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Research Article

BSA Interference in Immunoassays in Individuals with Egg Allergy

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Abstract

Aim: The aim of current study was to determine interference by bovine serum albümine (BSA) as blocking agent in enzyme-linked immunosorbent assay (ELISA) carried out in individuals with egg allergy.

Material and Methods: 14 people diagnosed with egg allergy and 7 people without allergy were included. The sample were studied with an indirect ELISA method for egg-white IgG antibody developed in our laboratory. Effect BSA on interference was studied by manipulating antigene coating (none vs. egg white extract), blocking (1% BSA vs. Tween 80), and sample diluent (PBS vs. PBS + 0.5% BSA).

Results: In wells that were blocked with 1% BSA without being coated with antigen, positive samples cross-reacted with BSA to give an optical density (OD) of 0.99 ± 0.16, while negative samples gave an OD of 0.08±0.01 ($p<0.05$). However, when the same samples were diluted with 0.5% BSA, the OD of positive samples decreased (from 0.99±0.16 to 0.08±0.01), and the statistical difference with negative samples disappeared. It was observed that tween, which was used as a blocking and diluting agent, did not cross-react with the samples. Positive samples gave an OD of 0.66±0.07 in antigen (egg white extract) coated and tween-blocked wells, and 1.01±0.11 OD in BSA blocking ($p<0.05$). When the samples were diluted with 0.5% BSA, positive samples gave 0.18±0.01 OD on the antigen coated plate, while negative samples gave 0.12±0.04 OD ($p<0.05$).

Conclusion: Ovalbumin, which is found in high levels in eggs, has a similar molecular structure to BSA, and some antibodies produced against ovalbumin in people with egg allergy may also cross-react against BSA. Therefore, it was concluded that the use of BSA in both dilution and blocking solution should be avoided if the samples of individuals with egg sensitivity are to be analyzed by ELISA method. It has been observed that Tween can be easily applied as an alternative blocking agent in allergy ELISA tests.

Keywords: Egg, allergy, BSA, IgG

INTRODUCTION

Food allergy is an important public health problem that adversely affects the lives of allergic patients and their families, may occur in adulthood or childhood, and its prevalence is increasing in the world (1,2). Allergic diseases are an immune disorder that reacts to certain types of allergens (3). Most allergens are proteins or glycoproteins with molecular weights ranging from 5000 to 100,000 Da. In addition, polysaccharides and low molecular weight substances may be allergenic (4).

All techniques using enzymes to demonstrate antigen-antibody reactions are generally referred to as the enzymatic immunoassay EIA/ELISA method (5). ELISA is a versatile, sensitive and quantitative technique that requires very little equipment (6). There are 4 different techniques when applying the ELISA test. These; direct, indirect, sandwich and competitive ELISA (7). In the indirect ELISA, the microplate is plated to the bottom of the wells. Blocking agent is used to prevent non-specific binding to spaces between antigens. Samples are diluted with dilution buffer and added to the wells. Afterwards, the secondary antibody, substrate and stop solution that recognize the antibody are added respectively and the reactions are stopped. The microplate is read on a microplate reader set to 450 nm (8).

CITATION
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The most preferred blocking agents are proteins (BSA, milk powder, etc.) and Tween (9). Researchers often use BSA as a blocking agent to prevent non-specific binding of antigens and antibodies to the microtiter well (10).

ELISA is one of the approved and routinely used immunological tests in allergy research, in various industries, in allergy-related quality control, and in allergy diagnosis (11). Food allergy is a pathological, potentially fatal immune reaction triggered by normally harmless food protein antigens (12). Egg allergy is one of the most common food allergies in infants and young children, and egg whites contain more than 20 different proteins and glycoproteins. Ovomucoid (Gal d 1), ovalbumin (Gal d 2), conalbumin (ovotransferrin) (Gal d 3) and lysozyme (Gal d 4) have been identified as the main allergens in chicken eggs (13). Since ovalbumin (OVA) and BSA have some immunologically similar epitopes, the antibody produced against one of them usually cross-reacts against the other (14). Therefore, if the samples of people with egg sensitivity are to be analyzed by ELISA method, the use of BSA may be inconvenient. Because if the antibodies in the sample bind to the BSA used in the blocking, a similar result occurs as if they were bound to the antigen (Figure 1). This actually leads to non-specific high optical density. In this context, the aim of our study is; The aim of this study is to investigate the effect of BSA use on the results in the ELISA test to determine the IgG level in egg-sensitive individuals.

MATERIAL AND METHOD

Before the study, ethical approval was obtained from the Inonu University Health Sciences Non-Interventional Clinical Research Ethics Committee (No: 2022/3017).

As a positive control in our study; In our laboratory, 14 people whose samples were previously found and diagnosed with egg allergy were used. Samples of 7 people without any allergic disease were used in the negative control group.

A 96-well ELISA plate was used in the study (Thermo Scientific Maxisorp Nunc 96 well Plate) and the test protocol was designed according to the indirect ELISA method (Figure 2). Wells were coated with egg white extract as antigen (Figure 2. ”1. process”). Blocking was done with 1% BSA or 0.5% Tween 80 (Figure 2. ”2. process”). Samples were diluted 1/100 with solutions containing PBS or 0.5% BSA and added to the wells (Figure 2. ”3. process”). Then to all wells in order; secondary antibody (Biotinylated anti-human IgG,) and streptavidin peroxidase were added (Figure 2. ”4. and 5. process”). The color formed after the addition of chromogen substrate (3,3',5,5'-Tetramethylbenzidine (TMB)) was stopped with a stop solution (11% H2O2) and read at 450 nm wavelength (Figure 2.”6. process”). A total of 6 different experimental protocols studied are given in Table 1.

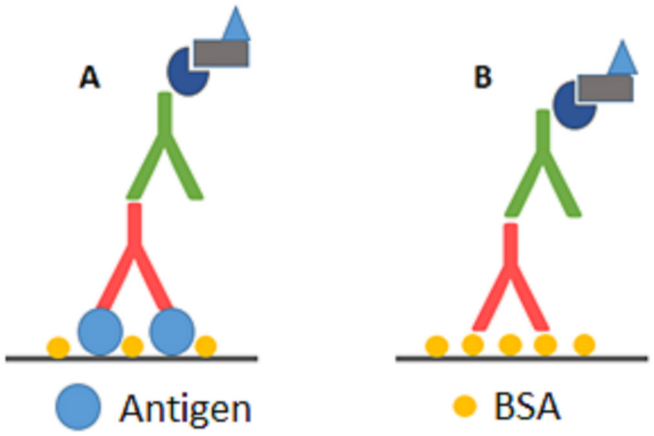

Figure 1. The binding of antibodies found in the samples of children with egg allergy to the antigen (A) and the BSA-blocked surface (B) provides similar results

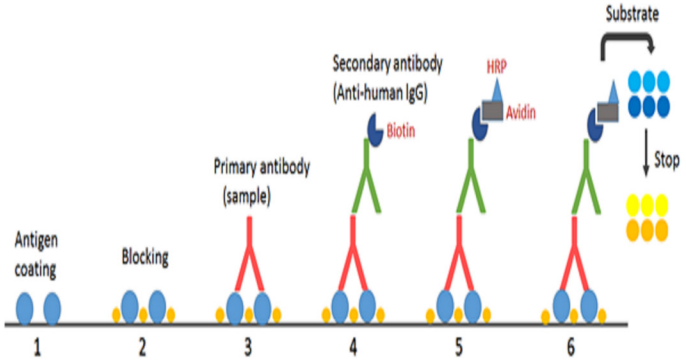

Figure 2. The indirect ELISA working principle that we used in our study

In Protocols 1, 2 and 3, direct blocking was performed without coating the wells with antigen. Protocol 1 was designed to determine the binding levels of samples to BSA. In the second protocol, the samples were diluted with BSA to measure the extent to which BSA in the dilution solution would bind the antibodies in the sample. In protocol 3, the affinity of the samples for a blocking agent other than BSA was measured by blocking the empty well with a BSA-free agent (Tween 80).

In protocols 4, 5 and 6, wells were coated with egg white extract as antigen. BSA was not used in protocol 4. Thus, IgG levels against egg white were determined in the samples. Unlike the previous experiment in protocols 5 and 6, BSA was used and how BSA affected the reference result in protocol 4 was evaluated.

Statistical Analysis

Since the data obtained did not meet the parametric conditions, Friedman test was used for dependent groups and Kruskal Wallis-H test was used for independent groups in statistical comparison. Post hoc evaluations were made with Bonferroni corrected Wilcoxon (dependent) or Mann-Whitney U test (independent). The cut off (threshold) value used to evaluate the binding adequacy of the samples to BSA or egg extract was determined by the ROC curve (Receiver Operating Characteristic Curve).

Table 1. Six different protocols of the study (The applications made after adding the samples were not included in the table since they were common in all trials)

	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Protocol 5	Protocol 6
Antigen coating	-	-	-	Egg extract	Egg extract	Egg extract
Blocking	1% BSA	1% BSA	0.5% Tween80	0.5% Tween80	1% BSA	1% BSA
Sample dilution	PBS	0.5% BSA	PBS	PBS	PBS	0.5% BSA

Table 2. Descriptive statistical data of allergic and non-allergic samples (Results are given as OD)

	ALLERGİC SAMPLES		NON-ALLERGİC SAMPLES		
	Mean±S deviation	Median (min-max)	Mean±S deviation	Median (min-max)	P value
Protocol 1	0.99±0.16	0.86 (0.20-2.39)	0.08±0.01	0.08 (0.06-0.09)	0.00*
Protocol 2	0.08±0.01	0.08 (0.06-0.10)	0.08±0.01	0.08 (0.07-0.10)	0.64
Protocol 3	0.08±0.01	0.08 (0.07-0.10)	0.08±0.00	0.09 (0.08-0.09)	1.00
Protocol 4	066±0.07	0.66 (0.28-1.27)	0.13±0.04	0.12 (0.08-0.19)	0.00*
Protocol 5	1.01±0.11	0.92 (0.50-1.85)	0.12±0.04	0.12 (0.08-0.20)	0.00*
Protocol 6	0.18±0.01	0.17 (0.15–0.34)	0.12±0.04	0.11 (0.08-0.20)	0.00*

P values represent the difference between the results of allergic and non-allergic subjects for each protocol. (*) Indicates that there is a statistically significant difference

RESULTS

Determination of affinity for BSA

In our study, six different protocols were used in which allergic and non-allergic samples were tested. Binding levels of samples to BSA were analyzed in protocol 1. The cut off value was calculated as 0.146 Optical Density (OD) by ROC analysis, and samples that gave results above the cut off were considered to have affinity for BSA. All 14 allergic samples tested in protocol 1, where blocking with 1% BSA without coating with antigen, were found to be above the cut-off value (Figure 3A). In protocol 2, allergic samples were diluted with 0.5% BSA. Because BSA in the dilution solution binds antibodies with affinity to it, the antibodies did not reach the bottom of the wells and no color was formed in the wells. In protocol 3, allergic samples were tested against a BSA-free blocking agent (0.5% tween 80) and similar results were obtained with protocol 2 (Figure 3A). In the same protocols, 7 non-allergic samples were also tested and, unlike the allergic samples, showed no affinity for BSA (Figure 3B).

Determination of IgG levels of samples against egg extract

IgG levels of allergic samples against egg white extract were tested in protocol 4 without BSA. Cut off value was determined as 0.236 OD. Allergic samples gave positive results above the cut-off (0.66±0.07), while non-allergic samples remained below the threshold value (0.13±0.04) (Table 2).

Effect of BSA use on results

The data obtained from protocol 4 were accepted as the reference value and the effect of BSA on the experiment was evaluated. A statistically significant increase in OD values for allergic samples was seen in protocol 5 where blocking was done with BSA compared to protocol 4 (p=0.001). When looking at protocol 6, in which BSA is used both in blocking and dilution of the samples, a significant decrease was observed in the OD values of the allergic samples compared to protocol 4 and 5 ([p=0.001], [p=0.001]). The use of BSA did not change the results, since non-allergic samples did not show affinity for BSA.

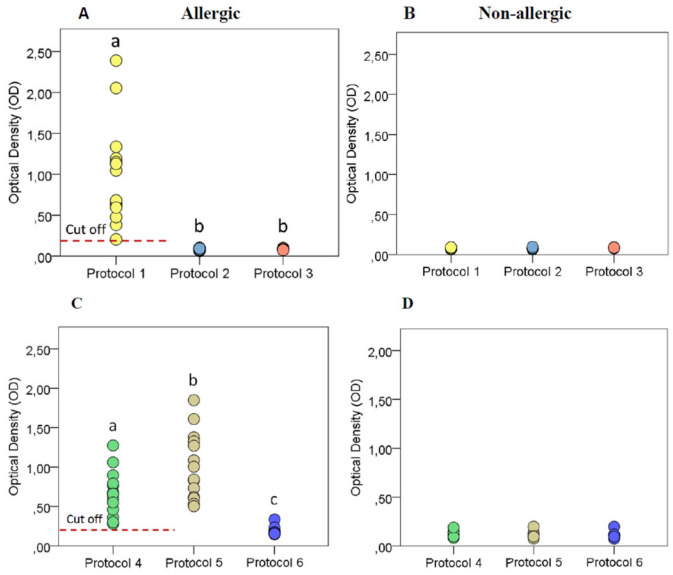

Figure 3. ELISA egg white specific IgG results of different protocols applied in allergic and non-allergic individuals

DISCUSSION

The ELISA method is a frequently used method in allergy research (11). For allergy ELISA tests to give accurate results, antibodies only need to recognize the antigens in the wells. Substances such as tween and BSA as blocking agents are used to close the gaps left after coating with antigens (9). BSA is frequently used as a blocking agent in egg allergy-specific IgG studies (15-18).

Tomicic, et al. (15) used BSA as a blocking agent for

ELISA IgG1 and IgG4 measurement against food allergens (including OVA). In another study, Zhang et al. (16) used 3% BSA as a blocking agent in the ELISA method to measure the IgG level specific to egg components Gal d 1, Gal d 2 (OVA), Gal d 3, Gal d 4 and Gal d 5. McKendry, et al. (18) in a study; used 1% BSA for the measurement of raw peanuts, egg whites and cow's milk specific IgG ELISA. However, according to the findings we obtained in our study, it was seen that the use of BSA as a blocking agent can cause false results.

Zhang and Mine (1998), on the other hand, used 3% BSA as a blocking agent for the measurement of ovomucoid IgG ELISA, but performed it with 1% BSA as serum dilution. In this study, dilution of samples from subjects allergic to ovalbumin was done with BSA. This dilution eliminated non-specific binding to BSA used as the blocking agent, resulting in ovomucoid (not ovalbumin) specific IgG measurement. This study design is compatible with the results of our study.

In some studies, although BSA was used as the blocking agent in allergy-specific IgG ELISA tests, some wells were coated with BSA as a control to eliminate specific binding. The results of the absorbances on the BSA-coated plate were subtracted from the results on the allergen-coated plates, following a different protocol (19,20). However, since antibodies formed against ovalbumin in these protocols will give a certain optical density as a result of cross-reaction in the well coated with BSA as a control, the result obtained in the applied subtraction process; will give the total optical density of those other than ovalbumin. Instead of these protocols, the use of tween 80 as a blocking agent, which we have shown not to bind specifically in our study, is thought to be a more reliable protocol.

In a study by Hermanson (2013); It has been noted that ovalbumin (OVA) and BSA have some immunologically similar epitopes, and antibodies produced against one of them will often cross-react against the other. In our study, it was seen that the antibodies formed against ovalbumin by the ELISA method cross-reacted with BSA, and this result is consistent with the statements of Hermanson (2013).

CONCLUSION

BSA is not suitable for use as a blocking agent because it cross-reacts with antibodies against ovalbumin. The use of tween as a blocking agent instead of BSA is important in terms of obtaining more accurate results. When serum dilutions of people with egg allergy are made with BSA, it is recommended not to use BSA in serum dilution, since antibodies against ovalbumin will bind to BSA and will not bind to the coated antigen.

At the same time, the present results indicate that there will be false high optical density in people with egg allergy as a result of using BSA as a blocking agent in ELISA tests other than allergy ELISA tests. Therefore, it is recommended not to use BSA as a blocking agent in ELISA tests other than allergy ELISA tests. Even if it will be used, it is recommended to take a history of past or current allergic conditions in the people who will be included in the study.

Studies have shown that ovalbumin and BSA have similar epitopes and cross-react. Whether this situation causes erroneous results in the ELISA method has not been examined. In the current study, it was observed that the use of BSA in ELISA measurements in egg allergic individuals caused interference and affected the test result. It seems essential to investigate BSA interference in individuals with allergies to different foods.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Before the study, ethical approval was obtained from the Inonu University Health Sciences Non-Interventional Clinical Research Ethics Committee (No: 2022/3017).*

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Research Article

Histopathological Analysis of Tongue Lesions and Distribution by Age Groups

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Abstract

Aim: To retrospectively evaluate the histopathological characteristics of biopsies taken from tongue lesions and compare the results with current literature.

Material and Methods: Histopathological results of biopsies taken from 163 patients with suspected tongue lesions between January 2017 and January 2022 were retrospectively reviewed. The results were evaluated in different age groups.

Results: Seventy-two (44.2%) of the patients were male and 91 (55.8%) were female. The mean age of the patients was 47.76±17.33 years. The lesion was benign in 135 (82.8%) patients, precancerous in 4 (2.5%), and malignant in 24 (14.7%).

Of the 135 patients with benign findings, squamous papilloma was detected in 33 (24.4%) patients and irritation fibroma in 19 (14.1%). Ulcer/inflammatory granulation tissue was detected in 18 (13.3%) patients, parakeratosis/acanthosis in 14 (10.4%), fibroepithelial polyp in 14 (10.4%), and pseudoepitheliomatous hyperplasia in 9 (6.7%). Less frequently, lymphoid hyperplasia, neurofibroma, mucocele, and verruca vulgaris were detected in 1 (0.7%) patient each. Squamous cell carcinoma was detected in all patients with malignancy. When evaluated according to age groups, it was found that the patients were most commonly in the 50–59 (27.6%) age group, followed by the 40–49 (19.6%) age group.

Conclusion: The results show that most tongue lesions are benign. It should be noted that not every mass in the tongue is cancerous, but leukoplakic and ulcerated areas can be malignant. Due to the rapid metastasis of tongue lesions, early diagnosis and treatment may contribute significantly to the prognosis of patients.

Keywords: Biopsy, tongue, histopathology, carcinoma, leukoplakia

INTRODUCTION

The tongue is a muscular organ located on the lower surface of the oral cavity; it consists of a freely movable body and a root that forms part of the anterior wall of the pharynx and is attached to the base of the pharynx. It is lined with stratified keratinized squamous epithelium on the dorsal surface and non-keratinized epithelium on the ventral surface, which contains numerous nerve endings, fat cells, minor salivary glands, and lymphoid tissues with a richly vascularized network. Each of these structures may be the origin of benign, malignant, or precancerous lesions (1).

Oral cavity cancers are observed in the tongue the second most frequently after the lips. Squamous cell carcinoma (95%) is observed most frequently, whereas adenoid carcinoma is rarely observed (1%) (2). Although squamous cell tongue cancers are common in male patients over 50 years of age, there has been an increase in its incidence in younger patients as well (3). It has been reported that tongue cancer progresses more aggressively in young patients than in the elderly patient group, and the recurrence and survival rates are worse (4). With regard to etiology, smoking and alcohol use are blamed in the elderly patient group. In young patients, genetic predisposition comes to the fore rather than smoking and alcohol (5,6).

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Although lesions in the oral cavity and tongue can be easily examined, their diagnosis and treatment remain challenging. In studies involving lesions in the tongue, clinical appearance and prevalence studies of the tongue are seen more frequently (7,8). In our study, the histopathological results of biopsies performed for suspicious lesions on the tongue and their distribution by age were retrospectively reviewed. We aim to contribute to the early diagnosis and treatment of tongue tumors prone to metastasis.

MATERIAL AND METHOD

The histopathological results of biopsies of 163 patients who underwent tongue biopsies for suspicious lesions at the Antalya Training and Research Hospital between January 2017 and January 2022 were retrospectively reviewed. The distribution of the data by age groups was examined. Outpatient consultation cases were excluded from the study.

The demographic data of patients and pathological findings were obtained from pathology reports and hospital data systems. Demographic data such as age and gender as well as histopathological findings of the patients were evaluated. The patients were categorized into nine groups by age: 0–9 years, 10–19 years, 20–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70–79 years, and ≥80 years. Histopathological findings were divided into three groups: benign, precancerous, and malignant.

All data were evaluated using Statistical Package for Social Sciences 18.0. The data were presented with descriptive statistics, frequency, percentage, mean and standard deviation, and minimum–maximum values.

RESULTS

The study included 163 patients with tongue lesions. Of the patients, 72 (44.2%) were male and 91 (55.8%) were female. The mean age was 47.76±17.33 (2–87 years). Benign findings were detected in 135 (82.8%) patients, precancerous in four (2.5%) patients, and malignant findings in 24 (14.7%) patients. Of patients with benign lesions, 78 (57.8%) were female and 57 (42.2%) were male, whereas for those with malignant lesions, 12 (50%) were female and 12 (50%) were male.

Of 135 patients with benign findings, it was revealed that 33 (24.4%) patients had squamous papilloma; 19 (14.1%), irritation fibroma; 18 (13.3%), ulcer/inflammatory granulation tissue development; 14 (10.4%), fibroepithelial polyps; 14 (10.4%), parakeratosis/acanthosis; nine (6.7%), pseudoepitheliomatous hyperplasia; seven (5.2%), chronic inflammation findings; six, (4.4%), lichen planus; four (3.0%), pyogenic granuloma; three (2.2%), hemangioma; two (1.5%), lymphoid hyperplasia; one (0.7%), lymphoepithelial cyst; one (0.7%), neurofibroma; one (0.7%), mucocele; one (0.7%), verruca vulgaris; one (0.7%), fungus; and one (0.7%), benign fibrous histiocytoma (Table 1).

Table 1. Distribution of benign findings in tongue biopsies

Benign Findings	Total n (%)
Benign Fibrous Histiocytoma	1 (0.7)
Fibroepithelial Polyp	14 (10.4)
Fibroma	19 (14.1)
Ulcer/Inflammatory Granulation Tissue	18 (13.3)
Chronic Inflammation	7 (5.2)
Lymphoepithelial Cyst	1 (0.7)
Lymphoid Hyperplasia	2 (1.5)
Lichen Planus	6 (4.4)
Hemangioma	3 (2.2)
Fungal Infection	1 (0.7)
Mucocele	1 (0.7)
Neurofibroma	1 (0.7)
Parakeratosis/Acanthosis	14 (10.4)
Pseudoepithelial Hyperplasia	9 (6.7)
Pyogenic Granuloma	4 (3.0)
Squamous Papilloma	33 (24.4)
Verruka Vulgaris	1 (0.7)
	135 (100.0)

Of four patients with precancerous findings, one (25%) patient had actinic keratosis and three (75.0%), low-grade dysplasia. Squamous cell carcinoma was detected in all patients with malignancy.

Table 2. Distribution of histopathological findings by age groups

Age Groups	Number of patients			Total n (%)
	Benign lesions n	Malignant lesions n	Precancerous lesions n	
< 9	3	0	0	3 (1.8)
10-19	10	0	0	10 (6.1)
20-29	9	0	0	9 (5.5)
30-39	22	3 (12.5%)	0	25 (15.3)
40-49	28	2 (8.3%)	2	32 (19.6)
50-59	40	4 (16.7%)	1	45 (27.6)
60-69	13	7 (29.2%)	0	20 (12.3)
70-79	9	5 (20.8%)	1	15 (9.2)
≥ 80	1	3 (12.5%)	0	4 (2.5)
Total	135 (82.8%)	24 (14.7%)	4 (2.5%)	163 (100.0)

While the youngest patient in our study was 2 years old and showed pyogenic granuloma, the oldest patient was 87 years old for whom the development of ulcer/inflammatory granulation tissue was observed. When we look at the distribution of patients according to age groups, the patients were in the 50–59 age group most frequently with a rate of 27.6%, and they were in the 40–49 age group second most frequently with a rate of 19.6% (Table 2).

DISCUSSION

The tongue is the most common area of oral cavity cancers after the lips. Tongue lesions can lead to serious life-threatening oral cavity cancers. A biopsy is the first step in diagnosing tongue lesions. Many factors such as age, sex, socioeconomic level, and genetic predisposition can affect the frequency of tongue lesions. Various studies have shown different results in different geographical regions (9,10). In a prevalence study conducted by Avcu et al. with 5150 patients in Turkey, the incidence of tongue lesions was found to be 44.2% in women and 62.0% in men (7).

In our study, demographic data, pathological findings, pathology reports, and hospital data systems of 163 cases with only tongue lesions between 2017–2022 were obtained. We analyzed patients demographic data, such as age and sex, as well as histopathological findings. While the youngest patient in our study was 2 years old who showed pyogenic granuloma, the oldest patient was 87 years old for whom development of ulcer/inflammatory granulation tissue was detected. Considering the distribution of the patients by age groups, the patients were in the 50-59 age group with 27.6% of the patients. The frequency of distribution by age groups is similar to the studies in the literature. In the study by Shamloo et al., most patients were in the 40–60 age group, and in the study by Alaeddini et al,. a majority of the patients was in the 45–64 age group (9,11). In our country, in the study by Aydın et al., the most frequent age range in years was found to be 40–59 for benign lesions and 60–79 for malignant lesions (12). In our study, the age range was the same in both benign and malignant lesions.

Of 135 patients with benign lesions, 78(57.8%) were female and 57(42.2%) were male. According to the frequency distribution, the most common findings were squamous papilloma in 33 (24.4%) patients; irritation fibroma, 19(14.1%); ulcer/inflammatory granulation tissue development, 18 (13.3%); fibroepithelial polyps, 14 (10.4%); parakeratosis/acanthosis, 14(10.4%); pseudoepitheliomatous hyperplasia, nine (6.7%); and chronic inflammation, seven (5.2%). Considering existing literature, Lasisi et al. found pyogenic granuloma (12.1%), fibroma (8.1%), and fibroepithelial polyps 4% (13) most frequently. Furthermore, Shamloo et al. found fibroma most frequently (42.2%) and pyogenic granuloma the second most frequently (40%) (9). In our country, in the study by Aydın et al., acanthosis/parakeratosis was observed most frequently with a rate of 20.9%, followed by fibroepithelial polyp (20.5%) and papilloma (20%) (12). As seen in studies from different regions, it is noteworthy that lesions secondary to trauma are the most common.

Of the 24 patients with malignant lesions, 12 (50%) were female and 12 (50%) were male. In all patients with malignancy, squamous cell carcinoma was found on histopathological diagnosis. In addition, one of four patients with precancerous findings had actinic keratosis and three, low-grade dysplasia. When 24 patients with malignancy were analyzed according to age groups, it was seen most frequently in the 60-69 age group with 7 (29.2%) cases. It was seen in the 70-79 age group with 5 (20.8%) cases in the second frequency. In our study, 12 (50%) of malignant lesions were seen in the 60-79 age group. In our literature review, squamous cell carcinoma constitutes more than 95% malignant tumors of the tongue. The most frequently affected part is the middle 1/3 of the free lateral border of the tongue (1,14). Azakli et al. reported that squamous cell carcinoma was the most common malignant lesion in their study that included the entire oral cavity (15). One of the suspicious lesions in the development of tongue cancer is lichen planus. Barnard et al. reported cases of squamous oral carcinoma developing on the basis of lichen planus and reported a 5% malignancy risk (16).

CONCLUSION

Lesions on the tongue may be challenging for clinicans in terms of diagnosis and treatment. Clinical examination and histopathological analysis may help categorize the type of lesion. According to our study results, it may be concluded that not every lesion on the tongue is cancer; most of them are benign. However,diagnosis and treatment must not be delayed in the case of suspicious lesions. Given that oral cavity cancers are most frequently found on the tongue after the lips and they metastasize rapidly, we believe that early diagnosis and treatment of oral cavity cancers will contribute to the prognosis of the patient.

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Research Article

Effects of Uremic Pruritus on Dermatological and Kidney Disease Quality of Life in Patients Receiving Hemodialysis

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Abstract

Aim: This study was conducted in order to determine the dermatological and kidney disease quality of life of patients with uremic pruritus receiving hemodialysis treatment.

Material and Methods: The present study was a descriptive study. The sample comprised 200 of HD patients. The participants were required to complete patient information form, 5-D-Itch scale, Dermatological Quality of Life Index (DLQI) and Kidney Disease Quality of Life Form (KDQOL-36) were used to collect the data of the study.

Results: It was determined that the DLQI score average of the patients with pruritus was 11.57±4.74 and that the dermatological quality of life of 60.6% of the patients was largely or extremely affected. It was revealed that the average KDQOL-36 score of the patients with pruritus was 59.36±12.27, and their kidney disease quality of life was of moderate level. Pruritus severity explained a moderate amount of the variability of DLQL (crude R^2 =.181) and KDQOL-36 (crude R^2 =.184).

Conclusion: It was determined that as the severity of uremic pruritus increased, DLQI and KDQO-36 decreased. Therefore, timely treatment, nursing care and counseling are recommended to monitor level of pruritus, dermatological and general quality of life to improve the quality of life in HD patients.

Keywords: Dermatology quality of life, general quality of life, hemodialysis, itch

INTRODUCTION

In addition to affecting all systems, chronic kidney diseases (CKD) and Hemodialysis (HD) used in its treatment cause serious dermatological problems. HD prolongs the life of patients, but on the other hand, it leads to various dermatological symptoms and complications and affects dermatological and kidney disease quality of life (1,2). Patients receiving HD treatment suffer from some skin problems such as dryness, uremic itching, hyperpigmentation, pallor, skin ulcers, purpura, ecchymosis, and uremic frost. Such skin problems also lead to deterioration in skin structure and physical, psychosocial, and emotional distortion in the general health perception of patients (3-6).

Uremic pruritus is not only one of the most typical and disturbing skin problems of CKD and HD, but also it is a common and severe skin-related symptom affecting 40-60% of patients receiving HD treatment (7-8). Pruritus, which is not encountered in acute kidney diseases cases and is treated after kidney transplantation in CKD, occurs in various severity in patients receiving HD treatment. Although it is thought that uremic pruritus is caused as a result of eliminating toxins from the body through the skin, it is stated that the reason behind it might be secondary hyperparathyroidism, hyperphosphatemia, calcium-phosphate deposition in the skin, changes in skin pH, dry skin due to atrophy in sweat and sebaceous glands, the proliferation of mast cells in the skin and consequently increased plasma histamine level, inadequate dialysis, anemia, iron deficiency, dialysis with devices that have low hypersensitivity, as well as antiseptic solutions used

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for disinfection, nickel-containing needles, epoxy resin, colophony, formol, and thiuram, as well as other allergenic substances. Due to the obscurity of the pathogenesis of uremic pruritus, the use of limited therapeutic methods is not enough to take the pruritus under control (8,9).

Pruritus is described as the worst and most disturbing symptom by patients receiving HD treatment (3,6,9). Uremic pruritus can cause physical, psychosocial, and emotional deterioration in general health perceptions of patients (1,6). Uremic pruritus generally reduces the dermatological quality of life of HD patients due to the continuous sense of itching and lesions and dryness occurring due to itching (10). Uremic pruritus causes sleeplessness, fatigue/exhaustion, anger, anxiety, depression as well as decreased self-esteem, social isolation, a decline in self-care and quality of life, along with the scratching behavior during the day and especially at night, making the disease management more difficult (4-8,11-13). Due to these itching-related problems, patients have a 17% higher mortality rate, along with pruritus and sleep problems, than regular HD patients (14). In a study conducted on HD patients, it was indicated that the dermatological quality of life of patients with uremic pruritus was low (4,10,15).

The impact of uremic pruritus on quality of life, which affects at least half of HD patients, is a critical issue that should be properly addressed by clinicians. No study was found regarding dermatological and general quality of life and their relationship in patients with uremic pruritus. The effects of HD on the dermatological and general quality of life of the patients are not adequately assessed, especially in Turkey. The results of assessments can help the HD healthcare team question and treat the pruritus experienced by patients so that dermatological and kidney disease quality of life of patients can be improved. The HD healthcare team plays an important role particularly in the identification of the presence and severity of uremic pruritus, its care, and treatment, as well as in the holistic assessment of patients and improvement of their dermatological and kidney disease quality of life.

MATERIAL AND METHOD

Patient selection

The study was carried out in a descriptive fashion to determine the dermatological and kidney disease quality of life of patients with uremic pruritus receiving haemodialysis treatment.

The study population consisted of 224 patients treated in a private provincial HD center and an HD unit of a university hospital. No sample selection method was used in the study; the entire universe, 200 patients, were included in the sample (89.2% of the invited participants). After the study, post-hoc power analysis was conducted. The power of the test was measured using the G*Power 3.1 tool. According to the relational findings of the study, the effect size was found to be 0.42, and the power of the study was measured as 99%.

Inclusion and exclusion criteria; Patients who were 18 years of age or older, had cognitive competence to answer questions, had been receiving HD treatment for at least 6 months due to CKD, did not have a chronic skin disease, and who gave their verbal and written consent to participate in the study were included in the sample.

Data collection and measures

Before collecting data, the patients were informed about the purpose of the study, and their written and verbal informed consent was acquired. The questionnaire was filled out in an average of 15-20 minutes for an average patient immediately after the HD treatment was initiated. The research was carried out between January-30 November 2020.

The related literature was examined when creating the patient information form. The form consists of 8 questions regarding socio-demographic characteristics of the patient, 11 questions regarding characteristics of the disease, CKD and HD duration, patient's compliance to diet and treatment, presence and intensity of itching, 19 questions in total (1-6).

5-D Itch Scale was developed by Elman, Hyman, Gabriel, and Mayo in 2010. Five dimensions of itching beginning with letter “D” are included in 5-D pruritus scale and are evaluated during the previous 2 weeks. The first three items (duration, degree, direction) are single-item domains and are scored from 1 (‘less involvement according to the item’) to 5 (‘most involvement according to the item’). Disability represents a multiple-item domain and includes the effects of itching on daily activities such as sleep, leisure/social activities, housework/errands and work/school. The score for the disability domain is obtained by obtaining the highest score within four evaluated sub domains. The fifth item (Distribution) evaluates the presence of itching within 16 body parts over the previous 2 weeks. Regarding the number of affected parts, five scoring bins are constructed. 5-D scores range from 5 (no pruritus) to 25 (most severe pruritus). Altınok Ersoy and Akyar performed the validity and reliability analyses of the scale in Turkey, and its internal consistency was ensured with Cronbach's Alpha coefficient of 0.608. The items and dimensions of the scale remained the same following the validity and reliability analyses, and no changes were made in the scoring or calculation (16,17). In this study, Cronbach's Alpha of the scale was found to be 0.853.

The Dermatological Quality of life Index, DLQI, created by Finlay and Khan in 1994, is a simple, short, understandable test and is the most important and commonly used across dermatology-related tests. Its Turkish validity and reliability analyses were performed by Öztürkcan et al. in 2006. It consists of 10 questions with possible answers each and is based on the patient's feelings regarding skin-related symptoms, their daily activities, free time

occupations, school/work life, personal relationships, and treatment. DLQI is scored between 0-30 points. A higher score means quality of life is affected negatively. Öztürkcan et al. (2006) measured Cronbach's Alpha of the scale as 0.85 in their study (18,19). In this study, Cronbach's Alpha of the scale was measured as 0.766.

Kidney Disease Quality of Life Form (KDQOL-36) is the most commonly used scale among the disease-specific scales in CKD cases. KDQOL-36 was developed in the USA in 1994 by Ron Hays et al. This is a scale used to monitor patients with CKD and various treatment effects, and well-being of the patients are evaluated in the light of their own expressions. The scale originally consisted of 134 items. However, since it took too much time to complete the scale, it was reduced to 80 items by expert researchers (20). The scale was revised by the Kidney Disease Quality of Life Development Center (Research and Development-RAND) in 2000, reducing the scale to 36 items and naming it KDQOL-36. The questionnaire contains 36 items divided into 5 dimensions. SF-12 (12 items); Items related to kidney disease (5 dimensions/24 items): List of symptoms/issues (12 items), Effects of the kidney disease (8 items), Burden of the kidney disease (4 items), SF12 physical component (6 items), SF12 mental component (6 items). The scale is used to determine the quality of life of individuals with CKD between stages 1-5 and receiving HD treatment (https://www.rand.org, access date: January 1, 2020). Yıldırım et al. performed the Turkish validity and reliability study of the scale, and Cronbach's Alpha value of the study was measured to be 0.84-0.91. Scores range from 0 to 100 in each dimension, and higher scores reflect better health quality in life (21). Cronbach's Alpha of the scale was found to be 0.868 in our study.

Data analysis

The data were evaluated using the Statistical Package for Social Science 22.0 (SPSS) software. Descriptive statistics were given as the number of units (n), percent (%), average and standard deviation values. The conformity of the data to normal distribution was determined by Shapiro-Wilk and Kolmogorov-Smirnov tests. Parametric tests were used for normally distributed data, and nonparametric tests were used for non-normally distributed data. Cronbach's α values of the scales used were calculated. $p<0.05$ value was considered statistically significant in the comparisons.

