip and palate (24). There is no correlation between "Viewing Rate" and "Interaction Index" in our study. In the "Viewing Rate" and "Interaction Index" equations, the "Total number of views" parameter is the numerator in one equation and the denominator in the other. In addition, the continuous increase in the time from the day the videos are uploaded to the present reduces the possibility of correlation between these two evaluation parameters. However, another study investigating a similar relationship found a correlation between these two variables in some subgroups (12).

Most of the videos in this study were uploaded from the accounts of dentists and professional institutions (hospitals, universities and clinics). The number of videos from non-professional uploaders is less. Among the videos that we did not include in the study, there are quite a lot of lay user videos that mention dental amalgam-mercury toxicity. However, since the videos in our study are operational videos, it is normal for professional uploaders to be in the majority._There are similar studies with the same parallel results (13,24,25). In this study, the video with the highest interaction index belongs to a non-professional uploader. Conversely, the video with the lowest interaction index was uploaded from a dentist's account. There are also studies which differ in terms of interaction levels between sources (13,22). In addition, there are studies in the literature indicating that non-professional uploaders share more videos on related health issues (12,23). Since the interaction values depend on the liking criteria of the viewers, we can say that whether the uploader is a dentist or non-professional does not directly affect the viewers' liking or watching the videos.

In the evaluation made according to the scores they got from the SMART protocol, more than half of the videos were in the "Slightly useful" category. In parallel with our research, although there are studies in which low information videos are more common (21–23), there are also some studies in which moderate and high information videos are more (12,13,24). There is also a positive correlation between the "Interaction Index" of the videos in the study and their SMART scores. It can be said that the videos that receive more interaction are those that contain more information about preventive measures during amalgam removal. The use of detailed equipment and the explanation of protective measures in the videos for dental amalgam removal may attract the attention and appreciation of the viewers; however, they are supposed to know all protective measures in order for the viewers to notice the videos that do not contain these protective measures. This is not something expected from the ordinary audience. Measures against mercury vapor toxicity are rare in video content. It is thought that the results of the study on mercury vapor emitted from dental amalgam particles have not yet increased the awareness of dentists on this issue (15, 29).

We can say that the audio-visual quality of the videos in our study is in correlation with the "Viewing Rate" and "Interaction Index" rates. This indicates that features, such as video resolution and sound quality, good flow, and presence of subtitle, directly affect the viewing and interaction of the users. These positive features can attract the attention of ordinary viewers, regardless of the content of the video and the accuracy of the information it contains. The ranking according to the number of videos in the groups was determined as Moderate> Poor> Excellent. This ranking is different from two studies in which the same assessment method was used before (14,24). In the other two studies, the least number of videos are in the "Poor" category. Also, in the present study, the most watched video and the video with the highest "Interaction Index" are in the "Poor" audio-visual category. The videos with the highest "Viewing Rate", the most liked video, and the most commented video are in the "Moderate" audio-visual category. Only 4 of the 16 videos that received "Excellent" rating are in the "Maximal useful" group in the SMART classification. In addition, of the 57 videos with moderate and above audio-visual quality, 30 of them have a SMART score of moderate and above. This situation reveals that the number of useful videos in the study is insufficient in terms of the criteria evaluated.

The "Slightly useful" part of the videos uploaded by dentists constitutes the subgroup with the highest number of videos (n= 34). More than half of the "Excellent" and "Maximal useful" videos were uploaded to Youtube™ by dentists. 17% of the videos uploaded by dentists are in the "Excellent" group, whereas 9 % are in the "Maximal useful" group. Besides, the majority of the videos in the "Poor" and "Minimal useful" groups were also uploaded to YouTube™ by

dentists. The distribution in these rates (the fact that dentists have more videos with both high and low scores compared to other uploaders) is related to the fact that the majority of the uploaders of the videos are dentists.

YouTube™ video content is highly variable. Therefore, search results can constantly change as interests and video viewing times change over time. There may be inconsistencies in the search results because new videos are added every day or old videos are deleted. Also, the order of search results changes with interaction and time. Therefore, the limitation of the current study is that the data collection method is instantaneous, as in similar studies. Another limitation is the keywords used in the research. In this study, we performed two independent searches using the keywords "Removal of dental amalgam fillings" and "Replacement of amalgam fillings". However, some patients or physicians may use other search terms and reach different results. In addition, although the process of removing amalgam restorations is common all over the world, the evaluation of the videos only in English is among our limitations. The oldest video in our study is 12 years ago. One of our limitations is that it is practically difficult to evaluate the videos of the old years according to the literature knowledge and technical possibilities of their own period. However, establishing temporally separate evaluation criteria does not match the purpose of our study. The fact that viewers still have access these videos requires them to be evaluated in terms of today's conditions. Another limitation of our study is the SMART rating scale created for the study. It is used for an overall assessment of compliance with the precautions to be taken during operations. Perhaps in other studies, related videos can be evaluated better with different evaluation criteria. In addition, only operational videos were evaluated in our study. The information contained in non-operational videos should also be analyzed.

5. CONCLUSION

Within the limitations of this study, although social media provides a great advantage in terms of reaching a wide audience, it can also cause the misinformation to be easily spread to a wide audience with the same method. Operational videos about the removal of dental amalgam fillings should be uploaded to YouTube™ after approval by the experts of the subject. In addition, the technical features of operational videos are supposed to be better. In the study, there are videos containing current literature information on the preventive measures recommended during amalgam filling replacement. However, the rate of these videos should increase. More studies are needed to investigate the quality of the content of related videos on the change of dental amalgam fillings on different social media platforms.

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Conflicts of interest

The authors have no conflicts of interest to report.

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Clinical and Experimental Health Sciences

Perceived Stress and Perceived Vulnerability at Healthcare Workers during COVID-19 Pandemic

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ABSTRACT

Objective: This study evaluated the psychological effects of the COVID-19 pandemic on healthcare workers (HCWs) and determined several risk factors.

Methods: An online cross-sectional survey was administered to 244 HCWs recruited via the Google Docs platform. The 36-item questionnaire comprised three domains: demographic details, the Perceived Stress Scale-10 (PSS-10), and the Perceived Vulnerability to Disease Questionnaire (PVDQ).Multiple linear regression analysis was used to determine the risk factors for adverse psychological responses.

Results: Overall, 244 HCWs aged between 20 and 60 years old participated in the survey and the mean scores for perceived stress (PS), perceived infectability (PI), and germ aversion (GA) were 20.15, 28.83 and 47.78, respectively. Additionally, they were positively associated with gender. Females' score on PS (p=0.001), PI (p=0.017), and GA were also significantly higher than men (p=0.001).Scores on PSS-10 showed a significant difference between age groups (p=0.010) in contrast to GA (p=0.515) or PI (p=0.346).The regression model showed that the PI scores were higher among men (B=3.145) than among women (p=0.019).The analysis showed significant effects working during COVID-19 on PI scores (B=3.101; p=0.006).Furthermore, GA was also significantly related to worsening of the COVID-19 pandemic (B=2.73; p=0.004) and was higher among females (B=4.622; p<0.001).

Conclusion: According to the results of the study, gender, age, professional experience and knowledge, and working during a pandemic were important factors for PS and PVD. Additionally, supporting the mental health for HCWs obtaining adequate support and taking precautions are essential.

Keywords: Perceived stress, perceived vulnerability, COVID-19

1. INTRODUCTION

The pandemic has been difficult for some healthcare workers (HCWs) because of the high number of COVID-19 patients and long working hours. On the other hand, others worked less because of public health-related precautions (1). Initially, most countries spread the pandemic but the pandemic is running quickly and includes few waves (2).

Some studies have found that long working hours cause anxiety, depression, and disrupts mental health among physicians and nurses (3-5). Several studies in the previous literatüre, have examined the relationship between work-related stress and impaired mental health, especially among in doctors and nurses. This is a significant factor because it includes either providing quality service to patients or being professional workers (5-8). During the pandemic, observing and protecting mental health are the primary significant factors for HCWs. Therefore, they should be aware of the factors that affect their mental health and take preventive measures (9).

With the spread of COVID-19 and under the guidance of the World Health Organization (WHO), people have begun to wash their hands more frequently. Concomitantly, we have become more afraid of infection than ever before. People have begun to disinfect various places and objects using alcohol and to wear masks. These behaviors are based on a heightened perceived vulnerability to disease (PVD). A psychological scale has been developed to measure this tendency (10). Therefore, perceived stress (PS) has been defined as a person's feelings or thoughts about how much stress he or she is under over a given time period. Gonzales et al. (11) explained that the high levels of vulnerability to contracting COVID-19 as perceived by the population over 60 years of age with a baseline disease at the beginning of the

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epidemic in Spain and their avoidance of dental care. Zhang et al. (12) explained that quarantine did not increase the perceived stress of participants with existing

chronic diseases. However, it can be more difficult to access medical treatment and medication during quarantine, which may lead to higher stress. It produces a series of mental health problems, such as anxiety, depression, and psychosomatic illness, as well as cardiovascular, metabolic and immune regulatory function damage (13). Additionally, it can severely diminish the quality of one's life.

This study evaluated the levels of PS and PVD among HCWs during the COVID-19 pandemic in Turkey to assess the psychological impact of the pandemic in Turkey and focus on the HCWs' for psychological reverse signs, and provide a guide for implementing measures to prevent public health crises in other countries.

2. METHODS

The ethics committee of the Bakirkoy Dr. Sadi Konuk Training and Research Hospital (approval number 2020-179) approved this study. Informed consent was obtained from all participants after informing them about the study based on the provisions of the Declaration of Helsinki regarding research on human subjects.

2.1. Study Design and Population

An online cross-sectional survey was administered to HCWs' during the COVID-19 pandemic. The link to the online survey (Google Forms) was provided on behalf of the researcher. Participants' information in the survey was processed anonymously. The survey was sent through e-mails and WhatsApp messages to participants who provided voluntary consent and their personal identifying information was excluded. Data was collected from across various hospitals of Istanbul because of the higher number of COVID-19 cases during the pandemic. Power of the sample size was determined using http://sampsize.sourceforge.net/iface/. The power analysis gave a power of 0.95, which showed that the estimated sample size was adequate (n=226).

2.2. Instruments

The questionnaire consisted of 36 items under three domains: demographic details, the perceived stress scale-10 (PSS-10), and the perceived vulnerability to disease questionnaire (PVDQ).

The independent variables included in this study were gender, age, health profession (physician, nurse, dentist), years of working experience (0–10, 11–20, 21–30, and 31–40 years), and whether they worked related to COVID-19 (no/yes).

2.3. Perceived Stress Scale (PSS-10)

The PSS-10 has been widely used to evaluate perceived stress (14). The Turkish version of the PSS-10 (15) was used to measure the degree of stress perception on a 5-point Likert scale (from 0=never to 4=very often). The total scores ranged from 0 to 40, with higher scores corresponding to higher PS (0–13: low stress level; 14–26: moderate stress level; 27–40: high stress level) (16). In this study, the minimum and maximum scores on this scale were 0 and 35, respectively. Cronbach's coefficient of reliability for the PSS-10 was 0.893.

2.4. Perceived Vulnerability to Disease Questionnaire (PVDQ)

The 15-item PVDQ (15) measures two factors: perceived infectability (PI; 7 items), which is related to the beliefs of one's own suspicions of flu, colds, and other infectious diseases and Germ Aversion (GA; 8 items), which is related to the cognizance of disturbance in situations with infection or a pathogen (9). Figure 1 lists each of the 15 items, along with the factor loadings for each factor. The internal consistency (Cronbach's alphas) of these subscales in this study were 0.792 for PI with minimum and maximum scores of 8 and 49, respectively, and 0.727 for GA with minimum and maximum scores of 18 and 56, respectively.

2.5. Data Analyses

This cross-sectional study included individuals aged between 20 and 60 years, while considering gender. Analysis of variance (ANOVA) was used since the data was normally distributed. Data analysis was done using SPSS® Statistics for Windows, version 22.0 (IBM, NY, USA). The differences between groups in all variables were analyzed using the Spearman rank correlation, LSD test, Student's *t*-test, and Dunn test. Regression analyses was used for categorical data. Furthermore, Cronbach's α was calculated for validity and reliability of the scales.

3. RESULTS

The final sample consisted of 244 HCWs aged between 20 and 60 years after excluding two participants who provided incomplete or insufficient information. Therefore, the sample comprised 50 (20.5%) men and 194 (79.5%) women.

Table 1 shows the basic demographic characteristics of the HCWs. Three quarter of the study participants were female (79.5%), 77% were married, and 38.1% had 11–20 years of experience. The highest age range of the participants was 41–50 years who were also married. The mean scores for PS, PI, and GA were 20.15, 28.83, and 47.78, respectively. Of these, 68 (27.9%) were general dental practitioners, 67 (27.5%) were nurses, 41(16.8%) were specialist dentists, and 28 (11.5%) were specialists (Table 1).

Table 1. *Demographic characteristics of participants*

Characteristic		N	(%)
Gender	**Male**	**50**	**20.5**
	Female	**194**	**79.5**
Age	20-30	32	13.1
	31-40	90	36.9
	41-50	103	42.2
	51-60	19	7.8
Married	**no**	**56**	**23.0**
	yes	**188**	**77.0**
Have Children	no	67	27.5
	yes	177	72.5
Healthcare Workers Profession	Specialist	28	11.5
	Physicians	8	3.3
	Specialist Dentists	41	16.8
	General Dental Practitioner	68	27.9
	Nurse	67	27.5
	Others	32	13.1
Clinical experience (years)	**0-10**	**59**	**24.2**
	11-20	**93**	**38.1**
	21-30	**79**	**32.4**
	31-40	**13**	**5.3**
Employed	University	14	5.7
	Hospital	41	16.8
	Pandemia hospital	23	9.4
	Center for Oral Health Care	96	39.3
	Private practice	17	7.0
	Other	**53**	**21.7**
Worked during COVID-19 pandemic	**No**	**94**	**38.7**
	Yes	149	61.3
Place of work during COVID-19 pandemic	Filiation	79	48.8
	Intensive care units (ICU)	13	8.0
	Emergency	10	6.2
	Other	47	31.5
COVID-19 test	**No**	**156**	**63.9**
	Yes	**88**	**36.1**
Exposed to coronavirus disease	No	69	76.7
	Yes	21	23.3
Psychological response			
Perceived Vulnerability to infection	**Mean ±SD**		
Infectability subscale	28.83 ± 8.47		
Germ-aversion subscale	47.78 ± 7.34		
PSS-10*	20.15 ± 6.46		

**Perceived Stress Scale*

Significant gender differences were observed in the assessment of the participants. GA, PI, and PS were positively associated with gender. Additionally, females' scores on the PS (p=0.001), PI (p=0.017), and GA (p=0.001) were significantly higher than those of men. A statistically significant difference was found between the levels of PS according to gender (Table 2). Scores on the PSS-10 showed significant differences between the age groups (p=0.010). The scores on the PSS-10 also decreased with age. The PSS-10 scores of the 20–30 and 31–40 years age groups were significantly higher than those aged 51 years and above (p=0.022; p=0.010). The PSS-10 scores were positively associated with age. There were no significant differences between age groups for either GA (p=0.515) or PI (p=0.346) (Table 2). The mean PSS-10, PI, and GA scores of particiopants who worked during the COVID-19 pandemic were 21.41±6.40, 29.91±8.51, and 48.87±6.11, respectively. All scores were higher in the group of HCWs who worked during the COVID-19 pandemic compared to the non-working group (p<0.05) (Table 2).

We found that most HCWs reported moderate levels of stress 138 (56.6%); however, 104 (42.6%) participants reported high stress levels, with scores ranging from 0 to 34. In this study, scores ranging between 14 and 26 were deemed as moderate PS associated with COVID-19. The scores of 27 and above were regarded as high PS associated with COVID-19. About 104 participants (42.6%) scored high on PS, which was significantly related to the HCWs who worked during the COVID-19 pandemic (OR=2.21 %95CI: [1.26–3.89]; p=0.006). After adjusting for COVID-19 test positivity, the relationship remained significant (OR=3.11 %95CI: [1.01–9.54]; p= 0.047). PI was affected by the type of HCW's profession (p=0.007) (Table 3). However, there were no statistically significant differences in the GA (p=0.266) and PSS-10 scores (p=0.103). No significant difference was observed in the evaluation of the place who worked during the COVID-19 pandemic (p>0.05) (Table 3).

Accordingly, PI and GA were positively and significantly related with gender. PS was positively associated with PI in both genders; however, there was no association between GA and PS among men (Table 4). Accordingly, PI and GA were positively and significantly related to COVID-19 test negativity. PS was negatively associated with GA in the COVID-19 test-positive group; however, the difference was not statistically significant (p=0.267). There was no positive correlation between PI, GA, and PSS-10 for COVID-19 test positivity (Table 4).

The regression model showed that the PI scores were higher among men (B=3.145) than among women (p=0.019). The analysis showed significant effects of working during COVID-19 (B=3.101) on the PI scores (p=0.006). Accordingly, PI scores were negative but not significantly affected by age. Furthermore, GA was significantly related to worsening of the COVID-19 pandemic (B=2.733, p=0.004) and was higher among females (B=4.622; p<0.001) (Table 5). Moreover, regression analysis showed significant effects of gender and worsening COVID-19 pandemic on PSS-10 scores (p=0.001 and p<0.001, respectively) (Table 5).

Table 2. *Differences in gender, age groups, and worked during the pandemic in PSS-10 and PVDS among HCWs*

	GENDER			AGE GROUPS					WORKED DURING THE PANDEMIC		
	male	female		20-30	31-40	41-50	51+		Yes	No	
	(n=50)	(n=194)	p	(n=32)	(n=90)	(n=103)	(n=19)	p	(n=149)	(n=94)	p
	Mean(SD)	Mean(SD)		Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)		Mean(SD)	Mean(SD)	
PI	26.28±8.88	29.48±8.26	0.017	27.78± 9.66	30.10± 8.14	28.09± 8.54	28.58±7.31	0.346	29.91±8.51	27.07±8.19	0.011
GA	44.22±8.56	48.70±6.71	0.001	47.06± 7.94	48.27± 6.88	47.65± 6.70	47.42± 11.30	0.515	48.87±6.11	46.22±8.61	0.051
PSS-10*	17.38±7.04	20.86±6.13	0.001	21.63± 5.93	21.37± 5.97	19.11± 6.96	17.53± 5.26	**0.010***	21.41±6.40	18.12±6.10	0.001

Note.PSS-10 Perceived Stress Scale (PSS-10)*

Table 3. *Type of HCWs profession and place of worked differences in PSS-10 and PVDS during the COVID-19 pandemic*

HCWs Profession	n	PI	GA	PSS
Specialist	28	26.32±9.46	45.96±10.13	17.89±6.80
Physicians	8	31.00±8.98	50.38±4.69	23.00±5.35
Specialist Dentists	41	26.59±8.51	47.83±7.32	20.05±5.26
General Dental Practitioner	68	26.53±7.26	46.78±6.48	19,04±6,51
Nurse	67	30.91±8.00	48.45±7.88	20.94±6.61
p		0.007*	0.27	0.103
Place of work				
Filiation	79	29.92±8.14	48.75±5.20	21.59±6.06
Intensive care units	13	29.92±10.68	50.38±6.16	21.00±6.73
Emergency units	10	31.40±10.72	49.00±11.96	23.90±7.19
p		0.841	0.28	0.551

Table 4. *Spearman correlations for all categorical data, gender, and COVID-19 case correlations during pandemic*

ALL CATEGORICAL DATA			PI	GA	PSS
PI		r		0.416	0.42
		p		0	0
GA		r	0.416		0.269
		P	0		0
PSS		r	0.42	0.269	
		p	0	0	
GENDER					
	PI	r		0.433	0.389
N=50		P		**0.002**	**0.005**
	GA	r	0.433		0.273
		P	**0.002**		**0.055**
	PSS	r	0.389	0.273	
		P	**0.005**	**0.055**	
	PI	r		0.383	0.386
N=194		P		**0**	**0**
	GA	r	0.383		0.203
		P	**0**		**0.005**
	PSS	r	0.386	0.203	
		P	**0**	**0.005**	
COVID-19					
negative case	PI*	r		0.639	0.552
N=69		p		0	0
	GA**	r	0.639		0.516
		P	0		0
	PSS ***	r	0.552	0.516	
		p	0	0	
positive case	PI*	r		0.015	0.099
N=69		p		**0.947**	**0.668**
	GA**	r	0.015		-0.254
		p	**0.947**		**0.267**
	PSS***	r	0.099	-0.254	

*Note. *Perceived Infectability (PI) **Germ Aversion(GA) ***Perceived Stress Scale (PSS-10)*

Table 5: *Regression model for all categorical scores*

PI	Unstandardized Coefficients		Standardized Coefficients	P	95.0% Confidence Interval for B	
	B	Std. Error	Beta		Lower Bound	Upper Bound
age	-.085	.740	-.008	.908	-1.544	1.373
gender	3.145	1.335	.150	.019	.515	5.776
Worked in pandemic	3.101	1.117	.178	.006	.900	5.301
a. Dependent Variable: infectability subscale						

GA	Unstandardized Coefficients		Standardized Coefficients	P	95.0% Confidence Interval for B	
	B	Std. Error	Beta		Lower Bound	Upper Bound
age	.266	.623	.030	.670	-.961	1.493
gender	4.622	1.123	.257	.000	2.409	6.834
Worked in pandemic	2.733	.940	.183	.004	.882	4.584
a. Dependent Variable: Germ-aversion subscale						

PSS-10	Unstandardized Coefficients		Standardized Coefficients	P	95.0% Confidence Interval for B	
	B	Std. Error	Beta		Lower Bound	Upper Bound
age	-.609	.541	-.077	.261	-1.674	.456
gender	3.199	.975	.200	.001	1.278	5.119
Worked in pandemic	3.006	.816	.227	.000	1.400	4.613
a. Dependent Variable: PSS						

4. DISCUSSION

Pandemics are periodic facts that have private characteristics in terms of causality, progression, and control precautions (17). All HCWs provide care for patients with COVID-19. The rapid spread of COVID-19 and the severity of symptoms have been extremely stressful for HCWs due to the limits of the healthcare system (9). Therefore, HCWs face a greater risk of exposure, excessive workloads, ethical dilemmas, and a rapidly evolving practice that greatly varies from what they are familiar with (18–20). Additionally, all other problems and challenges continued during the vaccination period.

The results of our study showed that levels of distress among HCWs were high during the COVID-19 pandemic. Increasing anxiety levels can lead to increased recurrent behaviors and precautions among people during the COVID-19 pandemic. People often ignore their psychological health while only supporting their physical health. Therefore, individuals with low PVD levels may prefer to protect their mental well-being at the expense of their physical health. On the other hand, individuals with high PVD levels show high avoidance behaviors that prevent their anxiety (21–23).

The study population comprised 244 HCWs, of which 79.5% were women. Several studies in the literature have unequal gender distribution (24–27). Additionally, our findings indicate that women reported more PS than men. Furthermore, regression analysis was used to reduce the effect of unequal gender distribution and we found results similar to the previous studies.

Our study results showed that PS, PI, and GA were found more frequently among females than males. One study found no significant gender differences in PI; however, differences existed in the GA (11). Another study reported that either GA or PI was positively associated with both genders (28). When we analyzed our results on the basis of subgroups, we found that similar to previous studies (10, 29, 30), females' scores on PI and GA were significantly higher than those of men.

Coninck et al. (31) reported that age differences were found for GA only: older age categories reported significantly higher GA than younger ones. Another study explained that people with a higher education degree showed higher anxiety levels about the COVID-19 exposure (32). Younger groups are much less likely to have experienced difficult life events compared to older age groups because of which they can have higher stress levels; however, middle-aged and older age groups can manage the current process due to having experienced stressful life events previously. Our study results showed that younger HCWs may have higher PS levels during the COVID-19 pandemic compared with older HCWs. In Turkey, the Ministry of Health declared that older HCWs who had a chronic disease or/and had a threatening illness did not have to work during the pandemic. For this reason, older HCWs who worked during the pandemic had more experience in their jobs and had no illness. Because of that, our study results found that PS decreased with age.

We found that HCWs who worked during the pandemic had significantlly higher PS values than those who did not work during the pandemic. Ehrenstein et al (33) found 28% of professionals may abandon work in favour of protecting themselves and their family. Quereshi et al (34) said that the most significant barrier to HCWs' willingness to work was fear for their own and their families' health. Balicer et al (35) anticipate up to 50% of HCWs being unwilling to work, with clinical staff more likely to attend than non-clinical ones.

We also found that PI was affected by the type of HCWs' profession; physicians and nurses were more affected than others. However, GA and PSS-10 scores were not affected by the type of their profession. Therefore, it was a stronger trigger when a pathogen threat was not seen as an immediate environmental threat (36). Du et al. (37) found that frontline HCWs had moderate to severe levels of PS (PSS scores≥14), and depressive and anxiety symptoms were more common among women.

Lai et al. (24) reported that HCWs from 34 hospitals in different regions of China with direct contact with COVID-19 patients were at significantly higher risk of experiencing symptoms related to post-traumatic stress, depression, anxiety, and insomnia. Another Chinese study (12) reported that non-medical HCWs had a lower prevalence of anxiety, insomnia, depression, and obsessive-compulsive symptoms than HCWs, and they emphasized the need for attention and recovery programs.

Weilenmann et al. (1) reported that female nurses at the frontline who were exposed to COVID-19 patients had higher stress levels compared with male non-frontline physicians who were not exposed to COVID-19 patients. Lai et al. (24) reported that being a woman, having an intermediate professional title, and working in the frontline directly treating patients with COVID-19 were associated with severe symptoms of depression, anxiety, and distress compared with working in second-line positions and working in a tertiary hospital. They found it to be an independent risk factor for all psychiatric symptoms after adjustment. Li et al. (25) declared that the general public and non-frontline nurses had significantly higher vicarious traumatization scores than frontline nurses. Moreover, frontline nurses had higher psychological endurance while non-frontline nurses were more likely to suffer from psychological problems. Finally, the frontline nurses were more knowledgeable about the pandemic, were selected voluntarily and were the primary staff with working experience and psychological capacity compared with non-frontline nurses and the general public. Our study results showed that all scores were higher in the group of HCWs who worked during the COVID-19 pandemic compared to the not-working group. Furthermore, PS score was higher in the emergency unit than the intensive care unit and filiation. Females had higher PS score than males. Our results supported the results of these two previous studies.

In our study, PI was more predictive of both genders whereas GA was more predictive of only women On the other hand, PI and GA were positively related with COVID-19 test negativity. One study found that the moderate and low PVD groups did not show significantly less preventive behavior or significantly less knowledge and emotional distress than the high PVD group (32). It was observed that individuals with high PVD scores were more cautious, had more information about the disease, and experienced higher stress than the other groups. PI scores provides information about the disease prevention behaviors of individuals. GA scores provide information about the increase in preventive behaviors and decrease in risky behaviors. In the light of these findings, individuals with high PVD scores are more cautious and more knowledgeable about the issues that may lead to the disease, so that it is possible to avoid the disease.

PS was negatively associated with GA in the COVID test-positive group, but there was no statistical significance. Our results showed that females experienced more PS than men and paid more attention to disease prevention. In the literature, post-traumatic stress, depression, insomnia, and (mental) distress are generally significantly associated with the fear of infection and perception of risk (38–43).

Brier et al. (44) identified four factors that may protect HCWs from developing mental health problems during the pandemic. First, being informed and receiving support from the manager prevented the development of mental health problems. Second, being in quarantine worsened mental health. Third, there was no relationship between job stress and mental health. Fourth, HCWs may suffer from mental health problems because of the risk or fear of becoming infected or infecting others.

Because the pandemic is ongoing and its progress is undetectable, one study suggested the use of monitoring systems for HCWs' mental health and prevention to protect their wellness during the pandemic (45).

Limitations

This study has a few limitations. The F/M ratio was the first limitation of this study. The regression analysis was used for the reduction of the bias. Future studies should consider the F/M participant ratio.

Second, this study relied on a self-report questionnaire, which is a subjective source of data collection. Therefore, participants may have not provided objective responses to the questionnaire items. However, the results of this study may be useful for special groups.

Third, in this study, participants did not have a history of psychiatric illness; however, we had no opportunity to control the psychological and physical conditions of HCWs due to extraneous factors.

5. CONCLUSION

This study assessed the levels of PS and PVD during the COVID-19 pandemic among HCWs. Gender, age, professional experience and knowledge, and working during a pandemic

were important factors for PS and PVD. According to the results of this study, support programs by hospital organizations, social support by colleagues, and a sense of control and coping ability are essential to support the mental health of HCWs. Further research will be required at the end of the pandemic to verify these results among HCWs.

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Conflicts of interest

None.

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Clinical and Experimental Health Sciences

Effect of High Cholesterol Diet and α-Tocopherol Supplementation on Endoplasmic Reticulum Stress and Apoptosis in Hippocampus Tissue

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ABSTRACT

Objective: Neurodegenerative diseases are life-threatening disorders that occur when cells in peripheral nervous system or brain lose function over time and ultimately die. Our study aimed to establish a high cholesterol fed rabbit model to investigate the status of endoplasmic reticulum (ER) stress, proteasome activity and apoptosis as well as the beneficial role of α-tocopherol on hippocampus.

Methods: Hypercholesterolemic rabbit model was established by feeding on 2% cholesterol diet for 8 weeks, and α-tocopherol supplementation was applied with intramuscular injections. Alterations of Grp78, Grp94, Bax, Bcl-2 and BACE1 levels in hippocampus tissue were determined by western blotting, while proteasome activity was measured by fluorometric detection.

Results: Cholesterol containing diet for 8 weeks increased well-known parameters of ER stress and apoptosis, together with no chance in proteasome activity. On the other hand, α-tocopherol supplementation in rabbits fed two percent cholesterol containing diet significantly down-regulated certain ER stress and apoptosis parameters along with an increase in proteasome activity.

Conclusion: Our results underline a novel function of α-tocopherol in reducing cholesterol induced ER stress and apoptosis by increasing proteasome activity in the hippocampus.

Keywords: Hypercholesterolemia, Endoplasmic reticulum stress, Proteasome, Apoptosis, Hippocampus

1. INTRODUCTION

Neurodegenerative diseases are progressive neurologic disorders characterized by neuronal loss and affecting millions of people each year (1). Hypercholesterolemia, a well-defined risk factor for neurodegeneration, is a common feature in general population. (2). Animal studies reported that cholesterol containing diets induce neuronal accumulation of amyloid β (Aβ) and also activates oxidative stress, inflammation and apoptosis signaling (3-5), while clinical studies revealed the positive correlation between plasma cholesterol levels and Alzheimer's disease (AD) (6, 7). Despite all these studies, the underlying mechanisms by which cholesterol causes the development of neurodegenerative diseases remain unclear.

Accumulation of free cholesterol is known to enhance the induction of oxidative and endoplasmic reticulum (ER) stress signalings (8). ER is a crucial organelle in folding of proteins, homeostasis of calcium and signaling mechanisms of cell death. Unfolded protein response (UPR) is a preventive process triggered by ER dysfunction leading to ER stress. UPR induction enhances the ER resident chaperones including glucose-regulated proteins 78 (Grp78) and Grp94 which eliminate misfolded or unfolded proteins (9). Therefore, ER plays a crucial role in neurodegenerative disorders which are defined by misfolded protein accumulation (10). Proteasome has a crucial part in the degradation of intracellular misfolded and damaged proteins. Ability of oxidative stress mediated protein aggregates to inhibit proteasome activity has been identified in many studies and accepted as a primary cause in neurodegenerative disease development (11).

Increased production, oligomerization and aggregation of Aβ plaques formed by the cleavage of amyloid β-precursor protein (APP) by the β-secretase 1 (BACE1) enzyme is one of the major hallmarks of AD (12). Recent studies have shown that ER stress involves in AD development via increasing BACE1 levels (13). However, activation of UPR in the hippocampus suggests the increased presence of Grp78 and Grp94 in AD neurons. Prolonged ER stress developed in neurodegenerative diseases also causes an increase in

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neuronal apoptosis by activation of Bcl-2 family proteins (14). The Bcl-2 protein family consists of proapoptotic (Bax and Bak) and anti-apoptotic (Bcl-2, Bcl-xL and Mcl-1) members. Members of the proapoptotic Bcl-2 family, known as BH3 proteins, activate the intracellular apoptotic pathway in the presence of cellular stress.

Alpha-tocopherol is the most active form of vitamin E. Although the mechanisms of action of α-tocopherol in neurodegenerative diseases are unclear, its critical role in maintaining neurological health has been reported (15). Multiple studies have shown the suppressive role of α-tocopherol in neurodegeneration development via regulating various genes (16-18). For instance, oxidative stress and neuronal cell death in hippocampus tissue were significantly reduced in epileptic rats supplemented with α-tocopherol (19). Another *in vivo* study found that α-tocopherol reduced amyloid oligomers, oxidative stress, and interleukin-1 (IL-1) and IL-6 production (20). α-Tocopherol also acts as a regulator of inflammatory pathways in neuroglia and a cytoprotective factor in hippocampal neurons (21). This literature information support the hypothesis that α-tocopherol might be a critical protective agent in neurodegenerative disorders. However, the effects and mechanisms of α-tocopherol in cholesterol induced brain injury remain poorly understood.

Therefore, the aim of our study is to identify the impact of high cholesterol diet on ER stress, proteasome activity and apoptosis in hippocampus tissue, which are related to pathological hallmarks for neurodegenerative diseases. Furthermore, the influence of a-tocopherol on these signalling was also observed.

2. METHODS

2.1. Rabbits and Treatments

All animal experiments were approved by Marmara University Experimental Animals Research and Implementation Committee (protocol number 49.2015. mar). Starting at 2-3 months old, male albino rabbits were fed with α-tocopherol poor diet or α-tocopherol poor diet containing 2% cholesterol and divided into following groups; i) control, ii) cholesterol, iii) cholesterol + α-tocopherol and iv) α-tocopherol. Intramuscular injections of 50 mg/kg of α-tocopherol (Evigen, Aksu Pharmaceutical) were applied daily to cholesterol+α-tocopherol and α-tocopherol groups. α-Tocopherol concentration used in the study was determined in accordance with our previous studies (22, 23). Rabbits were sacrificed eight weeks after feeding/treatment period, and blood was collected to measure cholesterol and α-tocopherol. Hippocampus was rapidly frozen and stored at – 80°C for immunoblotting.

2.2. Cholesterol and α-Tocopherol Measurement in Serum

Cholesterol levels in serum was identified by using Roche-Hitachi Modular P800 Analyzer. To measure α-tocopherol levels in serum, high-performance liquid chromatography (HPLC) was established following the lipid extraction according to Nierenberg and Nann (24). α-Tocopherol was obtained by C18 column (5µm, 4.6×250mm), while MeOH:dH2O (95:5, v/v) was the mobile phase and a UV detector (Thermo) at 294 nm was used. α-Tocopherol level was calculated as µg/mL following the comparison with the relative peak areas of standard.

2.3. Immunoblot Analysis

Fifteen mg of hippocampus was lysed in RIPA buffer (Cell Signaling) via Ultra Turrax homogenizator in accordance with manufacturer's protocol. After the measurement of total protein amount with Bradford using iMark Microplate Reader (Bio-Rad), twenty µg of protein was loaded onto SDS-PAGE gels, and immobilized on nitrocellulose membrane. After the blocking with 5% skim milk in TBS, membranes were incubated with primary antibodies as follows: Grp94 (Cell Signaling, 2104), Grp78 (Cell Signaling, 3177), Bcl-2 (Cell Signaling, 2870), Bax (Abcam, ab115193), BACE1 (LifeSpan Biosciences Catalog No: LS-A8893-50) and β-actin (Cell Signaling, Catalog No: 4967). Following the HRP-conjugated secondary antibody and chemiluminescence substrate incubations, blots were photographed by ChemiDoc (Bio-Rad) instrument and quantified by Image J.

2.4. Proteasome Activity Measurement by Fluorometry

Proteasome activity measurement was applied according to our previous study (23). Briefly, hippocampus was lysed in 1mM dithiothreitol at 4°C. After the centrifuge at 14000g, supernatants were collected and chymotrypsin-like proteasome activity was determined by using fluorogenic peptide succinyl-LLVY-methyl coumarin as substrate. Following the incubation at 37 °C for 30 min, liberation of methyl coumarin was determined by Enspire 2300 Multilabel Reader (PerkinElmer) at 360 nm excitation and 485 nm emission. Proteasome activity was determined after the comparison with free methyl coumarin as standard. Lactacystin was used in excluding other protease activities.

2.5. Statistical Analysis

Prism 4 (Graph-Pad) software was used in performing statistical analysis. Statistical signifance was estimated using One-Way ANOVA followed Student-Newman-Keuls test for multiple comparisons. P-value less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Cholesterol and α-Tocopherol Levels in Serum

Dietary supplementation of two percent cholesterol in rabbits increased cholesterol levels in serum compared to control (Fig. 1A). Similarly, intramuscular injections of α-tocopherol increased α-tocopherol levels in the serum of cholesterol+α-tocopherol and α-tocopherol rabbits. In accordance with our previous studies (23), α-tocopherol level in serum was

also elevated in cholesterol group, which might be related to increased LDL fraction transporting α-tocopherol in the circulation (Fig. 1B).

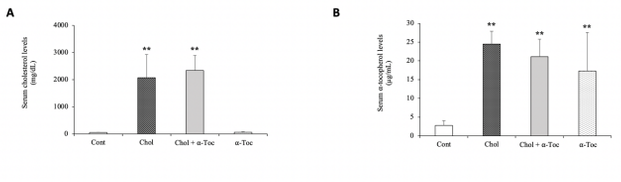

Figure 1. *Serum cholesterol and α-tocopherol levels*
Serum cholesterol (A) and α-tocopherol (B) levels in rabbits receiving eight weeks of 2% cholesterol diet and/or α-tocopherol.
***p < 0.01 vs. control group (n=5).*

3.2. ER Stress and Proteasome Activity in Hippocampus Tissue

After the verification of our *in vivo* model, we next tried to gain insight into the status of ER stress and proteasome. Grp78 and Grp94 are the most well-characterized ER chaperones and well-defined ER stress parameters that participate the correct folding of proteins. In the present study no significant change in Grp94 protein level was observed between groups (Fig. 2A). Despite unchanged Grp94 levels, Grp78 was increased in cholesterol group. Supplementation of α-tocopherol prevented this cholesterol driven Grp78 activation (Fig. 2B). Besides the enhancement of protein folding, activation of protein degradation by proteasome is another process in maintaining protein quality control (25). In this scope, we measured the proteasome activity in rabbit hippocampus tissues by fluorometric detection method. As a potential beneficial effect against high cholesterol induced ER stress, rabbits of cholesterol+α-tocopherol group exhibited significantly increased proteasome activity, while no significant change was determined in α-tocopherol group (Figure 2C).

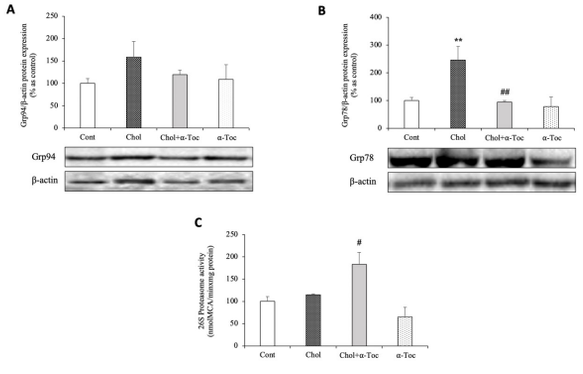

Figure 2. *ER Stress and Proteasome Activity in Hippocampus Tissue*
Hippocampus tissues were homogenized and expressions were measured by western blotting. Protein bands were analyzed and normalized to β-actin against Grp94 (A) and Grp78 (B) antibodies. 26S proteasome activity in hippocampus tissue lysates (C).
Data are expressed as mean ± S.D.
***p < 0.01, vs. control group,*
##p < 0.01, and #p < 0.05 vs. cholesterol group (n=5).

3.3. Bax, Bcl-2 and BACE protein expressions in the hippocampus tissue

As the proteins of Bcl family that induce apoptotic cell death, Bcl-2 and Bax are well identified neuroapoptosis markers (26). Therefore, we determined the levels of Bcl-2 and Bax by immunoblotting. As a parameter of apoptosis induction, high cholesterol diet rabbits elevated Bax and Bcl-2 levels (Figure 3A-B). Alpha-tocopherol supplementation was significantly inhibited the induction of Bax (Figure 3A). We also examined the brain injury in hippocampus tissue by measuring BACE expression. As shown in Fig. 3C, high cholesterol diet induced BACE levels, while α-tocopherol supplementation reversed. We also evaluated the changes in Bax/Bcl-2 ratio and observed a higher Bax/Bcl-2 ratio in cholesterol group compared to control. In agreement with Fig. 3A and 3B, α-tocopherol supplementation reversed this increase (Figure 3D). These results propose the effect of α-tocopherol on high cholesterol diet mediated apoptosis and brain injury in hippocampus tissue.

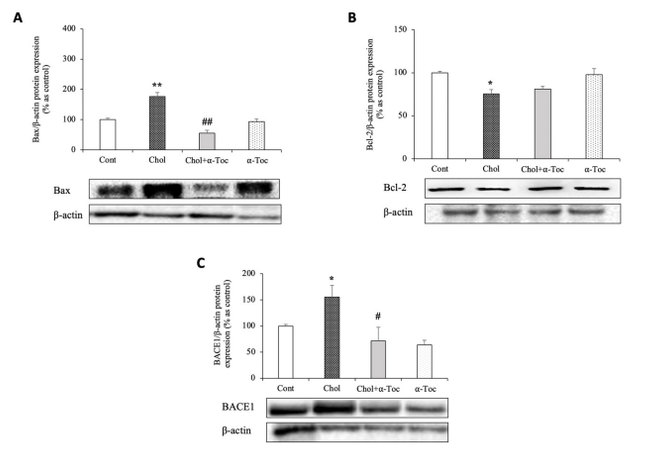

Figure 3. *Bax, Bcl-2 and BACE protein expressions in the hippocampus tissue*
Hippocampus tissues were homogenized and expressions were measured by western blotting. Protein bands were analyzed and normalized to β-actin against Bax (A), Bcl-2 (B) and BACE1 (C) antibodies. Based on the densitometric quantification of the bands for Bcl-2 and Bax, Bax/Bcl-2 ratio was calculated (D).
Data are expressed as mean ± S.D.
***p < 0.01, and *p < 0.05 vs. control group,*
##p < 0.01, and #p < 0.05 vs. cholesterol group (n=5).

4. DISCUSSION

A variety of studies has been reported hypercholesterolemia as a serious risk factor in numerous disorders including neurodegeneration and cardiovascular disease (27). Multiple studies using in-vivo models of AD have found the capacity of high cholesterol diet in increasing Aβ accumulation (28, 29). In this scope, rabbits fed high cholesterol diet exhibited a considerable number of findings of AD similar to those observed in patients with AD such as Aβ accumulation in the hippocampus, apoptosis and microglia activation (30, 31). Therefore, in the present study we established

a hypercholesterolemic rabbit model to determine the protective role of α-tocopherol against ER stress, BACE1 activity and apoptosis in hippocampus. Due to its lipophilic ability, most of the α-tocopherol in serum is bound and carried by circulating LDL and HDL. In our study, elevated α-tocopherol levels determined in serum of cholesterol group are possibly related to its enhanced release from adipose tissue and uptake of LDL fraction (32). However, α-tocopherol in cholesterol group was observed to be reduced by rationing serum α-tocopherol to serum cholesterol, in accordance with our previous studies performed in different rabbit groups (22, 33).

A variety of stress conditions may enhance the misfolded protein accumulation and trigger the ER stress. *In vivo* and clinical findings have determined the interaction between cholesterol and ER stress in the development of metabolic disorders (8). ER contains abundant concentrations of folding sensors and chaperone molecules involved in the quality control process of proteins. Upregulation of Grp78 and Grp94 in brain samples of patients with AD revealed the importance of UPR in disease development (34). Additionally, Lu and colleagues (35) demonstrated the presence of ER stress in the hippocampus of mice fed high cholesterol diet. Similarly, Cai and colleagues (36) determined that rats fed high-fat diet exhibit symptoms of neuronal plasticity together with the induction of hippocampal ER stress. Tunicamycin, an ER stress inducer, is also associated with the induction of Grp78 and Grp94 in cerebral cortex, hippocampus and cerebellum of mice (37). Moreover, Onoda and colleagues (38) revealed that particles derived from air pollution such as carbon black induce misfolded protein accumulation and ER stress in mice brain. In our study, despite the increase of Grp78 in rabbits fed high cholesterol diet, no change was determined in Grp94. This Grp78 increase emphasizes the possible impact of high cholesterol diet in inducing ER stress response and protein damage status. As a response to ER stress, UPR enhances two major degradation systems, proteasome and autophagy, that reduce misfolded/unfolded protein accumulation. Recent studies have determined the reduction of proteasomal activity associated with neurodegenerative diseases that contributes to the failure of misfolded protein degradation and the accumulation of protein aggregates (39). Our data exhibited the increase of proteasome activity in hippocampus tissue when α – tocopherol was supplemented with high cholesterol diet. Thus, α-tocopherol may be effective in the suppression of ER stress through mediating proteasome mediated elimination of damaged proteins.

Uncontrolled and overactivated ER stress in neurodegenerative disorders leads to an increase in neuronal apoptosis, one of the pathological characteristics of neurodegenerative diseases, via activating Bcl-2 family proteins (40, 41). El-Sayyad and colleagues (42) observed an increased neuronal apoptosis in rats fed high cholesterol diet. Increased production and aggregation of Aβ in extracellular area is considered as the major hallmark in AD development. BACE1 is the major enzyme involve in the production of Aβ from amyloid β-precursor protein (APP) which further induces neuronal apoptosis. Related to this, BACE1 protein expression was found to be induced in the majority of AD patients. (43). Zhao and colleagues (14) determined increased protein levels of Bax, Bcl-2 and BACE1 in hippocampus of hyperlipidemic mice. In our study, we observed that the hippocampus of rabbits fed high cholesterol diet exhibited Bax and BACE1 induction. Early studies in animal models were also demonstrated that α-tocopherol is an essential molecule during the development and maintenance of nervous system (44). Accordingly, α-tocopherol has been shown to protect the synaptic plasticity and function by preventing apoptotic cell death in the hippocampus (19). In a study with SH-SY5Y neuroblastoma cells, α-tocopherol was reported to downregulate Bax/Bcl-2 ratio (45). Despite all these studies, the effect of α-tocopherol on cholesterol-mediated apoptosis in brain tissue is unknown. In the present study, Bax and BACE1, two well-studied parameters of apoptosis and AD, were reduced by α-tocopherol supplementation in hippocampus tissue. Our findings suggested that alpha tocopherol may be important molecule in the development of therapeutic strategies against neurodegenerative disorders by targeting apoptotic cell death and BACE1 levels.

5. CONCLUSION

In conclusion, we demonstrate an *in vivo* evidence that the ER stress status and apoptotic activity in hippocampus is likely to be induced in rabbits fed high cholesterol diet. Our results identified the beneficial role of α-tocopherol in cholesterol induced ER stress and apoptosis, possibly via enhancing proteasome mediated degradation of damaged proteins. However, further studies are needed to determine the underlying mechanisms that enhance cholesterol mediated ER stress activation, and the molecules in which α-tocopherol increase proteasome activity in hippocampus.

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Conflict of Interest

The authors declare no conflicts of interests.

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Clinical and Experimental Health Sciences

Anxiety Levels of Dental PhD and Specialty Program Students in Turkey About COVID-19 Pandemic Process

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ABSTRACT

Objective: The aim of this study was to evaluate the anxiety levels about education, clinical practice and professional development of dental PhD and specialty program students in Turkey during pandemic process.

Methods: In this study, a scale consisting of two parts was developed by researchers and an online survey portal was used to reach the participants. The first part included personal information of participants. In the second part, there were 5-point scale items with three sub-dimensions to determine the anxiety levels about COVID-19. Item pool was constructed with 23 scale items. For the construct validity, exploratory factor analysis and confirmatory factor analysis was conducted. After the exploratory factor analysis, 3 items exluded from the scale and it resulted in three-factor structure. According to the theoretical background, factors were called as “clinical practice process”, “education process” and “professional development process”. When the items related to the sub-dimensions were examined, it was seen that education process includes 8 items, clinical practice process includes 9 items and professional process includes 3 items. Then, this three-factor structure with 20 items by the way one factor model based on these three subfactors were tested through confirmatory factor analysis and higher order confirmatory factor analysis. After exploratory factor analysis and confirmatory factor analysis, it was concluded that there was enough evidence for the construct validity. For the reliability study Cronbach alfa coefficients and corrected item-total correlations was calculated. It was observed that the Cronbach alpha coefficients for the education process sub-dimension, the clinical practice process sub-dimension and the professional development process sub-dimension were 0.87, 0.86 and 0.70, respectively. The stratified Cronbach alpha coefficient calculated for the overall scale was 0.90. It was referred that the scores obtained from the sub-dimensions of the scale and by the way for general score were reliable. For the corrected item-total correlations, education process sub-dimension was in the range of 0.559-0.661, clinical practice sub-dimension was in the range of 0.407-0.812, and professional development process sub-dimension was in the range of 0.487-0.534. It was concluded that all items were sufficiently distinctive and served its purpose. Moreover the data based on the scale were analyzed with t-test and Kruskal Wallis H test. With the statistically significant results of Kruskal Wallis H test, Mann-Whitney U test were conducted as post hoc.

Results: A total of 208 students (156 females, 52 males) participated. There was no significant difference among anxiety levels of the groups in terms of gender and department variables ($p > 0.05$). However, there was statistically significant difference in terms of age and education year. The students with the ages in the interval of 22-24 had significantly lower anxiety levels regarding the clinical practice process ($p < 0.05$). The anxiety levels of students with more than 4 years in education were significantly lower than other groups regarding education process ($p < 0.05$). Younger students had lower anxiety levels about clinical practice process in PhD or specialty programs.

Conclusion: Increase of the year in education leads reducement in the anxiety level about education process.

Keywords: Covid-19, anxiety level, student, education process, clinical practice

1. INTRODUCTION

The coronavirus disease (COVID-19) outbreak that emerged in Wuhan, China in December 2019 has become a major public health problem for the global community. The World Health Organization (WHO) declared COVID-19 as a Public Health Emergency of International Importance on January 30, 2020 (1), and then declared a pandemic on March 11, 2020 (2).

COVID-19 may be asymptomatic for some patients or may cause symptoms ranging from various flu-like symptoms to pneumonia with a relatively high mortality rate (3). The disease is mainly transmitted by respiratory droplets and contact. Therefore, to be closeness of dentists to the oral cavity of patients, and to occur aerosols and droplets during majority of dental treatments increase the risk for dentists,

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dental staff, and patients (4-6). Under these circumstances, dentists may be worried about infection during dental treatments.

WHO publishes reports that provide temporary guidance on the prevention, control and management of infections related to the pandemic and these guidelines are constantly updated. During the COVID-19 pandemic period, in parallel with the developed countries of the world, measures and emergency treatment application protocols for the control of cross infection risk between dentists and patients were determined in Turkey (6,7). Dental treatments, primarily emergency treatments, have been applied by considering these protocols and taking measures.

The education of PhD and specialty students in faculty of dentistry is both theoretical and practical process. The spreading of COVID-19 has led to the closure of institutions all over the world, and the education to be conducted on the internet. This situation has significantly affected the mission of practical education. At the same time, setback was observed in scientific studies conducted during PhD and specialty programs (8).