Ethical considerations

To be able to conduct the study in institutions, permission (no: 20/232) was acquired from a University Non-Invasive Clinical Research Ethics Committee (Approval no: 20/114). Written permissions were acquired from the institutions where the research was conducted after submitting a written application with a form including information regarding the purpose and scope of the study. Throughout the study, the ethical principles of the Declaration of Helsinki were complied with, and the confidentiality of individual information was maintained. Out of the consideration of the willingness to participate and voluntariness principles, the process took off by acquiring the verbal and written consents of the participants.

RESULTS

It was determined that the age average of patients with pruritus was 56.83±12.55 years, 46.6% of them were between 51-64 years old, 61.2% were male, 30.1% were primary school graduates, 84.5% were married, 85.4% were unemployed, 65% were from the middle class, 58.3% of them did not receive social support. The average age of the patients without pruritus participating in the study is 52.69±13.69 years, 44.3% of them were between 51-64 years old, 55.7% were male, 26.8% were high school graduates, 79.4% were married, 77.3% were unemployed, 63.9% were from the middle class, 67% did not receive social support. It was stated that patients with and without pruritus had similar sociodemographic characteristics ($p>0.05$). It was found that 87.4% of the patients with pruritus had a secondary chronic disease, 19% had diabetes as their secondary chronic disease, 11.7% did not comply with treatment, 54.4% did not comply with their diet, 46.6% had been receiving dialysis treatment for 1-5 years. As for patients without pruritus, it was stated that 86.6% of them had a secondary chronic disease, 22.4% had diabetes and hypertension as their secondary chronic disease, 14.4% did not comply with the treatment, 47.4% did not comply with their diet, 39% had been receiving dialysis treatment for 1-5 years. It was stated that the patients with and without pruritus had similarities in terms of the presence of the secondary diseases, types of secondary chronic diseases, the average number of drugs used, and compliance with their treatments and diets ($p>0.05$; Table 1).

It was revealed that 51.5% of the patients with pruritus began itching after initiation of the HD treatment and had pruritus and itching in the two weeks prior to this study, and 37.9% of them still suffered from itching on the day of the study. It was revealed that 36.9% of the patients intensely experienced pruritus during the evening prior to the day they underwent HD on their back (10.9%), abdomen (8.8%), and upper arm (7.6%). 70% of the patients were found to use medication to cope with pruritus, and 58.9% of them benefitted from the medication (Table 3). 5-D Itch scale score average of the patients measured to be 13.05±4.29, and it was revealed that patients experienced moderate severity itching (Table 2).

It was determined that the DLQI score average of the patients with pruritus was 11.57±4.74 and that the dermatological quality of life of 60.6% of the patients was largely or extremely affected. The average DLQI score of the patients without pruritus was measured to be 11.95±5.86, and in this regard, it was determined that the dermatological quality of life of 49.3% of the patients

Table 1. Distribution of socio-demographic characteristics of the patients (n=200)

Characteristics	Itching (n=103)		No itching (n=97)		Test
	n	%	n	%	
Gender					
Female	40	38.8	43	44.3	x^2=0.621 p=0.431
Male	63	61.2	54	55.7	
Age (year) X±SD (min-maks.)	56.83±12.55 (30.00 - 90.00)		52.69±13.69 (21.00 - 82.00)		t=2.228 p=0.270
Educational level					
Illiterate	7	6.8	8	8.2	x^2=7.614 p=0.179
Literate	12	11.7	4	4.2	
Primary school graduates	31	30.1	20	20.6	
Secondary school graduates	21	20.4	25	25.8	
High school graduates	22	21.3	26	26.8	
University	10	9.7	14	14.4	
Marital status					
Married	87	84.5	77	79.4	x^2=0.875 p=0.350
Single	16	15.5	20	20.6	
Employment					
Employed	15	14.6	22	22.7	x^2=2.183 p=0.140
Unemployed	88	85.4	75	77.3	
Income status					
Well class	23	22.3	23	23.7	x^2=0.054 p=0.973
Middle class	67	65.0	62	63.9	
Low class	13	7	12	12.4	
Receive social support					
Yes	43	41.7	32	33.0	x^2=1.635 p=0.201
No	60	58.3	65	67.0	
Secondary chronic disease					
Yes	90	87.4	84	86.6	x^2=0.027 p=0.870
No	13	12.6	13	13.4	
Number of drugs used X±SD (min.-maks.)	6.41±2.21 (1.00-12.00)		6.27±2.58 (2.00-17.00)		t=0.412 p=0.681
Compliance to treatment					
Yes	91	88.3	83	85.6	x^2=0.342 p=0.559
No	12	11.7	14	14.4	
Diet compliance					
Yes	47	45.6	51	52.6	x^2=0.962 p=0.326
No	56	54.4	46	47.4	
Dialysis time					
1 years and less	15	14.6	30	30.9	x^2=10.519 p=0.033
1- 5 years	48	46.6	38	39.2	
6- 10 years	18	17.4	19	19.5	
11-15 years	11	10.7	5	5.2	
16 years and more	11	10.7	5	5.2	

X^2= chi square test, t= t test

Table 2. Distribution of pruritus characteristics of hemodialysis patients (n=103)

Characteris	n	%
Itch duration, presence (month) X±SD (min.-max.)	14.40±9.75 (3-48)	
Presence of itch in the last two weeks		
Yes	103	51.5
No	97	48.5
The presence of itch today (n=103)		
Yes	39	37.9
No	64	62.1
Time the itch of most intense (n=103)		
During hemodialysis	22	21.4
The night of hemodialysis	38	36.9
The day left hemodialysis	13	12.6
Continually	30	29.1
Locations of itch*		
Back	67	10.9
Abdomen	54	8.8
Upper arms	47	7.6
Lower legs	46	7.5
Chest	42	6.8
Soles	38	6.2
Tops of feet/toes	38	6.2
Tops of hand/fingers	36	5.8
Forearms	35	5.7
Face	34	5.5
Buttocks	33	5.3
Points of contact w/ Clothing (e.g waistband, undergarment)	33	5.3
Head and scalp	31	5.0
Thighs	29	4.7
Groin	27	4.4
Palms	27	4.4
Medication for itch (n=103)		
Yes	73	70.9
No	30	29.1
Benefit status of drugs (n=103)		
Yes	43	58.9
No	30	41.1

* More than one answer was given

was largely or extremely affected. The dermatological quality of life of the patients with pruritus was determined to be 20% worse on average. It was revealed that the average KDQOL-36 score of the patients with pruritus was 59.36±12.27, and their kidney disease quality of life was of moderate level. The average KDQOL-36 score of the patients without pruritus was measured to be 60.08±12.97, and the kidney disease quality of life of the patients was found to be of moderate level (Table 3).

Table 3. Distribution of 5-D itch, DLQI and KDQOL-36 scales scores of hemodialysis patients (n=200)

Scale and subsections	X±SD (min-max)	
5-D Itch Scale	13.05±4.29 (6.25-25.00)	
Duration	2.14±1.36 (1.00-5.00)	
Degree	3.01±0.90 (2.00-5.00)	
Direction	3.11±1.16 (1.00-5.00)	
Disability	2.39±0.98 (1.00-5.00)	
Distribution	2.41±1.61(1.00-5.00)	
	Itching X±SD (min-max)	**No itching X±SD (min-max)**
DLQI	11.57±4.74	11.95±5.86
Dermatological quality of life impact states	n (%)	n (%)
No effects (0-1)	0 (0)	1 (1)
Small effects (2-5)	3 (3.1)	9 (8.7)
Moderate effects (6-10)	36 (35.0)	41 (42.3)
Very large effect (11-20)	55 (53.4)	45 (46.4)
Extremely large effect (21-30)	7 (7.2)	3 (2.9)
	X±SD (min-max)	**X±SD (min-max)**
KDQOL-36	59.36±12.27	60.08±12.97
Subsections		
Physical component	44.30±28.18	53.57±26.66
Mental component	48.10±21.03	47.81±23.68
Burden of the kidney disease	41.50±19.86	41.43±20.40
List of symptoms	73.89±11.37	75.73±11.17
Effects of the kidney disease	66.23±12.47	60.02±13.97

It was stated that the average DLQI score of the patients who had been consistently suffering from pruritus and was also experiencing pruritus on the day of the study ever since the initiation of the haemodialysis treatment was significantly higher ($p<0.05$). It was found that the average KDQOL-36 score of the patients who experienced consistent pruritus since the initiation of haemodialysis treatment was statistically significantly lower ($p<0.05$).

When general scores of the scales are correlated, it was discovered that there was a moderately positive and significant correlation between the average 5-D itch scale score and the average DLQI score ($r=0.425$, $p=0.001$). It was determined that there was a moderately negative

Table 4. Correlation between 5-D Itch, DLQI, and KDQOL-36 scales score of patients with Itching (n=103)

		5-D itch scale 1 duration	5-D itch scale 2 degree	5-D itch scale 3 direction	5-D itch scale 4 disability	5-D itch scale 5 distribution	5-D itch score
KDQOL-36 physical component	r	-0.251*	-0.133	-0.053	-0.297**	-0.081	-0.212*
	p	0.011	0.179	0.596	0.002	0.414	0.031
KDQOL-36 mental component	r	-0.353**	-0.123	-0.237*	-0.354**	-0.138	-0.299**
	p	0.001	0.215	0.016	0.001	0.163	0.002
KDQOL-36 burden of the kidney disease	r	-0.367**	-0.167	-0.261**	-0.383**	-0.219*	-0.355**
	p	0.001	0.091	0.008	0.001	0.026	0.001
KDQOL-36 list of symptoms	r	-0.301**	-0.273**	-0.289**	-0.257**	-0.153	-0.345**
	p	0.002	0.005	0.003	0.009	0.123	0.001
KDQOL-36 effects of the kidney disease	r	-0.198*	-0.089	-0.149	-0.096	-0.055	-0.128
	p	0.045	0.369	0.133	0.334	0.580	0.197
KDQOL-36 score	r	-0.401**	-0.207*	-0.229*	-0.347**	-0.158	-0.429**
	p	0.001	0.036	0.020	0.001	0.112	0.001
DLQI score	r	0.268**	0.304**	0.117	0.287**	0.234*	0.425**
	p	0.006	0.002	0.241	0.003	0.017	0.001

r= spearman rank correlation coefficient *significant correlation at the 0.05 level **significant correlation at the 0.001 level

and significant correlation between the average 5-D itch scale score and the average KDQOL-36 score (r=-0.429, p=0.001). It was revealed that, as the severity of pruritus increased, dermatological and kidney disease quality of life accordingly decreased (Table 4).

Effects of itching on dermatological and kidney disease quality of life: regression analysis table 5. Model 1-2 The analysis of the t test results for the significance of the regression coefficients revealed that itch was significant predictors for dermatological quality of life (R^2=.181) and kidney disease quality of life (R^2 =.184).

Table 5. Effect of itch severity on DLQI and KDQOL-36 in patients receiving hemodialysis: regression analysis (n=103)

Variables	B	SE	B	t	p
Model 1					
Constant	21.955	1.904		11.528	<.001
Kidney disease quality of life	.150	.031	.429	4.776	<.001
R=.429 R^2=.184				F=22.811 p<0.001	
Model 2					
Constant	8.590	1.019		8.431	<.001
Dermatological quality of life	.385	.082	.425	4.723	<.001
R=.425, R^2=.181, F=22.307, p<0.001					

DISCUSSION

Uremic pruritus, which is considered a symptom that is an important health problem that affects the dermatological and general quality of life of patients receiving HD. It was determined that 51.5% of the patients experienced pruritus in the last two weeks and 37.9% of the patients on the day of the study. In a study focused on skin problems of HD patients, it was revealed that 65.1% of the participants experienced itching stated that 49.1% of the patients stated that they experienced itching in a study they conducted on patients receiving HD treatment (1,13). Our results are in parallel with those of these studies. More than half of patients receiving HD treatment were seen to experience pruritus on various severities. We determined in our study that the 5-D Itching scale score of HD patients was moderate severity and the patients experienced moderate itching. Other studies concluded that HD patients experience moderate itching, similar to our findings (12,22). We observed in our study that HD patients mostly experienced severe pruritus on their back region. When other studies in the literature are examined (24,25), it is seen that Kılıç Akça and Taşcı (2014) concluded in their study that patients experienced pruritus on their arm and back where the fistula is located (23). Other studies revealed that pruritus was mostly localized on the extremities and back (22,24). We observed in our study that HD patients mostly experienced pruritus in the evening prior to the day they would undergo haemodialysis (36.9%). When other studies in the literature are examined,

it is seen that they reveal that pruritus reaches its peak in the evening prior to the day of HD treatment and continues during HD (5,6). Our results are in parallel with those of these studies.

The dermatological quality of life of more than half of the HD patients was found to be low, and those who experienced consistent itching were found to have even lower quality of life as a result of severe and widespread itching. It was revealed in our study that the DLQI of the patients with pruritus was largely and extremely affected by 60.6%. In a study conducted in Turkey, it was concluded that the dermatological quality of life of the patients was low after the initiation of the haemodialysis treatment (1). Küçükünal et al. stated in their study that the dermatological quality of life of patients undergoing HD was low.10 Adejumo et al., (2016) reported in their study that the dermatological quality of life of 12.4% of the patients with ESRD was moderately affected, and 3.8% of the patients' quality of life was severely affected (2). Satti et al., (2019) found in their study that the DLQI score of the patients was 9.8±1.7, 34.1% of them were moderately affected, and they exhibited depressive symptoms (13). Similarly, studies examining the quality of life of dialysis patients with uremic pruritus revealed that the dermatological quality of life decreased significantly in pruritus cases (15,25). Some sources support our findings. However, the dermatological quality of life of HD patients in studies conducted in other countries was reported to be higher compared to our study. One can say that the reason behind this difference is cultural differences that shape treatment compliance, living conditions and quality of life perceptions of HD patients.

In our study, when the KDQOL-36 scale was examined in terms of pruritus, it was observed that the general quality of life the patients was moderate, but it was found that the general quality of life decreased as the severity of pruritus increased. In a study conducted in Turkey in 2015, which supports our results, it has been observed that pruritus significantly decreases the quality of life of HD patients.10 Similarly, in other studies, it was determined that HD-related pruritus reduced the quality of life patients (2,4-6). The literature also points out that pruritus affects the overall quality of life HD patients (26-29).

CONCLUSION

In this study, it was concluded that more than half of the HD patients experienced moderate uremic pruritus. It was determined that uremic pruritus decreased the dermatological and kidney disease quality of life of the patients. In this regard, it is recommended that patients with uremic pruritus receiving HD treatment should be thoroughly evaluated for the reasons behind pruritus and relative problems. It can additionally be recommended to carry out more comprehensive studies by ongoing counseling HD patients in order to help them cope with this situation and to increase their dermatological and general quality of life.

Limitations of the study

The results of this study are valuable in terms of discussing in dermatological and general quality of life HD and influential factors such as pruritus. However, there were some limitations in the study. Firstly, the study was completed with relatively small samples and may only be generalized for its of population. Secondly, a cross-sectional study design is limited in establishing a causal association between dermatological quality of life and pruritus. In order to be able to show causality, longitudinal studies are needed.

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Research Article

"Cadavers with a Soul": A Qualitative Study of Individuals Who Donate Their Body

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Abstract

Aim: The study was conducted to determine the thoughts of individuals who donate their body.

Material and Methods: This study was qualitative research conducted using the case study design. The sample was determined with the purposeful sampling method and 9 individuals who stated that they would donate their body were interviewed. The data were collected between March and April 2022 with an information form and a semi-structured interview form and analyzed with the content analysis method.

Results: The mean age of individuals was 67.11±4.10 years. Five of the participants were male and four were female. Eight out of nine people were married, all were university graduates and retired, and eight had good income level. Two main themes and five sub-themes were revealed in the research. The themes were determined to be support *(contribution to health education, chain of immortality)* and obstacles *(pressure from family and religion, awareness, uncertainty)*.

Conclusion: Individuals want to donate their bodies to contribute to health education by leaving a legacy for science and think they will be immortal by offering their cadaver for science. Additionally, they feel pressure due to families and religious beliefs and think that society is ignorant about body donation and that the functionality of the body donation system is inadequate.

Keywords: Cadaver, body donation, thoughts, qualitative study

INTRODUCTION

Cadaver dissection is an integral part of medical education (1). Basic education obtained by observing the organization, structures and tissues of the human body in the real environment is very important for professional development of individuals training in the field of health. Therefore, education and training with cadavers has unique aspects (2-3). Today, with the advances of technology, although there are many visual education methods (4), studies on cadavers are still needed in clinical education and especially surgical sciences in order to improve the dexterity of health professionals (5). It is critical to have enough donors who donate their body to support health education (6). Voluntary body donations made by the general public in England and America meet the needs of health education institutions (7). In other countries such as Nigeria, India and Iran, the bodies of orphans are donated to health institutions as cadavers (8-10). This situation is caused by many factors such as people's religious beliefs, social taboos, and cultural characteristics (11-14). In order to increase the number of individuals who donate their body, it is emphasized that the perception of the individual about whole body donation and what affects this perception should be known in detail (15). However, it was reported that people should be encouraged to donate because there is a shortage of cadavers all over the world (16).

In this context, it is important to determine the thoughts of individuals who plan to donate their body. In addition, determining the thoughts of individuals who plan to donate their body can contribute to increasing body donation in health education and research studies and awareness

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studies about this subject. In addition, studies that explain the thoughts of individuals who plan to donate their body will contribute to the literature and will also be beneficial to readers. For this reason, this study was carried out to determine the thoughts of individuals who plan to donate their body.

Questions of Research

- What do individuals who plan to donate their body think about body donation?

MATERIAL AND METHOD

Study Type

This research was a qualitative study using a case study design to determine the thoughts of individuals who will donate their body.

Study Population and Sample

The population of this research consists of individuals who applied for body donation in the anatomy department of a faculty hospital in a province of Kayseri in 2022. The sample was determined by the purposeful sampling method and 9 individuals who agreed to participate in the study constituted the sample.

Inclusion criteria for the study

Individuals over the age of 18, who applied for body donation (signed an agreement), talked and explained the subject of body donation with their heirs (family) and volunteered to participate in the study were included. Since having a mental illness would affect the results of the study, individuals with a diagnosis of mental illness, who made any payment or financial demands and did not volunteer to participate in the study were not included in the study.

Data Collection Tools

Data collection tools consist of two parts; "Information Form" with introductory information about individuals and the semi-structured "Interview Form".

Information Form: This consists of questions that include the introductory characteristics of individuals regarding their age, gender, marital status, employment status, income level, education level and body donation (Ten Questions).

Semi-structured Interview Form: This consists of open-ended questions evaluating the thoughts about body donation of individuals who plan to donate their body (Table 1).

Collection of Data

The data were collected by the researchers between March and April 2022 using the personal information form and a semi-structured interview form. The questions included on the semi-structured interview form are listed in Table 1. In the interviews, the questions were asked in the same order and additional explanations were made when necessary. All of the stated opinions were evaluated as qualitative data. The data were recorded as interview notes with the permission of the participants who refused a voice recorder. Interviews were continued until data saturation was reached and were terminated when data saturation was reached. Interviews lasted an average of 40-45 minutes. The interviews were conducted in an environment where the researcher and participant could see each other easily, where there was no noise and disruption, and where comfortable communication was possible.

Table 1. Semi-structured İnterview questions

1. What do you think about body donation?
2. What does body donation mean to you?
3. What do you think influenced your decision to donate your body?
4. What do you think are the positive and negative aspects of body donation?
5. Is there anything you want to add that I didn't ask about and you think is important?

Evaluation of the Data

Quantitative data on the Personal Information form were evaluated in the computer environment and expressed as numbers and percentages. Qualitative data were analyzed by content analysis and written as raw data. Qualitative data were first written down in the computer environment by the researchers and then analyzed by content analysis. The themes and sub-themes were formed by revealing the relationships between the categories in the research data. Expert opinion was obtained from two independent researchers with qualitative research training and experience regarding the validity of the themes and sub-themes. After the expert opinions were received, unnecessary coding was removed, the connections between them were regrouped, the main idea in the expressions was discovered and the themes and sub-themes were finalized. Themes were supplemented with direct quotes when necessary. Citations are shown as participant number, gender and age (P1-Female 50, P2-Male 60).

Credibility and Trustworthiness of Qualitative Data

In this study, long-duration interviews, participant confirmation and expert review methods were used to ensure credibility. Before the in-depth interview, necessary information was provided for the establishment of secure communication between the researcher and participant, and data were collected on the planned day, time and place. For participant confirmation, at the end of the interview the data obtained by the researcher were summarized for the individuals and the individuals were

asked to state their thoughts on the accuracy. In addition, individuals were asked whether they had any final opinion that they would like to add. The interview was terminated by recording the additional explanations made. During the planning phase of the study, expert opinion was obtained regarding the questions on the interview form and the themes created. Thus, attempts were made to ensure credibility by obtaining expert opinions from the beginning to the conclusion of the research. Researcher triangulation was used to ensure reliability in the study. In the name of confirmability, interview notes were taken as raw data and notes about the statements of the participants during the interview, and the statements of individuals were directly included in the research report. It is thought that the research results obtained from the interviews with this sample group can be used in similar sample groups in different environments, and thus the transferability criterion was met.

Reflexivity

The self-reflexive knowledge of the researchers in this study is as follows, the first researcher (Ph.D) completed their doctorate in psychiatric nursing and has scientific research experience in qualitative research. The fact that the researcher conducting the interviews was a psychiatric nurse enabled effective communication with the participants. The second researcher (Ph.D) completed their doctorate in anatomy. They have scientific research experience in anatomy and body donation.

Ethical Aspects of the Study

The study protocol was carried out in accordance with the Helsinki Declaration of 1975 and approval was obtained from the Nevşehir Hacı Bektaş Veli University Scientific Research Ethics Committee (Decree no: 2022.03.028). Institutional permission was obtained from the anatomy department of the Erciyes University in the province where the research was conducted (Decree no: 220297). Written informed consent was obtained by explaining the purpose of the study to all participants. The names of the participants were kept confidential, and the participant number, gender and age were indicated as codes instead of names for the statements of the individuals (P1-Female 50, P2-Male 60).

RESULTS

Interview and sample characteristics

The mean age of the individuals was 67.11±4.10 years. Five of the participants were men and four were women. It was determined that eight of the nine people were married, all of them were university graduates and retired, and eight of them had good income level.

Themes emerging from the interviews

In the research, two themes and five sub-themes related to the thoughts of individuals who plan to donate their body emerged. The themes and sub-themes obtained in the research are presented in Table 2.

Table 2. Themes emerging from the interviews

Main Theme	Sub-Themes
Support	Contribution to Health Education Chain of Immortality
Barriers	Pressure from Family and Religion Awareness Uncertainty

Theme 1. Support

Most of the participants stated that they thought that the knowledge of students who used their body health/medical education would be improved. Individuals stated that their bones can contribute not only to the development of the students, but also to the professions of the students, the patients they treat and care for, society and humanity. However, they emphasized that they believed that the contribution of their body, as a cadaver, would be immortal and that they would live forever as cadavers with souls.

a. Contribution to Health Education

All of the participants stated that the main reason for body donation was that it is difficult to obtain cadavers in health/medical education, and therefore they wanted to support medical education and contribute to science with their body. They also stated that they believed their body would be useful to assist in medical science and teaching, and would be used for teaching and research.

"I learned that it is difficult to find cadavers for the education of medical students, that the cadavers are worn out and it is difficult to work on them. I think body donation is a very good decision. I believe it is necessary for medical school students to receive a better education." (P1, Male - 67)

"At least medical students will benefit from my body, which would just rot under the ground... I believe that many students will complete their education in this way. In addition, I will contribute to the development of not one but two, but hundreds of health/medical students and therefore thousands of patients they will treat" (P2, Female - 61)

b. Chain of Immortality

Almost all of the participants described body donation as a legacy left to science. They believed that they would become immortal in science with the legacy of their body. In addition, they stated that they were influenced by people around them in making body donations, wanted to be immortal in their impact, and that a chain of immortality would be formed with the widespread use of body donation.

"My body is a legacy I leave to science. While people are alive, they have buildings built, water fountains opened or schools built and named after them. To me, body donation

is the same thing, their name, my soul will be immortal!

"The transformation of my body into a cadaver is a symbol of immortality, isn't it? The people around me had a great influence on my decision to donate my body. I feel strong, happy, and well, as if holding hands in a science circle."

Themes 2. Barriers

Most of the participants stated that it was not easy to decide on the issue of body donation, especially that family members objected, and they were able to convince them after long struggles. They stated that there was not enough awareness-raising activities about body donation, and they had to deal with many time-consuming procedures when they decided to donate. In addition, they ambiguously criticized the different systems for body donation in each country and emphasized the necessity of an advanced system for body donation.

a. Pressure from Family and Religion

Most of the participants stated that the pressure of their families and religion was a major obstacle to body donation. They stated that family members associated body donation with worthlessness and opposed it, and they perceived it as a difficulty when they had to convince them otherwise. However, they stated that people around them tried to discourage them because of their intense religious beliefs and that they were uncomfortable with this situation.

"At first, my family was surprised… they didn't want me to donate my body and they were totally against it. I told them they had to respect my decision, although reluctantly they agreed after the last argument."

"Religiously, they say that the body will come to life again in the future. That's why I was put under a lot of pressure. Frankly, there is no meat on the bone when it is under the ground, if it will come to life, it can also come to life when used as a cadaver, right?"

b. Awareness

Most of the participants stated that there was not enough awareness in society about body donation. They stated that when they started to think about body donation, their decision was not clear, but the fact that there were people around them who planned to donate their bodies and they could exchanged opinions with them enabled them to make a firm decision to donate their body.

"I had a chance at body donation; While I was studying at university, I participated in an event for body donation. That's when the lights came on in my mind and soul. I said I could do it too."

"There are many people who ask what body donation is, I wish they knew what they can contribute as a cadaver…"

c. Uncertainty

Almost all of the participants criticized ignorance about body donation and the fact that there are systems that differ from country to country. They expressed that they were concerned about the possibility that their body would not be used as a cadaver even though they donated it, and thought that the functionality of the body donation system was insufficient.

"There are huge gaps in the law. So I have concerns about whether I will be used as a cadaver after I die. I'm also afraid that instead of my body being donated to medical school I will be buried in a paupers grave. I don't think this system has been developed enough."

"I was given an identity card, but I don't know how clearly it is stated in the population registration. I have the identity card given to me in my pocket, but I am worried if I die at home or die in a traffic accident, if I don't have the identity card with me or it gets lost. Since it is not systematized, this situation gives me anxiety."

DISCUSSION

Cadavers form the basis of morphological research at all levels of health science education (17). In one study, it was found that participants wanted to further medical education/research (primary cause), want their body to have meaning after life (secondary cause), and donate their body to be useful (18). In another study, the majority of the participants were willing to donate their body for medical/research and learning, and about half of them found body donation contrary to their religious beliefs.16 Similar to the literature, the participants in this study stated that their bones could contribute not only to the development of the students, but also to the professions of the students, the patients they will treat and care for, society and humanity.

From ancient times to the present, both cultural and religious aspects and legal regulations have effectively limited the use of corpses for scientific research and teaching (19). Religious factors, socio-economic status, education, marital status or age may be the main factors affecting body donation (20). In a study, although body donation was thought to be a tool for learning surgical practices, it was perceived as an act contrary to belief (21). In another study, it was reported that body donation creates communication anxiety about death, and at the same time, religiosity or belonging to a certain religion negatively affects the decision to donate (22). The reluctance to donate is mostly affected by religion, psychological disability and family reasons (23). In a study, while most of the participants were in favor of donating their body in the future, very few of their families and relatives agreed (7). As a matter of fact, most of the participants in this study stated that despite their determination about body donation, they were pressured by their families due to their religious beliefs and this pressure was a major obstacle to body donation.

In order to train health professionals, it is necessary to raise awareness in society about the ongoing need for and value

of body donation (24). Although body donation is a very controversial issue, it is discussed very little by religions, traditions or society, so there is a need for awareness campaigns about body donation (25). Ignorance of body donation programs, lack of knowledge and religious beliefs are barriers that limit body donation (26). Similarly, most of the participants in this study stated that society was ignorant about body donation and they thought that the functionality of the body donation system was insufficient, so they had anxiety and fears about the completion of their donation.

In the study, the participants stated that when they started to think about body donation, their decisions were not clear. It was found that the participants were encouraged toward body donation after motivation through the media (family members, faculties, celebrities, etc.). It was also emphasized that there is a need for body donation laws to facilitate medical teaching, advance knowledge and expand the field of medical science (27). In this context, awareness studies about body donation may contribute to increasing donations.

Limitations of the Study

The country where the study was conducted is a country with regional differences in terms of culture and religious beliefs. In addition, all of the participants in the study had higher education (undergraduate and graduate) levels. These cases were considered to be limitations.

CONCLUSION

This study provides important findings about determining the thoughts of individuals who plan to donate their body and the approach to body donation. In the study, individuals plan to donate their body to contribute to health education by leaving their body to science and think that they will become immortal with the support they offer as cadavers. However, in the study, individuals emphasized that they were pressured by their families and religious beliefs about body donation, that society was ignorant and they thought that the functionality of the body donation system was insufficient, and therefore they had anxiety and fears. Activities such as body donation week can be organized in order to raise awareness in society about body donation. Donation programs can be organized by learning and taking into account the perspectives and characteristics of the society.

Limitations of the study

The results of this study are valuable in terms of discussing in dermatological and general quality of life HD and influential factors such as pruritus. However, there were some limitations in the study. Firstly, the study was completed with relatively small samples and may only be generalized for its of population. Secondly, a cross-sectional study design is limited in establishing a causal association between dermatological quality of life and pruritus. In order to be able to show causality, longitudinal studies are needed.

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Research Article

The Effects of Taste Changes on the Quality of Life of Patients Receiving Chemotherapy Treatment

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Abstract

Aim: The aim of this study is to determine the effect of taste changes on quality of life in cancer patients taking chemotherapy

Material and Methods: This descriptive study was conducted between January 2020 and September 2020 with cancer patients receiving chemotherapy in the inpatient and outpatient unit of a university hospital. The sample consisted of 466 cancer patients who met the inclusion criteria and received the same treatment at the same university hospital. Data were collected using the Introductory Information Form, the European Organization for Cancer Research and Treatment Quality of Life Scale Version 3 (EORTC QLQ-C30 Version 3.0), and the Chemotherapy-Induced Taste Change Scale (CiTAS).

Results: While the mean total score of EORTC QLQ-C30 was 62.97±13.31, the mean total score of CiTAS was found to be 40.43±17.84. Statistically significant correlations were found between total scores of EORTC QLQ-C30 and CiTAS scales and sub-dimension scores ($p<0.001$). In the regression analysis, it was found that the EORTC QLQ-C30 total score average of the individuals had a statistically significant and negative effect on the CiTAS total score average.

Conclusion: As a consequence, it was determined that taste changes in cancer patients receiving chemotherapy treatment negatively affect the quality of life.

Keywords: Cancer, chemotherapy, taste changes, quality of life

INTRODUCTION

Cancer is a set of diseases that emerge as a result of the mutation or abnormal activation of genes that control the growth and proliferation of cells and affect the person in terms of many psychological, physiological, economic, and social aspects (1). In Turkey and the rest of the world, cancer is in second place following cardiovascular diseases (2,3). According to the 2020 data of the World Health Organization (WHO), 19.3 million new cancer cases were diagnosed, and the yearly number of cancer diagnoses is anticipated to reach 30.2 million in 2040 (2).

Chemotherapy, which is applied to kill cancer cells or control their growth, has a significant place in cancer treatment (4). In individuals who are receiving chemotherapy treatment, in addition to symptoms such as pain, fatigue, insomnia, neutropenia, thrombocytopenia, bleeding, hiccups, dyspnea, mucositis, nausea, vomiting, anorexia, cachexia, diarrhea, constipation, itching, alopecia and skin and nail changes, changes in the sensation of taste may also be seen highly frequently (5,6). The prevalence of taste changes varies based on the type of antineoplastic agent, the localization of the tumor, and its type (7,8). According to recent studies, the prevalence of taste alterations in patients who are receiving chemotherapy treatment varies in the range of 20-86% (9-11).

Changes in taste affect the individual negatively in the psychological (stress, depression, reduced treatment adjustment, dysfunctional coping mechanisms, dislike/disgust for some foods), physiological (loss of appetite, weight loss, malnutrition, dry mouth, olfactory dysfunction, weakened immune system), and social (prolonged hospitalization) sense (7,10,12,13). Taste changes, which affect the life of the patient in many ways, also influence their quality of life negatively (7,9,13). It is important for cancer patients who are receiving chemotherapy treatment

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to live a quality life. Nurses assume important roles in the management of taste changes in cancer patients and the impacts of these changes on their quality of life (14). In this sense, a nurse should evaluate the taste change, its type, and its severity in their patients. They should support the patient in coping with the adverse effects of the treatment, plan interventions relevant to taste changes and implement these interventions (15). In the review of the literature on taste changes in chemotherapy patients, it was seen that there are very few studies conducted in Turkey on this topic. Hence, this study was carried out with a large sample to search the effects of taste changes on the quality of life of patients taking chemotherapy treatment.

MATERIAL AND METHOD

Research Type

This is a descriptive study.

Settings, Time, and Location

This study was carried out between January 2020 and September 2020 with patients who were receiving cytotoxic treatment at the medical oncology inpatient clinic and the outpatient chemotherapy unit of the Inonu University Turgut Ozal Medical Center Research and Training Hospital.

Population and Sample

The population of the study consisted of oncology patients who were taking chemotherapy treatment as inpatients and outpatients at the Inonu University Turgut Ozal Medical Center Research and Training Hospital. The sample consisted of those who were taking chemotherapy at the medical oncology inpatient clinic and the outpatient chemotherapy unit between the dates given above and met the inclusion criteria of the study. The minimum required sample size was calculated as 452 oncology patients in a 95% confidence interval, with a 0.05 error margin, and 0.95 power to represent the population (n=452). The sample included 466 patients.

Inclusion Criteria

The study included patients who were conscious, able to communicate verbally, over the age of 18, literate, volunteering to participate, taken at least one course of chemotherapy and experienced chemotherapy-induced taste changes.

Exclusion Criteria

The study excluded patients who were receiving radiotherapy in addition to chemotherapy and those who did not meet the inclusion criteria.

Data Collection

The data were collected using an Introductory Information Form that was developed by the researcher, the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Version 3 (EORTC QLQ-C30 Version 3) and the Chemotherapy-Induced Taste Alteration Scale (CiTAS). Data collection took place in face-to-face interviews with the patients after they were individually informed about the study and provided consent. Each interview took approximately 20-25 minutes.

Data Collection Instruments

Introductory Information Form

The form, included nine questions on the patients' sociodemographic characteristics (age, sex, height, weight, BMI, education status, working status, income status, marital status, number of children, person/people living with them, problem/problems affecting the oral mucosa), habits (smoking and alcohol consumption status, daily oral care status and frequency), and disease-related characteristics (clinical diagnosis and stage, chronic diseases, treatment protocol, time since diagnosis, history of previous chemotherapy, duration of chemotherapy treatment, status of using other medication, problem/problems experienced other than taste changes).

Chemotherapy-Induced Taste Alteration Scale (CiTAS)

The scale, which aims to reveal the effects of taste changes that are observed in relation to chemotherapy on the person, was developed by Kano et al. in 2013 (10). It was made for validity and reliability in Turkish in 2014 by Sozeri and Kutluturkan (16). The 5-point Likert-type scale consists of 18 items and 4 subscales. The Decline in Basic Taste subscale assesses the status of sweet, salty, bitter, sour, and umami tastes to be sensed by the person; the Discomfort subscale assesses the relationship between changes in the sensation of taste and having difficulty in eating hot foods/fatty foods/meat, experiencing changes in the sensation of smell, loss of appetite, and nausea-vomiting; the Phantogeusia and Parageusia subscale assesses the status of the patient to experience phantogeusia and parageusia and the General Taste Alterations subscale assesses the status of the patient to experience cacogeusia, hypogeusia, and ageusia. The minimum and maximum scores of each subscale are 1 and 5 higher scores indicate the higher severity of the taste changes experienced by the patient and their increased discomfort associated with these changes (10). In the original Turkish article of the scale, its reliability coefficient was determined as 0.869, while this coefficient was calculated as 0.864 in this study (16).

European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Version 3 (EORTC QLQ-C30 Version 3.0)

The scale, which is used in cancer patients and has been performed to have validity, reliability, and applicability in large patient populations in 12 different countries, was made for validity and reliability in Turkish in 2008 by Cankurtaran et al. (17,18). It incorporates the categories

of Functional Scales, Global Health Status and Symptom Scales. The scale includes 30 items in total, and the first 28 items are 4-point Likert-type items. The last two items assess the patient's health status and general quality of life in the last week on a scale of 1 to 7. The minimum and maximum subscale scores in each of the three categories are 0 and 100. The first 28 items are related to the Functional Scales and Symptom Scales categories, and lower scores demonstrate higher quality of life levels, whereas higher scores demonstrate lower quality of life levels. The last two items are related to the Global Health Status category, and higher scores demonstrate higher quality of life levels, whereas lower scores demonstrate lower quality of life levels. Cankurtaran et al. reported the reliability coefficients of the scale in the range of 0.56 to 0.85, while the reliability coefficient of the scale was calculated as 0.910 in this study (18).

Data Analysis

The Statistical Package for the Social Sciences (SPSS) 25 program was used in the analyses of the data. The level of statistical significance was accepted as $p<0.05$. The analyses included percentage, frequency, mean and standard deviation values, Spearman's Correlation Analysis, and Linear Regression Analysis.

Ethical Approval

To conduct the study, ethical approval was got from the Inonu University Scientific Research and Publications Ethics Committee (2019/367), and written institutional permissions were obtained from the Chief Physician's office at the Inonu University Turgut Ozal Medical Center Research and Training Hospital and the Directorate of the Medical Oncology Department. Permissions to use the scales were obtained via e-mail from the authors who developed the scales. Consent was got from the patients who voluntarily agreed to participate in the study.

RESULTS

It was found that 57.9% of the patients were female, 51.5% were between the ages of 45 and 64, 87.3% were married, 41.6% were primary school graduates, and the incomes of 51.9% were equivalent to their expenses. The most frequently observed type of cancer was breast cancer (36.9%), the clinical stage of 40.3% of the patients was stage 2, their mean diagnosis duration was 18.60±29.13 months, and the mean duration of their current chemotherapy course was 2.82±2.01 months (Table 1).

While 72.3% of the patients reported a condition affecting the oral mucosa, the most frequently reported condition was dryness in the mouth at 57.6%. It was determined that 97.2% of the patients practiced oral hygiene daily, the mean number of their oral hygiene practices per day was 2.19±1.37, and the most frequently practiced oral hygiene method was brushing teeth at 51.4% (Table 2).

Table 1. Sociodemographic and disease-related characteristics of the patients (n=466)

Sociodemographic and Disease-Related Features		n	%
Gender	Male	196	42.1
	Female	270	57.9
Age	between the ages of 18 and 30	10	2.1
	between the ages of 31 and 44	77	16.5
	between the ages of 45 and 64	240	51.5
	65 years and older	139	29.8
Marital Status	Single	59	12.7
	Married	407	87.3
Education Status	Literate	92	19.7
	Primary School	194	41.6
	Middle School	60	12.9
	High School	82	17.6
	University	37	7.9
	Master's and Doctorate	1	0.2
Income Level	Income Less Than Expenses	207	44.4
	Income Equivalent to Expense	242	51.9
	Income More Than Expenses	17	3.6
Clinical Diagnosis	Lung Cancer	85	18.2
	Breast Cancer	172	36.9
	Hematological Cancers	21	4.5
	Genitourinary System Cancers	79	17.0
	Gastrointestinal System Cancers	86	18.5
	Other	23	4.9
Clinical Stage	Stage 1	65	13.9
	Stage 2	188	40.3
	Stage 3	162	34.8
	Stage 4	51	10.9
		Mean±Standard Deviation	
Diagnosis Duration (Months)		18.60±29.13	
Current Chemotherapy Cycle Duration (Months)		2.82±2.01	

Table 2. The characteristics of the oral mucosa and care of the patients (n=466)			
Oral Mucosa and its Care-Related Features		**n**	**%**
Is there a condition that affects the oral mucosa?	Yes	337	72.3
	No	129	27.7
What condition affects the oral mucosa? (You can mark more than one)	Dry Mouth	235	57.6
	Mouth Wound	167	40.9
	Intraoral Bleeding	6	1.5
Do you do daily oral care?	Yes	453	97.2
	No	13	2.8
How do you perform your oral care? (You can mark more than one)	Brushing Teeth	317	51.4
	Rinsing mouth with water	177	28.7
	Mouthwash	123	19.9
		Mean±Standard Deviation	
Daily Oral Care Frequency		2.19±1.37	

The mean total EORTC QLQ-C30 score of the patients was 62.97±13.31, while their mean total CiTAS score was 40.43±17.84. Among the categories of EORTC QLQ-C30, the mean scores of the patients were 8.31±2.95 in the Global Health Status category, 29.72±8.66 in the Functional Scales category, and 25.49±7.41 in the Symptom Scales category. Among the subscales of CiTAS, the mean scores of the patients were 2.04±1.30 in the Decline in Basic Taste subscale, 2.41±0.96 in the Discomfort subscale, 2.16±1.20 in the Phantogeusia and Parageusia subscale, and 2.32±1.23 in the General Taste Alterations subscale (Table 3).

Statistically significant relationships were found between EORTC QLQ-C30 and CiTAS and between the subscales of EORTC QLQ-C30 and the subscales of CiTAS ($p<0.001$). The total EORTC QLQ-C30 scores and the EORTC QLQ-C30 Global Health Status category scores of the patients were significantly and negatively related to their total CiTAS scores and their scores in all subscales of CiTAS, while the EORTC QLQ-C30 Functional Scales and Symptom Scales category scores of the patients were significantly and positively related to their total CiTAS scores and their scores in all subscales of CiTAS (Table 4).

The results of the regression analysis showed that the total EORTC QLQ-C30 scores of the patients had a negative and statistically significant affect their total CiTAS scores. Accordingly, a rise in the quality of life levels of the patients led to more favorable outcomes regarding their taste changes (Table 5).

Table 3. EORTC QLQ-C30 and CiTAS total score and sub-dimension score averages		
		Mean±Standard Deviation
EORTC QLQ-C30	**Scale Total Score**	62.97±13.31
	Global Health Status	8.31±2.95
	Symptom Scales	25.49±7.41
	Constipation	1.72±0.93
	Pain	3.98±1.82
	Diarrhea	1.35±0.75
	Nausea/Vomiting	3.85±1.37
	Fatigue	7.15±2.41
	Appetite Loss	2.13±1.08
	Sleep Disturbance	2.11±1.11
	Dyspnea	1.53±0.81
	Financial Impact	1.68±0.94
	Functional Scales	29.72±8.66
	Physical Functioning	11.44±3.69
	Emotional Functioning	7.67±3.38
	Role Functioning	3.61±1.59
	Social Functioning	3.75±1.81
	Cognitive Functioning	3.26±1.42
CITAS	Scale Total Score	40.43±17.84
	Discomfort	2.41±0.96
	General Taste Alterations	2.32±1.23
	Phantogeusia and Parageusia	2.16±1.20
	Decline in Basic Taste	2.04±1.30

EORTC QLQ-C30: European Organization for Research and Treatment of Cancer Quality of Life Scale, CiTAS: Chemotherapy-induced Taste Alteration Scale

Table 4. The relationship between EORTC QLQ-C30 and CiTAS total score and sub-dimension score means

			EORTC QLQ-C30				CITAS				
			Global Health Status	Functional Scales	Symptom Scales	Total	Decline in Basic Taste	Discomfort	Phantogeusia and Parageusia	General Taste Alterations	Total
EORTC QLQ-C30	Global Health Status	r	-	-0.580	-0.640	-0.508	-0.291	-0.413	-0.257	-0.366	-0.423
		p		**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
	Functional Scales	r		-	0.808	0.945	0.452	0.502	0.381	0.488	0.549
		p			**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
	Symptom Scales	r			-	0.921	0.474	0.585	0.397	0.523	0.616
		p				**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
	Total	r				-	-0.496	-0.579	-0.418	-0.529	-0.616
		p					**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
	Decline in Basic Taste	r					-	0.524	0.497	0.771	0.829
		p						**<0.001***	**<0.001***	**<0.001***	**<0.001***
	Discomfort	r						-	0.476	0.564	0.814
		p							**<0.001***	**<0.001***	**<0.001***
	Phantogeusia and Parageusia	r							-	0.563	0.715
		p								**<0.001***	**<0.001***
	General Taste Alterations	r								-	0.868
		p									**<0.001***
CITAS	Total	r									-
		p									

EORTC QLQ-C30: European Organization for Research and Treatment of Cancer Quality of Life Scale, CiTAS: Chemotherapy-induced Taste Alteration Scale, r: Spearman Correlation coefficient, *p<0.05: There is a statistically significant relationship between the scores

Table 5. CiTAS total score estimation regression analysis

					%95 Confidence Interval	
	B	β	t	Sig.	Lower Limit	Upper Limit
Constant	7.487		0.636	0.525	-15.659	30.634
EORTC QLQ-C30 Total Score Average	-0.789	-0.588	-14.570	**<0.001***	-0.682	-0.895

B: Non-standardized Beta Coefficient, R(Correlation Coefficient)=0.637, R2(Explanatory Coefficient) = 0.406, Adjusted R2(Standardized Explanatory Coefficient)=0.382, β: Standardized Beta Coefficient, F= 16.980, p*<0.05: t test result for the significance of the regression coefficients

DISCUSSION

Although taste changes do not constitute a life-threatening symptom, they lead to a reduced quality of life in cancer patients who receive chemotherapy treatment because they affect these patients in many respects (7,9).

In our study, most of the patients had a condition that affected their oral mucosa, and the most frequently reported condition was dryness in the mouth. Most patients performed daily oral care practices, and the most common oral care method was brushing teeth. Sozeri (n=184) also reported the most common oral care method as brushing teeth (7). In the literature, taste changes have been observed more frequently in patients with dry mouth and mouth sores (16,19). In their study on chemotherapy patients (n=120), Berk et al. reported that 51.7% of the patients experienced mild taste changes due to their mouth sores (19). Chemotherapy-induced reductions in saliva secretion and changes created in the oral mucosa by chemotherapy can affect the sensation of taste (15,20-22). Patients with dryness in the mouth are at risk of taste dysfunctions because foods have to be dissolved for their contact with taste receptors. This is why nurses are recommended to provide education to patients regarding this issue, perform their oral care, and increase their fluid intake (15). It was reported that special education and training programs on oral care affected the clinical practices of nurses positively (23).

Considering the maximum possible scores of CiTAS and

its subscales, it may be stated that the patients who were included in our study experienced moderate levels of taste changes, and they had discomfort associated with these taste changes. A previous study that was conducted with lung cancer patients revealed that the patients experienced moderate levels of taste changes and moderate discomfort associated with these taste changes (24). In the study that was carried out by Celik et al. (n=196), it was found that patients experienced moderate levels of taste changes, as well as moderate levels of phantogeusia and parageusia among types of taste change (25). In other studies using CiTAS and examining taste changes in different types of cancer, CiTAS subscale scores have usually been reported in the range of 1-3 (7,26,27). In addition to the direct physiological effects of chemotherapy, other symptoms of the disease itself and the adverse effects of chemotherapy also affect the sensation of taste. Due to all these factors, patients experience taste changes (7,28). The finding in our study that the "Discomfort" subscale scores of the patients were higher can be explained by the possibility that these patients experienced discomforting symptoms such as nausea, vomiting, and loss of appetite.

In our study, the scores of the patients in the "Functional Scales" category of EORTC QLQ-C30 were higher than their scores in the other categories. High scores in the "Functional Scales" and "Symptom Scales" categories of EORTC QLQ-C30 indicate low quality of life levels (17). In line with this information that is used to assess the scores of the scale, the quality of life levels of the patients in our study were low. Other studies in the literature have shown that chemotherapy has negative effects on quality of life and lowers the quality of life of patients (29-31). In a study that was performed to compare the quality of life levels of cancer patients in two different chemotherapy cycles (n=50), the scores of the patients in both groups in the "Functional Scales" category of EORTC QLQ-C30 were found higher than their scores in the other categories (32).

In our study, statistically significant relationships were identified between EORTC QLQ-C30 and CiTAS and between the subscales of EORTC QLQ-C30 and the subscales of CiTAS. As the CiTAS scores of the patients increased, their scores in the "Global Health Status" category of EORTC QLQ-C30 decreased, and their scores in the "Functional Scales" and "Symptom Scales" categories of EORTC QLQ-C30 increased. In other words, as the taste changes scale scores of the patients increased, their quality of life decreased. The consequence of the regression analysis in this study supported the results of the correlation analysis. According to other studies in the literature, taste changes affect the quality of life of patients negatively (8, 9, 33). In another study that was carried out with cancer patients receiving cytotoxic treatment (n=197), it was reported that the patients showed symptoms of loss of appetite and fatigue due to taste changes, and these symptoms affected their quality of life negatively (9). In the study by Gamper et al. that was conducted with breast cancer patients and gynecologic cancer patients who were receiving chemotherapy (n=109), the authors showed a statistically significant relationship between the taste changes of the patients and their symptoms of fatigue and loss of appetite. They stated that these symptoms affected the quality of life of the patients negatively (8). In their study on patients undergoing chemotherapy treatment (n=214), Kano et al. found that taste changes affected the patients' activities of daily living negatively (10). In another study that included patients who were taking chemotherapy treatment (n=289), the quality of life levels of the patients who experienced taste changes were lower compared to those who did not experience taste changes (34). Spotten et al. (n=40), on the other hand, determined that taste and olfactory changes reported by patients with solid tumors did not significantly affect their quality of life (35). The results of our study were in parallel with those in the literature. Taste changes, which are a commonly overlooked symptom, disrupt the well-being and quality of life of patients and affect their daily lives and social and emotional statuses adversely (36,37).

Limitations

As the study was carried out during the ongoing COVID-19 pandemic period, during data collection at the hospital, some patients did not want to be included in the study to avoid the prolongation of their hospital stay and to prevent infection. The exclusion of these patients was among the limitations of this study.

CONCLUSION

In our study, statistically significant relationships were found between the total and subscale scores of the patients in the taste changes and quality of life scales. It was found that as the scores of the patients in the taste changes scale increased, their quality of life decreased. Based on the results of this study, nurses are recommended to provide the cancer patient and their family with education on their disease, treatment, complications, and symptoms, assess their symptoms of taste change and take these symptoms under control. It is also recommended to plan nursing interventions to increase the quality of life of patients experiencing taste changes associated with chemotherapy and organize educational programs.

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Research Article

Evaluation of Prolonged Rt-Pcr Positivity and Viral Load in COVID-19 Patients

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Abstract

Aim: Real-time reverse transcription polymerase chain reaction (RT-PCR) test is used in the diagnosis of COVID-19. It was aimed to evaluate the factors affecting the viral conversion time, to examine the relationship between viral load, and to determine other factors that may be associated with viral load.

Material and Methods: Patients were hospitalized between 15.03.2020-01.08.2020, and viral conversion detected were evaluated retrospectively. Patients were divided into two according to viral conversion time (0-14 days vs >14 days).

Results: 349 patients were included in the study (284 vs 65 patients). The age and gender characteristics were similar. Prolonged PCR positivity group had more death (p=0.036) and lower cycle-threshold (CT) value (p=0.017). In the examination of CT values of 246 patients, 228 patients with viral conversion and 18 patients without viral conversion due to death, the CT value was found to be lower, therefore the viral load was higher in patients over 60 years of age (p=0.006), in the presence of cardiovascular system disease (p<0.001) and in patients who died (p<0.001).

Conclusion: Prolonged PCR positivity may indicate excess viral load and adverse outcomes. An evaluation including the patient's age, CT value, comorbid conditions, and viral conversion time can give an idea about the prognosis.

Keywords: Clearance, cycle threshold (CT) value, negative conversion, SARS-CoV-2, viral load

INTRODUCTION

Coronavirus Disease 2019 (COVID-19), which is caused by a new virus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), first appeared in China in late 2019 and spread all over the world in a short time, causing a global pandemic (1,2). Although serological diagnoses are possible, real-time reverse transcription polymerase chain reaction (RT-PCR) testing is mainly used for the diagnosis of COVID-19, and it is recommended to evaluate combined swab samples taken from the nasopharynx and oropharynx (3,4). RT-PCR also allows the detection of the cycle threshold (CT) value as well as the diagnosis of the disease. The CT value is defined as the number of amplification cycles required for the target gene to exceed a certain threshold and can be used indirectly to measure viral load (5).

Various studies have been conducted to correlate virus shedding time with disease prognosis, viral load, or other factors. In these studies, it was reported that virus excretion takes a long time in advanced age, in patients with underlying disease or in severe cases (6-8). Despite these data in the literature, viral dynamics related to the disease have not been clarified yet.

In this study, it was aimed to evaluate the factors affecting the viral clearance time, to examine the relationship between viral load and viral clearance time, and to determine other factors that may be associated with viral load.

MATERIAL AND METHOD

This study was planned retrospectively in our center. Ethics committee approval was obtained from the Kirikkale University and the Non-Interventional Studies Ethics Committe with number and date of 2021.03.28

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and 25.03.2021. The patients were informed and a signed consent form was obtained from each patient for inclusion in the study. Patients aged 18 years and older were hospitalized in our center between 15.03.2020 and 01.08.2020, with positive SARS-CoV-2 RT-PCR test and viral conversion detected during follow-up were included in the study. Patients were divided into two groups according to viral conversion time; 0-14 days and >14 days (prolonged polymerase chain reaction -PCR- positivity). The data of the patients were determined from the hospital system and archive files. The age, gender and comorbid conditions of the patients were noted. Complete blood count, creatinine, electrolytes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP), ferritin were evaluated at the time of admission to the hospital. Thorax computed tomography images of the patients were categorized according to the COVID-19 Reporting and Data System (CO-RADS) classification evolved by The Radiological Society of the Netherlands (NVvR) and classified between CO-RADS 1-5 (9). Antiviral and antibacterial treatments given to the patients during the hospitalization period were examined from the patient files. Treatment outcomes of the patients were noted as discharge or death. Symptom duration was not included in the study because the information provided was not clear and the data could not be evaluated optimally.

Evaluation of samples and determination of conversion time

Evaluated swabs were taken from both the nasopharynx and oropharynx of the patients in a combined manner. The swabs taken were tested with the RT-PCR method. Bioeksen Bio-Speedy® SARS-CoV-2 Double Gene RT-qPCR Kit and its versions were used for RT-PCR. All of the kits were run on the Biorad CFX96 Touch Real-Time PCR Detection System device. All of the study was completed with the same kit and device.

SARS-CoV-2 specific N and ORF1ab gene regions were targeted during PCR method. The recommended threshold level in the kit insert for calculating the number of cycles was 200 RFU for Biorad CFX96 instruments. The shape of the amplification curves was examined according to the kit package insert, and sigmoidal curves with assigned CT value and CT value ≤ 33 in the Fam channel were considered positive. If a sample was assigned a CT value but the curve was not sigmoidal, the result was recorded as negative. During the determination of these CT values, attention was paid to the evaluation of all samples by the same person. CT values were evaluated at diagnosis, regardless of patient outcome. Therefore, the CT values of the cases that resulted as dead were also determined.

Internal control (IC) was used in line with the kit recommendations while working the samples. However, due to the retrospective nature of the study, no correction was made according to the internal control value during the analysis of the data. For all that, according to the sampling method of the test, all samples were taken by a predetermined and trained team to prevent false negative results and to ensure that the samples were of similar quality.

When calculating the viral conversion time; the day on the virus was detected by the RT-PCR method was determined as the first day. Viral conversion was defined as two consecutive negative RT-PCR results evaluated at least 24 hours apart. The first day of consecutive tests with a negative result was recorded as the day when viral shedding ended. The control RT-PCR test was taken on the 5th day at the earliest. If the result was positive, control RT-PCR tests were repeated every day or every other day.

Statistical analysis

IBM SPSS for Windows (version 23.0; SPSS Inc., Chicago, IL, USA) program was used for data analysis. Shapiro Wilk test was used for normality assessment. For non-normally distributed continuous variables, Mann-Whitney U and Kruskal Wallis tests were used to compare the two groups. Chi-square test was used to evaluate categorical data. A p value less than 0.05 was considered statistically significant.

RESULTS

349 patients who were hospitalized in our center between 15.03.2020 and 01.08.2020 with the diagnosis of COVID-19 and were found to have viral conversion during the follow-up were included in the study. It was determined that there were 284 and 65 patients in the groups with a viral conversion period of 0-14 days and >14 days, respectively, and the age and gender characteristics of the two groups were similar. CT values of 185 patients in the group with viral conversion duration of 0-14 days and 43 patients in the group with >14 days were evaluated. Since the study was retrospective, the ct value of 121 patients with viral conversion could not be reached. CT value was found to be significantly lower in the group with prolonged PCR positivity compared to the other (28.31 (17.89- 38.38) vs 26.16 (18.77- 34.55) days respectively, p=0.017). The comparison of demographic characteristics, comorbid conditions, radiological images and treatments used according to the viral conversion time of the patients is given in Table I.

AST and lymphocyte levels were found to be lower and sodium levels were higher in those with prolonged PCR positivity in the blood tests. The difference in AST and sodium values was statistically significant, but was not considered clinically significant. Detailed analysis of the blood values of the two groups is given in Table II.

In order to examine the relationship of CT values with age, gender, comorbid conditions, radiological involvement, and clinical outcome, an analysis consisting of 246 patients was performed (Table III). When analyzing the factors related to the CT value; The data of 228 patients with known ct value in the group with viral conversion were analyzed. In addition, the data of 18 patients whose

Table 1. Comparison of demographic characteristics, comorbid conditions, radiological images and treatments used according to viral conversion time

		All patients (n= 349)	Viral conversion time		p value
			0-14 days (n=284)	>14 days (n=65)	
Median (min- max)					
Age (years)		42 (18-87)	42 (18-85)	43 (21-87)	0.708
Viral conversion time (days)		9 (5-21)	8 (5-14)	16 (15- 21)	<0.001
Hospital stay (days)		9 (5-36)	9 (5-19)	16 (6-36)	<0.001
Intensive Care Unit stay (days)		0 (0- 13)	0 (0-13)	0 (0-3)	0.934
CT value		27.95 (17.89-38.38)	28.31 (17.89-38.38)	26.16 (18.77-34.55)	0.017
n (%)					
Sex (male)		173 (49.5%)	143 (50.3%)	30 (46.1%)	0.541
Smoking		68 (19.4%)	56 (19.7%)	12 (18.5%)	0.739
Pregnancy		3 (0.8%)	3 (1.1%)	0 (0%)	0.405
Comorbid condition (≥1 comorbidity)		126 (36.1%)	103 (36.2%)	23 (35.4%)	0.894
Cardiovascular diseases	Hypertension	51 (14.6%)	43 (15.1%)	8 (12.3%)	0.842
	Coronary artery disease	41 (11.7%)	33 (11.6%)	8 (12.3%)	
Respiratory diseases	Chronic obstructive pulmonary disease	14 (4%)	12 (4.2%)	2 (3%)	0.881
	Asthma	9 (2.5%)	7 (2.5%)	2 (3%)	
Diabetes mellitus		35 (10%)	29 (10.2%)	6 (9.2%)	0.812
Chronic kidney disease		3 (0.8%)	1 (0.3%)	2 (3%)	**0.032**
Neurological diseases		16 (4.5%)	14 (4.9%)	2 (3%)	0.519
Malignancy		3 (0.8%)	3 (1.1%)	0 (0%)	0.405
Radiological findings (Computed tomography)	None	12 (3.4%)	7 (2.5%)	5 (7.7%)	0.087
	CO-RADS 1	207 (59.3%)	167 (58.8%)	40 (61.5%)	
	CO-RADS 2	0 (0%)	0 (0%)	0 (0%)	
	CO-RADS 3	24 (6.8%)	19 (6.7%)	5 (7.7%)	
	CO-RADS 4	9 (2.5%)	6 (2.1%)	3 (4.6%)	
	CO-RADS 5	97 (27.8%)	85 (29.9%)	12 (18.5%)	
Medical treatment	Hydroxychloroquine	339 (97.1%)	275 (96.8%)	64 (98.4%)	0.477
	Favipiravir	17 (4.9%)	10 (3.5%)	7 (10.8%)	**0.014**
	Lopinavir/ritonavir	3 (0.8%)	2 (0.7%)	1 (1.5%)	0.511
	Azithromycin	132 (37.8%)	115 (40.5%)	17 (26.1%)	**0.032**
	Moxifloxacin	8 (2.3%)	6 (2.1%)	2 (3%)	0.639
	Oseltamivir	46 (13.2%)	39 (13.7%)	7 (10.8%)	0.524
Clinical outcome (death)		1 (0.3%)	0 (0%)	1 (1.5%)	**0.036**

Chi-square and Mann Whitney U test was used
CT: Cycle Threshold

Table 2. Comparison of blood tests according to viral conversion time

Laboratory tests Median (min- max)	All patients (n= 349)	Viral conversion time		p value
		0-14 days (n=284)	>14 days (n=65)	
AST (U/L)	25 (12- 331)	25 (12- 331)	21 (13- 98)	0.046
ALT (U/L)	19 (5- 266)	19 (5- 266)	19 (6- 153)	0.884
Creatinine (mg/dL)	0.7 (0.3- 2.1)	0.7 (0.4- 1.9)	0.7 (0.3- 2.1)	0.794
Sodium (mmol/L)	136 (128- 143)	136 (128- 143)	137 (132- 142)	0.045
Potassium (mmol/L)	4.0 (3- 6.2)	4.0 (3.2- 6.2)	4.1 (3- 5.4)	0.981
CRP (mg/dL)	0.5 (0.01- 16.3)	0.5 (0.01- 16.3)	0.5 (0.02- 13.80)	0.177
White blood cell (10^9/L)	5.7 (1.6- 18.8)	5.7 (1.6- 18.8)	6.2 (2.7- 10.7)	0.249
Lymphocyte (10^3/uL)	1.7 (0.35- 6)	1.7 (0.46- 6)	1.36 (0.35- 4)	0.024
Hæmoglobin (g/dL)	14 (6.8- 18.2)	14.1 (6.8- 18.2)	13.7 (7.6- 17.3)	0.379
Platelet (10^9/L)	216 (54- 446)	211 (54- 446)	218 (99- 419)	0.759
Ferritin (µg/L)	84 (0.5- 1002)	86 (0.5- 1002)	71 (9- 849)	0.162

Mann Whitney U test was used. AST: Aspartate Aminotransferase. ALT: Alanine Aminotransferase. CRP. C-reactive Protein

Table 3. Comparison of CT values according to age, gender, radiological involvement, comorbid conditions, and clinical outcome

All patients (n= 246)		CT value (Median (min-max)	p value
Age	≤ 60 years (n=193)	27.94 (18.75-38.38)	**0.006**
	>60 years (n=53)	25.15 (14.40-35.74)	
Sex	Male (n=129)	27.64 (14.40-36.62)	0.642
	Female (n=117)	27.68 (16.22-38.38)	
Radiological findings	CO-RADS 1 (n=145)	27.68 (18.77-38.38)	0.570
	CO-RADS 5 (n=70)	27.61 (14.40-37.50)	
Comorbid condition	No (n=147)	28.22 (18.83-38.38)	**0.020**
	≥1 comorbidity (n=99)	26.97 (14.40-35.74)	
Cardiovascular diseases	No (n=171)	28.23 (18.77-38.38)	**<0.001**
	Yes (n=75)	25.27 (14.40-35.74)	
Diabetes mellitus	No (n=216)	27.94 (18.71-38.38)	0.408
	Yes (n=30)	27.51 (17.89-35.64)	
Respiratory diseases	No (n=227)	27.94 (17.89-38.38)	0.230
	Yes (n=19)	24.51 (18.77-35.74)	
Chronic kidney disease	No (n=241)	27.95 (17.89-38.38)	0.555
	Yes (n=5)	24.36 (21.51-35.08)	
Neurological diseases	No (n=234)	27.89 (17.89-38.38)	0.947
	Yes (n=12)	27.51 (18.77-34.61)	
Clinical outcome	Discharged (n=227)	28.01 (17.89-38.38)	**<0.001**
	Death (n=19)	21.36 (14.40-35.08)	

Chi-square and Mann Whitney U test was used
CT: Cycle Threshold

CT values were determined but we could not detect viral conversion because they died and we could not include them in the study were also included in the study. CT value was found to be statistically significantly lower in patients over 60 years of age, in the presence of at least 1 comorbid condition, in the presence of cardiovascular system (CVS) disease, and in patients who died.

DISCUSSION

In this study was determined that patients with a viral conversion period longer than 14 days had a longer hospital stay, a lower CT value, and a higher death rate. In addition, it was found that the CT value was lower in cases older than 60 years of age, with at least one comorbid condition, with CVS disease and resulting in death, and this result indirectly suggested that the viral load in these patients was higher.

Viral conversion time is variable, and studies have shown that viral RNA mostly becomes negative after the 7th day. In a study by Hu et al., while the PCR test became negative in 10.2% of patients at the end of the 1st week, this rate was found to be 62.7% and 91.2% at the end of the 2nd and 3rd weeks, respectively (10). In another study, the detection time of virus in respiratory samples was determined as 18 days (13-29 days) (6). In our study, the duration of viral

conversion was 9 days (5-21 days), which was interpreted as shorter than the studies in the literature. This result is thought to be due to the fact that the duration of viral conversion is determined from the date of the first positive RT-PCR, not from the onset of symptoms.

The duration of viral conversion is associated with age, and it has been shown in various studies that this period is prolonged in older age (8,10,11). In a study by Bhattacharya et al., the time from symptom onset to PCR negativity was higher in patients aged 60 years and over (mean 21 days, p=0.004) (8). However, when this time was evaluated from the date of the first positive RT-PCR, no significant difference was observed according to age groups (p=0.18) (8). In our study, the ages were similar in both groups, and no difference was found. This result is considered to be obtained because the viral conversion time was evaluated from the date of the first positive RT-PCR, similar to the study of Bhattacharya et al.

There is an inverse relationship between the CT value and the viral conversion time. In the study of Aranha et al., it was reported that the viral conversion period was longer in those with low CT values (12). While viral conversion was frequently observed in the first week in patients with a CT value of 31 and above, it occurred between 2-4 weeks in 79.2% of those with a CT value of 25 and below (12). Similarly, in our study, the CT values of patients who developed viral conversion after 14 days were found to be significantly lower than the others. It is thought that RT-PCR negativity is delayed due to the high viral load in patients with low CT values.

Viral conversion time is associated with disease severity, and it has been found that this period increases in severe disease (6). Although the severity of the disease was not directly evaluated in our study, it was found that mortality was higher in cases whose conversion period exceeded 2 weeks. From a radiological point of view, no relationship was found between radiological involvement and viral conversion time, and a similar result was obtained in our study (10).

When the patients followed up with the diagnosis of COVID-19 were analyzed in terms of blood tests, low lymphocyte levels were found to be associated with severe illness and the need for intensive care (13). In addition, we determined that patients with prolonged PCR positivity had significantly lower lymphocyte counts. In our study, the low lymphocyte count in patients with a longer viral conversion period suggests that viral load is higher in this patient group and adverse outcomes may be more.

Factors associated with CT value

Although the CT value is not directly related to age, it has been shown in various studies that the viral conversion period is longer in elderly patients (8,10,11). In addition, when we look at the literature, it is seen that patients with a long conversion period have low CT values and high viral loads (12,14). In our study, the CT values of patients over 60 years of age were found to be significantly lower, and the viral load is thought to be high in these patients. In our study, the age of the patients was found to be associated with the CT value but not with the conversion time. Because data of 18 patients whose viral conversion could not be detected due to death were included in the analyzes related to the CT value. Therefore, the thought that patients who died had a lower CT value and a higher viral load could explain this finding.

Although it has been reported in various case series that the CT value decreases as the lung damage increases, no significant relationship was found between the presence of pneumonia and the CT value in a study conducted in our country (15,16). Similarly, in our study, no correlation was found between the CT values of the group whose computed tomography findings were classified as CO-RADS 1 (normal or noninfectious findings) and the groups classified as CO-RADS 5 (typical COVID-19 findings).

There is an interaction between viral load, mortality and CVS diseases in the course of COVID-19 (17-19). In a study by Huang et al.; myocardial enzymes were found to be higher in patients with high viral load (17). In another study, it was reported that troponin and CT values showed a negative correlation (18). Evaluation of these enzymes at the time of diagnosis strengthens the possibility of underlying CVS disease rather than CVS involvement due to COVID-19. Similar to these studies, we found a correlation between the presence of underlying CVS disease and the CT value and viral load.

There is an inverse relationship between the CT value and the severity of the disease. Studies have reported that the CT value is lower in cases with severe disease (18,20). Considering the relationship between CT value and mortality, in a study conducted by Huang et al., was reported that the CT value of the cases resulting in mortality was lower than the survivors (p<0.001) (17,21). In our study, the CT value of the cases that resulted in death was found to be significantly lower, and it is thought that these patients have higher viral loads and therefore result in severe disease and mortality.

The strengths of our study are that it considers the CT value and indirectly the viral load as well as viral conversion, and the evaluation of a large population in terms of number of patients. In addition, the definition of viral conversion as the detection of two consecutive negative RT-PCR results in samples taken at least 24 hours apart prevented false-negative results and increased the reliability of the study results. The weakness is that the conversion time is evaluated from the date of the first positive RT-PCR and does not include the symptoms of the patients.

Although it was determined in our study that there was a relationship between CT value and advanced age, presence of comorbid conditions, presence of CVS disease and mortality, in the RT-PCR test performed with different samples taken from the same patient, there may be a difference in the internal control CT values of the samples, which indicates the virus load. It should be noted that it may

affect the CT value. Since our study was retrospectively designed, no correction was made according to the IC value, but all of the samples were taken by a team who had previously been trained in this subject in order to ensure that the samples were similar. In addition, when the studies in the literature were examined, it was determined that no correction was made according to the IC value (17-21).

The strengths of our study are that it considers the CT value and indirectly the viral load as well as viral conversion, and the evaluation of a large population in terms of number of patients. In addition, the definition of viral conversion as the detection of two consecutive negative RT-PCR results in samples taken at least 24 hours apart prevented false-negative results and increased the reliability of the study results. The weakness is that the conversion time is evaluated from the date of the first positive RT-PCR and does not include the symptoms of the patients. In addition, the fact that there is no correction according to the IC value can be considered as another weakness.

CONCLUSION

Patients with a viral conversion duration longer than 14 days have a higher viral load and more adverse outcomes like death. In addition, the viral load is higher in cases older than 60 years of age, with at least one comorbid condition, CVS disease and death. An evaluation including the patient's age, CT value, comorbid conditions, and viral conversion time can give an idea about the prognosis of the disease. Therefore, the development of a scoring system that includes these values may help clinicians in approaching patients. However, further studies are needed for this. We believe that our study will be a guide for future scoring and prediction studies.

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MEDICAL RECORDS-International Medical Journal

Research Article

Assessment of Periodontal Disease and the Presence of Pulp Stone: Retrospective Radiologic Study

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Abstract

Aim: To evaluate the relationship between the severity of periodontal disease and the presence of pulp stones using panoramic radiographies.

Material and Methods: The study included 96 individuals aged 18-45 years. The patients were divided into four groups as control (periodontally healthy), gingivitis, stage 1-2 periodontitis and stage 3-4 periodontitis. The patients' panoramic radiographs and dental examination records were evaluated retrospectively and any pulp stones were recorded. The Chi-square test was used to determine the relationship between the presence of pulp stones and other parameters.

Results: Evaluating the findings on a patient basis, the highest pulp stones were seen in the stage 3-4 (79.2%) group, and the least pulp stones were observed in the control group (20.8%). There was a statistically significant difference between the control group and the gingivitis group ($p=0.017$), the control group and the stage 3-4 group ($p<0.001$), and the stage 1-2 and stage 3-4 groups ($p=0.001$). On a tooth basis, the most pulp stones were observed in the stage 3-4 (14.9%) group, and the least pulp stones were observed in the control group (1.7%).

Conclusion: The severity of periodontitis was associated with increased pulp stones. The diagnosis and treatment of patients with endodontic-periodontal problems should be carefully planned accordingly. The presence of pulp stones, which can complicate accessing root canals in endodontic treatment, should be comprehensively investigated.

Keywords: Pulp stone, periodontitis, panoramic radiography, periodontal disease, calcification

INTRODUCTION

The pulp and periodontium are anatomically connected through the apical foramen and lateral or accessory canals. Human and animal studies have shown that pathologic changes in dental pulp can cause periodontal changes (1). However, it is not yet established whether periodontal disease can cause pulpal disease. Previously, it was believed that blood flowed from the pulp to the periodontium and that inflammation spread via venous blood; therefore, it was presumed that periodontal disease did not affect the dental pulp. More recent studies have shown that chronic periodontitis was associated with pathologic changes in the pulp, including inflammatory changes, localized necrosis, root resorption, calcification, and secondary dentin formation. These changes occur due to the reverse flow of inflammatory substances through the lateral and accessory canals (1,2).

Pulp stones are calcified masses in the dental pulps of healthy, diseased, and unerupted teeth (3). They may exist freely within the pulp or be attached to or embedded in dentin (4). Although its etiology is not fully understood, several factors have been associated with pulp stone formation, including inductive interactions between epithelium and pulp, pulp degeneration, orthodontic tooth movement, pulp circulation disorders, idiopathic factors, trauma, periodontal disease, genetic predisposition, age, deep fillings, caries, chronic inflammation, anemia, acromegaly, certain medications, atherosclerosis, and

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Marfan syndrome (5,6).

Periodontitis has been reported to negatively affect pulpal circulation due to bone loss and increased pocket depth (7). In reference to literature indicating that pulpal circulation disorders are effective in pulp stone formation, this study investigated the prevalence of pulp stones among individuals with periodontal disease (7).

Pulp stones can be detected using radiographic and histologic methods (7). Histologic examination gives accurate and clear results, but is not clinically applicable due to its invasive nature (8). The pulp stone must have reached a certain size (>200 μm) and mineralization to be detected on radiography. Radiographic examination is often preferred because it is the only method that can non-invasively detect pulp stone in clinical studies (7).

In a study that radiographically investigated the effect of aggressive periodontitis on pulp, the prevalence of pulp stones was found to be higher in the aggressive periodontitis group compared with the periodontally healthy control group (9). We believe that other periodontal diseases can also induce pulp stone formation due to damage to neighboring structures. Therefore, in this study, we aimed to compare the prevalence of pulp stones in individuals with different periodontal disease and periodontally healthy individuals.

MATERIAL AND METHOD

Study groups

This study was approved by the Ethics Committee of Recep Tayyip Erdoğan University. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. The purpose and content of the research were explained to the individuals included in the study, and voluntary consent forms were signed.