In the current literature, there is no comprehensive study about anxiety levels of PhD and specialty program students. The aim of this study was to evaluate the anxiety levels about education, clinical practice and professional development of dental PhD and specialty program students in Turkey during pandemic with developed scale by researchers.

2. METHODS

This study was conducted among PhD and specialty program students of dentistry in Turkey from January to February 2021. Ethical approval was received from the Ethics Committee of Gazi University (Ref No: 2020 – 731).

In this study, an online survey was structured into two main parts (Table 1). The questions in the first part were about demographic variables: gender, age, department and year in education of participants. In the second part, the Anxiety Scale of COVID-19 developed by the researchers was used. For the development of the scale, the eight-stage scale development process suggested by DeVellis was taken into consideration (9). Accordingly, a 5-point Likert-type item pool with 23 items was created for Anxiety in Pandemic Process. In order to determine the opinions of PhD and specialty program students on the items, a Likert type rating scale was used such as 5 = strongly agree, 4 = agree, 3 = partially agree, 2 = disagree, 1 = strongly disagree.

2.1. Statistical Analysis

The exploratory factor analysis (EFA) was conducted with Kaiser-Meyer-Oklin's (KMO) test to evaluate the suitability of the sample and KMO value was obtained as 0.853. KMO value greater than 0.6 indicates that the sample is suitable for factor analysis (10). In the exploratory factor analysis, the value of 0.40 was taken as the lower limit of factor loadings of the items in the relevant factor, considering the sample size (11). Due to the insufficient loading of the items on the relevant factor and the cross-loading situation, analyzes were repeated after each item was removed. Besides the EFA, the proof of the construct validity was also provided with the confirmatory factor analysis (CFA) in terms of the suitability of the obtained structure. After the study of construct validity, a reliability study was conducted. Cronbach's alpha internal consistency coefficient and corrected item total correlations were examined for the reliability. The scale developed with three sub-dimensions would be also used as total score and accordingly the investigations were made through the total score and sub-dimensions. It was tested whether the group means for each variable differ statistically, taking into consideration the demographic variables of gender, age, department and education year. For comparisons, the independent samples t-test for the two-level gender variable and one-way ANOVA for age, department and education variables that have more than two levels were envisioned. In order to carry out parametric analysis, it was checked whether the assumptions of normality and homogeneity of variances were ensured. When examining the levels of the gender variable in the context of each sub-dimension and general score, it was observed that there were deviations from normality, but the assumptions of homogeneity of variances were provided. The mean comparisons for the gender variable were made using independent samples t-tests. When the variables of age, department and year of education which have more than two levels were examined, the analyzes were carried out with the Kruskal Wallis H test. Mann-Whitney U test was used as a post-hoc test.

3. RESULTS

A total of 208 PhD and specialty dental students (156 females, 52 males) from 8 departments were participated in the survey.

3.1. Exploratory factor analysis (EFA)

The analyses were carried out with the orthogonal rotation (varimax) technique using the principal factor axis method due to the fact that 3 sub-dimensions were envisaged according to theoretical studies in factor analysis**.** When the factor loadings obtained as a result of the rotation were examined, the 14th item, which was not loaded any dimension, and then the 17th item and the 21st item, which were overlapped, were removed one by one and the analyses were repeated, respectively. The scree plot of the results of EFA performed on the remaining 20 items is presented (Figure 1).

According to the scree plot graph it was observed that three factors have emerged from the first break point and it would be inferred that there is a three-factor structure. In addition to the scree-plot graph, explained variances for the factors with an eigenvalue above 1 obtained as a result of EFA based on 20 items are presented in the Table 2. It was seen that three factors with eigenvalues above 1 emerged.

Table 1. *The survey form*

Cinsiyetiniz?

( ) Kadın ( ) Erkek

Yaşınız?

( ) 22-24 ( ) 25-27 ( ) 28-30 ( ) 31+

Hangi bölümdesiniz?

( ) Ağız, diş, çene cerrahisi ( ) Pedodonti

( ) Ağız, diş, çene radyolojisi ( ) Restoratif diş tedavisi

( ) Endodonti ( ) Periodontoloji

( ) Ortodonti ( ) Protetik diş tedavisi

Doktora eğitim programınızın kaçıncı yılındasınız?

( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 4+

1.Pandemi nedeniyle, uzmanlık ve doktora programlarının eğitim kalitelerinin olumsuz etkilenme ihtimali ile ilgili endişeleniyorum.
2.Pandemi nedeniyle tez çalışmamın ilerleyişinde olumsuzluk yaşanacağı düşüncesiyle kaygılanıyorum.
3.Pandemi sürecinde eğitimimde yıl kaybı yaşayacağımdan tedirgin oluyorum.
4.Pandemi sürecinde verilen eğitimlerde edindiğim bilgilerin kalıcı olmayacağını düşünüyorum.
5.Pandemi sürecinde eğitimimdeki aksaklıklar nedeniyle maddi kayıp yaşayacağım düşüncesi beni huzursuz ediyor.
6.Pandemi süreci sonrasında eğitimimle ilgili eksikliklerin telafi edilemeyeceğini düşünüyorum.
7.Pandemi sırasında aerosol oluşturacak diş tedavilerini yapmak ile ilgili tedirginlik duyuyorum.
8.Pandemi sürecinde gelinen noktada, üniversite kliniklerine geri dönerek hasta bakmak ile ilgili endişeleniyorum.
9.Covid-19 enfeksiyonu için aşı olmayan bir hastanın rutin diş hekimliği işlemlerini uygulamada olabildiğince hızlı davranırım.
10.COVID-19 için aşı olan bir hastada koruyucu önlemler alarak rutin diş hekimliği işlemlerini uygularken dahi pandemiden önceki gibi rahat davranamam.
11.Pandemi sürecinde hastalara uygun tedavi planlaması yapmak ile ilgili endişeleniyorum.
12.Bir hastadan Covid-19 bulaşma riski beni tedirgin ediyor.
13.İş arkadaşımdan Covid-19 bulaşma riski beni tedirgin ediyor.
14.Dental tedavileri uygularken Covid-19 enfeksiyonunu aileme taşıyabileceğimden çok korkuyorum.
15.Bir hastanın rutin dental tedavi sırasında COVID-19'a yakalanma olasılığı ile ilgili rahatsızlık duyuyorum.
16.Dental tedavileri için kliniklere gelen hastaların Covid-19 enfeksiyonu ile ilgili farkındalıklarının yüksek olmaması ile ilgili endişeleniyorum.
17.Pandemi sürecinde yaptığım diş tedavilerinde hasta takibi yapamayacağımı düşünüyorum.
18.Pandemi süreci ve diş hekimlerinin içinde bulunduğu risk düzeyi göz önüne alındığında diş hekimliği mesleğinin geleceğini düşündüğümde kendini kötü hissediyorum.
19.Pandemi süreci sonrasında doktora/ uzmanlık eğitimimi tamamladığımda iş bulma konusunda endişeleniyorum.
20.Pandemi süreci ve diş hekimlerinin içinde bulunduğu risk düzeyi göz önüne alındığında mesleğimi değiştirme fikri kendimi kötü hissettiriyor.
21.Pandemi nedeniyle kongrelerin ve bilimsel toplantıların iptal edilmesi, ertelenmesi nedeniyle mesleki gelişimimin etkileneceği ile ilgili endişeleniyorum.
22.Pandemi sürecinde eğitimde görülen aksaklıklar nedeniyle mesleki özgüvenimin olumsuz etkileneceği düşüncesi bana huzursuzluk veriyor.
23.Pandemi nedeniyle bilimsel çalışmalarımın olumsuz etkileneceği ile ilgili tedirginlik duyuyorum.

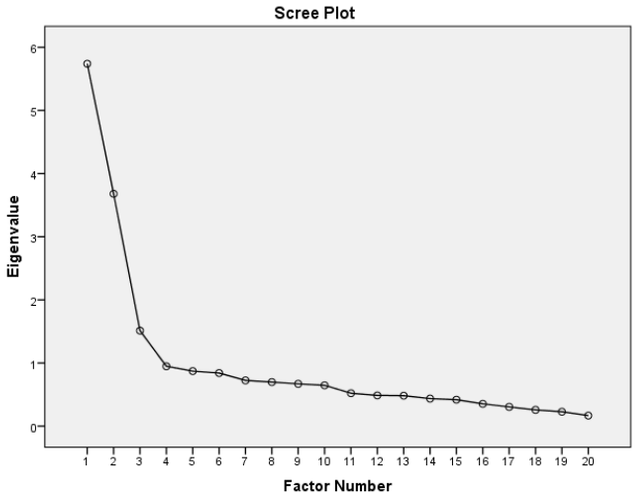

Figure 1. *Scree Plot Graph of EFA*

Table 2. *Eigenvalue and explained variance ratios*

	Eigenvalue	Explained variance ratio (%)	Total explained ratio (%)
1	5.740	20.190	20.190
2	3.680	18.291	38.481
3	1.513	8.517	46.998

Factor loadings matrix of the three-factor structure are presented in the Table 3. The factor loadings for Factor 1 were in the range of 0.427-0.876, for Factor 2 were in the range of 0.603-0.708 and for Factor 3 were in range of 0.593-0.635, and each item has sufficient factor loading on the relevant dimension. According to the theoretical background, Factor 1 was named as "clinical practice process", Factor 2 was named as "education process" and Factor 3 was named as "professional development process". When the items related to the dimensions are examined, it was seen that there were 8 items in the education process sub-dimension, 9 items in the clinical application process sub-dimension and 3 items in the professional development sub-dimension. The total explained variance was 46,998%. When the explained variance ratios of sub-dimension were examined, it was observed that the education process sub-dimension explained 18.291% of the variance, the clinical practice process sub-dimension explained 20.190% of the variance and the professional development process sub-dimension explained 8.517% of the variance. Accordingly, it was concluded that the ratio of explained variance were sufficient.

3.2 . Confirmatory Factor Analysis (CFA)

After the exploratory factor analysis, the structure of the developed scale was tested through the confirmatory factor analysis. CFA provides statistical evidence for a predetermined or constructed structure (12). CFA was applied to three sub-dimensional scale which were theoretically predetermined. The unweighted least squares method (ULS) was used in CFA analysis. In order to provide evidence for the summability of scale scores, three sub-dimensional theoretical structures were tested with higher order CFA that three dimensions were associated to a single factor. Evaluations regarding the tested structure were carried out with t-values, standardized factor loadings and fit indices.

The significance of the observed variables was examined with the t-values obtained as a result of higher order CFA, and all items were above the critical value and were significant (13). In addition, a path diagram was obtained to examine the standardized factor loadings (Figure 2). The factor loadings of the items related to the education process sub-dimension were in the range of 0.54-0.77, the factor loadings of the items related to the clinical practice process were in the range of 0.45-0.88, and the factor loadings of the items related to the professional development sub-dimension were in the range of 0.57-0.76. It was referred that each item was loaded on the relevant dimension sufficiently. In addition to the factor loadings, some fit indices, which also provide information about model-data fit, were used. chi-square in the absolute fit category, RMSEA in the parsimony correction category, and the comparative fit index (CFI), normalized fit index (NFI), non-normalized fit index (NNFI), goodness of fit index. (GFI) and adjusted goodness of fit index (AGFI) in the comparative fit category were used. These indexes regarding the general fit of the scale are presented in the Table 4. It was seen that the value related to the section of the square's degrees of freedom is less than 2. This value indicates perfect fit (14). When the RMSEA value was examined, it was observed that this value was 0.065 and was indicated good fit (15). CFI, NNFI, GFI and AGFI values were 0.95 and above which indicated perfect fit (16-17). On the other hand, NFI index was above 0.90 which indicated good fit (17). According to the fit indices in three different fit categories, it was concluded that the model fits the data, and the three-dimensional structure was verified.

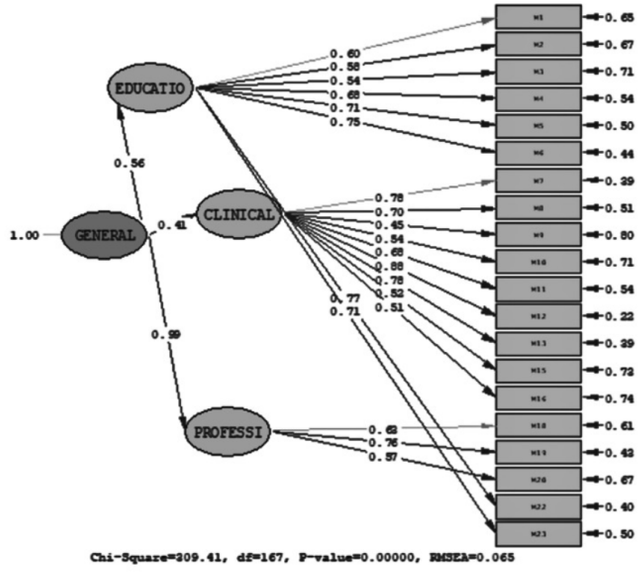

Figure 2. *Path diagram*

Table 3: *Factor load values of the three-factor*

		Factor		
		1	2	3
I1	I am concerned about that quality of education of PhD and specialty programs can be affected adversely due to pandemic.		.680	
I2	I worry that there will be negativity in progress of my thesis due to pandemic.		.708	
I3	I am worried that I will lose time in my education process due to pandemic.		.628	
I4	I think that the education I have acquired during pandemic will not be permanent.		.694	
I5	The thought that I will have financial loss due to educational problems during the pandemic disturbs me.		.603	
I6	I think that the deficiencies in my education cannot be compensated after pandemic.		.670	
I7	I worry about performing dental treatments included aerosol droplets during pandemic.	.768		
I8	At the point reached during pandemic, I am concerned about going back to university clinics and treating patients.	.727		
I9	I act as quickly as possible for routine dental treatments of a patient who is not vaccinated for COVID-19 infection.	.427		
I10	I cannot behave comfortably as before the pandemic, even when performing routine dental procedures by taking preventive measures in a patient vaccinated for COVID-19.	.561		
I11	I am worried about making suitable treatment planning for patients during the pandemic.	.572		
I12	The risk of Covid-19 transmission from a patient worries me.	.876		
I13	The risk of Covid-19 transmission from my colleague worries me.	.778		
I15	I am uncomfortable due to the possibility of transmission of COVID-19 to patient during routine dental treatment.	.561		
I16	I am concerned that patients who come to clinics for dental treatments do not have high awareness of Covid-19 infection.	.495		
I18	Considering the pandemic process and the level of risk dentists are in, I feel bad when I think about the future of the dentistry profession.			.593
I19	After the pandemic process, when I complete PhD/specialty education, I worry about finding a job.			.614
I20	Considering the pandemic process and the risk level of dentists are in, the idea of changing my profession makes me feel bad.			.635
I22	The thought that my professional self-esteem will be affected negatively due to the disruptions in education during the pandemic gives me uneasiness.		.638	
I23	I am worried that my scientific studies will be negatively affected by the pandemic.		.606	

Table 4. *Fit indexes obtained as a result of CFA*

Chi-squared	Sd	Chi-squared/sd	RMSEA	CFI	NFI	NNFI	GFI	AGFI
309.41	167	1.85	0.065	0.96	0.91	0.95	0.96	0.95

3.3. Reliability

In order to examine the reliability of the survey results, Cronbach alpha internal consistency coefficient was used. Due to the fact that scale consists of three sub-dimensions, the Cronbach alpha coefficient for each dimension was calculated, and then the reliability coefficient for the overall scale was calculated using the stratified Cronbach alpha formula (Table 5). It was observed that the Cronbach alpha coefficient for the education process sub-dimension of the scale was 0.87, the Cronbach alpha coefficient for the clinical practice process sub-dimension was 0.86, and the Cronbach alpha coefficient for the professional development process sub-dimension was 0.70. The stratified Cronbach alpha coefficient calculated for the overall scale was 0.90. Considering the levels of the reliability coefficients, the professional development sub-dimension of the scale was considered to be medium, the coefficients related to the education process, clinical application process and overall scale were considered to be high (18). In this respect, it would be referred that the scores obtained from the sub-dimensions

of the scale and by the way for general score were reliable. In addition to the Cronbach's alpha reliability coefficients, the corrected item total correlations for each item on the basis of the relevant dimension were calculated and presented with the item numbers (Table 6). For corrected item-total correlations, education process sub-dimension was in the range of 0.559-0.661, clinical practice sub-dimension was in the range of 0.407-0.812, and professional development process sub-dimension was in the range of 0.487-0.534. Therefore, it was concluded that all items were sufficiently distinctive and served its purpose.

Table 5. *Cronbach Alpha Coefficients regarding the sub-dimensions and the general of the scale*

	Education process	Clinical practice process	Professional development process	General
Cronbach alfa	0.87	0.86	0.70	0.90

Table 6. *Total correlations of corrected item*

Dimensions	First For Item No	New Form Item No	Corrected Item – Total Correlation
Education process	I1	I1	,602
	I2	I2	,607
	I3	I3	,559
	I4	I4	,661
	I5	I5	,612
	I6	I6	,657
	I22	I19	,649
	I23	I20	,607
Clinical practice process	I7	I7	,728
	I8	I8	,668
	I9	I9	,407
	I10	I10	,528
	I11	I11	,567
	I12	I12	,812
	I13	I13	,719
	I15	I14	,508
	I16	I15	,476
Professional development process	I18	I16	,512
	I19	I17	,487
	I20	I18	,534

3.4. Anxiety Levels of Students Depending on COVID-19 Pandemic

Independent sample t test was used to determine whether the anxiety levels of all PhD and specialty program students in the study differed by gender according to the dimensions of the scale and the total score (Table 7). There was no significant difference between female and male participants in terms of anxiety levels about education process, professional development process, clinical practice process and in the general anxiety scores (p>0.05).

According to the age of participants, the anxiety levels about the education process, professional development process and general anxiety scores did not show a statistically significant difference (p>0.05) (Table 8). However, it was observed that anxiety levels regarding the clinical practice process showed a statistically significant difference (p<0.05). When post-hoc tests were carried out, it was observed that the participants with the ages in the interval of 22-24 had lower anxiety levels compared to other groups.

Table 7. *Independent sample t-test results comparing anxiety levels according to gender variable*

	Gender	n	$\bar{x}$	ss	sd	t	p
Education process	F	156	28.76	6.48	206	.444	.658
	M	52	28.31	6.17			
Clinical practice process	F	156	35.74	5.89	206	1.251	.212
	M	52	34.50	7.09			
Professional development process	F	156	9.96	2.69	206	1.420	.157
	M	52	9.37	2.40			
General score	F	156	74.47	10.53	206	1.281	.201
	M	52	72.17	12.97			

Table 8. *Kruskal Wallis H test results comparing anxiety levels according to age variable*

	Age	n	$\bar{x}$	ss	Mean rank	sd	χ^2	p
Education process	22-24	7	6.99	2.64	110.64	3	1.449	.694
	25-27	99	6.59	.66	109.09			
	28-30	73	6.67	.78	98.28			
	31+	29	4.78	.89	103.02			
Clinical practice process	22-24	7	4.86	1.84	38.07	3	9.976	.019
	25-27	99	5.77	.58	102.31			
	28-30	73	6.64	.78	110.58			
	31+	29	6.18	1.15	112.71			
Professional development process	22-24	7	2.51	.95	76.64	3	2.037	.565
	25-27	99	2.62	.26	107.49			
	28-30	73	2.69	.31	105.34			
	31+	29	2.57	.48	98.90			
General	22-24	7	13.35	5.04	70.57	3	2.438	.487
	25-27	99	10.75	1.08	105.23			
	28-30	73	11.76	1.38	104.80			
	31+	29	10.59	1.97	109.43			

In terms of the department variable, anxiety levels about the education process, clinical practice process, professional development process, and general anxiety scores did not show a statistically significant difference (p>0.05) (Table 9).

There was no significant difference among anxiety levels about clinical practice process, professional development process and general anxiety scores in terms of year in education of participants (p>0.05) (Table 10). However, it was observed that the anxiety levels of the participants with more than 4 years in education were significantly lower than other participants regarding education process (p <0.05).

Table 9. *Kruskal Wallis H test results comparing anxiety levels according to department variable*

	Department	n	$\bar{x}$	ss	Mean rank	sd	χ^2	p
Education process	Oral and Maxillofacial surgery	18	30.83	7.47	129.28			
	Dento-Maxillofacial Radiology	16	27.69	7.21	95.84	7	5.741	.570
	Endodontics	26	27.73	5.94	92.25			
	Orthodontics	14	26.93	8.35	90.64			
	Pedodontics	10	29.10	4.93	105.05			
	Restorative Dentistry	16	28.56	7.73	106.97			
	Periodontology	28	29.71	4.47	112.41			
	Prosthodontics	80	28.54	6.26	103.73			
Clinical practice process	Oral and Maxillofacial surgery	18	33.17	6.73	83.64			
	Dento-Maxillofacial Radiology	16	34.13	4.84	86.56	7	13.72	.56
	Endodontics	26	34.69	6.62	96.58			
	Orthodontics	14	33.14	6.44	82.82			
	Pedodontics	10	35.20	3.58	96.70			
	Restorative Dentistry	16	36.00	6.70	110.66			
	Periodontology	28	33.75	8.21	95.34			
	Prosthodontics	80	37.35	5.09	122.10			
Professional development process	Oral and Maxillofacial surgery	18	9.44	2.97	101.36			
	Dento-Maxillofacial Radiology	16	9.00	2.42	87.50	7	7.947	.337
	Endodontics	26	9.31	2.31	91.71			
	Orthodontics	14	9.79	3.19	99.86			
	Pedodontics	10	10.40	2.72	117.55			
	Restorative Dentistry	16	9.75	2.74	105.34			
	Periodontology	28	9.29	2.77	90.50			
	Prosthodontics	80	10.35	2.48	116.68			
General	Oral and Maxillofacial surgery	18	73.44	13.02	106.03			
	Dento-Maxillofacial Radiology	16	70.81	11.35	85.66	7	8.457	.294
	Endodontics	26	71.73	12.73	91.81			
	Orthodontics	14	69.86	13.57	83.57			
	Pedodontics	10	74.70	8.67	103.75			
	Restorative Dentistry	16	74.31	13.63	107.88			
	Periodontology	28	72.75	10.91	98.13			
	Prosthodontics	80	76.24	9.45	117.36			

Table 10. *Kruskal Wallis H test results comparing anxiety levels according to year in education variable*

	Year in education	n	$\bar{x}$	ss	Mean rank	sd	χ^2	p
Education process	1	42	30.69	6.14	124.55			
	2	62	28.63	6.28	103.34			
	3	74	28.88	5.91	105.62	4	13.820	.008
	4	20	26.55	6.91	88.05			
	4+	10	22.70	6.98	52.10			
Clinical practice process	1	42	34.45	6.22	93.35			
	2	62	35.87	6.66	109.45			
	3	74	36.09	5.11	108.86	4	7.030	.134
	4	20	35.80	7.65	115.65			
	4+	10	31.20	6.91	66.10			
Professional development process	1	42	9.79	2.34	105.12			
	2	62	10.03	2.64	108.58	4	6.715	.152
	3	74	10.08	2.56	109.68			
	4	20	9.05	3.33	92.70			
	4+	10	8.10	2.08	61.85			
General	1	42	74.93	11.07	109.45			
	2	62	74.53	10.91	105.66			
	3	74	75.05	9.26	108.86	4	8.923	.063
	4	20	71.40	14.91	101.55			
	4+	10	62.00	12.88	50.10			

4. DISCUSSION

COVID-19 which has been spread rapidly on the worldwide since late December 2019 caused human mortality. Besides this result, it also negatively affects the social behaviors. The governments have taken serious measures about social areas, healthcare systems and economy for control and mitigation of pandemic (19). As a part of measures, the dental clinics were closed, or dental treatments were restricted for a while, since healthcare professionals are exposed to a high risk of being infected due to potential contact with infected patients (5). Especially the dentists work in close contact with patients and expose to aerosol and droplets splashing out of oral cavity of patients (5,6). Besides, the dentists have spreading potential of infection to their families, peers and other patients. WHO suggests that the priority is given to emergency dental treatments and the other dental treatments are deferred until a time when outbreak goes into recession (20).

The clinical practice, which is a part of dental education, is important for postgraduate students as well as undergraduate students in dentistry to gain experience and to make suitable treatment planning. In this study, the PhD and specialty students with the ages in the interval of 22-24 showed lower anxiety levels regarding the clinical practice process compared to other groups. It can be concluded that this group, who is at the beginning of the postgraduate education, wants to return to the clinics due to deficiency of clinical practice.

The dentistry faculties switched to online education like other educational institutions to ensure continuity of theoretical education. Although online education provides opportunity to learn anywhere and at any time, the interruption of education and problems related digital platforms may decrease motivation or may cause increase in anxiety tendency of students (6,21). In this study, the anxiety levels of the students with more than 4 years in education were significantly lower than other students regarding education process. It could be assumed that theoretical knowledge and clinical experience increase, and postgraduate students make progress in thesis and scientific researches with the increasing of year in education. Thus, the students with more than 4 years in education had lower anxiety levels about quality of education and losing time in education process.

Özdede et al (21) measured the anxiety levels of dental students and it was observed that the students were anxious about the COVID-19 pandemic. Also, there was no difference between gender and anxiety levels significantly. In the present study, it was demonstrated that the gender variable is not caused difference among anxiety levels about education process, professional development process, and clinical practice process. Similar results were observed for department variable as well. Additionally, the concerns about the future of the profession, finding a job or the thought of changing the profession due to COVID-19 were questioned, and no difference was found among the anxiety levels of the students.

Unfortunately, COVID-19 pandemic is a long period and destructive results of pandemic about education will be detected in the future. Therefore, new education models must be developed for PhD and specialty dental students such as video records including different case series and their treatments. Online seminars and congresses must be organized, and the students must be promoted to attend organizations. Even though online education and online seminars cannot compensate lack of clinical practice, self-esteem of dentists can be supported by increasing their theoretical knowledge and improving their perspectives.

5. CONCLUSION

For clinical practice process, the lowest anxiety levels were detected in students with the ages in the interval of 22-24. Regarding education process, students with more than 4 years in education have the lowest anxiety levels. The variables of gender and department did not lead statistically significant difference among anxiety levels of groups about education process, clinical practice process and professional development process.

Conflict of interest: No author of this article has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in the manuscript.

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Clinical and Experimental Health Sciences

Perceived Individualized Care and the Satisfaction Levels of Patients Hospitalized in Internal Medicine Departments: A Cross-Sectional and Correlational Survey

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ABSTRACT

Objective: Individualized nursing care, which indicates the belief on the uniqueness and worthiness of human beings, improves the quality of healthcare and contributes to patient satisfaction. The aim of this study is to determine the relationship between the perceived individualized care and the level of satisfaction with nursing care for patients hospitalized in internal medicine departments.

Methods: This study was carried on 250 patients hospitalized in internal medicine department of a university hospital in Turkey between December 2019 and February 2020. Patient information form, individualized care scale and the Newcastle satisfaction with nursing scale were used for data collection. Mann-Whitney U, Kruskal-Wallis H and Spearman's rho correlation test were used for data analysis.

Results: Participants believed that the nursing interventions supported their individuality and had positive perceptions about the individuality in their own care. They were highly satisfied with the nursing interventions. Besides there was a positive correlation between the scores obtained from the individualized care scale and Newcastle satisfaction with nursing scale. Finally, age and education levels of the patients had a positive impact on perceived individualized care and the level of satisfaction with nursing care.

Conclusion: The findings suggest that individuality of each patient should be prioritized during the nursing interventions in order to increase patient satisfaction and improve the quality of nursing care.

Keywords: Individualized care, patient satisfaction, nursing care, nursing

1. INTRODUCTION

Nursing is a scientific discipline primarily related with the healthcare provided to patients (1). Healthcare is closely associated with the discipline of nursing and the primary responsibility of all nurses around the world (2,3). The aim of healthcare is to meet patient needs in an individualized way while protecting personal integrity (4,5). Patient-centered healthcare plans nursing interventions after determining the unique needs of each patient (6,7). Focusing on the patients during the process of caregiving is the most important element of individualized nursing care, which emphasized on the uniqueness and worthiness of all patients and improves the quality of care (8, 9).

The concept of individualized nursing care includes the elements of focusing on care needs by supporting the autonomy of the patients and providing healthcare in a holistic and cooperative way (3). While providing individualized care, nurses should take patient satisfaction into consideration. Patient satisfaction, which is closely related with the quality of healthcare services, is a multidimensional concept that occurs during the interaction with nurses and as a consequence of the adequacy of nursing services (10). Reflecting the balance between the expected and received quality of care, patient satisfaction is considered as an important indicator to evaluate the quality of healthcare (10,11).

Individualize care and patient satisfaction, which are widely used in the field of nursing, are considered as the indicators of actual healthcare received by the patients (12). Nurses, who adopt an individualized care and satisfaction approach, take the experiences, behaviors, opinions and the perceptions of patients into account, are aware of the uniqueness of each patient and plan nursing interventions together with the patients. In this way, they can quickly identify the situation of the patients and realize their health problems. (13,14).

Existing studies in the literature have reported that the care needs are higher for the patients hospitalized in internal medicine departments (15). Compared to other

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patients, the need of dependent patients for nursing care, and consequently the nurses, are higher. This situation may increase patient expectations from the nurses and influence their perceptions about nursing care (16). Internal medicine clinics provide healthcare services to the patients, who are mostly aging and dependent, have chronic diseases, and require long-term hospital stay. Patient-nurse interaction among the internal medicine patients is higher since most of these patients have a longer hospital stay due to chronic diseases. An efficient interaction between these patients and the health professionals is required to improve the quality of care and manage their diseases. Consideration of these factors indicate the importance of individualized care and the level of satisfaction with nursing care for patients hospitalized in internal medicine departments (17,18).

Academic interest on the issues of individualized care and patient satisfaction has gradually increased in recent years. The study of Karayurt et al. (2018) reported that the participant nurses were aware of the importance of individualized care but faced with difficulties due to problems, such as the inadequate number of health professionals, lack of cooperation, communication problems and insufficient education (19). Kayrakçı and Özşaker (2014) found that the satisfaction of surgical patients with nursing care was moderate but could be improved (10). Altınbaş and İster (2020) noted a relationship between professional satisfaction and the attitudes of nurses towards the nursing care and stated that the nurses, who were satisfied with their profession, had better attitudes towards their roles as caregivers (3).

Recent studies noted that individualized care and satisfaction level should be evaluated from the perspective of the patients (19). Given the fact that the individualized care is directly related with the satisfaction of the patients with nursing care, it is highly important to reveal the relationship between individualized care and patient satisfaction with nursing care. Determining the level of patient satisfaction with nursing care may empower the communication between the patients and nurses, maintain the involvement of patients in their care, and help the nurses to evaluate the quality of care so that the points to be improved may be determined (20). Within this context, this study aims to determine the relationship between the patient perceptions about individualized care and their satisfaction with the nursing care.

2. METHODS

2.1. Research Design

This study had a descriptive, cross-sectional and correlational design.

2.2. Population and Sampling

The population of the study comprised patients, who received treatment at the departments of internal disease, neurology, chest diseases, nephrology, endocrinology and gastrology of a university hospital in Turkey between December 2019 and February 2020. Sample of the study comprised 250 patients over the age of 18 years, who could communicate, read and write in Turkish, had been in hospital for at least five days and agreed to participate. Patients with a psychiatric history or unconscious patients were excluded.

2.3. Data Collection Tools

Patient information form, Individualized Care Scale and the Newcastle Satisfaction with Nursing Scale were used for data collection. After obtaining permission, we asked the participants, who were decided to be discharged, to complete the data collection forms. Data collection process took approximately 25 minutes.

Patient Information Form

This form was prepared by the researchers by using the relevant literature. The patient information form included questions on the socio-demographic characteristics of the patients, such as age, gender, marital status, educational level, place of residence, smoking, alcohol consumption and duration of hospitalization.

Individualized Care Scale (ICS)

ICS, which was developed by Suhonen et al. (2005) to evaluate the perceptions of patients about individualized care, was adapted into Turkish by Acaroğlu et al. (21,22). The scale had two subscales, which measured patients' views on how individuality was supported through specific nursing interventions (ICA) and how they perceived individuality in their own care (ICB). Each subscale had 17 items and three dimensions, namely, clinical situation (7 items), personal life situation (4 items) and decisional control over care (6 items). Cronbach's alpha of the ICA and ICB were 0.92 and 0.93, respectively. Cronbach's alpha of the ICA and ICB in our study were also 0.92 and 0.93, respectively. We obtained the permission to use the scale via e-mail.

Newcastle Satisfaction with Nursing Scale (NSNS)

NSNS was originally developed by Thomas et al. (1996) in order to measure the satisfaction of the English patients with the nursing care (23). Validity and reliability of the Turkish version of NSNS was tested by Uzun (2003) on 280 patients in Turkey (24). The NSNS had 19 items, which were scored on a 5-point Likert scale (1=not at all satisfied, 2=barely satisfied, 3=quite satisfied, 4=very satisfied, 5=completely satisfied). Total score obtained from the items was summed and transformed to yield an overall satisfaction score of 0-100, with higher scores indicating higher level of satisfaction. Cronbach's alpha of the original scale and our study were 0.94 and 0.974, respectively. We obtained permission to use the scale via e-mail.

2.4. Data Analysis

Collected data were analyzed with SPSS v. 24.00 statistical software. Frequency analysis was performed to analyze sociodemographic characteristics of the patients and descriptive statistics were used to analyze the scores obtained from the ICA, ICB and the NSNS. Kolmogorov-Smirnov test was used to examine the normal distribution of data and Mann-Whitney U test and Kruskal-Wallis H test were used for those data that did not follow a normal distribution. Correlation between the ICA. ICB and the NSNS was analyzed with Spearman's rho test. Statistical significance was set at $p < 0.05$.

2.5. Limitations

There were two limitations of this study. Firstly, it was conducted at a single hospital and only the internal medicine units. Secondly, data collection process was terminated due to the COVID-19 pandemic.

2.6. Ethical considerations

We obtained permission from the Drug and Non-medical Device Research Ethics Committee of Meram Faculty of Medicine at Necmettin Erbakan University (No: 2019/2174).

3. RESULTS

Mean age of the participants was 54.12±18.21 years, 44.4% of the participants were at 61 years and above, 55.2% were male, 84% were married and 60.8% were graduates of primary school. 94% of the participants did not live alone, 60.8% lived at provincial centers. Duration of hospital stay was 5-7 days for 42.8% of the participants (Table 1).

Mean scores obtained from the clinical situation, personal life situation and decisional control over care dimensions of the ICA were 4.57±0.83, 3.87±1.23 and 4.38±0.92, respectively. On the other hand, mean scores obtained from the clinical situation, personal life situation and decisional control over care dimensions of the ICB were 4.62±0.83, 4.21±1.09 and 4.73±0.62 respectively. Mean NSNS score was 94.86±11.38 (Table 2).

Table 3 compared the mean scores obtained from the NSNS and the dimensions of the ICA and the ICB according to descriptive characteristics. Although the difference between the age groups and the scores obtained from the clinical situation and personal life situation dimensions of the ICA was not significant ($p>0.05$), the difference between age and the decisional control over care dimension of the ICA was statistically significant ($p<0.05$). Mean ICA-decisional control over care scores of the participants aged 40 years and below were significantly lower than older participants. Besides, the difference between age groups and the mean scores obtained from the clinical situation and personal life situation dimensions of the ICB was statistically significant ($p<0.05$). That is, mean scores obtained by the participants aged 40 years and below from the clinical situation and personal life situation dimensions of the ICB were significantly lower than older patients. However, there was no statistically significant difference between age groups and mean ICB-decisional control over care scores ($p>0.05$). The difference between age groups and the NSNS scores was also statistically significant with patients aged 61 years and above obtained higher scores from the NSNS ($p<0.05$) (Table 3).

Table 1. *Descriptive characteristics*

	Number (n)	Percentage (%)
Age ($\bar{x}$ =54.12±18.21)		
40 and below	62	24.80
41-60	77	30.80
61 and above	111	44.40
Gender		
Female	112	44.80
Male	138	55.20
Marital status		
Married	210	84.00
Single	40	16.00
Educational level		
Literate	30	12.00
Primary school	152	60.80
High school	33	13.20
University	35	14.00
Lives alone		
No	15	6.00
Yes	235	94.00
Lives in		
Provincial center	152	60.80
Central district	56	22.40
Village/town	42	16.80
Smoking		
Yes	29	11.60
No	221	88.40
Alcohol consumption		
Yes	6	2.40
No	244	97.60
Duration of hospitalization		
5 – 7 days	107	42.80
8-10 days	89	35.60
11 days and above	54	21.60

Table 2. *Scores obtained from ICA, ICB and NSNS*

	n	$\bar{x}$	s	Min	Max
ICA-Clinical situation	250	4.57	0.83	1	5
ICA-Personal life situation	250	3.87	1.23	1	5
ICA-Decisional control over care	250	4.38	0.92	1	5
ICB-Clinical situation	250	4.62	0.83	1	5
ICB-Personal life situation	250	4.21	1.09	1	5
ICB-Decisional control over care	250	4.73	0.62	1	5
NSNS	250	94.86	11.38	35	100

We found a statistically significant difference between education level and the mean scores obtained from the

clinical situation, personal life situation and the decisional control over care dimensions of both the ICA and the ICB (p<0.05). Mean scores obtained by the literate participants and the graduates of primary school from the clinical situation and the decisional control over care dimensions of the ICA and the ICB were significantly lower than the graduates of high school and university. Besides, mean scores obtained by the literate participants from the personal life situation dimensions of the ICA were significantly lower than the graduates of primary school (Table 3). On the other hand, we also found a statistically significant difference between the level of education and the mean scores obtained from the NSNS (p<0.05). Mean NSNS scores of the graduates of primary school were significantly higher than the graduates of university (Table 3).

Table 3. *Comparison of the ICA, ICB and the NSNS scores according to the descriptive characteristic*

Characteristics	ICA-Clinical situation	ICA-Personal life situation	ICA-Decisional control over care	ICB-Clinical situation	ICB-Personal life situation	ICB-Decisional control over care	NSNS
Age							
40 and below	4.32± 1.05	3.50 ±1.43	4.04 ±1.12	4.35±1.04	3.74± 1.34	4.54 ±0.84	91.76±14.12
41-60	4.57± 0.84	3.92 ±1.19	4.44 ±0.89	4.67 ±0.80	4.38± 0.96	4.73± 0.63	93.87±12.21
61 and above	4.70 ±0.65	4.05± 1.11	4.54 ±0.77	4.73 ±0.70	4.35± 0.95	4.83 ±0.43	97.27± 8.23
Statistical Analysis	p=.084	p=.066	p =.001*	p =.013*	p =.007*	p =.102	p =.010*
Gender							
Female	4.53±0.86	3.73 ±1.27	4.33 ±0.95	4.61 ±0.82	4.09 ±1.19	4.72 ±0.65	94.53±11.97
Male	4.60 ±0.82	3.99 ±1.20	4.43± 0.90	4.62 ±0.85	4.30 ±1.00	4.74 ±0.61	95.12±10.92
Statistical Analysis	p=.309	p=.075	p=.086	p=.324	p=.140	p=.660	p=.334
Marital status							
Married	4.60 ±0.81	3.86 ±1.22	4.41 ±0.90	4.64 ±0.83	4.23 ±1.08	4.74± 0.61	95.26±11.22
Single	4.39 ±0.91	3.95± 1.33	4.25 ±1.05	4.51± 0.88	4.11 ±1.16	4.70 ±0.69	92.75±12.12
Statistical Analysis	p=.084	p=.451	p=.318	p=.125	p=.347	p=.461	p=.127
Education							
Literate	4.29 ±0.87	3.48 ±1.33	4.19±1.00	4.39±0.93	3.89 ±1.22	4.72± 0.61	93.33±13.10
Primary school	4.22± 1.19	3.51 ±1.37	3.94±1.12	4,26± 1,16	3.79±1.24	4.41±0.96	95.85±10.09
High school	4.75± 0.62	3.77±1.30	4.40±1.03	4.77± 0.61	4.08± 1.24	4.82±0.40	95.15±10.95
University	4.66± 0.73	4.06± 1.14	4.52±0.80	4.71 ±0.74	4.40± 0.95	4.79±0.55	91.57±14.83
Statistical Analysis	p=.001*	p=.042*	p=.001*	p=.003*	p=.003*	p=.071	p=.048*
Lives alone							
Yes	4.24 ±1.01	3.97±1.30	4.40±0.83	4.55±0.80	4.20±1.15	4.66±0.69	93.93±12.75
No	4.59±0,82	3.87±1.23	4.38 ±0.93	4.62±0.84	4.21±1.09	4.73±0.62	94.91±11.32
Statistical Analysis	p=.107	p=.748	p=.887	p=.296	p=.851	p=.789	p=.886
Lives in							
Provincial center	4.55 ±0.82	3.69±1.29	4.31±0.94	4.59±0.86	4.12 ±1.12	4.72±0.59	94.04±12.56
Central district	4.63± 0.87	4.20±1.11	4.48±0.95	4.67±0.79	4.40±1.04	4.74±0.61	95.86±10.70
Village/town	4.56± 0.83	4.09±1.09	4.52±0.83	4.65±0.80	4.27±1.05	4.73±0.77	96.48±6.73
Statistical Analysis	p=.495	p=.026*	p=.086	p=.620	p=.194	p=.777	p=.733
Duration of hospital stay							
5 – 7 days	4.57 ±0.91	3.85±1.20	4.39±0.88	4.63±0.87	4.20±1.10	4.71±0.75	95.77±10.26
8-10 days	4.52± 0.81	3.81±1.25	4.33±0.98	4.59±0.78	4.21±1.10	4.72±0.56	93.57±12.90
11 days and above	4.64±0.70	4.01±1,27	4.47±0.94	4.63±0.86	4.23±1.09	4.77±0.43	95.17±10.82
Statistical Analysis	p=.471	p=.476	p=.471	p=.482	p=.952	p=.475	p=.117

**p<0,05, Mann-Whitney U, Kruskal-Wallis H*

Thirdly, there was no significant difference between the place of residence and the mean scores obtained from the clinical situation and the decisional control over care dimensions of the ICA (p>0.05). However, we found a statistically significant difference between the place of residence and the personal life situation dimension of the ICA (p<0.05). Compared to the participants, who lived in central districts, the participants living in provincial center obtained higher scores from the ICA-personal life situation dimension. On the other hand,

there was no significant difference between the place of residence and the scores obtained from the NSNS and the dimensions of the ICB (p>0.05). Finally, the difference between gender, marital status, living alone, duration of hospitalization and the mean scores obtained from the NSNS and the dimensions of the ICA and the ICB were not statistically significant (p>0.05) (Table 3).

We found a positive and statistically significant correlation between the mean scores obtained from the clinical situation, decisional control over care, and personal life situation dimensions of the ICA and the NSNS, indicating that the increase in the scores obtained from these dimensions brought an increase in NSNS scores (p<0.05). There was also a positive and statistically significant correlation between the mean scores obtained from the clinical situation, decisional control over care and personal life situation dimensions of the ICB and the NSNS, indicating that the increase in the scores obtained from these dimensions brought an increase in NSNS scores (p<0.05) (Table 4).

Table 4. *Correlation between the scores obtained from the ICA, ICB and NSNS*

		NSNS
ICA-Clinical situation	r	0.667
	p	0.000*
ICA – Personal life situation	r	0.347
	p	0.000*
ICA – Decisional control over care	r	0.455
	p	0.000*
ICB-Clinical situation	r	0.620
	p	0.000*
ICB – Personal life situation	r	0.389
	p	0.000*
ICB – Decisional control over care	r	0.591
	p	0.000*

**p<0.05,* Spearman's rho correlation test

4. DISCUSSION

Maintaining and sustaining individualized care is closely related with the participation of the patients in the decisions about his/her care, and the patient satisfaction with nursing care, which occurs during the patient-nurse interaction (21). Participants of this study obtained high scores from the clinical situation ($\bar{x}$=4.57±0.83), decisional control over care ($\bar{x}$=4.38±0.92) and personal life situation ($\bar{x}$=3.87±1.23) dimensions of the ICA, which measured the patients' views on how individuality was supported through specific nursing interventions. This finding indicated that the participants were aware of the nursing interventions supporting individuality. Similar to our findings, studies on Turkish orthopedic, internal diseases and surgical patients found that the ICA scores were relatively high (26,27). On the other hand, the study of Seyyed Rasooli et al. (2013) on Iranian patients found that the ICA scores were at moderate levels and the patients obtained the highest and lowest scores from the decisional control over care and personal situation dimensions, respectively (8). In our study, the participants were primarily concerned with their clinical situation and placed less emphasis on personal situation. In the patient-centered care, nurses should adapt a holistic approach and take individual differences into consideration. The findings of this study showed the importance of nursing interventions, which are sensitive to all aspects of the patients' lives and show this sensitivity to the patients.

The participants also obtained higher scores from the clinical situation ($\bar{x}$=4.62±0.83), personal life situation ($\bar{x}$=4.21±1.09) and decisional control over care ($\bar{x}$=4.73±0.62) dimensions of the ICB, which measured how the patients perceived individuality in their own care. The study of Rose on radiation oncology patients in Australia found that the ICB scores were high (28). Another study by Tekin on orthopedic patients in Turkey also found that the ICB scores were high (26). Patients in our study and the studies of Rose obtained the highest score from the decisional control over care dimension of the ICB (28). These findings indicated that the patients believed that they held the control during the process of nursing care and their individuality was supported by caregivers.

An important indicator to evaluate the nursing care quality is patient satisfaction (29,30). Nursing care is the primary factor that increases the quality of care (31). Mean scores obtained by the participants of our study from the NSNS was $\bar{x}$=94.86±11.38, indicating a high level of satisfaction with all dimensions of nursing care. Similarly, the study of Kersu et al. on surgical patients found that the participants were highly satisfied with the nursing care (32). The study of Yeşil et al. on intensive care patients also found high level of patient satisfaction (33). On the other hand, gynecology patients in the study of Akbaş expressed moderate levels of satisfaction with nursing care (31). Besides, 77% of the patients in the study of Olewe and Odeyemi expressed high level of satisfaction (34).

Our findings indicated that age was an important factor determining the satisfaction with nursing care. Elder participants were more satisfied with the nursing care. Cerit (2016) reported that age was not a significant factor influencing patient satisfaction with nursing care (12). The study of Yanık and Ateş (2018) on patients hospitalized in internal medicine clinics found a positive correlation between age and patient satisfaction (17). Similar to our findings, Akbaş reported that patient satisfaction increased as the age of the patients increased (31). Positive correlation between age and patient satisfaction with nursing care may be explained with reference to the tendency of older patients to avoid imposing themselves on others (12,17). Besides, older people are mostly less demanding, more tolerant and respectful towards health professionals and have more experiences with the nursing care, which, in turn, may have contributed to the positive correlation between age and patient satisfaction in our and other studies.

Decisional control over nursing interventions supporting individuality was higher for the participants aged 40 years and below. Besides, the participants aged 40 years and below obtained lower scores from the clinical situation and personal life situation dimensions of the ICB. Köberich et al. (2016) found no significant difference between age and individualized nursing care of hospitalized patients in Germany (35). Other studies reported that individualized nursing care perceptions of patients did not differ according to their age group (2,37). On the other hand, the study of Suhonen and Leino-Kilpi (2012) on orthopedic patients reported that the older patients were more positive in their evaluation of individualized care (36). Positive perceptions of individualized care among the older patients may be related to the increase in the number of chronic diseases due to aging, which, in turn, may increase the need for support to meet daily activities.

Level of education may be a factor determining patient satisfaction and the perception of individualized care (31). In our study, participants with lower level of education obtained lower scores from the clinical situation and the decisional control over care dimensions of the ICA and the ICB. Contrary to our findings, Ceylan and Eser (2016) reported that ICA and ICB scores of the patients with lower level of education were higher (38). Similarly, Köberich et al. (2016) found that individualized care perceptions of the patients with lower level of education were higher (35). Due to this reason, further qualitative studies may be conducted to evaluate the effects of education level on individualized care perceptions.

Participants, who graduated from primary school, had higher level of satisfaction with nursing care. Similarly, Yeşil et al. found that patient satisfaction was higher for the patients with lower level of education (33). On the other hand, Yanık and Ateş (2018) did not find any relationship between the levels of education and patient satisfaction (17). The study of Cerit (2015) on internal medicine and surgical patients reported that the level of education was negatively associated with patient satisfaction (12). Alasad et al. (2015) noted that education level had a significant impact on patient satisfaction and patients' expectations about the quality of care increased as their education levels increased (39). Since patients' expectations may increase parallel to the increase in their levels of knowledge, we may suggest that the participants with lower level of education had higher patient satisfaction as their expectations from the nursing care were lower.

Positive correlation between the ICA and the NSNS scores in our study indicated that the satisfaction of the participants with nursing care increased as they believed that their individuality was supported through specific nursing interventions. The study of Kersu et al. found a positive and significant correlation between the perceptions about and the patient satisfaction with the nursing care (32). The study of Tekin and Yıldız Fındık on orthopedic surgery patients in Turkey also found a positive correlation between satisfaction and the level of awareness about nursing interventions (26). On the other hand, the study of Olewe and Odeyemi at a university hospital in Nigeria reported that nurses should be sensitive to the emotions, opinions an autonomy of the patients in order to increase patient satisfaction with nursing care (34).

Existing studies noted that the respect for patients while providing nursing care had a significant impact on patient satisfaction (29). Positive and significant correlation between the ICB and NSNS scores in our study also indicated that the level of patient satisfaction increased parallel to the increase in perceived individuality in their own care. The study of Tang et al. on patients in Malesia found that the participants were least satisfied with the decisional control over care dimension of the ICB (29). Another study on Turkish intensive care patients found that the participants obtained high scores from the ICB and the NSNS (33). Higher satisfaction parallel to the perception of autonomy and individuality in nursing care was an expected finding of our study.

5. CONCLUSIONS

In conclusion, participants hospitalized in internal medicine departments perceived that the nursing interventions supported their individuality and had positive perceptions about the individuality in their own care. They were highly satisfied with the nursing interventions. Besides, patient satisfaction increased as the scores obtained from the ICA and the ICB increased. These findings revealed the importance of individualized care in increasing patient satisfaction and the quality of nursing care. Therefore, we may suggest that nurses might take the individuality of each patient into consideration while dealing with them. Besides, nursing interventions might be planned and implemented by protecting the individuality of patients and considering every aspects of their lives. Finally, further qualitative studies on the relationship between age, education level, perceived individualized care and patient satisfaction with nursing care might be carried out.

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Clinical and Experimental Health Sciences

The Complex Role of Oxytocin in Major Depressive Disorder

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ABSTRACT

Objective: One proposed mechanism to subclassify depressive illness relates oxytocinergic dysregulation, via its effect on social behavior and Hypothalamic-pituitary-adrenal (HPA) axis inhibition. To further investigate the role of oxytocin in Major Depressive Disorder (MDD), we compared plasma oxytocin levels in patients with MDD to healthy controls.

Methods: Plasma samples from 12 healthy controls and 33 MDD patients were collected at baseline, 8 weeks, and 12 weeks of treatment and oxytocin was measured by enzyme-immunoassay. Depression and anxiety scales were administered at screening, baseline, and at weeks 2, 4, 8, and 12 of treatment. Additionally, we investigated possible associations between blood concentrations of inflammatory biomarkers and oxytocin.