The study included patients aged 18-45 years without systemic diseases who presented to the Recep Tayyip Erdoğan University Faculty of Dentistry between January 2014 and June 2020. Power analysis revealed that at least 96 subjects were required for a study with four groups for an effect size of 40%, a confidence interval of 95%, and 90% power.

We retrospectively examined patient files and recorded periodontal parameters (plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD), clinical attachment loss (CAL), and panoramic radiographs. The subjects were divided into four groups according to the 2017 classification of periodontal diseases (10). The groups were defined as follows: control group (group 1, n = 24), periodontally healthy, no CAL, PD ≤3 mm, minimal BOP (≤10%), no radiographic bone loss; gingivitis group (group 2, n = 24), no CAL, PD ≤3 mm, BOP ≥10%, no radiographic bone loss; periodontitis stage 1-2 group (group 3, n = 24), interdental CAL ≤4 mm, PD ≤5 mm, radiographic bone loss ≤ coronal 1/3, no tooth loss due to periodontal disease; periodontitis stage 3-4 group (group 4, n = 24), interdental CAL ≥5 mm, PD ≥6 mm, history of multiple tooth loss, presence of deep periodontal lesions extending to the apical portion of the root.

The exclusion criteria were as follows: panoramic radiographs with poor image quality, history of tooth clenching (due to potentially causing pulp stone formation), orthodontic treatment, systemic disease (e.g. hypercalcemia, gout, cardiovascular disease), inability to radiographically evaluate the pulp chamber (e.g. due to caries, filling, crown restoration).

Radiographic examination

All panoramic radiographs were obtained using a Planmeca Promax 2D S2 (Planmeca Oy; Helsinki, Finland) device with parameters of 66 kVp, 8 mA, 16.6 s. The maxillary and mandibular first and second premolars and first and second molars were evaluated for pulp stones. Pulp stones were defined as distinct radiopaque masses inside the pulp cavity and evaluated as "1" (present) or "0" (absent) (Figure 1).

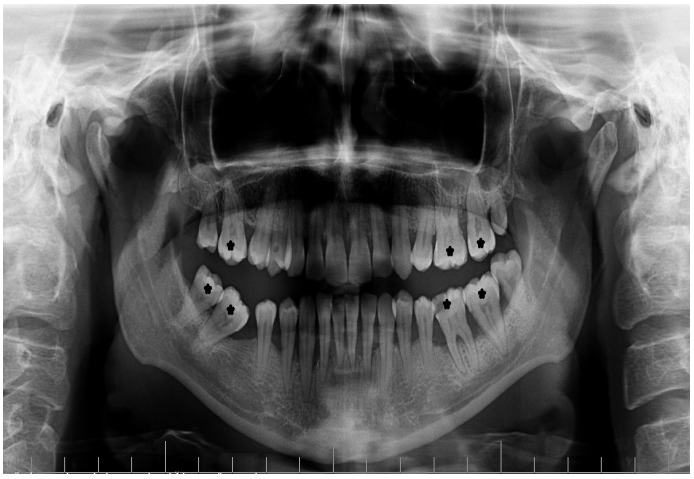

Figure 1. Pulp stones observed in patients with stage 3-4 periodontitis (pulp stones in teeth marked with *)

The presence of pulp stones was checked by an oral and maxillofacial radiologist with 10+ years' experience (T.E.K). Twenty percent (n = 20) of the radiographs were re-evaluated to determine the intra-observer agreement.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (IBM Corp., version 23.0, Armonk, USA). $P<0.05$ was considered statistically significant. The chi-square test was performed to compare the prevalence of pulp stones per tooth between the groups according to sex and the severity of periodontal disease. For evaluation of intra-agreement, Cohen's kappa was used.

RESULTS

The study included a total of 96 patients and 1269 teeth. The mean age of the patients was 32 years. Among the subjects, 61 were female and 35 were male. When the

images were re-evaluated, it was seen that Cohen kappa was quite good according to the statistics (k = 0.872). The patients' periodontal parameters are presented in Table 1.

Table 1. All groups' probing pocket depth (PPD), clinical attachment loss (CAL), bleeding in probing (BOP), plaque index (PI), and gingival index (GI) values

	Group 1 (n=24)	Group 2 (n=24)	Group 3 (n=24)	Group 4 (n=24)
PI	0.37±0.17	1.79±0.36	2.22±0.42	2.31±0.51
GI	0.11±0.05	1.77±0.34	1.97±0.37	2.27±0.64
BOP	6.27±2.27	69.87±14.95	75.87±15.45	79.86±12.26
PPD	1.35±0.38	2.17±0.34	3.03±0.40	4.27±0.51
CAL	0±0	0±0	3.38±0.46	4.80±0.70

The highest values of PI, GI, BOP, PPD and CAL parameters were observed at group 4, respectively the values were 2.31±0.51, 2.27±0.64, 79.86±12.26, 4.27±0.51, 4.80±0.70. The lowest values of these parameters were observed at group 1, respectively the values were 0.37±0.17, 0.11±0.05, 6.27±2.27, 1.35±0.38, 0±0. On a patient basis, pulp stones were the most common in group 4 (79.2%) and the least common in group 1 (20.8%) (Table 2).

Table 2. Frequency of pulp stones in all groups according to the number of patients

Dependent variable	Present	Absent	Total	X^2	p
Group 1	5 (20.8%)	19 (79.2%)	24	5.69	0.017*
Group 2	13 (54.2%)	11 (45.8%)	24		
Group 1	5 (20.8%)	19 (79.2%)	24	0.95	0.330
Group 3	8 (33.3%)	16 (66.7%)	24		
Group 1	5 (20.8%)	19 (79.2%)	24	16.33	<0.001*
Group 4	19 (79.2%)	5 (20.8%)	24		
Group 2	13 (54.2%)	11 (45.8%)	24	2.12	0.146
Group 3	8 (33.3%)	16 (66.7%)	24		
Group 2	13 (54.2%)	11 (45.8%)	24	3.37	0.066
Group 4	19 (79.2%)	5 (20.8%)	24		
Group 3	8 (33.3%)	16 (66.7%)	24	10.24	0.001*
Group 4	19 (79.2%)	5 (20.8)	24		

*Statistically significant at p<0.05

An intergroup comparison revealed a significant difference between groups 1 and 2 (p=0.017), groups 1 and 4 (p<0.001), and groups 3 and 4 (p=0.001) (Table 2). On a tooth basis, pulp stones were the most common in group 4 (14.9%) and the least common in group 1 (1.7%) (Table 3).

Table 2. Frequency of pulp stones in all groups according to the number of teeth

Dependent variable	Present	Absent	Total	X^2	p
Group 1	6 (1.7%)	343 (98.3%)	349	23.76	<0.001*
Group 2	32 (10.8%)	265 (89.2%)	297		
Group 1	6 (1.7%)	343 (98.3%)	349	7.31	0.007*
Group 3	17 (5.6%)	284 (94.4%)	301		
Group 1	6 (1.7%)	343 (98.3%)	349	39.36	<0.001*
Group 4	48 (14.9%)	274 (75.1%)	322		
Group 2	32 (10.8%)	265 (89.2%)	297	5.22	0.022*
Group 3	17 (5.6%)	284 (94.4%)	301		
Group 2	32 (10.8%)	265 (89.2%)	297	2.34	0.126
Group 4	48 (14.9%)	274 (75.1%)	322		
Group 3	17 (5.6%)	284 (94.4%)	301	14.27	<0.001*
Group 4	48 (14.9%)	274 (75.1%)	322		

*Statistically significant at p<0.05

We further analyzed our results based on sex. For women, pulp stones were the most common in group 4 (69.2%) and the least common in group 1 (17.6%). For men, pulp stones were the most common in group 4 (90.9%) and the least common in group 3 (21.4%) (Table 4).

Table 4. Frequency of pulp stones in groups according to sex

Dependent variable	Pulp stone	Female	Male	Total	X^2	p
Group 1	Present	3 (17.6%)	2 (28.6%)	5	0.36	0.549
	Absent	14 (82.4%)	5 (71.4%)	19		
Group 2	Present	10 (47.6%)	3 (100%)	13	2.90	0.089
	Absent	11 (52.4%)	0 (0%)	11		
Group 3	Present	5 (50%)	3 (21.4%)	8	2.14	0.143
	Absent	5 (50%)	11 (78.6%)	16		
Group 4	Present	9 (69.2%)	10 (90.9%)	19	1.70	0.193
	Absent	4 (30.8%)	1 (9.1%)	5		

*Statistically significant at p<0.05

DISCUSSION

The communication between pulp and periodontium has developmental, iatrogenic, and pathologic components, but the major communication canals are the accessory canals and the apical foramen (2). Clinical evidence suggests that periodontal disease affects dental pulp mainly via its anatomic relationship and circulation. Periodontal disease causes inflammatory lesions in apical and radicular areas of pulp. Usually, the communication with the periodontal

lesion is via the lateral canals, potentially leading to secondary pulpitis (1).

Studies show that pulp stone formation is associated with various systemic diseases and syndromes, including type1 diabetes, osteitis deformans, kidney diseases, cardiovascular diseases, dentinal dysplasia, Marfan syndrome, van der Woude syndrome, Saethre-Chotzen syndrome, elfin facies, and familial expansile osteolysis (4,11-13). Hence, we excluded individuals with systemic diseases.

In the literature, it has been indicated that periapical radiography, bitewing radiography, and cone-beam computed tomography offer more accurate results than panoramic radiography in the detection of pulp stones (9). However, since our study was a retrospective study and we have panoramic radiographs of all patients, the presence of pulp stone was evaluated by a panoramic imaging method. In this study, panoramic radiographs that were taken for routine dental examination were used when evaluating pulp stones. Additional radiographs were not taken to prevent unnecessary radiation exposure. Another advantage of panoramic radiography is the overall coverage of the dental arches. Current technology also allows enlarging images with minimal quality loss. Image quality is also dependent on using the correct device settings and the experience of the X-ray technician (5). The radiographs included in the study were assessed by radiologists for image quality and correct positioning.

The literature indicates that the prevalence of pulp stones increases with age (14,15). Therefore, we chose patients from similar age groups.

Turkish studies report varying prevalence rates for pulp stones. Şener et al. (16) reported that 4.8% of teeth and 38% of all patients had pulp stones. Sisman et al. (17) found these rates as 15% and 57.6%, Gulsahi et al. (14) as 5% and 12%, Colak et al. (15) as 27.8% and 63.6%, and Ilday et al. (5) as 0.5% and 3.5%, respectively. In our study, 46.8% of patients and 8.1% of teeth had pulp stones, and pulp stones were most common in group 4 (79.2%) and the least common in group 1 (20.8%). Our results support the hypothesis that pulp degeneration is associated with periodontal disease (2,18,19). The variance between the studies can be ascribed to differences in populations, radiography methods, evaluated teeth, and the experience of the examiner.

Several studies have investigated the relationship between pulp stones and sex. Ilday et al. (5) reported that pulp stones were significantly more common among men. Gulsahi et al. (14), Kannan et al. (20), and Sisman et al. (17) found no significant correlation between sex and pulp stones. Colak et al. (15) indicated that pulp stones were more common among women. In our study, we found no significant relationship between sex and pulp stones in any of the groups.

Gutman evaluated 102 molars and found that 28.4% of all samples had accessory canals in the furcation region (21). Zuza et al. reported 37 of 40 third molars had accessory canals in the furcation region (22). In their study, Dammaschke et al. detected accessory foramina in 79% of permanent molars (23). Communication between the pulp chamber and the external surface was determined through dentin tubules. The high prevalence of accessory canals in the furcation region and root surface is prominent in the relationship between periodontal and endodontic diseases (24).

The literature reports that the prevalence of pulp stones is different for every tooth, and several studies found that pulp stones were more common in molars (14,15,17,25). We also found that pulp stones were the most common in molars. This may be because molar teeth are more likely to sustain damage due to being the first permanent teeth to erupt and increased occlusal load, and that molars may have more accessory canals in the furcation region.

Histologic studies suggest that individuals with periodontal disease have increased dystrophic calcification (19, 26). One study radiographically examined the relationship between pulp stones and periodontal disease and found more pulp stones among subjects with aggressive periodontitis than in controls (9). In our study, pulp stones were the most common in group 4 and the least common in group 1. We think that the reason for this is that pulpal blood flow was negatively affected due to the damage in surrounding tissues because increased bone loss and probing depth associated with periodontal disease, and exposed root surface due to attachment loss may become vulnerable to more pathologic changes in pulp. Periodontal disease can result in several degenerative changes in the dental pulp, including odontoblastic degeneration, fibrosis, reticular atrophy, hyperemia, calcifications (pulp stones, diffuse calcifications), inflammation, and necrosis (26).

In this study, panoramic radiographs of individuals with different periodontal disease were evaluated retrospectively. Further studies, examining the prevalence of pulp stones in a larger population using periapical radiography or tomography, which would provide more detailed examination possibilities, can elicit more comprehensive data.

CONCLUSION

Our results indicate that pulp stones were more common among people with advanced periodontal disease than healthy controls. Physicians should consider the presence of pulp stones, which may complicate accessing root canals and the pulp chamber, during endodontic treatment in individuals with periodontal disease.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *This study was approved by the Ethics*

Committee of Recep Tayyip Erdoğan University. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

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MEDICAL RECORDS-International Medical Journal

Research Article

Comparison of Platelet Parameters and Electrocardiogram Data in Patients with Generalized Anxiety Disorder with Healthy Control Group

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Abstract

Aim: Generalized anxiety disorder (GAD) refers to the intense stress and tension felt in the face of various life events. Various studies have shown that cardiovascular diseases are more common in patients with anxiety. Frontal QRS-T (fQRS-T) has been shown to be elevated in cardiovascular diseases. In this study, the fQRS-T, hemogram, and biochemistry values of patients with GAD were compared with healthy controls (HC) and the cardiovascular risk status of GAD patients was evaluated.

Material and Methods: Seventy patients with a diagnosis of GAD and no comorbidity followed in the district state hospital's psychiatry outpatient clinic were included in this study. Sociodemographic data, disease severity, hemogram, biochemistry values, electrocardiogram (ECG) data of these patients were recorded. Disease severity was evaluated with The Generalized Anxiety Disorder Test-7 (GAD-7). These values were compared with 80 HCs without any psychiatric or organic disease. The correlation of fQRS-T value with platelet parameters and age was investigated in GAD patients.

Results: According to the statistical analysis, fQRS-T was wider in patients with GAD than in HC (p<.001). Accordingly, basophil count was statistically lower in patients with GAD (p<.001). Eosinophil count and mean platelet volume (MPV) were significantly elevated in patients with GAD (p=.019 and p=.003 respectively). Accordingly, fQRS-T and MPV are highly correlated (p<.001). The GAD-7 score and fQRS-T were positively correlated (p=.001). According to the linear regression analysis for fQRS-T, MPV and GAD-7 scores positively and significantly predict fQRS-T (p<.001 and p=.036 respectively).

Conclusion: This study is the first in the literature to examine fQRS-T in patients with GAD. In this study, we discovered that MPV predicts fQRS-T in GAD. Future studies are essential in predicting cardiovascular risk using methods demonstrating platelet dysfunction in anxiety disorders.

Keywords: Generalized anxiety disorder, mean platelet volume, frontal QRS-T angle

INTRODUCTION

Generalized anxiety disorder (GAD) is marked by psychological and physical signs. Persistent anxious mood, irritability, difficulty concentrating, and feeling restless are psychological symptoms. Although most patients report memory problems, this often develops secondary to difficulty concentrating. Repetitive anxious thoughts consist of everyday events and physical complaints. For example, in the case of autonomic hypersensitivity, the patient begins to worry that he will have a heart attack if he notices the heartbeat. Physical complaints originate from muscle tension and autonomic hypersensitivity. Tension in the muscles can cause tension-type headaches, especially in the frontal or occipital regions, and tremors and pain in the back and shoulder region. Autonomic hypersensitivity can cause various somatic complaints by affecting all systems in the body. For example, the respiratory system is affected, causing a feeling of tightness in the rib cage and excessive breathing. Cardiovascular complaints are in the form of palpitations and chest pains (1).

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In general practice, patients with GAD refer to physicians because of physical rather than psychological symptoms. According to studies, these patients seek treatment from non-psychiatrists twice as often as they seek treatment from psychiatrists. Most patients with non-cardiac chest pain complaints have GAD (2). Patients with GAD apply to cardiology clinics at almost the same rate as panic disorder patients. However, panic disorder is recognized more than GAD due to the increasing media attention, especially in recent years. Other presenting complaints of GAD include chest pain, irritable bowel syndrome, hyperventilation syndrome, and fatigue. Therefore, examinations such as exercise electrocardiogram (ECG), echocardiogram, coronary angiogram, and endoscopy performed to evaluate most of these patients unnecessarily increase the cost on the healthcare system. There are limited data on the increased risk of cardiovascular disease in patients with GAD. In several studies, it has been reported that the risk of cardiovascular disease is elevated in patients with GAD (3).

Hypothalamic-pituitary-adrenal dysregulation, changes in platelet functions, impaired immune system and decreased heart rate variability are blamed in the pathophysiology of GAD. In addition, cardiac risk factors such as sedentary life, smoking, diabetes, dyslipidemia, alcoholism and hypertension are detected more frequently in GAD patients. These pathophysiological mechanisms and risk factors suggest that there may be a relationship between GAD and cardiovascular disease (3).

Frontal QRS-T angle (fQRS-T) is a non-invasive and simple ECG parameter that can be simply computed from ECG without requiring any special software. The fQRS-T is the definite angle diffraction between the QRS and T axes. This ECG parameter is quite recent and provides valuable insight into the process of myocardial repolarization (4). Researchers have documented that fQRS-T can forecast upcoming cardiovascular events in diverse populations, and this parameter has been linked to arrhythmias and sudden death (5).

Although the risk of cardiovascular disease in GAD patients has been evaluated in longitudinal studies in the literature, there is no similar study evaluating fQRS-T like this study. In this study, it is aimed to evaluate the risk of cardiovascular disease in GAD patients through the evaluation of fQRS-T and hemogram parameters and to provide treatments to prevent cardiovascular disease development in these patients.

MATERIAL AND METHOD

Study Design

The current research is a comparative and interpretive study. The Local Ethics Committee accepted the research protocol (Approval date: 2021-12-14; IRB Number: 2021/10-10). Permission was secured from all subjects before the project, and the examination was implemented following the Declaration of Helsinki.

Sample Size

The sample size was calculated as a result of the evaluation of the study by Almis et al. (6) According to the power analysis we performed, when $p<0.05$, power of 0.80, enrollment ratio=1, and effect size of 0.5 were accepted. The standard t-test was applied according to the study of Almis et al. (6) (group 1: mean 7.50, standard deviation 1.24; group 2: mean 8.15, standard deviation 1.41; mean difference between groups: $p<0.05$). As a result, it was determined that at least 57 people must be in each group.

Study Group

The patients included in this study were followed by the same psychiatry specialist in the district state hospital psychiatry clinic. Cardiovascular examination of all patients was performed by a specialist cardiologist. Eighty-three patients with GAD, determined through The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), were involved in the examination (7). The Structured Clinical Interview for DSM-5-Clinician Version (SCID-5/CV) was used to diagnose patients in the study. Those with hypertension (n=2), coronary artery disease (n=1), valve disease (n=1), and arrhythmia (n=2), and those not within the age range of 18 to 65, were not included in the study. The symptom checklist 90 (SCL-90) was used to evaluate additional psychiatric disorders of GAD patients. Seven patients with depression were not included in the study. A total of 70 GAD patients were included as eligible for the study. Eighty healthy controls (HC) without any psychiatric or organic disease, who applied to the cardiology outpatient clinic to obtain a driver's license, employment report, and health report, were included in the study. Those with known coronary artery disease, valve disease, diabetes, hypertension, arrhythmia, thyroid gland disease, kidney disease, iron deficiency anemia were not included in the study. Age, gender, smoking status, hemogram, biochemistry, blood pressure measurements, and ECG parameters of the participants were used. Hemogram, biochemistry and ECG examinations of GAD patients were performed at the time of their first application to the psychiatry outpatient clinic.

The Generalized Anxiety Disorder Test-7 (GAD-7)

GAD-7 is a quick self-report instrument in line with DSM-IV-TR guidelines for assessing GAD (8). This is a scoring scale (0=none, 1=several days, 2=over half the days, 3=nearly every day) composed of seven questions that evaluate the feelings associated with the items on the scale over the last two weeks. The aggregate scores of 5, 10, and 15 on the scale are cut-off scores for mild, moderate, and severe anxiety, correspondingly. The Turkish validity and reliability study of the GAD-7 scale was performed by Konkan et al. (9). Konkan et al reported that the most acceptable value of sensitive and specificity for the diagnosis of GAD was 8 in GAD-7 scale. The GAD-

7 scale was administered to the patients during their first application to the psychiatry outpatient clinic.

Electrocardiogram Examination

The 12-lead ECG (Nihon Kohden, Tokyo, Japan) was monitored for each subject. QRS and QT interval parameters were generated systematically. QRS duration was measured between Q wave onset and S wave finish. QT interval was described as the duration between QRS onset to T wave end. QT interval differs with the heart rate (with a rise in heart rate, QT interval reduces, while with a drop in heart rate, QT interval lengthens). Thus, it needs to be adjusted accordingly to the heart rate. The corrected QT interval (QTc) represents the QT interval at a constant heart rate of 60. QRS and T axes were readily accessible in the documentation of the ECG machine. The report checked them, and the fQRS-T was determined by the actual difference between the QRS and T axes. ECG examination was performed by an 8-year-experienced cardiologist.

Laboratory Analyses

A venous blood sample was collected on admittance to the hospital. An electronic hematology testing device CELL-DYN Ruby (Abbott Diagnostics, Abbott Park, IL, USA), was utilized to measure white blood cells (WBC), including neutrophils and lymphocytes. We also measured hemoglobin, mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW), and platelet counts.

Statistical Analysis

Data were evaluated through SPSS software 26.0 (SPSS Inc., Chicago, IL, USA). Means and standard deviations were computed to represent numerical parameters, and percentages were computed to represent qualitative parameters. An elucidation of data patterns was done via the Kolmogorov-Smirnov test. Independent sample t-tests were applied when comparing continuous measures, while Mann-Whitney U tests were employed for non-continuous numerical measures. Chi-square tests were conducted to compare qualitative variables within the study group. Spearman correlation test was run to examine the relationship between fQRS-T, platelet parameters, disease severity score, and age. Linear regression analysis was performed to assess the impact of platelet parameters, disease severity score, and age on the fQRS-T.

RESULTS

According to the statistical analysis, fQRS-T was wider in patients with GAD than in HC (p<.001) (table 1). The comparison of laboratory parameters is presented in table 2. Accordingly, basophil count was statistically lower in patients with GAD (p<.001). Eosinophil count and MPV were significantly elevated in patients with GAD (p=.019 and p=.003 respectively). The correlation between fQRS-T with age, laboratory parameters, and GAD-7 score in patients with GAD is shown in Table 3. Accordingly, fQRS-T and MPV are highly correlated (p<.001). GAD-7 and fQRS-T were significantly and positively correlated (p=.001). Linear regression analysis was applied to evaluate the effect of age, GAD-7 score, MPV, PCT, and PDW on fQRS-T. Accordingly, the regression model was significant since p<.001 for ANOVA test. According to the linear regression analysis for fQRS-T, MPV and GAD-7 scores positively and significantly predict fQRS-T (p<.001 and p=.036 respectively) [F(5.58)=11.894, p=<.001, adjusted R square: .464].

Table 1. Comparison of sociodemografic and ECG parameters of patients with GAD and HC

	GAD Patients (n=70)	HC (n=80)	p
Age	32.34±6.87	32.53±6.29	.866[1]
Gender			
Female (n/%)	39 (55.7)	41 (51.2)	.585[3]
Male (n/%)	31 (44.3)	39 (48.8)	
Smoking (n/%)	23 (32.9)	22 (27.5)	.475[3]
Heart rate, bpm	79.79±13.90	78.71±12.93	.587[2]
QRS, msec	87.17±8.69	87.90±8.41	.603[1]
QT, msec	362.11±31.86	363.43±29.02	.792[1]
QTc, msec	404.49±28.84	404.51±27.15	.995[1]
fQRS-T (o)	40.84±26.82	23.85±19.44	**<.001[2]**

[1]Independent t test was used. [2]Mann-Whitney U test was used. [3]Chi square test was used. p<.05 was accepted as statically significance.
GAD: Generalized anxiety disorder, HC: Healthy controls, QTc: Corrected QT interval, fQRS-T: Frontal QRS-T angle

Table 2. Comparison of laboratory parameters of patients with GAD and HC

	GAD Patients (n=70)	HC (n=80)	p
Hemoglobin, mg/dL	14.78±1.84	14.29±1.98	.119[1]
WBC, 10^3/µL	7.65±1.81	7.90±1.62	.374[1]
Neutrophil, 10^6/µL	4.41±1.34	4.63±1.26	.325[1]
Lymphocyte, 10^3/µL	2.42±.76	2.54±.79	.377[1]
Monocyte, 10^3/µL	.53±.17	.50±.23	.829[2]
Eosinophil, 10^3/µL	.21±.17	.15±.12	**.019[2]**
Basophil, 10^3/µL	.04±.04	.08±.05	**<.001[2]**
Platelet, 10^3/µL	247.29±78.96	253.25±75.66	.540[2]
PDW, fL	19.93±1.25	20.14±2.01	.810[1]
MPV, fL•	8.34±1.21	7.73±1.47	**.003[2]**
PCT, %	.21±.05	.19±.05	.097[1]

[1]Independent t test was used. [2]Mann-Whitney U test was used. p<.05 was accepted as statically significance
GAD: Generalized anxiety disorder, HC: Healthy controls, WBC: White blood cells, PDW: Platelet distribution width, MPV: Mean platelet volume, PCT: Plateletcrit

Table 3. Correlation analyses of frontal QRS-T angle with age and inflammatory parameters in patients with GAD

	fQRS-T Angle
Age	r=.211
	p=.698
MPV	r=.666
	p=<.001
PCT	r=.119
	p=.328
PDW	r=.013
	p=.917
Platelet	r=.047
	p=.698
GAD-7	r=.420
	p=.001

Spearman corelation analyses was used. p<.05 was accepted as statically significance.
GAD: Generalized anxiety disorder, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, PLR: Platelet to lymphocyte ratio, GAD-7: Generalized anxiety disorder-7 score

Table 4. Lineer regression analyses of frontal QRS-T angle in GAD patients

	B	Std. Error	Beta	t	p	95 % CI	
						Lower	Upper
Constant	-122.116	51.292		-2.381	.021	-224.789	-19.444
GAD-7	1.723	.803	.214	2.145	.036	.115	3.330
MPV	13.344	2.203	.604	6.056	<.001	8.934	17.754
Age	.187	.405	-.044	-.462	.797	-.998	.623
PCT	13.994	43.796	.030	.320	.750	-73.672	101.660
PDW	1.548	2.062	.071	.751	.456	-2.580	5.676

Lineer regression analyses was used. p<.05 was accepted as statically significance.
GAD: Generalized anxiety disorder, GAD-7: Generalized anxiety disorder-7 score, MPV: Mean platelet volüme, PCT: Plateletcrit, PDW: Platelet distribution width

DISCUSSION

According to the results of our study, fQRS-T were wider among patients with GAD. In addition, MPV, positively correlated with fQRS-T, was higher in the group with generalized anxiety disorder.

fQRS-T shows the difference in the frontal plane projections of the QRS and T wave vectors showing ventricular repolarization and can be easily measured electrocardiographically. Aro et al., the first to analyze the connection between fQRS-T angle and cardiac mortality, stated that a wide fQRS-T angle was predictive of arrhythmic deaths (10).

Numerous studies have examined the increased cardiovascular risk associated with GAD. It is typical for patients with cardiovascular disease to suffer from anxiety disorders and related diseases, which can adversely affect cardiac mortality at the beginning and progression of their illness. The links between anxiety disorders and cardiovascular disease can be explained both physiologically (autonomic dysfunction, inflammation, endothelial dysfunction, alterations in platelet aggregation) and behaviorally. Because anxiety disorders and poor heart health are associated, it is crucial that these conditions are identified and treated promptly and accurately. The good news is that pharmacological and psychotherapeutic interventions are generally safe and effective for managing anxiety disorders (11,12).

There is a high incidence of GAD in people with heart disease. Meta-analysis found that cardiac patients were more likely to experience GAD at a point frequency of 11% and over a lifetime frequency of 26%. GAD frequency was reported at 14% in a meta-analysis of patients with coronary artery disease and heart failure. Comparatively, the general US population has a lifetime GAD frequency of 3 - 7% (13,14).

The cardiovascular risk of anxiety disorders is increased by a number of mechanisms. Inflammatory mechanisms have a significant contribution in the emergence and progress of heart disease (15). The development of heart diseases has been linked to inflammation pathways such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP). CRP levels have been linked to increased mortality in unstable angina patients. As a result, in patients with heart failure, inflammation is related to worsening of function, higher hospitalization rates, and poorer survival rates. It has been demonstrated that anxiety disorders are characterized by elevated inflammatory biomarkers (16,17). Research on healthy adults revealed high levels of anxiety to be correlated with increased levels of CRP, TNF-α, IL-6, homocysteine, and fibrinogen. Anxiety disorders, such as GAD, posttraumatic stress disorder (PTSD), and panic disorder (PD), have been implicated in inflammation, specifically CRP (18-20). It has been found that PTSD can also increase levels of TNF-α, IL-1, IL-6, interleukin-1β, and interferon-γ. There may be a link between anxiety disorders and inflammation that might be involved in the initiation to cardiovascular disease in anxiety disorders (21,22).

Regulating platelet activity, thrombosis, vascular tone, and leukocyte adhesion is important for maintaining vascular endothelial health (23). In patients with heart failure, endothelial dysfunction increases the likelihood of hospitalization, heart transplantation, and death (24,25). It has been found that anxiety disorders alter the endothelium of the vessels. Anxiety impairs the flow-mediated dilatation of the vasculature, suggesting more significant endothelial dysfunction (26-28). Patients with GAD, PD, and obsessive-compulsive disorder have lower levels of circulating endothelial progenitor cells,

which are essential for proper endothelial function and for preventing coronary artery disease progression (29). Further, in patients with PTSD, soluble tissue factor and von Willebrand factor levels are raised, which is related to thrombosis and atherosclerosis. Activation of platelets has been shown to play a major role in thrombosis and myocardial ischemia (30). There is evidence that serotonin increases platelet aggregation. There is proof that anxiety disorders are linked to disorders of the serotonin system, and this leads to increased cardiovascular disease risk (31,32).

By binding to 5-hydroxytryptamine-2 (5HT-2) receptors on platelets, serotonin increases the secretion of factors that boost platelet aggregation. Vasodilation is achieved by the production of nitrous oxide by endothelial cells in healthy vessels in order to prevent thrombus formation. Atherosclerosis damages the endothelial cells, causing the vessels to dilate improperly, and exposure to serotonin causes vasoconstriction (33). There is an underlying mechanism linking increased blood serotonin levels to cardiac events in coronary artery disease (34). Platelet aggregation is generally higher in patients suffering from anxiety and acute stress (35,36). The function of platelets may also be affected in anxiety disorders, such as PTSD and PD. Platelet activation may increase in PTSD patients due to fluctuations in circulating catecholamines and hyperactive sympathoadrenal system (37). Earlier studies have shown that patients with PD have abnormal nitrous oxide and homocysteine levels in their blood. Patients with PD showed increased MPV, indicating increased platelet activity (38).

Psychiatric conditions can cause biochemical changes in platelets. MPV, a indicator of platelet sizing, is viewed as a marker of platelet functioning (39). A higher MPV is strongly related to cardiovascular diseases, such as acute myocardial infarction, ischemic heart disease, and congestive heart failure (40). In hospitalized congestive heart failure patients, MPV is a predictive factor for hospital admission and 6-month mortality. Research has indicated that MPV is an independent determinant of atherosclerosis (41,42).

Ozdemir et al. found high MPV levels in patients with acute myocardial infarction and found a positive correlation between sympathetic activity and MPV. They explained this with the activation of alpha 2 receptors of the adrenergic system, activation of peripheral platelets and increased thrombocytopoiesis in the bone marrow (43). In a study comparing MPV values before and after cardioversion in patients with atrial fibrillation, Makowski et al. showed that there was a significant decrease in MPV levels and platelet levels indicated by fluorescent antibodies 4 weeks after sinus rhythm restoration with sinus rhythm restoration. They pointed out that MPV is inexpensive and easy to study as a predictor of thromboembolism in patients with atrial fibrillation (44). Chu et al. (2010) found that high MPV was a cardiovascular risk factor in a meta-analysis that included more than 6000 people and collected from 24 studies. They stated that this was due to the higher MPV levels in patients with acute myocardial infarction compared to the group without myocardial infarction, the higher mortality rates in patients with myocardial infarction with high MPV, and the higher incidence of restenosis in patients with high MPV in patients who underwent coronary angioplasty (45).

Uysal et al. investigated the relationship between anxiety levels and the dose of MPV and propofol in preoperative patients and found that MPV levels were higher in the group with high anxiety levels, and they found that the need for propofol dose was higher in the group with high anxiety levels (46). Almış et al found MPV higher and platelet count lower in GAD patients compared to the control group. According to the ROC analysis, they showed that those with MPV cut-off value above 7.5 fl had 56% sensitivity and 87.72% specificity for GAD (6). In a study by Mukta et al. with 144 patients, they found MPV levels to be higher in GAD patients compared to the control group (47). Gul et al found MPV levels lower in panic disorder patients compared to the control group. They thought that this might be due to abnormalities in 5-HT receptor functions (48).

In the present study, the high MPV in patients with GAD and the positive correlation of MPV with fQRS-T may explain the platelet dysfunction and excessive platelet aggregation that increase the risk of cardiovascular disease in GAD.

The present study has drawbacks. To validate these results, there could be a larger number of participants and multicenter studies. Laboratory reserches at the molecular level and microscopic examinations (peripheral smear), which can show platelet functions more clearly, may show better results. Since coronary angiography or myocardial scintigraphy was not performed in GAD patients or healthy controls, the presence of cardiovascular disease cannot be completely ruled out. Evaluation of the lipid panel could be used to assess participants' cardiovascular disease risk.

CONCLUSION

In this study, fQRS-T was found to be elevated in GAD patients. Evaluation of fQRS-T in GAD patients may identify GAD patients at high risk of cardiovascular disease. It may be recommended that these patients be evaluated by a cardiologist. Providing these patients with more effective psychiatric treatment and avoidance of tricyclic antidepressants or antipsychotics with high cardiac side effects may be helpful.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *The current research is a comparative and interpretive study. The Adıyaman University Local Ethics*

Committee accepted the research protocol (Approval date: 2021-12-14; IRB Number: 2021 / 10-10).

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Research Article

COVID-19 Infection in Patients with Gaucher Disease

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Abstract

Aim: Coronavirus disease 2019 (COVID-19) is a severe acute respiratory syndrome with a high mortality rate and has been labeled a global pandemic in March 2020. Gaucher Disease (GD) is one of the rare inherited lysosomal storage diseases (LSDs). We aimed to call attention to the frequency, susceptibility of COVID-19 infection, and the factors that prevent this infection in patients with GD as compared to other LSDs.

Material and Methods: The study was conducted retrospectively between September and December 2020. Participants were divided into two groups: GD group (19 patients) and the control group (19 patients, those with other LSDs). All patients were contacted by phone to collect data about their health status, and any possible contact with Covid-19 patients.

Results: Six of the GD patients (36.8%) had contacted a confirmed COVID-19 infected person but only three (15.8%) had developed a mild COVID-19 with fever and fatigue that did not require hospital admission. Four of the control group patients (21.1%) had experienced contact with a person with a confirmed COVID-19 infection. Three of the control group patients, that comprised of patients with various LSDs other than GD (15.8%) were positive on COVID-19 PCR tests and two of them had developed a mild COVID-19 infection. One of these (with Mucopolysaccharidosis type 1) had severe symptoms and required hospitalization.

Conclusion: There is no consensus on the management of rare diseases such as lysosomal storage diseases during the COVID-19 pandemic. Developing plans regarding the management of COVID-19 infections in LSDs will be useful when drawing up consensus guidelines.

Keywords: Gaucher disease, lysosomal storage disorders, COVID-19

INTRODUCTION

Lysosomal storage disorders (LSDs) are metabolic disorders that are caused by the storage of various substrates in the lysosomes. LSDs are mostly inherited autosomal recessively and the prevalence is approximately 1 in 4000 live births (1). The relevant clinical features are associated with substrate storage in the organs and systems including the heart, kidneys, skin, upper respiratory tract, lungs, and intestines. Symptoms may appear anytime from the newborn period to late adulthood (2).

Gaucher Disease (GD) is a genetically inherited LSD that occur due to homozygous variations in the GBA1 gene that causes diminished function of the lysosomal glucocerebrosidase enzyme and thus, glucocerebrosides accumulate in macrophages of various tissues (2). The disease is categorised as the non-neuronopathic (GD type 1) and neuronopathic (GD type 2 and GD type 3) types. Some patients that have previously undergone splenectomy due to hypersplenism can develop pulmonary vascular complications (3,4).

Many treatment options that are useful on many of the systemic non-neurological manifestations of GD are available including substrate reduction therapy (SRT) and enzyme replacement therapy (ERT) (5,6).

Chronic inflammation, immune dysfunction, coagulopathy and fibrinolysis have recently all been shown to be the underlying pathogenic mechanism in GD (7). The presence of activated macrophages and pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 and 10 (IL6, IL10) and have been reported in patients with

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GD (8). In addition, elevated serum angiotensin converting enzyme (ACE) is thought to be the result of production by Gaucher cells, reflect disease activity in GD (9).

Coronavirus disease 2019 (COVID-19) is a severe disease caused by the COVID-19 virus (10). People with chronic diseases are at higher risk of severe effects during this pandemic (11).

In this study, we aimed to analyze the frequency of COVID-19 infection, susceptibility to COVID-19 infection, and the preventive factors in patients with GD as compared to other LSDs.

MATERIAL AND METHOD

Permission to conduct the study was obtained from the local ethics committee (Approval date: 16.09.2020, approval number: E1-20-1173). The study was compatible with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000. Written consent was obtained from all participants. The subjects were divided into two groups as those with GD (named as Gaucher group) and those with other LSds (named as control group). Data were collected from the hospital records. All patients were contacted by phone to collect data about their health conditions, and any possible contact with Covid-19 patients. Laboratory tests including enzyme activity measurement, molecular genetic analysis, and angiotensin converting enzyme level determination were conducted.

Lysosomal enzyme activities were evaluated in leukocytes extracted from whole blood samples. Molecular genetic analysis was performed on DNA isolated from EDTA blood samples. Angiotensin converting enzyme levels were analyzed with the turbidimetric method from a biochemistry blood sample.

Descriptive statistics were used to express the results as the median and range. The Kolmogorov-Smirnov test was used to test the distribution of the variables, and the Mann-Whitney U test for the analysis of quantitative independent data. The chi-square test was used for the analysis of qualitative independent data, while the Fisher test was used when the chi-square test conditions were not met. The SPSS 27.0 software was used for the analyses.

RESULTS

The study was conducted retrospectively between September and December 2020. The Gaucher group consisted of 19 patients with GD (Table 1), with 8 males (42.1%) and 11 females (57.9%). Most of the patients in this group had type 1 GD (15/19, 78.9%) while 4 patients had type 3 GD (21.2%). The GBA analysis revealed that the most common genotype was detected as c.1226A>G (N370S) in type 1 GD and c.1448T>C (L444P) in type 3 GD. All patients were receiving ERT at the hospital but 6/19 (31%) patients reported that they had missed few doses at the beginning of the pandemic, without experiencing any bone-related or metabolic crisis.

Table 1. The gaucher disease patients' general characteristics

Gaucher Group		Min-Max	Median	Mean SD±(n%)
Age		2.0 - 38.0	22.0	20.6±11.1
Age at diagnosis		1.0 - 30.0	7.0	8.4±7.9
ACE enzyme level		25.0-82.6	35.0	44.7±21.5
		N (%)		
Gender	Female	11 (57.9%)		
	Male	8 (42.1%)		
Subtype of GD	Type I	15 (78.9%)		
	Type III	4 (21.1%)		
Genetic analysis	L444P	5 (26.3%)		
	N370S	14 (73.7%)		
Disruption of enzyme therapy?	Yes	13 (68.4%)		
	No	6 (31.6%)		
History of COVID-19 infection?	No	16 (84.2%)		
	Yes	3 (15.8%)		
Contact with COVID-19 infected person?	No	12 (63.2%)		
	Yes	7 (36.8%)		
Splenectomy status	No	16 (84.2%)		
	Yes	3 (15.8%)		

ACE: Angiotensin converting enzyme

Six of our patients with GD (36.8%) had contacted a confirmed COVID-19 infected person but only three (15.8%) were PCR-positive for COVID-19 with a nasal swab test and had developed mild symptoms with fever and fatigue. Two of these affected patients had undergone splenectomy. The median ACE level was 35 U/L and the mean 44.7±21.5 U/L (range 8-52). ACE levels of patients before the initiation of ERT were unavailable.

The control group included 19 patients consisting of 10 females (52.6%) and 9 males (47.4%) with a median age of 9 years and mean age of 14.7±17.1 years (Table 2). These patients had other LSDs such as Pompe disease, Metachromatic Leukodystrophy (MLD), Fabry disease, and Mucopolysaccharidosis (MPS). The diagnoses consisted of Fabry disease (4 patients), Pompe disease (3 patients), MLD (2 patients), Mucopolysaccharidosis (5 patients consisting of 1 patient with type 1, 2 patients with type

Table 2. The control group's general characteristics

Control Group		Min-Max	Median	Mean
Age		2.5-59.0	9.0	14.7±17.1
		N (%)		
Gender	Female		10 (52.6%)	
	Male		9 (47.4%)	
Subtype of LSD	Fabry		4 (21.1%)	
	Pompe		4 (21.1%)	
	MLD		2 (10.5%)	
	Krabbe		1 (5.3%)	
	MPS Tip 1		1 (5.3%)	
	MPS Tip 2		2 (10.5%)	
	MPS Tip 3		2 (10.5%)	
	Niemann-Pick Type B		1 (5.3%)	
	Niemann-Pick Type C		1 (5.3%)	
	Wolman		1 (5.3%)	
Disruption of enzyme therapy or substrate reduction therapy	Yes		8 (61.5%)	
	No		5 (38.5%)	
History of COVID-19 infection?	No		16 (78.9%)	
	Yes		3 (21.1%)	
Contact with COVID-19 infected person?	No		15 (78.9%)	
	Yes		4 (21.1%)	

MLD: Metachromatic leukodystrophy, MPS: Mucopolysaccharidosis

Table 3. The comparison of the characteristics of gaucher group and control groups

		Control Group		Gaucher group		P
		Mean ± SD	**Median**	**Mean ± SD**	**Median**	
Age		20.6±11	22	14.7±17.1	9.0	0.029m
		N (%)		**N (%)**		
Gender	Female	11 (57.9%)		10 (52.6%)		0.744X²
	Male	8 (42.1%)		9 (47.4%)		
Disruption of enzyme therapy or substrate reduction therapy	Yes	13 (68.4%)		8 (61.5%)		0.734X²
	No	6 (31.6%)		5 (38.5%)		
History of COVID-19 infection?	No	16 (84.2%)		16 (78.9%)		0.738X²
	Yes	3 (15.8%)		3 (21.1%)		
Contact with COVID-19 infected person?	No	12 (63.2%)		15 (78.9%)		0.283X²
	Yes	7 (36.8%)		4 (21.1%)		

m: Mann-Whitney u test / X² Chi-square test

2 and 2 patients with type 3), Krabbe disease (1 patient), Niemann-Pick disease type B (1 patient), Niemann-Pick disease type C (1 patient), and Wolman disease (1 patient). 12/19 of our patients in control group (68.4%) (with diagnoses of Fabry disease, MPS type1 and type 2, Pompe disease, and Wolman disease) were routinely receiving ERT while one patient with Niemann-Pick disease type C was receiving SRT. Eight patients in the control group had missed several doses during the pandemic.

Four patients in control group (21.1%) had experienced contact with a person with a confirmed COVID-19 infection. Three patients in control group (15.8%) were positive on COVID-19 PCR tests and two of them had developed a mild COVID-19 infection. One of these (with MPS type 1) had severe symptoms such as high fever, pneumonia, and ARDS. This patient later required intubation, followed by extubation after one week of hospitalization. She was discharged from the hospital after one month of treatment.

The age of the patients in the GD group was significantly higher than in the control group ($p<0.05$). Gender distribution and the rate of enzyme replacement therapy disruption in the Gaucher and control groups did not differ significantly ($p>0.05$). There was also no significant difference between the COVID-19 infection rate ($p>0.05$) or the contact history rate ($p>0.05$) in the Gaucher and control groups (Table- 3).

DISCUSSION

Lysosomal storage disorders (LSDs) are characterized by the accumulation of storage materials in the lysosomes. leading to severe multisystemic effects (19). The treatment of many LSDs consist of ERT or other oral drugs (20).

GD is caused by variants in the GBA1 gene, that is located on 1q21, and inherited autosomal recessively. The mutation leads to disturbed activity of the lysosomal glucocerebrosidase, that is responsible for the hydrolyzation of glucosylceramide (GlcCer) into ceramide and glucose. Three subgroups have been defined for GD. Type 1, the most common form, does not cause neurological damage, on the contrary, types 2 and 3 lead to neurological dysfunction (21).

Sphingolipids are known to be involved in inflammation and apoptosis, and glucosylceramide has been suggested to have direct effects on macrophage function. Several markers of macrophage activation (chitotriosidase, CCL18, and angiotensin-converting enzyme) have been detected in the plasma of patients with GD (22).

COVID-19 is the coronavirus that has caused the current pandemic (23,24). Angiotensin-converting enzyme 2 is a homologue of angiotensin-converting enzyme (ACE), which is the functional receptor of COVID-19.

COVID-19 infection has been recognized to develop in three stages. In stage I, the virus particles bind to an ACE2 receptor and endolysosomal processing starts. Early symptoms that include fatigue, cough, and fever can develop in this stage (12,13). Most patients recover at this stage. Progression to the second stage with pulmonary disease (with or without hypoxemia) is seen in 15% of the patients and chest X-ray or CT scan will reveal diffuse pulmonary infiltrates or ground glass opacities (14). The third stage occurs in 5% of the patients and can cause systemic inflammation, multiorgan involvement, and acute respiratory distress syndrome (ARDS) (15,16).

ACE2 is the functional receptor of COVID-19 and plays an important role in the pathogenesis of COVID-19 as it enables viral entry into human cells (17). ACE2 is a zinc-metallopeptidase that acts as an antagonist of ACE. ACE converts angiotensin I to angiotensin II which is a vasoconstrictor. ACE also breaks down bradykinin, a vasodilator. The main role of ACE2 is to convert Ang II to Ang I, thus counteracting the effects of Ang II as a pressor, proliferative agent, and pro-fibrotic agent (18).

The similarities between GD and COVID-19 infection are striking. Both diseases show lysosomal involvement, destruction, activated hypercytokinemia pathway, and a proinflammatory response.

The study population included 19 patients with Gaucher disease consisting of 8 males (42%) and 11 females (58%) with a median age of 19 years (range 2-38). Fifteen of our patients with Gaucher disease (78.9%) had type 1 disease while the remaining four cases were type 3. The GBA analysis revealed that the most common genotype was detected as c.1226A>G (N370S) in type 1 GD and c.1448T>C (L444P) in type 3 GD.

All subjects with GD were receiving ERT at the hospital. Six of the 19 patients (31%) reported that they missed few doses at the beginning of the pandemic but they had not experienced any bone-related or metabolic crisis. A study from Spain has reported that 25% of Gaucher patients that received ERT missed several doses during the pandemic, and again did not suffer any bone-related complaints or acute bone pain. However, the worldwide enzyme production has caused bone crisis, bone pain, anemia, and thrombocytopenia some patients around the world (24).

The Spanish Gaucher Disease Foundation has surveyed 113 GD patients. Six of the patients reported to contact with a confirmed COVID-19 infected person. Two previously splenectomized GD patients developed COVID-19 infection. One developed severe symptoms and died due to multiorgan failure while the second had mild symptoms and did not require hospitalization (25). Zimran et al. have studied 550 adult GD patients and found only one patient with confirmed COVID-19 infection, in this case with a mild clinical course (2).

Several blood biomarkers such as angiotensin-1-converting enzyme (ACE), chitotriosidase, and acid phosphatase have been identified for GD (24). The angiotensin converting enzyme levels were high in only 2 of our 19 GD patients (10%). These generally lower ACE levels in our group are thought to be related by receiving regular enzyme replacement therapy.

Although Ballout et. al have proposed that the inherent cellular and biochemical abnormalities of LSDs, especially Niemann-Pick disease type C (NPC) create possibly "unfavorable" environments for COVID-19 infectivity in the corresponding host cells (25), patients with LSDs are at high risk of severe symptoms because of multisystem involvement. The tests of 3 patients in the control group were positive for COVID-19, and two of them developed mild COVID-19 infection. One case with the diagnosis of MPS type 1 experienced severe symptoms such as high fever, pneumonia, and ARDS requiring intubation. The other two patients were mother and son who had both mild infection. None of the remaining patients had symptoms of Covid-19 and therefore, PCR tests were not performed. Eight patients (61,5%) in the control group had missed several doses during the pandemic. A study of 102 patients in Italy interviewed during the pandemic found that 77.5% were receiving ERT at the hospital and 22.5% were on home therapy, of which 100% continued to take their medication except one, while 49% of the patients receiving ERT at the health care facilitites experienced treatment interruptions although no one was infected, which may be explained by the attention of these patients to hygiene and infection prevention measures (26). The control group of our study consisted of patients with LSDs other than GD. Although this situation might be considered as a limitation, the main principle of our study was to evaluate the effects of COVID-19 infection on GD with respect to other LSDs. Our study has clearly showed that GD patients may have a better prognosis of SARS-CoV-2, when compared to other LSDs.

CONCLUSION

In conclusion, there was no sign of severe infection in our Gaucher Disease patients, probably because they received their ERT treatment regularly and paid more attention to the hygiene rules due to their underlying diseases with compared to the normal population.The ACE levels were not very high in our patients with GD because they had been receiving ERT treatment regularly for more than a year and none of our patients had been recently diagnosed. There is no consensus management of rare diseases such as lysosomal storage diseases during the COVID-19 pandemic. Developing plans regarding complications of lysosomal storage diseases as related to COVID-19 infections will be useful when drawing up consensus guidelines.

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Conflict of Interest: *The authors declare that they have no competing interest.*

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Research Article

Cellular Response of Cancer to Music: Mirror, Mirror, on the Wall, Which is the Most Effective of Them All?

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Abstract

Aim: Cancer is one of the most common health problems in the world. Music is a therapy method that creates positive changes in human behavior. This study aims to examine the effects of Turkish and Western classical music on MCF-7, SKOV-3, PC-3, U87, and COLO741 cancer cell lines.

Material and Methods: Group 1: No sound, Group 2: Turkish classical music (ney), Group 3: Western classical music (piano), and Group 4: Western classical music (violin). After listening to the cancer cells for 40 min, the audio files were interrupted for 1h, and then music was played for another 40 min. The effect of music on the proliferation of cancer cells was determined by WST-1 cell viability analysis. Statistical analyzes were performed with the SPSS program.

Results: There was no statistically significant difference between the groups that were applied music once in only the MCF-7 breast cancer cells and the control group ($p>0.05$). A statistically significant decrease was detected in cell viability when the control group and the other groups that were applied to music twice were compared ($p<0.05$). For other cancer cell lines except (please the SKOV-3 ovarian cancer cells, it was determined that music application once also caused a statistically significant decrease in cell viability ($p<0.05$).

Conclusion: We determined that classical music effectively reduced and suppressed the number of cancer cells. According to the data, Turkish and Western classical music can be used to support the treatment in of cancer patients.

Keywords: Breast cancer, prostate cancer, colon cancer, ovarian cancer, glioblastoma, turkish classical music, western classical music

INTRODUCTION

Cancer is one of the most important clinical issues in the world. According to the Turkey Cancer Statistics 2017 report, prostate and colon cancer in men and breast, colon, ovarian, and brain cancer in women are the most common cancer types in all age groups (1). According to the World Health Organization (WHO) data, 10 million people died of cancer worldwide in 2020 (2).

Cancer patients experience physical problems such as pain, loss of appetite, cachexia, hair loss, nausea, vomiting, fatigue, and psychological problems such as sleep disorders, depression, and anxiety due to the side effects of chemotherapy and radiotherapy. Music is a therapy method that changes human behavior and reduces pain and anxiety (3). Music has been used in various clinical treatments from the past to the present. In recent years, music therapy has been used as an option in many medical fields such as relieving symptoms associated with chemotherapy in cancer patients (4). Turkish-Islamic physicians such as Er-Razi, Farabi, Ibn-i Sina (Avicenna) were used music therapy for psychological diseases. They used medicine and music treatment, and these methods were developed by both Seljuk and Ottoman physicians until the 18th century (5).

Studies have shown that Western classical music can reduce the number of cancer cells (6), and there is no publication in the literature examining the effects of

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Turkish classical music on cancer cells using in vitro cell culture techniques. In our study, we aimed to find out whether Turkish classical music has a lethal effect on cancer cells, and if so, on which cancer cell type it is more effective. In vitro studies in the literature have been carried out only with Western classical music, and no publication that examines Turkish classical music comparatively.

MATERIAL AND METHOD

Cell culture

This study was carried out in the cell culture laboratory of Mugla Sitki Kocman University Faculty of Medicine, Department of Histology and Embryology. Before starting the laboratory experiments, "Laboratory Safety Rules" were explained to the lead student by the advisors and all experiments were performed under the supervision of the advisors. In this study, MCF-7 human breast cancer cell line (HTB-22™), PC-3 human prostate cancer cell line (CRL-1435™), SKOV-3 human ovarian cancer cell line (HTB-77™), U87 human glioblastoma cell line (HTB-14™), and COLO741 human colon cancer cell line (93052621) were used (Figure 1). These cells were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, USA) and the European Collection of Authenticated Cell Cultures (ECACC, UK Health Security Agency, Salisbury, UK).

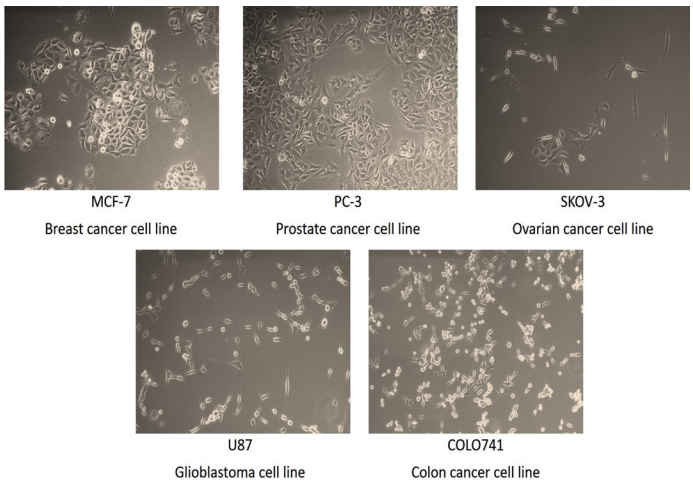
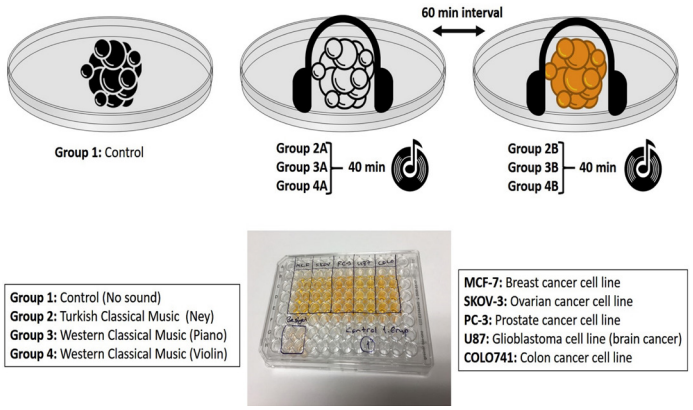


Figure 1. Inverted light microscope images of the cell lines used in the study. X20 magnification

Study design

Cancer cell lines used in the study were grown by culturing in a humid incubator at 37°C and 5% CO_2 conditions using Roswell Park Memorial Institute1640 (RPMI1640) medium containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 1% amphotericin B, and 1% L-Glutamine. After the cells seeded in cell culture dishes were incubated overnight, four experimental groups were formed. Group 1 (Control): No sound. Group 2 (Ney): Pesrevs played with ney from Turkish classical music were chosen for this group. An instrument called "Ney" is one of the musical instruments in Turkish history. Ney is the reed flute, especially played in Mevlevi (Sufi) music (7). Group 3 (Piano): The compositions of Western classical music played with the piano were selected for this group. Group 4 (Violin): The compositions of Western classical music played with the piano were selected for this group. The audio files of the compositions were limited to 40 min and combined for each group. The combined audio files were played for 40 min (Group 2A, Group 3A, Group 4A), after a break of 1 hour, they were played again for 40 min for the second time (Group 2B, Group 3B, Group 4B). Audio files were played in the Class II Biosafety cabinet using LG speakers, in the range of 70-100 dB (6,8).



Frequency spectra

The frequency spectra of all combined audio files were determined using Audacity version 3.1.3 software for Windows (Figure 2). Sound pressure levels were kept between 70 and 100 dB for all configurations. The compositions used in this study are shown in Table 1.

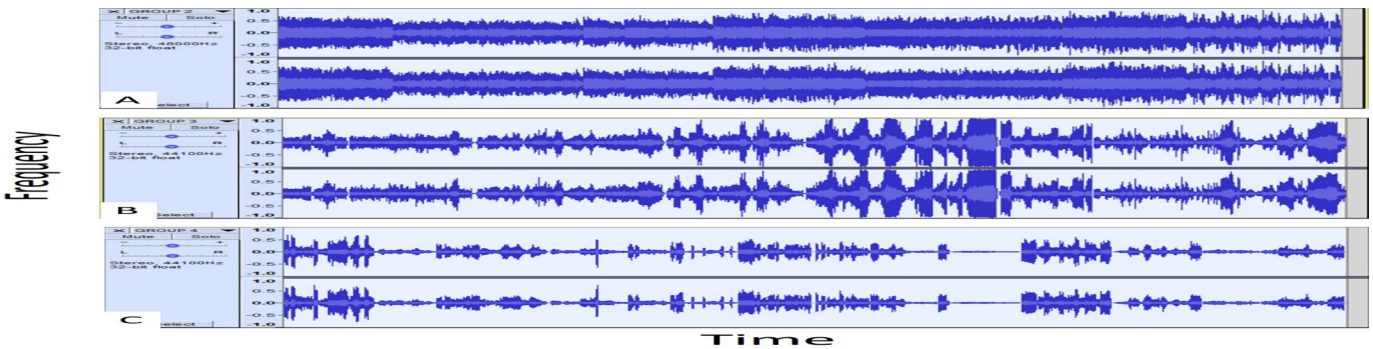


Figure 2. Frequency spectra of the compositions used in the study, obtained by the software Audacity version 3.1.3 for Windows. A. Group 2: Turkish classical music (ney), B. Group 3: Western classical music (piano), C. Group 4: Western classical music (violin)

Table 1. Compositions of music used as treatment on cancer cells

Groups	Compositions	Compositions
Group 1 (Control)	No sound	No composer
	Turkish Classical Music	
Group 2 (Ney)	Buselik Peşrev	Kemençeci Nikolaki (e. -1915)
	Segah Peşrev	Neyzen Yûsuf Paşa (e. 1821-1884)
	Yegah Peşrev	Râuf Yektâ Bey (e. 1871-1935)
	Nihavend Peşrev	Tanbûrî Refik Fersan (e. 1893-1965)
	Uşşak Peşrev	Neyzen Salih Dede (e. 1823-1886)
	Hüseyni Peşrev	Kemençeci Nikolaki (e. -1915)
	Hüzzam Peşrev	Şehzade Seyfeddin Osmanoğlu (e. e1874-1927)
	Western Classical Music	
Group 3 (Piano)	Prelude I in C Major	Johann Sebastian Bach (e. 1685-1750)
	Rondo Alla Turca "Turkish March"	Wolfgang Amadeus Mozart (e.1756-1791)
	Bagatelle No. 25 in A Minor "Für Elise"	Ludwig van Beethoven (e. 1770-1827)
	Swan Lake, Op. 20: Scene	Pyotr Ilyich Tchaikovsky (e. 1840-1893)
	Nocturne No. 2 in E-Flat Major, Op. 9 No. 2	Frédéric François Chopin (e. 1810-1849)
	Schwanengesang "Serenade"	Franz Schubert (e. 1797-1828)
	Arabesque No. 1 in E Major	Claude Achille Debussy (e. 1862-1918)
	Hungarian Rhapsody No. 2	Franz Liszt (e. 1811-1886)
Group 4 (Violin)	The Four Seasons (Spring, Summer, Autumn, Winter)	Antonio Vivaldi (e. 1678-1741)

Measurement of cell viability

MCF-7, PC-3, SKOV-3, U87, and COLO741 cancer cell lines were grown in 25 cm^2 flasks in an appropriate medium at 37°C and a 5% CO_2 environment, and the medium was changed 3 times a week. When the cells grown in the flask were 90% confluent, they were separated from the flasks with the trypsin-EDTA solution and counted. Cells counted at ~1x10^5 cells/ml in each well were transferred to 96-well culture dishes and incubated at 37°C and 5% CO_2 to be confluent. After the confluent cells were taken into the Class II Biosafety cabinet according to the order of the experimental groups. WST-1 measurements were made at 24h. Stock WST-1 at pH 7.4 by adding 25 mg of WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium] into 5 ml of sterile Phosphate-Buffered Saline (PBS) solution was prepared. The media on the cells were withdrawn without touching the cells, and the cells were washed with 100 µl of PBS. 10 µl of the prepared WST-1 solution was put into each well and kept in the incubator (37°C, 5% CO_2) for 2-4h. After incubation, 420-460 nm absorbance was measured in a microplate reader. Each experiment was repeated 3 times. Proliferation was determined by proportioning the absorbance values compared to the control group. The absorbance values of the control group were accepted as 100% viable cells.

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) 17.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean cell viability values of the groups were compared using the Student's t-test. The statistical significance value was accepted as $p<0.05$.

RESULTS

Effects of music on MCF-7 human breast cancer cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 97.307%, Group 3A was 95.918%, and Group 4A was 96.199%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the control group was 100%, Group 2B was 85.728%, Group 3B was 86.593%, and Group 4B was 80.728%. When the control group and Group 2A, Group 3A, and Group 4A were compared, there was no statistically significant difference ($p>0.05$). When the control group was compared with Group 2B, Group 3B, and Group 4B, a statistically significant decrease was detected ($p=0.001$, $p=0.002$, $p=0.002$, respectively). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease in cell viability was detected in Group 2B, Group 3B, and Group 4B, compared to the groups

that applied music once (p=0.038, p=0.045, p=0.15, respectively). When Group 2, Group 3, and Group 4 were compared with other, there was no statistically significant difference (p>0.05) (Figure 3).

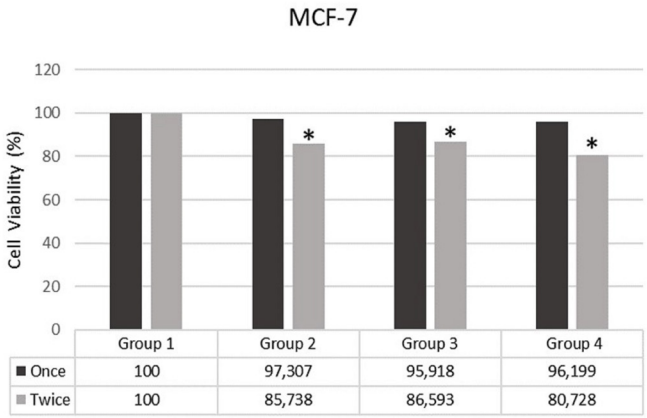


Figure 3. Effects of Turkish and Western classical music on MCF-7 breast cancer cell line after 24h. *Significant difference when compared with control p<0.05

Effects of music on PC-3 human prostate cancer cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 93.371%, Group 3A was 86.772%, and Group 4A was 87.048%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the control group was 100%, Group 2B was 89.112%, Group 3B was 77.443%, and Group 4B was 75.291%. When the control group and Group 2A, Group 3A, and Group 4A were compared, there were statistically significant differences (p=0.01, p=0.000, p=0.000, respectively). When the control group was compared with Group 2B, Group 3B, and Group 4B, a statistically significant decrease was detected (p=0.002, p=0.000, p=0.015, respectively). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease in cell viability was detected in Group 3B and Group 4B, compared to the groups that applied music once (p=0.000, for all). When the application numbers for Group 2 were compared, there was no statistically significant difference (p>0.05). When Group 2, Group 3, and Group 4 were compared with each other, there was a statistically significant difference between Group 2B and Group 3B, and between Group 2B and Group 4B (p=0.000, for all) (Figure 4).

Effects of music on SKOV-3 human ovarian cancer cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 125.312%, Group 3A was 120.72%, and Group 4A was 125.574%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the control group was 100%, Group 2B was 105.306%, Group 3B was 92.943%, and Group 4B was 91.542%. When the control group was compared with Group 2A, Group 3A, and Group 4A, a statistically significant increase was detected (p=0.000, for all). When the control group and Group 2B were compared, a statistically significant increase was detected (p=0.038). When the control group was compared with Group 3B and Group 4B, a statistically significant decrease was detected (p=0.003, for all). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease was detected in Group 2B, Group 3B, and Group 4B compared to the groups that applied music once (p=0.000, for all). When Group 2, Group 3, and Group 4 were compared with each other, there was a statistically significant difference between Group 2B and Group 3B, and between Group 2B and Group 4B (p=0.000, for all) (Figure 5).

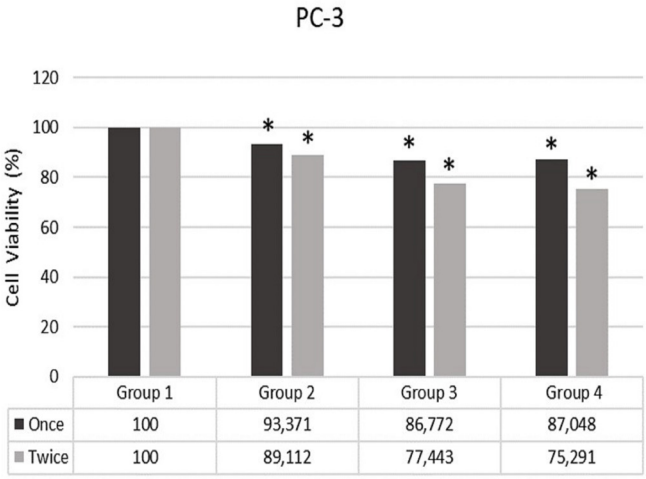


Figure 4. Effects of Turkish and Western classical music on PC-3 prostate cancer cell line after 24h. *Significant difference when compared with control p<0.05

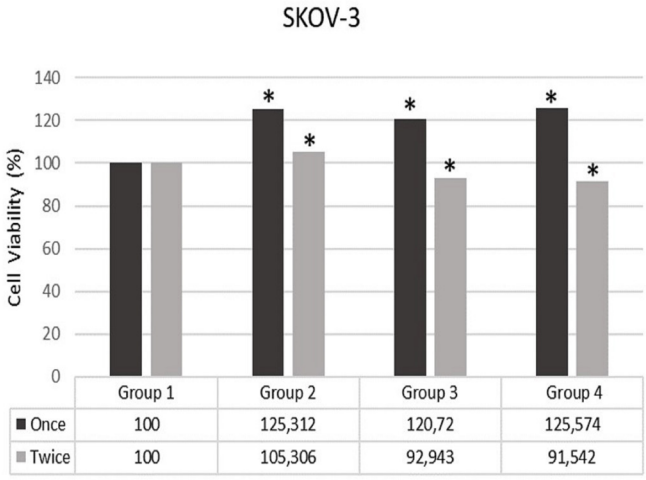


Figure 5. Effects of Turkish and Western classical music on SKOV-3 ovarian cancer cell line after 24h. *Significant difference when compared with control p<0.05

Effects of music on U87 human glioblastoma cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 92.817%, Group 3A was 89.179%, and Group 4A was 90.178%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the

control group was 100%, Group 2B was 74.396%, Group 3B was 70.054%, and Group 4B was 83.321%. When the control group was compared with Group 2A, Group 3A, and Group 4A, a statistically significant decrease was detected (p=0.003, p=0.000, p=0.000, respectively). When the control group was compared with Group 2B, Group 3B, and Group 4B, a statistically significant decrease was detected (p=0.000, p=0.000, p=0.005, respectively). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease in cell viability was found in Group 2B, Group 3B, and Group 4B, compared to the groups that applied to music once (p=0.000, p=0.000, p=0.038, respectively). When Group 2, Group 3, and Group 4 were compared with each other, there were statistically significant differences between Group 2A and Group 3A, and between Group 3B and Group 4B were detected (p=0.028, p=0.001, respectively) (Figure 6).

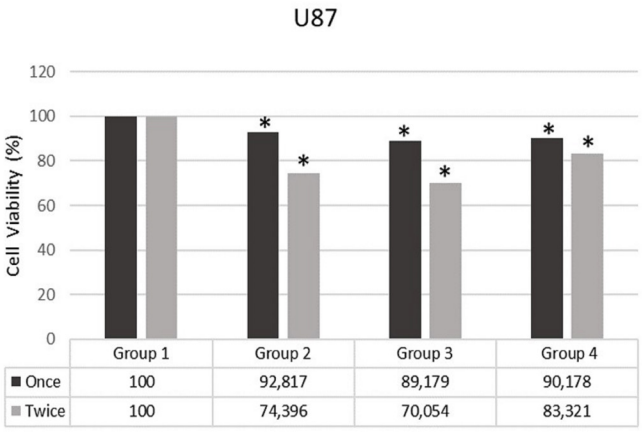


Figure 6. Effects of Turkish and Western classical music on U87 glioblastoma cell line after 24h. *Significant difference when compared with control p<0.05

Effects of music on COLO741 human colon cancer cells

In the WST-1 analysis of cells that were applied music once, the cell viability rate of the control group was 100%, Group 2A was 76.850%, Group 3A was 76.417%, and Group 4A was 84.958%. In the WST-1 analysis of cells that were applied music twice, the cell viability rate of the control group was 100%, Group 2B was 73.647%, Group 3B was 69.631%, and Group 4B was 72.006%. When the control group was compared with Group 2A, Group 3A, and Group 4A, a statistically significant decrease was detected (p=0.000, for all). When the control group was compared with Group 2B, Group 3B, and Group 4B, a statistically significant decrease was detected (p=0.000, for all). When the groups were compared among themselves in terms of the number of applications (once-twice), a statistically significant decrease in cell viability was detected in Group 3B and Group 4B, compared to the groups that applied music once (p=0.002, p=0.007, respectively). There was no statistically significant difference for Group 2 (p>0.05). When Group 2, Group 3, and Group 4 were compared, a statistically significant difference was detected between Group 3A and Group 4A (p=0.021) (Figure 7).

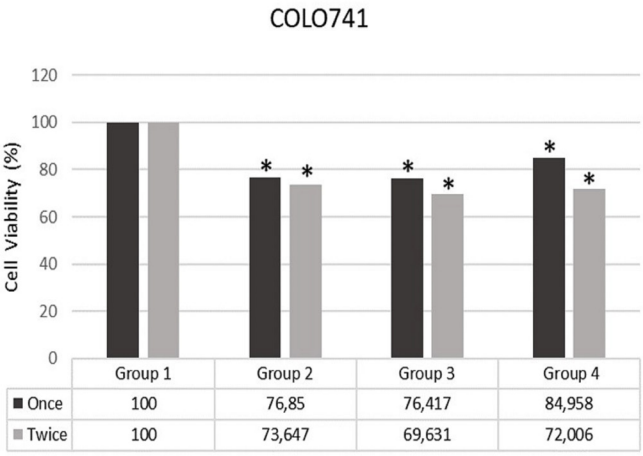


Figure 7. Effects of Turkish and Western classical music on COLO741 colon cancer cell line after 24h. *Significant difference when compared with control p<0.05

DISCUSSION

Music has physiological and psychological effects on individuals. It can help to restore physiological markers such as pulse, blood pressure, and breathing (3,4). Studies have shown that music has a positive effect on hormones such as serotonin, dopamine, and adrenaline, which regulate people's emotions (9). Marshall and Tomcala (1981) studied the effects of different types of music on stress. They found that subjects had varying stress levels and that all types of music had the same psychotherapeutic effects (10). Akpinar et al. (2020) investigated the effects of Turkish and Western classical music on the level of examination stress of university students. They found that students who listened to music experienced a significant decrease in exam anxiety. They concluded that Turkish and Western classical music can be used to reduce stress (4). Yıldırım and Gurkan (2007) reported that music reduced anxiety levels in patients undergoing chemotherapy (11).

Music also has antiproliferative properties in cancer cells (6,8,12). Ramirez-Rivera and Bernal (2019) applied Western classical music (Ludwig Van Beethoven) and metal music (Cannibal Corpse) to the AGS human gastric cancer cell line. The cell viability analysis was performed 12 h after the music applications. The researchers reported that metal music increased the number of cancer cells, but this increase was not seen in Western classical music. There was an increase in gene signals that caused cell death in Western classical music, thus reducing the number of AGS gastric cancer cells. In this study, high sound frequency (0-15 kHz) and sound peaks in metal music, low and medium sound frequencies (~3 kHz) in classical music were determined. The researchers suggested that high frequency and peaking sound may cause an increase in cell viability (12). Lestard (2013) et al. studied that MCF-7 breast cancer cells were applied to compositions of Mozart, Beethoven, and Ligeti for 30 min. While the compositions of Beethoven and Ligeti reduced cell viability, Mozart did not make any changes to cancer cells (8). In another study, Lestard (2016) et al. reported

that music activates cell death signaling pathways 48h after being applied to the Mozart, Beethoven, and Ligeti on MCF-7 and MDA-MB-231 breast cancer cell lines for 30 min. In Lestard's studies, while the compositions of Beethoven and Ligeti had a high frequency of sound (15 and above kHz), it was determined that the compositions of Mozart had a low frequency of sound (0-5 kHz). It was reported that the most effective group on breast cancer cells was the Beethoven group (6). It is an expected finding that more cells will die due to the negative physical effect of high sound frequency on cells. It is a remarkable finding that music with more sound peaks also increases the number of cancer cells.