Results: The average baseline oxytocin level was 429 pg/ml in MDD patients and 392 pg/ml in healthy control subjects. A significant negative correlation was found between baseline oxytocin and BMI. Treatment responders had significantly lower baseline oxytocin levels than non-responders. After stratifying patients into *low* and *high* oxytocin groups based on a median split, within the *high* oxytocin group, patients with no prior depressive episodes had significantly higher baseline oxytocin levels. A Chi-square distribution test revealed that African American patients were more likely to belong to the *high* baseline oxytocin group while Caucasian and Hispanic patients were more likely to belong to the *low* baseline oxytocin group. We found significant correlations between oxytocin and Von-Willebrand Factor (VWF) and Epidermal Growth Factor (EGF), only within the *high* oxytocin subgroup. There were no other significant correlations between baseline oxytocin and any other biomarkers.

Conclusion: Within our limited patient cohort, our data adds to the mixed literature regarding the role of oxytocin in MDD. Oxytocinergic dysregulation and confounding factors may play a role for a subset of depressed patients.

Keywords: Depression, oxytocin, social behavior, HPA axis, inflammation

1. INTRODUCTION

Major depressive disorder (MDD) affects a significant portion of the population and is estimated to impact over 300 million people worldwide. Despite intense basic and translational research efforts, unraveling the etiopathology of depression remains a major challenge. This, in large part, is due to the heterogeneity of the disorder, that is further compounded by the complex interactions between genetic, epigenetic, inflammatory, endocrinological, and environmental factors (1,2). Dysregulation of multiple systems has been causally associated with depression, including immunological alterations, metabolic changes, endocrinological disturbances, and variability in the stress response. Stress and stress vulnerability vs. resilience remain significant causative factors. Current treatment regimens often consist of a combination of antidepressant medications and psychotherapy, along with other adjunctive methods to optimize outcome, resulting in variable success rates. Despite the multitude and variety of antidepressants currently available, approximately one-third of patients on the most commonly prescribed antidepressants, SSRIs or SNRIs, achieve the gold-standard of remission, while 30-40% of patients do not respond to their pharmacologic treatment regimen (3). Consequently, diagnostic subclassification and personalized treatment of depressive illness, based on presumptive multifactorial etiopathological mechanisms, underlies current and future research endeavors.

Oxytocin (OXT) is synthesized primarily in the magnocellular neurons of the paraventricular nucleus (PVN) and the

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supraoptic nucleus (SON) within the hypothalamus, and is subsequently secreted into the periphery via the posterior pituitary (2,3). OXT binds to the oxytocin receptor (OXT-R) which mediates its peripheral effects on milk ejection in lactation, cardiovascular control, and uterine contractions during labor. In addition, OXT has been associated with multiple social behaviors including social interactions, decision making during social interactions, mother-infant pair bonding, pair bonding for mating, and emotional reactivity (3,4). Thus, dysregulation of the oxytocinergic system is thought to play a role in multiple psychiatric disorders including autism, eating disorders, schizophrenia, and mood disorders (1). Considering the overlap between depressive symptoms and OXT's involvement in social behavior, it is plausible that aberrations in OXT levels may reflect this hormone's involvement in mediating at least some aspects of depressive illness, as previously reported in the literature. Therefore, alterations in both peripheral and central OXT levels may be observed in this illness (2).

A well-documented endocrine change in Major Depressive Disorder (MDD) is hypothalamic pituitary adrenal (HPA) axis dysregulation with an associated decrease in negative feedback (5). OXT and arginine vasopressin (AVP) are nine amino acid neuropeptides involved in the regulation of the HPA axis, with only a two amino acid structural difference (3,6). Whereas increased AVP levels are associated with stimulation of the HPA axis, OXT has a reciprocal function in that it is associated with inhibiting the HPA axis in response to stress (1,7). Consequently, a disrupted balance of these neuropeptides in MDD, and shifting of this balance to favor OXT thereby inhibiting AVP, may alleviate depressive symptoms (8).

Given its multifactorial nature, the precise role OXT plays in depression has yet to be elucidated. Current limited data regarding the relationship between peripheral OXT levels and depression are mixed, with studies having demonstrated both increased or decreased oxytocin levels in individuals with depression, as well as increased variability in oxytocin levels, suggesting overall dysregulation in oxytocinergic systems (9). Accordingly, we examined the relationship between plasma OXT levels in MDD patients as compared to healthy control subjects.

Over the past decades, an association has been established between inflammation and depression, and generically stress-related disorders, and inflammation-mediated cytokine release has been documented. Thus, a correlation between inflammatory biomarkers and OXT would be expected (10). Since growth factors and other neurotrophins may play a pathophysiological role in depressive illness, we measured inflammatory biomarkers, growth factors, chemokines and cytokines to further examine their role in depression and their possible association with blood levels of OXT.

2. METHODS

2.1. Study Design

Plasma samples (N=33) were obtained from MDD patients (ages 20-65 years) enrolled in two consecutively conducted studies approved by the Institutional Review Board (IRB) of Loyola University Medical Center. Patients with bipolar depression were not included in this study. Potential candidates were pre-screened with the 17-item Hamilton Depression Rating Scale (HAM-D) to establish the minimum severity requirement for study participation. Prospective subjects with a HAM-D 17 score ≥18, underwent complete screening assessment as stipulated in the study protocol.

The screening process consisted of a structured interview using the Mini International Neuropsychiatric Interview (MINI), the Diagnostic Interview for Genetic Studies (DIGS) and select rater-administered and self-rating scales. Blood chemistries, urinalysis, and toxicology screens were used to rule out any concomitant illnesses or drug use. Exclusion criteria included history of heart or thyroid disease, hypertension, diabetes, current tobacco use, illicit drug use within the preceding 12 months. Eligible patients on anxiolytic or hypnotic medications were permitted to continue at the discretion of the investigators. However, eligible patients on antidepressant or antipsychotic agents at time of enrollment underwent a 4-week washout period prior to beginning the study. Details of the screening and assessment instruments are provided in a related publication from our team (11).

Upon successful enrollment into the studies, blood samples for biomarker analyses were drawn between 9:00 and 10:00 hours to establish a baseline and patients were subsequently started on either Escitalopram (SSRI) monotherapy or Quetiapine (atypical antipsychotic) monotherapy. The latter was used to determine whether Quetiapine monotherapy could exert antidepressant efficacy in doses not exceeding 300 mg/day (12). Antidepressant effect of Quetiapine was secondary to its major metabolite, norquetiapine, being a norepinephrine reuptake inhibitor (12). Escitalopram dosing was initiated at 10 mg/day and maintained in the range of 10–30 mg/day. Quetiapine dosing was initiated at 25 mg/day and titrated up to a 300 mg/day at the investigator's discretion. Enrolled study subjects did not receive any other form of therapy during the entire study period. Follow-up blood draws and assessments using the aforementioned clinician-rated depression and anxiety scales were performed at weeks 2, 4, 8, and 12. Blood was drawn only if patients were deemed free of an active infectious process or bleeding (including menses), as well as recent disruptive life stressors (11).

To be regarded as study completers, patients were required to complete at least 8 weeks of treatment. Those who withdrew on or after 8 weeks of treatment had to complete the final assessment and their results were carried forward for data analysis.

2.2. Healthy Controls

Healthy control subjects (N=12), matched to mean age of patients, were recruited by word of mouth and the posting of campus advertisements. The screening protocol was the same as the one used for MDD subjects (11). Healthy control subjects who met eligibility criteria provided blood samples for biomarker analyses.

2.3. Plasma Oxytocin Assay

Plasma samples were centrifuged at 1600 x g for 15 minutes at 4°C, aliquoted, and stored in a – 80°C freezer. Samples were thawed at room temperature immediately prior to the assay. Plasma OXT was measured using an Enzyme Immunoassay (EIA) purchased from Enzo Life Sciences, Inc. (Farmingdale, New York). The assays were performed according to the manufacturer's instructions. As reported by the manufacturer, the EIA is highly sensitive (minimal detection rate = 15.6 pg/ml) with very little antibody cross-reactivity with other neuropeptides. Samples were diluted 1:8 in assay buffer to give reliable results within the linear portion of the standard curve and assayed in duplicate. The inter – and intra-assay coefficients of variation were <6.8% and <7.9%, respectively, for the assays (13).

Validation of these assays, as well as challenges associated with the measurement of OXT in bodily fluids are detailed elsewhere (13,14). The decision to measure OXT in unextracted samples is based on the fact that the majority of OXT in plasma is discarded during extraction procedures, leaving values in the low and unstable portion of the standard curve. Two recent studies from other laboratories have directly compared values in extracted versus non-extracted samples; in those studies, significantly stronger relationships between plasma OXT levels and behavioral outcomes were obtained in samples that were not extracted prior to assay (15,16). It is important to note that studies using enzyme-based (or radio-immunoassays) assay kits routinely give OXT values in widely different ranges (17). The sources of this variation remain poorly understood, but this is likely due, in part, to different assay procedures and differences in the antibodies used in these kits. In our studies, within-study associations in humans between plasma OXT, behavior, and other biomarkers are often significant regardless of the procedures employed (extraction or not, or different assay kits). This supports the usefulness of comparisons within a given study, but makes comparisons across studies difficult, even when those studies are done in the same laboratory.

2.4. Biomarker Measurement

Subsequent to an overnight fast, subjects rested in a reclined position for 30 minutes prior to venipuncture. Blood draws were scheduled between 9:00 and 10:00 hours. To separate plasma and serum, whole blood samples were spun, and immediately frozen and stored at – 80 degrees centigrade until analyzed. "Using "Evidence Investigator™" by Randox Technologies, multiple cytokines, chemokines, and growth factors were measured in a single sample (11). We have validated this Biochip array technology for blood samples in our previous studies. For each individual parameter, Biochip results are reliable and comparable to results obtained via individual ELISAs.

3. RESULTS

No significant difference was found between baseline OXT in MDD patients and healthy controls. At baseline the average OXT level was 429 pg/ml in MDD patients and 392 pg/ml in healthy controls (p=0.74). Male MDD patients (N=15) had average baseline levels of 457 pg/ml in comparison to female MDD patients (N=18), who had an average level of 406 pg/ml (p=0.69). No significant difference was found in baseline OXT levels between premenopausal and postmenopausal patients (p=0.2662). Within premenopausal patients, there was no significant difference between baseline OXT in healthy and depressed patients (p = 0.7459). MDD patients younger than 40 (N=14) had average baseline OXT levels of 372 pg/ml in comparison to MDD patients 40 and older (N=19), who had an average level of 470 pg/ml (p=0.43) although a trend may be evident. Compared to baseline OXT levels, MDD patients at week 8 (N=20) had OXT levels of 404 pg/ml (p=0.78) and week 12 (N=22) levels of 413 pg/ml (p=0.85).

Since baseline OXT levels showed a wide distribution of values, we sought to determine whether subgroups may exist. Accordingly, OXT levels were split into a *high* baseline group (N=17) and a *low* baseline group (N=16) based on the median value of the distribution. We also assessed possible confounding factors and determined that baseline OXT and BMI were significantly negatively correlated (r=-0.359, p=0.04) (Figure 1). A Chi-square distribution test revealed that African American patients were more likely to belong to the *high* baseline OXT group (9 high: 0 low) whereas Caucasian (6 high: 10 low) and Hispanic patients (1 high: 6 low) were more likely to belong to the *low* baseline OXT group (p=0.001).

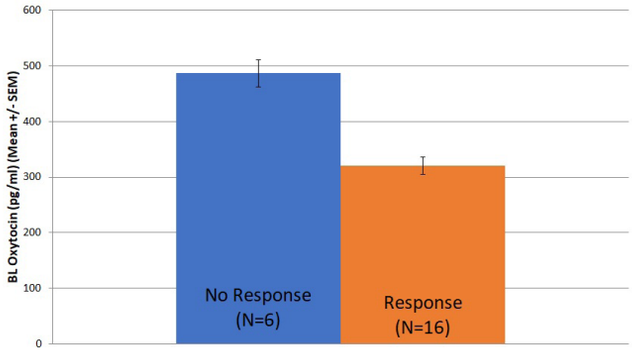

Figure 1. *Baseline oxytocin vs. BMI (R=-.359, p=0.04). Captions: Correlational analysis between baseline oxytocin levels and BMI revealed a significant negative correlation (r=-0.359, p=0.04)*

In the high OXT group, patients who had no prior depressive episodes (1141 pg/ml, N=3) had significantly higher baseline OXT levels than patients who had at least one prior depressive episode (472 pg/ml, N=14) (p=0.009) (Figure 2).

A Chi-square distribution test revealed no significant relationship between response to treatment and prior depressive episodes (p=0.47). However, total cohort analysis of baseline OXT revealed that MDD patients who were treatment responders had significantly lower OXT levels (320 pg/ml, N=16) than MDD patients who did not respond to treatment (486 pg/ml, N=6) (p=0.047) (Figure 3).

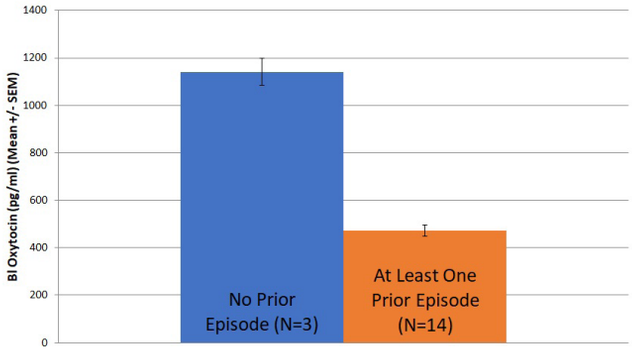

Figure 2. *Relationship between baseline oxytocin levels and depressive episodes (p=0.009). Captions Subjects were divided into high and low oxytocin groups via median split. For the high oxytocin group, patients who had no prior depressive episodes (1141 pg/ml, N=3) had significantly higher baseline oxytocin levels compared to patients who had at least one prior depressive episode (472 pg/ml, N=14) (p=0.009)*

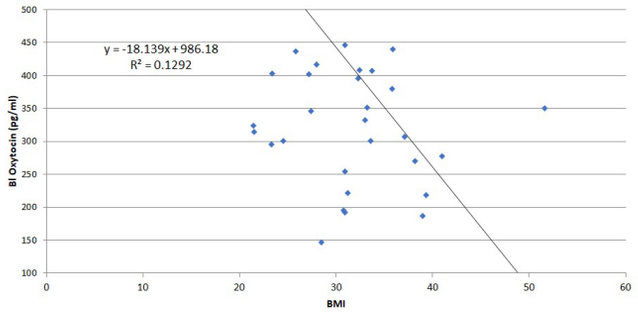

Figure 3. *Baseline oxytocin & response to treatment (p = 0.047). Captions: Analysis of baseline oxytocin levels of the total cohort found that MDD patients who were treatment responders had significantly lower oxytocin levels (320 pg/ml, N=16) than MDD patients who did not respond to treatment (486 pg/ml, N=6) (p=0.047)*

Correlational analyses in the total cohort between OXT and biomarkers revealed a trend toward statistical significance between OXT and Von Willebrand factor (VWF) (r=0.305, p=0.09) (Table 1). Correlational analysis between the *high* OXT group and biomarkers revealed significant correlations between OXT and VWF (r=0.577, p=0.015) and EGF (r=0.503, p=0.04) (Table 1).

Overall, no significant correlations were found between baseline OXT levels and scales for depression or anxiety. However, correlational analysis between the *high* OXT group and rating scales revealed a trend toward significance between OXT and HAM-D 17 (r=0.461, p=0.06).

Table 1. *Plasma Oxytocin and Biomarkers. Biomarkers were compared to baseline oxytocin levels. No significant relationship was found between any inflammatory biomarker and baseline oxytocin levels upon overall comparison of values. Overall analysis revealed a trend toward significance between oxytocin and VWF (r=0.305, p=0.09). After a median-split division of oxytocin into low and high oxytocin subgroups, significant correlations (*) were found between baseline oxytocin and VWF (r=0.577, p=0.015) and EGF (r=0.503, p=0.04) in the high oxytocin group.*

Overall		CRP	VWF	IL-2	IL-4	IL-6	IL-8	IL-10	IFNG	TNF-α	EGF
Baseline Oxytocin	Pearson Correlation	-0.045	0.305	-0.266	-0.090	0.064	-0.113	-0.123	-0.080	-0.124	0.013
	Sig. (2-tailed)	0.806	0.089	0.141	0.650	0.729	0.538	0.540	0.669	0.497	0.943
	N	32	32	32	28	32	32	27	31	32	32
Low Oxytocin Group		CRP	VWF	IL-2	IL-4	IL-6	IL-8	IL-10	IFNG	TNF-α	EGF
Baseline Oxytocin	Pearson Correlation	0.217	-0.051	0.261	0.021	-0.141	0.180	0.320	0.304	0.190	-0.243
	Sig. (2-tailed)	0.419	0.858	0.347	0.942	0.617	0.521	0.265	0.291	0.498	0.382
	N	16	15	15	14	15	15	14	14	15	15
High Oxytocin Group		CRP	VWF	IL-2	IL-4	IL-6	IL-8	IL-10	IFNG	TNF-α	EGF
Baseline Oxytocin	Pearson Correlation	0.057	0.577*	-0.477	-0.093	0.169	-0.081	-0.112	-0.179	-0.227	0.503*
	Sig. (2-tailed)	0.833	0.015*	0.053	0.751	0.515	0.758	0.716	0.491	0.380	0.040*
	N	16	17	17	14	17	17	13	17	17	17

4. DISCUSSION

4.1. OXT and Depression

The current literature is mixed regarding the association between OXT levels and depressive illness (2,18–21). While decreased peripheral OXT levels have been correlated with increased depressive symptoms, interestingly, patients with worsening depression (as well as other clinical disorders) have sometimes shown increased and/or dysregulated OXT levels (22). Given the broad functions of OXT, including HPA axis and autonomic regulation, social behavior, and a role in inflammation, multiple confounding factors likely play a mechanistic role. Another proposed explanation for the inconsistent data is that multiple prior studies were conducted without a proper control group, relied on a single biological sample, or consisted of a mixed population of patients afflicted by other disorders, such as fibromyalgia or bipolar disorder (18,19).

We found no statistically significant differences between baseline plasma OXT levels in MDD patients and healthy controls. Van Londen et al. (1997) reported similar findings, although they noted that patients trended towards higher OXT levels (21). Scantamburlo et al. (2005) compared concentrations of neurophysin-I in plasma and cerebrospinal fluid and found no significant difference between patients and healthy subjects in either sample. Neurophysin-I is the prohormonal carrier protein associated with OXT and more structurally stable, and thus proposed as indicative of chronic neurohormonal secretion (20). Furthermore, it has been suggested that multiple samples of OXT levels should be drawn due to diurnal variation of OXT (23,24). Single samples may be especially problematic in depressed patients, whose OXT level is reportedly variable than in healthy controls and depressed women are more likely than controls to display a dysregulated pattern of peripheral OXT release (22).

Some studies have reported decreased OXT levels in depressed patients. According to Ozsoy et al. (2009), not only did patients exhibit lower serum OXT concentrations, but this was true at both pre-treatment and post-treatment. This trend pertained mostly to women, however, the study included few men (18). Yuen et al. (2014) found significantly lower plasma OXT levels in female depressives compared to healthy female controls, yet some post-hoc statistical tests for males revealed no significance due to higher variability in male OXT levels.

To further complicate the issue, several studies have reported increased plasma OXT concentrations in depressive illness and that these increases peak most significantly during the night (1,19). Parker et al. (2010) measured plasma OXT levels over a 16-hour period and found a significantly increased nocturnal plasma OXT level in depressed patients. They noted this elevation occurred in the absence of corresponding depression-related alterations in AVP and cortisol levels, indicating an effect independent of the HPA axis. It was postulated that in these patients, oxytocinergic dysregulation manifests itself symptomatically as distress and impaired social behavior (19). Increased OXT in depression has been further supported by a study of OXT mRNA levels in depressed patients. Notably, the trend of increased OXT mRNA levels was only seen in autopsy measures of OXT in the PVN of patients with melancholic depression, not when comparing the entire cohort of depressed patients to controls (26). This suggests that a dysregulated oxytocinergic system may represent a subset of depressed patients, thereby possibly contributing to the mixed findings in the literature. This further supports the notion of a dysregulated oxytocin-vasopressin system in MDD patients, and may also reflect changes to the system over the lifespan, secondary to stressful and emotional experiences that may epigenetically modify OXT, vasopressin, and their receptors (9,22).

We also examined the relationship between baseline OXT and past depressive episodes. Within the *high* OXT subgroup, patients with no previous depressive episodes had significantly higher baseline OXT levels than patients who had suffered at least one prior episode ($p=0.009$). Given this strong statistical significance, it is plausible that OXT is a physiological diagnostic marker indicative of first episode of depressive illness, an observation warranting further exploration. In the current study particularly high levels of OXT were measured in the small group of patients who did not have a prior history of depression (Figure 2). it is possible that in these individuals increases in OXT were associated with early compensation for disease-associated processes, including inflammation (9). Notably, this relationship was not found in the *low* OXT group or for overall baseline OXT comparisons. Within the general population, variable OXT concentrations have been reported (5). Given the antagonistic effect of OXT on the HPA axis and its positive role in social functioning, it is plausible that higher OXT levels may confer protection against depressive episodes. This is further supported by a study that examined the relationship of OXT with social support and degree of loneliness (27). This study found a negative correlation between levels of loneliness and social support within the high OXT subgroup, further suggesting that in the presence of adequate OXT levels, the presence of good social support may diminish loneliness in depressed patients, and thus attenuate depressive symptoms. Interestingly, high OXT has been reported as predictive of greater post-partum depression (PPD) severity in women previously diagnosed with MDD. Furthermore, a higher plasma OXT concentration during the third trimester was associated with more severe symptomatology for PPD in females with a history of depression (28). Dose-dependent effects of OXT have been suggested by several experiments, with high doses of OXT even hypothesized to activate the vasopressin receptor, which may counteract OXT's protective role against stress. If confirmed, this would result in making the effects of OXT more difficult to predict, as chronically increased OXT levels may be secondary to vasopressin receptor stimulation and corresponding downregulation of the OXT receptor, and thus paradoxically result in more defensive responses (7).

4.2. OXT and Rating Scales

Given the proposed association of OXT with depressive illness, a correlation should be expected between OXT levels and depressive symptomatology, as reflected in depression and anxiety rating scales. While we found no significant correlations between baseline OXT levels and depression or anxiety scores, correlational analysis between the *high* OXT group and rating scales revealed a nearly significant positive correlation between plasma OXT and HAM-D 17 scores. This is supported by a study of sub-clinical depression in which an intervention group received "warm touch" from their spouses. This study found a positive correlation between greater depressive symptomatology and plasma and salivary OXT during the pre-intervention period. Interestingly, for this intervention group, salivary OXT levels did not remain elevated during the intervention. In the post-intervention phase, there were no longer any significant differences in plasma OXT levels in relation to depressive symptomatology. It was postulated that increased OXT levels were secondary to excessive stress levels, thus the "warm touch" intervention successfully lowered salivary OXT levels (29).

A direct association between OXT levels and Beck Depression Inventory (BDI) scores has been reported (23,30,31). In a study of HIV-positive African American and Caribbean women, those with very-high or very-low plasma OXT levels exhibited increased depressive symptomatology, as measured by the BDI (23). Furthermore, in the outpatient setting, a positive correlation was found between plasma OXT levels and temperament and character inventory (1,32). The study by Bell et al. (2006) further specified that the reward dependence sub-score accounted for 17% of the variability in plasma OXT levels.

However, there are also reports of negative correlations between plasma OXT and depressive symptomatology (6,33). These findings were based predominantly on the HAM-D, and the Spielberger State-Anxiety Inventory (1,33). In another study, females with fibromyalgia had a negative correlation between BDI scores and OXT levels in depressed versus to non-depressed patients. Subgroup analysis revealed a negative correlation between OXT levels and pain, stress, and depression scores. Interestingly, a positive correlation has been observed between OXT levels and happiness scores, suggesting that OXT may play a role in improving depressive symptoms such as anhedonia and impaired social reward responsiveness (34,35). The discrepant relationship between depressive symptoms and OXT levels further suggests that a dysregulated oxytocinergic pathway not only plays a role in depression but may also explain why there are mixed reports in the literature (31). This non-linear relationship could also help to explain why overall baseline OXT levels may not correlate with baseline scales for depression or anxiety (18).

4.3. Oxytocin and Treatment

We observed no statistically significant changes in plasma OXT levels over the course of treatment. Normalization of the HPA axis has been a major target of recent treatments for stress-related diseases, such as depression and anxiety, as their etiology is often multifactorial, including a dysregulated stress response and other factors such as genetic predisposition (5,36). Consequently, several clinical studies have examined the relationship between OXT and antidepressant treatment, often demonstrating that treatment does not significantly affect OXT levels (5,20). In a study of acute apomorphine or clonidine injections on OXT neurophysin levels in depressed patients there was no effect of ether medication (20). This was further supported by Ozsoy et al. (2009), who found that neither antidepressant drug treatment nor ECT had a significant effect on serum OXT levels (18). Of note, the patient population for this study consisted of patients with bipolar disorder and MDD patients. Similarly, Keating et al. (2013) found no significant change in OXT and cortisol concentrations in pre-SSRI and post-SSRI conditions (5). The relationship between OXT and SSRI is of interest, as serotonin is known to increase plasma OXT and plasma vasopressin levels (37). Consequently, it is plausible that OXT administration would also augment the effectiveness of antidepressant treatment. This has been demonstrated by one open trial study that found an improvement in depressive symptomatology with the combined administration of intranasal OXT and oral escitalopram. It is important to note that this trial consisted of a small patient cohort and did not include a control group. Consequently, it could not be differentiated whether the positive effect on depressive illness was secondary to the medication combination or due to a placebo effect (36). Unfortunately, while animal models of depression have suggested an antidepressant effect from OXT, this effect does not seem to be an effective alternative treatment, at least at this point. While intranasal OXT does not demonstrate a significant side effect profile, it has been ineffective in and of itself in ameliorating symptoms of depression or anxiety (38). However, research regarding OXT as adjunctive treatment and long-term use of OXT merits further investigation. In conjunction with pre-clinical data demonstrating increased OXT mRNA in the magnocellular PVN subsequent to acute citalopram administration, further research is warranted to examine this relationship. It is also important to consider that pre-clinical data did not demonstrate an increase in OXT mRNA levels subsequent to chronic citalopram (39).

After stratifying into *low* and *high* OXT subgroups, we found that responders to treatment had recorded significantly lower OXT levels at baseline compared to non-responders. Additional statistical analysis demonstrated that MDD patients who responded to treatment were no more likely to fall into the *low* OXT group than those who did not respond to treatment. A study by Lien et al. (2017) found that in the post-treatment condition, OXT levels in bipolar patients remained elevated whereas MDD patients demonstrated a slight decrease. Comparatively, MDD patients had significantly lower OXT levels, compared to bipolar patients, after treatment (6). Interestingly, one study of African American male veterans found a significant increase in psychiatric

medication use among those with higher OXT levels, even after adjusting for BMI (40).

4.4. Oxytocin and Metabolic Effects

We found a significant negative correlation between plasma oxytocin and BMI. Obesity has been associated with inflammation specifically characterized by a chronic state of systemic inflammation that contributes to insulin resistance, dyslipidemia, diabetes and cardiovascular disease (41). The physiologic effects of OXT on metabolism and its therapeutic potential for metabolic disorders have been discussed in a review article by McCormack et al. (42). In model systems, OXT promotes weight loss by decreasing energy intake. Pre-clinical studies have suggested that OXT plays a critical role in glucose and lipid metabolism, controlling weight, increasing motivation for being physically active, and decreasing consumption of food (43). This was supported by a study of African American male veterans that found lower HbA1c levels, BMI, and weight in those whose oxytocin levels were most elevated. Overall, these observations suggest a positive metabolic role of OXT (40). In the literature OXT has been referred to as a "satiety hormone" and has been demonstrated to be anorexigenic by affecting the reward pathway (18,44,45). Furthermore, preclinical studies have shown reduced plasma OXT levels in mice with diet-induced obesity and a subsequent increase in OXT levels in synatpotagmin-4 deficient mice that are protected against diet-induced obesity (46,47). It has also been shown that patients afflicted by Type 2 Diabetes and obesity are more likely to have lower oxytocin levels (40,47). Interestingly, the literature is mixed, as one study reported a weak positive correlation between plasma OXT levels and BMI in male patients only (18). Given the overlap between OXT's metabolic effects and the symptoms of decreased appetite and weight changes that may be seen in depression, it is plausible that these symptoms may be, at least partially, induced by a dysregulated oxytocinergic pathway (26).

4.5. OXT and Demographics

We found that African Americans were more likely to belong to the *high* OXT subgroup, whereas Caucasians and Hispanics were more likely to belong to the *low* OXT subgroup. In the context of pain tolerance, differences have been demonstrated between OXT, an inhibitor of the pain response, and ethnicity. African American women exhibited lower tolerance to ischemic pain as well as lower plasma OXT levels. In this study Grewen et al. (2008) also reported that African American females had significantly lower plasma OXT levels at baseline compared to non-Hispanic White females. A subset of study participants also underwent the Trier Social Stress Test (TSST), which elicits cardiovascular and neuroendocrine stress responses. Subsequent to the TSST stressor, a strong correlation was found between elevated baseline OXT and elevated OXT levels after stress. Interestingly, this study also noted a significant negative correlation between OXT levels and systolic blood pressure only in African American participants (48).

While we found no statistically significant differences between male and female patients, there is literature suggesting a gender disparity in OXT levels. In a study by Holt-Lunstad et al. (2011), females with high depressive symptomatology demonstrated significantly elevated plasma OXT levels compared to males and non-depressed females (29). Some, but not all, studies have shown that females have higher OXT levels and increased expression of the OXTR (49,50). Furthermore, this sexual dimorphism has been observed in areas of the brain where OXT exhibits behavioral effects, such as the ventromedial hypothalamic nucleus (50,51). Notably, the literature is mixed regarding the relationship between gender and OXT, as several studies have demonstrated no significant difference or even decreased OXT levels in females (6,18,25). It has been hypothesized that one contributor to variation in female OXT levels may be gender-specific features, such as lactation history and birth history. Peripheral OXT levels also fluctuate during various phases of the menstrual cycle, and may also vary in those who use oral contraceptives, making it more difficult to interpret baseline OXT levels (52). It has also been postulated that females may be more sensitive to the interplay between OXT and stress, as ovarian hormones, such as estrogen, may serve as an additional factor involved in regulating the HPA axis (18).

4.6. Oxytocin and Measured Biomarkers

Inflammation-mediated cytokine release in depressive illness is well established. A correlation between inflammatory biomarkers and OXT is plausible given literature regarding an inflammatory pathophysiology of depression in conjunction with HPA axis overactivation and oxytocinergic dysregulation in depressed patients. Interestingly, while we did not find any significant relationships between plasma OXT levels and measured interleukins, we found a nearly significant positive relationship between baseline OXT and Von Willebrand factor (VWF). After stratifying patients into *high* and *low* OXT subgroups, there was a significant positive relationship between plasma OXT level and VWF and Epidermal Growth Factor (EGF) within the *high* OXT group. Within the limited literature relating OXT to inflammation, especially in the context of depressive illness, there are also mixed data (47,53). EGF has been discussed in relation to OXT, in the context of the inflammatory cascade during pregnancy. EGF is a growth factor that accumulates in amniotic fluid and can be measured in maternal and fetal blood. EGF binds to the EGF receptor, activating tyrosine kinase and several subsequent signaling cascades to induce prostaglandin production. OXT is an agonist of prostaglandin production, with both EGF and OXT stimulating cyclooxygenase-2 (COX-2) expression in myometrial cells. COX-2 inhibition produces an augmented antidepressant response and even reversal of treatment resistance in both MDD and bipolar disorder (54,55). Furthermore, it was found that this signaling requires

protein kinase c activity (53). Interestingly, OXT has also been postulated to have anti-inflammatory effects. Animal studies found a negative correlation between serum OXT levels and High Sensitivity C-Reactive Protein (*hs*-CRP), in the context of dyslipidemia. Treatment with OXT resulted in decreased expression of Interleukin-6 and Monocyte Chemoattractant Protein-1 (MCP-1) and elevated Adiponectin, however, these trends did not reach statistical significance (47). In summary, further investigation is warranted to elucidate the role of these biomarkers and their possible relationship with OXT in the context of depressive illness.

5. CONCLUSION

While there were no significant correlations between baseline OXT levels and any rating scores, a significant positive relationship was found between MDD patients in the *high* OXT group and HAM-D 17. Treatment responders had significantly lower OXT levels at baseline than non-responders, indicating that comparatively low OXT may be a predictor of response to treatment. Within the high OXT group, patients who had no previous depressive episodes had significantly higher baseline OXT levels than those with one or more prior episodes, suggesting OXT may be a potential diagnostic marker for depressive illness. Significant positive relationships were found between patients in the *high* OXT group and VWF and EGF. There was a significant negative relationship between OXT and BMI.

One potential limitation of our study is the use of plasma OXT, as peripheral blood levels may not be an accurate representation of central OXT levels. Furthermore, we did not account for temporal variations in OXT.

Ethical Statement

1. This material has not been published in whole or in part elsewhere
2. The manuscript is not currently being considered for publication in another journal
3. Informed consent was obtained for experimentation with human subjects

Declaration of conflicting interests

The authors declare no conflict of interest with respect to the research, authorship, or publication of this article. The authors are responsible for the content and writing of the paper.

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Clinical and Experimental Health Sciences

Mutations in the SARS CoV2 Spike Gene and Their Reflections on the Spike Protein

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ABSTRACT

Objective: In this study, it was aimed to determine the mutation frequency in spike *(S)* genes of SARS CoV2 from six different regions of the world, their distribution on the gene and reflections of these mutations to the S protein.

Methods: SARS CoV2 *S* gene sequences originating from Asia, Africa, Europe, South America, Oceania and North America were obtained from NCBI virus database. The sequences were analyzed with *Geneious* and *BioEdit* multiple sequence alignment programs.

Results: 865 distinct mutations on the *S* genes were detected in the virus samples. Among these, 59 variants with numbers of 10 and above in the virus population were detected. The D614G(A1841G) substitution was found to be the most common with an average of 88.6%. Furthermore, it was determined that S477N(G1430A) substitution in the viruses of Oceania differed from other regions with a rate of 86.7%. The average mutation frequency of the *S* genes from different regions was calculated as $3{,}93x10^{-5}$.

Conclusion: The significant differences among the mutation frequencies in SARS CoV2 *S* genes isolated from different regions was identified. At least five distinct amino acid substitutions with high ratios in the population were detected in the RBD domain, which is involved in the binding of the viruses to the ACE2 receptor. These substitutions are T1355G (L452R), G1430A (S477N), C1433A (T478K), G1450A (E484K) and A1501T (N501Y). Among these, the S477N is the most predominant in the population. However, the importance of these mutations needs to be demonstrated both *in silico* and experimental studies.

Keywords: SARS CoV2, Coronavirus, Covid-19, Spike protein, Mutation

1. INTRODUCTION

Coronaviruses, which are usually the cause of mild respiratory tract infections, did not attract much attention until the outbreaks of SARS CoV(1) and MERS CoV(2,3) that caused the severe acute respiratory disease in China in 2002 and Saudi Arabia in 2012. In December 2019, a new type of coronavirus, SARS CoV2, emerged in Wuhan, China Hubei province (4), spread all over the world in a very short time, and caused a global pandemic. The SARS CoV2 causing Coronavirus Disease-2019 (COVID-19) in humans have been classified in the order *Nidovirales*, family *Coronaviridae* (5,6). According to the World Health Organization (WHO), more than 168 million people were infected with this virus in one and a half years, and about 3.5 million people died due to COVID-19 (https://covid19.who.int/ access: 27.05.21). The SARS CoV2 has a single-stranded, positive-sense RNA genome with an average size of 30 kb. This genome encodes four structural proteins including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (7). Generally, viruses that carry the RNA genome mutate at higher frequencies than DNA viruses. The RNA viruses typically have mutation rates that range between 10^{-6} and 10^{-4}. The mutation rates of DNA viruses are about 10^{-8} to 10^{-6} substitutions per nucleotide site per cell infection (8,9). The high rates of mutation in the RNA viruses can be explained by RdRP (RNA dependent RNA polymerase) that replicates the viral genome. Unlike many DNA polymerases, the RdRP enzyme has no proofreading activity and therefore, cannot correct the errors that occurred during replication. For this reason, influenza A viruses among the RNA viruses, which cause epidemics much more commonly than the coronaviruses in humans, have a very high mutation frequency and show a wide variation (10). Due to these high mutation rates and the variation, these viruses have a wide range of hosts (11,12). Unlike other RNA viruses, the viruses classified in the order *Nidovirales*, including coronaviruses, have proofreading capabilities that are independent of RdRP enzymes (13). This proofreading is thought to be an important factor in explaining how these viruses have much larger genomes (average 30 kb) when compared to other

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RNA viruses (14). Although the mutation frequency is lower than the other RNA viruses, mutations in the SARS CoV2 cause great concerns due to the possibility of the emergence of new variants that can be transmitted more easily from person to person and have higher mortality. One of the most important factors affecting the spreading rate of the SARS CoV2 is the spike glycoprotein, which is responsible for the attachment of the virus to the host cell receptors and its entry into the cells (15). SARS CoV2 spike protein consists of extracellular domains, a transmembrane domain (TM) and a short intracellular tail (CP). This protein is located as a homotrimeric structure on the surface of the viral envelope. The extracellular domain of the spike protein consists of S1 and S2 subunits responsible for binding to the host cell receptor and membrane fusion, respectively. The S1 subunit of the protein contains an NH_2-terminal domain (NTD) and the carboxyl terminal domain (CTD), also called the receptor binding domain (RBD) (16). The RBD of spike protein plays a crucial role in the binding of the viruses to the host cell receptor, the angiotensin converting enzyme 2 (ACE2) that is required for viral entry (17). The mutations causing amino acid substitution in the spike protein, which has a crucial role in both the attachment and entry to the cells, can significantly alter the rate of the transmission and pathogenesis of the virus.

The COVID-19 pandemic has massively accelerated the whole-genome analysis of SARS CoV2, and increased the virus genome database. In this study, the cDNAs encoding the spike proteins of the SARS CoV2 originating from Asia, Africa, Europe, South America, Oceania and North America were analyzed in terms of mutations based on the reference SARS CoV2 spike gene, which was first identified in Wuhan, China, and the amino acid substitutions caused by these mutations in the spike protein the distribution of the variations and their possible effects on virus pathogenesis are discussed.

2. METHODS

The full-length spike gene sequences of SARS CoV2 isolates from 6 different geographic regions in the world were obtained in the FASTA format from the NCBI virus database (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/) on 21 April 2021. The multiple sequence alignment of the cDNAs was achieved using *Geneious* and *BioEdit* software. The spike gene cDNA of the Wuhan-Hu-1 variant (NCBI accession code: NC_045512.2/YP_009724390.1) was used as the reference sequence. The samples with uncertainty in the nucleotide sequence were excluded from the analysis. Based on the Wuhan-Hu-1 variant, the nucleotide differences and positions, and the mutation frequencies in the genes, were determined. Similarly, amino acid substitutions in the spike proteins encoded by the genes were compared with the reference protein (NCBI accession code: YP_009724390.1). The ratio of the amino acid substitutions and the positions of these changes in the spike proteins encoded by the viruses that were isolated in different geographic regions were determined. The possible effects of amino acid replacement in the spike proteins encoded by different SARS CoV2 variants on the spread of the virus in humans were evaluated.

3. RESULTS

3.1. The ratios of single nucleotide changes in SARS CoV2 Spike genes

The mutations in the SARS CoV2 spike gene that cause amino acid substitutions in the protein have the potential to affect the transmission rate and pathogenicity of the virus. In this regard, the SARS CoV2 spike genes isolated in different geographical regions of the world were aligned using multiple sequence alignment programs, and both single nucleotide changes in the genes and amino acid substitution in the spike glycoproteins were analyzed. As of 21 April 2021, the sequencing data of the 5760 SARS CoV2 spike genes from six geographical regions of the world collected in genome databases were examined in terms of nucleotide changes. The mutation rates in the spike genes of SARS CoV2 isolates in different regions were determined (Table 1). Among the SARS CoV2 variants isolated in Africa, South America, or Europe until 21 April 2021, all samples except those with errors in the nucleotide sequences were evaluated. 2265, 9900 and 75630 samples isolated in Asia, Oceania and North America were defined in the SARS CoV2 genome database collected between 13 January 2020-21 April 2021, respectively. A total of 1000 samples within the SARS CoV2 isolates from Asia were evaluated in proportion to the sample number collected over 16 months. No data was found in Oceania between January and April 2020. In this region, a total of 1150 samples determined in proportion to the data collected between May 2020-April 2021 were analyzed. A total of 149 genome sequences of the SARS CoV2 isolates in North America were found in January-March 2020. In this region, 1649 samples, including the 149-genome sequence, and randomly selected 100 samples among the collected sequences in each month were evaluated. Regardless of the number of variants in the virus populations, the number of distinct nucleotide changes in the spike genes isolated from each region, was determined, and the mutation frequencies in the SARS CoV2 spike gene were calculated relative to the total number of nucleotides sequenced (Table 1).

Table 1. *The number of SARS CoV1 spike genes analyzed from different geographical regions in the world, the mutation frequencies and the number of substitutions in the genes.*

Geographic Regions	Number of Samples	Mutation Frequencies	Number of Nucleotide Substitution
Africa	721	$8{,}27x10^{-5}$	228
N.America	1649	$7{,}20x10^{-5}$	454
Europe	800	$5{,}56x10^{-5}$	170
Asia	1000	$5{,}94x10^{-5}$	227
S.America	440	$4{,}10x10^{-5}$	69
Oceania	1150	$2{,}37x10^{-5}$	104
Average (All Geographic Regions)	**5760**	**$3{,}93x10^{-5}$**	**865**

Table 2. *The average distribution rates of spike variants with numbers 10 or more in virus populations of different geographical regions in the world.*

Nucleotide Changes	Amino Acid Changes	The rates of amino acid substitutions in The SARS CoV2 Spike proteins from different geographic regions (%)							Phenotypic Effect
		Africa	Asia	Europe	North America	South America	Oceania	Total	
C13T	L5F	-	1,4	-	2,12	-	-	0,85	Missense
C35T	S12F	5,55	-	-	-	-	-	0,69	Missense
G38T	S13I		-	-	1,52	-	-	0,43	Missense
C52T	L18F	-	-	-	1,09	-	-	0,31	Missense
C59T	T20N	-	-	-	0,91	-	-	0,26	Missense
C76T	P26S	-	-	-	1,15	-	-	0,33	Missense
G162T	L54F	-	7	-	-	-	-	1,22	Missense
C249A	V83V	-	-	-	0,85	-	-	0,24	Silent
T363C	N121N	-	-	-	-	-	1,65	0,33	Silent
G412T	D138Y	-	-	-	1,03	-	-	0,30	Missense
G456T	W152C	-	-	-	1,33	-	-	0,38	Missense
G570T	R190S	-	-	-	0,73	-	-	0,21	Missense
T600C	Y200Y	-	-	-	1,33	-	-	0,38	Silent
C665T	A222V	2,08	-	2,38	-	-	-	0,59	Missense
A693T	I231I	-	-	-	2,79	-	-	0,80	Silent
A758G	D253G	-	-	-	2,06	-	-	0,59	Missense
C882T	D294D	-	22,5	-	-	-	-	3,91	Silent
G906T	T302T	2,22	1,6	1,63	-	-	-	0,78	Silent
C915T	S305S	-	1,7	-	-	-	-	0,30	Silent
C918T	F306F	-	-	-	-	-	17	3,40	Silent
C1062T	N354N	5,55	-	-	-	-	-	0,69	Silent
C1151T	P384L	1,94	-	-	-	-	-	0,24	Missense
A1250C	K417T	-	-	-	0,73	-	-	0,21	Missense
T1355G	L452R	1,94	-	-	1,58	-	-	0,69	Missense
A1358T	Y453F	-	-	1,63	-	-	-	0,23	Missense
G1430A	S477N	3,88	-	-	-	-	86,7	17,80	Missense
C1433A	T478K	-	-	-	1,7	-	-	0,49	Missense
G1450A	E484K	-	-	-	0,91	-	-	0,26	Missense
A1501T	N501Y	-	-	-	0,85	-	-	0,24	Missense
C1565T	A522V	-	1,5	-	-	-	-	0,26	Missense
C1629T	F543F	-	-	-	0,79	-	-	0,23	Silent
G1839A	Q613Q	-	-	-	-	-	86,7	17,31	Silent
A1841G	D614G	90,6	89,7	83,5	82,8	95,2	95,6	88,56	Missense
C1895A	T632N	-	-	-	-	-	1,39	0,28	Missense
C1963T	H655Y	-	-	-	0,91	-	-	0,26	Missense
T1986C	C662C	-	-	-	-	-	1,48	0,30	Silent
A2030C	Q677P	-	-	-	0,67	-	-	0,19	Missense
G2031T	Q677H	22,5	-	-	2,12	-	-	3,42	Missense
C2042G	P681R	6,8	-	-	-	-	-	0,85	Missense
C2042A	P681H	-	-	-	3,64	-	-	1,04	Missense
C2093T	S698L	-	-	-	-	-	0,96	0,19	Missense
G2101A	A701T	-	1,3	-	-	-	-	0,23	Missense
T2133A	S711S	-	-	1,5	-	-	-	0,21	Silent
C2169T	T723T	30,2	-	5,38	-	11,4	-	5,40	Silent
A2194G	T732A	-	-	-	3,09	-	-	0,89	Missense
C2367T	Y789Y	-	1,8	-	-	-	-	0,31	Silent
C2435T	P812L	-	3,3	-	-	-	-	0,57	Missense
C2472T	N824N	-	-	-	1,52	-	1,04	0,64	Silent
T2514C	G838G	-	-	-	1,58	-	-	0,45	Silent
G2515T	D839Y	-	-	2,13	-	-	-	0,30	Missense
T2787C	S929S	-	-	-	1,09	-	-	0,31	Silent
C3080T	T1027I	-	-	-	1,15	-	-	0,33	Missense
A3132T	G1044G	-	-	-	0,97	-	-	0,28	Silent
T3249A	H1083Q	-	1,7	-	-	-	-	0,30	Missense
C3342T	I1114I	-	-	-	1,64	-	-	0,47	Silent
G3371T	G1124V	-	-	-	-	-	3,39	0,68	Missense
G3526T	V1176F	-	-	-	1,15	-	-	0,33	Missense
C3782T	S1261F	-	-	-	-	4,09	-	0,31	Missense
G3790T	V1264L	5,96	-	-	-	-	-	0,75	Missense

The highest mutation frequency was defined in the spike genes with a value of $8.27x10^{-5}$ in the SARS CoV2 isolates from Africa when compared to other regions. The SARS CoV2 population from North America followed the African population with a mutation rate of $7.20x10^{-5}$. The lowest mutation frequency ($2.37x10^{-5}$) in the spike genes among the SARS CoV2 populations evaluated as defined in the viruses from Oceania. This mutation ratio was followed by mutations in the spike gene of the virus populations originating from South America with $4.10x10^{-5}$, Europe with $5.63x10^{-5}$ and Asia with $5.94x10^{-5}$. The average mutation frequency in the spike genes of the 5760 SARS CoV2 samples collected from different geographic regions was calculated as $3.93x10^{-5}$.

3.2. The amino acid substitutions and distribution in the SARS CoV2 Spike protein due to mutations in the gene

We performed multiple alignments of the amino acid sequence of the spike proteins encoded by the genes collected from different geographic regions in the world, defined the amino acid substitutions and compared them with each other. The nucleotide changes of the variants with numbers 10 or more in different virus populations and the corresponding amino acid substitution rates in the spike proteins are given in Table 1. Among the spike genes analyzed in the virus populations, the total distinct 59-nucleotide changes at different positions were detected in the variants with numbers 10 and above. It was defined that 20 of these nucleotide changes were silent mutations and did not cause amino acid substitution in the spike protein. Among all the virus populations, the most common amino acid substitution in the spike proteins is the D614G substitution comparing the reference Wuhan-HU1 (Spike cDNA: NC_045512.2 / Spike Protein: YP_009724390.1) virus. The ratio of variants having this substitution in the virus populations from different geographic regions varies between 82.8% and 95.6%. The average of the D614G substitution in spike proteins in all the viruses analyzed is about 89.6%. It draws attention to the variants having G1430A (S477N) substitution in the spike gene as high as 86.7% in the Oceania virus population. This mutation was defined with a rate of 3.88% only in the virus population originating from Asia, and was not found in the viruses from other regions. Another mutation with a high rate of 86.7% in the Oceania virus population is the G1839A (Q613Q), which is a silent mutation. Both of these mutations

likely emerged in the early stages of the pandemic. The most common mutations on the spike gene in the African-origin virus population are the G2031T (Q677H) and C2169T (T723T) with 22.5% and 30.2% respectively. The ratio of the C2169T silent mutation is 11.4% in the South American virus population, while it is 5.38% in Europe. This mutation was not defined in the virus populations from the other three geographic regions. The C882T (D294D) silent mutation has the second highest mutation rate in the Asian population with 22,4%. This mutation has not been found in other virus populations in the world.

In SARS CoV2 viruses, as a result of missense mutations in virus genes, especially caused by the viral RdRP, a large number of amino acid substitutions occur in the viral protein. The viral surface antigens such as the spike and influenza HA proteins have the most flexibility to change with missense mutations. These changes offer a wider range of host organisms to RNA viruses for replication. Therefore, the amino acid substitutions that occurred in the SARS CoV2 surface antigens have greater importance for the spread of the viruses and increasing their host diversity. The amino acid substitutions resulting from the missense mutations and their distributions in the spike protein consisting of 1273 amino acid residues are given in Figures 1-7. Figure 1 shows the distinct amino acid substitutions, in total 5760 spike proteins of SARS CoV2 viruses from different geographic regions analyzed in this study. The distribution of the amino acid substitutions in the spike protein caused by the missense mutations detected at least once in all virus populations is shown in Figure 1A. Although the homogeneous amino acid substitutions covering almost all parts of the spike protein is observed, it appears that the variations are slightly more intense in some regions. For instance, more amino acid substitutions are seen in regions spanning 1-100., 225-300., and 650-725. amino acid residues are found on the amino terminal half of the spike proteins. There are also some intense variations in regions corresponding to the 1150-1273 amino acid residues involving the HR2, TM and CP domains at the carboxyl-terminal end of the protein. On the other hand, most of the spike gene variants seen in the SARS CoV2 populations from different geographical regions consist of one or two samples. The number of variants with a rate of 0.25% and above in the virus populations and their positions on the spike protein is given in Figure 1B. Among the functional regions on the spike protein, the intensity of the amino acid substitutions in the amino terminal end of the spike including signal peptide draws attention. In this region, C13T (L5F), C35T (S12F), G38T (S13I), C52T (L18F), C59T (T20N) and C76T (P26S) substitutions with a ratio of 0.25% and above were detected.

At least five distinct amino acid substitutions with high ratios in the population were detected in the RBD domain, which is involved in the binding of the SARS CoV2 to the ACE2 receptor on the host cells. These are T1355G (L452R), G1430A (S477N), C1433A (T478K), G1450A (E484K) and A1501T (N501Y) substitutions. Among these, the S477N is the most predominant in the population. The mutations that cause the amino acid substitutions in the RBD of the spike protein may have a negative effect on the replication and spread of the viruses as well as facilitate the virus replication. The prevalence of G1430A (S477N) substitution in the virus population suggests that this mutation positively affects virus replication.