In our study, we examine the effects of Turkish and Western classical music on cell viability in different cancer cells. First, there was no statistically significant difference between the groups that applied music once only in the MCF-7 breast cancer cells and the control group ($p>0.05$). A statistically significant decrease in cell viability was detected when the control group and the other groups that were applied to music twice were compared ($p<0.05$). It was determined that the most effective application time in breast cancer cells was 40 min twice. For other cancer cell lines, except for SKOV-3 ovarian cancer cells, it was determined that music application once also caused a statistically significant decrease in cell viability ($p<0.05$). However, in these cell lines, it was determined that the application of music twice for 40 min was more effective in reducing the number of cells. On the basis of these findings, we believe that cancer cells exposed to sound frequency for a longer period of time are physically affected more negatively and react in the form of increased cell death. Turkish and Western classical music were effective in reducing the number of cells on the MCF-7 breast cancer cells. Although the Western classical music violin group had the highest decrease in cell number, there was no statistically significant difference when the groups were compared ($p>0.05$). Second, it was determined that the most effective application time on PC-3 prostate cancer cells was 40 min twice for the groups in which Western lassical music was applied. Turkish and Western classical music were effective in reducing the number of cells. When Turkish and Western classical music were compared, Western classical music was statistically more effective in reducing cell number ($p>0.05$). The Western classical music violin group was the most effective type of music. On the contrary, the application of music did not have reducing effects on cell viability in SKOV-3 ovarian cancer cells. Turkish and Western classical music increased the number of SKOV-3 cancer cells. It was observed that the cell increase was less when music was applied twice. So, it is possible to suppress the number of cells with more repetitive music applications.

On the other hand, it was determined that the most effective application time on U87 glioblastoma cells was 40 min twice for all groups. Turkish and Western classical music were effective in reducing the number of cells. It was the Western classical music piano group that reduced the number of cells the most. Turkish classical music was most effective in U87 brain cancer cells. The U87 glioblastoma cell line consists of neurons and glial cells. Compared to other cell lines used in our study, this is the only cancer cell line associated with the nervous system. Because the sound is perceived by the sensory neurons in the inner ear, it is not surprising that U87 glioblastoma cells is also more sensitive to the sound we applied in this study. Finally, it was determined that the most effective application time on the COLO741 colon cancer cells was 40 min twice for the groups in which Western classical music was applied. Turkish and Western classical music were effective in reducing the number of cells on the colon cancer cell line. The groups with the highest decrease in the number of cells were Turkish and Western classical music piano group.

In conclusion, activities that support non-pharmacological treatment such as dance therapy, music therapy, art therapy, meditation have been recommended for cancer patients in recent years. Music is known as a potential tool to improve the quality of life of cancer patients. In developed countries, music therapy in cancer treatment is an emerging field. We suggest that the use of music therapy together with pharmacological applications for cancer patients can increase the response to treatment by reducing the number of cancer cells. Despite the limitations, this study is the first to demonstrate a reduction in the number of cancer cells using Turkish classical music. It is also the first study to examine the effects of music on ovarian, prostate, colon, and brain cancers. We determined that Turkish and Western classical music were effective in reducing and suppressing the number of cancer cells. Turkish classical music showed the highest antiproliferative effects on U87 glioblastoma and COLO741 colon cancer cell lines. Considering that intestinal cells also produce hormones in the nervous system and communicate with them, it is not surprising that the cells most sensitive to music are those that are in constant communication. According to the data, Turkish and Western classical music can be used to support the treatment in cancer patients.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Ethical approval is not required, because this article does not contain any studies with human or animal subjects. The cancer cell lines used in this study were obtained from an accredited commercial provider.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Can the Hydrocele Sac Dissection Technique Affect the Surgical and Cosmetic Satisfaction Results of Conventional Hydrocelectomy?

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Abstract

Aim: In this study, we applied the classical technique and modified open pull-through procedure (OPtP) for hydrocele sac dissection in excisional Winkelmann hydrocelectomy procedure (WHP) to retrospectively compare the surgical and cosmetic satisfaction results of the two techniques.

Material and Methods: Sixty-two patients underwent excisional WHP from 2017 to 2020. The modified OPtP and classical technique groups included 30 and 32 patients, respectively. The intraoperative and postoperative parameters of the patients in both groups were evaluated statistically until the postoperative second month. Cosmetic satisfaction was evaluated statistically at the postoperative sixth month.

Results: Postoperative infection and recurrence were not observed in either group. The patients in both groups had large hydrocele sacs. There was no statistically significant difference between the groups in terms of the hydrocele sac volume, operation time, length of hospitalization, postoperative scrotal edema, and postoperative scrotal pain scores ($p>0.05$). The postoperative hematoma rate was 15.6% in the classical technique group. The statistical difference in incision length and cosmetic satisfaction was significant in favor of the modified OPtP group ($p<0.05$).

Conclusion: Excisional WHP was determined to be safe in the treatment of large hydroceles. In the modified OPtP group, shorter scrotal incision length and low risk of postoperative scrotal hematoma were achieved, and cosmetic satisfaction was also increased.

Keywords: CHP, WHP, OPtP, SMRI

INTRODUCTION

Testicular hydrocele is formed by fluid accumulation at a pathological rate between the visceral and parietal layers of the tunica vaginalis. Surgical treatment is applied due to cosmetic or pain complaints related to the scrotum. Conventional hydrocelectomy procedures (CHPs) or minimally invasive procedures are preferred for surgical treatment (1).

CHPs are known as Winkelmann, Bergman, and Lord techniques. Minimally invasive procedures include percutaneous aspiration and sclerotherapy, endoscopic resection, and open pull-through procedure (OPtP) (2). Studies have shown a lower risk of complications, such as wound infection, epididymo-orchitis, scrotal hematoma, and epididymo-vas deferens trauma due to minimally invasive procedures. Only the recurrence risk has been observed to be higher in these procedures than in CHPs (3).

Hydrocele sac (HS) dissection in hydrocelectomy can be applied in intrascrotal or extrascrotal areas totally or partially. The blind intrascrotal total dissection of HS is the classical technique used in CHPs. OPtP, a minimally invasive technique, refers to the extrascrotal partial dissection of HS (3,4). In the endoscopic resection technique, HS is resected in the scrotum without dissection (5).

CITATION

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In CHPs, HS can be excised totally or partially. Plication is applied to HS in the Lord technique. In OPtP or other minimally invasive procedures, HS can only be excised partially (5). The excised amount of HS can affect the hydrocele recurrence risk (3,5).

In this study, we applied the classical technique and modified OPtP for HS dissection in excisional Winkelmann hydrocelectomy procedure (eWHP). Then, we retrospectively compared the surgical and cosmetic satisfaction results of the two groups.

MATERIAL AND METHOD

This retrospective study was conducted with 62 male patients who underwent eWHP for idiopathic hydrocele between September 2017 and September 2020. The patients had scrotal swelling and pain complaints. After the physical examination, complete urine analysis and scrotal ultrasonography examinations were performed. eWHP was planned for patients with a pre-diagnosis of hydrocele. According to the technique used in eWHP, two groups were formed, with 30 patients in the modified OPtP and 32 patients in the classical technique group. Two different urologists performed the procedures in the two groups. All the patients underwent surgery under spinal anesthesia after 1 g of ceftriaxone administration in the preoperative period. The patients were followed up at the postoperative first week, first month, second month, third month, and sixth month. Postoperative complications and related treatments were recorded. The intraoperative and postoperative parameters of the patients were statistically compared between the groups until the postoperative second month. Patients' cosmetic satisfaction was evaluated at the postoperative sixth month using the following question: "Are you satisfied or dissatisfied with your scrotal cosmetic appearance after surgery?" Patients with chronic systemic diseases, inguinal surgery, or epididymo-orchitis were excluded from the study.

Statistical analysis

IBM SPSS Statistics 22.0 program was used for statistical analysis while evaluating the findings obtained in the study. In addition to descriptive statistical methods (mean and standard deviation), the Student t-test was used to compare quantitative data between the two groups. Fisher's exact test and the continuity correction (Yates) test were used to compare qualitative data. Significance was evaluated at the $p<0.05$ level.

Surgical technique of modified OPtP in eWHP

A scrotal median raphe incision (SMRI) was applied, and the scrotum layers were cut through to reach the parietal layer of the tunica vaginalis, and the surrounding area was slightly dissected and then aspirated. HS and the testis were pulled out of the scrotum, and the sharp HS dissection was completed by preserving the gubernaculum. Then, the layers of HS were resected and mutually sutured posterior to the testis. Finally, the testis was pulled into the scrotal compartment without suturing. The right hydrocele and incision line are shown in Figure 1, the pulled right testis within HS in Figure 2, the whole dissected HS and gubernaculum in Figure 3, a residual parietal layer of tunica vaginalis in Figure 4, and the end of the operation in Figure 5.

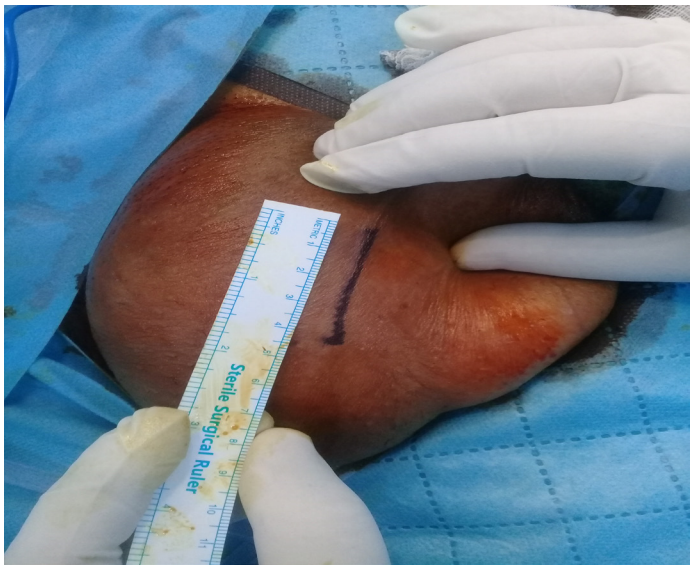

Figure 1. Incision line in a right hydrocele

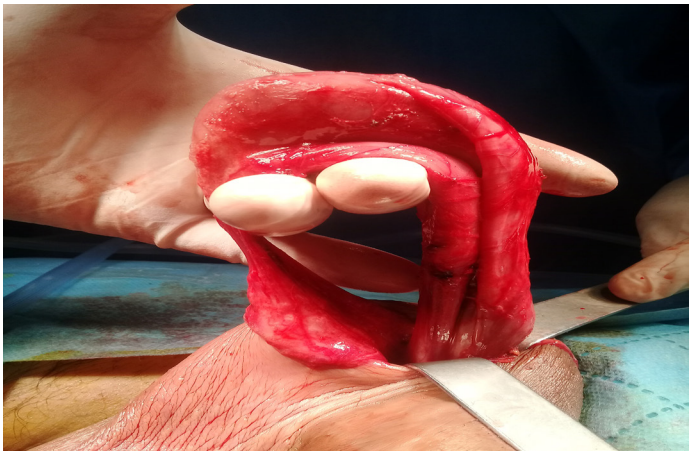

Figure 2. The pulled right testis within HS

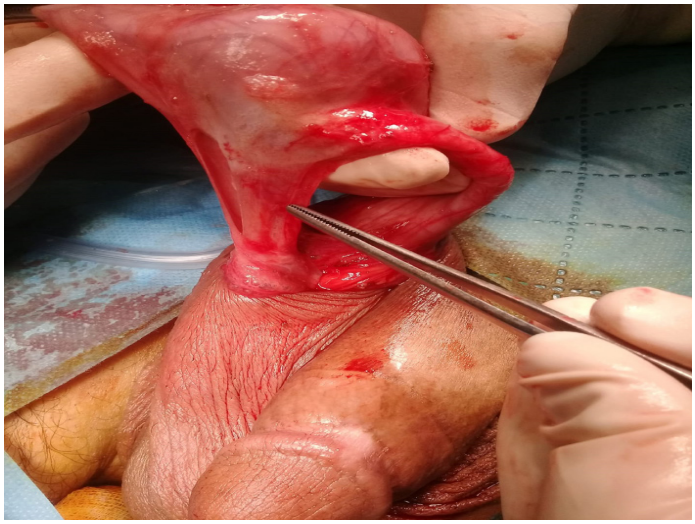

Figure 3. Whole dissection of HS and the gubernaculum

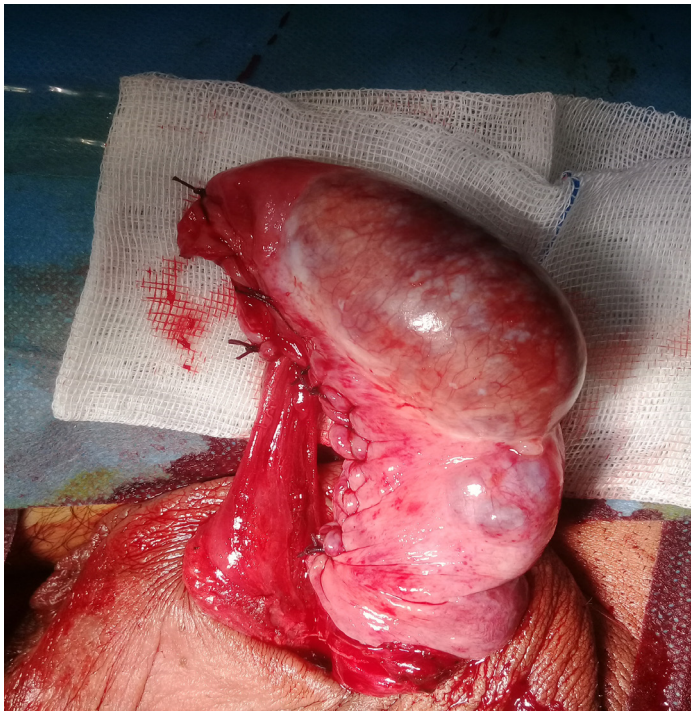

Figure 4. Residual parietal layer of the tunica vaginalis

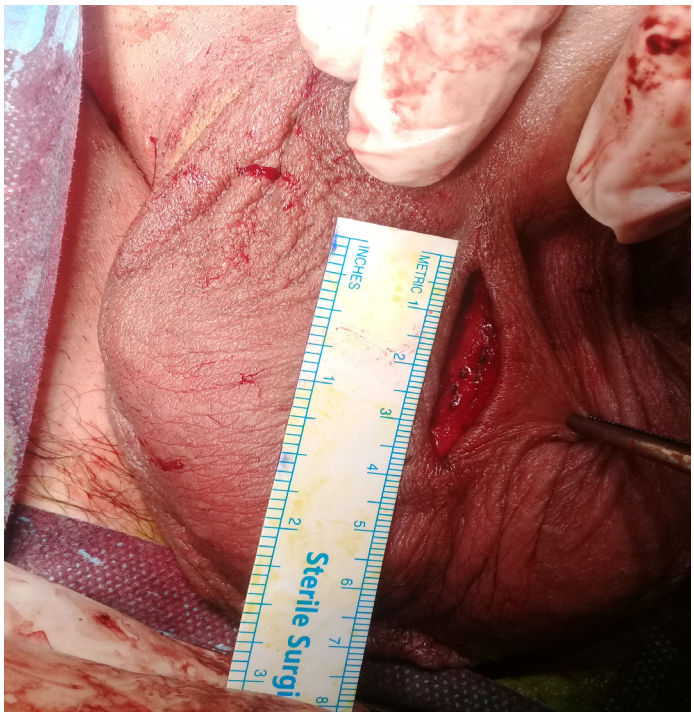

Figure 5. End of operation

Ethics committee approval

The study was approved by the ethical committee of Harran University Faculty of Medicine [Number: HRU-21/02/2012]. All the procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Consent and permission were obtained from all the patients who participated in the study.

RESULTS

The ages of the 62 patients included in the study ranged from 20 to 72 years, with a mean value of 43.60±11.08 years. There was no statistically significant difference between the OPtP and classical technique groups ($p<0.05$).

All the patients' scrotal complaints of edema, pain, and hematoma during the postoperative period were observed to have improved at the second month follow-up. Postoperative infection and recurrence were not observed in either group. Both groups had large HSs. There were no statistically significant differences between the two groups in terms of the HS volume, operation time, length of hospitalization, postoperative scrotal edema, and postoperative scrotal pain scores ($p>0.05$). The detailed results are shown in Table 1.

The incision length of the classical technique group was significantly higher than that of the modified OPtP group ($p<0.01$). There was no statistically significant difference in the incidence of postoperative hematoma between the groups ($p>0.05$). It was remarkable that the rate of scrotal hematoma observed in the classical technique group (15.6%) was significantly higher compared to the modified OPtP group. There was a statistically significant difference between the cosmetic satisfaction levels of the two groups ($p<0.01$). The cosmetic satisfaction rate of the modified OPtP group was significantly higher than that of the classical technique group (Table 1).

Table 1. Intraoperative and postoperative parameters of the patients

		Modified OPtP group	Classical technique group	p
		Mean±SD	Mean±SD	
Incision length (cm)		3.35±0.48	6.0±0.67	[1]**0.001****
Hydrocele sac volume (cc)		178.67±63.88	190.31±74.64	[1]0.513
Operation time (min)		31.50±5.12	29.44±4.01	[1]0.082
		n = 30; %100	n = 32; %100	
Length of hospitalization	1 day	22;%73.3	18;%56.3	[2]0.255
	2 days	8;%26.7	14;%43.8	
Postoperative scrotal Edema	Present	8;%26.7	14;%43.8	[2]0.255
	Absent	22;%73.3	18;%56.3	
Postoperative scrotal Pain	Present	4;%13.3	9;%28.1	[2]0.264
	Absent	26;%86.7	23;%71.9	
Postoperative scrotal hematoma	Present	0;%0	5;%15.6	[3]0.053**
	Absent	30%100	27;%84.4	
Cosmetic Satisfaction	Satisfied	30;%100	25;%78	[3]0.005**
	Unsatisfied	0;%0	7;%22	

[1]Student t-test [2]Continuity correction (Yates) test [3]Fisher's exact test
**p < 0.01 SD: Standard deviation

DISCUSSION

Testicular hydrocele is most frequently observed in male patients over the age of 40 years due to idiopathic causes (6). Operation time is around 30 minutes in all open hydrocelectomy procedures. Although hospitalization is generally one day, patients undergoing hydrocelectomy can also be discharged on the same day (7). In our study, the mean patient age was over 40 years for both groups. The mean operation time and postoperative length of hospitalization were also consistent with the literature.

Trans-scrotal incision (transverse or paramedian) is generally preferred for CHPs. The incision length is around 6 cm (8,9). Studies using SMRI are limited (10). In addition, both inguinal canal pathology and hydrocelectomy treatment are performed simultaneously through the inguinal route (9,11). In minimally invasive procedures, the scrotal incision length is less than 2 cm (12). In our study, SMRI was applied at an average of 3.35 cm in the modified OPtP group and 6 cm in the classical technique group, which indicated a statistically significant difference. The targeted minimum SMRI length in the modified OPtP group was sufficient to remove the aspirated HS with the pulling of the testis into the extrascrotal area.

WHP and the Bergmann hydrocelectomy procedure are used in the excisional surgical treatment of large, long-standing, or multi-located hydroceles. The inadequate excision of HS can increase the postoperative recurrence risk. The classical HS dissection technique is generally applied, in which the testicular gubernaculum is not preserved in the excisional hydrocelectomy procedure (13). In OPtP, the aspirated HS is pulled piece by piece into the extrascrotal area for partial dissection and resection (3). There are studies that applied scrotoscopy prior to OPtP. However, HS is excised with a resectoscope without dissection in Su-Wang's procedure (5). In our study, the size of HSs was large in both groups. In the modified OPtP technique we used, the scrotal incision was longer than in the classical OPtP technique. In addition, HS was total dissected and widely resected compared to the classical OPtP. In the literature, we did not find any study performing WHP by preserving the testicular gubernaculum.

Scrotal edema, wound infection, testicular pain, recurrence, epididymis injury, and orchiectomy are complications that can be seen after hydrocelectomy (14). In studies in which the complications of CHPs were compiled, the overall complication rate including scrotal hematoma, testicular pain and infection incidence was 11.4%, and the recurrence rate was 6.2% (13,15). In addition, in similar studies, the success of cosmetic satisfaction was 75% (7,8). In some studies in which aspiration and sclerosing agent injections were applied to patients with high morbidity and mortality risk, the success and complication rates were low (16,17). The postoperative complication rates of minimally invasive procedures were reported to be 10% for scrotal edema, 6.4% for testicular infection, and 4% for recurrence. Cosmetic satisfaction was also over 95% (12). In our study, no infection or recurrence was observed in either group. In the modified OPtP group, scrotal edema was observed at a rate of 26% and scrotal pain at 13.3%. In the classical technique group, scrotal hematoma was seen in 15.6% of the patients, scrotal edema in 43.8%, and scrotal pain in 28%. Although the differences in the rates of postoperative complication parameters of the two groups were not statistically significant, the rate of scrotal hematoma in the classical technique group was remarkable. Although the cosmetic satisfaction of both groups was at a high level, the difference between the groups was statistically significant in favor of the modified OPtP group.

CONCLUSION

In this study, eWHP was determined to be a safe procedure in the surgical treatment of large HSs. Neither HS dissection technique was superior to the other in terms of postoperative complication parameters (recurrence, scrotal pain, and scrotal edema). However, the use of the modified OPtP technique in eWHP resulted in a shorter incision length, preservation of the gubernaculum, and lower risk of postoperative hematoma and increased the cosmetic satisfaction of the patients.

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Research Article

Effects of Nd: YAG Laser Capsulotomy on Visual Acuity, Central Macular Thickness, Intraocular Pressure and Refraction in Patients with Diabetes Mellitus

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Abstract

Aim: This study aimed to evaluate the effects of Nd: YAG laser posterior capsulotomy (YAG PC) on best-corrected visual acuity (BCVA), central macular thickness (CMT), intraocular pressure (IOP), and refraction in patients with diabetes mellitus (DM).

Material and Methods: This retrospective study included patients who underwent YAG PC due to posterior capsular opacification (PCO). BCVA, refraction examination results, IOP, and CMT of the patients were evaluated. All patients were examined before, one day, and one month after the treatment.

Results: The study included 56 eyes from 48 diabetic patients (diabetic group, Group 1) and 61 eyes from 50 nondiabetic patients (nondiabetic group, Group 2). In Group 1, a significant increase was observed between pre-treatment BCVA and the first-day and first-month BCVAs. Similarly, a significant increase was observed on the first day and first-month CMT compared to the pre-treatment CMT. On the other hand, a significant increase was observed in Group 2 between pre-treatment BCVA and the first-day and first-month BCVAs. However, no significant increase was observed between pre-treatment and the first-day and first-month CMT.

Conclusion: Although CMT has increased in patients with DM after YAG PC, applied for PCO treatment, this increase did not affect the visual recovery.

Keywords: Central macular thickness, diabetes mellitus, intraocular pressure, laser capsulotomy, refraction

INTRODUCTION

The most common postoperative complication of cataract surgery is PCO (1); its incidence after cataract surgery varies between 10%-50% (2,3). The main reason for PCO development is the proliferation of the lens epithelial cells remaining in the capsule and their settlement on the posterior capsule (4). Regarding patients undergoing cataract surgery, PCO development has been reported to be significantly higher in patients with DM than in those without DM. However, there is no correlation between the stage of diabetic retinopathy, the systemic involvement of DM, and the severity of PCO (5,6). YAG PC treatment, preferred for PCO, is a non-invasive method; it does not require patient hospitalization and can be administered quickly (7). In addition, this treatment method creates a central opening in the thickened posterior capsule, obtaining effective results immediately (8). However, complications such as maculopathy and increased IOP may occur after YAG PC (9-11).

This study aimed to compare CMT, IOP, BCVA, and refraction changes observed on the first day and one month after YAG PC in patients with and without DM who developed PCO after cataract surgery.

MATERIAL AND METHOD

PCO is a disease that causes a decrease in visual acuity. It can be treated with YAG PC (12). YAG PC was applied for the patients who came to our clinic with decreased

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visual acuity and who were detected to have PCO in the slit-lamp examination. Patients who underwent phacoemulsification and intraocular lens implantation were included in this retrospective study. It was approved by Istanbul Training and Research Hospital Clinical Trials Ethics Committee. Patients treated with YAG PC for PCO at Istanbul Training and Research Hospital between March 2018 and May 2019 and whose PCO was sufficient for macular examination with pre-treatment OCT were included in the study.

Patients who had severe ocular pathologies, such as complications in cataract surgery in their history or examination, a follow-up period of less than one month, corneal haze, a history of eye surgery other than cataract, a systemic disease that will increase inflammation after YAG PC, retinal diseases, glaucoma, and uveitis were excluded. BCVA, refraction examination, biomicroscopy and IOP measurement were performed in all control. CMT was then measured by spectral-domain OCT (RVTue 100-2; Optovue, Fremont, CA). IOP measurement was performed three times with Topcon CT-80 non-contact tonometer (Topcon, Japan), taking their average. In addition, 1% tropicamide and 2.5% phenylephrine were administered before capsulotomy for pupil dilation. 0.5% proparacaine hydrochloride drops were applied to the eyes 5 minutes before the treatment for anaesthesia. YAG PC was performed by focusing on the posterior capsule, creating an opening of approximately 4 mm. According to the capsule thickness, the power was set between 1 and 2.4 mJ. Each capsulotomy was completed in a single session. One surgeon performed the treatment using a Zeiss Visulas Yag II Laser (Zeiss, Germany). After the treatment, patients were prescribed brinzolamide 1%+ timolol 0.5% combination topical antiglaucomatous drops (twice a day) and prednisolone acetate 1% (four times a day) to be used for one week. All patients were examined before, one day, and one month after the treatment.

The patients included in the study were divided into two groups: Group 1, patients with DM, and Group 2, patients without DM.

Statistical analysis

SPSS version 22.0 was used in statistical analysis. A paired t-test was used to compare BCVA, IOP, CMT, and spherical and cylindrical values before and after the treatment in Group 1. In contrast, the repeated measures ANOVA test was used in Group 2. The independent samples t-test was used to compare groups. As a result of the comparison, the effect size was determined as 0.5, using Gpower (version 1.3.9.7). The power of the analyses was calculated as 0.873.

RESULTS

56 eyes of 48 patients (diabetic group, Group 1) and 61 eyes of 50 patients (nondiabetic group, Group 2) were included in the study. Patients' demographic characteristics are shown in Table 1. BCVA, IOP, CMT, spherical and cylindrical values of Group.

Table 1. Demographic characteristics of patients

	Group 1 n:48	Group 2 n:50	p
Age	69.8±4.9	69.1±9.9	0.874 (Independent samples t test)
Gender (female/male)	26/22	28/22	0.435 (Chi-square test)
Follow-up time (Months)	1	1	

1 before and after the treatment are shown in Table 2. Comparing pre-treatment BCVA and first-day and first-month BCVAs shows a significant increase. Besides, significant increases are observed between pre-treatment IOP and first-day IOP (in 2 patients); IOP was brought under control with anti-glaucomatous drop therapy. There is no significant change between pre-treatment IOP and first-month IOP. A significant increase is observed between pre-treatment CMT and first-day and first-month CMT. There is no significant difference in spheric value on the first day or one month after the treatment. No significant change occurred in the cylindric value the day after the treatment; however, there is a significant decrease one month after the treatment.

BCVA, IOP, CMT, spherical and cylindrical values of Group 2 before and after the treatment are shown in Table 3. There is a significant increase between pre-treatment BCVA and first-day and first-month BCVAs. However, there is no significant change between pre-treatment IOP and first-day and first-month IOPs. Similarly, no significant change is observed between pre-treatment, first-day, and first-month CMTs. Regarding spheric and cylindric values, there is no significant change between pre-treatment and first-day values, whereas a significant decrease is observed in the first-month value.

The comparison of BCVA, IOP, CMT, spherical value, and cylindrical values between groups before and after the treatment is shown in Table 4. There is no statistically significant difference in BCVA, CMT, spherical value, and cylindrical values between the two groups before and after the treatment. In contrast, first-month IOP is significantly higher in Group 1.

The day after YAG PC, +1 cell was detected in the anterior chamber in 4 patients in Group 1 and 6 in Group 2. Anterior chamber reaction disappeared on the third day after topical steroid treatment, which was started routinely, and then the treatment was tapered and discontinued.

In addition, macular edema was detected in 1 patient in Group 1 and Branch Retinal Vein Occlusion (BRVO) in 1 patient in Group 2 during the first month of follow-up. The macular edema in the patient with DM returned to normal after intravitreal bevacizumab treatment. The BRVO patient underwent focal laser photocoagulation and was further followed.

Table 2. BCVA, IOP, CMT, spherical and cylindrical values of the Group 1 before and after the treatment		
	Group 1 n:56	**P (Paired t test)**
BCVA (logMAR)		
pre-treatment	0.44±0.36	
first-day	0.14±0.19	**<0.001**
first-month	0.09±0.15	**<0.001**
IOP (mmHg)		
pre-treatment	15.9±2.2	
first-day	18.6±8.3	**0.002**
first-month	16.0±2.2	0.431
CMT (µm)		
pre-treatment	262.7±37.2	
first-day	266.5± 38.2	**0.031**
first-month	267.0± 37.3	**0.036**
Spherical values (diopter)		
pre-treatment	+0.23±1.27	
first-day	+0.32±1.03	0.772
first-month	+0.21 ±1.10	0.101
Cylindrical values (diopter)		
pre-treatment	-1.07±0.70	
first-day	-0.88± 0.36	0.192
first-month	-0.71±0.73	**0.004**

Table 3. BCVA, IOP, CMT, spherical and cylindrical values of the Group 2 before and after the treatment		
	Group 2 n:61	**P (Repeated measures ANOVA test)**
BCVA (logMAR)		
pre-treatment	0.48±0.30	
first-day	0.14±0.11	**<0.001**
first-month	0.06±0.08	**<0.001**
IOP (mmHg)		
pre-treatment	15.8±2.4	
first-day	15.5±2.6	0.321
first-month	15.0±3.7	0.485
CMT (µm)		
pre-treatment	259.7±19.8	
first-day	255.8±23.4	0.218
first-month	258.7±27.3	0.642
Spherical values (diopter)		
pre-treatment	+0.61±1.0	
first-day	+0.42±0.93	0.153
first-month	+0.33.±0.96	**0.021**
Cylindrical values (diopter)		
pre-treatment	-1.08±0.61	
first-day	-0.89±0.57	0.241
first-month	-0.72±0.49	**0.012**

Table 4. The comparison of BCVA, IOP, CMT, spherical value, and cylindrical values between groups before and after the treatment

	Group 1 N:56	Group 2 N:61	P (Independent samples t test)
BCVA (logMAR)			
pre-treatment	0.44±0.36	0.48±0.30	0.227
first-day	0.14±0.19	0.14±0.11	0.329
first-month	0.09±0.15	0.06±0.08	0.960
IOP (mmHg)			
pre-treatment	15.9±2.2	15.8±2.4	0.619
first-day	18.6±8.3	15.5±2.6	0.056
first-month	16.0±2.2	15.0±3.7	0.014
CMT (µm)			
pre-treatment	262.7±37.2	259.7±19.8	0.398
first-day	266.5± 38.2	255.8±23.4	0.557
first-month	267.0± 37.3	258.7±27.3	0.760
Spherical values (diopter)			
pre-treatment	+0.23±1.27	+0.61±1.0	0.382
first-day	+0.32±1.03	+0.42±0.93	0.962
first-month	+0.21 ±1.10	+0.33.±0.96	0.850
Spherical values (diopter)			
pre-treatment	-1.07±0.70	-1.08±0.61	0.653
first-day	-0.88± 0.36	-0.89±0.57	0.981
first-month	-0.71±0.73	-0.72±0.49	0.942

DISCUSSION

YAG PC is the standard treatment for PCO (7). Although it is a reliable treatment method, complications such as IOP changes, refraction changes, and macular edema may occur afterwards (9-11). The causes of macular edema developing after YAG PC are the deterioration of the perifoveal inner blood-retinal barrier with the increase of inflammatory mediators such as prostaglandin and leukotrienes released to the posterior segment, vitreous damage, and vitreous activation (13,14). It is known that the risk of developing cystoid macular edema is high in patients with DM due to functional damage and necrosis of the retinal capillaries (15,16). Macular edema was detected in 1 patient in Group 1 in the first month of follow-up. This study showed a significant increase in CMT the day after and one month after YAG PC in Group 1. However, no significant change was observed in Group 2. Many studies in the literature state that CMT increases or remains unchanged after YAG PC (17-21). There was no statistically significant difference in CMT between the groups after the treatment in this study. Only one study compared CMT in patients with and without DM in the literature, and its results were similar to this study (22). In addition, BRVO was detected in 1 patient in Group 2 in the first month of follow-up. In the literature, 1 case of central retinal vein occlusion developing after YAG laser capsulotomy has been reported (23).

A significant increase was observed in BCVA in both Group 1 and Group 2 at the end of the 1-month follow-up. However, there was no statistically significant difference in BCVA between groups. The study conducted by Türkoglu et al. showed similar results; however, Awan et al. reported that BCVA increased in both groups, but the BCVA increase was better in the nondiabetic patients (5,22).

The most common complication of YAG PC is IOP increase (24). Prophylactic antiglaucomatous drugs are used because of this IOP increase after the treatment. Studies in the literature reported an increase of 15-30% in IOP despite prophylactic treatment (25-26). However, Ozkurt et al. reported no significant change in IOP after YAG PC (27). In this study, despite prophylactic treatment, IOP was measured between 40-46 mmHg in 2 cases in Group 1; it was brought under control at the end of the first month. Regarding post-treatment IOP changes of the groups, there was no significant difference in Group 2. In contrast, there was a significant increase in the mean IOP in Group 1 on the first day after treatment; it returned to normal in the first month. The possible reason for this IOP increase in 2 patients in Group 1 may be the total number of shots used in the treatment and the high total energy, as stated in previous studies (28).

Although effective results have been obtained in PCO treatment with YAG PC, many different results have been reported regarding refractive changes. Akmaz et al. reported a significant myopic shift after YAG PC (29); Oztas et al. found both a significant myopic shift in spherical equivalent and a significant decrease in cylindrical refractive power (30). Zaidi et al. and Polat et al. reported a significant hyperopic shift in spherical equivalent after YAG PC (31,32). Hu et al. reported no significant changes in spherical equivalent, but they found a significant decrease in cylindrical refractive power (33). Chua et al., on the other hand, did not observe any significant change in spherical values after YAG PC (34). This study showed a statistically significant myopic shift in spherical equivalence and a decrease in cylindrical refractive power at the first-month control in Group 2. In Group 1, there was a shift in spherical equivalent to myopia on the first day and first month after the treatment, but it was not statistically significant. In addition, a significant decrease was observed in the cylindrical refraction power in the first month. Therefore, after YAG PC, it is necessary to wait one month for optical refractive correction.

Study Limitations

The limitation of the study is its retrospective nature and the failure to group according to the amount of energy used in YAG PC.

CONCLUSION

In conclusion, despite the increase in CMT in diabetic patients after the administration of YAG PC for PCO treatment, this increase did not affect visual recovery. YAG PC has similar effects in increasing visual rehabilitation in diabetic and nondiabetic patients, but more studies are needed to confirm this. Prospective studies with more patients and a more extended follow-up period are needed.

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Research Article

Polypharmacy in the Elderly: A Double-Edged Sword

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Abstract

Aim: Polypharmacy is a very important geriatric syndrome related to critical health conditions. The purpose of this study is to research the association of polypharmacy with comprehensive geriatric assessment's (CGA) various parameters which are indicators of health and life quality in older individuals.

Material and Methods: 515 older adults admitted to a university hospital were included in this cross-sectional study. Along with CGA, European Quality of Life (EQ-5D) was performed on the participants. Geriatric Depression Scale (GDS) was used to evaluate depressive symptoms. Daily living activities were assessed by using Lawton & Brody index of Instrumental Activities of Daily Living (IADL) and Katz Index of Activities of Daily Living (ADL). The Full Mini-Nutritional Assessment (MNA) questionnaire was performed to evaluate nutritional status.

Results: The participants' mean age was 72.2±6.3 years and 58.6% of them were female. 242 participants using 5 or more drugs were included in the polypharmacy group. The polypharmacy group had lower IADL, MNA, EQ-5D, Hand Grip Strength (HGS), and gait speed scores and higher GDS scores compared to those without polypharmacy. According to the correlation analysis results, the number of the medications had a moderate positive correlation with GDS scores and a moderate negative correlation with EQ-5D and MNA.

Conclusion: As the number of medications increases; patients tend to have depression, malnutrition, and a decline in functional status. Polypharmacy also impairs the quality of life. We should address polypharmacy as a crucial health problem, optimize the number of medications and thereby make the health condition better.

Keywords: Polypharmacy, depression, daily living activities, malnutrition, quality of life

INTRODUCTION

The aging population has been constantly rising around the world and multimorbidity and geriatric syndromes are increasing consequently. Polypharmacy due to morbidity, which is a component of geriatric syndromes, is also increasing and turning into a serious health problem (1). Some physiological changes arising with aging make elderly susceptible to the drugs. Therefore, polypharmacy leads to more often unintended consequences among older individuals through drug-drug interactions and adverse reactions (2). Falls, frailty, impairment in cognition and physical function, increased hospitalization and recurrent readmission, prolonged hospital stay, decreased drug adherence, reduced medication efficacy and increased mortality have been linked to polypharmacy which is also a great economic burden (3).