The amino acid substitutions in the spike proteins of SARS CoV2 were also evaluated regionally within themselves. In the viruses isolated from Asia, there is no amino acid substitution with a ratio of 0.5% or more in the spike proteins, affecting the RBD located in the amino terminal half, and the CP, TM, HR1 and HR2 domains at the carboxyl terminal of the protein (Figure 2). In contrast, four distinct changes in the RBD are seen in the spike proteins of viruses originating in Africa. Three amino acid substitutions with a ratio of over 0.5% in the SP located at the amino terminal end of the spike proteins of the viruses from Africa were defined (Figure 3). The number of SARS CoV2 spike gene sequencing data from South America was less than from the other regions. In the virus samples examined, there are no amino acid substitutions in the RBD of spike protein with a significant ratio comparing to the reference protein (Figure 4). Three amino acid substitutions with a ratio of more than 0.5% in the RBD of the proteins were determined within the SARS CoV2 isolates from Europe. In this virus population, two amino acid substitutions in the SP domain and one in the CP domain were determined in the spike proteins (Figure 5).

It was observed that the mutation resulting in the replacement of aspartic acid (D) to glycine (G) at the 614th position (D614G) of the spike protein had the highest ratio in all the virus populations. In addition to A1841G (D614G) missense mutation, a high ratio of the S477N substitution was detected in the virus isolates from Oceania. This amino acid substitution occurred in the RBD of the protein and was detected in 997 of the 1150 samples examined (Figure 6). However, the S477N substitution in the spike protein was not found within the viruses isolated in Europe, America, and Africa.

Most of the genome sequence data for the SARS CoV2 originated from North America. As of April 21, 2021, the SARS CoV2 genome sequencing data was determined as 75630. Here, the spike gene/protein belonging to 1649 samples of this data was evaluated. Four different amino acid substitutions with a ratio of 0.5% and more were detected in both the SP and RBD of the spike protein originating from North America (Figure 7).

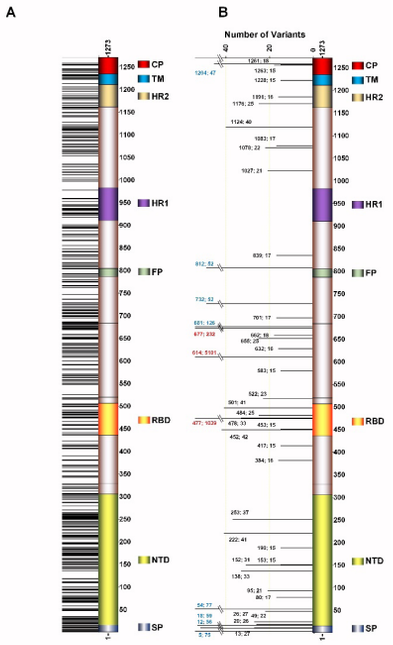

Figure 1. *Distribution of the amino acid substitutions on the spike proteins of SARS CoV2 isolates in the six geographic regions of the world and the number of distinct variants in the virus population. A. Distribution of amino acid substitutions on the spike protein, which is determined in all virus samples regardless of their number in the population. B. The number of variants with a ratio of higher than 0.25% (15 and more) in the virus population and the positions of amino acid substitutions. CP: cytoplasmic domain, TM: transmembrane domain; HR1 and HR2: heptad repeat, FP: fusion peptide RBD: receptor-binding domain NTD: N-terminal domain; SP: signal peptide.*

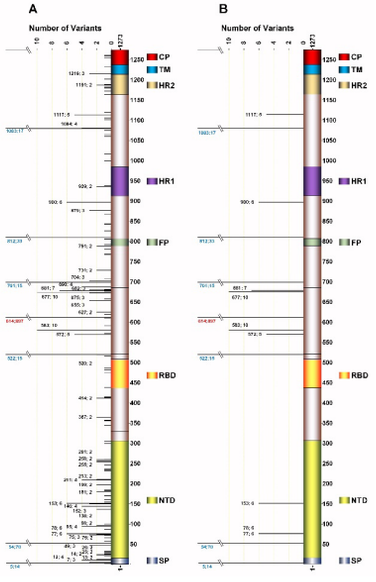

Figure 2. *Distribution of the amino acid substitutions on the spike proteins of SARS CoV2 isolates in Asia and the number of distinct variants in the virus population. A. Distribution of amino acid substitutions on the spike protein, which is determined in all virus samples regardless of their number in the population. B. The number of variants with a ratio of ≥ 0.5% (5 and more) in the virus population and the positions of amino acid substitutions.*

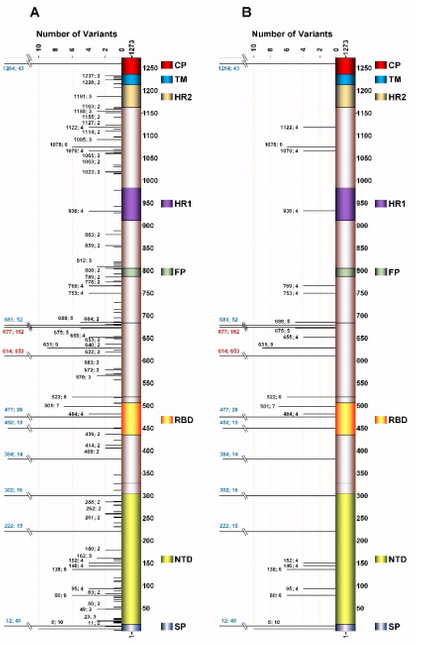

Figure 3. *Distribution of the amino acid substitutions on the spike proteins of SARS CoV2 isolates in Africa and the number of distinct variants in the virus population. A. Distribution of amino acid substitutions on the spike protein, which is determined in all virus samples regardless of their number in the population. B. The number of variants with a ratio of ≥ 0.5% (4 and more) in the virus population and the positions of amino acid substitutions.*

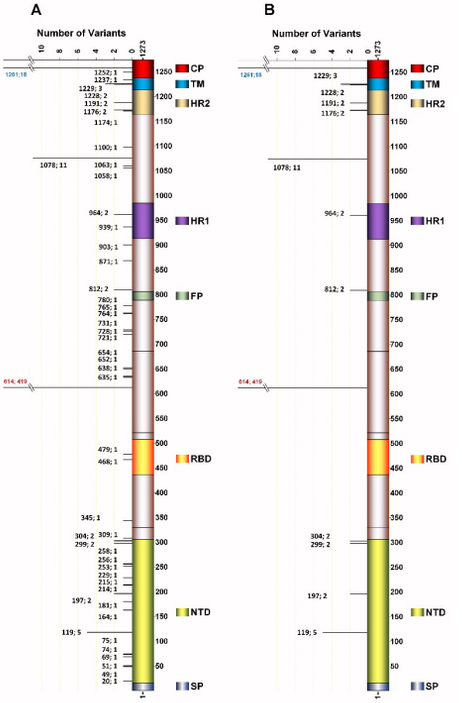

Figure 4. *Distribution of the amino acid substitutions on the spike proteins of SARS CoV2 isolates in South America and the number of distinct variants in the virus population. A. Distribution of amino acid substitutions on the spike protein, which is determined in all virus samples regardless of their number in the population. B. The number of variants with a ratio of ≥ 0.5% (2 and more) in the virus population and the positions of amino acid substitutions.*

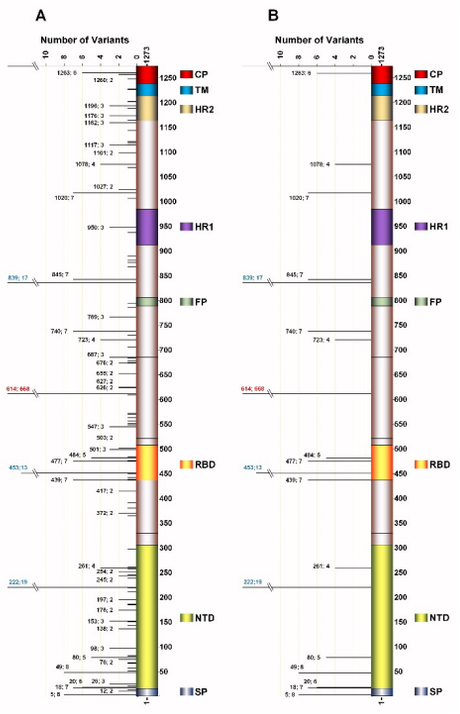

Figure 5. *Distribution of the amino acid substitutions on the spike proteins of SARS CoV2 isolates in Europe and the number of distinct variants in the virus population. A. Distribution of amino acid substitutions on the spike protein, which is determined in all virus samples regardless of their number in the population. B. The number of variants with a ratio of ≥ 0.5% (4 and more) in the virus population and the positions of amino acid substitutions.*

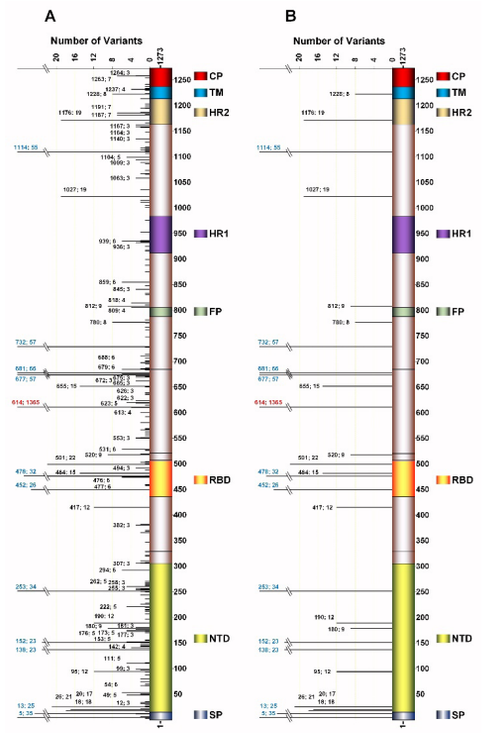

Figure 7. *Distribution of the amino acid substitutions on the spike proteins of SARS CoV2 isolates in North America and the number of distinct variants in the virus population. A. Distribution of amino acid substitutions on the spike protein, which is determined in all virus samples regardless of their number in the population. B. The number of variants with a ratio of ≥ 0.5% (8 and more) in the virus population and the positions of amino acid substitutions.*

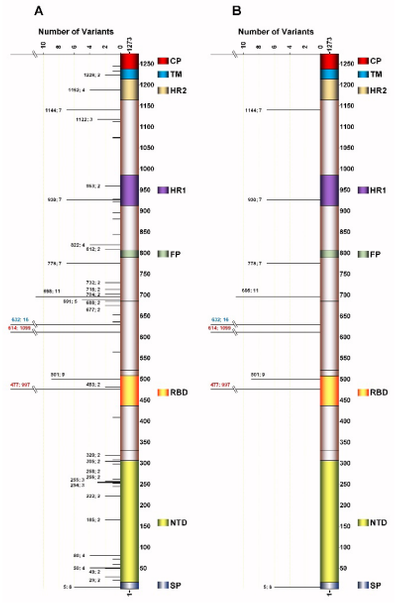

Figure 6. *Distribution of the amino acid substitutions on the spike proteins of SARS CoV2 isolates in Oceania and the number of distinct variants in the virus population. A. Distribution of amino acid substitutions on the spike protein, which is determined in all virus samples regardless of their number in the population. B. The number of variants with a ratio of ≥ 0.5% (6 and more) in the virus population and the positions of amino acid substitutions.*

4. DISCUSSION

The spike protein, which allows the SARS CoV2 to attach to the ACE2 receptor, is of great importance for the transmission of the virus and immune response in humans. In this study, the nucleotide/amino acid substitutions seen in the spike gene/protein were evaluated. It was found the significant differences between the mutation frequencies in the virus populations isolated from different geographical regions suggesting that the reason may be caused by the deficiencies in the experimental data rather than the ability of the virus to mutate. Considering the possible changes in the nucleotide sequences that occur during genome analyses, it can be asserted that the mutation frequency in the SARS CoV2 spike gene, which is largely due to the errors of the viral RdRP enzyme, is lower than $3.93x10^{-5}$. It can be argued that much lower rates of nucleotide changes occur in the genes of the SARS CoV2 virus compared to the $1.8x10^{-4}$ – $2.5x10^{-4}$ mutation frequency of the influenza A viruses carrying a single-stranded segmented RNA genome (18). It is suggested that the low mutation ratios in the SARS CoV2 genes may be the result of the viral repair mechanism that is independent of the RdRP enzyme (19,20). The mutations in the SARS CoV2 spike gene result in a lower rate of phenotypic changes in the spike protein due to silent mutations. Therefore, it would not be wrong to say that the immune response to the SARS CoV2 virus in humans will be longer than expected.

5. CONCLUSION

The mutation rate of the SARS CoV2 is lower compared to the other RNA viruses with high mutation frequency, such as the influenza A virus. However, the mutations in the spike genes causing the amino acid changes in the RBD of the protein can affect the pathogenesis and spreading rate of the SARS CoV2. At least six mutations causing amino acid substitutions in the RBD of spike protein with a ratio of over 0.5% were detected in the SARS CoV2 isolates from different geographic regions. These mutations resulted in the L452R, Y453F, S477N, T478K, E484K, and N501Y substitutions in the RBD of the proteins. The S477N substitution in the spike protein in the Oceania virus population draws attention with a high ratio of 86,7%. Whether these amino acid substitutions in the RBD of the spike protein are important in terms of their effect on the virus adsorption to the host cell via ACE2 receptor, remains the subject of both *in silico* and experimental research. However, the results will be useful in predicting the spread of the virus in the human population, the persistence of acquired immunity against these viruses, and the protective effects of vaccines.

Conflict of interest

All the authors declare no conflict of interest.

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Clinical and Experimental Health Sciences

Factors Affecting Fear, Anxiety, and Depression during COVID-19 in Turkey: A Cross-sectional Study

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ABSTRACT

Objective: The aim of this study was to research levels of fear, anxiety and depression related with the COVID-19 outbreak and the potential risk factors contributing these facts within the population of Turkey.

Methods: 377 people participated in this study. This study conducted from September to the end of December 2020. An online survey was performed by using the Individual Information Form, The Fear of COVID-19 Scale (7-35 points) and Hospital Anxiety and Depression (HAD) Scale; HAD-A (0-3 points, ≥10), HAD-D (0-3 points, ≥7).

Results: When Hospital Anxiety and Depression Scale are examined, the anxiety scores of 15.9% (> 10, n = 60) and depression scores of 34.2% (> 7, n = 129) of the participants are higher than the cut-off points. HAD-A, HAD-D and COVID-Fear data were positively significantly correlated with each other ($p < 0.001$). In regression analysis, females, those with a relative who has at least one chronic illness, those with mental disorders, and those receiving psychological support were determined as risk factors.

Conclusions: Interrelationships of mental wellbeing, and health status changing at an individual basis must be taken into consideration while evaluating psychological effects of COVID-19.

Keywords: Anxiety, COVID-19, Depression, Fear

1. INTRODUCTION

Adults in Wuhan, the capital of Hubei province, applied to local hospitals with severe pneumonia symptoms for unknown reasons in December 2019. The People's Republic of China reported the epidemic to the World Health Organization (WHO) on December 31, 2019, and then it rapidly affected the whole world in a short time. The situation was declared as a pandemic. In those who suffered from this disease (which is called coronavirus disease of 2019,COVID-19), pathophysiological diseases such as acute respiratory distress syndrome and pneumonia can also be seen (1). This virus, which causes major respiratory infections, not only threatens the physical health of individuals but also can have acute and long-term effects on mental health (2). Individuals have been observed to suffer greater depression, anxiety, and stress, particularly as the pandemic spreads and the number of cases rises (3) Previous studies have shown that infectious diseases such as severe acute respiratory failure syndrome (SARS) can increase individuals' anxiety, depression, and stress levels (4). In addition, basic precautions against pandemic, changing daily routine, separation from family and friends, the thought of food and medicine shortages, loss of income, social isolation, and school closures have created fear in individuals. Other factors that create fear in individuals during the pandemic are the fear of being infected and infecting their beloved ones. Especially before the curfews, people flocking to markets and increasing the risk of transmission by not maintaining social distance increased fear during the COVID-19 in Turkey (5).

Depression and anxiety disorders are common mental illnesses that affect a large part of the population. The reason for examining anxiety and depression together is that both diseases are seen at a very high rate (6, 7). Depression is a constant sadness, loss of interest, hopelessness, concentration disorder, interrupted sleep or food.In anxiety, situations such as worry, fear, restlessness, irritability and social isolation are seen. 322 million people suffer from depression while 264 million people suffer from anxiety at the global (8). In the emergence of the anxiety and depression in humans, the disease may occur after external factors other than medical factors. Pandemic is one of the factors (9, 10).

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COVID-19 pandemic is a stressful situation for people and nations (11). Significant links have been found between viral diseases and anxiety-depression (12). The fear of COVID-19 was significantly associated with depression and anxiety according to Italian study which applied Hospital Anxiety and Depression scale (13). Chronic patients who experience pathophysiological stress may also be under psychological stress during the COVID-19. Studies showed that chronic disease symptoms worsen in the presence of anxiety and depression (14, 15). Individuals with chronic diseases are more susceptible to vulnerable from depression, and depression is more common with people having chronic diseases than the healthy population worldwide (16). The purpose of this study is to evaluate the factors affecting psychological variables such as fear, anxiety, and depression of individuals during the COVID-19 pandemic in Turkey. Our aim is to shed light on developing strategies and ongoing COVID-19 researches to prevent fear, anxiety, and depression.

2. MATERIAL AND METHODS

2.1. Design and Participants

This descriptive study was carried out to evaluate the factors affecting psychological variables such as fear, anxiety, and depression during the COVID-19 pandemic. Participants were recruited via social media channels (Facebook, Instagram, Twitter, WhatsApp) from September to the end of December 2020. Participants were over age of 18, literate in Turkish, had internet access and agreed to participate. The research data were collected through a web-based online survey conducted to decrease face to face interaction due to the COVID-19 isolation strategy. Participants completed the questionnaires by connecting to the website with a computer or smartphone.

2.2. Sampling method and sample size

Sample size was calculated according to 23.6% expected prevalence (17), Type 1 error of 5% and statistical power of 95% in a similar study. It was predicted that at least 277 people will be included in the study. Rate of waste was accepted as 30%. The study was completed with the participation of 377 people. OpenEpi, Version 3, open-source calculator was used for sampling size.

2.3. Measurements

The data were collected using the Individual Information Form, The Fear of COVID-19 Scale and Hospital Anxiety and Depression (HAD) Scale.

2.3.1. Individual Information Form

There are 20 questions –all of which has been developed by researchers – in questionnaire about socio-demographic characteristics (age, gender, marital status, etc.), information about chronic disease (whether there is any chronic disease and/or any relative with any chronic disease), information about whether the participants suffer from any mental disorder.

2.3.2. The Fear of COVID-19 Scale

Fear scale was firstly defined by Ahorsu et al and modified by Bakircioglu et al for Turkish version (13, 14). The scoring system for this 7-item scale is a 5-point Likert-type rating system. Participants use a 5-point Likert-type rating system to answer the questions as “strongly disagree” (1), “disagree” (2), “neither agree nor disagree” (3), “agree” (4), and “strongly agree” (5). This scale does not include reverse coding items. The total score obtained from all items of the scale reflects the level of the fear caused by COVID-19. The scale yields scores ranging from seven to thirty-five. A high score on the scale indicates that participants are afraid of COVID-19.The internal consistency of the scale is 0.88 (18).

2.3.3. Hospital Anxiety and Depression (HAD) Scale

The scale was developed by Zigmond&Snaith (19) and adapted to Turkish context by Aydemir et al. (20). It has subscales for anxiety and depression. It’s a 14-item self-assessment scale with seven items investigating depressive symptoms (even numbers) and seven items investigating anxiety symptoms (odd numbers). Answers are graded on a four-point Likert scale, with scores ranging from 0 to 3. The goal of the scale is to determine the risk group by screening anxiety and depression in a short amount of time, rather than to make a diagnosis. Despite the fact that the scale’s name includes the term “hospital,” it can also be utilized in research. As a result of the ROC analysis, the cut-off points of the Turkish HAD scale were determined as 10 for the anxiety subscale (HAD-A) and 7 for the depression subscale (HAD-D) (20).

2.4. Ethical Considerations and Consent

The study was approved by the Gumushane University Scientific Research and Publication Ethics Board (approval number 2020/6). In addition, the necessary permission for COVID-19 scientific research studies was obtained from the Republic of Turkey Ministry of Health (approval number: 2020-06-03T17_26_53) and Ankara Provincial Health Directorate (approval number 36198255). Before taking part in the study, all of the participants signed an electronic informed consent form.

2.5. Data analysis

Data were analyzed using SPSS 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). $p<0.05$ was considered statistically significant. Descriptive statistics were performed to evaluate our data. Data shows numbers and percentages for categorical variables, mean and standard deviation for numerical variables. One Sample Kolmogorov Smirnov test was performed to adjust normal distribution, Mann Whitney U test was performed to compare numerical variables between two independent groups since assumption of normality was not met. Spearman test was performed for correlation analysis. Participants were divided into anxiety and depression groups according to the HAD scale cut-off points. The factors of anxiety and depression were determined by using logistic regression. The researchers utilized multiple linear regression analysis to find factors linked to COVID fear. Odds ratio (OR) and 95% confidence intervals (95% CI) are reported.

3. RESULTS

The average age of 377 participants in the study is 41.3±14.7, 52.5% (n = 198) of them are women. 68.2% of the participants are married (n = 257), 88.6% (n = 334) live with their families. The rate of those having a full-time job was 60.5% (n = 228). There are 75 (19.9%) active smokers. When the presence of chronic disease is questioned, 36.3% of the participants (n = 137) stated to have a chronic disease, 60.5% (n = 228) of the participants are those with a relative who has at least one chronic illness. The rate of those with mental disorders was 4.2% (n = 16), and the rate of those who received psychological support was 24.9% (n = 94). When the presence of individuals diagnosed with COVID in the family was questioned, 28 people (7.4%) answered "yes". The average HAD-A score of participants is 7.3 ± 3.9, the HAD-D score is 6.2 ± 4.0 and the COVID-fear score is 17.7 ± 6.4 **(Table 1).**

Table 1. *Sociodemographic and clinical characteristics of the participants*

Variables	Number (N=377)	%
Gender		
Male	198	52.5
Female	179	47.5
Marital Status		
Married	257	68.2
Single	120	31.8
Civil Status		
Living with family	334	88.6
Single	43	11.4
Type of employment		
Employment full-time	228	60.5
Unemployed	149	39.5
Use of tobacco products		
Current smoker	75	19.9
Lifetime abstainer	212	56.2
Former smoker	90	23.9
Presence of a chronic disease		
Yes	137	36.3
No	240	63.7
Being with a relative who has at least one chronic illness		
Yes	228	60.5
No	149	39.5
Presence of a mental disorder		
Yes	16	4.2
No	361	95.8
Receiving psychological support		
Yes	94	24.9
No	283	75.1
The Presence of an Individual Diagnosed with COVID in the Family		
Yes	28	7.4
No	349	92.6
Age,Mean±SD	41.3±14.7	
HAD-A, Mean±SD	7.3±3.9	
HAD-D, Mean±SD	6.2±4.0	
COVID Fear, Mean±SD	17.7±6.4	

Anxiety scores of women, those with a relative who has at least one chronic illness, and respondents supported psychologically were significantly higher. Depression scores of respondents with chronic diseases, those with a relative who has at least one chronic illness, respondents with psychiatric diseases and respondents supported psychologically were also higher. When we look at results of COVID-fear scale, scores of women, respondents with chronic diseases, those with a relative who has at least one chronic illness, and respondents supported psychologically were significantly higher **(Table 2).** When the cut-off points of HAD scale are observed, the anxiety scores of 15.9% (n = 60) of the participants and 34.2% (n = 129) of the participants are higher than cut-off points.

Table 2. *Comparison of participants' HAD-A, HAD-D and Fear scores*

	HAD – A Mean±SD	HAD-D Mean±SD	Fear Mean±SD
Age			
18-49	7.7±3.8	6.3±3.9	17.9±6.5
>50	6.1±4.1	6.0±4.3	17.3±6.1
P value	**<0.001**	0.412	0.573
Gender			
Male	7.8±4.2	6.7±4.2	18.6±6.6
Female	6.7±3.6	5.7±3.6	16.6±6.0
P value	**0.021***	**0.041***	**0.004***
Marital Status			
Married	7.2±4.0	6.4±4.1	18.0±6.3
Single	7.4±3.8	5.9±3.8	17.0±6.5
P value	0.654	0.205	0.143
Civil Status			
Living with family	7.3±3.9	6.3±4.0	17.6±6.3
Single	7.1±4.0	5.8±3.9	18.1±7.2
P value	0.821	0.468	0.818
Type of employment			
Employment full-time	7.1±3.7	6.2±3.9	17.3±6.4
Unemployed	7.5±4.2	6.3±4.1	18.2±6.3
P value	0.488	0.830	0.176
Use of tobacco products			
Yes	7.4±3.9	6.7±4.3	18.2±6.9
No	7.2±3.9	6.1±3.9	17.6±6.3
P value	0.950	0.324	0.643
Presence of a chronic disease			
Yes	7.6±3.7	6.8±4.1	18.3±6.1
No	7.0±4.0	5.9±3.9	17.3±6.5
P value	0.126	**0.032***	0.101
Being with a relative who has at least one chronic illness			
Yes	7.9±3.8	6.7±4.0	18.6±6.4
No	6.3±3.9	5.6±3.9	16.3±6.1
P value	**<0.001***	**0.002***	**0.001***
Presence of a mental disorder			
Yes	9.2±5.3	10.2±5.8	18.9±6.9
No	7.2±3.8	6.1±3.8	17.6±6.4
P value	0.135	**0.005***	0.420
Receiving psychological support			
Yes	8.5±4.3	7.4±4.8	19.5±6.9
No	6.9±3.7	5.9±3.6	17.1±6.1
P value	**0.003***	**0.017***	**0.009***
The Presence of an Individual Diagnosed with COVID in the Family			
Yes	7.5±4.4	6.7±3.7	18.6±6.9
No	7.2±3.9	6.2±4.0	17.6±6.3
P value	0.999	0.595	0.465

HAD-A: Hospital Anxiety and Depression, Anxiety Subscale

HAD-D: Hospital Anxiety and Depression, Depression Subscale

**p <0.05*

Gender (OR=2.362, 95% CI = (1.289,4.328)) and receiving a psychological support (OR=1.865, 95% CI = (1.013,3.435)) are risk factors for anxiety; being with a relative who has at least one chronic illness (OR=1.939, 95% CI = (1.205,3.120)) and presence of mental disorder (OR=3.813, 95% CI = (1.246,11.667)) are determined as risk factors for depression according to the findings of a multiple binary logistic regression analysis of depression and anxiety risk variables **(Table 3).**

Table 3. *Results of logistic regression analysis on factors significantly associated with depression and anxiety.*

	Anxiety according to HAD-A		Depression according to HAD-D	
	OR (95% CI)	P value	OR (95% CI)	P value
Age	0.980 (0.958, 1.002)	0.074	0.992 (0.976, 1.008)	0.327
Gender	2.362 (1.289, 4.328)	**0.005***	1.273 (0.818, 1.982)	0.285
Presence of chronic disease	0.928 (0.488, 1.765)	0.820	1.213 (0.740, 1.989)	0.444
Being with a relative who has at least one chronic illness	1.830 (0.968, 3.460)	0.063	1.939 (1.205, 3.120)	**0.006***
Presence of a psychiatric disease	1.669 (0.482, 5.780)	0.419	3.813 (1.246, 11.667)	**0.019***
Psychological support	1.865 (1.013, 3.435)	**0.045***	1.533 (0.926, 2.538)	0.096

HAD-A: Hospital Anxiety and Depression, Anxiety Subscale

HAD-D: Hospital Anxiety and Depression, Depression Subscale

**p <0.05*

Female gender (β = 0.142, p = 0.005), being with a relative who has at least one chronic illness (β = 0.151, p = 0.003), and psychological support status (β = 0.130, p = 0.010) were independent predictors for COVID-19 fear to estimate scores according to multiple linear regression analysis and displayed in **Table 4.**

Table 4. *Results of multiple linear regression analysis of clinical variables for predicting COVID-fear scales*

	COVID-19 Fear			
	B	β	95% CI for B	P value
Gender (Male and Female)	1.816	0.142	(0.557,3.076)	**0.005***
Presence of chronic disease (yes-no)	0.448	0.034	(-0.880,1.775)	0.508
Being with a relative who has at least one chronic illness	1.971	0.151	(0.659,3.284)	**0.003***
Psychological support (yes-no)	1.923	0.130	(0.460,3.386)	**0.010***

**p <0.05*

The correlation of HAD-A, HAD-D and COVID-Fear scales is shown in **Table 5.** All data were positively significantly correlated with each other (p <0.001). The correlation coefficients ranged from r = 0.322 to r = 0.610.

Table 5. *Correlation of HAD-A, HAD-D and Fear scales*

	CD		None-CD		Total	
	HAD-A	HAD-D	HAD-A	HAD-D	HAD-A	HAD-D
HAD-D	r= 0.447*		r= 0.695*		r= 0.610*	
Fear	r= 0.486*	r= 0.302*	r= 0.452*	r= 0.342*	r= 0.466*	r= 0.322*

** p<0.001, CD:Chronic disease, None-CD: Absence of chronic disease*

4. DISCUSSION

The COVID-19 pandemic has been an unexpected and uncertain situation during which our country and other countries had difficulty in understanding and discerning, especially at the beginning. Fear (getting sick, dying, being separated from beloved ones due to quarantine, losing beloved ones due to virus), feeling helpless and stressed due to social isolation, feeling lonely and depression symptoms come to the fore by looking at the symptoms of those directly or indirectly affected by the pandemic (21, 22).

The aim of this study is both to examine the interaction amongst fear, anxiety, depression levels and the factors affecting fear, anxiety, depression levels of individuals related to the COVID-19 pandemic in Turkey. The first notable finding of our research is the differentiation of some psychological variables according to gender during the COVID-19 pandemic. The anxiety and fear levels of female respondents were higher compared to male respondents. This finding is consistent with previous studies showing that women experienced some psychological problems more negatively during pandemic periods (17, 23-28). The scores of anxiety and fear of female respondents being higher can be explained by the presence of a gender perception in which duties and responsibilities within the home environment are expected from women to a large extent (29). In the COVID-19 pandemic, due to social isolation measures, spending more time with family at home may represent more responsibility and duty that are needed to be overcome, especially for women. In addition, we believe that genetic, biological and hormonal characteristics of women, controlling their emotions and behavior less than men in society, and experiencing their emotions more intensely than men affected anxiety and fear results (24).

The COVID-19 pandemic has resulted in a slew of significant etiological global mental diseases that have impacted all aspects of life and thrown the societal fabric into disarray. COVID-19 is a condition that can expose a variety of fears (contagion, the future, financial difficulties, xenophobia, and agoraphobia) as well as cause anxiety and fear-related aspects. (30). In addition to gender, other findings of the present study are COVID-fear and depression with those with a relative who has at least one chronic illness, COVID-fear and anxiety with those receiving psychological support, and depression scores with those with mental disorder(s) were found to be significantly higher. In a study conducted with 1304 people in Turkey, the relationship between fear of COVID-19 and depression, anxiety and stress variables were examined. It has been determined that fear of COVID-19 mediates these variables (22). In another study conducted with university students in Turkey, depression and anxiety were found to be predicted positively by fear (21). 78 percent of participants in an Australian study conducted during the COVID-19 outbreak showed that their mental health had deteriorated since the outbreak, a quarter said they were too or extremely worried about contracting COVID-19, and half said they were worried about family and friends contracting COVID-19. In addition, the COVID-19 fear score was found to be higher in those who previously received psychological support in that study (31). In a study conducted in South Africa, the relationships between perceived COVID-19 risk and depression were examined over two waves of COVID-19. Especially adults with a history of childhood trauma were twice as likely to experience depressive symptoms. Participants also had a high level of anxiety. It was also determined that the participants' fear of COVID-19 was due to financial insecurity and fear of contamination (32). In a study conducted during the COVID-19 outbreak in Hong Kong, the depression score was found to be high (33). Other studies investigated support our research (34, 35). A prolonged and unknown threat can become chronic and draining. Fear of the unknown causes anxiety in both healthy people and people with mental illnesses (36). Such a high infection and mortality rate is disturbing for individuals. Fear and anxiety can arise both by obtaining more information about the virus and by fearing from both the uncertainties and various mutations of the virus.

Chronic diseases involve challenging processes that fundamentally affect habits and quality of life. Scientists, health professionals, and people who pulled through the coronavirus state that the treatment processes were carried out under harsh conditions, and many difficulties were experienced by patients. Therefore, additionally, for individuals dealing with the difficulties of chronic illnesses, it may be frightening to hear about those aforementioned conditions and difficulties. Another important finding of the study is related with the fear of coronavirus and depression of the individuals according to their chronic illnesses. It has been observed that those with a history of chronic disease experience the psychological effects of the epidemic such as stress, anxiety, and depression at higher levels (23). The results of similar studies support our research (17, 18, 35, 37-39).

In the present study, a positive correlation was found between fear, depression and anxiety. Studies of the COVID-19 outbreak and other previous global infections have been found to cause health problems such as fear, anxiety and depressive symptoms (27, 28, 35). One of the studies, through which it was determined that the fear of COVID-19 can be exacerbated because of the coexistence of depression and anxiety disorders, is in parallel with our research results (13). Many factors have affected the level of public distress during COVID-19. Protection measures, curfews, social distancing, and especially restrictions cause negative effects on those who cannot sustain themselves. This leads to increased levels of anxiety and depression (23). While putting the measures to reduce the spread of the virus at the center of management

strategies, it is necessary to adopt practices that support the physical and psychological health of individuals. Knowing the psychiatric aspects of infectious diseases will guide clinicians for fast and appropriate psychological interventions.

The study had some limitations to consider. First, the participants' responses were constrained by items. Second, because the study used a web-based online survey, the participants' computer skills might have influenced how they replied to the survey. Third, because this is a descriptive study, the individuals' mental health could not be tracked over time. Fourth, our study findings may not be generalizable to other populations because factors such as the prevalence of COVID-19 and differential mortality can alter the effects of COVID-19 on mental health.

5. CONCLUSION

In conclusion, this study demonstrates the factors affecting generalized anxiety, depression, and fear associated with COVID-19 in Turkey. One of the main implications of this study is that governments should provide psychological support to citizens during a pandemic. In brief, home and web-based psychological interventions should be developed to reduce the negative effects of COVID-19 on mental health.

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Conflict of Interest

None

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Clinical and Experimental Health Sciences

Validity And Reliability of the Turkish Version of the Cultural Competence Assessment Tool in Nursing Students

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ABSTRACT

Objective: Increased cultural diversity in society like Turkey is becoming increasingly important to give a holistic nursing care to meet cultural requirements. It is important to assess cultural competence in order to provide appropriate care for cultural needs. The purpose is to adapt the Cultural Competence Assessment Tool (CCATool) for nursing students into the Turkish language and to determine its validity and reliability.

Methods: A total of 400 nursing students were included in the methodological study in Ankara, Turkey. In the validity study of the CCATool were performed language validity, content validity, construct validity, tool response bias and in the reliability study were performed test-retest reliability and internal consistency analysis.

Results: In the content validity analysis the Item Content Validity Index was .91 and the Scale Content Validity Index was .90. The tool Cronbach's α value is .876 and the Cronbach's α values of the sections vary between .706-.821. The scale was determined there is a statistically positive relationship between test-repeat test score averages of the scale.

Conclusions: The results showed that CCATool adapted to Turkish is a valid and reliable scale in determining the cultural competence level of nursing students.

Keywords: Cultural competence, nursing student, validity, reliability, Turkish

1. INTRODUCTION

In this increasingly globalized world, people are migrating for a better standard of living, travel, education, diplomatic asylum (1). In this context, a growing number of people from different cultures are coming to Turkey from abroad. According to the Turkish Statistical Institute, 51,860,042 foreign tourists visited our country in 2019 (2). In addition, according to international patient statistics published by the Turkish Ministry of Health 662,087 patients received health services within the scope of health tourism in 2019 (3). Turkey is importing patients from neighboring countries such as Germany, Azerbaijan, and Iraq. According to the International Organization for Migration's World Migration Report 2020, Turkey, where 3.7 million refugees have taken refuge, is "the country hosting the most refugees"(4).

Health workers who play a role in the delivery of health services to individuals, families, and groups; are expected to take primary responsibility for individuals from different cultures for receiving health care in accordance with the cultures and with an approach that does not exclude their culture (5). Therefore nurses should be educationally prepared to provide culturally congruent health care. To ensure adequate preparation of nursing students, these variables must be fully integrated throughout the nursing curriculum (6). In this way, nursing students can have values and beliefs that meet the cultural needs of patients (7).

Nurse researchers have made significant contributions over the past three decades through the continuous development and improvement of many models and assessment tools to advance their knowledge of the cultural competence literature. These models and tools have raised awareness, understanding and sensitivity that are important in providing culturally competent care to nurses and improving the quality of care for clients of diverse cultural backgrounds (8). Over the years, application of several major cultural competence models in nursing practice, education, research and administration has been used by numerous researchers (9-12).

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Papadopoulos, Tilki ve Taylor (PTT) model was developed in the UK in the 1990's to improve cultural competence. According to the model, cultural competence is defined as "ensuring that people are provided effective health care services taking into account their cultural beliefs, behaviors, and needs" and is seen as both a developing process and output throughout life. The model consists of four stages as cultural awareness, cultural knowledge, cultural sensitivity, and cultural practice (competence). Cultural awareness is the first stage in the development of cultural competence. Nurses need to examine their own personal values and beliefs, be aware of and understand the impact of the care they provide to increase their knowledge of cultural identity and ethnic centeredness. The cultural knowledge phase includes nurses gaining multidisciplinary knowledge on their health beliefs and behaviors in various ways, having relationships with individuals from different cultures, understand differences, inequalities, and base different health problems and behaviors on culture. The cultural sensitivity phase includes the development of appropriate communication skills. Finally, cultural competence requires the synthesis and implementation of all previous stages (12).

Cultural competence is an important component of culturally compatible nursing care. In order to reduce healthcare inequalities and identify improvement potentials in nursing practices, researchers need to be able to accurately assess cultural competence (13). There are many tools for the assessment of cultural competence and the Cultural Competence Assessment Tool (CCATool) based on the PTT model used in this study was developed by Papadopoulos, Tilki and Lees. They have determined cultural awareness, cultural knowledge, cultural sensitivity, and cultural practice areas on a scale and focused on these concepts for the development of cultural competence (14-15). This scale measures the levels of cultural competence of nursing students, which can be the basis for planning and implementing educational programs. An evaluation tool is needed to accurately assess the cultural competence of Turkish nursing students. The creation of a valid and reliable tool will facilitate the conduct of relevant studies that will enrich the literature on the cultural competence of nursing students.

2. METHODS

2.1. Study Design and Participants

This study was based on methodological design. The study was conducted between January and December 2018 in the city of Ankara been the capital of Turkey. The research was carried out in two state universities in Ankara city center; 750 3rd and 4th year nursing students in Department of Nursing. Both universities are in the city center and their education curricula and course application areas are similar. It was administered to 400 nursing students who agreed to participate in the study, without choosing a sample. The sample size was estimated based on the criterion that at least 10 participants per item were required for conducting an exploratory factor analysis of an instrument (16). Accordingly, since there were 40 items in CCATool, the study needed to include 400 subjects and the study was carried out with a sample size of 400.

2.2. Research Instrument

The data were collected by using the Personal Information Form and Cultural Competence Assessment Tool (CCATool).

2.2.1. The Personal Information Form: The form prepared by the researcher consists of ten questions including questions such as class, age, gender, family type, place of residence, foreign language knowledge, willingness to work in a different culture, willingness to provide care to patients from different cultures and the necessity of health practices of different cultures in the nursing curriculum.

2.2.2. Cultural Competence Assessment Tool: Data were collected using the CCATool Student version is based on PTT model developed by Papadopoulos, Tilki & Lees. In the reliability study conducted by Papadopoulos, Tilki and Lees (2004) in England, Cronbach's Alpha coefficients of both the scale and the sub-dimensions were determined as greater than 0.80 (15). The scale of four sections including cultural awareness, cultural knowledge, cultural sensitivity and cultural practice. Each cultural section contains 10 statements on a 4-point scale (1=completely disagree, 2=disagree, 4=agree, 5=completely agree). There is no neutral option (3) in the scale, since students have to either agree or disagree with the statements. The maximum total number of points that can be achieved in each section is ten (10) points (1 point per each correct statement). When all four sections of the CCATool are completed the total maximum number of points that can be awarded is forty (40) points. A person is considered to be culturally competent if they achieve a total score of forty (40) points, comprising a score of ten (10) in all of the four sections. The CCATool determines four levels of cultural competence development. The level of cultural competence is calculated by the software formula which then assigns the level according to the following criteria:

Cultural Incompetence: A person is culturally incompetent if they receive a score of less than 5 in the cultural awareness domain. This applies regardless of their score achieved in the other three sections of the CCATool.

Cultural Awareness: A person is culturally aware if they have achieved a score of 5 or more in cultural awareness without necessarily having all of the generic statements in the other areas correct.

Cultural Safety: A person is considered culturally safe if they have achieved a score of 5 or more in cultural awareness and have all the generic statements correct in the other sections.

Cultural Competence: A person is culturally competent if they have achieved a score of 10 in all of the four stages (total of 40 points).

2.3. Translation of the Scale

At the first phase of the validity study of the scale, studies were conducted to ensure language validity. Translation back translation method was used in the language validity phase. In order to ensure language equivalence of the original scale, a team of three experts in their field was formed and the scale was translated from English to Turkish by the team. The original language and translated forms of the scale were compared with each other and the scale was rearranged by the researcher and the consultant. No item was removed from the measurement scale by remaining loyal to the original, as it was not an item that was not suitable for coverage and reduced appearance validity. In the next step of the scale which was translated into Turkish, was translated back into English by three experts in different fields. An English scale was obtained by choosing the most appropriate expressions by comparing the translations made. The final form of the scale was given by comparing the original language and the translated forms with each other and by re-examining the items by the researcher consultant. The scale which took its final form was presented for expert opinion. After the language validition of the scale, the opinions of eight experts (seven experts in cultural competence in nursing and one in the field of Social Anthropology were consulted to ensure content validity. Experts evaluated whether the scale served the specified purpose and in terms of its suitability to Turkish culture. The scale was finalized with appropriate word arrangements in line with expert opinions.

2.4. Pilot Study

The pilot study of the scale whose language and content validity was applied to a group representing the sample. It is generally recommended to apply to a number of people representing 10% of the sample (16). The pilot study was conducted with 82 students and since each item was found to be understandable, no changes were made to the scale. pilot study data were not included in the research sample.

2.5. Data Collection

Data were collected from 400 nursing students who agreed to work two weeks after the pilot study. The scale was applied to 71 students for test-retest analysis five weeks after the first application. Data collection was collected by the researcher in the classroom environment. The administration of the scales took 20-25 minutes.

2.6. Statistical Analysis

Statistical Package for the Social Sciences (SSPS) 20.0 and Analysis of Moment Structures (AMOS) 23.0 statistical program were used for the statistical analysis of the data. Sociodemographic features were analyzed using descriptive statistical analysis. Content validity, construct validity and scale response bias were examined in the validity study of the scale. Content Validity Index was used to evaluate the expert opinions in contect validity. Confirmatory Factor Analysis and Explanatory Factor Analysis methods were used for construct validity. In the Explanatory Factor Analysis, Kaiser-Meyer Olkin (KMO) was used to decide whether the sample size was sufficient or not, and Bartlett test was applied to determine whether the variables correlated with each other. Explanatory factor analysis, principal component and varimax axis rotation method were used and factors with eigen values > 1.0 were determined. Goodness of fit indices were used in confirmatory factor analysis. Computed goodness of fit indices, Pearson's chi square of freedom, approximate root mean square error (RMSEA), root mean square error (RMR), comparative goodness of fit (CFI), goodness of fit index (GFI), adjusted goodness of fit index (AGFI)) includes the increasing fit index (IFI). In the reliability analysis, internal consistency reliability and test-retest reliability were made. The correlation coefficient (r value) was calculated using the Cronbach's Alpha for the internal consistency reliability and the Pearson Product-Moment Correlation test for the test-retest reliability. Hotelling T^2 test was used to calculate the scale response bias. For all statistical analyzes, two-sided $p<0.05$ was considered statistically significant.

2.7. Ethical Considerations

Permission was obtained from Rena Papadopoulos for the use of CCATool in validity and reliability study. Official permission was obtained from Universities to conduct the research. All procedures in the study were carried out in accordance with the Helsinki Declaration. The study has been approved by the Ankara University Ethical Committee (2018 and 02/28). Written consents were obtained to the participants after the purpose of the study was explained.

3. RESULTS

3.1. Characteristics of the Participants

The average age of the students was 20.88±1.09 years (min:19, max:28), most of students were women (82.5%) and nuclear family (87.5%). Approximately half of the students spent most of their lives in the urban area (53.3%). About half of students reported that they knew any foreign language (47.3%). Most of the students stated that they wanted to work in a different culture (72.8%), provide care to patients from different cultures (88.5%) and health practices related to different cultures should be included in the nursing curriculum (81%) (Table 1).

3.2. Validity Analysis

Content validity, construct validity (explanatory factor analysis and confirmatory factor analysis), scale response bias were performed in the validity analysis.

Content Validity Index was used to evaluate the expert opinions in contect validity. Item Validity Index (I-CVI) and the Scale Validity Index (S-CVI) was calculated for the whole

scale. Eight experts suggestions were consulted for contect validity and it was calculated as I-CVI= 0.91, S-CVI=0.90. Kaiser-Meyer Olkin (KMO) was used to decide whether the sample size was sufficient or not. In the analysis of the scale, KMO value was found 0.847 and Barlett test (c2= 4841.682; p = 0.000). According to this result, the KMO value is in the "very good" range and the Barlett test's significance indicates that the scale is suitable for factor analysis.

Table 1. *Descriptive characteristics of students (N=400)*

Characteristics	n	%
Sex		
Female	330	82.5
Male	70	17.5
Types of Families		
Nuclear Family	350	87.5
Extended Family	50	12.5
The residence where they maintained their lives for the longest time	213	53.3
Urban	187	46.8
Rural		
Foreign Language Knowledge		
Yes	189	47.3
No	211	52.8
Willingness to Work in a Different Culture		
Yes	291	72.8
No	109	27.3
Willingness to Care for Patients from Different Cultures	354	88.5
Yes	46	11.5
No		
The Necessity of Health Practices of Different Cultures in the Nursing Curriculum	324	81.0
Yes	76	19.0
No		

In the explanatory factor analysis of this research, principal component and varimax axis rotation method were used. It was determined that the scale consists of four factors with eigenvalues above 1.00, explaining 40.34%. Cultural awareness section explains 10.124% of total variance, cultural knowledge section explains 9.505% of total variance, cultural sensitivity section explains 9.883% of total variance, cultural practice section explains 10.922% of total variance. As a result of the analysis, the load values of factor 1 are 0.462-0.673; the load values of factor 2 are 0.465-0.650; load values of factor 3 are .401-.690; load values of factor 4 had values varying between 0.342-0.705. These factor loads were found to be at a significant level.

For the construct validity of a scale, the goodness of fit indexes made in the confirmatory factor analysis should be at an appropriate level. Data of the study were applied to confirmatory factor analysis with 40 items, and results were showed in the Table 2. These values showed that the data were compatible with the model, they confirmed the four-factor structure, the items and sections of the scale were related to the scale, and the items in each section defined their factor sufficiently. These results support the construct validity of CCATool-T.

Another important indicator in confirmatory factor analysis is the significance of the regression coefficients. Accordingly, factor loadings are important since the "p" values are less than .001. If factor loadings are significant, it means that items are loaded correctly on factors. In this study, the absolute z value calculated for the parameters is greater than the critical ratio 1.96, and it is seen that all parameters are significantly different from zero (Table 3).

In the study, whether the responses of the students to the scale items were equal or not was evaluated with the Hotelling T^2 test. As a result of this test Hotelling $T^2 = 534.276$, p = 0.000 and it was determined that there was no response bias in the scale.

3.3. Reliability Analysis

The reliability of CCATool-T was determined by internal consistency analysis and test-retest reliability analysis.

The Cronbach's correlation Coefficient of the scale and its sections were calculated to test its internal consistency. The reliability of the scale was examined by calculating Cronbach's alpha coefficient for the total scale (0.876) and for each subsection cultural awareness (0.806), cultural knowledge (0.785), cultural sensitivity (0.783), and cultural practice (0.821) (Table 4).

The scale was re-applied four weeks after the first application and the test-re-test confidence coefficient obtained because of the two measurements applied was evaluated by Pearson Product-Moments Correlation Coefficient. Accordingly, it was determined there is a statistically positive relationship between test-repeat test score averages of the scale. Paired samples T-test was carried out to determine whether there was a difference between the first and second measurement score averages of the scale; no statistically significant difference was found (p =0.000) (Table 4).

Table 2. *Goodness of fit ındex values of the scale*

Index	Good Fit	Acceptable Fit	Output	Assesement of Fit
χ2/sd	0 £ χ2/ sd £ 3	3 £ χ2/ sd £ 5	1.489	Good Fit
RMSEA	0 £ RMSEA£ .05	0.05 £ RMSEA £ 0.08	.037	Good Fit
RMR	0 £ RMR £ .05	0.05 £ RMR £ 0.10	.025	Good Fit
CFI	.97 £ GFI £ 1.00	0.90 £ GFI £ 0.97	.900	Acceptable Fit
GFI	.95 £ GFI £ 1.00	0.90 £ GFI £ 0.95	.869	Acceptable Fit
AGFI	.90 £ AGFI £ 1.00	0.85 £ AGFI £ 0.90	.850	Acceptable Fit
IFI	.95 £ IFI £ 1.00	0.90 £ IFI £ 0.95	.901	Acceptable Fit

Table 3. *Confirmatory factor analysis results of the scale*

Scales	Item	Standardized Regression Coefficients	Construct Reliability	p
Cultural Awareness	Item 1	.488		.000
	Item 2	.437	6.129	.000
	Item 3	.531	6.998	.000
	Item 4	.385	5.622	.000
	Item 5	.484	6.597	.000
	Item 6	.568	7.273	.000
	Item 7	.481	6.549	.000
	Item 8	.671	7.923	.000
	Item 9	.590	7.429	.000
	Item 10	.606	7.531	.000
Cultural Knowledge	Item 11	.578		.000
	Item 12	.495	9.966	.000
	Item 13	.356	5.612	.000
	Item 14	.570	9.207	.000
	Item 15	.612	8.193	.000
	Item 16	.562	7.690	.000
	Item 17	.567	8.122	.000
	Item 18	.487	6.843	.000
	Item 19	.616	7.859	.000
	Item 20	.350	5.300	.000
Cultural Sensitivity	Item 21	.300		.000
	Item 22	.300	3.553	.000
	Item 23	.300	3.986	.000
	Item 24	.676	4.722	.000
	Item 25	.677	4.722	.000
	Item 26	.408	4.137	.000
	Item 27	.718	4.763	.000
	Item 28	.453	4.285	.000
	Item 29	.376	3.998	.000
	Item 30	.426	4.166	.000
Cultural Practice	Item 31	.521		.000
	Item 32	.547	9.022	.000
	Item 33	.513	7.155	.000
	Item 34	.629	8.109	.000
	Item 35	.616	7.933	.000
	Item 36	.451	6.531	.000
	Item 37	.581	7.742	.000
	Item 38	.412	6.102	.000
	Item 39	.635	8.157	.000
	Item 40	.548	7.367	.000

Table 4. *Reliability analysis results of the scale*

Scales	Cronbach's Alfa (N=400)	Test-Retest (N=71)			
		r	P	t	p
Cultural Awareness	.806	.967	.000	-3.188	.002
Cultural Knowledge	.785	.993	.000	-8.597	.000
Cultural Sensitivity	.783	.983	.000	-9.695	.000
Cultural Practice	.821	.985	.000	-4.029	.000
Cultural Competence	.876	.992	.000	-9.606	.000

4. DISCUSSION

The adaptation of a pre-developed measurement tool to a different culture is a methodological process and it is desirable to have two features such as validity and confidence. Validity refers to whether an instrument measures what it was designed to measure (17). Language validity, content validity, construct validity and scale response bias were performed in CCATool-T validity analysis.