The definition of the polypharmacy is using potentially inappropriate drugs and/or concurrently multiple medications including prescribed and over-the-counter drugs (4). Even though the exact number of drugs in polypharmacy is still not clear and has been defined variously, using five or more drugs per day is generally considered polypharmacy, given the number of drugs alone (5). "Excessive polypharmacy" is defined as using ≥10 medications concurrently (6). Older adults are more susceptible to side effects and interactions of the drugs because of the high rates of the comorbidities in comparison with the younger adults (7). Prescribing numerous drugs may cause inappropriate drug use, an increase in adverse reactions, decreased medication adherence, repetition of therapy, and interactions between drugs. Thus, polypharmacy is associated with impairment in nutrition and physical function, decline in cognitive functions and

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increase of geriatric syndromes (2,8). Status of the nutrition, cognitive and functional capacity are markers of health quality in older ages (9,10). Sarcopenia is known as one of the major risk factors for frailty, and polypharmacy is also known to be associated with sarcopenia (11,12). Frailty in the elderly is a multi-dimensional geriatric syndrome that may cause a decline in functional ability, falls, delirium, hospitalization, and death (13). There is a close relationship between polypharmacy and multimorbidity, and both these conditions independently increase the frailty and hospitalization risk. A decline in physical performance is also a consequence of polypharmacy (14). A poorer cognitive capability and disability in instrumental activities are also shown to be negative outcomes of polypharmacy (15,16).

Various health-related conditions influencing life quality such as polypharmacy, comorbidities, and malnutrition, and disability trigger depression (17). Late-life depression is a common health problem and affected by gender, socioeconomic status, physical activity, and nutrition, is associated with serious consequences ending up morbidity and mortality (18). There is a relationship between polypharmacy and depression, and depression may be a better independent predictive factor for polypharmacy than the other comorbid diseases (19). Lower depression scores have been shown to increase adherence to medication in the general population (20).

The aim of this study is to research the association of polypharmacy with CGA's various parameters that are indicators of health status and quality of life in the elderly.

MATERIAL AND METHOD

This cross-sectional study was conducted with 515 older individuals aged 65 and over who applied to geriatric outpatient clinic of a university hospital between the dates May 2022 and August 2022. Patients who have cognitive disorders impairing cooperation, cancer, aphasia, inflammatory and neuromuscular diseases, and comorbidities severely restricting to assess muscle functions like severe osteoarthritis, neuropathy and peripheral artery disease were excluded. The medical histories of the participants including comorbid diseases and polypharmacy were questioned. Concurrently use of five and more different drugs in a day was described as "Polypharmacy" (5). Epi Info software was used to calculate the sample size. The minimum sample size was 298 participants at the level of $\alpha = 0.05$ with 95% power. We conducted the study with the approval of the local ethics committee.

The components of the comprehensive geriatric assessment consist of evaluating the nutritional status, fall risk, instrumental activities of daily living, activities of daily living, sarcopenia, mental status, and depressive symptoms.

All of the questionnaires were performed using the validated forms and in the Turkish language. In order to evaluate depressive symptoms, the GDS was used which is coded according to responses to questions. Negative responses were coded as 0 point whereas positive responses were coded as 1 point. Some questions were reversely coded. The scores 14 points and over were considered as "depression", those between 0 and 10 as "no depression" and those between 11 and 13 as "possible depression".

The criteria of the Working Group on Sarcopenia in Older People (EWGSOP2) were used in order to diagnose sarcopenia. The bioelectric impedance analyzer branded Tanita SA165 A-0950U3 was used to measure muscle mass and handgrip strength (muscle strength) was measured by Jamar hydraulic hand dynamometer. The values lower than 32 kilograms for males and 22 kilograms for females were considered as low hand grip strength (HGS). The values lower than 0.823 for females and 1.049 for males were considered as low muscle mass after calculating skeletal muscle mass index (SMMI) adjusted for body mass index (BMI). The speed of the gait lower than ≤ 0.8 m/s was considered as low gait speed.

The dependence of the participant in physical activities including feeding, transferring, dressing, continence, toileting, and bathing was evaluated by the questionnaire of Katz Index of Activities of Daily Living (ADL). Scores were given 0 to 6 and higher scores were considered as greater independence. The dependence of the participant in physical activities including using the telephone and public transportation, housekeeping, food preparation, managing money, shopping, doing laundry, and taking routine medicines was evaluated by the questionnaire of Lawton & Brody index of Instrumental Activities of Daily Living (IADL) Scores were given 0 to 8 and higher scores were considered as greater independence.

The Mini Nutritional Assessment questionnaire (MNA) was used to evaluate the status of nutrition. Scores above 24 were considered as "normal nutritional status", those below 17 were considered as "malnutrition" (MN), and those between 17 and 23.5 as "risk of malnutrition" (MNR).

The cognitive status of the participants was evaluated by using the mini-mental state examination questionnaire (MMSE). In the test maximum score for orientation is 10 points, attention and calculation is 5 points, registration is 3 points, language/visuospatial construction is 9 points, and recall is 3 points. The scores 24 and below were considered as "suggesting dementia". In the "orientation" item, the participants were asked questions like “Which city do you live in?”, “Which year?” or "What building are you currently in?" etc. In the item "attention and calculation" the participant were asked to count backwards; in the "registration" and "recall" they were asked to listen, repeat and then recall certain words, and in the "language/visuospatial construction" they were asked to implement some orders and try to draw the same what they see on the page.

In order to evaluate life quality, the European Quality of Life-5 Dimensions questionnaire (EQ-5D) was used. 5 aspects of health status including anxiety/depression,

usual activities, mobility, pain/discomfort, and self-care were questioned and the index score was calculated. Negative values scores mean that person is unconscious, bedridden, and dependent while the score "0" means death and the score "1" shows flawless health.

The independent samples t-test and Mann–Whitney U test were used to compare two independent groups of variables. The relationship between categorical variables was assessed with the $\chi 2$ test and numerical variables with Spearman's rank correlation coefficient. SPSS for Windows version 22.0 was used and a p-value of <0.05 was accepted as statistically significant.

RESULTS

The mean age of the participants was 72.2±6.3 years and 58.6% were female. Of the participants, 242 were considered as having polypharmacy based on the number of the drugs they used. The rate of chronic diseases was higher in the polypharmacy group, except cerebrovascular disease. Polypharmacy group had lower IADL, MNA, EQ-5D, HGS, and gait speed scores and higher GDS scores and antidepressant user rates compared to those without polypharmacy (Table 1). The frequency of sarcopenia was higher in the polypharmacy group, although there was no statistically significant difference.

Table 1. Socio-demographic characteristics and CGA results of the participants (n=515)

Variables	Polypharmacy (-) (n=273)	Polypharmacy (+) (n=242)	p	Total (n=515)
Gender				
Female	158 (57.9%)	144 (59.5%)	0.708	302 (58.6%)
Male	115 (42.1%)	98 (40.5%)		213 (41.4%)
Age†	72.2±6.3	72.2±6.4	0.942	72.2±6.3
Number of comorbidities#	2 (0-6)	3 (1-8)	<0.001*	2 (0-8)
Number of medications#	2 (0-4)	6 (5-20)	<0.001*	4 (0-20)
Comorbidities				
Hypertension	132 (48.4%)	165 (68.2%)	<0.001*	297 (57.7%)
Diabetes mellitus	96 (35.2%)	147 (60.7%)	<0.001*	243 (47.2%)
Coronary artery disease	52 (19.0%)	93 (38.4%)	<0.001*	145 (28.2%)
Asthma/COPD	21 (7.7%)	42 (17.4%)	<0.001*	63 (12.2%)
Neurodegenerative diseases	25 (9.2%)	49 (20.2%)	<0.001*	74 (14.4%)
Cerebrovascular disease	9 (3.3%)	13 (5.4%)	0.245	22 (4.3%)
Antidepressant use	6 (2.2%)	20 (8.3%)	0.002*	26 (5.0%)
ADL†	4.0±1.7	4.0±1.7	0.913	4.0±1.7
IADL†	5.9±2.0	5.4±2.3	0.004*	5.7±2.2
GDS#	6 (0-30)	9 (2-30)	<0.001*	7.5 (0-30)
MMSE†	24.2±5.1	23.4±5.7	0.093	23.8±5.4
MNA†	23.1±5.2	21.6±5.3	0.002*	22.4±5.3
EQ-5D#	0.70 (-0.27-1.00)	0.41 (-0.53-1.00)	<0.001*	0.52 (-0.53-1.00)
BMI†	29.2±5.2	29.9±5.4	0.167	29.5±5.3
Sarcopenia	85 (32.4%)	89 (38.7%)	0.148	174 (35.4%)
HGS (kg)†				
Female	20.7±6.5	18.2±6.4	0.001*	19.5±6.6
Male	27.8±7.0	27.2±6.8	0.380	27.5±6.9
SMMI (BMI)†				
Female	0.80±0.16	0.80±0.16	0.896	0.80±0.16
Male	1.18±0.19	1.18±0.17	0.880	1.18±0.18
Gait speed †	0.84±0.28	0.76±0.28	0.001*	0.80±0.28

*p<0.05; †Data are presented as Mean±SD. #Data are presented as median (min-max)
CGA, comprehensive geriatric assessment; COPD, Chronic obstructive pulmonary disease; ADL, Katz Index of Activities of daily living; IADL, Lawton & Brody index of Instrumental Activities of Daily Living; GDS, The Geriatric Depression Scale; MMSE, Mini Mental State Examination; MNA, The Mini Nutritional Assessment Tool; EQ-5D; European Quality of Life-5 Dimensions; HGS handgrip strength; SMMI skeletal muscle mass index

Number of the medications had a negative moderate correlation with EQ-5D and MNA scores, a negative weak correlation with IADL, HGS and gait speed scores, and a positive moderate correlation with GDS scores according to the correlation analysis results (Table 2, Table 3).

Table 2. Correlation analysis results between the variables

		Number of medications	Age	Number of diseases	ADL	IADL	EQ-5D
Number of medications	r		0.047	0.513**	-0.070	-0.224**	-0.423**
	p		0.290	0.000	0.114	0.000	0.000
Age	r	0.047		-0.008	-0.079	-0.256**	-0.306**
	p	0.290		0.853	0.073	0.000	0.000
Number of diseases	r	0.513**	-0.008		0.114**	-0.123**	-0.343**
	p	0.000	0.853		0.010	0.005	0.000
ADL	r	-0.070	-0.079	0.114**		0.537**	0.545**
	p	0.114	0.073	0.010		0.000	0.000
IADL	r	-0.224**	-0.256**	-0.123**	0.537**		0.655**
	p	0.000	0.000	0.005	0.000		0.000
EQ-5D	r	-0.423**	-0.306**	-0.343**	0.545**	0.655**	
		0.000	0.000	0.000	0.000	0.000	

r: Spearman rank correlation coefficient; ** Significant at 0.01 level. ADL, Katz Index of Activities of daily living; IADL, Lawton & Brody index of Instrumental Activities of Daily Living; EQ-5D; European Quality of Life-5 Dimensions

Table 3. Correlation analysis results between the variables

		Number of medications	GDS	MMSE	MNA	HGS	Gait speed
Number of medications	r		0.313**	-0.086	-0.305**	-0.153**	-0.145**
	p		0.000	0.051	0.000	0.001	0.001
GDS	r	0.313**		-0.286**	-0.315**	-0.335**	-0.215**
	p	0.000		0.000	0.000	0.000	0.000
MMSE	r	-0.086	-0.286**		0.104*	0.297**	0.222**
	p	0.051	0.000		0.018	0.000	0.000
MNA	r	-0.305**	-0.315**	0.104*		0.233**	0.240**
	p	0.000	0.000	0.018		0.000	0.000
HGS	r	-0.153**	-0.335**	0.297**	0.233**		0.502**
	p	0.001	0.000	0.000	0.000		0.000
Gait speed	r	-0.145**	-0.215**	0.222**	0.240**	0.502**	
		0.001	0.000	0.000	0.000	0.000	

r: Spearman rank correlation coefficient; ** Significant at 0.01 level. ADL, Katz Index of Activities of daily living; IADL, Lawton & Brody index of Instrumental Activities of Daily Living; EQ-5D; European Quality of Life-5 Dimensions

DISCUSSION

In our study we found that polypharmacy is significantly related to depression, decline in instrumental daily activities, impaired nutritional status, and worsening in life quality. The number of the comorbidities was higher in the polypharmacy group. Having multiple comorbid diseases is the most important reason for polypharmacy. On top of that, polypharmacy increases the risk of geriatric syndromes and the rates of morbidity and mortality in older people (3). Increasing rates of multimorbidity is closely related to a decrease in life quality and decline in functional capacity (21).

Previous studies have shown that polypharmacy is associated with impaired physical functioning in older adults (14,16), and this close relationship is thought to be a bidirectional cause-effect relationship (22). In the

current study, gait speed and IADL scores were lower in the polypharmacy group whereas ADL scores found to be similar. “Polypharmacy” itself and “excessive polypharmacy” were shown to be related to disabilities in IADL (15,23). However, in another study in which polypharmacy was defined as using six and more drugs, there was not any association between polypharmacy and ADL scores (24). There may be a reasonable explanation of this situation: IADL includes activities such as using the telephone, preparing food or doing laundry those are more complicated than ADL because of the dependence of instruments. Due to the same reason, it also requires superior cognitive functions than ADL that includes basic living activities like feeding, bathing or toileting. "Excessive polypharmacy" has shown to be closely associated with a decrease in cognitive capacity (15). More drugs mean more dependence in basic living activities (25). Therefore using fewer drugs may only affect the IADL while excessive numbers of drugs can impair both of IADL and ADL.

Functional independence is rather strong predictor of life quality and a worsening in functional status is closely related to depressive symptoms (26). Depression in the elderly is still highly under-recognized syndrome and has a rate as 40%–60% of cases (27), and depressive symptoms among community-dwelling older adults have a substantial ratio ranging from ~8 to 16% (28). The relationship between polypharmacy and depression has been shown previously (17), and moreover, depression may be a better independent predictor of polypharmacy than the other comorbid diseases (19). Depression has also shown to be associated with drug nonadherence in general population (20). Thus, in depressive patients with additional comorbidities, having polypharmacy and medication nonadherence can make the treatment process more complicated and lead to worsening the condition of the patient’s diseases.

In our study we found that MNA scores were significantly lower in polypharmacy group, and there was also a moderate negative correlation between MNA scores and the number of medications (Table 3). Although an explicit relationship has been found between polypharmacy and malnutrition in the previous studies (15,29,30), the mechanism of it is not obvious. Nutritional status of elderly is a very important subject that should be definitely addressed because of its influence on the pharmacology of a lot of drugs (31). In addition, a wide variety of drugs may cause malnourishment through a lot of side effects including nausea, anorexia, early satiety, reduced feeding ability, dysphagia, constipation and diarrhea (32,33). Regulating pharmacotherapy and decreasing the number of drugs may be a reasonable way in order to improve patient's nutritional status (34).

With the modern pharmacotherapy, increasing variety of drugs caused increased adverse reactions and drug interactions, and polypharmacy itself has started to show detrimental effects on the various aspects of health. In the current study, our results have shown that polypharmacy has negatively affected the quality of life in line with previous studies (35).

We depicted that there was no difference regarding sarcopenia and MMSE scores in both groups. Although there was no difference, HGS was lower in polypharmacy group. Although there was not significantly difference, the frequency of sarcopenia was higher in the polypharmacy group. The precise association between polypharmacy and sarcopenia remains unknown. Although some studies in the literature are supporting the relationship of polypharmacy with sarcopenia (36), a recent study has shown that the strength and mass of muscle in sarcopenic patients with stroke was not positively affected after "deprescribing" (34). Sarcopenia is a consequence of a various of predisposing factors, such as physiological changes by getting older, deficiency in nutrition, chronic comorbid diseases, and insufficient physical activity (37). These predisposing factors may be the reason of these conflicting results.

One of the limitations of the study is that the participants' number is relatively limited. Second, since the study is cross-sectional, we cannot mention a cause-effect relationship. Despite these limitations, the study has also some strengths. First, a comprehensive geriatric evaluation was performed, thereby investigating other conditions that could affect the results. Second, along with the CGA, quality of life was also evaluated. Thus, a multidimensional assessment of the health status of older adults included the study was provided.

CONCLUSION

In conclusion, depending on the outcomes of our study we can conclude that as the number of the medications used by patients increase; the detrimental effects of the drugs on the health emerges a tendency to depression, malnutrition, and a decline in functional status. Polypharmacy also impairs the quality of life which is an overall health term and indicator of optimal aging. It will be wise to address the polypharmacy as a crucial health problem, optimize the number of the medications and thereby make the health condition better of an older patient having multiple comorbidities.

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Ethical approval: *All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval for the study was granted by the Gaziantep University Medical Faculty ethical committee (no. 2022/155).*

Authors' contributions: *Author EÖ designed the study,*

performed manuscript writing; Authors SG performed data collection and analysis; Author ZAO reviewed the manuscript and provided critical revisions. All authors have approved the final article.

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Research Article

Retrospective Investigation of Brainstem Volume and Craniovertebral Junction Morphometry in Migraine Patients

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Abstract

Aim: Migraine, a complex, multifactorial neurovascular brain disorder, might cause several functional and morphometric changes in the brain. Despite many studies, a consensus has not emerged on its pathophysiology, and it is not fully elucidated so far. Recently, changing brain structures in migraine with aura has been attracting the attention of the scientific periphery. The brainstem is a critical region in the pathogenesis of migraine. Another transition point is the craniovertebral junction. Regional pathologies might trigger off the pathogenesis of neurodegenerative and neurological diseases by affecting hydrodynamics. Moreover, there is insufficient data on the relationship between migraine and the craniovertebral junction. The present study aimed to make the volumetric analysis of brainstem volume in migraine with and without aura, perform some angular and linear measurements of the craniovertebral junction, and evaluate the effects of these parameters in migraine patients.

Material and Methods: The study retrospectively analyzed the brain Magnetic Resonance Images of 108 migraine patients (aged 18 to 65). Their brainstem volumes were measured using volBrain (online brain MRI volumetry system). Also, the angular and linear parameters of craniovertebral junctions were derived from the images. The obtained data were transferred to the SPSS 22 package program and analyzed.

Results: The mean brainstem volume was 17.21±2.79 cm^3 in the migraine with aura group, 17.33±2.48 cm^3 in the migraine without aura, and 19.27±2.76 cm^3 in the control group. There was no statistically significant difference between migraine with and without aura groups ($p>0.05$). There was a statistically significant difference between the control and both migraine groups ($p<0.05$). Furthermore, the clivus-canal angle was significantly different between the control and patient groups.

Conclusion: The study found that the brainstem volume was lower in the migraine groups (with and without aura) than in the control group. Also, the different clivus-canal angles between the control and patient groups show that this issue should be more comprehensively studied.

Keywords: Migraine, brainstem volume, craniovertebral junction

INTRODUCTION

Pain sense experienced by human beings–at least once in their life–significantly affects the quality of daily life but protects the body against possible dangers. Headache is a crucial protective mechanism for our brains. Migraine, in which autonomic, neurological, gastrointestinal, cognitive, vestibular, and emotional symptoms induced by genetic and environmental factors can accompany recurrent headache attacks, is a neurovascular headache that develops from excessive trigeminovascular system activations. The International Headache Society (IHS) classified migraine as a primary headache. The worldwide prevalence of migraine is 15%, with a higher prevalence in women, which might depend on fluctuations in female sex hormone levels (1). Although there are many subgroups according to the complications it accompanies, there are two main types: with and without aura. Auras are transient neurological deficits that occur in 20% of the cases, usually before (sometimes after) a headache, and gradually increase in severity within five minutes. Harold G Wolff suggests aura symptoms relate to cerebral vasoconstriction and headaches to cerebral vasodilation (2). In addition to positive visual symptoms, such as zig-zag patterns and photopsia, and negative visual symptoms, such as hemianopsia and quadrantopsia, unilateral

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positive or negative somatosensory pictures and less common speech/language disorders can appear as the aura. There are many subtypes according to the symptoms seen. Despite many studies on migraine pathophysiology, there is still no consensus (1-7). Research on the aura mechanism has focused on cortical spreading depression and changes in brain structures (2). In the past, researchers considered no morphological change in brain structures in neurological imaging and examination of chronic migraine. However, as a result of increasing human studies over time, morphometric changes in the brain structures of migraine patients have been reported (6,8-11). It has been emphasized that there might be volumetric changes in gray and white matter structures. Despite many functional neuroimaging studies in the literature to understand the pathophysiology of migraine, very few studies have investigated structural changes in the brain. Besides, these studies could not reach a consensus. While some studies reported a statistically significant difference in the gray and white matter, cerebellum, and brainstem volumes (BV) in the migraine patient group, others documented no statistically significant difference in the control and migraine groups (7,10-12). Some studies report that vertigo, vomiting, and autonomic dysfunctions prevalent in migraine originate from the brain stem (2), a transition region that contains the main structures in the pathogenesis of migraine (10). Another critical neurovascular transition is the craniovertebral junction (CVJ), also known as the skull base. Being the most mobile region of the axial skeleton and containing important neural and vascular structures explains the region's importance (13). CVJ is a potential bottleneck for craniospinal hydrodynamics. Malformations or deformations in the region cause obstruction in the cerebrospinal fluid (CSF) and blood flow. Reportedly, this may be the cause or a contributing factor in the pathogenesis and progression of neurodegenerative and neurological diseases (14,15). Therefore, changes in some angular and linear parameters might indicate migraine pain triggers. Migraine and other headache diseases make up 20% of the patients who visited the neurology outpatient clinic (6). The fact that migraine affects most of the population and that there is still no consensus in the studies reveals that more studies are necessary on the macro anatomy and morphometry of the brain. Therefore, the current study aimed to conduct a volumetric analysis of the brainstem volume in migraine with and without aura and contribute to the literature by researching the relationship between migraine and some angular and linear parameters in CVJ, a crucial transition point and potential bottleneck between the cranium and spinal canal.

MATERIAL AND METHOD

Related University Non-Interventional Clinical Research Ethics Committee granted necessary permissions (Nr: 2021/02-16) for the current study, carried out between 01.01.2012 and 31.12.2020 in Related University Training and Research Hospital Neurology Headache Outpatient Clinic. This research covering 108 individuals (female: 80, male: 28), aged 18-65 years (mean 34.78±10.83), diagnosed with migraine according to the International Classification of Headache Disorders (ICHD)-3 beta criteria, retrospectively analyzed participants' brain Magnetic Resonance Images (MRI) and divided migraine patients into two groups: migraine with aura and migraine without aura. Those with neurological diseases such as multiple sclerosis, cerebrovascular disease, Parkinson's disease, Alzheimer's disease, epilepsy, and systemic diseases such as chronic liver disease, chronic renal failure, diabetes, hypertension, and the ones with head trauma, intracranial surgery, and malignancy were excluded from the study. Besides these, the control group had to meet additional criteria, such as not having a migraine or having a different primary headache diagnosis, and so forth. The control group had a brain MRI for other reasons (somatization, benign positional paroxysmal vertigo, etc.) Brain T1-weighted MRI of the with-aura, without-aura, and control groups that met the criteria were taken in DICOM format. Then, DICOM files were converted to nifti format with a converter named dcm2niigui. Images of each individual converted to nifti were uploaded to the online brain MRI volumetry system (volBrain) by entering age and gender information. VolBrain reports brainstem volume measurements in cm3.

The following parameters on Sagittal T1 weighted MRI were measured using the program in the radiology department:

1. McGregor Line Length (MGL): The line drawn from the posterior edge of the hard palate to the lowest point of the os occipitale (Figure 1) (16).

2. McRae Line Length (MRL): The line drawn from basion to opisthion. (Figure 1) (17).

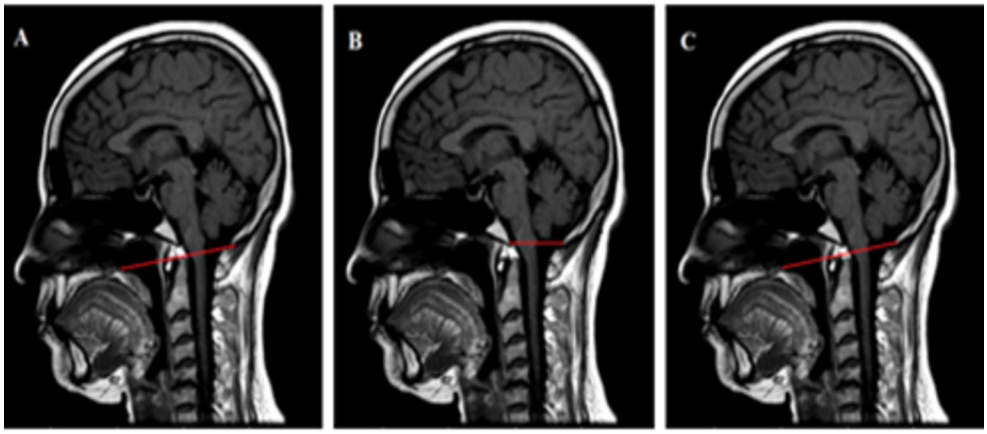

Figure 1. Demonstration of linear parameters. A: McGregor line (MGL), B: McRae line (MRL), C: Chamberlain line (CL)

3. Chamberlain Line Length (CL): The line drawn from the posterior edge of the hard palate to the opisthion (Figure 1) (17).

4. Craniocervical Tilt Angle (CTA): The angle between the line drawn upwards from the anterior face of the dens of axis and the line drawn from the anterior aspect of the clivus (Figure 2) (18).

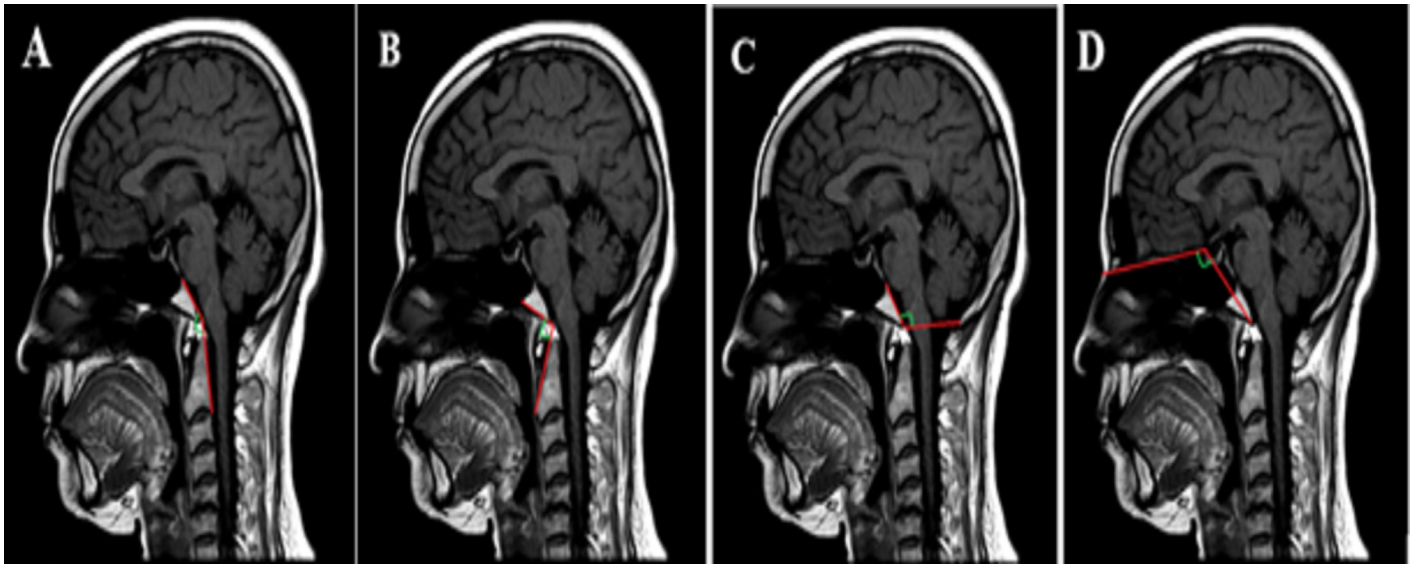

Figure 2. Demonstration of angular parameters. A: Clivus canal angle (CCA), B: Craniocervical tilt angle (CCT), C: Boogard angle (BA), D: Welcher basal angle (WBA)

5. Clivus Canal Angle (CCA): The angle between the line extending from the posterior of the dens of axis on the upper surface of the clivus to the vertebral canal (Figure 2) (16).

6. Boogard Angle (BA): The angle between a line drawn from the upper face of the clivus and the opisthion (Figure 2) (19).

7. Welcher Basal Angle (WBA): The angle between a line extending "from the nasion to the tuberculum sella" and a line extending "from the basion to the tuberculum sella" (Figure 2) (16).

The obtained data were transferred to the SPSS 22 package program. The normal distribution of the data was checked with skewness and kurtosis values. Values between -2 and +2 were accepted in a normal distribution and used in parametric tests (20). Descriptive statistical analyzes were performed using the mean and standard deviation. One Way ANOVA test, one of the parametric tests, was used for comparisons between groups. Among the post hoc tests, the Tukey test served for homogeneous variances, and the Games-Howell test for non-homogeneous variances. Pearson correlation analysis was performed to determine the relationship between all parameters. In the study, $P<0.05$ was statistically significant.

RESULTS

The study was carried out on the brain MRI images of 108 participants (aged 18-65) in the Control and Patient groups, consisting of 80 females (mean age: 34.25±10.13) and 28 males (mean age: 36.29±12.72). In the study, the patient group was divided into two subgroups: migraine with- and without-aura. Table 1 shows the distribution of participants by group and gender.

Table 1. The distribution of participants by group and gender

	Gender		
Groups	**(F)**	**(M)**	**Sum**
MA	21	7	28
MO	38	11	49
C	21	10	31
Sum	80	28	108

MA: Migraine with aura, MO: Migraine without aura, C: Control, F: Female, M: Male

In the study, of the participants, 74.1% were female (n=80), and 25.9% were male (n=28). Despite the retrospective analysis of brain MRI across the entire data range in the current study, due to the less prevalence of migraine with aura in the community and failure to convert some files to a proper format, only the images of 28 individuals (male:7, female:21) have been possible to convert into the volBrain-compliant file format. The number of male participants in the control and migraine without aura groups was kept low to balance the difference between the groups' sample numbers. Therefore, the study could not examine the inter-gender variations in the parameters. Table 2 shows the mean and standard deviation values of the angular, linear and volumetric parameters of the data obtained from 80 women and 28 men by gender. The mean BV of the control group was higher than the other two patient groups. According to Pearson correlation analysis, no relationship existed between BV and age ($r=-0.006$, $p=0.954$).

Table 2. Findings of the parameters of the patient and control groups

Para meters	Groups	N	Total Mean±SD	N	Female Mean±SD	N	Male Mean±SD
MGL (mm)	MA	28	81.28±5.44	21	81.24±5.67	7	81.41±5.11
	MO	49	82.18±4.97	38	82.56±5.28	11	80.88±3.57
	C	31	83.44±3.92	21	82.59±3.66	10	85.23±4.02
MRL (mm)	MA	28	36.92±3.46	21	36.89±3.71	7	37.02±2.82
	MO	49	37.44±3.57	38	37.44±3.65	11	37.44±3.45
	C	31	41.51±9.92	21	38.49±4.20	10	39.40±4.18
CL (mm)	MA	28	78.04±6.13	21	78.00±6.40	7	78.18±5.73
	MO	49	78.50±5.38	38	79.09±5.74	11	76.44±3.32
	C	31	77.88±12.67	21	79.47±4.57	10	83.02±3.80
CCA (°)	MA	28	149.00±10.05	21	149.66±9.18	7	147.04±12.95
	MO	49	149.72±8.07	38	150.27±8.52	11	147.79±6.18
	C	31	154.77±10.15	21	152.23±9.66	10	160.11±9.47
CTA (°)	MA	28	118.35±9.86	21	117.15±8.55	7	121.94±13.18
	MO	49	115.86±8.16	38	115.61±7.81	11	116.71±9.63
	C	31	121.57±12.30	21	119.45±12.12	10	126.02±12.06
BA (°)	MA	28	130.44±9.34	21	129.94±9.25	7	131.94±10.16
	MO	49	126.77±8.32	38	127.39±8.00	11	124.63±9.45
	C	31	125.68±8.34	21	125.24±8.19	10	126.62±9.01
WBA (°)	MA	28	129.58±6.90	21	129.94±6.72	7	128.50±7.86
	MO	49	131.11±5.51	38	131.72±5.70	11	128.97±4.37
	C	31	131.84±5.29	21	133.16±3.31	10	129.66±2.58
BV (cm^3)	MA	28	17.27±2.79	21	16.84±2.99	7	18.58±1.58
	MO	49	17.33±2.48	38	16.83±2.06	11	19.05±3.13
	C	31	19.27±2.63	21	18.15±1.72	10	21.61±2.76

MA: Migraine with aura, MO: Migraine without aura, C: Control, SD: Standard deviation, MGL: McGregor Line Length, MRL: McRae Line Length, CL: Chamberlain Line Lenght, CCA: Clivus-canal angle, CTA: Craniocervical tilt angle, BA: Boogard angle, WBA: Welcher basal angle, BV: Brainstem volume

Table 3. Comparison of brainstem volume according to patient (1,2) and control (3) groups

Groups	N	Mean±SD(cm^3)	Sources of variance	SS	df	MS	F	p	Significance
MA (1)	28	17.27±2.79	Between group	84.663	2	42.332	6.201		
MO (2)	49	17.33±2.48	Within group	716.777	105	6.826		0.003*	3 to 1.2
C (3)	31	19.27±2.63	Total	801.440	107				

MA: Migraine with aura, MO: Migraine without aura, C: Control, SS: Sum of squares, df: Degree of freedom, MS: Mean squares, SD: Standart deviation, *signifcant difference ($p<0.05$)

Table 4. Correlation values between all parameters investigated in the MA group

	1) Age	2) BV	3)MGL	4) MRL	5) CL	6) CCA	7) CTA	8) BA	9)WBA
2	-0.320								
3	0.054	-0.171							
4	-0.217	0.111	0.308						
5	0.001	0.000	0.950**	0.335					
6	0.389*	0.049	-0.221	-0.153	-0.216				
7	0.223	-0.208	-0.359	-0.351	-0.414*	0.375*			
8	-0.318	0.334	-0.248	0.017	-0.186	-0.415*	-0.030		
9	-0.181	0.304	0.206	0.317	0.237	-0.304	-0.363	0.388*	1

*p<0.05 ve **p<0.01

Table 5. Correlation values between all parameters investigated in the MO group

	1) Age	2) BV	3)MGL	4) MRL	5) CL	6) CCA	7) CTA	8) BA	9)WBA
2	0.126								
3	0.112	0.136							
4	0.092	0.281	0.337*						
5	0.054	0.109	0.948**	0.357*					
6	0.039	0.037	-0.167	0.086	-0.194				
7	0.049	0.272	-0.062	0.246	-0.062	0.395**			
8	0.123	-0.090	-0.129	-0.218	-0.073	-0.108	-0.169		
9	-0.157	-0.170	0.061	0.018	0.072	-0.319*	-0.192	-0.309*	1

*p<0.05 ve **p<0.01

Table 6. Correlation values between all parameters investigated in the control group

	1) Age	2) BV	3)MGL	4) MRL	5) CL	6) CCA	7) CTA	8) BA	9)WBA
2	0.097								
3	-0.057	-0.136							
4	-0.241	-0.046	-0.046						
5	0.150	0.041	-0.521*	-0.812*					
6	0.236	0.430*	0.099	-0.095	0.262				
7	0.077	0.453*	0.314	0.225	0.102	0.646**			
8	-0.073	0.296	-0.351	0.217	-0.380	-0.139	-0.187		
9	0.214	-0.337	-0.93	-0.104	0.016	-0.393*	-0.593**	0.308	1

*p<0.05 ve **p<0.01

Post Hoc analyses revealed that, among angular and linear parameters, only CCA showed a statistically significant difference between control and migraine with aura (p=0.048) and migraine without aura (p=0.049) groups (p<0.005). While there was a moderate negative correlation between CCA and BA in migraine with aura, no correlation was found in migraine without aura (table 4-5). Correlations of all parameters according to the groups are given in tables 4,5, and 6.