Content Validity Index was used to evaluate the expert opinions. Polit and Beck have recommended the use of a content analysis index in nursing studies to see the distribution of responses from all specialists and the distribution of each item. I-CVI; it is recommended that if expert opinion of five and below is obtained, the item content validity value should be 0.90 and above if there are six or more experts 0.78 and above and S-CVI; scale content analysis value is recommended to be 0.80 and above (16). In this study, the recommendations of eight experts were applied, I-CVI was found to be 0.91, and S-CVI was found to be 0.90 and are above the recommended values. These results show that the scale and every item on the scale measures the desired concept and does not contain different concepts except for each concept that is desired to be measured.

The structure validity of the scale was tested with descriptive factor analysis and validator factor analysis. The Kaiser-Meyer Olkin (KMO)'s sampling adequacy measurement technique is used to determine whether the sample size is adequate. As the value obtained by this technique approaches 1, the sampling adequacy increases, and as it moves away, it suddenly decreases. A sample size of 0.90 and above is excellent, between 0.80 and 0.90 is very good, and it should be at least over 0.60 (17). In this study, the KMO value is in the "very good" range and the significance of the Barlett test shows that the scale is suitable for factor analysis.

After factor analysis, the rotation technique is used to determine the size under which the items are collected, to increase the load of items in a factor, to make the factors find the item in the highest relationship with them, and to facilitate interpretation. If the eigenvalue of the factors is greater than 1 and above 1, it is considered significant and the analysis is carried out with these factors. The percentages at which the variance of factors with an eigenvalue greater than 1 explains the total variance are calculated. It is considered appropriate that the variance described is over 40% (18). In the explanatory factor analysis, the basic component and varimax axis rotation method were used and it was determined that the scale consisted of four factors with eigenvalues above 1.00 explaining 40,34% of the total variance.

A factor loading value is a coefficient that describes the relationship of items to factors. The loading values of items in the factor in which they are located are expected to be high. If there is a cluster of items that relate highly to a factor, this finding means that those items together measure a concept-structure-factor. In general, the factor loading value

of 0.60 and above is high, regardless of its sign; the loading value between 0.30-0.59 is defined as moderate in size (18). As a result of the analysis, it was determined that the factor loadings were significant.

Confirmatory factor analysis is an extension of descriptive factor analysis. It is one structural equation modeling procedure designed to assess construct validity and is based on previous research and theory, the development of a proposed model, and then testing this model against real-world data (19). In the confirmatory factor analysis carried out in this study, it was determined that it had acceptable harmony with goodness of fit values. These values showed that the data was compatible with the model, confirmed the four-factor structure, that the items and sub-dimensions of the scale were associated with the scale, and that the items in each sub-dimension defined their factor as sufficient. These results support the construct validity of CCATool-T and reveal that it is a valid tool.

In the analysis of the confirmatory factor analyses, another important indicator is the meaningfulness of the regression coefficients. Accordingly, factor loadings are important because the "p" values are smaller than 0.001. Significant factor loadings mean that the items are loaded into the factors correctly. In the confirmatory factor analysis, the C.R. value is divided by the standard error of the parameter estimate and is dispersed as a z-statistic, thus expressing the statistical significance of the parameter (20). In this study, the calculated absolute z value for parameters is greater than the critical ratio of 1.96 and all parameters are significantly different from zero.

One of, he characteristics that the scale should carry is the reliability of the measurement to produce the same results under the same conditions (17). The reliability of the scale was determined by internal consistency analysis and test-retest reliability analysis.

Internal consistency describes the extent to which all the items in a test measure the same concept or construct and hence it is connected to the inter-relatedness of the items within the test (21). Cronbach Alpha Reliability Coefficient of the scale and its sub-dimensions is calculated to test the internal consistency of the scale. Cronbach Alpha Reliability Coefficient is considered reliable in cases where it is 0.70 and above, and the values in this study show that the scale is reliable (16). Papadopoulos, Tilki and Lee reliability study in the UK, Cronbach Alpha set the Reliability Coefficients as greater than 0.80 (15). Vasiliou et al., conducted a reliability analysis in Southern Cyprus in 2013, found Cronbach Alpha Reliability Coefficients for cultural awareness for sub-dimensions as 0.786, for cultural knowledge as 0.734, for cultural sensitivity as 0.643, and for cultural practice as 0.826 (22). Internal consistency analysis is similar to these studies, and accordingly, it can be said that the items are interrelated within themselves and serve the entire measurement tool, are equally weighted to each other, in other words, the scale is homogeneous.

The scale was determined there is a statistically positive relationship between test-repeat test score averages of the scale. Vasiliou et al., conducted test-re-test reliability in 2013 with the same scale in Southern Cyprus and found that the scale had good internal consistency and was sufficient for group comparisons (22). The absence of a significant difference after repeated measurements in both studies shows that the scale is reliable.

5. CONCLUSIONS

In this study, the Turkish version of CCATool was found as a valid and reliable measurement tool for evaluating the cultural competence of nursing students. Evaluating the cultural competence of nursing students will contribute to the delivery of quality appropriate care to patients of different cultures. The scale evaluates not only cultural competence but also cultural awareness, cultural knowledge, cultural sensitivity, cultural practice, which are the four sub-dimensions of the scale. This scale will be the first in Turkey evaluated in terms of cultural competence and four sub-dimensions. Adapting this scale will also help create strategies to improve education in multicultural care and allow practitioners to provide beneficial care that respects the individual's culture.

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Clinical and Experimental Health Sciences

Comparison of Reaction Time, Manual Dexterity, and Working Memory Levels of Adolescent Video Game Players and Non-Players

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ABSTRACT

Objective: This study aims to examine differences between adolescent video game players and non-players in terms of their reaction time, manual dexterity, and working memory levels.

Methods: The sample of the study, which has a comparative cross-sectional design type, consists of 432 adolescents at the grades between 9 and 12. Non-video game players, and video game players were subjected to simple visual and auditory reaction time tests, manual dexterity tests, matrix, and digit span working memory test.

Results: Compared to non-video game players, video game players were found to have shorter visual and auditory reaction times. Also, several motor dexterity subtest skills of video game players were found to be lower, while working memory did not differ between the two groups.

Conclusion: Our findings support the idea that playing video games seem to improve some aspects of cognitive and motor skills but reduce several other aspects.

Keywords: adolescent, manual dexterity, reaction time, working memory, video game

1. INTRODUCTION

The video game industry, which constitutes an important part of the media with a consumption figure of $160 billion across the world, has continued to grow each passing day (1). Among the reasons for this growth are many factors such as interaction, and interactive communication, experiencing the feeling of power and success, and easy access. Although there are players from all age groups, the use of video games by children and young people has been increasing day by day, while the playing age has been decreasing (2). Particularly, there is a significant increase in the percentage of young people who follow up technological developments for using the technological tools and possessing them, and the games that contain physical activities have been replaced by passive/video games played while sitting at a desk.

In the literature, playing video games without overdoing has been considered normal as part of a healthy lifestyle and having positive effects such as emotional relaxation (3). However, in some studies, it is stated that playing video games intensely and uncontrollably causes problems in the individual's social and family relationships, academic performance, and psycho-social functions (4). Although there are several discussions in the literature about the effects of video games, there is no consensus (5).

Notably, the interest in the potential effects of these games on our daily lives, particularly, on cognitive skills has increased with the increasing popularity of video games. These studies focused particularly on how action and first-person shooter games change/affect the basic sensory and perceptual processes that support cognition (6-8). Some studies reported that video game players (VGPs) demonstrated better performance than non-video game players (NVGPs) in selective attention, working memory, visual-spatial attention, and attentional control tests (9-11). On the other hand, other studies stated that there was no difference between groups considering the comparison between the cognitive skill variables of VGPs and NVGPs (12, 13). Although several studies in the literature examined the relationship between playing video games and cognitive and

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motor skills, a clear perspective has not emerged. The data show that playing video games has positive effects on some cognitive functions while negative effects on others (13, 14).

Also, some researchers have emphasized that the studies comparing VGPs and NVGPs had some methodological and statistical problems. They reported that the possibility of Type 1 errors may be high since many of these studies were carried out with very small samples (e.g. groups with less than 7-8 samples) (13, 15). Moreover, Unsworth et al. (13) emphasize the importance of simultaneous measurement of multiple cognitive functions in their article, which examines the relationship between video game experience and cognitive skills. More research is needed to test whether there is a relationship between playing video games and cognitive functions (8,13). Due to the methodological inadequacies in the studies and their inconsistent results and since several cognitive skills have not been studied yet, there are large gaps on this issue in the literature (8, 9). This study compares adolescent video game players and non-players in terms of their reaction time, manual dexterity, and working memory levels. The present research seeks to find an answer to the following two questions: "Is there a difference between adolescent VGPs and NVGPs in terms of their reaction time, manual dexterity, and working memory levels?" and "Do time spent playing video games and the duration of regularly playing video games in years have a correlation with adolescents's reaction time, motor skill, and working memory levels?".

2. METHOD

2.1. Design and Participants

The study, which has a comparative cross-sectional design type, population consists of 17 schools affiliated to the Provincial Directorate of National Education in Tekirdağ, Turkey. These schools are listed by numbering. These schools are listed by numbering. Then, a school was determined by random selection from these schools using the https://www.random.org/ online program. In this school, 1122 students' study at the grades between 9 and 12. The study included 487 students whose families gave explicit consent. The students, who voluntarily participated in the study, had no neurological or orthopedic disease, and no upper extremity injuries or surgeries. Existing vision and hearing problems of students may prevent them from showing their real performance in the tests to be done in the research. For this reason, before starting the study, the eyes test with the Snellen Chart, the whisper test and the hearing test with the Rinne test were applied to the students. Fifty-five students were excluded from the study based on the results while students whose problems were not detected in these tests were included in the study. The video-game playing time of the remaining 432 students was determined using a sociodemographic and gaming-related behaviors survey form. Students were asked the following questions: "How many hours do you have to play on a weekday?" and "How many hours do you play games in a weekend day? According to the answers, students were grouped as VGPs and NVGPs. Those who played seven hours a week or more (277 students – 62 females and 215 males) were classified as VGPs. On the other hand, those playing one hour a week or less (155 students – 134 females and 21 males) were classified as NVGPs. Students who played between 1-7 hours a week (as the VGP cutoff values determined by several previous studies (16,17) were not included in the study. Recruitment and testing took place between January-June 2019.

2.2. Instruments

Sociodemographic and Gaming-Related Behaviors Survey Form: The form was created by researchers by examining the relevant literature (16,17). The form includes questions about the student's age, gender, daily video game playing time on weekdays and weekends, and the duration of regularly playing video games in years.

Simple Visual and Auditory Reaction Time: The participants' visual reaction time (VRT) and auditory reaction time (ART) were determined using special online applications developed for reaction time measurement (VRT: www.humanbenchmark.com; ART: cognitivefun.net) (as used in several previous studies: Brewin et al. (18); Pancar et al. (19). In the visual reaction time measurement, the participants were asked to turn off the light by pressing the mouse with the index finger of the dominant hand as soon as possible when a green color was displayed on the screen. In the measurement of auditory reaction time, the participants were asked to press the mouse as soon as they heard the sound. Five tests were carried out to measure both reaction times, and the average time was recorded in milliseconds as per Brewin et al. (18), and Pancar et al. (19).

Manual Dexterity Test: Manual dexterity of the participants was evaluated using the Purdue Pegboard Test (Layfayette instruments, model 3202). The Purdue Pegboard Test, which was developed by Tiffin and Asher, measures the pin placement speed and the number of hands and fingers used to determine the manual dexterity level. In this study, the following four manual dexterity subtests were used 1 – right hand (30 sec.), 2 – left hand (30 sec.), 3 – both hands (30 sec.), and 4 – assembly (60 sec.). The right-hand and left-hand subtest requires the participant to place as many pins as possible in the slot within thirty seconds. The score indicates the number of pins correctly placed. The final score is the number of pins inserted. In both hands subtest, the time is limited to thirty seconds, and the left and right hands are used simultaneously to insert pins into the slots in both columns. The score shows the number of pin pairs inserted. The total time for the assembly subtest is one minute and the participant is required to assemble four parts to complete the assembly. This score represents the number of pieces assembled (i.e., pin, washer, collar, second washer). The implementation of the tests was carried out according to the principles in the user manual (retrieved from http://www.limef.com/downloads/MAN-32020A-forpdf-rev0.pdf).

Working Memory Test (WM): The computer-aided online Matrix Memory Test and Digit Span Test were used to determine the working memory capacity of the participants (www.humanbenchmark.com). Matrix memory test is used to measure visual-spatial working memory relying on passive storage. A computerized version (www.humanbenchmark.com) of the matrix memory task was included. White and black grid patterns are displayed in a random order for 1000 milliseconds each. The participants are required to remember the locations of the black squares. Subsequently, the black squares disappear before the grid appears. Then, the participants are required to determine whether black squares are located in the same positions. The evaluation is completed when they cannot remember the pattern correctly (they have the right to make 3 mistakes); then, the test level of the participant is determined. A high level indicates that visual-spatial working memory is high (20). On the other hand, the Digit Span Test is used to measure working memory's number storage capacity. Participants see a sequence of numerical digits and are tasked to recall the sequence correctly. They are tested with increasingly longer sequences in each trial. The participant's span is the longest number of sequential digits that s/he can accurately recall (https://www.cambridgebrainsciences.com/science/tasks/digit-span).

2.3. Data Collection

A free and quiet room that would not distract students was arranged to apply the tests. The students participating in the study were accepted to room one by one. Students first completed reaction-time and working-memory tests. These tests were controlled by a PC attached to a 17-inch monitor (96 dpi with a refresh rate of 120 Hz). Participants were seated approximately 0.5 m from the screen. They were informed about the implementation of each test and asked to try it once. The test was completed after three inaccurate responses, and the average visual and auditory reaction times were determined in milliseconds. Participants were allowed to take a short break (maximum 5 min) between tests. In the second stage, the participant moved to the area where the Purdue Pegboard Test would be performed. Each subtest (30 sec for right/left hand and both hands; 60 sec for assembly subtest) was performed using a stopwatch. After each test, participants were told how many pins s/he had placed. Then, the researchers recorded the results.

2.4. Data Analysis

The data were analyzed using IBM SPSS Statistics 22 software package for Windows. Descriptive statistics, mean comparisons, and bivariate correlations were conducted. The normality of the parameters was evaluated using the Kolmogorov-Smirnov Test and Shapiro-Wilk Test. Since the sample was found to be normally distributed, the Student's t-test was carried out to analyze the possible significant differences between the groups. The correlations between variables, such as video game playing time, duration of regularly playing video games in years, visual and auditory reaction time, manual dexterity, visual working memory, were analyzed using Pearson`s product-moment correlation coefficient. The significance level was set $p = .05$.

2.5. Ethics

After ethical approval was granted by the Namik Kemal University Faculty of Medicine Ethical Committee (Project Date/Number: 2018.177.12.11), permission to carry out the study was given by the Provincial Directorate of National Education. The researchers and the students who participated in the study did not know which VGP or NVGP group they were in.

3. RESULTS

The average age of VGPs (n=277) is 15.71±1.20, 77.6% of them are male (n=215), and their average playing time is 5.13±3.07 h per day. The average duration of regularly playing video games is 5.18±3.32 years. On the other hand, the average age of NVGPs (n=155) is 15.93±1.21, 86.5% of them are female (n=134), and their average playing time is .004±.03 h per day. No significant difference was found between groups in terms of the age variable (*t: 1.778, p=.076*, N.S.) (Table 1).

Comparing the reaction time, working memory, and motor skills of adolescents, a significant difference was found in favor of VGPs for visual (341.21±36.73, $t = 3.942$, $p < .01$) and auditory reaction time (214.97±44.06, $t = 3.368$, $p < .01$*).* Manual dexterity subtest scores of NVGPs were found to be significantly higher than those of VGPs right hand (14.92±1.81, $t = 3.057$, $p < .05$) and both hands (22.43±3.02, $t = 2.220$, $p < .05$) (Table 2). No significant difference was found between groups according to matrix memory test ($t = – .991$, $p = .322$, N.S.), digit span test ($t = – 1.600$, $p = .110$, N.S.), and left hand ($t = .766$, $p = .444$, N.S.) and assembly manual dexterity subtest ($t = 1.573$, $p = .116$, N.S.).

A negative correlation was found between the average time that adolescents devote to playing video games per day, and the subtest scores of VRT ($r(430) = – .112$, $p < .05$) and ART ($r(430) = – .146$, $p < .01$), right hand ($r(430) = – .173$, $p < .01$) and both hands ($r(430) = – .123$, $p < .05$) motor dexterity. Adolescents' duration of regularly playing video games in years was found to have negative correlations with their subtest scores of visual ($r(430) = – .156$, $p < .01$) and auditory reaction times ($r(430) = – .118$, $p < .05$), right hand ($r(430) = – .114$, $p < .05$), both hands ($r(430) = – .160$, $p < .01$), assembly ($r(430) = – .140$, $p < .01$), and motor dexterity. Moreover, a positive correlation was found between WM-Matrix Memory test ($r(430) = .102$, $p < .05$) scores and the duration of regularly playing video games in years by adolescents (Table 3).

Table 1. *Features of adolescents about sociodemographic and gaming-related behaviors of VGPs and NVGPs (n=432)*

	VGPs		NVGPs				
	n(%)	Mean±SD	n(%)	Mean±SD	*t*	*p*	*d*
Age		15.71± 1.20		15.93± 1.21	*1.778*	*0.076*	*0.18*
Gender							
Female	62 (22.4%)		134 (86.5%)				
Male	215 (77.6%)		21 (13.5%)				
Video game playing time (hours per day)		5.13± 3.07		.004 ±.03	-28.07	.000	-22,80
Duration of regularly playing games (years)		5.18± 3.32		0	-17.83	.000	-5.07

** p < .05, ** p < .01, t: Independent t-test. NVGPs: non-video game players; VGPs: video game players*

Table 2. *Comparison of VGPs and NVGPs in terms of their reaction time, memory and motor skills. (VGPs n=277, NVGPs. n=155).*

	NVGPs Mean±SD	VGPs Mean±SD	*t*	*p*	*d*
Visual Reaction Time (ms)	356.41±41.29	341.21±36.73	3.942	**.000**	.39
Auditory Reaction Time (ms)	231.97±53.50	214.97±44.06	3.368	**.001**	.35
WM-Matrix Memory Test (Score)	8.70±1.23	8.82±1.29	-.991	.322	.10
WM-Digit Span Test (Grade)	7.95±1.23	8.16±1.32	-1.600	.110	.16
MD-Right Hand[a]	14.92±1.81	14.38±1.72	3.057	**.002**	.31
MD-Left Hand[a]	13.70±1.64	13.57±1.65	.766,	.444	.08
MD-Both Hands[a]	22.43±3.02	21.78±2.86	2.220	**.0027**	.22
MD-Assembly[a]	29.26±4.42	28.57±4.32	1.573	.116	.16

** p < .05, ** p < .01, t: Independent t-test*

[a] Number of pins placed in 30 sec is reported for left, right, and both hands' motor dexterity test, and the number of pins placed in 60 sec for assembly motor dexterity test. NVGPs: non-video game players; VGPs: video game players; WM: working memory; MD: motor dexterity; ms: milliseconds

Table 3. *The correlation of duration of regularly playing video games in years and the playing time per day with adolescents' reaction time, and memory and motor skills (n=432)*

	Visual Reaction Time	Auditory Reaction Time	WM-Matrix Memory Test	WM-Digit Span Test	MD-Right Hand	MD-Left Hand	MD-Both Hands	MD-Assembly
Duration of regularly playing video games in years	-.156**	-.118*	.102*	.012	-.114*	-.073	-.160**	-.140**
Video game playing time per day	-.112*	-.146**	,066	.026	-.173**	-.071	-.123*	-.090

** p < .05, ** p < .01, Pearson`s correlation coefficient*

WM: working memory; MD: motor dexterity

4. DISCUSSION

In particular, whether people learn anything useful from playing video games is an important question in the context of many children and adolescents who play video games for a long time. A clear conclusion cannot be confirmed due to inconsistencies in previous studies, which often involve the correlation between playing video games and cognitive and motor skills. The present research was conducted to determine whether there were differences between VGP and NVGP adolescents in terms of their visual and auditory reaction times, manual dexterity, and working memory levels. In contrast to previous studies with small sample size, the results obtained in this study are noteworthy due to the high sample size. Age and gender are two important factors that affect playing video games. In this study, while there was no difference between VGP and NVGP groups in terms of their age, the groups have a significant difference in terms of gender distribution. Several studies have revealed that males played video games more than females (e.g.; 2,4,7, 21). In the sample of the study, the VGP group predominantly consists of males while the NVGP group predominantly consists of females, which is in line with the literature. Contrary to studies reporting that gender does not affect executive functions (22), there are also studies reporting that males have faster and less variable reaction times compared to females (23).

When adolescents were asked the names of the games they preferred to play in their daily lives, they stated that they played many types of games. Thus, in this study, the groups were not compared by classifying them based on game types. Unlike previous studies, one of the most distinctive aspects of this research is that it focuses on the adolescents'

experience of playing all types of video games throughout their lives, rather than a certain video game genre.

In the present research, visual and auditory reaction times of VGPs were found to be significantly shorter than those of NVGPs. Moreover, it was found that the increases in the duration of regularly playing video games in years and the playing time were correlated with the decrease in reaction times. This result is consistent with previous studies revealing that VGPs have faster reaction times than NVGPs (24-27). In a video game, several visual and auditory events can occur almost simultaneously, such as popping balls appearing on the screen, driving vehicles fast without hitting any obstacles, or defending against suddenly emerging enemies/creatures, etc. As a result, player's various perceptual, cognitive, and motor skills such as the speed of perceiving potential threats, problem-solving skills, planning, and visuomotor coordination can develop.

Some studies suggest that video games improve working memory (10, 11, 29), whereas others do not report this effect (13). Previous studies that suggest a strong correlation between video game experience and cognitive abilities highlight the need to efficiently store and change information about video game rules in working memory for a successful game. In this study, although there was no difference between the groups, it was found that adolescent's WM test scores increased with an increase in the duration of regularly playing video games in years. This finding is consistent with the findings of Unsworth et al.(13), Green et al.(30), and Waris et al. (31).

The impact of video games on adolescents' fine manual dexterity and bimanual coordination is not definitive either. Another important finding of this study is that NVGPs have higher/ better motor dexterity subtest skills (right hand and both hands) compared to VGPs. This result can be attributed to the fact that activities such as real-life games, music, sports, and arts require more muscle strength, coordination, and dexterity than virtual actions in video games. Increased time spent by children due to screen exposure may cause them to limit the time they allocate to other activities, and to physical activity (2, 32), which may result in reduced dexterity. Also, according to the results of the correlation analysis conducted in the study, it was found that some motor skills (right hand, both hands, and assembly) weakened with the increase in the duration of regularly playing video games in years and the playing time. This finding can be interpreted as the increase in the frequency and duration of playing video games leads to an increase in the interest and sensitivity to visual information, rather than tactile information, which results in a decrease in manual dexterity.

As a result, it was found that VGPs had shorter visual and auditory reaction times, and there was difference between the two groups. Also, some motor dexterity subtest skills were lower in high-school adolescents with normal development. Besides, the duration of regularly playing video games in years and the total time allocated for playing video games have a negative correlation with reaction time and some motor dexterity subtests. On the contrary, there is a positive correlation between working memory test scores and the duration of regularly playing video games in years. On the other hand, our cross-sectional study cannot provide causal inferences, and longitudinal and experimental studies are needed for strong recommendations.

5. CONCLUSION

In this study, it can be seen that video games can improve some aspects such as reaction time of cognitive and motor skills but weaken other aspects such as motor dexterity. As a result, the impact of playing video games on cognitive and motor skills is more complex than the positive/negative distinction with definitive limits. Personality structure, environmental stimuli, playing time, and experience of the player can affect this relationship. Adolescents are interested in video games, also, their playing time is quite remarkable. For this reason, ex post facto or experimental studies should be conducted to determine the possible effects of video games on the development of cognitive and motor skills. Thus, the selection of instructional and constructive video games used in different applications such as education, entertainment, and sports may contribute to the motor and cognitive development. Also, future studies are required for additional research on the correlation between physical activity, playing video games, and motor skills. Experts working in this field should be vigilant about children and adolescents who use video games uncontrollably, and it may be advisable to provide counseling to both children and families on positive and negative cognitive effects.

Limitations

This research has several limitations. As in similar studies in the literature, VGPs consisted of more male participants, and the NVGPs was composed of more female adolescents. It is important to note that the disproportionate gender distribution is consistent with previous studies and reflects the differences in population similarly. Another limitation is that the specified playing time and duration in years are based on the participants' statements. Moreover, differences such as the individual's past learning, skills, personality structure, and individual interests may affect the speed of cognitive and motor skills. These factors were not considered in the study. The research findings can only be generalized to the high schools that are included in the population of this study.

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Clinical and Experimental Health Sciences

The Theory-Practice Gap in Nursing Education During the Pandemic Period from the Perspective of Stakeholders: A Qualitative Study

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ABSTRACT

Objective: The purpose of this study was to understand the experience of theoretical and practical nursing education carried out by distance education during the COVID-19 pandemic and the theory-practice gap in nursing education in Turkey.

Methods: This qualitative study used a descriptive qualitative study. This research was conducted with nursing lecturers and students from two universities in two different geographical regions. Lecturers and undergraduate students participated in the research. The data for the research were collected using the online Zoom program during four focus-group discussions.

Results: Three main themes emerged for the categories of theoretical and practical education emerged during the study: strong structural conditions (technological integration and accessibility) both strong and weak structural conditions (asynchronous participation and changing comfort levels/routines) and weak structural conditions (cold contact and inequality of opportunity).

Conclusion: This study revealed the strengths and weaknesses in the experience of distance nursing education and will guide future planning of nursing education programs and clinical fields.

Keywords: COVID-19 pandemic, distance education, nursing education.

1. INTRODUCTION

The theory-practice gap is defined as the discrepancy between what students acquire through theoretical classroom lessons and what they experience in a clinical setting (1, 2).

The pandemic, which has affected all areas of life, has required a reassessment of perspectives, especially in terms of education. It has also been necessary to evaluate social equality, community support, and the philosophy of openness in education, as well as the economic dimension of education (3-7).

During the pandemic, many countries have switched to a distance education system (4, 8-9). Nursing education, an academic system based on both theory, clinical practice that supports students' cognitive, sensory and psychomotor skills, and students graduate as future professional nurses with a high level of competence and confidence (10). Although there are many advantages to distance education, such as reducing the workload of educational staff, saving time, increasing academic achievement, reducing cost, improving quality, increasing the prestige of a university, overcoming physical distance and providing flexibility, there are also disadvantages such as lack of social and academic activities in school, lack of interaction, barriers to access, infrastructure costs, and the inability of students to acquire affective and psycho-motor behaviors (6-7, 10-13).

The field of practice provides excellent opportunities for students to convert theory into clinical practice and gain competence in nursing skills prior to graduation (14), but during the COVID-19 pandemic, it was not possible to use either the hospital or the skills laboratories. There are growing concerns about clinical experience and psychomotor skills competencies in nursing students who will graduate without having been able to apply them in practice. To ensure an efficient nursing education, all parties need to plan together and set out action plans for the future.

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2. METHODS

2.1. Study Purpose

This case study aimed to understand the experiences of nursing lecturers and students who used distance education during the COVID-19 pandemic to examine the effects of such distance education and to determine what action plans are needed for the future and the theory-practice gap in nursing education.

2.2. Study Design

This research used the case study method from among the qualitative research designs (15, 16).

2.3. Settings

The research was conducted with 21 nursing students and 14 lecturers studying at one state (N= 355) and one foundation (N=411) university. The average number of students per lecturers in these two schools, which both offered face-to-face education before the pandemic, was 27. Both universities switched to distance education through online platforms (8,9) as a result of the decision taken by the Turkish National Higher Education Institute on 26 March 2020. Although the distance education infrastructure of both universities existed before the pandemic, it was not actively used until the advent of COVID-19. During the pandemic, theory and practical training have been carried out through the Learning Management System (LMS) at the state university where the research was conducted, and the Blackboard Learning Management System at the foundation university. Lecturers and students received institutional training in the use of this platform. Not all students had the tools and technical infrastructure required for distance education. Neither the lecturers nor the students had any prior distance education experience for vocational courses.

2.4. Participants

In the study, purposive multilevel sampling was used (17, 18). The representation of perceptions and views of different groups about the subject of investigation is important (15,19, 20) The research was carried out by two female lecturers who had completed PhDs in nursing principles and public health nursing. During data analysis, the lecturers and students were coded by letters and numbers. The demographic characteristics of the participants are given in Table 1 and 2. In determining the participants in the study, the basic criterion was for lecturer, the criteria were that they were teaching practice-oriented nursing courses and had completed a PhD. The exclusion criteria from the study were refusal to participate in the study, the participants having internet problems, the home environment being unsuitable for the interview and screen recording not being allowed. A teach staff who suddenly had a health problem and 3 students who lost the internet were excluded from the study.

Table 1. *Socio-demographic characteristics of lecturers (n=14)*

Lecturers	Gender	Age	Profession	Clinical experience period Year/ month (Y/M)	Academic experience period Year/ month (Y/M)	Institution working time Year/ month (Y/M)	University
TS1	Female	43	Nursing fundamentals	6 M	20 Y	20 Y	State
TS2	Female	47	Publich health	-	21 Y	21 Y	State
TS3	Female	43	Child health and diseases	2 Y	22 Y	22 Y	State
TS4	Female	33	Nursing fundamentals	-	10 Y	3 Y	State
TS5	Male	31	Child health and diseases	1 Y	7 Y	1 Y	State
TS6	Female	39	Management in nursing	8 Y	7 Y	7 Y	State
TS7	Female	39	Psychiatric nursing	5 Y	12 Y	4 Y	Foundation
TS8	Female	36	Publich health	5 Y	13 Y	5 Y	Foundation
TS9	Female	31	Nursing fundamentals	3 Y	3 Y	3 Y	Foundation
TS10	Female	39	Women's health and diseases	5 Y	12 Y	6 Y	Foundation
TS11	Female	47	Surgical diseases	24 Y	3 Y	6 M	Foundation
TS12	Female	45	Internal diseases	8 Y	18 Y	6 Y	Foundation
TS13	Female	29	Surgical diseases	4 Y	5 Y	3 Y	Foundation
TS14	Female	36	Management in nursing	11 Y	3 Y	3 Y	Foundation

Table 2. *Socio-demographic characteristics of students (n=22)*

Students	Gender	Age	Class	Grade average	University
S1	Female	20	4	3.18	State
S2	Female	20	3	2.43	State
S3	Female	21	3	3.84	State
S4	Female	20	2	3.24	State
S5	Female	20	3	2.94	State
S6	Male	19	1	2.7	State
S7	Female	19	1	2.26	State
S8	Female	20	2	2.86	State
S9	Female	20	2	3.48	State
S10	Female	21	4	3.29	State
S11	Female	21	4	4.00	State
S12	Female	20	1	4.00	Foundation
S13	Male	21	2	3.73	Foundation
S14	Female	20	2	3.15	Foundation
S15	Female	20	4	3.01	Foundation
S16	Female	21	3	3.55	Foundation
S17	Female	21	4	3.70	Foundation
S18	Male	22	4	3.88	Foundation
S19	Female	22	4	3.58	Foundation
S20	Female	20	3	2.98	Foundation
S21	Female	20	3	3.57	Foundation

*Note. * According to the students' grade point average of 4 Overall Academic Grade Point Average (OAGPA) AA:4.00, BA:3.50, BB:3.00, CB:2.50, CC:2.00*

2.5. Data Collection

Research data was collected by focus-group interview via the Zoom online meeting program. A pilot study was conducted with one student and one lecturer to review the content of the interview questions (21). The purpose and process of the research were explained to the participants and from the online platform oral and written consent was obtained from those who agreed to participate in the study. Research data was collected through a semi-structured interview form prepared by researchers in line with the literature and a socio-demographic form (15, 22-24). A total of four focus-group interviews were conducted. Each focus group interview lasted an average of 145 minutes. The interviews were terminated when data saturation was reached (19, 25).

2.6. Data Analysis

The procedures carried out during the data analysis stage were as follows:

The maxqda 2020 software program, which is used in qualitative data analysis and helps analyze various data such as focus group, video, audio file, literature, visuals, was used (26).

Redacting the entire dataset to text	◄	The audio-visual recordings were transcribed by the researchers using the Microsoft Office Word program.
Reading the entire dataset	◄	The data set for sub-theme operations of the interview transcripts was transferred to the MAXQDA Plus 2020 Program from qualitative software. Two researchers checked the accuracy of the audio-visual recordings and transcripts.
Encoding data	◄	The entire data set was independently encoded by two researchers who were experts in their field.
Coding the parts	◄	Both researchers created 30-35 sub-themes from the data sets.
Combining relevant sub-themes and creating sub-themes	◄	The MAXQDA Plus Program was used to combine sub-themes. Both researchers decided on two main categories, four themes and eight sub-themes.
Presentation of common themes for the opinion of two experts in their field	◄	Two experts in qualitative research were asked to evaluate the common themes created.
Participant checking	◄	Control of themes and sub-themes was approved as a Member Check for lecturer and student.

2.7. Ethical Considerations

Consent for the research was obtained from the Noninvasive Clinical Research Ethics Committee, Faculty of Medicine, Dicle University (Number: 302, July 2020). Institutional permission was obtained from the Faculty of Health Sciences, Mardin Artuklu University (Number: 53920853-200-, December 2020) and the Maltepe University School of Nursing (Number: E-87517843-605.01-000.001.25440, December 020).

3. RESULTS

The themes that emerged regarding distance nursing education during the COVID-19 pandemic were developed in two stages. In the first stage, they were divided into two categories: "structural conditions for theory education" and "structural conditions for practice education. Then the "structural conditions for theory education" were gathered under three themes: strong structural conditions, both strong and weak structural conditions and weak structural conditions in the "structural conditions for practice education" category, only the theme of weak structural condition was obtained. A total of eight sub-themes was found.

Structural conditions for theory education

Theme 1: strong structures

In the strong structural conditions theme, the sub-themes most mentioned by both academics and students were technological integration (n=43) and accessibility (n=23).

- ***Sub-theme 1: technological integration***

Both teachers and students have acquired new technological skills in distance education during the period of the pandemic in Turkey (27-29). Distance education is essential in times of emergency situations like pandemics. The students stated that their use of databases had increased, they had followed professionals in the field, discovered many online sites, and actively used YouTube. One student said: "I discovered many academics and many sites during this period. I began studying foreign sources more in this period" (S3). Lecturers, on the other hand, learned computer technologies. One lecturer said: "We had to support our classes with extra videos because we couldn't guide them [in person]. Naturally, we learned about technology. We learned how to fix videos, edit and use animation programs" (TS13).

- ***Sub-theme 2: accessibility***

It has been reported that in Turkey it actually became easier for students to communicate with their instructors than before the pandemic (27,29). Students focused on the benefits of access to lecturers and lecture videos and the ability to rewatch them. One student said: "All our lessons are being recorded. We can open them again and again and watch them and take notes whenever we want. We have the opportunity to research and learn the subject from the computer without any delay" (S14).

Theme 2: both strong and weak structures

The second theme in the "structural conditions for theory education" category was the theme of both strong and weak structural conditions, with sub-themes of asynchronous participation (n=25) and changing comfort levels/routines (n=22).

- ***Sub-theme 1: asynchronous participation***

Asynchronous participation is the opportunity for individuals to create work plans according to their own speed, ability, and interest in the educational process. However, asynchronous participation also reduces interactivity among participants in education. The students who participated in our research stated that they preferred asynchronous participation because it sometimes provides a sense of comfort and sometimes because the home environment is not suitable. One student said: "I'm the only girl in the house. I have to do a lot of house chores. At the same time, I may have to do different things while listening to lessons. So, I don't turn on the camera, this can be also called 'feeling comfortable'" (S3).

- ***Sub-theme 2: changing comfort levels/routines***

Students noted that they experienced changes in their daily routines along with distance education (n=22). Students returned to their family homes during the pandemic. During this period, they stated that they had problems with sleep, nutrition, changes in physical activity, inability to adapt, their home being overcrowded, inability to get up in the morning and lack of motivation to attend class. One student said: "When I came to school during the face-to-face education period, at least it was clear what time I had to get up. Now I'm taking the class as soon as I get up. Eating and sleeping are also very affected." (S16). Lecturers, on the other hand, noted that they had difficulty balancing their roles in the home and roles in the work environment: " I go running around and cook, then I come back and start the lesson. My sleep pattern is disturbed. I am experiencing a conflict of roles. I don't have five minutes for myself. This role confusion is really tiring me out" (TS1).

Theme 3: weak structures

The most common sub-themes of the weak structural conditions theme in the "structural conditions for theory education" category were cold contact (n=41) and inequality of opportunity (n=26).

- ***Sub-theme 1: cold contact***

Students and lecturers stated that distance education led them to understand the value of face-to-face education. One student said: "We're stuck in our house right now. Actually, it's not an educational period that makes us happy. We have a lot of being distant from our environment and not being able to communicate one-to-one with our teachers" (S1). Lecturers, on the other hand, stated that they had a very difficult time teaching to the screen without students: "During distance education, we try to reach out the students without being able to make eye contact, without using gestures and facial expressions, without entering into a one-to-one relationship" (TS7).

- ***Sub-theme 2: inequality of opportunity in education***

It has previously been determined that most students and lecturers studying at universities in Turkey have electronic devices and internet services that allow them to access distance education (27,28). However, the majority of the students who participated in the study stated that they could not participate in the course synchronously due to financial difficulties and lack of infrastructure. Distance education created inequality of opportunity, especially among students living in rural areas. A lecturer at state university said: "Those living in the village in particular experience connection problems. Internet quotas are very insufficient, and they have trouble accessing the classes" (TS3). A student at the foundation university said: "I am very glad that our university. My friends, who were studying at the state university, were constantly upset that their connection kept breaking all the time during the course" (S18).

Structural conditions for practice education

In the category of "structural conditions for practice education", the sub-themes most commonly mentioned by both academics and students in the weak structural conditions theme were an increased sense of inadequacy (n=19), and dissatisfaction (n=13).

Theme 1: weak structures

- ***Sub-theme 1: increased sense of inadequacy***

Students stated that they felt inadequate in terms of clinical practice during the pandemic, and that learning only through visual tools was not enough. One student said: "It will be almost a year and a half. We'll start our employment before we've had a chance to practice. We haven't gained enough experience" (S10).

- ***Sub-theme 2: dissatisfaction***

The dissatisfaction sub-theme was expressed only by lecturers. They stated that they were caught off guard by having to provide distance practice education, that both they and the institutions had not been able to adapt, and the students they were educating were perceived as inadequate by society in general. One lecturer said: "We were not ready for this process. We don't have an efficient infrastructure. We don't have virtual reality classes or online simulation classes. Therefore, we couldn't connect theory and practice" (TS4). Another lecturer said: In the comic, a patient asked a student if they were a nurse student who graduated in 2020, and the student says yes. The patient told the student to 'Stay away from me! You didn't get a good education!'. Society does not have confidence in the students we have educated during this period" (TS3).

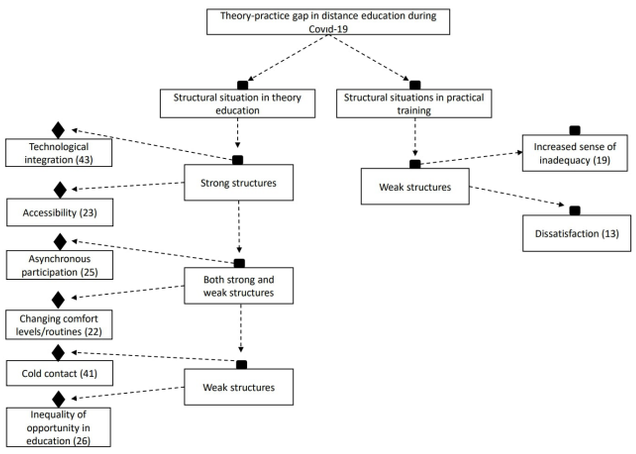

Figure 1. *Theme and sub-theme map for theory and practice.*

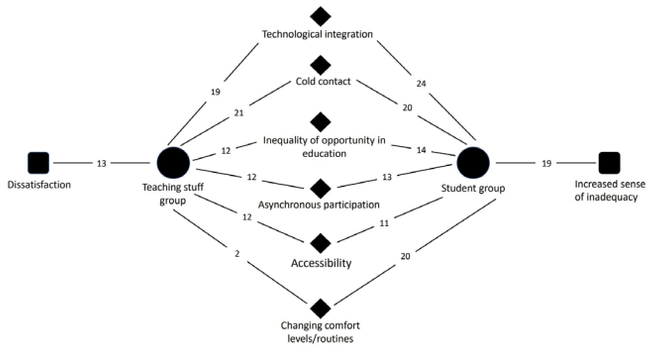

Figure 2. *Two case analyses of teaching staff and students.*

4. DISCUSSION

In this study, during the pandemic, the inability of nursing students to integrate their theoretical knowledge with practice seems to have deepened the default gap between theory and practice.

In the institutions where the research was conducted, theoretical education was provided through the Blackboard Learning Management System and the Learning Management System (LMS) programs. It has been determined that these programs are sufficient in theory. Simulation laboratories, case solutions, games and art programs in order to properly deliver practice education (30-33).

Well-structured virtual reality simulations for practice education is a teaching method that enables asynchronous participation (34). Cloud-based case simulations allow the student to visualize thought processes and re-observe actions as a result of repeating clicks throughout a case. Cloud-based simulation products such as EHR Go and NovEx can be used to improve clinical reasoning (5). Practice nourishes and strengthens theory. To deliver adequate practice education, universities must provide the necessary infrastructure, and lecturers must specialize in technology-based simulation applications.

In this research, accessibility was a strong structural factor in theoretical education. In their study of 220 college students, Civril et al. (12) similarly mentioned the theme of accessibility. Accessibility to educators allows students to set their own learning goals and develop their professional competence (5,35-36). Kaya and Akin Isik (6) in their qualitative research with in Turkey, they mentioned the theme of "disconnection of communication with the trainer". These differences may be due to differences in infrastructure and faculty competencies.

The current study found that synchronized participation in the lesson by students was poor. Other studies have also determined that the synchronized participation of nursing students in the course was low (10,37). Collaborative learning methods with peers encourage dialogue and provide deeper

learning. They also facilitate the development and application of knowledge and skills (13,38). This finding suggests that there is a need for teaching methods and motivational tools to support students' synchronized participation in lessons.

Work and life balance is being able to fulfil one's personal responsibilities and desires while also maintaining one's role in the business and/or professional fields (39). The current study found that participants were unable to maintain a balance between the work and home environment. In the literature, nursing students stated that their home environments were only partially suitable for distance education (6,11,37). In the interviews conducted by Suliman et al. (33) with 18 nursing students it was determined that female students in particular had difficulties in balancing their family responsibilities and school lessons during online classes. The students who participated in our research stated that their sleep patterns were disturbed. The reason for this difference may have been due to their domestic lives, changes to their routines and habits in order to adapt to the new situation, and the stress experienced because of the pandemic.

The participants stated that cold contact was a weak structural factor in theoretical education. Similar to our research, other studies also determined that students studying in health professions during the pandemic preferred this kind of education (10,27,33, 40-41). In distance education there is no immediate communication or touch, it is hard to model or teach compassion and empathy (42). In Seah et al.'s (42) study an online "communication and cultural diversity" lesson module was created to strengthen student-patient communication, and virtual group discussions and therapeutic communication was carried out with patients and students in a clinic. Students' use of such interactive classroom practices can be efficient in improving their skills and eliminating the emotional gap in care.

Financial insufficiencies and lack of infrastructure may reveal the inequality of opportunity in distance education during the pandemic. Research has revealed that nursing students experience internet connection/infrastructure problems (6,10,37,41). It has been shown that those with high incomes have more convenient access to online education and databases than those who do not, and are also more successful in learning (5,13,30,32-33,43). Private and public institutions should offer opportunities to students to eliminate inequality of opportunity in education. University libraries should provide free internet access, and databases should be free and made easier to access.

As practical training was not provided to the nursing students who participated in our research under the necessary conditions during the pandemic, their sense of inadequacy with regard to their professional skills was more intense during this period. It has been reported that nursing students feel inadequate both in their theoretical and in their practical education (6,11,36-37). Virtual practical learning environments are defined as supportive for developing knowledge, skills and attitudes to improve competence (44,45,46). Proposed implementing effective transition programs such as peer support, supervision, in-service training, simulations, mentoring and feedback to strengthen the experience of newly graduated nurses in health care settings and support the learning process.

The lecturers who participated in the research stated that they were unprepared for distance education, they were not able to provide sufficient guidance to the student, especially in practice education, and they were therefore dissatisfied. Today, the rapid development of technological software and hardware for education leads educators to constantly question their own technological competence (47,48). It is extremely important that educational institutions provide the necessary educational and infrastructure services to lecturers to help them develop in these areas.

5. CONCLUSION

The possibility for asynchronous participation in theoretical courses allowed students to participate when they were ready to learn. However, it also decreased lecturers' interactions with students and had a negative effect on their motivation. This indicates the need for teachers to make more efforts to provide interactive learning environments in online education. Distance education caused significant negative change in the routine health behaviors of both students and lecturers. There is a need to work to resolve the problem. There is a need to increase infrastructure investment to promote the use of augmented reality, virtual reality and web-based simulations, and game-based teaching tools in order to create interactive classroom environments with real cases for practice education. Policies aimed at eliminating the inequality of opportunity in distance education are also necessary. Intensive internships should be provided in the summer months in nursing programs in order to reduce the sense of professional inadequacy. Conducting more intensive and long-term in-service training programs for nurses who graduated during the pandemic period may help to replace the deficiencies in this period.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

Effectiveness of Diabetes Nursing Course Designed With Hybrid Learning Pedagogy: A Pilot Study

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ABSTRACT

Objective: This study aimed to examine the effectiveness of hybrid learning pedagogy in a redesigned diabetes nursing course for senior nursing students in enhancing knowledge and skills related to diabetes education.

Methods: A single-group pre-test and post-test quasi-experimental design were used. The study was conducted between January-June 2018 in a state university's nursing department in Istanbul, Turkey. Sixteen senior nursing students were included in the sample group of the study.

Results: It was detected that the Insülin Injection Skill Checklist (II-SC) post-test score, Blood Glucose Measurement Skill Checklist (BGM-SC) post-test score, and Educational Skills Checklist (ESC) post-test score were significantly higher than the pre-test scores. There was a significant difference between the Mini Exams (ME) pre-test and post-test scores of participants for each online lesson.

Conclusions: The study results showed that a hybrid learning-based diabetes nursing course effectively increases the knowledge and skills of students regarding diabetes education.

Keywords: Diabetes education, hybrid learning, nursing education, nursing student.

1. INTRODUCTION

Diabetes mellitus is a significant health problem in Turkey and throughout the world because of the frequency of occurrence (1). Nurses play an essential role in the self-management training of individuals with diabetes in health care settings (2). However, studies show that nurses and nursing students have inadequate knowledge about diabetes and its management (3-8). Whereas, healthcare professionals should be owned with the basic knowledge and skills about diabetes care and management (9). Providing a significant role for nurses in training individuals with diabetes and their families to manage diabetes can only be achieved through an effective training program that will make nursing students acquire diabetes knowledge and skills during undergraduate education (10). Graduation of students from the undergraduate program with insufficient knowledge and skills will lead to inexpert nurses who provide inadequate education for patients with diabetes (9).

The rapid developments in science and internet technologies influence the methods and strategies used to increase training programs' effectiveness. Hybrid learning is just one of these innovations in teaching-learning processes (10). In the literature, the concepts of "blended learning", " hybrid learning" or "mixed mode teaching" are used interchangeably. The concept "hybrid learning" was used in this study.

Hybrid learning is defined as learning activities that involve a systematic combination of face-to-face and technology-based interactions between students and teachers, and it is an increasingly widespread approach in the education and training settings (11). This method intends to apply by combining the advantages of face-to-face learning and online learning and minimizing their disadvantages. Face-to-face lessons of the hybrid learning process are maintained through classroom activities, whereas a learning management system (LMS) is used in the distance education dimension. The LMSs are software that enables users to present learning material and course content over the web, evaluate students' performance and monitor their participation. In addition, these systems allow faculty members to assign homework, organize exams and give feedback to students (12,13). Hybrid learning pedagogy has been successfully used in nursing education (14,15) and in various courses such as ethics,

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clinical education, research, and evidence-based nursing (16-19). On the other hand, McCutcheon et al. (2015) emphasized that is the lack of strong evidence on the implementation of a hybrid learning approach in undergraduate nurse education, and they stated that more research is required to assess the effectiveness of the hybrid learning (20).

The Diabetes Nursing Course based on Hybrid Learning Pedagogy (HLP-DE) aims to improve or update senior nursing students' knowledge and skills who take an intern program to gain more competence in a field. Thus, this course will contribute to the education and counseling skills of nursing candidates who will be working in the care of people with diabetes after graduation. The following hypotheses were tested in the study;

- H1: Hybrid learning-based diabetes nursing course increases senior nursing students' knowledge of diabetes
- H2: Hybrid learning-based diabetes nursing course increases the diabetes related skills of senior nursing students

2. METHODS

2.1. Aim

This study aimed to examine the effectiveness of hybrid pedagogy in a redesigned diabetes nursing course for senior nursing students in enhancing knowledge and skills related to diabetes education.

2.2. Study design, setting, and sample

In this study, the single group pre-test post-test quasi-experimental design was used. The study was conducted between January-June 2018 in a state university's nursing department in Istanbul, Turkey. All students who preferred the diabetes nursing intern program in the spring semester were invited to the study. 16 senior nursing students were included in the study sample group. In this study, using the G*Power-3.1 program was tested after the study analysis whether the sample size was sufficient at the 95% confidence level. According to the results of posthoc power analysis, the power of the research is 0.76 according to the smallest difference value; it was determined to be 0.90 according to the largest difference value (Table 3, Table 4).

2.3. Instruments

***2.3.1. Achievement Test**:* The Achievement Test (AT) consisted of 40 multiple choice questions created by Okuroğlu and Alpar (2019) (21). The test evaluates the theoretical knowledge gain after completing the online module. Okuroğlu and Alpar calculated the reliability of AT with the Kuder Richardson-20 (KR-20) formula and reported KR value of AT for health professionals was 0,75. In this study, the reliability coefficient of the AT was higher (KR-20 = 0.84) (22).

2.3.2. Mini Exams (ME): Researchers prepared mini exams to evaluate learning after every online lesson. Mini-exams included ten multiple-choice questions that were implemented online before and after every lesson and assessed 100 points. Those who scored 80 points or higher were considered successful and could continue to the next lesson.

***2.3.3. Insulin Injection Skill Checklist (II-SC)**:* This tool was created by Okuroğlu and Alpar (2019) (21). The tool consists of 21 process steps. The Kendall Tau coefficient had calculated for the reliability of this form and indicated as 0.93 (22).

2.3.4. Blood Glucose Measurement Skill Checklist (BGM-SC): This checklist was created by Okuroğlu and Alpar (2019) (21). The tool consists of 12 process steps; the Kendall Tau coefficient had calculated for the reliability of this form and indicated as 0.87 (22).

***2.3.5. Educational Skills Checklist (ESC)**:* Researchers developed this tool to evaluate educational skills, including pre-training preparation, planning, implementation, and post-training evaluation of nursing students who take diabetes nursing course. The tool consisted of 14 items scored as 1 to 3 (must be improved-adequate-good). The Kendall Tau coefficient was calculated 0.79, indicating that the reliability of the form was "excellent" (22).

2.3.6. Hybrid Learning Satisfaction Questionnaire (HLSQ): This questionnaire has evaluated students' satisfaction with the course and their likelihood of taking future classes with hybrid instruction. The tool consisted of 5 items, and the items have scored from 5 (strongly agree) to 1 (strongly disagree).

2.4. Interventions

In the current curriculum, Diabetes Nursing Course are included within the intern nursing program's scope, theoretical lesson 2 hours and clinical practise at the hospital 24 hours a week. Theoretical lessons are carried out with face to face method. In this study, the Diabetes Nursing Course was redesigned based on hybrid pedagogy.

The online learning part of hybrid learning was carried out with the Online Diabetes Platform (ODP) (www.onlinediyabet.com), which has been developed by the researchers and proven to be effective. ODP includes five modules and thirteen lessons enriched with videos and animations. It also provides online exam opportunities (23). Face-to-face lessons were held in the classroom the week after each online module was completed. The Diabetes Nursing Course Based on Hybrid Learning Pedagogy (HLP-DNC) content was presented in Table 1.

The study process was completed in three stages. In the first stage, pre-test data were collected with AT, II-SC, BGM-SC, and ESC. Task trainers (models) were used in the skill lab to assess students' insülin injection and blood glucose measurement skills, and their performance was observed via checklists. Then, students were asked to prepare and present a patient education. During this performances, students' educational skills were observed via ESC.

Table 1. *The curriculum of the diabetes nursing course based on hybrid learning pedagogy*

Moduls	Weeks	Lessons	Subjects	Learning Method
MODUL I: Definition of Diabetes	1.Week	Lesson 1	Definition, Diagnosis and Classification	Online Learning
		Lesson 2	Pathophysiology, Screening and Prevention	Online Learning
	2.Week			Face to Face Learning
MODUL II: Diabetes Treatment	3.Week	Lesson 3	Self Monitoring	Online Learning
		Lesson 4	Medical Nutrition Therapy in Diabetes	Online Learning
		Lesson 5	Physical Activity and Exercise in Diabetes	Online Learning
	4.Week			Face to Face Learning
	5.Week	Lesson 6	Non-Insulin Antihyperglycemic Drugs	Online Learning
	6.Week	Lesson 7	Principles of Insulin Therapy	Online Learning
	7.Week			Face to Face Learning
MODUL III: Complications of Diabetes	8.Week	Lesson 8	Acute Complications of Diabetes	Online Learning
		Lesson 9	Chronic Complications of Diabetes	Online Learning
	9.Week			Face to Face Learning
MODUL IV: General Health Advices in Diabetes	10.Week	Lesson 10	Foot Care	Online Learning
		Lesson 11	Diabetes and Special Conditions	Online Learning
		Lesson 12	General Health Advices	Online Learning
	Week			Face to Face Learning
MODUL V: Diabetes Education	Week	Lesson 13	Patient Education and Counseling in Diabetes	Online Learning
	Week			Face to Face Learning
	Week	Presentation of Sample Patient Trainings		

In the second stage, a password was given to the students to access the online learning platform at www.onlinediyabet.com. Students completed MEs before and after each online course. Face-to-face courses were held the week after each online module. In the face-to-face courses, the topics were repeated as summaries; the questions that the students did wrong in the MEs were explained, the subjects that the students did not understand were asked and re-explained. The program completed in the thirteenth week, and students presented their diabetes patient training samples in the fourteenth week.

In third stage, post-test data were collected. The presentation performances of the students were evaluated via the ESC (post-test). Students's theoretical knowledge gain was measured via AT (post-test). Insülin injection and blood glucose measurement skills of students were observed using SCs in the skill lab (post-test). Also students' satisfaction with the HLP-DNC was evaluated with HLSQ. Finally, pre-tests and post-tests scores were compared for AT, SCs, and ESC. Besides, pre-test and post-test MEs scores were compared for each online lesson.

2.4. Statistical analysis

The Statistical Package for the Social Sciences program (IBM Corporation, Armonk, NY, USA) was used for the data evaluation. Besides basic statistical calculations, the Wilcoxon's Signed Rank test and Mann–Whitney U-test were used. All the results were considered meaningful at $p < 0.05$ and a confidence interval of 95%.

2.5. Ethical considerations

This study was approved by Ethics committee of Marmara University Medicine Faculty (approval date and no: 09.2018.363). Also, all the participants were informed about the study and written informed consent was obtained from the participants volunteering to participate in the study.

3. RESULTS

The sample group consisted of 16 senior nursing students, and their mean age was 20.31±0.79 years. Most of the participants were females 93.8% (n = 15). Only 25% (n=4) of participants had previous experience in diabetes education (course, symposium, congress). All of the participants had internet access (n = 16, 100%). Each participant spent an average of 3.12±1.54 hours daily using the internet. All of the participants completed the online lessons (n= 16, 100%) and most of the participants attended face-to-face lessons (n = 14, 87.5%). (Table 2).

The results of the analysis made to compare the pre-intervention and post-intervention AT, II-SC, BGM-SC, ESC and ME scores of the study group are presented in Table 3. There was a significant difference between the AT pre-test and post-test scores of participants ($Z = -3.522$; $p = 0.000$). It was detected that the II-SC post-test score was significantly higher than the II-SC pre-test score ($Z = -3.531$; $p = 0.000$). Similarly, it was determined that the BGM-SC post-test score was significantly higher than the BGM-SC pre-test score ($Z=-3.601$; $p = 0.000$). There was a significant difference between the ESC pre-test and post-test scores in the sample group. The ESC post-test score was significantly higher than the ESC pre-test score ($Z=-3.535$; $p = 0.000$) (Tablo 3). Also, there was a significant difference between the ME pre-test and post-test scores of participants for each online lesson ($p < 0.01$) (Table 4).

Table 2. *Demographic characteristics of the participants (n = 16)*

Characteristics	n (%)
Age	
Mean (SD)	20.31 (.79)
Range	19-22
Gender	
Female	15 (93.8)
Male	1 (6.2)
Previous experience with diabetes education (Lesson, symposium, congress)	
Yes	4 (25)
No	12 (75)
Access to the internet	
Yes	16 (100)
No	0
Number of hours spent on the internet daily	
Mean	3.12 (1.54)
Range	1-6
Completing of all online activities	
Yes	16 (100)
No	0
Attendance of all face-to-face lessons	
Yes	14 (87.5)
No	2 (12.5)
Level of Hybrid Learning Satisfaction	
Mean	4.93 (.25)
Range	4-5

Table 3. *Comparison of achievement test, insulin injection skill checklist, blood glucose measurement skill checklist, educational skills checklist pre-test post-test scores of the study group (n: 16)*

Results	Pre-test	Post-test			Post-hoc	
	Median (Q1-Q3)	Median (Q1-Q3)	*Z*	*p*	*Effect size*	*Power*
Achievement Test	25.00 (22.12-30.00)	90.00 (81.87-94.37)	-3.522	0.000*	0.88	0.89
Insulin Injection Skill Checklist	48.00 (42.00-53.50)	63.00 (60.00-63.00)	-3.531	0.000*	0.88	0.89
Blood Glucose Measurement Skill Checklist	24.00 (24.00-27.00)	36.00 (36.00-36.00)	-3.601	0.000*	0.90	0.90
Educational Skills Checklist	19.50 (13.50-24.00)	36.00 (33.00-39.00)	-3.535	0.000*	0.88	0.89

*Wilcoxon Signed Ranks Test was used; Q1: First quarter, Q3: Third quarter; *p<0,01*

When students' respond to the items in the HLSQ are examined, it was seen that the averages of the points are 4.93±0.25 (range=4.00-5.00) (Table 1).

Table 4. *Comparison of the mini exam scores of the study group before and after online lessons (n: 16)*

Subjects	Pre-test	Post-test			Post-Hoc	
	Median (Q1-Q3)	Median (Q1-Q3)	*Z*	*p*	*Effect size*	*Power*
Definition, Diagnosis and Classification	40 (60-80)	100 (100-100)	-3.10	0.002*	0.77	0.88
Pathophysiology, Screening and Prevention	60 (80-95)	100 (100-100)	-2.71	0.007*	0.67	0.80
Self Monitoring	25 (40-75)	100 (100-100)	-3.08	0.002*	0.77	0.88
Medical Nutrition Therapy in Diabetes	60 (80-95)	100 (100-100)	-2.72	0.006*	0.68	0.81
Physical Activity and Exercise in Diabetes	65 (80-100)	100 (100-100)	-2.58	0.010*	0.64	0.76
Non-Insulin Antihyperglycemic (Oral Antıdiabetıc and Insulin-Mimetic) Drugs	20 (40-75)	100 (100-100)	-2.95	0,003*	0.73	0.85
Principles of Insulin Therapy	40 (60-75)	100 (100-100)	-2.95	0.003*	0.73	0.85
Acute Complications of Diabetes	40 (40-55)	100 (100-100)	-2.91	0.004*	0.72	0.84
Chronic Complications of Diabetes	20 (20-40)	100 (100-100)	-3.13	0.020*	0.78	0.89
Foot Care	25 (60-100)	100 (100-100)	-2.55	0.011*	0.68	0.81
Diabetes and Special Conditions	25 (60-75)	100 (100-100)	-2.95	0.002*	0.73	0.85
General Health Advices in Diabetes	60 (80-80)	100 (100-100)	-3.13	0.002*	0.78	0.89
Patient Education and Counseling in Diabetes	65 (80-95)	100 (100-100)	-2.75	0.006*	0.69	0.82
Total	58.46 (53.07-65.38)	100 (100-100)	-3.06	0.002*	0.76	0.88

*Wilcoxon Signed Ranks Test was used; Q1: First quarter, Q3: Third quarter; *p<0,01*

4. DISCUSSIONS

Hybrid learning approach is defined as the integration of face-to-face and online learning environments (24,25). This study aimed to examine the effectiveness of hybrid learning pedagogy in a redesigned diabetes nursing course for senior nursing students in enhancing theoretical knowledge and skills related to diabetes education. When the mean scores of the students after the intervention were evaluated, it was seen that they had a score close to the maximum scores (Table 3, Table 4). Therefore, these mean scores met the instructor's expectations, and the learning outcomes of the course. So, it is possible to say that the students successfully completed this course.

In many studies conducted in different areas where the effectiveness of the hybrid learning model has been investigated, it has been stated that hybrid learning increases theoretical success (26,27). Kurt et al. (2017) said in a meta-analysis study that mixed learning significantly increased students' theoretical success in various fields such as medicine, education, and technology sciences (26). Farzi et al. (2020) stated that hybrid learning as a new educational strategy can improve nurses' performance and reduce medication errors (28). Liu et al. (2016) stated that hybrid learning appears to be more effective than nonhybrid instruction for knowledge acquisition in health professions (29). Zhan et al. (2017) stated that hybrid learning was more effective in increasing primary health care workers' theoretical knowledge about public health services (2017). Li et al. (2019) noted that hybrid learning effectively increases nursing students' knowledge in a meta-analysis study (31). Similarly, Sung, Kwon & Ryu (2008) evaluated the hybrid learning model's effectiveness to increase nursing students' knowledge and skills regarding medication management (32). They stated that the students' knowledge level increased after education. This study's results are consistent with those of the study of Sáiz-Manzanares, Escolar-Llamazares, & Arnaiz González (2020), in which the hybrid learning assesses the impact of nursing students on learning outcomes (33). In this study, the participants' diabetes theoretical knowledge score was higher than before the training (Table 3). Also, there was a significant difference between the ME pre-test and post-test scores of participants for each online lesson (Table 4). This finding showed that training based on the hybrid learning method effectively increases nursing students' theoretical knowledge about diabetes.

Knowledge and skill are two crucial parts of nursing education. Diabetes education includes many skills that should be gained by nursing students. In recent years, many limitations have arisen regarding clinical application areas. This situation has created the need to support the clinical skills of students further (20). In this study, the effects of hybrid learning on students' diabetes-related skills were also examined (Table 3). It was found that the hybrid learning increased the scores of nursing students' insulin injection and blood glucose measurement skills. Tokunaga, Yamaguchi & Yamamoto (2017) stated that the hybrid learning more effective than the face-to-face learning method in nursing skill practice in Japan (34). Alvarez, Dal Sasso & Iyengar (2017) and Verkuyl et al. (2016) used hybrid learning-based mobile applications in pain assessment courses. Both of the studies stated that hybrid learning was useful to increase nursing students' pain assessment skills (35,36). Shorey et al. (2018) explained that nursing students had enhanced communication skills after the hybrid learning process in their study (15). The study results of Strickland, Gray & Hill (2012) indicated that hybrid learning helped students understand and use their research skills in a practical way (37).

Nurses must have educational skills to perform effective diabetes education. For this reason, it is essential to provide nursing students with educational skills during undergraduate education. This study also focused on improving nursing students' educational skills by hybrid learning based diabetes training. The results showed that the post-training educational skills score of students was higher than the pre-training score (Table 3). Choi used hybrid learning and Kim (2018) in a study focused on building competency in nursing students' health education (38). Findings from this study indicate that a hybrid learning approach enhanced student ability to teach patients. Also, Wu et al. (2020) stated in their research that clinical teaching designed with hybrid learning increased nurse educators' clinical teaching competencies (27). These findings show that hybrid learning is useful in acquiring educational skills.

It was determined that students reported high levels of satisfaction with hybrid learning based diabetes nursing course (Table 1). This study concurred with some of the previous research (19,39).

4.1. Limitations

The study population was restricted to the nursing department's students of one state university in Turkey. Therefore, it may limit the generalizability of the study. One of the limitations of the study is the lack of a control group. The sample size may be small, as the students taking a one-semester course are included in the study. Hence, educators and management should exercise caution when applying the results of this study.

5. CONCLUSIONS

In this study, it was determined that a diabetes nursing course designed according to hybrid learning pedagogy effectively increased students' theoretical knowledge and skills about diabetes and gave them educational skills. It was also observed that the students reported a high level of satisfaction with this course designed with hybrid learning.

It is recommended to use hybrid learning in the design of different courses in the nursing curriculum. Also, it is recommended to conduct randomized controlled studies with larger sample sizes in the future. Moreover, the researchers

can conduct studies that were taken feedback from patients taught by the students.

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Clinical and Experimental Health Sciences

Randomized Clinical Trial of Heated HighViscosity Glass Ionomer Class II Restorations in Deciduous Molars: 12 Months Follow Up

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ABSTRACT

Objective: New generation High Viscosity Glass Ionomer Cements (HVGICs) have enhanced physical and mechanical properties. By effectively closing the restoration margin, it ensures that the restorations will last longer. The aim of this study was to investigate the clinical performances of heat-cured versus non heated HVGIC in class II restorations of deciduous molars.

Methods: This randomized, split mouth, multicentre study was performed in four different centres. A total of 250 deciduous molars from 88 individuals were randomly allocated to one of the following groups: 1) non-heated (*n* = 125) 2) heated (*n* = 125) and restored with a HVGIC using LED light for heat application. Restorations were clinically evaluated according to the modified USPHS at the baseline, 6 months and 12 months. The survival analysis was performed by Kaplan Meier and Life Tables. This study was retrospectively registered to the ClinicalTrials.gov with the ID number of NCT04291872 at 2nd March 2020.

Results: No statistically significant differences were found between the groups regarding to modified USPHS criteria ($p>0.05$). Success rate in retention criteria was 94.1% of the heat-cured and 92.6% of the non-heated restorations after 12 months. The mean survival time was 11.8 ±0.1 months in the heated group, while 11.9±0.1 months in the non-heated group.

Conclusion: The heat treated HVGIC for Class II restorations did not show any significant differences in 12 months' follow-up compared with the conventional technique.

Keywords: Dental caries, Glass ionomer cement, Deciduous tooth

1. INTRODUCTION

The conventional Glass Ionomer Cements (GICs) have numerous useful characteristics. For instance; chemical adhesion to structure of the tooth, a slow fluoride releasing, acceptable biocompatibility, good compressive (1, 2). On the other hand, the material is sensitive to water uptake within first 10 min after mixing which affect low wear resistance of GIC restorations (3).

Considering these disadvantages, manufacturers have developed new generation High Viscosity Glass Ionomer Cements HVGICs to improve physical and mechanical properties, as well as effectively seal the cavity restoration margin, resulting in higher longevity of restorations (4). Currently, the material of choice is HVGICs, due to its satisfactory survival in Class II restorations deciduous and permanent teeth (5, 6). Although HVGICs have certain advantages that present improved mechanical properties when compared to the earlier generation of conventional restorative GICs, some undesirable features limit to its clinical use. The major vulnerable feature of GICs is their poor fracture resistance. It is thought that this feature can improve with the process of the material maturation (7). In the previous research has shown that there are phrasal maturation processes in GICs. It takes over from the 4 to 6th weeks of settings (8). If dehydration occurs during the reaction, the GIC may present low surface strength, leading to have wear values lower and poor flexural and compressive strength at the early stage of setting (9).

Thermal-cured application to GIC was introduced to improve physical properties in recent studies (3, 10). It was thought that the change in temperature do not directly change the

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mechanic properties of the material and but increase the molecular kinetic energy to ensure adequate adhesion to dental tissues (11). Adding external energy through heat application, the setting of traditional GIC is needed to obtain improved initial mechanical features and marginal adaptation (12). The idea was tested to get shorter initial stage to improve the hardness of GIC that could increase physical-mechanical strength and decrease microleakage values. Heat application in order to setting the material faster and reach sufficient maturation can positively affect the mechanical properties, especially in encapsulated glass ionomer cements (10).

Dental setting lamps can be used as a source of heat for GIC (13). The newest LED light have higher power density and hence higher thermal emission (14, 15).

The objective of the current research was to compare the clinical success rates of heated versus non heated HVGIC in class II restorations of deciduous molars.

2. METHODS

The study reviewed and approved by The Human Research Ethics Committee of Yeditepe University, School of Medicine with the protocol number of 37068.608.6100-15-1081 (Date: 25.06.2015). This study was retrospectively registered to the ClinicalTrials.gov with the ID number of NCT04291872.

2.1. Study Design and Selection of Participants.

This randomized controlled, split–mouth multicentre study was carried out in Marmara University, Faculty of Dentistry, Department of Pediatric Dentistry, Istanbul University, Faculty of Dentistry, Department of Pediatric Dentistry, Karadeniz Technical University, Faculty of Dentistry, Department of Pediatric Dentistry, and Ege University, Faculty of Dentistry, Department of Pediatric Dentistry between December 2015 – September 2017. A total of 275 children who came to these centres' clinics were assessed for the eligibility of the study. According to inclusion and exclusion criteria 88 children with 250 teeth were included in the study (Figure1). Informed consent forms were obtained from parents/caregiver before their participation in the study. No children were excluded based on sex, social or economic status. The consort principles for Randomised Control Trials (RCTs) were used (16).

The inclusion criteria were:

- healthy children aged between 5-7 years old
- has at least two approximal carious in deciduous posterior teeth
- has an appropriate interdigital occlusal relationship
- has at least one adjacent contact tooth

The exclusion criteria were:

- non-cooperative children
- children with special needs
- cavity with restorations
- developmental and acquired defects due to teeth,
- abscess, pain, pulp exposure
- has Class III occlusal relationship, cross bite, occlusal interference

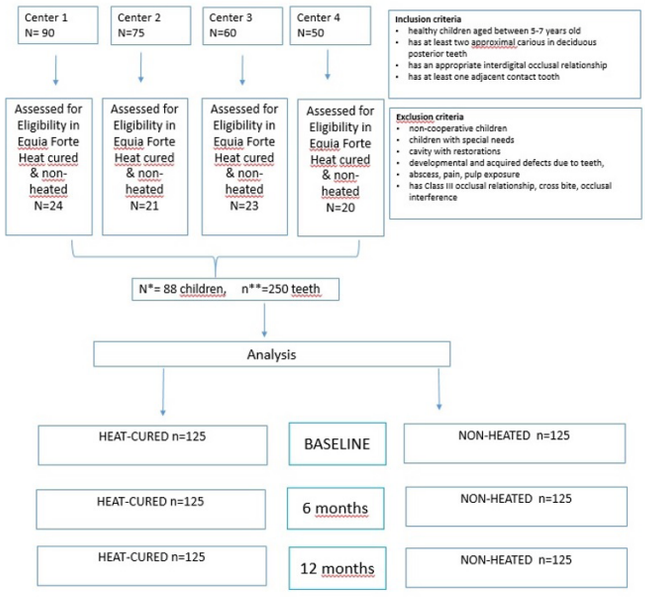

Figure 1. *Flow diagram of reporting trials of patient randomization*

2.2. Calibration

This randomized controlled, multicentre study was performed in four different centres. All pediatric dentists who took part in the study participated in the lecture and hands on course. Each pediatric dentist was educated and were familiar according to *Good Clinical Practise* (17). In each centre, one calibrated pediatric dentist made all the restorations accompanying with chairside assistant.

2.3. Sample Size Calculation

The sample size of this research was calculated according to previous study by Hubel et al. (18). The minimum sample size for the study was calculated as 78 participants at alpha error of 0.05 and beta error of 0.20. With possible drop outs 88 patients were included in the study.

2.4. Treatment Procedure

For randomisation, a lottery method used to distribute the groups (heated or non-heated) used for each patient's first restoration. Topical (10%Lidocaine – VEMCAINE, VEM İlaç San. Ankara/Turkey) and infiltrative local anaesthesia (Articaine Hydrocloride with epinephrine 0,01mg, VEM İlaç San. Ankara/Turkey) was applied respectively. To prepare the access cavity for proximal surface, a high-speed 801-012 diamond bur (FG Diamond-ADIA Dental Burs, Turkey) was used with under an air–water coolant. After obtaining access of the proximal site, Class II cavity was prepared using low speed considering the preparation of cavity according to non-selective caries removal technique.

Cavity was isolated by using cotton rolls and the sectional contoured metal matrices band (TOR VM, Russia) was placed interproximal and secured with a wooden wedge.

For split mouth study design, each restoration was randomly distributed to one proximal lesions until two treatment options were placed in each child with in equal number.

Non-Heated: Class II cavities were restored with Equia Forte (GC Corporation, Europe) according to manufacturer's instruction. For mixing the encapsulated Equia Forte Bulk Fill Glass Hybrid, Capsule Mixer CM-II (GC Europe) was used for 10 sec under +/ – 4000 rpm. The automatically mixed study material (10 s) was slowly injected into the cavity. The overflowing excesses were cleaned by hand tools. The restoration material was formed into shape after 45 sec.

Heated: Teeth were restored with the same protocol with non-Heated Group. LED light (GC – D-Light DUO) was used at standard mode 1200 m W /cm^2, at 50-60 ºC, for 60 sec. Immediately after heat application, occlusal interferences were checked. All the restorations both heated and non-heated were trimmed and polished after setting time (estimated setting time; net setting time=2 minute, total time (Average)= 3 minute, 25 seconds).

2.5. Clinical Evaluation and Follow-up

All restorations were evaluated by two calibrated examiners.

Marginal Integrity (MI), Marginal Discoloration (MD), Secondary Caries (SC), Anatomic Form (AF), and retention (R) were examined according to the modified USPHS at baseline and 6, 12 months. For each scale, there is an evaluation range from Alpha (perfect) to Delta (unsuccessful). Alpha (A) and Bravo (B) were used for clinically satisfying restorations (successful), while the Charlie (C) and Delta (D) scores were used to score clinically failures (unsuccessful) (19). The baseline evaluated initially after completing the restoration.

2.6. Statistical Methods

IBM SPSS Statistics 22 (SPSS IBM, Turkey) programs were used. Fisher's Exact Chi-square, Continuity (Yates) correction, Fisher Freeman Halton test and Mc Nemar test were used to compare the data with descriptive statistical methods (frequency). Survival analysis were evaluated using Kaplan Meier and Life Tables. Additionally, effects of selected such factors (heating condition: heated/ nonheated, jaw: maxilla/ mandibula, deciduous molar teeth: first/second molar, location: left/right,) were used to test their main effects on the dependent variable scores of 12 months' retention were redefined as binary variables (Alpha and Beta=Success and Charlie=Failure)) by using binary logistic regression enter method and $p<0.05$ was considered statistically significant.

3. RESULTS

3.1. Demographic Characteristics

The research was completed with 88 patients (boy=40, girl=48) with 250 teeth at the end of 12 months. The children's age ranged between 5–7 years (mean age=6.79±0.9). The distribution of the teeth according to the localization was described in Table 1.

Table 1. *The distribution of the treated teeth*

		N*	%
Molar type (N=250)	First Deciduous molar	134	53.6
	Second Deciduous molar	116	46.4
Jaws (N=250)	Upper jaw	111	44.4
	Lower jaw	139	55.6
Location (N=250)	Right side	126	50.4
	Left side	124	49.6

**indicates N= Number of teeth*

3.2. Clinical success

According to the Fisher Freeman Halton Test, the marginal integrity (p=0.007), marginal discoloration (p=0.027) and the retention criteria (p=0.001) showed significant differences for heat-cured and non-heated restorations in 6 months follow up period (Table 2).

While the success rates of anatomic form did not display any statistical significant differences between two groups, the heated group showed more acceptable success rates for 6 and 12 months' controls (Tables 2 and 3).

According to the retention criteria; the heat-cured was 94.1 % and the non-heated restoration was 92.6 % judged clinically as successful after 12 months (Table 3). The anatomical form and secondary caries showed no significant changes for both groups in one-year follow-up (Table 3).

The mean survival time in the heat-cured was 11.8±0.1 months and non-heated group was 11.9±0.1 months (Figure 2).

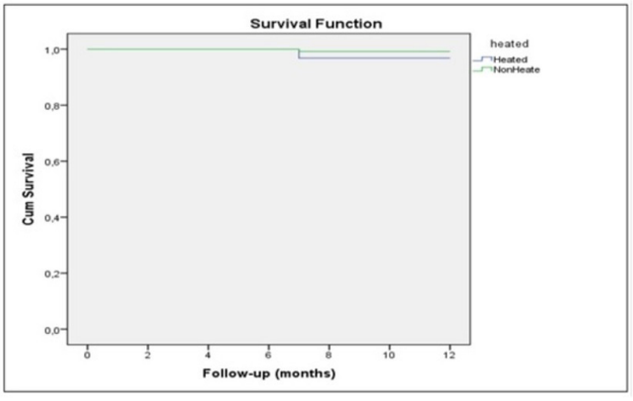

Figure 2. *Estimated cumulative survival curves of the restorations according to the retention codes*

The redefined ratios of success and failure were calculated as 89.2% (Success) and 7.2% (Failure), respectively. Also, the missing sample ratio was obtained as 3.6%. The binary logistic regression model revealed that any factors did not have significantly effects on the outcomes of 12 months retention (heating condition: p=0.307, jaw: p=0.620, deciduous molar: p=0.483, location: p=0.975, p>0.05 for all) (Table 4).

Table 2. *Comparison of baseline, 6 months and 12 months successes of groups*

		Baseline			6 months			12 months		
		Heatcured	NonHeated	p	Heatcured	NonHeated	p	Heatcured	NonHeated	p
Marginal Integrity	A	125 (100%)	125 (100%)		107 (86.3%)	93 (75%)		90 (75.6%)	77 (63.1%)	
	B	———-	———-	___	12 (9.7%)	24 (19.4%)	[1]0.007*	21 (17.6%)	27 (22.1%)	[1]0.087
	C	———-	———-		0 (0%)	5 (4%)		1 (0.8%)	6 (4.9%)	
	D	———-	———		5 (4%)	2 (1.6%)		7 (5.9%)	12 (9.8%)	
Marginal Discoloration	A	125 (100%)	125 (100%)		111 (89.5%)	102 (82.3%)		84 (70.6%)	73(59.8%)	
	B	———-	———-	___	9 (7.3%)	21 (16.9%)	[1]0.027*	26 (21.8%)	34 (27.6%)	[3]0.196
	C	———-	———-		4 (3.2%)	1 (0.8%)		9 (7.6%)	15 (12.3%)	
Seconder Caries	A	125 (100%)	125 (100%)		122 (98.4%)	118(95.2%)	[2]0.281	112 (94.1%)	108 (88.5%)	[4]0.147
	B	———	———	___	2 (1.6%)	6 (4.8%)		7 (5.9%)	14 (11.5%)	
Anatomic Form	A	125 (100%)	124 (99.2%)		109 (88%)	99 (79.8%)		88 (74%)	84 (68.9%)	
	B	———-	1 (0.8%)	1.000	11 (8.8%)	19 (15.3%)	[1]0.219	21 (17.6%)	25 (20.5%)	[3]0.665
	C	———-	———-		4 (3.2%)	6 (4.8%)		10 (8.4%)	13 (10.6%)	
Retantion	A	125 (100%)	125 (100%)		120 (96.8%)	112(90.3%)		105 (88.2%)	94 (77%)	
	B	———-	———-	___	1 (0.8%)	12 (9.7%)	[1]0.001*	7 (5.9%)	19 (15.6%)	[3]0.079
	C	———-	———-		3 (2.4%)	0 (0%)		7 (5.9%)	9 (7.4%)	

*[1]Fisher Freeman Halton Test, [2]Fisher's Exact Test, [3]Chi-Square, [4]Continuity (yates) test, *indicates p<0.05. A: Alpha, B:Bravo, C:Charlie, D:Delta.*

Table 3. *Clinical evaluation of heated and non-heated glass ionomer restorations with percentages values of clinically acceptable ratings (Alpha and Bravo) at baseline, 6 months and 12 months.*

		Baseline	6 months	12 months
		A+B	A+B	A+B
Marginal Integrity	H	100% (125/125)	95.2% (119/125)	88.8% (111/125)
	NH	100% (125/125)	93.6% (117/125)	83.2% (104/125)
	p	-	[a]0.767	[a]0.072
Marginal Discoloration	H	100% (125/125)	96% (120/125)	88% (110/125)
	NH	100% (125/125)	98.4% (123/125)	85.6% (107/125)
	p	-	0.370	[a]0.312
Anatomic Form	H	100% (125/125)	96% (120/125)	87.2% (109/125)
	NH	100% (125/125)	94.4% (118/125)	87.2% (109/125)
	p	-	0.540	[a]0.616
Retention	H	100% (125/125)	96.8% (121/125)	89.6% (112/125)
	NH	100% (125/125)	99.2% (124/125)	90.4% (113/125)
	p	-	0.370	[a]0.190

[a]Continuity (yates) correction A: Alpha, B: Bravo, C: Charlie, D: Delta; H: Heated, NH: Non-Heated

Table 4. *Binary logistic regression outcomes of the 12 months retention*

Factors	Std Error	Wald	p	Exp(B) – CI95% (Lower-Upper)
Heating condition (Heated/Non-Heated)	0.503	1.043	0.307	1.671 (0.624-4.48)
Jaw (Maxilla/Mandibula)	0.504	0.246	0.620	0.779 (0.29-2.092)
Deciduous molar (First/Second deciduous molar)	0.504	0.492	0.483	0.703 (0.262-1.885)
Location (Left/Right)	0.493	0.001	0.975	0.984 (0.375-2.586)
Consant	0.263	97.039	0	0.075

4. DISCUSSION

Glass-ionomer cements have been the subject of numerous studies regarding their clinical performance. Some researchers have tried to accelerate GIC setting reaction using ultrasonic waves, heat application with warm metal plates or heat application using Light / LED Cure dental devices in in-vitro studies (13, 20, 21).The efficacy of heat application deals with the thermal features of GICs. One of the favourable characteristics of the GICs is acceptable thermal properties, and these properties have not been studied many. Heat application is assumed to quicken the matrix-forming response of the cement and so at starting organize, the setting response will result in a more progressed and more noteworthy surface hardness. Applying heat moves forward the early properties of the GICs at a time when they are most vulnerable to harm (22). In this study, it was aimed to examine whether the durability of the material can be increased by heat application or not. Heat cure has been applied for different periods in different studies (10, 23). We applied 60 seconds in our study.

According to the conclusion of this research display that LED curing achieved significantly lower temperature rise than halogen lights for all test conditions (15). Clearly sufficient heat was not emitted from all LED curing lights. Thus, a special "heat application" LED light-curing unit has been manufactured. Its output temperature reaches 60 C in less than 60 seconds. The heat source is the LED light source also using for the polymerization of the composite resin material. Led cure dental devices can heat up to 50-60 degrees to a certain depth (3).

Molina et al. (2013) (24) compared Biaxial Flexural Strength (BFS) of different HVGICs after heat application with LED. They found that heating the GICs with an LED curing light 1400 Mw/cm2 during for setting for 30sec increased the BFS value for all GICs. In another in-vitro study; heating glass-ionomer restorative cements with an LED light-curing unit of 1200 mW/cm2 during 40 s improved marginal adaptation to enamel (13). However, there have been concerns that exposure to heat from these sources could lead to damage to tooth pulp. Van-Duinen et al. (2016) (25) concluded in their in-vitro study that heated GIC restoration setting does not have any harmful effect on pulp tissue and any pathological conditions. Gavic et al. (2015) (14) explained that glass-ionomer cements are capable of protecting the pulp for thermal damage. This issue cannot be concluded with just a few studies but more clinical studies are needed.

The recently introduced thermal-cured GIC have been used in restorative clinical studies. Skrinjaric et al. (2008) (23) evaluated the retention rate of heated GI sealant material for 1-year clinical follow up and concluded that heat treatment of GIC have had no effect on retention rate. In another clinical study, the success of light cured ART conventional HVGI fissure sealants were evaluated. Light cured conventional ART HVGI displayed more acceptable results in compared to those sealed with resin-composite and glass-carbomer (26). Tal et al. (2017) (3) concluded that heated HVGIC may be a better alternative restorative material for Class II restorations in deciduous molar.

Based on our 6 months' results, the retention of the restoration was more successful in the non-heated group, whereas in the 12-month follow-up, the heated group was more successful. While there was no significant difference between the groups according to the retention criteria, there was less loss of restoration in the heated group in 12 months. Within the statistical confines of survival analysis, although there was no statistically significant difference, heat application on GIC restoration had more successful results. According to the 6-month results, the marginal discoloration in the non-heated group was less observed than the heated group, whereas in the 12-month data, the number of the fail of restorations in the non-heated group was higher.

Although, we did not any obtain significant differences for all measurements during the study period, we have decided to clarify whether 12 months of retention outcomes would be affected by any other factors. The study focused on the deciduous and the young permanent dentition associated unsuccessful restorations with some variables such as patients' age (27). But the present clinical trial fulfilled this requirement since it evaluated the survival rates of restorations in only deciduous dentition. Also the split mouth design was followed so as to compare the two methods in the same child, in such a way so that all parameters and environment are kept constant (28). With respect to the potential data to obtain the main effects of such other factors (heating condition, jaws, deciduous molars and location of teeth), the binary logistic regression model (scores of 12 months were redefined as binary variables: Alpha and Beta=Success and Charlie=Failure) was used. Our regression model indicated neither factors revealed significant effects on the survival rates in 12 months' retention. Even though, this redefined variable is not much more clinically realistic, one year results might be promising regarding heat application for HVGIC. The heating condition (heated or unheated) of GICs could have an action on the retention of material if they could be tested in longer periods with more samples in later studies.

Although there are studies including CIS for one year follow up period (29-31), short follow up period is the limitation of our study as well. However, we first aimed to evaluate the efficiency of the heat application, and we focused on the success of the restorations. Eventually, we can suggest that this technique should be further investigated.

5. CONCLUSION

Our 12 months' findings suggested that the clinical success of HVGICs in class II cavity in posterior teeth are independent from the heat application. This study presents valuable results regarding the clinical application of GICs restoration in class II restorations in deciduous teeth.

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Clinical and Experimental Health Sciences

Compassion Fatigue and Satisfaction in Nurses and Midwives During the COVID-19 Pandemic in Turkey

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ABSTRACT

Objective: To examine the levels of compassion fatigue and compassion satisfaction of nurses and midwives during the COVID-19 pandemic and the influencing factors.

Methods: This descriptive and cross-sectional study was carried out with the participation of Turkish midwives and nurses. In the study using the convenience sampling method, data were collected online using an online questionnaire. The questionnaire, created through the Google form, was shared between May and June 2021 in the midwife and nurse groups (Facebook, Instagram, WhatsApp) on electronic platforms. Compassion fatigue (CF) and compassion satisfaction (CS) consisting of the burnout (BO) and secondary traumatic stress (STS) subcomponents were assessed by the Professional Quality of Life scale. The study was completed with 402 nurses and midwives from various units. Descriptive statistics, and the Mann-Whitney U and Kruskal-Wallis tests were used in the analysis of the data.

Results: Among the participants, the rate of dissatisfaction with the clinic worked was 24.6% and the turnover intention rate was 70.6%. During the pandemic period, 75.6% of the participants reported that they were exposed to verbal violence, 7.7% to physical violence, and 74.4% to mobbing behaviors. Low CS was found in 24.9% of the participants, a high level of BO in 27.1%, and high STS in 32.8%.

Conclusion: Violence and mobbing against nurses and midwives should be prevented in order to increase the professional quality of life.

Keywords: Quality of Life, Burnout, Compassion Fatigue, Personal Satisfaction

1. INTRODUCTION

According to the data of the World Health Organization, as of June 2021, more than 170 million people worldwide have tested positive for SARS-CoV-2 and more than 3.7 million deaths have occurred due to Coronavirus disease (COVID-19) (1). Midwives have witness deaths among the patients they cared for during the COVID-19 pandemic and continue to face ambiguity, pain and fear (2). Similarly, nurses working at critical units such as intensive care and emergency care could suffer severe emotional difficulties such as compassion-related and general exhaustion in the future as they witness the long-term suffering of the patients they provide care to (3). In addition, the nurses and midwives encountered various occupational health risks and difficulties when providing care for and protecting the patients during the COVID-19 pandemic (2-4). The COVID-19 pandemic has resulted in nurses losing their colleagues and falling ill after being affected personally (5). It is said that the healthcare staff working on the front lines have suffered physical and mental loss together with the financial, economic and social effects of COVID-19 on the population due to the increased number of health care procedures, irrespective of the country (6). This has resulted in an overstretched health care system working under inadequate conditions (6) and severe pressure on the health care professional, which could cause physical and mental exhaustion (2-4,6). All these factors could have influenced the professional quality of life of the nurses and midwives during the pandemic (4).

Persons helping subjects exposed to traumatic stressors have been reported to have a risk of developing negative signs related to exhaustion, depression and post-traumatic stress (7). The healthcare staff have worked in the front lines during the pandemic and witnessed pain and trauma, and the importance of monitoring the changes in their professional quality of life is therefore emphasized (4). Stamm has explained the professional quality of life (ProQoL) within a contextual framework. Both the positive and negative aspects of the work performed can have an effect on the professional quality of life in this framework. The professional quality of life consists

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of two parts: positive (compassion satisfaction) and negative (compassion fatigue). Compassion satisfaction (CS) defines the satisfaction with the work performed. CS enables the person to contribute more to his/her work and the society he/she lives in. Compassion fatigue (CF), which indicates the physical and emotional attrition of professionals providing health care service, is composed of the burn out (BO) and secondary traumatic stress (STS) components (7). BO is reflected with fatigue, anger, depressive mood, and decreased interest and enthusiasm towards the occupation at home (7,8). STS is the fear and trauma related to work (7). CF is associated with increased desire to quit work (9), emotional exhaustion, loss of sensitivity, and decreased personal success (8). CF is also said to have a negative effect not only on the employee but also on patient safety and the organization's financial profile (8). CS is reported to have a possible protective effect against unfavorable work conditions. CS is also said to have a partial softening affect on the relationship between the job demands and job strain, and high CS is said to decrease the perception of workload (10).

Some studies have reported an increased risk of CF or BO development with the stress created by the traumatic and complex work-related situations in health care workers (4,8). Increased rates of post traumatic stress disorder, anxiety, and BO have been reported among health care workers with the effect of the pandemic (11). It has therefore been emphasized that the indirect trauma caused by the COVID-19 pandemic should not be overlooked (12). Our country has reported more than 5 million confirmed cases and almost 50 thousand deaths since March 2020 but the effect of the pandemic on the professional quality of life of nurses and midwives is not clearly known. It is believed that determining the CS and CF (professional quality of life) of nurses and midwives is important in increasing the quality of care and its application. The aim of the study was to evaluate the CF and CS levels of nurses and midwives during the COVID-19 pandemic together with the influencing factors.

2. METHODS

2.1. Participants

This descriptive and cross-sectional study was carried out with the participation of Turkish nurses and midwives. This study was conducted with an online survey in order to facilitate the participation of nurses and midwives who have a busy lifestyle and to eliminate face-to-face interaction during the COVID-19 pandemic. The inclusion criteria of the study were working at a health care institution in Turkey, having at least six months of experience, and accepting to participate. The convenience sampling method was used. The survey to be completed via Google Forms was distributed to the midwife and nurse groups on electronic platforms (Facebook, Instagram, WhatsApp) during May and June 2021. The purpose of study, the inclusion criteria, and the informed consent information were clearly defined in this questionnaire form. The ProQoL 5 scores' standard deviations in the Turkish sample and the number of nurses and midwives working in Turkey were used to calculate the required sample size (13). Using a confidence interval of 95% and a margin of error of 5%, the minimum sample size was found to be 385. The study was completed with a total of 402 nurses and midwives from various units.

2.2. Data Collection

A questionnaire form and the Professional Quality of Life Scale were used to collect the data.

2.2.1. Questionnaire form

The questionnaire form was created by the study authors based on a review of the literature (4,9,13). The form contains 17 questions on the sociodemographic (age, educational status, occupation, years of experience, marital status etc.) and professional characteristics (the unit of employment and the satisfaction with it, being exposed to violence and mobbing, etc.).

2.2.2. The Professional Quality of Life Scale: Compassion Satisfaction and Compassion Fatigue (ProQoL) Version 5

This scale developed by Stamm is used to evaluate the professional quality of life. The Professional Quality of Life scale consists of the compassion fatigue (CF) and compassion satisfaction (CS) components. Compassion fatigue consists of the burnout (BO) and secondary traumatic stress (STS) sub-components (7). ProQoL is a 30-item self-report questionnaire and is designed to measure CS (the pleasure obtained from being able to do one's work well), BO (exhaustion, frustration, anger, and depression related to work), and STS (fear related to primary or secondary trauma associated with work) in order to help professionals. The five-item Likert-type scale is evaluated by adding the subscale item scores. The subscale scores can also be grouped as low, medium, and high based on the 25th-75th percentile values. A higher CS score means more satisfaction with the ability to be an effective caregiver while working. Higher BO and STS subscale scores indicate a higher risk for BO and STS (7).

The Turkish validity and reliability study of the scale has been conducted by Çınarlı and the Turkish form consists of 21 items (13). The specific items in the Turkish form of the ProQoL are distributed into the following subscales: CS (items 3,6,12,16,18,22,30), BO (items 1,10,19,21,26), and STS (items 5,7,9,11,13,14,23,25,28). Reverse scoring is used for only one item in the BO subscale of the Turkish ProQoL form. The CS score range is 7 to 35, BO score range 5 to 25, and STS score range 9 to 45. The following internal consistency coefficients have been reported for the original Turkish ProQoL 5 form subscales: α = .83 for CS, α = .68 for BO, and α = .78 for STS (13). The values calculated in the current study were α = .87 for CS, α = .83 for BO, and α = .87 for STS.

2.3. Ethical considerations

Ethical approval was obtained from the Ethics Committee of the relevant (Niğde Ömer Halisdemir University) University (decision 2021/10-14 dated May 27) and the Turkish Ministry of Health (decision no T20_52_34 dated May 16) before starting the study.

2.4. Data Analysis

The SPSS (24.0) software was used to evaluate the study data. Data were presented using descriptive statistics such as numbers (n) and percentages (%), mean±standard deviations (X±SS), and medians and 25th-75th percentiles. ProQoL reliability was evaluated with the Cronbach alpha value. Normal distribution and homogeneity of the values was evaluated with the Shapiro–Wilk test and the Levene test, respectively. The Mann-Whitney U and Kruskal-Wallis tests were used to compare the ProQoL subscale scores regarding the subject characteristics and occupational variables.

3. RESULTS

The study was conducted on a total of 176 midwives and 226 nurses, of which the majority (90.8%) were female. The mean age of the subjects was 32.4±8.39 (min-max: 21-59) years. According to the declarations of the subjects, 41.8% had a professional experience of ≤ 5 years, 55.2% were married, and 81.6% had received an education of bachelor's degree and above. The rate of experiencing a COVID-19 and losing a close one due to COVID-19 were 37.3% and 30.3%, respectively. The mean weekly work duration of the subjects was 47.89±10.14 (min-max: 8-80) hours, and 55.2% reported working in rotating day and night shifts. The place of work was emergency care in 21.4% of the subjects, primary care in 20.2%, the COVID-19 clinics in 15.2%, and the maternity ward in 10.4%. The rate of dissatisfaction with the clinic was 24.6% and the turnover intention rate 70.6%. Exposure to verbal violence during the pandemic was reported by 75.6% of the subjects, physical violence by 7.7%, and mobbing by 74.4%. The rate of needing to receive professional psychological support was found to be 6.7% among the subjects.

The ProQoL subscale scores are presented in Table 1. Low CS was detected in 24.9% of participants, and approximately eight out of ten participants had a moderate to high risk of BO and STS (CF).

Table 1. *Participants' ProQoL subscale scores (n=402)*

*ProQoL sub-scale	X̄±SD	Median (Min-Max)	Percentiles (25th-75th)	Low (%)	Medium (%)	High (%)
Compassion satisfaction	26.43±5.65	27(7-35)	22.8-31	24.9	53.2	21.9
Burnout	17.48±4.77	18 (5-25)	14-21	21.6	56.3	22.1
Secondary traumatic stress	26.45±7.61	27(9-45)	21-31	22.4	55.5	22.1

** ProQoL: The Professional Quality of Life Scale: Compassion Satisfaction and Compassion Fatigue (Version 5)*

Table 2. *The distribution of the ProQoL subscale scores by subject characteristics (n= 402)*

Characteristic	n (%)	Compassion satisfaction	Burnout	Secondary traumatic stress
Age				
21-34 years	264(65.7)	26.15±5.76	17.59±4.76	26.23±7.46
35 years ↑	138(34.3)	26.96±5.43	17.26±4.78	26.86±7.91
*P**		0.298	0.476	0.618
Sex				
Female	365(90.8)	17.50±4.72	26.47±5.64	26.74±7.57
Male	37(9.2)	17.24±5.32	25.97±5.88	23.59±7.58
*P**		0.518	0.954	**0.014**
Occupation				
Nurse	226(56.2)	26.04±5.76	17.82±4.87	26.71±7.86
Midwife	176(43.8)	26.93±5.49	17.04±4.62	26.11±7.29
*P**		0.140	0.076	0.709
Professional experience (years)				
≤ 5 years	168(41.8)	26.44±5.64	17.01±4.89	25.58±7.17
6-9 years	83(20.6)	25.80±6.00	18.46±4.74	27.49±8.23
≥ 10 years	151(37.6)	26.76±5.48	17.45±4.59	26.84±7.68
P**		0.542	0.076	0.290
Marital status				
Married	222(55.2)	26.96±5.13	17.35±4.65	26.69±7.58
Unmarried	180(44.8)	25.77±6.19	17.64±4.92	26.15±7.66
*P**		0.133	0.427	0.490
Educational level				
Associate degree and below	74(18.4)	26.68±5.65	18.79±4.75	26.47±8.05
Bachelor and above	328(81.6)	26.37±5.66	17.18±7.73	26.44±7.52
*P**		0.734	**0.005**	0.804

**Mann-Whitney Test **Kruskal-Wallis Test*

Table 2 presents the distribution of the ProQoL subscale scores by subject characteristics. The STS scores of the women were higher. The BO scores were higher in those with an educational level of associate degree or less. This result indicated that the CF risk was increased in women and those with an educational level of associate degree or less ($p < 0.05$). There was no statistically significant relationship between the ProQoL scores and the other variables evaluated ($p > 0.05$).

Table 3 presents the distribution of the ProQoL scores according to some occupational variables. We found dissatisfaction with the clinic of employment, turnover intention, and a history of exposure to mobbing to be associated with decreased CS and increased CF risk ($p < 0.01$). The risk of CF was increased in those who reported being exposed to physical or verbal violence and also in those who had needed psychological support (by a psychologist or psychiatrist) ($p < 0.05$). There was no statistically significant relationship between the ProQoL subscale scores and the other variables evaluated (clinic/unit of employment, work shift) ($p > 0.05$).

Table 3. *The distribution of the ProQoL scores according to some occupational variables (n= 402)*

Characteristic	n (%)	Compassion satisfaction	Burnout	Secondary traumatic stress
The place of work				
COVID-19 clinics/ intensive care	61(15.2)	26.78±5.43	17.57±4.88	26.80±8.97
Maternity ward	42 (10.4)	27.11±5.54	18.35±4.13	27.66±7.02
Primary care	81(20.2)	27.24±5.48	17.74±4.97	25.88±8.20
Emergency care	86(21.4)	25.33±5.64	17.04±4.80	25.90±6.84
Other hospitalization care	132(32.8)	26.26±5.86	17.28±4.78	26.60±7.26
*P***		0.165	0.665	0.633
Work shift				
Day and night shift rotation	222(55.2)	25.94±5.82	17.76±4.62	26.49±7.62
Fixed morning shift	180(44.8)	27.03±5.40	17.13±4.94	26.40±7.63
*P**		0.062	0.182	0.810
Satisfaction with the employed clinic/unit				
Yes	303(75.4)	27.47±5.11	16.59±4.73	26.11±7.64
No	99(24.6)	23.24±6.06	20.19±3.76	27.47±7.49
*P**		**<0.001**	**<0.001**	0.087
Turnover intention				
Yes	284(70.6)	24.55±5.16	18.78±4.30	27.57±7.30
No	118(29.4)	30.95±3.99	14.35±4.39	23.75±7.70
*P**		**<0.001**	**<0.001**	**<0.001**
Exposure to mobbing				
Yes	299(74.4)	25.95±5.77	18.19±4.66	27.24±7.84
No	103(25.6)	27.81±5.09	15.41±4.49	24.15±6.42
*P**		**0.007**	**<0.001**	**<0.001**
Exposure to physical violence				
Yes	31(7.7)	23.90±7.42	19.16±4.95	27.22±7.98
No	371(92.3)	26.64±5.44	17.34±4.73	26.38±7.59
*P**		0.067	**0.037**	0.344
Exposure to verbal violence				
Yes	304(75.6)	26.24±5.84	17.82±4.73	26.99±7.68
No	98(24.4)	27.02±5.03	16.42±4.76	24.78±7.17
*P**		0.418	**0.012**	**0.008**
Professional psychological support need				
Yes	27(6.7)	25.88±5.41	19.74±3.63	29.62±7.43
No	375(93.3)	26.47±5.68	17.32±4.80	26.22±7.58
*P**		0.519	**0.010**	**0.013**

**Mann-Whitney Test ** Kruskal-Wallis Test*

4. DISCUSSION

The CS and CF levels of the nurses and midwives during the pandemic were evaluated together with the effects of some individual and occupational variables in the current study. Our results indicated that the CS level was low in 24.9% of the participants, and approximately 8 out of every 10 subjects had a moderate or high level of CF risk. In addition, the CS and CF levels of the nurses and midwives were similar in this study. Similarly, a study conducted in Spain during the pandemic has found that the CS rate in health care professionals (physicians and nurses) was 15.6%, and approximately 9 out of every 10 subjects experienced a moderate or high level of CF (4). The results of this study and various previous studies (4,14) indicate that most of the nurses and midwives suffered from low CS and moderate to high levels of CF in their professional work during the pandemic.

CF and CS are influenced by many variables such as age, marital status, economic status, gender, and education (4,14,15). The CF level was higher in females in our study, similar to a study from Spain (4). This finding could be explained by the different stress regulation methods and reactions to stress in females and males. It has been reported that the stress factor creates an emotional response but the male and female participants show their response differently over time and this leads to greater changes in the females while males show significantly lower emotional variance (16). Our results show that the increase in the professional education level of nurses and midwives has a positive effect on their ability to develop strategies for coping with stress.

A higher rate of STS, CF, and psychological problems has been reported in health care staff working in departments directly related to COVID-19 (4,17-19). However, working with COVID-19 patients was not a factor that affected the professional quality of life of nurses and midwives in this study. Similarly, another study from Italy, a country severely affected by the pandemic, has shown that the work-life balance is not affected by providing care to a COVID-19 patient. The same study has postulated that this finding could be explained by the perceived support or the relationship within the team. However, the CF level was found to increase and the CS level to decrease in those who were not satisfied with the clinic they worked at in the same study (20). The possible reasons for this findings are the fact that COVID-19 is still not under control in our country, the need to provide care for a patients with a diagnosis/suspicion of COVID-19 at every step due to the structure of our health care system, and the changing of the units the nurses and midwives work in without asking for their permission. Having the nurses and midwives work in departments they do not want to be employed in has a negative effect on their professional quality of life.

Violence against healthcare employees is gradually increasing both in Turkey and globally. Liu et al. have found in their meta-analysis that 62% of healthcare workers had been exposed to at least one type of violence (21). The rate was 75% for verbal violence and 7% for physical violence during the pandemic in the current study. Unfortunately, these high rates of violence were similar to the findings of other studies from our country (22,23). The current study also demonstrated that the risk of CF increased in those exposed to violence. It was therefore not surprising that nurses and midwives who are exposed to such a high rate of violence experienced CF in this study, in accordance with previous reports (24-26). Violence against

healthcare professionals is reported to occur more frequently in underdeveloped countries like ours. Various initiatives by politicians are needed to tackle the problem of violence in healthcare, one of the causes of CF.

Mobbing refers to any kind of systematic unethical behavior with humiliation as its objective. Mobbing is reported to occur frequently among healthcare professionals. Being exposed to mobbing was declared by 75% of the participants in the current study. Previous studies (27-29) in nurses have also reported rates of up to 90% for being exposed to job-related bullying or mobbing. We were able to demonstrate a relationship between mobbing, BO, and STS. Similarly, other studies (9,30,31) have reported a negative effect of mobbing on the professional quality of life.

A previous study has found that being exposed to mobbing decreases CS and increases turnover intention in nurses (9). The turnover intention rate was similarly very high (70%) in our study. This rate has been reported to be 49% in Korea (32), 60% in Japan (33) and 53% in Switzerland (34). It is obvious that the pandemic process has had a negative affect on the nurses and midwives in our country. We also found that turnover intention decreased CS and increased the CF risk in this study. Other studies have reported similar findings (9,32). A study from Korea has reported significantly lower turnover intention levels in nurses with a high CS. The same study has determined STS to increase turnover intention 1.14 times and BO 1.54 time (32). The turnover intention and professional quality of life are therefore very closely associated. Current OECD data show Turkey to be in the fourth from last place for the number of nurses per 1000 persons, with a value of 2.3. Norway takes first place with a value of 18 (35). The high turnover intention rates in our study could be associated with the further increase during the pandemic of the workload that was already heavy beforehand.

The CF risk was found to be higher in the subjects who stated needing psychological support in this study. Other studies have reported a lower CF level (37) and increased CS (17) in nurses with higher perceived social support. It is also stated that the types of social support including the corporate culture and climate in addition to the support provided by peers and the supervisor can potentially protect employees faced with a risk of STS (36). Based on the current study and various previous studies (15,17,37) we believe that a supportive work environment plays an important role in the professional quality of life. Awareness-based activities (body screening, conscious movement, walking and sitting meditation, etc.) are said to be a tool for health care providers to fight the symptoms of compassion exhaustion. These activities are reported to positively influence the participants with lower stress and a more relaxed state, better awareness of the environment and their attitudes and emotions, and more positive thinking with higher awareness of their thoughts (38). Consistent with the literature (10,38), we suggest that managers should focus on activities that support better coping strategies (such as the use of organizational support, mindfulness-based stress reduction program, and provision of hospital counseling services), including improving their employees' social support system.

Limitations

The study is limited by the statements of the subjects. Conducting the study online has increased the access of subjects from various regions of Turkey and also increased the generalizability of the results. However, it may also have resulted in the exclusion of nurses and midwives who have no internet access and/or work in rural areas. The authors have only evaluated the current status of the professional quality of life of the nurses and midwives during the pandemic, and have not evaluated other traumatic events that could also have an influencing role.

5. CONCLUSION

This study has revealed that the professional quality of life of the nurses and midwives in Turkey was at a moderate level and 8 out of every 10 subjects had a tendency to experience moderate to high levels of CF while conducting professional work. There was a high rate of turnover intention and dissatisfaction with the clinic of employment among the nurses and midwives and this was associated with decreased CS and increased CF risk. Nurses and midwives were found to experience violence (both physical and verbal) and mobbing behavior at a level that could negatively affect their professional quality of life during the pandemic. The CF risk was also higher in the nurses and midwives who stated needing professional support.

Implications for Research, Policy and Practice

The first thing to do to increase the professional quality of life of nurses and midwives is to ensure their increased satisfaction (prevention of mandatory assignment to clinics they do not want to work in, decreasing the workload, etc.). It is important to take every precaution to protect health care staff from any kind of verbal, physical or emotional violence and enforce punitive measures. The issue of mobbing and its effects must be recognized by the administrators and internal audit mechanisms should be enforced with preventive measures. Mobbing should be checked for at regular intervals, mobbing-preventing measures put into place, and regular training provided for effective coping. Violence, mobbing and professional quality of life should be monitored regularly and a supportive environment and relevant events should be planned and put into action. We recommend urgently studying the reason for the increased turnover intention in nurses and midwives and decreasing their workload.

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Declaration of Competing Interest

None

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Conflict of Interest Statement

The authors report no actual or potential conflicts of interest.

Authors' contributions

Ozlem ASCI (ÖA) and Meltem Demirgoz BAL (MDB) designed the study. OA, MDB and Ferdane KOCOGLU (FK) recruited participants and collected data. Data analysis was performed in collaboration with OA, MDB and FK. MDB drafted the manuscript, which OA and FK critically revised. All authors read, contributed to, and approved the final manuscript.

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Clinical and Experimental Health Sciences

Quantitative Evaluation of Lung Parenchyma Changes after Treatment in COVID-19 Pneumonia with Volumetric Study in Computed Tomography

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ABSTRACT

Objective: COVID-19 pandemic, causing approximately 3 million deaths over worldwide, still continues. Effect of COVID-19 pneumonia after treatment on the lungs still not know. Although widely using computed tomography (CT) for diagnosing COVID-19 pneumonia, there is not enough study to determine damage of lung after treatment in COVID-19 pneumonia. In this study, our aim was to evaluate lung parenchyma changes in COVID-19 pneumonia after treatment with volumetric study, quantitatively.

Methods: 25 patients, who has CT at the time of diagnosis (CT1) and after 28±2 days (CT2), and positive polymerase chain reaction test, were included in this retrospective single center study. Total lung volüme (TLV) and emphysematous lung (ELV) volume of CT1 and CT2 were calculated automatically by using Myrian® XP-Lung and Percentage of emphysematous area (PEA) was calculated by dividing ELV by TLV. Differences between CT1 and CT2 in PEA and in TLV and ELV was determined by Wilcoxon and Paired sample t test, respectively.

Results: Although higher TLV was found in CT2 (4216,43 ± 1048,99 cm3) than CT1 (3943,22 ± 1177,16 cm3), there was no statistical significance difference (p=0.052) between CT1 and CT2. ELV was statistically (p=0.017) higher in CT2 (937,22 ± 486,89 cm3) than CT1 (716,26 ± 471,65 cm3). There was a strong indication that the medians were significantly different in PEA (p=0,009).

Conclusion: Our study showed that there were emphysematous changes in lung parenchyma after COVID-19 pneumonia with CT, quantitatively and in our knowledge, this is the first study that evaluating lung changes quantitative after COVID-19 pneumonia.

Keywords: COVID-19, Emphysema, Pneumonia, Quantitative, Tomography

1. INTRODUCTION

Novel coronavirus disease (COVID-19), named by The World Health Organization (WHO), was first reported in Wuhan, China, in December 2019. It spread rapidly all over the World (1). WHO has declared the outbreak on March 11 2020, a public health emergency of international concern (2). As of April 2 2020, 130,400,220 cases have been documented globally and 3,357,988 cases in Turkey. 31,713 deaths also were reported in Turkey (3). With the epidemic, the burden of hospitals and healthcare workers has increased, and it has had many social, psychological and economic effects in terms of health professionals and society. (4). Coronaviruses, belonging to the Coronoviridea family, are single-stranded, non-segmented, positive-strand, enveloped Ribonucleic Acid (RNA) viruses (5). Quantitative polymerase chain reaction (Qpcr) for quantitative detection of nucleic acid was used to diagnose COVID-19 (6). However, there were some limitations as a sample collection and transportation, and depending on these limitations, the total positive rate of RT-PCR for nasal and pharyngeal swab samples was informed to be approximate 30%–60% at first admitted (7).

The main route of transmission of this disease is respiratory infection (8). Fever, cough, dyspnea, headache are the main symptoms (2). However, other viruses, such as İnfluenza A and B, can cause the same clinical symptoms as COVID-19 (9). COVID-19 demonstrated apparent destruction of the pulmonary parenchyma, interstitial inflammation and consolidation and caused lesions characteristic of interstitial pneumonia (10).

Imaging plays an essential role in diagnosing and treating COVID-19 pneumonia, Computed Tomography (CT) is the gold standard imaging examination in COVID-19 pneumonia, and imaging-based scoring systems have been developed for diagnosis (2,11). Various lung changes patterns such as

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ground-glass opacities, consolidation, the reticular pattern was seen on CT (12). CT was used for many years to diagnose emphysema qualitatively, but special software programs have been developed to analyse emphysematous lung volume quantitatively (13).

The effect of COVID-19 pneumonia after treatment on the lungs is still not known. Although widely used CT for diagnosing COVID-19 pneumonia, there is not enough study to determine lung damage after treatment in COVID-19 pneumonia. The primary target of our study was to determine lung parenchyma changes in COVID-19 pneumonia after treatment with a quantitative volumetric study using a threshold value. To the best of our knowledge, this is the first study that evaluates the role of CT in lung parenchyma changes of COVID-19 pneumonia after treatment, quantitatively.

2. MATERIAL AND METHODS

The study was conducted in adherence to the Declaration of Helsinki. It was reviewed and approved by the institutional review board, and protocol review committee (Approval No: 07.07.2020/2020-12-04) and patient consent was waived by committee decision.

2.1. Patients

1297 patients admitted to the emergency room with suspicion of COVID-19 disease were evaluated between March 23 and June 30 2020. Patient selection was consecutive for this study. Nasal and pharyngeal swab specimens were taken from all patients, and the diagnosis of 895 patients was confirmed by one positive result of real-time reverse transcriptase-polymerase chain reaction (rRT-PCR). Exclusion criteria were; no thorax CT examination at the time of diagnosis (625 patients), no thorax CT after 1 month from first CT (233 patients) and patients with cardiac, lung and other systemic disease affecting lung parenchyma (12 patients). 25 patients were included in this single-centre, retrospective study.

2.2. CT acquisition

All CT examinations were performed by a 64-slice multi-detector row CT scanner (Somatom Go Now, Siemens Healthcare, Erlangen, Germany). All images were acquired at full inspiration in the supine position. No intravenous contrast media was used. The scanning range was C3 vertebrae to L2 vertebrae, including the apex and base of the lung. 512x512 matrix size, 1.0 mm slice thickness, and a sharp reconstruction kernel (KernelBr64) were used. A window level of – 600 Hounsfield Unit (HU) and width level of 1200 HU were used for standard window settings.

2.3. Image analysis

One radiologist with 6 years of experience assessed all images who was blinded to the clinical information. All images were transferred to the workstation, and Myrian (version 2.7.6) software was used for advanced analysis of images. The consistency of intragroup correlation was not checked because the software analysed all measurements automatically.

2.4. CT visual, quantitative evaluation

Total lung volumes (TLV) of all patients were calculated automatically with Myrianâ XP-Lung application (Fig. 1). Healthy and pathological areas were identified automatically with the threshold method by the COVID-19 application. Emphysematous lung volume (ELV) with threshold values under – 950 HU and TLV were recorded for each patient (Fig. 2). The percentage of the emphysematous area (PEA) was calculated by dividing ELV by TLV. All calculations were done for both first (CT1) and second CT (CT2) separately.

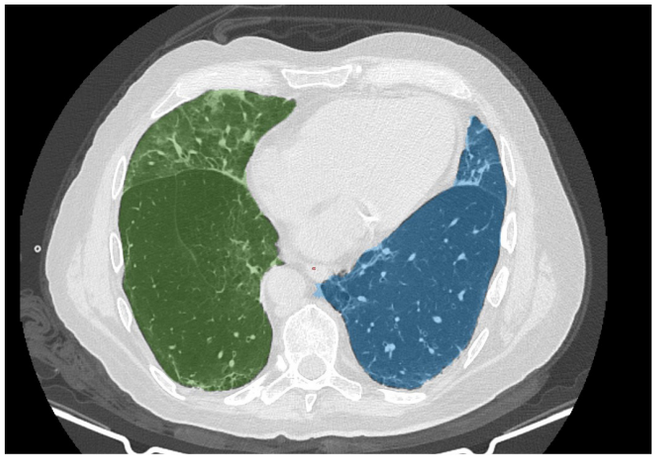

Figure 1. *60 years old men admitted to the emergency room with fever and cough. MyrianÒ software XP-Lung application calculates right and left lung volume separately from axial CT images.*

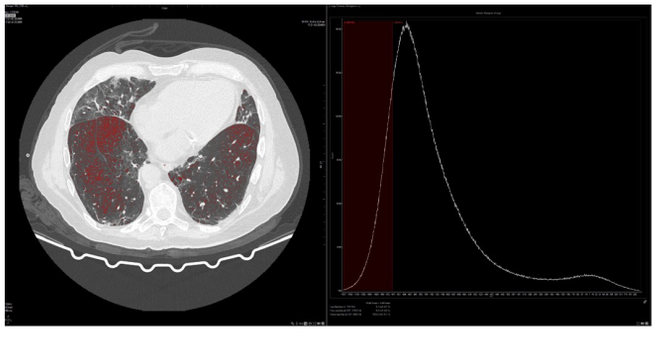

Figure 2. *The COVID-19 application evaluated the same patient. When threshold level choosing – 950 HU and under, the software calculated emphysematous lung volume automatically.*

2.5. Statistical analysis

IBM Statistical Package for the Social Sciences (SPSS version 25 for Mac OS) software was used for all statistical analysis. The Shapiro-Wilk test determined the fitness of the numeric

data set to the normal distribution. Descriptive analyses were determined for each data. Due to non-normal distribution, the Wilcoxon test was carried out to measure differences between PEA of patients. Paired sample t-test was used for measuring differences of TLV and ELV between CT1 and CT2. A p-value lower than 0.05 was considered statistically significant.

3. RESULTS

3.1. Patients demographics

13 patients (52%) were male, and 12 patients (48%) were female. The mean age and standard deviation was 51,31 ± 3,88 for male (range: 30-84 years) and 45,50 ± 4,97 for female (range: 24-73 years) (Fig. 3). During the study period, 5 males and 3 females were hospitalised, and no patient was died and required intensive care unit. 21 patients (84%) had a cough, 12 patients (48%) had a fever, 6 patients (24%) had dyspnea, and 4 (16%) patients had headaches. According to the COVID-19 treatment guideline of the Turkey health ministry, hydroxychloroquine sulfate 2x200 mg was taken by all patients for 5 days. 8 patients who were hospitalised took Favipiravir 2x600 mg for 5-7 days.

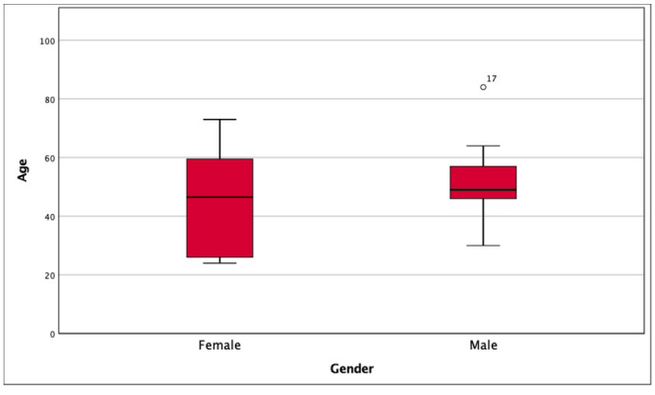

Figure 3. *The distribution of age according to gender.*

3.2. Radiological findings

The mean and standard deviation (SD) for TLV of CT1 was 3943,22 cm^3 ± 1177,16 cm^3 and 4216,43 cm^3 ± 1048,99 cm^3 for CT2. The mean of ELV for CT1 and CT2 were 716,26 cm^3 (SD; ± 471,65 cm^3) and 937,22 cm^3 (SD; ± 486,89 cm^3). According to gender, same measurements were summarized in table 1. PEA of CT1 and CT2 was showed non-normal distribution and median and interquartile range values were 16,51% (10,61% – 19,98%) and 22,14% (15,51% – 27,94%), respectively.

The mean value of TLV in CT2 was higher than CT1, but there were no statistically significant differences between the two measures. Nevertheless, p-value was very close to 0,05 (p=0,052). A statistically significant difference was found between ELV of CT1 and CT2 with a 95% confidence interval (p=0,017). After applying the Wilcoxon rank test to the percentage of CT1 and CT2, negative rank (mean rank; 9,83, the sum of ranks; 59) was found in 6 patients, and positive rank (mean rank; 13,39, the sum of ranks; 241) was found in 19 patients. There was a strong indication that the medians were significantly different, equal to a two-tailed p-value of 0,009.

Table 1. *The total lung volume and emphysematous lung volume of first and second computed tomography in males and females.*

	Male	Female
TLV CT1	4410 ± 1334	3476 ± 802
TLV CT2	4632 ± 1133	3800 ± 799
ELV CT1	863 ± 577	570 ± 291
ELV CT2	1064 ± 555	810 ±3 89

*Results were presented as mean ± SD * cm3*
Abbreviations; CT1, computed tomography at the time of diagnosis; CT2, computed tomography after treatment; ELV, emphysematous lung volume; TLV, total lung volume

4. DISCUSSION

In our study population, only 8 of 25 patients were hospitalised, and no one was needed intensive care unit. All patients had no severe pneumonia and complicated disease according to the COVID-19 treatment guideline of the Turkey health ministry. In our study, the most crucial finding was that ELV and PEA are statistically higher in follow-up CT with a threshold value of – 950 HU. The study designed by Bradley et al. reported that the main findings in COVID-19 pneumonia were alveolar damage and focal micro-thrombi (14). Moreover, autopsy studies have shown that alveolar damage is more widespread with disease progression, and fibrotic changes are revealed in the alveolar wall in two weeks (15). Therefore, we think that increased ELV and PEA in follow-up CT in our study were associated with diffuse alveolar damage caused by COVID-19 pneumonia consistently with the literature.

Lemmers et al. was reported that pneumothorax, pneumomediastinum and subcutaneous emphysema are the indicators of alveolar damage in severe disease and these complications are 7 times higher in COVID-19 patients, treated with mechanic ventilation than non-COVID-19 patients (16). Previous studies show that COVID-19 patients have similar mechanic ventilation parameters with non-COVID-19 patients, and pneumothorax is not associated with barotrauma. (17, 18). Pathophysiologic process in pneumomediastinum and subcutaneous emphysema was identified as Macklin effect, characterised by air dissection across bronchovascular sheaths and spreading interstitial emphysema into the mediastinum (19). These patients supported this theory by the absence of smoking history and no significant comorbidities predisposing air leak. Diffuse alveolar damage, cellular fibromyositis exudates, evident desquamation of pneumocytes, and hyaline membrane formation was shown in COVID-19 pneumonia similarly SARS (20). Wintermark et al. reported that increased pressure in the chest, such as cough episodes and Valsalva manoeuvre,

and severe diffuse alveolar damage leads to interstitial emphysema and causes alveolar rupture and air dissection along bronchovascular sheaths. Moreover, they specified that presence of pneumothorax and pneumomediastinum after progression of pulmonary lesions in thorax CT is associated with alveolar damage (21).

The value of qualitative visual evaluation of lung injury in thorax CT for predicting prognosis in COVID-19 was proved in previous studies (22). Although the recommendation of qualitative and visual evaluation score according to CT patterns in COVID-19 pneumonia, some limitations such as the absence of standardisation and lack of experience were reported (23). Some clinical studies also reported that CT densitometry reveals emphysematous areas qualitatively before spirometry test, there is a good correlation with pulmonary function tests, and there is a better correlation in determining emphysema with CT densitometry in inspiratory phase than visual evaluation (24, 25).

In the literature, qualitative CT studies focused on parenchymal lesions, such as GGO, consolidation, on CT between diagnosis and the 14th day of COVID-19 (26). To evaluate average lung volume, area of both consolidation and GGO quantitatively and objectively for measuring disease burden in follow-up examinations may provide information about disease progression and treatment response (23). In previous studies, evaluation of quantitative thorax CT was found successful in predicting clinical outcome in COVID-19. Furthermore, involved lung volume to total lung volume ratio showed high accuracy to predict oxygen support and need of mechanic ventilation. As a result, studies were revealed that quantitative CT was critical in COVID-19 triage (27). The other important finding in these studies was that quantitative evaluation of thorax CT at the time of diagnosis and the 4th day of COVID-19 might predict the risk of progression into severe disease better than clinical biomarkers (28). However, there is no study evaluating the literature on lung parenchyma changes after COVID-19 infection with quantitative CT.

Our study has several limitations. The first limitation of our study was designed as a retrospective study. Another limitation was the small study population, and no long term follow up of patients.

5. CONCLUSION

In conclusion, this is the first study showing that quantitative CT can reveal lung changes after COVID-19 infection. Additionally, the results of our study showed that mild COVID-19 pneumonia might cause emphysema, and this effect may reveal by CT quantitatively. Therefore, even though it is possible to infer that mild COVID-19 infection may cause emphysema after healing, the accuracy of the results obtained from our study should be supported by larger patient groups and with multi-centre studies.

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Clinical and Experimental Health Sciences

Synthesis, Characterization and Biological Evaluation of Novel Thiourea Derivatives

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ABSTRACT

Objective: A new series of 4-[3-(substitutedphenyl)thioureido]-*N*-(6-chloropyrazin-2-yl)benzenesulfonamide were synthesized from sulfaclozine.

Methods: All compounds were characterized by IR, ^{1}H-NMR spectroscopic methods and elemental analysis. In addition to the antioxidant activity of the synthesis series, enzyme inhibition activities such as anticholinesterase, tyrosinase, α-amylase and α-glycosidase were determined for the first time in this study.

Results: According to these biological activity test results, compound **2a** in the DPPH, **2c** in the $ABTS^{\cdot+}$ assay exhibited more antioxidant activity than reference standard. All thiourea derivatives demonstrated good BChE inhibitory activity than galantamine. Among the compounds, **2e** and **2f** showed the best tyrosinase enzyme inhibition activity, while **2g** had the best α-amylase and α-glucosidase enzyme inhibition activity. In addition, we evaluated the druglikeness properties of compounds and their oral bioavailability were also found to be high.

Conclusion: Thiourea derivatives exhibited remarkable antioxidant activity and enzyme inhibition activity against tyrosinase, cholinesterase, α-amylase and α-glucosidase.

Keywords: Thiourea, antioxidant, anticholinesterase, tyrosinase, α-amylase.

1. INTRODUCTION

Thiourea functional groups have numerous pharmacological activities such as antidiabetic, antibacterial, antifungal, antiviral, anticancer, anticonvulsant, antidiabetic (1-3). Thioureas play an important role in the regulation of various pharmacological activities by increasing potency and selectivity or by modulating of physicochemical properties. The pharmacological activity of thioureas results from specific interactions between proteins, enzymes, receptor targets and drugs. For example, the protons on the two nitrogens act as hydrogen bond donors, while the C=S fragment of thiourea acts as a hydrogen bond acceptor (4). Therefore, thiourea compounds are widely used in the search for new drug candidates (5). An another biologically active group is also sulfonamides (6). There are many active pharmaceutics used in different activities due to sulfonamide structure (7). For example, sulfadizine (antibacterial), darunavir (antiviral), celecoxib (anti-inflammatory), clopamide (diuretic), zonisamide (anticonvulsant) (8).

Kollu et al. demonstrated high antioxidant activities of thioureas after DPPH and FRAP experiments (9). In another study, Yiğit et al. indicated that compounds with thiourea structure show better activity than the reference drug tacrine against enzymes such as achethylcholinesterase (AChE) and butyrylcholinesterase (BChE) (10). Not only but also antidiabetic activity of sulfonamides and thioureas were reported in the literature (11,12). In our previous study, we also demonstrated the high anticancer activities of compounds with both thiourea and sulfonamide structures (13).

Keeping in view of significant biological activities of thiourea derivatives, we aimed to synthesize and show different biological activities of some novel thiourea derivatives. For this purpose, all synthesized compounds investigated for their

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antioxidant activity by DPPH, ABTS, β-carotene-linoleic acid and CUPRAC method, antidiabetic activity with α-amylase and α-glucosidase inhibitory assays, anticholinesterase activity against AChE and BChE, tyrosinase inhibitory activity.

2. METHODS

Sulfaclozine sodium monohydrate was granted by Medicavet A.Ş. All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). To monitor the reaction progress and determine the purities of the synthesized compounds were used thin-layer chromatography (TLC) (Merck, Darmstadt, Germany). Melting points were determined by SMP II melting point apparatus (Cole-Parmer Ltd. Staffordshire, UK). IR spectra were recorded on a Schimadzu FTIR-8400S spectrophotometer (Shimadzu Corp., Kyoto, Japan). ^{1}H-NMR spectra were recorded on a Bruker Avance DPX-400 spectrometer (Billerica, MA, USA). Tetramethylsilane as the internal standard and DMSO-d_6 as the solvent were used for ^{1}H-NMR spectrum. Elemental analyses were performed with GmbH varioMICRO CHNS (Langenselbold, Germany).

2.1. Chemistry

General procedure for the preparations of thioureas (**2a**-**2h**)

Firstly, sulfaclozine sodium monohydrate (1 mmol) was dissolved in 3 mL of water. When HCl (5%) was added dropwise, sulfaclozine began to precipitate and the precipitate was washed with plenty of water.

Then, sulfaclozine (1 mmol) was dissolved in anhydrous acetone (10 mL). 1 mmol of substituted phenylisothiocyanate was added on it. The mixture was refluxed for 10 hours. Excess solvent was evaporated under vacuum. The precipitated product was crystallized from methanol (14).

4-amino-*N*-(6-chloropyrazin-2-yl)benzenesulfonamide (**1**)

Yield: 85%; m.p. 235.2-235.3 °C. IR (n, cm^{-1}): 3348, 3234 (N-H), 3028 (aromatic =C-H), 1633 (C=N), 1325, 1139 (SO_2), 1089 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 6.17 (s, 2H, – NH_2), 6.60 (d, *J*=8.8 Hz, 2H, Ar-H), 7.65 (dd, *J*=8.6 Hz, 2H, Ar-H), 8.28 (d, *J*=16.4 Hz, 2H, pyrazine protons), 11.52 (s, 1H, – SO_2NH). Anal. Calcd for $C_{10}H_9ClN_4O_2S$: C 42.18, H 3.19, N 19.68. Found: C 42.23, H 3.19, N 19.67 %.

N-(6-Chloropyrazin-2-yl)-4-(3-phenylthioureido) benzenesulfonamide (**2a**)

Yield: 75%; m.p. 202.3-202.5 °C. IR (n, cm^{-1}): 3255 (N-H), 3043 (aromatic =C-H), 1585 (C=N), 1338, 1157 (SO_2), 1240 (C=S), 1087 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.15 (s, 1H, Ar-H), 7.42 (m, 4H, Ar-H), 7.84 (m, 4H, Ar-H), 8.34 (d, *J*=19.5, 2H, pyrazine protons), 10.17 (d, *J*=19.2 Hz, 2H, NH), 11.90 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{14}ClN_5O_2S_2$: C 48.63, H 3.36, N 16.68. Found: C 48.43, H 3.35, N 16.74 %.

N-(6-Chloropyrazin-2-yl)-4-(3-[4-fluorophenyl]thioureido) benzenesulfonamide (**2b**)

Yield: 78%; m.p. 249.5-249.9 °C. IR (n, cm^{-1}): 3205 (N-H), 3028 (aromatic =C-H), 1599 (C=N), 1325, 1149 (SO_2), 1226 (C=S), 1091 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.20 (d, *J*=8.4 Hz, 2H, Ar-H), 7.46 (s, 2H, Ar-H), 7.84 (m, 4H, Ar-H), 8.34 (d, *J*=19.8 Hz, 2H, pyrazine protons), 10.15 (d, *J*=36.6 Hz, 2H, NH), 11.92 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{13}ClFN_5O_2S_2$: C 46.63, H 2.99, N 15.99. Found: C 46.81, H 3.01, N 15.92 %.

4-[3-(4-Chlorophenyl)thioureido]-*N*-(6-chloropyrazin-2-yl) benzenesulfonamide (**2c**)

Yield: 70%; m.p. 173.0-173.4 °C. IR (n, cm^{-1}): 3205 (N-H), 3020 (aromatic =C-H), 1599 (C=N), 1323, 1151 (SO_2), 1230 (C=S), 1087 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.46 (m, 4H, Ar-H), 7.80 (m, 4H, Ar-H), 8.35 (d, *J*=19.3 Hz, 2H, pyrazine protons), 10.24 (d, *J*=23.6 Hz, 2H, NH), 11.93 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{13}Cl_2N_5O_2S_2$: C 44.94, H 2.88, N 15.41. Found: C 45.19, H 2.92, N 15.33 %.

4-[3-(4-Bromophenyl)thioureido]-*N*-(6-chloropyrazin-2-yl) benzenesulfonamide (**2d**)

Yield: 80%; m.p. 189.5-189.9 °C. IR (n, cm^{-1}): 3205 (N-H), 3030 (aromatic =C-H), 1599 (C=N), 1325, 1141 (SO_2), 1230 (C=S), 1087 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.54 (m, 4H, Ar-H), 7.69 (m, 2H, Ar-H), 7.89 (t, 2H, Ar-H), 8.34 (d, *J*=19.1, 2H, pyrazine protons), 10.23 (d, *J*=19.2 Hz, 2H, NH), 11.93 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{13}BrClN_5O_2S_2$: C 40.93, H 2.63, N 14.04. Found: C 40.81, H 2.62, N 14.10 %.

N-(6-Chloropyrazin-2-yl)-4-(3-*p*-tolylthioureido) benzenesulfonamide (**2e**)

Yield: 83%; m.p. 186.3-186.7 °C. IR (n, cm^{-1}): 3194 (N-H), 3012 (aromatic =C-H), 1593 (C=N), 1336, 1149 (SO_2), 1228 (C=S), 1089 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 2.30 (s, 3H, CH_3), 7.25 (m, 4H, Ar-H), 7.84 (m, 4H, Ar-H), 8.34 (d, *J*=19.1 Hz, 2H, pyrazine protons), 10.08 (d, *J*=16.0 Hz, 2H, NH), 11.91 (s, 1H, – SO_2NH). Anal. Calcd for $C_{18}H_{16}ClN_5O_2S_2$: C 49.82, H 3.72, N 16.14. Found: C 49.99, H 3.71, N 16.19 %.

N-(6-Chloropyrazin-2-yl)-4-[3-(4-methoxyphenyl)thioureido] benzenesulfonamide (**2f**)

Yield: 82%; m.p. 166.3-166.7 °C. IR (n, cm^{-1}): 3211 (N-H), 3007 (aromatic =C-H), 1583 (C=N), 1336, 1151 (SO_2), 1236 (C=S), 1089 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 3.77 (s, 3H, OCH_3), 6.96 (d, *J*=8.8 Hz, 2H, Ar-H), 7.37 (d, *J*=8.8 Hz, 2H, Ar-H), 7.83 (m, 4H, Ar-H), 8.30 (d, *J*=19.1 Hz, 2H, pyrazine protons), 10.00 (d, *J*=25.8 Hz, 2H, NH), 11.91 (s, 1H, – SO_2NH). Anal. Calcd for $C_{18}H_{16}ClN_5O_3S_2$: C 48.05, H 3.58, N 15.57. Found: C 48.19, H 3.60, N 15.49 %.

N-(6-Chloropyrazin-2-yl)-4-(3-(4-ethylphenyl)thioureido) benzenesulfonamide (**2g**)

Yield: 75%; m.p. 183.9-184.4 °C. IR (n, cm^{-1}): 3205 (N-H), 3022 (aromatic =C-H), 2962 (C-H), 1583 (C=N), 1338, 1138 (SO_2), 1230 (C=S), 1087 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 1.13 (t, 3H, CH_2CH_3), 2.43 (q, 2H, CH_2CH_3), 7.26 (m, 4H, Ar-H), 7.77 (m, 2H, Ar-H), 7.88 (m, 2H, Ar-H), 8.31 (d, *J*=19.5, 2H, pyrazine protons), 10.09 (d,

J=18.6 Hz, 2H, NH), 11.90 (s, 1H, – SO_2NH). Anal. Calcd for $C_{19}H_{18}ClN_5O_2S_2$: C 50.94, H 4.05, N 15.63. Found: C 51.08, H 4.02, N 15.66 %.

N-(6-Chloropyrazin-2-yl)-4-[3-(2,6-dichlorophenyl) thioureido]benzenesulfonamide (**2h**)

Yield: 73%; m.p. 148.3-148.6 °C. IR (n, cm^{-1}): 3211 (N-H), 3010 (aromatic =C-H), 1585 (C=N), 1336, 1149 (SO_2), 1232 (C=S), 1084 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.37 (m, 1H, Ar-H), 7.60 (t, 2H, Ar-H), 7.91 (m, 4H, Ar-H), 8.31 (d, *J*=20.2 Hz, 2H, pyrazine protons), 10.44 (d, *J*=25.8 Hz, 2H, NH), 11.94 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{12}Cl_3N_5O_2S_2$: C 41.77, H 2.47, N 14.33. Found: C 41.88, H 2.48, N 14.70 %.

2.2.Biological Activity

The all experiments were carried out in triplicate. The DMSO was used as a negative control to follow the reaction. The bleaching rate was calculated from the absorbance's differences versus time. The sample concentration providing 50% inhibition activity (IC_{50}) for other all assays while 0.5 absorbance ($A_{0.5}$) for CUPRAC assay were calculated from the graph of bleaching rate (%) against sample concentrations. All biological activity measurements were using a 96-well microplate reader (SpectraMax 340PC^{384}, Molecular Devices, USA).

In vitro Antioxidant Activity

The antioxidant activity of thiourea derivatives **2a-2h** was determined using four complimentary assays, namely, β-carotene bleaching method, DPPH free radical scavenging activity, ABTS cation radical scavenging activity, and cupric reducing antioxidant capacity (CUPRAC). The αlpha-tocopherol (α-TOC) and butylatedhydroxytoluene (BHT) were used as standard to compare the activity.

Determination of the lipid peroxidation inhibitory activity of the thiourea derivatives

The lipid peroxidation inhibitory activity was evaluated using the β-carotene-linoleic acid model assay system (15) with slight changes. The method is based on the occurrence of lipid peroxidation from linoleic acid in singlet oxygen saturated water. Then the radical degrades the color of b-carotene followed using 470 nm wavelength. The more color exhibits more powerful lipid peroxidation inhibitor capacity. Briefly, the reactive was prepared by mixing β-carotene (0.5 mg) in 1 mL of chloroform was added to 25 µL of linoleic acid, and 200 mg of Tween 40 emulsifier in a bottle. After chloroform evaporated, the mixture was dissolved using 100 mL pure water saturated with singlet oxygen. Briefly, 160 mL of prepared reactive was mixed with 40 mL of thiourea derivatives **2a-2h** dissolved in DMSO at different concentrations. The zero-time absorbance was measured at 470 nm, and the measurement was done in every 30 minutes up to the absorbance of control reduces under 0.1 absorbance in 96 well plate cell length. The measurement took almost 2 hours.

Determination of DPPH free radical scavenging activity of the thiourea derivatives

The DPPH free radical scavenging activity was performed according to Blois (16), with slight modifications. Briefly, 160 mL of 0.004% of DPPH in ethanol was mixed with 40 mL of thiourea derivatives **2a-2h** dissolved in ethanol at different concentrations. The absorbance was measured at 517 nm after 30 min incubation in dark.

Determination of ABTS cation radical scavenging activity of the thiourea derivatives

The ABTS cation radical scavenging activity was performed according to Re et al. (17), with slight modifications (18). The ABTS cation radical was prepared using the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, and then stored in the dark at room temperature for 12 h. Before usage, the occured ABTS cation radical solution was diluted 1:88 ration with ethanol to get 0.700 ± 0.025 absorbance at 734 nm. Then briefly, 160 mL of prepared reactive was mixed with 40 mL of thiourea derivatives **2a-2h** dissolved in DMSO at different concentrations. The absorbance was measured at 734 nm after 10 min incubation.

Determination of Cupric reducing antioxidant capacity of the thiourea derivatives

Cupric reducing antioxidant capacity (CUPRAC) assay was performed according to Apak et al. (19). Briefly, 50 µL 10 mM Cu (II), 50 µL 7.5 mM neocuproine, and 60 µL NH_4Ac buffer (1 M, pH 7.0) were added to each well in a 96 well plate. To the mixture, 40 µL of thiourea derivatives **2a-2h** at various concentrations were added. After 1 h, incubation absorbance was recorded at 450 nm.

In vitro Enzyme Inhibitory Activities

Galantamine for anticholinesterase, kojic acid and L-mimosine for tyrosinase, acarbose for α-amylase and α-glucosidase were used as positive standard to compare the inhibitory activity.

Determination of anticholinesterase activity of the thiourea derivatives

The acetylcholinesterase, (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg), enzyme obtained from electric eel, was bought ready-made from Sigma aldrich by the suppliers in Turkey. Horse reddish butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg) were used to determine the anticholinesterase activity of DMSO thiourea derivatives **2a-2h** where acetylthiocholine iodide and butyryl-thiocholine chloride were employed as substrates using spectroscopic method (20). Briefly, 130 mL sodium phosphate buffer (100 mM, pH 8.0), 10 mL thiourea

derivatives **2a-2h** at different concentrations, and 20 mL AChE or BChE enzymes in buffer were mixed. After incubation for 15 min at 25 °C, 20 mL 0.5 mM DTNB (5,50-dithiobis (2-nitrobenzoic acid) and 20 mL acetylthiocholine iodide (0.71 mM) or butyryl-thiocholine chloride (0.2 mM) were added. Then the absorbance was measured at 412 nm.

Determination of tyrosinase inhibitory activity of the thiourea derivatives

The mushroom tyrosinase (EC 232-653-4, 250 KU) was used to determine the inhibitory activity of the thiourea derivatives **2a-2h** where L-DOPA was substrate according to DOPAchrome method (21) with slight modifications. Briefly, 150 µL of 50 mM sodium phosphate buffer (pH 6.8), 10 µL of thiourea derivatives **2a-2h** in DMSO, 20 µL of tyrosinase enzyme solution (13.3 U/well) were added in a 96-well plate. After 10 min incubation at 37 °C, L-DOPA (0.5 mM) was added to start the enzymatic reaction. The enzymatic reaction formation of DOPA chrome was monitored using 475 nm wavelength at 37 °C for 10 min.

Determination of a-amylase inhibitory activity of the thiourea derivatives

a-Amylase inhibitory activity of the thiourea derivatives **2a-2h** was tested by using the spectroscopic method with slight changes Quan et al. (22). Briefly, 25 µL sample solution in different concentrations and 50 µL a-amylase solution (0.1 U/mL) in phosphate buffer (20 mM pH=6.9 phosphate buffer prepared with 6 mM NaCl) were mixed in a 96-well microplate. The mixture was pre-incubated for 10 min at 37 °C. After pre-incubation, 50 µL starch solution (0.05 %) was added and incubated for more 10 min at 37 °C. The reaction was stopped by addition of 25 µL HCl (0.1 M) and then 100 µL Lugol solutions were added for monitoring. 96-well microplate reader was used to measure absorbance at 565 nm.

Determination of a-glucosidase inhibitory activity of the thiourea derivatives

a-Glucosidase inhibitory activity of the thiourea derivatives **2a-2h** was determined using the spectroscopic method with slight modifications (23). Briefly, 50 µL phosphate buffer (10 mM pH=6.9), 25 µL PNPG (*p*-nitrophenyl-*a*-*D*-glucopyranoside) in phosphate buffer (10 mM pH=6.9), 10 µL sample solution and 25 µL a-glucosidase (0.1 U/mL) in phosphate buffer (10 mM pH=6.0) were mixed in a 96-well microplate. After 20 minutes incubation at 37 °C, 90 µL sodium carbonate (100 mM) was added into the each well to stop the enzymatic reaction. Absorbance of the 96-well microplate reader was recorded at 400 nm.

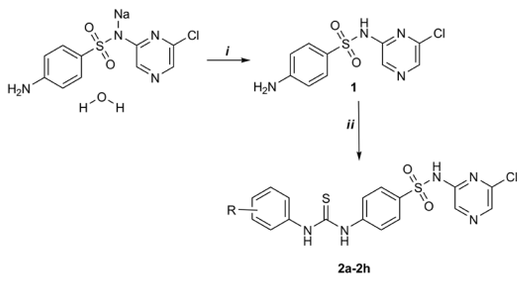
***In Silico* Prediction of Druglikeness**

The druglikeness properties such as Lipinski and Veber rules were calculated by using Swissadme online server (http://www.swiss adme.ch/).

3. RESULTS

3.1. Chemistry

In this study, novel thiourea derivatives were synthesized as indicated in Figure 1. Firstly, sulfaclozine was prepared by washing sulfaclozine sodium monohydrate with diluted hydrochloride acid (5%). Then, new thiourea derivatives were obtained by heating sulfaclozine with the same molar ratio of substituted isothiocyanate in the presence of anhydrous acetone. The confirmation of all thioureas was carried out by IR, ^{1}H-NMR spectroscopic methods and elemental analysis.



Comp.	R
2a	4-H
2b	4-F
2c	4-Cl
2d	4-Br
2e	4-CH_3
2f	4-OCH_3
2g	4-C_2H_5
2h	2,6-diCl_2

Figure 1. *The synthetic route of thiourea derivatives. Reagents: (i) 5% HCl, water; (ii) substituted phenylisothiocyanate, acetone.*

In the Infrared spectrum, the C=S stretching bands belonging to the thiourea group were detected in the range of 1226-1240 cm^{-1}. The thiourea N-H, aromatic =C-H and pyrazine C=N streching bands were observed in the region of 3194-3255 cm^{-1}, 3007-3043 cm^{-1} and 1583-1599 cm^{-1}, respectively. The asymmetric and symmetric SO_2 streching bands of the sulfonamide structure were assigned in the range of 1323-1338 cm^{-1} and 1138-1157 cm^{-1}, respectively.

In the ^{1}H-NMR spectrum, the observation of NH peaks belonging to the thiourea group with the 2H integral values in the range of 10.00-10.44 ppm supported the synthesis of thiourea groups. In addition, the disappearance of the amine peaks seen at 6.17 ppm was an another important indicator for thiourea synthesis. The aromatic protons in the pyrazine ring were detected in the range of 8.30-8.35 ppm, the other aromatic protons resonated in the range of 6.96-7.91 ppm. The sulfonamide NH peaks appeared at 11.90-11.94 ppm. Elemental analyses (C, H, N) were in accordance within ±0.4% of theoretical values.

3.2. Biological Activity

The antioxidant activity assays results of the synthesized thiourea derivatives were given in Table 1. The activity of the synthesis products was found to exhibit much better activity than the compound used for the synthesis of thiourea derivatives. According to the β-carotene-linoleic acid assay result, it was determined that compounds **2b** and **2e** exhibited best lipid peroxidation inhibition in the tested series. In the DPPH$^{\cdot}$ assay, all the thiourea derivatives exhibited better activity than BHT used as the positive standard of the assay,

while compounds **2a**, **2b**, **2c**, **2e** and **2f** were also found to exhibit tremendous activity than the α-TOC, which is other positive standard of the assay. In the $ABTS^{\cdot+}$ assay, among all the thiourea derivatives, compound **2c** showed greater activity than both standards with an IC_{50} value of 1.08±0.44 µM. In the CUPRAC assay, it was determined that all synthesized thiourea derivatives exhibited more effective activity than a-TOC. The most active compound in the series was found to be **2c** (IC_{50}=7.46±0.02 µM) in the tested activity.

The enzyme inhibition activity assays results of the synthesized thiourea derivatives were given in Table 2. It was determined that the anticholinesterase inhibitory activity of the synthesized thiourea derivatives showed better activity than AChE when the AChE and BChE assay results were compared with the galantamine, which is the standard of the assay, in general. In the AChE assay, compound **2c** showed the best activity within the thiourea series. In the BChE assay, on the other hand, all synthesized compounds were determined to be more active than galantamine. Also, in this assay, the thiourea derivative showed a tremendous activity of **2h** and was found to be approximately 3.5 times more active than galantamine. In the tyrosinase enzyme inhibition activity of the synthesized thiourea derivatives, compounds **2e** and **2f** came to the fore as the derivatives exhibiting the best activity.

In α-amylase and α-glucosidase enzyme inhibition activity, known as antidiabetic assay, compound **2g** is the most active within thiourea derivatives in both assays and competes with the acarbose being standard of the assays.

Table 1. *Antioxidant activity results of compound* **1** *and* **2a-2h**[a]

Compound	β-carotene-linoleic acid assay IC_{50} (µM)	$DPPH^{\cdot}$ assay IC_{50} (µM)	$ABTS^{\cdot+}$ assay IC_{50} (µM)	CUPRAC assay $A_{0.50}$ (µM)
1	>250	>250	>250	130.60±0.03
2a	55.62±0.97	2.25±0.01	26.52±1.84	8.88±0.01
2b	27.03±0.79	9.90±0.39	6.54±0.71	11.29±0.02
2c	34.10±0.95	11.21±1.07	1.08±0.44	7.46±0.02
2d	33.51±0.56	22.94±1.35	9.02±0.58	12.66±0.00
2e	27.84±0.14	10.29±0.69	56.71±0.95	7.73±0.01
2f	56.98±0.09	12.47±0.39	43.49±0.63	11.39±0.00
2g	37.64±0.39	19.54±1.03	12.58±0.24	7.60±0.00
2h	41.44±1.60	46.28±0.69	47.34±1.51	10.73±0.02
α-TOC[b]	4.79±0.14	12.90±0.57	5.14±0.29	40.45±0.01
BHT[b]	2.45±0.29	54.82±0.76	2.80±0.47	3.88±0.03

[a]*Values expressed are the mean ± SEM of three parallel measurements (p<0.05).*
[b]*Reference compounds.*

Table 2. *Enzyme inhibition activities of compounds* **1** *and* **2a-2h**[a]

Compound	Anticholinesterase Activity		Tyrosinase Activity IC_{50} (mM)	α-Amylase Activity IC_{50} (µM)	α-Glucosidase Activity IC_{50} (µM)
	AChE IC_{50} (µM)	BChE IC_{50} (µM)			
1	31.12±1.56	24.57±1.22	NA	>250	132.23±1.73
2a	10.58±0.11	20.03±0.57	14.56±1.72	115.35±0.05	110.66±1.90
2b	11.38±0.89	28.28±1.38	16.03±1.42	90.21±1.51	131.53±1.40
2c	8.58±0.48	21.89±0.27	11.86±0.70	100.41±1.19	119.49±0.73
2d	43.33±0.64	30.65±1.07	19.96±1.46	125.42±0.02	135.59±1.26
2e	13.38±0.50	17.47±1.34	6.71±0.89	110.66±0.79	132.13±1.44
2f	32.37±0.47	33.92±1.00	5.08±0.58	113.94±0.96	124.80±0.59
2g	29.25±0.46	15.37±0.77	15.64±0.78	65.41±0.34	106.06±0.31
2h	25.52±0.78	14.57±1.18	13.79±0.32	130.12±0.37	163.46±0.74
Galantamine[b]	5.07±0.62	48.02±0.21	NT	NT	NT
Kojic acid[b]	NT	NT	0.71±0.54	NT	NT
L-mimosine[b]	NT	NT	0.79±0.09	NT	NT
Acarbose[b]	NT	NT	NT	25.14±0.60	63.95±1.29

[a]*Values expressed are the mean ± SEM of three parallel measurements (p<0.05).*
[b]*Reference compounds. NT: Not tested. NA: Not active.*

Table 3. *Druglikeness properties of the synthesized compounds.*

Code	Lipinski rule of five				Veber rule	
	MW (g/mol)	cLog P	*n*-ON	n-OHNH	*n*-ROTB	TPSA
2a	419.91	0.94	4	3	7	136.48
2b	437.90	1.73	5	3	7	136.48
2c	454.35	1.85	4	3	7	136.48
2d	498.80	1.56	4	3	7	136.48
2e	433.93	1.58	4	3	7	136.48
2f	449.93	1.06	5	3	8	145.71
2g	447.96	1.81	4	3	8	136.48
2h	488.80	1.94	4	3	7	136.48

MW: molecular weight, cLog P: calculated partition coefficient, n-ON: number of hydrogen bond acceptors, n-OHNH: number of hydrogen bond donors, n-ROTB: number of rotatable bonds. TPSA: Topological polar surface area, Calculations were performed using SwissAdme online server (http://www.swissadme.ch).

In Silico Prediction of Druglikeness

To evaluate the druglikeness of a drug is used the Lipinski and Veber rules. A good strategy for predicting oral bioavailability if the compound meets Lipinski and Veber rules. Compounds that do not meet these rules may cause physicochemical and pharmacokinetic problems (24). According to Lipinski, compounds must meet at least three of following rules: (i) molecular weight (MW) ≤ 500 Da, (ii) Partition coefficient values (log P) ≤ 5, (iii) number of hydrogen bond donors (n-OHNH) ≤ 5, number of hydrogen bond acceptors (*n*-ON) ≤ 10 (25). On the other hand, the criteria of Veber rules are rotatable bonds (*n*-ROTB) ≤ 10 and polar surface area (TPSA) ≤ 140 Å^2 (26-28). These results were given in Table 3. The thiourea derivatives comply the Lipinski and Veber rules except compound **2f** (only one violation; TPSA>140).

The pink area in bioavailability radar indicates the optimum range for six physicochemical properties such as polarity, saturation, size, flexibility, lipophilicity and solubility. All physicochemical properties except for saturation of the compounds are in the pink area. The bioavailability radar of compounds **2a**-**2h** was given in Figure 2.

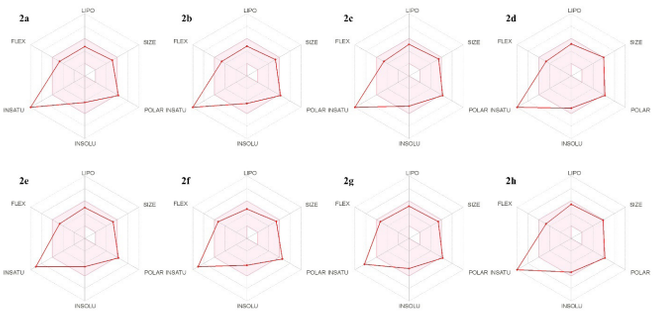


Figure 2. *The bioavailability radar of all compounds (The pink area represents the optimal range for each property. LIPO: Lipophilicity, SIZE: Molecular weight, POLAR: Total Polar Surface Area, INSOLU: Insolubility in water by log S scale, INSATU: Insaturation by carbon fraction in sp3 hybridization, FLEX: Flexibility as per rotatable bonds).*

4. DISCUSSION

In this study, the characteristic C=S bands in the IR spectrums demonstrated the synthesis of thiourea structures successfully. In addition, the disappearance of the free amine peaks and the observation of thiourea peaks with 2H integral value in the ^{1}H-NMR spectrum were other factors that proved the synthesis of thiourea structures.

According to antioxidant activity results, especially in DPPH and APTS$^+$ assays, more effective compounds were found than the reference standard. The evaluation of the anticholinesterase enzyme activity, all compounds showed higher activity than the standard against BChE. Compound **2c** was the most effective derivatives against the AChE enzyme, with an IC_{50} value of 8.58 µM (IC_{50} value for galantamine, 5.07 µM). Against the tyrosinase enzyme, compounds **2e** and **2f** were the most active compounds in this series. Against the enzymes α-amylase and α-glucosidase, which are related to antidiabetic activity, the compound **2g** showed the most promising activity. Therefore, thiourea derivatives exhibited remarkable biological activities in this study.

According to *in silico* prediction, the majority of all compounds did not violate druglikeness properties such as Lipinski and Veber rule. The bioavailability radar, defines a bioavailable drug candidate, showed that compounds are slightly outside the pink area on one side only, due to the inconformity of saturation. On the other hand, all thiourea derivatives have bioavailability score of 0.55, which means good pharmacokinetic properties.

5. CONCLUSION

As a result of the development of resistance to many drugs used in the clinic after a long period or the limited use of drugs due to various side effects, the need for the synthesis of more effective and selective drugs is increasing day by day. Therefore we synthesized some novel thiourea derivatives based on sulfaclozine and screened their antioxidant activity and enzyme inhibitory activity against cholinesterase (AChE and BChE), tyrosinase, α-amylase and α-glucosidase. The antioxidant activity results showed that compounds **2b** and **2c** may be candidate compounds for the elimination of free radicals in the body due to various reasons. The anticholinesterase activity results indicated that all compounds exhibited higher BChE inihibitory activity than galantamine. Compounds **2e** and **2f**, which exhibited the best activity in tyrosinase inhibition activity, remarked among the synthesis derivatives as potential compounds in the treatment of skin diseases related to melanin biosynthesis. The results of α-amylase and α-glycosidase enzyme inhibition activities also showed that thiourea derivatives could be a lead compound for antidiabetic activity.

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The authors declare that they have no competing financial interest

Conflicts of interest

The authors declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

Effect of Pruritus on Sleep Quality in Individuals Undergoing Hemodialysis Effect of Pruritus on Sleep Quality

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ABSTRACT

Objective: This study was conducted to evaluate uremic pruritus levels and sleep quality in individuals undergoing hemodialysis treatment and to determine the relationship between variables.

Methods: This descriptive study was conducted with 219 patients who underwent hemodialysis in State Hospital, University Hospital hemodialysis units and a private dialysis center and met the study criteria. Research data were collected using the Introductory Information Form, 5-D Pruritus Scale and Pittsburg Sleep Quality Index. In the analysis of the data, Kruskal-Wallis T test, Perason correlation analysis were used.

Results: In the study, the 5-D Pruritus Scale mean score of the patients was 12.70±3.35 points, and the Pittsburg Sleep Quality Index score was 12.82±2.42. A positive, moderately significant (r=.509, p<0.05) relationship was found between the mean scores of the two scales. It was determined that patients with unbearable itching, who defined itching prognosis as bad, stated that itching affected their daily activities and experienced widespread itching had worse sleep quality (p<0.05).

Conclusions: Patients undergoing hemodialysis experience moderate itching and their sleep quality is generally poor. However, there is a moderate relationship between itching and sleep quality.

Keywords: Hemodialysis, Pruritus, Sleep Quality

1. INTRODUCTION

Chronic Kidney Failure is a serious health problem that is common in the world and in Turkey due to its changing etiology, complex treatments and high economic costs (1). It is thought that approximately 100 million people in the world are affected by this disease, in other words, one out of every six adults has Chronic Kidney Failure (CKF) (2).

The problems experienced by CKF patients are generally seen as either the signs and symptoms of the disease that causes kidney failure or the signs and symptoms of the affected system. Fluid-electrolyte imbalances, nausea, vomiting, anemia, nutritional problems, pulmonary edema, signs and symptoms of cardiovascular diseases and uremic skin changes are frequently observed in patients. Skin problems related to CKF are xerosis, nail changes, seborrheic keratosis, pigmentation changes and uremic pruritus (3,4).

It is stated that the prevalence of itching associated with Chronic Kidney Failure in the world is between 30% and 50% (4,5). In a study conducted with peritoneal dialysis (PD) and hemodialysis (HD) patients, it was determined that 62.6% of PD patients and 48.3% of HD patients experienced uremic pruritus (6). In the study conducted by Du et al. (2016) using the Visual Analog Scale (VAS) to question itching in patients with end-stage kidney failure 78% of the patients stated that they experienced itching (7). In a study conducted with 194 patients based on two hemodialysis units in Turkey, this rate was found to be 54.1% (8).

Although uremic pruritus is not a life-threatening problem itself, it affects the quality of life and daily life activities of the patients, causes anxiety, depression, physical and mental fatigue and negatively changes the patient's adaptation to the disease (9,10,11). One of the important daily activities affected by itching is sleep. Sleep is one of the indispensable activities in human life, which is among the basic needs in nursing theories and is considered an important component of health that affects the quality of life and well-being of the individual (12). In a study conducted in Japan with 18.801 HD patients, the rate of uremic pruritus was found to be 42%, and it was reported that itching affected sleep quality and quality of life (13). In the study conducted by Akyol et al. with CKF patients, the rate of itching among the factors that prevent falling asleep was found to be 43.9% (1).

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Although uremic pruritus is an irritating symptom for patients with kidney failure, studies on its general definition and its effect on patients are limited (9,14,15). When these studies in the literature are evaluated, it is seen that uremic pruritus is often evaluated using one-dimensional scales, visual/numerical scales or questionnaire questions compiled by researchers from the literature. In this context, it is thought that the dimension of questioning the effects of itching on the individual is insufficient in studies. In this study, a comprehensive scale was used to question the itching levels of the patients in terms of duration, degree, direction, limitation of activities and distribution to body parts. The questions generally focus on the itching dimensions of the last two weeks and one month, avoiding the question of instant itching. In this context, it is thought that the planned study is important in terms of investigating the effects of itching symptoms in individuals with CKF in detail and examining its effect on sleep quality, which is an important daily life activity.

2. METHODS

2.1. Study Design and Sample

The study was designed as a descriptive study. The universe of the study consisted of 250 individuals who received hemodialysis treatment in the hemodialysis units of the University and State Hospital and in Private Dialysis Center. However, 12 individuals out of 250 did not want to participate in the study voluntarily. Moreover, 31 individuals who did not meet the inclusion criteria were not included in the study and the study was conducted with 219 individuals. The criteria for inclusion in the study were being on hemodialysis treatment for one year, being 18 years of age or older, and having no cognitive impairment or communication obstacle. In the study, it was aimed to reach all individuals who are receiving hemodialysis treatment in Sivas center and comply with the criteria, but the mentioned institutions gave permission to our study.

2.2. Data Collection Tools

Personel Information Form: This form, prepared by the researcher, consists of 14 questions questioning the sociodemographic characteristics of the patients and their knowledge about the disease.

5-D Pruritus Scale: The scale was developed by Elman et al. in 2010 (16). The scale consists of 5 dimensions and 8 variables: the duration of the itch, its degree, its direction, its effect on daily daily activities, and its distribution on the body. The total score of the scale ranges from a minimum of 5 points (no itching) to a maximum of 25 points (itching at the highest degree). The validity and reliability study of the scale for Turkey was conducted by Altınok Ersoy (2017) (10). In this study, the Cronbach's Alpha coefficient of the scale was determined as 0.806.

Pittsburgh Sleep Quality Index (PSQI): PSQI, developed by Buysse et al. in 1989, is a scale that provides information about sleep quality and the type and degree of sleep disorder in the last month (17). In the scale consisting of 24 questions in total, 19 questions are answered by the person, while 5 questions are filled by the person's bedmate. With 19 questions answered by the individual, 7 sub-dimensions are evaluated: subjective sleep quality, sleep latency, sleep duration, habitual sleep activity, sleep disturbance, usage of sleeping pills, and daytime dysfunction. Each item in the scale takes a value between 0 (no distress) and 3 (serious distress) points. The sum of the scores for the seven sub-dimensions gives the total PSQI score. The score of each sub-dimension ranges from 0 to 3. The total PSQI score varies between 0 and 21. The sleep quality of those with a total score of 5 or less is considered "good", and those with more than 5 points are evaluated as "bad". The Turkish validity and reliability study of the scale was conducted by Agargün et al. (1996) (18). In this study, the Cronbach's Alpha coefficient of the scale was determined as 0.732.

2.3. Procedure

Patients who met the sampling criteria in the dialysis unit and center where the study was conducted were informed about the study and the patients who accepted to participate in the study were evaluated. The data on the individuals were collected in a suitable interview room in the specified hemodialysis units, after filling the informed consent form of the individuals, and by making face-to-face interviews in the form of questions and answers. The average survey application time took between 10-15 minutes.

2.4. Ethical Considerations

This study was conducted in compliance with the principles of the Declaration of Helsinki. The permission for the study was received from the Chief Physician's Office at a University Research and Training Hospital, and approval was obtained from the Clinical Research Ethics Board at University (Decision no. 2019-04/31). Additionally, by complying with the principle of voluntary participation, written consent was obtained from the patients who were included in the study. Written permission was obtained from all institutions where the study would be conducted.

2.5. Data Analysis

The data were analyzed by using the SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). In the statistical analysis of the study, the mean value was taken, the Kolmogorov-Smirnov Z test was used to test the normal distribution of the scale means and the Kruskal-Wallis test was applied. To determine the relationship between itching and sleep quality, Pearson's correlation analysis was utilized. The statistically significant level was accepted as $p<0.05$.

3. RESULTS

3.1. Patients' characteristics

It was determined that of the individuals undergoing hemodialysis treatment, 52.1% were male, 60.7% were over 61 years old, 75.3% were married, 62.6% were primary school graduates, 47.9% were housewives, 39.3% were

retired, almost all (96.8%) did not work. When the clinical characteristics of the individuals undergoing hemodialysis treatment were examined, it was determined that 58% of the individuals had dialysis treatment for 1-5 years, 25.6% for 6-10 years, 16.4% for more than 10 years and 98.6% of them had hemodialysis 3 times a week.

3.2. Patients' itching Levels

In Table 1, which includes the 5-D Itch Pruritus total score and its components, 37.4% of the individuals stated that they experienced itching for 6-12 hours a day, nearly half (45.7%) had moderate itching, 50.2% months their itching was better compared to the last two, but still persisted, and 50.7% itching occasionally affected their life activities. More than half of the individuals (75.8%) stated that they had itching in the 0-5 area in the evaluation to determine the distribution of itching. The mean score of the 5-D scale was determined as 12.70±3.35.

Table 1. *5-D Pruritus Scale total score average and distribution of components of the ındividuals treated with hemodialysis (n:219)*

Components of the 5-D Itch Scale	n	%	Mean ±sd (Min-max)
Duration (past 2 week)			
Less than 6 h/day (1 point)	64	29.2	2.26±1.19 (1-5)
6–12 h/day (2 point)	82	37.4	
12–18 h/day (3 point)	42	19.2	
18-24 h/day (4 point)	12	5.5	
All day (5 point)	19	8.7	
Degree (past 2 week)			
Mild (2 point)	60	27.3	3.09±0.90 (2-5)
Moderate (3 point)	100	45.7	
Severe (4 point)	38	17.4	
Unbearable (5 point)	21	9.6	
Direction (last 2 weeks compared to last month)			
Completely resolved (1 point)	20	9.1	2.40±0.79 (1-5)
Much better, but still present (2 point)	110	50.2	
Little bit better, but still present (3 point)	72	32.9	
Unchanged (4 point)	14	6.4	
Getting worse (5 point)	3	1.4	
Disability			
Didn't affect at all (1 point)	7	3.2	2.61±0.66 (1-4)
Rarely affected (2 points)	86	39.3	
Occasionally affected (3 points)	111	50.7	
Frequently affected (4 points)	15	6.8	
Always impressed (5 points)	0	0.0	
Distribution			
0-5 part (1\2 point)	166	75.8	2.40±0.84 (2-5)
6-10 part (3 point)	33	15.1	
11-13 part (4 point)	4	1.8	
14-16 part (5 p point)	16	7.3	
5-D Itch Scale total score			12.70±3.35 (6-25)

3.3. Patients' itching Levels

PSQI mean scores of individuals who underwent hemodialysis treatment were given in Table 2. When the table is examined, it is seen that the mean PSQI scale sub-dimension mean scores were respectively sleep quality was 2.13±.59, sleep latency 2.82±.47, sleep latency 2.63±.64, habitual sleep activity 2.30±.92, sleep disturbance 1.61±.52, sleeping pill usage 0.41±.91, and daytime dysfunction 1.05±.58. PSQI total score average was determined as 12.82±2.42.

Table 2. *Distribution of PSQI scores of the individuals treated with hemodialysis (n:219)*

Components of PSQI	Mean± Sd	Minimum	Maximum
Sleep quality	2.13±.59	0	3
Sleep latency	2.82±.47	0	3
Sleep duration	2.63±.64	0	3
Habitual sleep efficiency	2.30±.92	0	3
Sleep disturbance	1.61±.52	1	3
Use of sleeping medication	0.41±.91	0	3
Daytime dysfunction	1.05±.58	0	3
PSQI total score	12.82±2.42	3	19

PSQI, Pittsburgh Sleep Quality Index

3.4. Correlation Between Itching and Sleep Levels of Patients

The correlation between the 5-D Pruritus Scale and the PSQI score averages of the individuals undergoing hemodialysis treatment was given in Table 3. When the table was examined, a positive, moderate, significant correlation was found between the Pruritus Scale scores and subjective sleep quality (r=0.526, p=0.001), usage of sleeping pill (r=0.530, p=0.001), daytime dysfunction (r=0.590, p=0.005) and PSQI total scores (r=0.509, p=0.002).

Table 3. *Correlation of 5-D Pruritus Scale and PSQI scores of individuals treated with hemodialysis*

Components of PSQI	5-D Itch Scale	
	r	p
Sleep quality	0,526	0,001
Sleep latency	0,039	0,569
Sleep duration	0,011	0,874
Habitual sleep efficiency	0,003	0,963
Sleep disturbance	0,024	0,719
Use of sleeping medication	0,530	0,001
Daytime dysfunction	0,590	0,005
PSQI total score	0,509	0,002

PSQI, Pittsburgh Sleep Quality Index

The comparison of 5-D Pruritus Scale components and the average PSQI total score of individuals who underwent hemodialysis treatment was given in Table 4. According to the table, it is seen that the sleep quality of the patients who described the itching degree as unbearable, who defined the itching prognosis as worse compared to the previous month, and who described generalized itching in the body parts in general, were significantly worse than the PSQI. Furthermore,

it was determined that individuals who stated that itching did not affect their daily activities had significantly higher sleep quality.

Table 4. *Comparison of 5-D Pruritus Scale components and PSQI total scores of individuals treated with hemodialysis*

Components of the 5-D Itch Scale	PSQI		Test
Duration(past 2 week)	**Ort**	**Sd**	
Less than 6 h a day	12.39	2.59	KW:3.488
6–12 h/day	13.03	2.03	P:0.480
12–18 h/day	12.83	2.36	
18-24 h/day	12.33	3.14	
All day	13.57	2.91	
Degree (past 2 week)			
Mild	12.53	1.76	KW:13.879
Moderate	12.61	2.31	**P:0.003**
Severe	12.81	3.13	
Unbearable	14.61*	2.47	
Direction (last 2 weeks compared to last month)			
Completely resolved	11.80	3.50	KW:10.509
Much better, but still present	12.07	2.00	**P:0.033**
Little bit better, but still present	12.85	2.37	
Unchanged	13.02	3.04	
Getting worse	16.66*	2.08	
Disability			
Didn't affect at all	9.00*	3.51	KW:12.018
Rarely affected	12.67	2.24	**P:0.007**
Occasionally affected	13.11	2.23	
Frequently affected	13.20	2.83	
Distribution			
0-5 part	12.48	2.26	KW:8.126
6-10 part	12.69	2.84	**P:0.043**
11-13 part	14.00	2.94	
14-16 part	14.43*	2.52	

** Different group*
PSQI, Pittsburgh Sleep Quality Index

4. DISCUSSION

4.1. Discussion of 5-D Pruritus Scale and PSQI Scores in Individuals Undergoing Hemodialysis

CKF is a serious health problem with increasing frequency all over the world, and it significantly affects the quality of life and daily life activities due to the symptoms it causes. Among these symptoms, itching is accepted as a symptom that should be evaluated in detail.

In this study using the 5-D Pruritus Scale, the mean scale score of the patients was determined as 12.70±3.35 (min=5, max=25). According to the result obtained in this context, it can be said that the individuals in the study sample experienced moderate itching. In the study where Ersoy and Akyar (2017) made an evaluation with the same scale, the mean value of the scale was determined as 13.97±4.11 (10). The result of this study conducted in Turkey was close to the finding we obtained. In the study of Güder et al. (2012) in which skin changes were examined with 100 hemodialysis patients, it was found that the rate of uremic pruritus was 49% (19). Du et al. (2016), in their study using the Visual Analog Scale (VAS) to question itching in ESKF patients, stated that 78% of the patients experienced itching (7). In the study conducted by Ko et al. (2014) with 173 hemodialysis patients, this rate was found to be 34.8% (20). In another study involving individuals who received hemodialysis and peritoneal dialysis, the itching rate of HD patients was found to be 62% (6). When the studies in Turkey and the world literature are evaluated (5,9,10), the rates are different, but itching is a common symptom that should be at the basis of nursing care because of the feeling of discomfort, especially in patients with CKF and dialysis treatment. It can be said that besides determining this symptom only as a rate, the evaluation of its dimensions is also important in terms of symptom management.

In the study, the duration of itching was evaluated and it was determined that 37.4% of the patients in the sample experienced itching for 6-12 hours a day. In the sample group in which the validity and reliability study of the 5-D Pruritus Scale was conducted for Turkey, the rate of patients experiencing itching for 6-12 hours a day was found to be 40.3% (10). In both studies, it is observed that nearly half of the sample experienced itching for 6-12 hours. Considering that patients experienced itching in almost half of their daily life, it can be said that this symptom negatively affected the daily activities of individuals.

In the study, almost half of the patients (45.7%) described the degree of itching as moderate, and 27.0% as severe and unbearable. In the study of Ersoy and Akyar (2017), the rate of patients experiencing moderate itching was 40.3%, and the rate of patients experiencing severe and unbearable itching was 29.3% (10). In another study conducted in Turkey, the rate of moderate and severe uremic pruritus was reported at a rate of 42% (21). In different studies conducted with hemodialysis patients outside Turkey, the rate of patients experiencing severe itching was found to be 37.7% and 29% (22,23). When the findings are evaluated, it is seen that the patients generally experienced moderate and severe itching. This finding again emphasized the degree of itching in patients and showed that it should be focused on itching by healthcare personnel and caregivers. In the study, itching degree mean score was determined as 3.09±0.90 (min=2, max=5). In a study conducted by Shavit et al. (2012) on twelve patients with uremic pruritus, the itching level of the patients was evaluated with VAS and the value was found to be 9.7±0.9 (min=0, max=10) (24). In the study comparing three different dialysis methods, it was found that the itching rate seen in HD patients was 24%, while it was found that the itching rate in HD patients increased in the following sessions (15). When these rates were evaluated, it was determined that an irritating symptom such as itching was experienced intensely both in duration and in degree. When the findings are evaluated, it becomes more important to evaluate this symptom in more detail in patients and to plan and implement

nursing interventions that offer a holistic approach to how to cope with itching and adaptation to this symptom.

In the study, half of the patients (50.7%) stated that itching occasionally affected their life activities. When the findings regarding the duration and degree of itching are evaluated, it is inevitable that the symptom will affect daily activities. In similar studies, it was determined that dermatological symptoms such as itching affect daily activities, school/work life and personal relationships negatively (21,25). Itching is an irritating situation. It is thought that when it is felt continuously and at a certain level during the day, it may affect the daily performance, social life and work/school environment of the person. Although the findings obtained determined the presence of itching, it shows that a comprehensive evaluation with its various dimensions is important. It is thought that this study conducted with this feature will contribute to other studies on uremic pruritus in hemodialysis patients.

In addition to the psychological changes observed in patients, with problems caused by additional diseases, physiological changes, and complications after dialysis, sleep disorders occur in patients. In our study, the mean PSQI score was 12.82±2.42, and it was concluded that there were serious disturbances in sleep quality in patients who had hemodialysis. In the study of Indrarini et al. (2019), the sleep quality of patients with ESKF was found to be poor in 94.3% of the patients (26). Shen and He (2015) found this rate as 69.1% (27). In the study of Sert et al. (2015), poor sleep quality was found at 81.5% in individuals who underwent hemodialysis, and it was found that sleep disorders negatively affected the quality of life by reducing self-care power (28). Sleep is an important daily life activity and affects an individual's quality of life and well-being. It is reported that changes in sleep structure such as periodic leg movements during sleep, sudden awakenings, pain, dietary restrictions, fatigue, dyspnea, cramp and obstructive sleep apnea (OSA) can be observed in dialysis patients and these conditions will affect the sleep process (29). However, it is also accepted that patients with ESKF tend to sleep due to abnormal cellular interleukin production, and hemodialysis causes sleep problems by eliminating these substances that cause sleep (1).

In a study conducted by Elder et al. (2008) with the inclusion of seven countries, the rate of sleep disturbance in hemodialysis patients was determined to be 49%, and it was stated that the rate of morbidity increased with the increase in the rate of sleep disturbance (30). In the study of Ricardo et al. (2017), it was found that low GFR values are associated with sleep disorders (31). When the results obtained from the study and the literature findings are evaluated, it can be said that the sleep quality of the patients who underwent hemodialysis was negatively affected by the disease and the treatment process. Sleep is an important daily life activity within the scope of nursing service and affects the illness process and well-being of the patients. Therefore, it is important to identify sleep disorders and their causes and to plan nursing interventions to increase sleep quality in the specified patient group.

4.2. Discussion of Findings Regarding the Relationship Between Itching and Sleep Quality in Individuals Undergoing Hemodialysis

Although itching is an important problem on its own, it causes negative experiences on the patient with its effect on other systems. The most common problem is the change in sleep patterns that patients complain of itching (1,13,14). In the study, a positive, moderate, and significant relationship was found between the 5-D Pruritus Scale score and PSQI total score and the components of subjective sleep quality, sleeping medication, and daytime dysfunction. In a study conducted by Akyol et al. (2017) with 198 hemodialysis patients, factors affecting sleep were asked and itching was found to be at a rate of 46% (1). In the study of Ko et al. (2014), the rate of uremic pruritus was found to be 34.8%, and it was concluded that hemodialysis patients with sleep disorders had higher pruritus VAS scores (20). In a study conducted with 18.801 hemodialysis patients in Japan, the rate of uremic pruritus was found to be 42%, and it was reported that itching affects sleep quality and quality of life and increases the risk of depression (13). Min et al. (2016) found the rate of itching as 45% in patients who underwent hemodialysis and peritoneal dialysis, and found that the sleep time of the patients experiencing itching decreased and they woke up frequently (6). Similarly, in the study of Ersoy and Akyar (2017), it was determined that itching delayed falling asleep by 50.8% and caused occasional awakenings from sleep (10). In general, it is reported that the rate of sleep disturbance due to itching associated with CKF varies between 28% and 90% (14,32).

5. CONCLUSION

In this study, which was conducted to determine the effect of itching on sleep quality in individuals undergoing hemodialysis treatment, the mean score of the 5-D Pruritus Scale was 12.70±3.35 and the total PSQI mean score was 12.70±3.35. In the study, a positive, moderate, significant relationship was found between the 5-D Pruritus Scale and subjective sleep quality, usage of sleeping pill, daytime dysfunction and PSQI Scale. Furthermore, as a result of the comparison of the 5-D Pruritus Scale and PSQI mean scores, it was determined that the sleep quality of the patients who described the itching degree as unbearable, defined the itching prognosis as worse compared to the previous month, and who described generalized itching in their body parts was significantly worse than the PSQI.

In line with these results, comprehensive and routine evaluation of itching in individuals undergoing hemodialysis treatment, close monitoring of sleep, determination of other factors affecting sleep, planning interventional studies to reduce itching and improve sleep quality in individuals undergoing hemodialysis treatment, by taking a holistic approach to patients, to continue the necessary training and

counseling to patients and their relatives in order to alleviate all their symptoms can be recommended.

Limitations

CKF is a disease that has high mortality and morbidity rates and a condition where the desired goals in treatment cannot be reached despite the developments in treatment methods. The findings of this study are valuable in terms of discussing sleep quality in CKF and influential factors such as pruritis. However, there were some limitations in the study. Firstly, the study was carried out with relatively small samples and it is an important limitation that its results may only be generalised for its of population. Secondly, a cross-sectional study design is limited in establishing a causal association between sleep quality and pruritis. In order to be able to show causality, future longitudinal prospective studies are needed.

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Clinical and Experimental Health Sciences

The Role of Omega-3 and Antioxidant Nutrients in Age-Related Macular Degeneration: A Review Article

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ABSTRACT

Abstract Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss worldwide. The cause of the disease is not well explained; studies previse a multifactorial etiology. Various results of studies suggest that omega-3 fatty acids may have beneficial effects in AMD. Besides the omega-3, clinical evidence showed that specific micronutrients (antioxidant vitamins and minerals) protect against AMD. The definition of risk factors for the development and progression of AMD is important for understanding the causes of the disorder and for the determination of its prevention strategies. In this study, the relationship between omega 3 and antioxidant nutrients and the incidence and progression of AMD were evaluated.

Keywords age-related macular degeneration, omega 3 fatty acids, nutrition, dietary supplementations

1. INTRODUCTION

Age-related macular degeneration (AMD) is one of the major causes of irreversible vision loss in the USA, Europe, and other developed countries (1, 2). Approximately 11 million people in the USA and 170 million people worldwide are reported to be diagnosed with AMD, which is expected to reach 22 million in the USA in 2050 and 288 million in the world. AMD prevalence increases with age, and genetic predisposition and environmental factors affect the occurrence of AMD (3-5).

AMD is defined by the existence of specific changes in the macula, notably the deposition of focal yellow extracellular deposits entitled drusen (6). The classification of AMD is divided into dry type (known as nonexudative or non-neovascular) and wet type (known as exudative or neovascular). Dry AMD refers to the presence of drusen in the disease and atrophic variation of dry AMD, where geographic atrophy is prevalent (7). Wet AMD is characterized by the presence of neovascularization within the macula. The developing neovascularization results in hemorrhage and leakage of fluid into the internal retinal layers or subretinal space (8).

Generally, the visual loss in dry AMD develops in years while wet AMD progresses more rapidly. Dry AMD accounts for about 90% of all AMD cases. However, over time exudative AMD type of development is observed in 10-20% of patients. Wet AMD is the type that is observed in quick vision loss (9, 10).

AMD treatment includes medication, smoking cessation, blood pressure control, combined medication, photodynamic therapy, thermal laser photocoagulation, radiation therapy, surgery, and nutrition (11). Laboratory and animal studies indicate that particular micronutrients may have a beneficial effect in filtering short-wavelength light, reducing oxidative stress, inflammation damage, apoptosis, and angiogenesis in the eye. However, clinical and epidemiological studies, which are involved in both interventional and observational studies for dietary nutrients and supplements provide a directly beneficial effect on patients (12). The National Eye Institute of the National Institutes of Health has started The Age-Related Eye Disease Study (AREDS) study as the number of studies investigating the therapeutic effect of nutrition on eye diseases has steadily increased (13). In AREDS and AREDS2 trials, the effect of high-dose antioxidant vitamin combinations (vitamin C, E, beta-carotene, zinc, copper) on the progression of AMD and its association with vision loss were studied. The AREDS formulations consist of pharmacologic doses of vitamins and minerals withstood the recommendations of expert biochemists, ophthalmologists,

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and nutritionists. Although there is consensus on the potential benefits of lutein/zeaxanthin and omega 3, no conclusive results have been obtained for other micronutrients and their combinations. Studies show that vitamin and mineral supplementation with dietary antioxidants (especially lutein and zeaxanthin) could be overtaken by AMD risks (13, 14). There are numerous studies on the intake of dietary carotenoids, antioxidants, and omega-3, which reduce the risk of developing AMD substantially (15-17). The nutrient content of the AREDS and AREDS2 formulations is given in Table 1.

Table 1. *Nutrient content of the Age-Related Eye Disease Study (AREDS) and Age-Related Eye Disease Study 2 (AREDS2) formulation (13, 45)*

Nutrient content of the AREDS formulation			Nutrient content of the AREDS2 formulation	
Nutrient	Daily dosage	% Daily value	Daily dosage	% Daily value
Vitamin C	500 mg	754	500 mg	754
Vitamin E (d-α-tocopherol)	400 IU	1334	400 IU	1334
Zinc	80 mg	464	25 mg	464
Copper	2 mg	80	2 mg	80
Vitamin A (beta-carotene)	28,640 IU	572	15 mg	572
Lutein*	-		10 mg	
Zeaxanthin*	-		2 mg	
Docosahexaenoic acid*	-		350 mg	
Eicosapentaenoic acid*	-		650 mg	

**No daily recommended dietary intake established.*

2. CLASSIFICATION OF AMD

Although there are many classifications of AMD, the classification proposed by the AREDS is now typically used (18-20). There are four stages of AMD progression consistent with clinical examination. A few (<20) medium-size drusen or retinal pigmentary abnormalities are observed in early (dry) AMD. Intermediate AMD is characterized by at least one large druse, numerous medium-size drusen, or geographic atrophy that does not extend to the center of the macula. Advanced or late AMD can be either dry (non-neovascular, atrophic, or nonexudative) or wet (neovascular or exudative). Drusen and geographic atrophy spread out to the center of the macula in advanced dry AMD. Advanced wet AMD is characterized by choroidal neovascularization and its sequelae. Dry AMD accounts for about 80% of cases affecting both eyes. Dry AMD usually slowly progresses and causes only mild loss of vision. But for unknown reasons, it can become a neovascular form of this disease (9).

3. PATHOPHYSIOLOGY OF AMD

The retina changes with AMD is associated with pathological changes in the retinal pigment epithelium, choriocapillaris-choroid complex, and Bruch's membrane (a collagen-rich extracellular matrix between the RPE and choroidal vasculature) (21). Pathogenesis of AMD includes drusen accumulation (extracellular deposits of debris), lipofuscin formation, local inflammation, angiogenesis, and oxidative stress (Figure 1) (22, 23). However, the primary damage of AMD is still unknown, genetic and environmental factors related to primary RPE senescence, alterations in the metabolic pathway, increased inflammation, oxidative stress, imbalance of growth factors, and excessive lipofuscin accumulation may cause harm (24).

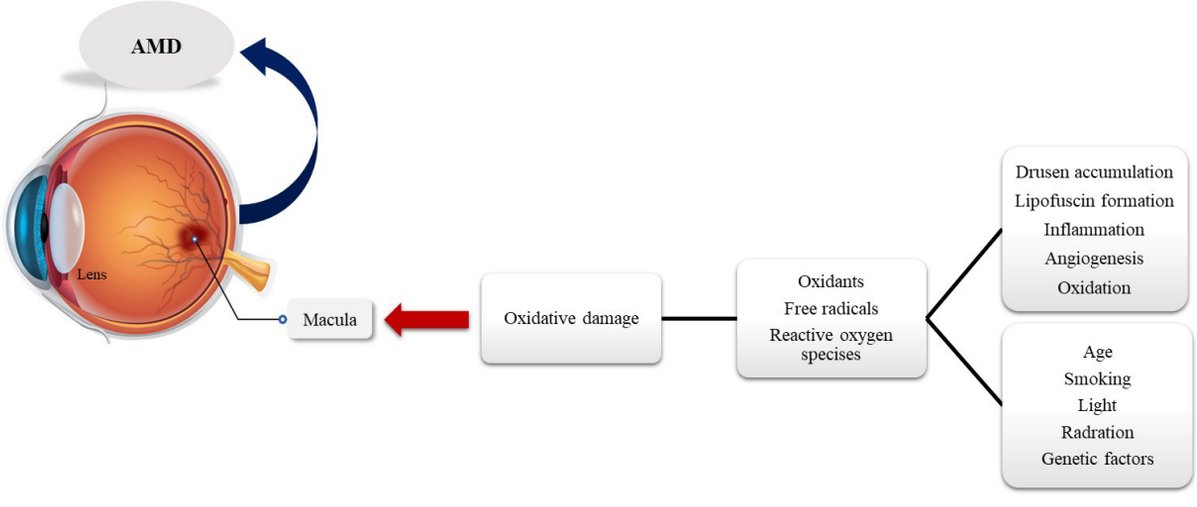


Figure 1. *Pathogenesis of AMD (64)*

There are many risk factors associated with AMD development. Some risk factors, such as family history, ethnicity, gender, cannot be modified, while other risk factors, like smoking, alcohol intake, nutrition, decreased dietary lutein, zeaxanthin, omega 3 and antioxidants, concomitant diseases such as vascular disease, Alzheimer's disease, cardiac disease, and hypertension can be modified (25, 26). The factors that accelerate AMD progression are given in Figure 2.

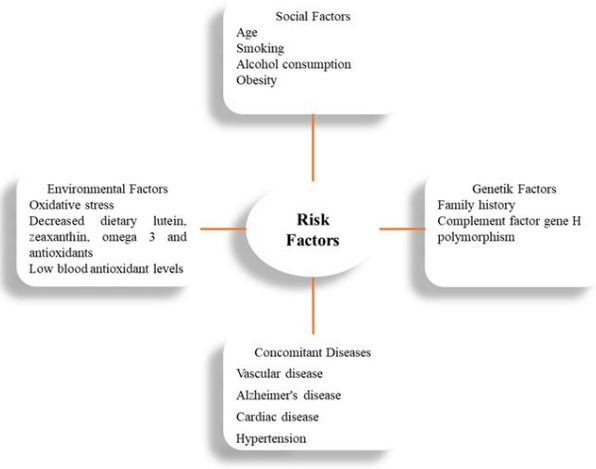


Figure 2. *Factors that accelerate AMD progression (26)*

4. THE MECHANISM OF OMEGA-3 IN AMD

Research has shown that omega-3 fatty acids have potential benefits in patients with AMD and AMD risk (27, 28). Omega 3 is a particularly important component of membrane phospholipids of tissue lipids (29). It is the main fat content of the retina. Omega 3 ensures the fluidity, permeability, and density of the photoreceptor membrane (30). In addition, omega-3 fatty acids can increase the concentration of macular pigment; this pigment is important to filter blue light and locally shows anti-inflammatory and antioxidant activity (31). Dietary omega-3 polyunsaturated fatty acids sources are docosahexaenoic acids (DHA) and eicosapentaenoic acids (EPA). DHA, which transmits visual signals, plays an active role in the regeneration of rhodopsin. Depending on the DHA deficiency, the retinal function has been proven to change. EPA, which is a precursor of DHA, also affects the same metabolic pathway (31-33). EPA and DHA reduce $CD4^+$ T activation and provide an anti-inflammatory environment by transmitting pro-inflammatory into the anti-inflammatory environment. Omega-3 fatty acids can alter inflammation-associated signal cascade and plasma membrane organization by enhancing the molecular organization of lipids and inhibiting lipid seconder messengers, and proteins are needed for activation of T-cells. The anti-inflammatory effect of omega-3 suppresses the formation of new choroidal vessels in exudative AMD (34, 35). Dietary omega-3 fatty acids in the retina macular stimulating the antiangiogenic effect of the derivative is stated to be protective against degeneration (31). Walnut, salmon, sardine, mackerel, flaxseed, flaxseed oil, tuna, and caviar are good sources of omega-3. Omega-3, EPA, and DHA content of walnut, flaxseed, flaxseed oil, and sea products are given in Figure 3 (36).

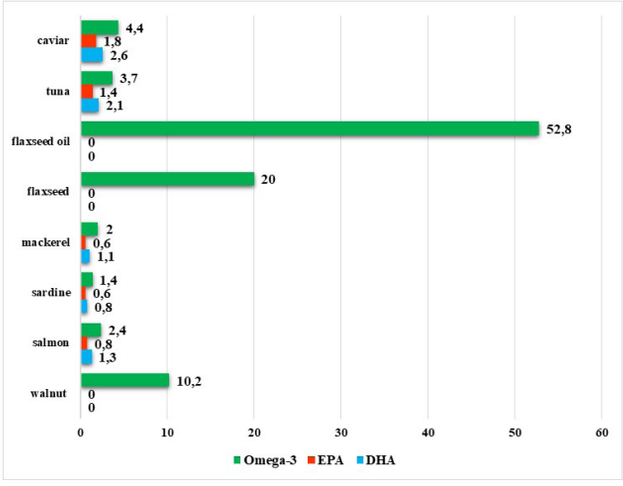


Figure 3. *Omega-3, EPA, and DHA content of foods (g/100 g) (36)*

4.1. Omega-3 Fatty Acids and Fish Intake

The major dietary source of omega-3 fatty acids is fish. The cross-sectional and case-control

studies have shown that EPA, DHA, and fish intake were associated with a significantly decreased risk of incident AMD (37-40).

The AREDS study has been also used to examine the relationship between dietary fat intake and AMD. The AREDS was a double-blind placebo-controlled trial involving 3640 participants from 11 clinics. Omega 3 intakes with patients were estimated by a self-administered semi-quantitative FFQ. The study found that total dietary omega-3 fatty acid intake was inversely related to the incidence of neovascular AMD at baseline (41, 42). In addition, the data obtained from a multivariate analysis of 2132 participants from the AREDS study showed that AMD progression over more than 6 years was inversely associated with EPA or EPA+DHA intake (41).

In The Blue Mountains Eye Study, the relationship between dietary fatty acid intake and AMD development risk within 10 years was evaluated. According to the results obtained from the study, 1 serving of fish per week was associated with a reduced risk of an incident early AMD (RR, 0.69, 95% CI, 0.49-0.98). Similar to the fish intake, 1 to 2 servings of nuts per week was associated with a reduced risk of the incident early AMD (RR, 0.65, 95% CI, 0.47-0.91) (43).

In the meta-analysis and systematic review of prospective cohort studies, once per week fish consumption significantly decreased the risk of AMD (RR, 0.89, 95% CI, 0.83-0.96). Analysis by fish type showed that dark meat fish (RR, 0.68, 95% CI, 0.46–0.99), especially tuna fish (RR, 0.58; 95% CI, 0.47–0.71) intake, was associated with reduced AMD risk (44).

4.2. Omega-3 Supplementation

The AREDS2 is one of the most important randomized, double-blind controlled trials which investigated the effect of omega-3 in the prevention of AMD. The AREDS2 tried to determine the effect of the addition of the carotenoids lutein and zeaxanthin, the omega-3 fatty acids on the risk of developing advanced AMD and/or moderate vision loss in people at moderate to high risk for progression. Similar to AREDS, AREDS2 was a multicenter, double-blind, placebo-controlled trial. It included 4203 patients between the ages of 50 and 85 who had intermediate AMD according to the classification system described for AREDS. The results of the study showed that omega 3 did not prevent the development of AMD (45).

The nutritional AMD Treatment 2 (NAT-2) study is another randomized, case-controlled study to evaluate the efficacy of DHA-enriched oral supplementation (840 mg/day DHA and 270 mg/day EPA from fish oil capsules) in preventing exudative AMD (46). The incidence of neovascularisation was not significantly different when the DHA-enriched supplemented group was compared with the placebo group. However, the study mentioned that the DHA-enriched supplemented group had a significantly lower risk of developing neovascularization in red blood cells membranes over 3 years.

Patients with dry AMD were given omega-3 supplements (3.4 g EPA and 1.6 g DHA) for 6 months. There was a significant improvement in visual acuity in all patients within the fourth and sixth months after omega-3 supplementation (47). In the LUTEGA study, individuals were divided into 3 groups, and the first group (D1) was given supplements containing 10 g lutein, 1 mg zeaxanthin, 100 mg DHA, 30 mg EPA and antioxidant once a day; the second group (D2) was given supplements containing 10 g lutein, 1 mg zeaxanthin, 100 mg DHA, 30 mg EPA and antioxidant twice a day, and the third group was given placebo (P). The volume of macular pigment optical density increased significantly in D1 and D2 and decreased significantly in P. Best-corrected visual acuity improved significantly in D1and D2 groups at the end of 12 months compared with the P (48).

Despite similar main results, the studies have methodological differences. The formulations of supplements (e.g., DHA / EPA ratio), duration of the study, sample size, nutritional parameters of patients (e.g., serum DHA / EPA), and bioavailability of supplements (e.g., ethyl-esters form or triglyceride form) are among the differences of the studies. There is a need for detailed and comprehensive studies using different formulations.

In addition to the protective effect of omega-3 fatty acids on AMD, the omega-6/omega-3 ratio is important. Čaljkušić Mance et al. suggested that decreased omega-6/omega-3 ratio protects against neovascular AMD. The data showed that the dietary omega-6/omega-3 ratio in patients with neovascular macular degeneration was about 11/1, while this ratio in mild and moderate form AMD was about 7-7.5/1, and the control group was about 7/1 (49).

These results support the hypothesis that omega-3 fatty acids could prevent or delay the onset of macular degeneration. While increasing the intake of omega-3 rich nutrients in the diet, it is important to consider the omega-6/omega-3 ratio as well (50).

5. ANTIOXIDANT NUTRIENTS AND AMD

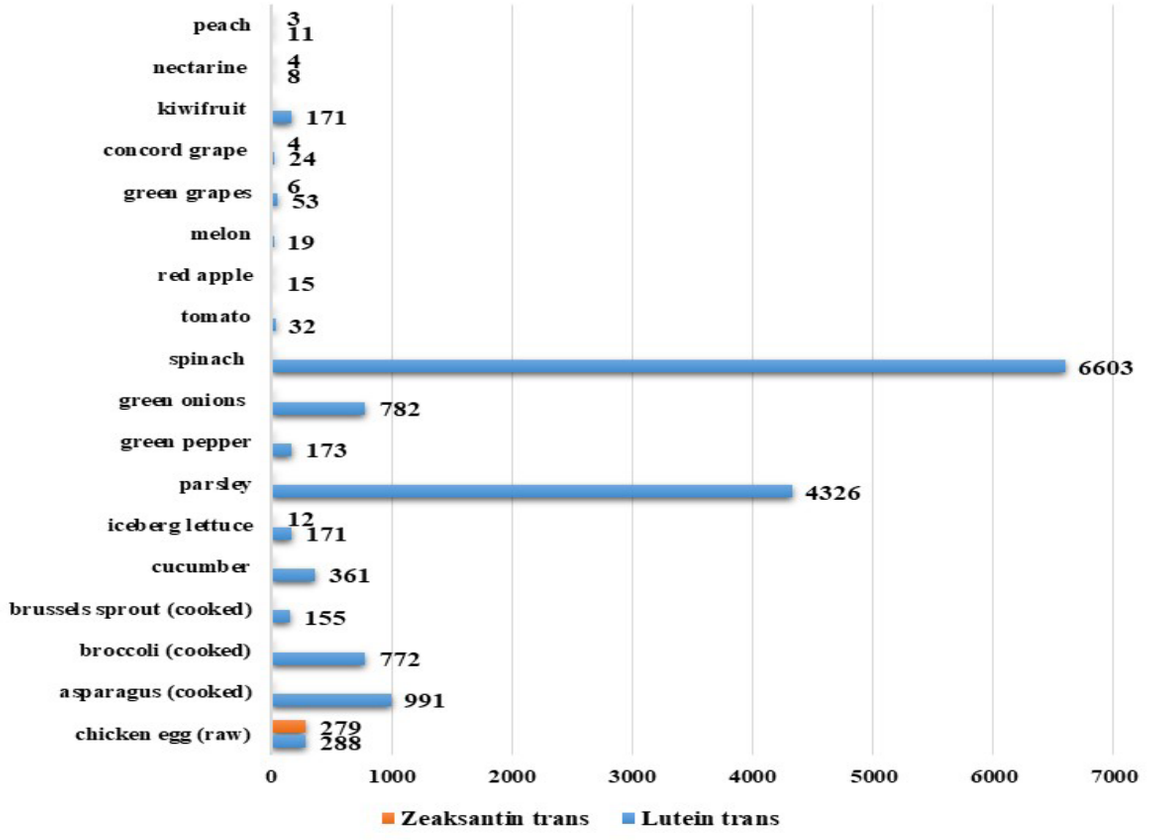
Oxidative stress, which refers to cellular damage caused by reactive oxygen species, has been supposed to contribute to the development of AMD (51). Oxygen consumption of the retina is higher than many other tissues, and the retina is exposed to intense light conditions. Retinal constituents are rich in polyunsaturated fatty acids that oxidize easily, and retinal pigment epithelium contains an excess of photosensitizers. The reasons cited are the formation of reactive oxygen species in the retina and the development of oxidative damage (52).

As oxidative stress could cause the accumulation of drusen, intake of antioxidants with diet or supplements may be beneficial in AMD. An appropriate diet or supplementation with vitamins, minerals, and carotenoids can prevent the development and progression of AMD (53, 54).

5.1. Carotenoids

Although there are more than 600 varieties of carotenoids, 6 carotenoid species are found in diet and serum: lutein, zeaxanthin, α-carotene, β-carotene, lycopene, and β-cryptoxanthin (55). Lutein, zeaxanthin, and their common metabolite are known as meso-zeaxanthin macular pigments (56). Meso-zeaxanthin comes from the lutein and zeaxanthin pigments in the yellowish color structure of the macula. Lutein and zeaxanthin protect the retina against photo-oxidative damage (57, 58). Several studies determined that a higher intake of carotenoids in diet, especially lutein/zeaxanthin, was associated with a lower risk for AMD (16, 55, 59). In the AREDS study, supplement formulation containing antioxidant vitamins (vitamin E, C) and minerals (zinc and copper) and β-carotene but not lutein and zeaxanthin reduced the risk of AMD progression (13).

It should be noted that high beta carotene intake can cause lung cancer in smokers. Results of the prospective cohort studies on this subject are needed (12). Lutein and zeaxanthin content of the chicken egg, some fruits, and vegetables are given in Figure 4.



For all foods, it is the predominant form of xanthophylls trans isomeric forms.
***Figure 4**. Lutein and zeaxanthin content of foods (µg/100 g) (36)*

5.2. Vitamin C

Vitamin C is an important water-soluble antioxidant. It supports the regeneration of vitamin E (60). In a population-based cohort study, it was reported that vitamin C intake was not associated with AMD (61). In a systematic review of five studies searching for antioxidant supplements in the development of AMD, there was no evidence that vitamin C prevented or delayed the onset of AMD (62).

5.3. Vitamin E

Vitamin E is a lipid-soluble antioxidant that maintains the cell membrane by scavenging the reactive oxygen species (63). A case-controlled study was conducted with people aged ≥ 50 years, and it revealed that a high dietary intake of vitamin E was found to substantially reduce the risk of developing AMD (64). The Rotterdam study also showed a decreased risk for AMD in subjects with high dietary intakes of vitamin E, but a prospective randomized placebo-controlled trial showed that daily vitamin E supplements did not prevent the development or progression of early/late stages of AMD (65). At the same time, vitamin E supplementation has an anticoagulant effect and may cause heart attacks. There are studies suggesting that an increase in vitamin E intake increases the risk of prostate cancer (66, 67)

5.4. B Vitamins

High plasma homocysteine concentrations cause vascular endothelial dysfunction that induced the development of AMD. Several studies show the relationship between plasma homocysteine levels and AMD risk. A meta-analysis of eight homocysteine-lowering trials showed that vitamin B_6 and vitamin B_{12} and folic acid reduced homocysteine levels (68), and vitamin B_6 and vitamin B_{12} improved endothelial dysfunction (69). In a randomized, double-blind, placebo-controlled study with females, combined folic acid, vitamin B_6, and vitamin B_{12} therapy was associated with a 34% lower risk of AMD and a 41% lower risk of visually significant AMD (70). B vitamins both reduce the levels of homocysteine and protect the blood vessels in the eye by creating an antioxidant effect.

5.5. Zinc

Zinc has antioxidant functions and acts as a cofactor in several enzymes (e.g., retinol dehydrogenase, superoxide dismutase) (71). The retina contains high amounts of zinc; therefore, zinc is an important mineral for the optimal metabolism of the eye (72).

In the AREDS clinical trial, patients were divided into 4 categories based on disease severity. The first group received antioxidant daily oral tablets which contained vitamin C 500 mg; vitamin E (as d-α-tocopherol) 400 IU; beta carotene 15 mg, the second group was given zinc 80 mg (as zinc oxide) and copper 2 mg (as cupric oxide) the third group was given antioxidants+zinc; the fourth group was given a placebo. The results revealed that the risk of AMD progression was reduced by 25%. No such reduction was detected in antioxidant vitamins alone (12). However, compared to high-dose (80 mg) in the AREDS2 study (45), no significant benefit was found in low-dose zinc (25 mg), Findings of the Rotterdam Study showed that dietary intake of zinc was inversely associated with incident AMD and reduced the risk of AMD in elderly people (73).

6. CONCLUSIONS

The risk of AMD is increasing all over the world. In particular, exudative AMD gives rise to the types of irreversible vision loss. The results of studies suggested that there was a positive effect of dietary omega-3 and lutein/zeaxanthin intake on the development and progression of AMD. To reduce the incidence and severity of disease, it is necessary to increase the intake of omega-3, lutein/zeaxanthin by the age group and requirements of the individual's diet. Dietary sources of omega-3 are oily fish, seafood, walnut, flaxseed, and their oils. Lutein and zeaxanthin are obtained from green leafy vegetables (peas, spinach, kale, lettuce, and broccoli), carrots, eggs, and cheese. To increase the dietary omega 3 intakes, nutritionists recommend that fish be consumed twice a week (500 g). Regarding antioxidant micronutrients, only the AREDS 2 formulation can currently be recommended to patients for reducing the risk of AMD.

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Author Contribution *(Authors initials)*

Research idea: EK

Design of the study: EK, NAT

Drafting the manuscript: EK

Revising it critically for important intellectual content: NAT

Final approval of the version to be published: EK, NAT

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