DISCUSSION

In migraine, volumetric changes can appear in many brain structures, especially in the cerebellum and brain stem (10,21). Techniques such as positron emission tomography (PET) and functional MRI can detect which brain parts show higher activation during migraine attacks. In migraine that develops from the excessive activation of the trigeminovascular system, a high

activation is evident in the brainstem structures such as the nucleus spinalis nervi trigemini, the tegmentum pontis and the substantia grisea centralis in the mesencephalon, especially during attack periods (21,22). Gray matter amounts can decrease, especially in active areas during migraine pain attacks. Some researchers thought that one of the underlying causes of migraine pathophysiology might be the volumetric changes in some structures in the brain stem (21). However, more morphometric studies are necessary on this subject because the studies are scant in the literature, and no consensus has been reached (1-4,6,8,21). There is no specific laboratory or radiological evaluation method for the diagnosis of migraine. Therefore, morphometric studies that provide objective data in addition to the patient's history will guide physicians for "normal" and "early" diagnosis. The current study found brainstem volume lower in the migraine patient 3 groups with- and without-aura than in the control group but no statistically significant difference between them. In a study consisting of a patient group with chronic migraine (n=24) and a control group (n=24), Bilgiç et al.(10), found that the brainstem volume was statistically lower in the migraine patient group than in the control group, similar to our study. In the current study too, the brainstem volume of the migraine groups with aura (17.27±2.79 cm^3) and without aura (17.33±2.48 cm^3) differed statistically significantly from the control group (19.27±2.63 cm^3) ($p<0.05$). Both studies have revealed that brainstem volumes decrease in migraine. In their work, Bilgiç et al. (10) did not divide the migraine patients into groups as with- and without-aura. Chong et al. (23) calculated the brainstem volumes of the migraine (n=55) and control (n=58) groups–as a whole and part by part. They reported no statistically significant difference between the total brainstem volume of the migraine patient group and the control group. In another study consisting of the patient (n=25) and control groups (n=25), researchers reported that the brainstem volume was statistically significantly lower in the patient group ($p<0.005$) (11). The literature shows that research on migraine and volumetric changes in brain structures is generally on the brain's total volume, gray or white matter, cerebellum, and brain lobe volumes (7,9,11,12,24). However, very few studies investigate the relationship of brainstem volume with migraine with-aura and without-aura. In the neutral position, the clivo-axial angle varies between 145° and 160°. An angle of less than 150° causes deformations in the upper cervical vertebrae and creates pressure on the brain stem. In addition to neurological problems, this pressure also deteriorates regional craniospinal hydrodynamics due to obstruction (25). The clivo-axial angle refers to the CCA angle in this research. In the current study, the CCAs of patients with aura and without aura were less than 150°, but no statistically significant difference emerged between the control and patient groups (Table 2). The clivo-axial angle has recently appeared as a prominent angular parameter in evaluating CVJ instability and deciding on stabilization surgeries (25). Asal et al. reported that the clivo-axial angle exhibited a statistically significant difference between migraine (142.65±8.73) and control (153.66±6.35) groups and that skull base angles changed in migraine disease (14). MGL, MRL, and CL are linear parameters used for evaluating basilar invagination. Odontoid protrusion above this line is always pathological and indicates basilar invagination. MRL also refers to the anterior-posterior diameter of the foramen magnum and should be greater than 19 mm (13,19). The current study found the control group's MRL value as 41.51±9.92 mm. Yüksel et al., in their research examining Chiari Malformation Type-1 and CL and MRL values of the control group, reported the CL and MRL values of the control group as 74 mm and 38.2 mm, respectively (26). The current study found the CL and MRL values as 79.47 mm and 38.49 mm, respectively. In their research, Chandra et al. measured the CTA angle from BT images of 70 patients diagnosed with atlantoaxial dislocation and basilar invagination. They documented the value of CTA in the control group as 119.8°±9.2° (27). The current study revealed the value of CTA in the control group as 121.57±12.30. These results suggest that different results might stem from separate studies with various exclusion criteria and patient groups.

WBA is an important angular parameter in the evaluation of the platybasia and should be less than 140° (13, 16). It was less than 140° in the current study, and no statistically significant difference emerged between the groups (Table 2). Nascimento et al. measured the WBA, CCA, and BA of the control group and patients with basilar invagination and reported statistically significant differences between the groups (28). As stated above, despite retrospectively scanning MRI across the entire data range in this research, the sample size of this group could not increase further due to the low prevalence of migraine with aura. Therefore, the sample size of migraine without aura and control groups remained the same to balance the sample size difference in all groups. A review of the literature did not demonstrate any other studies investigating the angular and linear parameters–considered by this study–which are significant in evaluating CVJ anatomy in patients with migraine. Since migraine is affected by several factors, such as its phase, attack frequencies, and disease duration, abnormalities in the brain are dynamic (24). Therefore, measurements and evaluations performed on MR, PET, or fMRI images taken instantly in volumetric and functional changes during an attack will produce more accurate results. However, the current study could not perform a dynamic measurement because of its retrospective nature. Studies have shown that migraine is not a simple trigeminovascular but a complex neurovascular disease affecting many neural structures, such as cortical, subcortical, and brainstem (24). Knowing both functional and morphometric changes of the brain stem in migraine is likely to provide important clues in understanding the pathophysiology of migraine and in early diagnosis.

CONCLUSION

The present study found that the brainstem volume of migraine patient groups was statistically significantly

lower than the control group (p<0.05). Based on these data, we have concluded that migraine affects the brainstem morphometry, where our crucial vital functions are managed. However, the scant number of relevant studies and the lack of a consensus reveal that more studies are necessary. Volumetric MRI examinations in further studies will guide clinicians in migraine diagnosis, treatment, and complication follow-up.

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Research Article

Oxidative Stress Elevates eNOS Expression and VSMCs Proliferation of the Umbilical Vein of GDM Mothers

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Abstract

Aim: Gestational diabetes mellitus (GDM) is associated with an increased risk of fetal and maternal complications, such astype 2 DM (diabetes mellitus) and cardiovascular disease (CVD). This study aimed to predict the potential for future vascular complications in mothers with GDM by evaluating oxidative stress, endothelial NO synthase (eNOS) expression, and vascular smooth muscle cell (VSMC) proliferation in the umbilical vessels of mothers with GDM.

Material and Methods: Subjects were divided into two groups: the normoglycemic control (NGC) group (n = 10) and the GDM group (n = 12). Expression of eNOS and production of reactive oxygen species (ROS) in human umbilical vein endothelial cells (HUVECs) were determined. The mitochondrial mass of HUVECs was evaluated by spectrofluorometry. VSMC proliferation was ascertained *in vitro* with an EdU cell proliferation assay. Advanced glycation end products (AGEs) accumulation was measured by ELISA and assessed by immunohistochemical staining

Results: VSMC proliferation, eNOS expression, and ROS production in HUVECs were significantly increased, and greater immunohistochemical staining to AGEs was observed in endothelium in GDM.

Conclusion: Increased oxidative stress, which elevates eNOS expression and VSMC proliferation in the umbilical vessels of mothers with GDM, may be a sign that mothers have a high potential for developing diabetes or cardiovascular disease in the future.

Keywords: GDM, oxidative stress, VSMCs, ROS, eNOS

INTRODUCTION

Gestational diabetes mellitus (GDM) is a condition of glucose intolerance with onset or first recognition during pregnancy (1). With the increase in obesity and diabetes mellitus (DM) cases, an elevation in the incidence of GDM is observed (2). Understanding the pathogenesis of GDM is essential for the precaution against the progression of type 2 DM and cardiovascular disease (CVD). In a long-standing hyperglycemic state in DM, protein glycation reactions lead to the formation of advanced glycation end-products (AGEs), which are thought to be the major causes of different vascular complications in DM (3). Oxidative stress generation in a variety of cells is induced by AGEs' interaction with a receptor for AGEs (RAGE) (4). The intermolecular collagen cross-linking caused by AGEs results in diminished arterial compliance and increased vascular stiffness (5). The deposition of AGEs has been known to progress at an accelerated rate under DM. AGEs are hardly broken down and remain in diabetic tissue for a long time, even with improved glycemic control (4). Cellular exposure to high glucose as seen in DM induces reactive oxygen species (ROS) production (6). The release of ROS and the generation of oxidative stress are considered critical factors for the

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pathogenesis of DM (7). ROS production in the vessel wall is considered a risk factor for atherosclerotic CVD as well (8). A marked increase in oxidative stress characterized by the overproduction of ROS has been observed in DM. A study has shown that oxidative stress brings about the downregulation of occludin (9). It has been reported that the downregulation of β-catenin in placental vessels is significantly associated with GDM (10).

Endothelial dysfunction is a common feature in GDM (11). Endothelial dysfunction determines future vascular disease complications (12) as well. Vascular smooth muscle cells (VSMCs) are crucial to maintaining the integrity of the arterial wall. VSMC proliferation contributes to vascular remodeling in CVD and diabetic vascular complications (13).

Studies have confirmed that endothelial nitric oxide synthase (eNOS) is an important factor in endothelial function. eNOS is involved in vascular development by promoting angiogenesis (14). A high level of glucose exposure increases eNOS expression (15). eNOS is the main weapon of endothelial cells to overcome vascular diseases. This study aimed to predict the potential for future vascular complications in mothers with GDM by evaluating oxidative stress, eNOS expression, and VSMC proliferation in the umbilical vessels of mothers with GDM.

MATERIAL AND METHOD

Subjects

The subjects were recruited from the Department of Gynecology and Obstetrics of Kocaeli University Hospital. The study protocol was approved by the Clinical Research Ethical Committee of Kocaeli University. An informative written consent was signed by all the subjects. The normoglycemic control women were selected as pregnancies without any history of illness, and no risk factor of GDM including a normal oral glucose tolerance test (OGTT) result. Women aged between 18-40 years, with a body mass index (BMI) of less than 30 kg/m^2 at the beginning of pregnancy were selected (Table 1). Diagnosis of GDM was made according to American Diabetes Association (ADA) criteria at the end of the OGTT after 75 g oral glucose load between 24 and 28 weeks of pregnancy (16).

Collection of Blood Plasma, Placenta, and Umbilical Cord Samples

Placentas and the umbilical cord samples were collected and transferred to the laboratory immediately. Fetal umbilical cord blood samples were collected from the umbilical veins and placed into heparinized tubes. An equal volume of Histopaq®-1077(SIGMA-ALDRICH®, USA) reagent was added and centrifuged at 20 ºC, 400 g for 30 min. The plasma samples were frozen at -80 ºC.

Isolation of Human Umbilical Vein Endothelial Cells

HUVECs were isolated through enzymatic digestion. Umbilical cords were stored at 4 ºC in Hank's Balanced Salt Solution (HBSS) with 1% Pen/Strep for a night. The umbilical cords were washed extensively with Dulbecco's Phosphate Buffered Saline (DPBS), one end of the vein was gripped with a clamp and the vein was filled with 0.1% collagenase (Gibco) prepared in HBSS. After incubating at 37 °C for 25 minutes, the endothelial cells in the enzyme solution were transferred into a falcon tube. After centrifugation (1500 rpm, 5 min), the cell pellet was washed with DPBS and finally resuspended in EGM-2 (Lonza) medium containing 5% fetal bovine serum (FBS) before being drawn into a gelatin-coated T25 culture flask. HUVECs were cultured until forming a 70% confluent monolayer and passaged afterward. HUVECs were immunostained for CD31 to prove the culture purity. HUVECs were seeded in 8-well slides (Ibidi, ibiTreat), and fixed with formaldehyde. For the primary antibody, anti-CD31 (Dako Denmark A/S, Glostrup, Denmark) antibody, and for the secondary antibody, Alexa Fluor 488 labeled secondary antibody (Molecular Probes, Life Technologies) were used. At last, for nuclear staining, DAPI was applied. The negative controls were cells that were treated with the same protocol but without the primary antibody.

Vascular Smooth Muscle Cell Isolation Through Explant Culture

The umbilical cord vein was opened with scissors, and the endothelial layer on its surface was removed. Then smooth muscle fibers are mechanically pulled out and immediately added to gelatin-coated culture wells. Vascular smooth muscle fibers are cultured in a minimal amount of culture medium (10% FBS, 1% Pen/Strep, and DMEM-F12 with 2 ng/ml bFGF) with keeping tissue attached to the surface. VSMCs were immunostained for alpha-smooth muscle actin (α-SMA) to prove purity. For the primary antibody, anti-α-SMA antibody (Abcam; ab5694), for the secondary antibody, Alexa Fluor 568 labeled secondary antibody (Molecular Probes, Life Technologies) were applied.

Collagen Isolation from Umbilical Cords

Umbilical cord tissues were cut into small pieces and treated with 0.2% sodium chloride for 3 days at +4°C with shaking at 90 rpm. After incubating, the liquid part was discarded by centrifugation at 10000 rpm for 10 minutes. Umbilical cord tissues were then treated with 0.1% pepsin prepared in 0.2 M acetic acid for 4 days at + 4°C. After centrifuging, the supernatant was drawn into the new tubes, and an equal volume of 1.8 M sodium chloride was added and left undisturbed overnight. The collagen fibers were retrieved by centrifugation at + 4ºC and 12000 g for 45 min. The collagen pellets were disintegrated in 0.5 M acetic acid and dialyzed at + 4 °C against 0.1 M acetic acid for 3 days. 0.1 M acetic acid was refreshed daily and then the collagen samples were dialyzed against distilled water at + 4 °C at 90 rpm for the whole day. Collagen solutions were collected and stored at + 4 °C in tubes for further analysis.

Detection of Reactive Oxygen Species

Intracellular production of ROS was measured using 2',7'-dichlorofluorescein diacetate (H2DCF-DA Sigma-

Aldrich). This non-fluorescent compound rapidly oxidizes to highly fluorescent DCF by interacting with cellular ROS. H2DCF-DA was added to the HUVECs seeded in a 96-well plate (10^4cells/well) at a final concentration of 5 µM and the cells were incubated at 37 ºC for 1h. Cells from all experimental groups were seeded on the same plate and treated simultaneously with the same H2DCF-DA stock solution. Cellular fluorescence was measured in a Flex Station3 spectrofluorometer with excitation and emission wavelengths set at 490 nm and 530 nm, respectively. The DNA content of each well was ascertained with Quant-iT PicoGreen dsDNA assay kit (Molecular Probes, Life Technologies, Eugene, Oregon, USA), and fluorescence emission/DNA content was calculated for normalization of the values.

Determination of Mitochondrial Mass

Mitochondrial mass was determined by incubating the HUVECs seeded in 96-well plate (10^4cells/well) with 100nM Mito Tracker Orange (MTO) (Molecular Probes, Life Technologies) prepared in EBM-2 medium for half an hour at 37 ºC. It was washed with warm DPBS and immediately fluorescence was read on a Flex Station3 spectrofluorometer with excitation and emission wavelengths set to 554 nm and 600 nm, respectively. The DNA content of each well was determined with the same dsDNA assay kit mentioned above.

Quantitative Real-time PCR for eNOS Expression in HUVECs

RNA was isolated from HUVECs by using the High Pure RNA Isolation Kit (ROCHE) and subsequently converted to cDNA with Transcriptor High Fidelity cDNA Synthesis Kit (ROCHE) according to the manufacturer's instructions. Quantitative real-time PCR analysis was performed on the Light Cycler 480-II (Roche Diagnostics, Rotkreuz, Switzerland) with appropriate cycle conditions using the Light Cycler 480 SYBR Green I Master kit (Roche Diagnostics) for amplification of eNOS and Real-Time Ready Single Assay (Roche) and Light Cycler 480 Probes Master kit for amplification of β-actin. Primer sequences used for the amplification of eNOS were as follows: forward 5'-AGGAACCTGTGTGACCCTCA-3', reverse 5'-CGAGGTGGTCCGGGTATCC-3'. Real-Time Ready probes (Roche Diagnostics, Assay ID: 143636, Config. No. 100069730) were used to amplify β-actin in each sample. Expression levels of eNOS were calculated using the 2^-ΔCt (ΔCt = Target Gene – Reference Gene) formula. β-actin was used as a reference gene.

Determination of Vascular Quality by Immunohistochemistry

Placentas and umbilical cords were fixed with formalin and embedded with paraffin, then sections were cut 4-5 µm thick. AGE, RAGE, occluding, and β-catenin in the placenta and umbilical cord tissues were determined by immunostaining. Placenta and umbilical cord sections were incubated with the primary antibodies against RAGE (Abcam; ab3611), AGE (Abcam; ab23722), Occludin (Abcam; ab31721), and β-catenin (Abcam; ab1605). After washing with DPBS, Biotinylated Goat Anti-Polyvalent, Streptavidin peroxidase, and AEC Chromogen were applied. The negative controls were sections that were treated with the same protocol but without the primary antibody. Immunohistochemical staining assessment was performed blindly. Immunohistochemically positive stained areas were scored by two pathologists by semi-quantitative method (0: no staining, 1: weak, 2: moderate, 3: high).

Vascular Smooth Muscle Cell Proliferation

EdU cell proliferation assay was performed to determine VSMC proliferation. VSMCs were seeded in 8-well slides (Ibidi, ibiTreat) at a density of 1.5 × 10^4cells/well and cultured in presence of EdU (Click-iT® EdU Alexa Fluor® 488 Imaging Kit, Invitrogen) with, a final concentration 1µl/mL for 5 days and fluorescently labeled for EdU after fixation in 4 % paraformaldehyde. Fluorescent images were obtained by fluorescence microscopy from 6 different areas of each sample and EdU positive cell number/total cell number was determined by counterstaining all cell nuclei with Hoechst. The proliferation index was analyzed according to the following formula:Proliferation Index=(EdU positive cell number/total cell number)x100

ELISA for Determination of Ages in the Umbilical Cord Blood and Umbilical Cord Collagen

Determination of the average level of AGEs in the umbilical cord blood and the accumulation number of AGEs in the umbilical cord collagen was performed by ELISA. The number of AGEs in the umbilical cord blood and the umbilical cord collagen were determined with OxiSelect™ Advanced Glycation End Product (AGE) Competitive ELISA Kit (Cell Biolabs, STA-317), respectively, according to manufacturers' instructions.

Statistical Analysis

The sample size of the present study was determined considering a power of 80% to detect the effect of a given test at the desired level of significance (based on a two-tailed alpha level of 0.05). Values for clinical parameters and *in vitro* assays were given as mean ± SD. Statistics were performed with the number of different biological samples and corresponding cell cultures with 2–4 replicates per experiment. Student's unpaired t-test and Fisher's Exact test were applied. JMP Start Statistics version 9 (Statistical Discovery Software SAS Institute, Cary, NC, USA) was used for analysis. $p<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics for GDM and normoglycemic control groups are summarized in (Table 1). There were slight differences identified in maternal ages and gestational weeks at partum between GDM (n=12) and NGC group (n=10) ($p<0.05$). However, there were no significant differences in newborn weight and APGAR score between the two groups ($p>0.05$).

Effects of Maternal Diabetes on the Proliferation of VSMC

The proliferation of diabetic VSMC was higher than control VSMC cultured in DMEM-F12 with 10% FBS (p<0.001) (Figure 1). Both EdU positive and Hoechst-stained total nuclei numbers were counted and (EdU positive cell number/total cell number)x100 was calculated as the proliferation index.

Table 1. Comparison parameters between groups

Parameters	GDM (n=12) Mean±SD	NGC (n=10) Mean±SD	Statistical significance
Maternal age, years	35.83±4.43	29.80±4.48	p<0.05*
Gestational weeks at partum	37.30±0.35	38.70±0.39	p<0.05*
Newborn weight, g	3077±129	3166±135	p>0.05
APGAR score	7.90±0.25	8.20±0.27	p>0.05
AGEs in umbilical cord blood (µg/mL)	8.53±1.84	7.70±1.25	p>0.05
AGEs in umbilical cord collagen (µg/mg)	89.4±29.1	79.7±24.2	p>0.05

Abbreviations: APGAR, appearance, pulse, grimace, activity, and respiration; AGEs, advanced glycation end products; GDM, gestational diabetes mellitus; NGC, normoglycemic control; SD, standard deviation; $p<0.05$* was considered statistically significant

Maternal Diabetes Induces Oxidative Stress in HUVECs

The average ROS of HUVECs was significantly increased in the GDM (n=12) compared to the NGC (n=10) group ($p<0.05$) (Figure 2A).

Effects of Maternal Diabetes on the Mitochondrial Mass of HUVEC

The average mitochondrial mass of HUVECs was determined in the GDM (n=12) and NGC (n=10) group. There were no significant differences identified between groups ($p>0.05$) (Figure 2B).

Effects of Maternal Diabetes on the Expression of eNOS in HUVECs

Quantitative real-time PCR analysis revealed that eNOS mRNA levels were significantly increased in the GDM (n=12) compared to the NGC (n=10) group ($p<0.001$) (Figure 2C).

Effects of Maternal Diabetes on the Accumulation of AGEs in Umbilical Cord

Blood and Umbilical Cord Collagen

The result of ELISA showed that there were no differences in terms of the average amount of AGEs in umbilical cord blood and umbilical cord collagen of GDM (n=12) and NGC (n=10) ($p>0.05$) (Table 1).

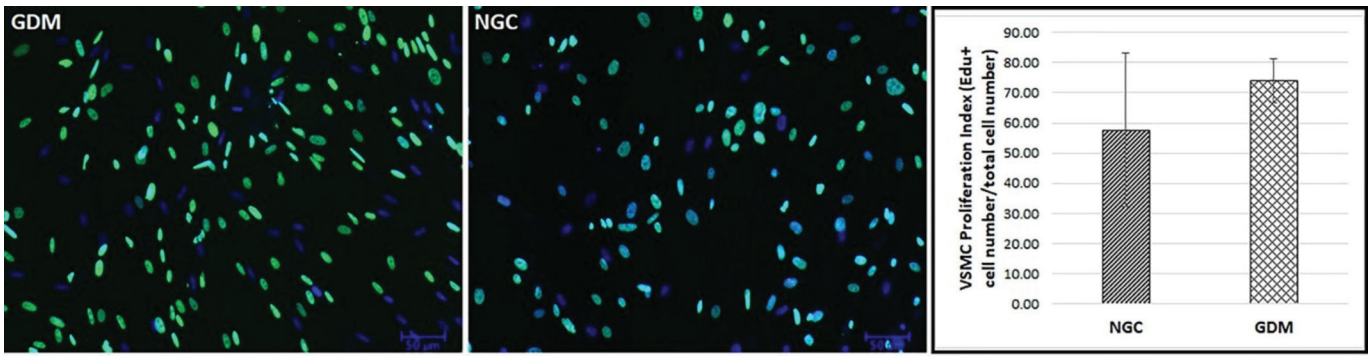


Figure 1. Proliferation index of VSMCs. Cell nuclei were stained for EdU and with Hoechst (Green: EdU positive proliferating cells, Blue: Hoechst stained nuclei). The proliferation index(EdU positive cell number/total cell number)x100 of VSMCs from the GDM group was determined to be significantly higher than the NGC group (p<0.05). Scale bars: 50 µm

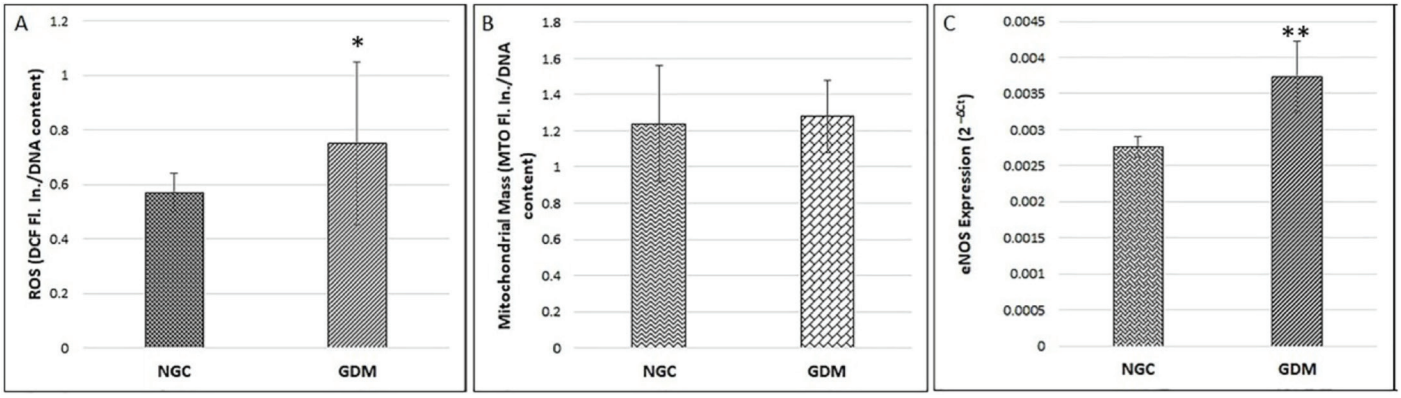


Figure 2. Comparison of ROS production, mitochondrial mass, and eNOS expression in HUVECs between groups. A. ROS production (DCF fluorescence intensity/DNA content), B. Mitochondrial mass (Mitotracker Orange fluorescence intensity/ DNA content), C. eNOS expression (2 −ΔCt). ROS production and eNOS expression were found to be significantly higher in HUVECs from the GDM group (*$p<0.05$ and **$p<0.001$, respectively)

Effects of Maternal Diabetes on the Expression of AGE, RAGE, and Vascular Junctional Proteins of Occludin and β-Catenin in the Placenta and Umbilical Cord

Immunohistochemical analysis showed that AGEs and RAGE (Figure 3) are localized in endothelial cells (shown by the arrows). The immunostaining positivity to AGEs and RAGE was present in endothelial cells of blood vessels in the chorionic villi of the placentas and umbilical cord. Higher immunohistochemical staining to AGEs was found in endothelial cells of umbilical veins in GDM ($p<0.05$). However, no significant differences were identified between the two groups concerning immunohistochemical positivity to RAGE (Table 2). Analysis of adherence and tight junctional proteins showed that β-catenin and occludin were immunolocalized to endothelial paracellular clefts of the placental and umbilical cord arteries and veins in both groups (shown by the arrows) (Figure 3). The result of the analysis showed a slight decrease in immunostaining for β-catenin and occludin in GDM. However, there were no significant differences identified in terms of immunohistochemical positivity to β-catenin and occludin between groups ($p>0.05$) (Table 2).

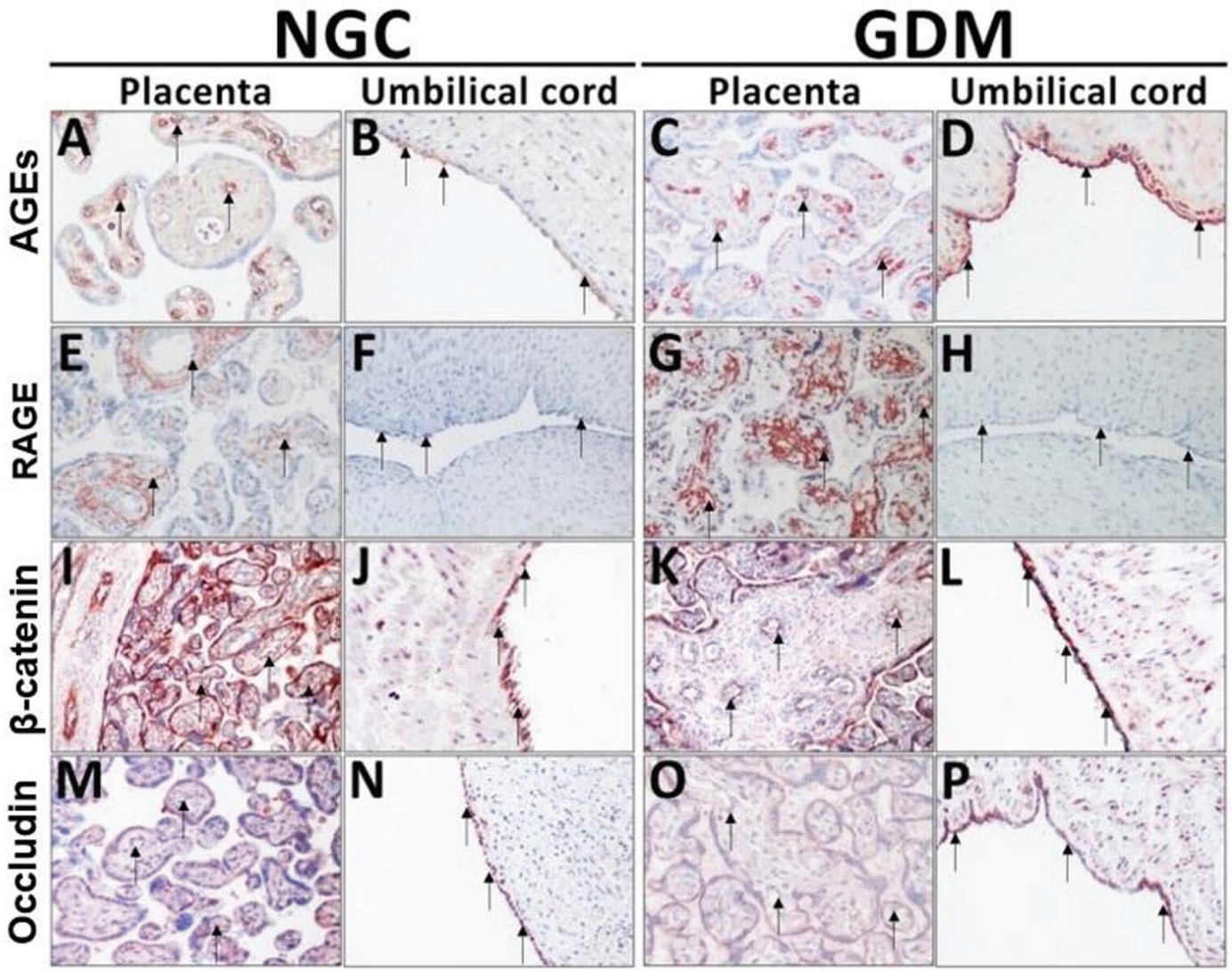
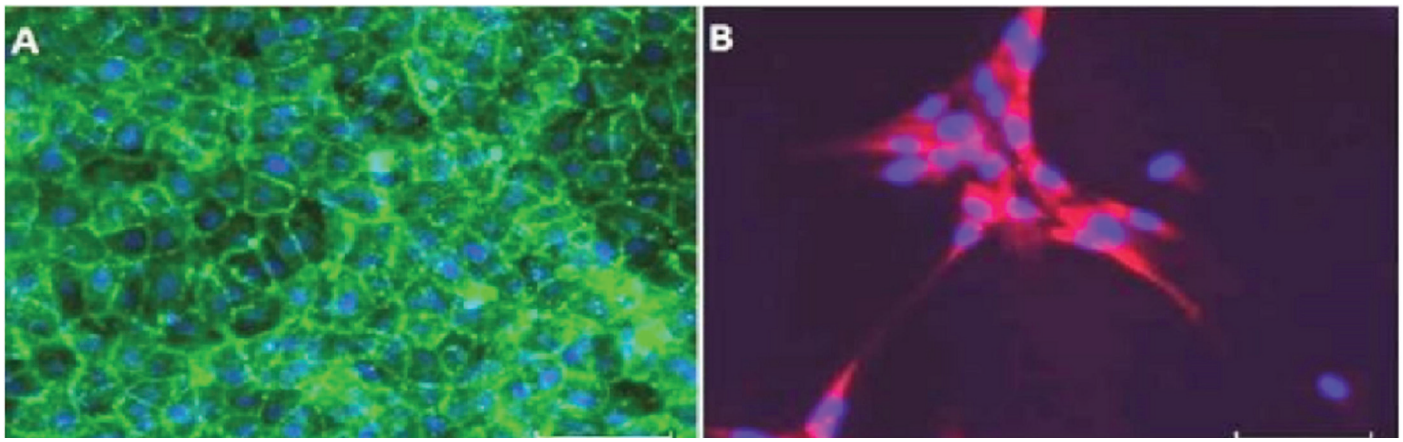


Figure 3. Representative immunohistochemical staining image of AGEs, RAGE, β-catenin, and Occludin in the placenta and umbilical cords. Anti-AGEs, RAGE, β-catenin, and Occludin immunohistochemical staining in endothelial cells of blood vessels in the chorionic villi of the placentas (A, E, I, M) and umbilical cord (B, F, J, N) in NGC (shown by arrow). Anti-AGEs, RAGE, β-catenin, and Occludin in endothelial cells of blood vessels in the chorionic villi of the placentas (C, G, K, O) and umbilical cord (D, H, L, P) in GDM (shown by arrow), respectively. Immunohistochemically positive stained areas were scored by the semi-quantitative method (0: no staining, 1: weak, 2: moderate, 3: high). All panels, 200× magnification

Table 2. Immunohistochemical staining data of the two groups

Variable		GDM group	NGC group	Statistical significance
Occludin staining (placenta)	≥ 1+ (positive)	8 (67%)	7 (78%)	p>0.05
	0 (negative)	4 (33%)	2 (22%)	
Occludin staining (artery)	≥ 1+ (positive)	9 (75%)	5 (63%)	p>0.05
	0 (negative)	3 (25%)	3 (37%)	
Occludin staining (vein)	≥ 1+ (positive)	9 (75%)	6 (67%)	p>0.05
	0 (negative)	3 (25%)	3 (33%)	
β-catenin staining (placenta)	≥ 1+ (positive)	9 (75%)	9 (100%)	p>0.05
	0 (negative)	3 (25%)	0 (0%)	
β-catenin staining (artery)	≥ 1+ (positive)	1 (75%)	6 (75%)	p>0.05
	0 (negative)	10 (25%)	2 (25%)	
β-catenin staining (vein)	≥ 1+ (positive)	3 (75%)	2 (67%)	p>0.05
	0 (negative)	8 (25%)	7 (33%)	
AGEs staining (placenta)	≥ 1+ (positive)	7 (70%)	5 (71%)	p>0.05
	0 (negative)	3 (30%)	2 (29%)	
AGEs staining (artery)	≥ 1+ (positive)	3 (25%)	0 (0%)	p>0.05
	0 (negative)	9 (75%)	8 (100%)	
AGEs staining (vein)	≥ 1+ (positive)	6 (55%)	0 (0%)	*p<0.05
	0 (negative)	5 (45%)	8 (100%)	
RAGE staining (placenta)	≥ 1+ (positive)	7 (70%)	5 (56%)	p>0.05
	0 (negative)	3 (30%)	4 (44%)	
RAGE staining (artery)	≥ 1+ (positive)	3 (33%)	4 (50%)	p>0.05
	0 (negative)	6 (67%)	4 (50%)	
RAGE staining (vein)	≥ 1+ (positive)	1 (11%)	1 (11%)	p>0.05
	0 (negative)	8 (89%)	8 (89%)	

Abbreviations: AGEs, advanced glycation end products; RAGE, the receptor for advanced glycation end products; GDM, gestational diabetes mellitus; NGC, normoglycemic control; *p<0.05 was considered statistically significant



Supplementary Figure. Fluorescence micrographs of HUVECs and VSMCs: A. HUVECs immunostained for CD31, B. VSMCs immunostained for α-SMA. Scale bar: 50 μm

HUVEC and VSMC morphology and culture purity

The morphology and purity of HUVECs and VSMCs were assessed through fluorescence microscopy. All cells in the HUVEC culture were positive for CD31 and all cells in the VSMC culture stained positive for α-SMA in both groups (Supplementary Figure).

DISCUSSION

We demonstrated in the present study that AGEs deposition was substantial within the umbilical cord vein of GDM women. It is supposed that maternal hyperglycemia-induced AGEs accumulation in the umbilical vein either takes place due to fluctuations in blood glucose level despite the insulin treatment or diet control applied afterward (17). In the present study, ROS production in HUVECs was identified as significantly higher in GDM. Our result of increased ROS production in response to hyperglycemia in GDM was consistent with previous reports (18). We also found that umbilical vein-derived VSMCs proliferation was considerably higher in GDM. Elevated ROS may be related to the proliferation and migration of VSMCs. This is parallel with recent studies that ROS promotes VSMCs growth by inducing autologous/paracrine growth mechanisms (19). In addition, ROS mediates the proliferation effect of hormones and growth factors on VSMCs (20). VSMC proliferation is associated with the pathogenesis of DM (21) and atherosclerotic CVD (22).

Moreover, in the current study, increased eNOS expression in HUVECs was identified as markedly different in GDM. ROS elevates the expression of eNOS through posttranscriptional and posttranslational modifications (23). Higher expression in eNOS in HUVECs associated with type 2 DM (24).

From another point of view, in the present study, there were indicators of oxidative stress such as elevated ROS generation and AGEs deposition in the tissue of the GDM mothers. Kostopoulou et al. showed that oxidative stress leads to the development of DM and its complications (25). Cristian E. indicated that GDM exposes the placenta to a hypoxic environment that would disturb vascular function on account of persistently increased oxidative stress. He suggested that hyperglycemia supports this pro-oxidant environment and leads to endothelial dysfunction. Regulation of vascular tone by endothelial cells could be distorted in favor of vasoconstriction and further tissue hypoxia. Continued hyperglycemia probably damages blood vessels and forces β-cells to secrete insulin intensively, causing metabolic and vascular disorders that predispose the mother to CVD in the long run (26).

Furthermore, we investigated whether GDM leads to alterations in the expression of tight and adherent junctional molecules involved in endothelial barrier function and angiogenesis (11). In this study, we found that there is a propensity for reduction of junctional protein expression. However, no significant decrease was observed. We suggest that three-dimensional visualization techniques may be required to confirm enhanced angiogenesis in the GDM placentae.

We characterized levels of RAGE protein expression in the umbilical vein during pregnancy. Interestingly, no difference was identified in the RAGE protein expression levels. Nevertheless, our results confirmed that there was a significant deposition of AGEs in an umbilical vein in GDM.

We could conclude that increased oxidative stress generated from ROS production and AGEs deposition probably elevates the predisposition to vascular endothelial dysfunction of the GDM mother in the long run.

Our study has been limited to a small number of participants. Thus, a high number of participants will result in a more sensitive analysis and more statistically significant conclusive results. Detailed evaluation of oxidative stress in GDM mothers would provide new targets for future research in the prevention of DM or CVD.

CONCLUSION

GDM mothers were under oxidative stress status when compared to the control group. In addition, high eNOS production by HUVECs and increase in proliferative capacity of VSMCs at time of delivery are indicative of a deepening of oxidative stress. Thus, there is a tendency to endothelial dysfunction and the probability of having CVD or type 2 DM increases in mothers with GDM in the long run.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *This study was conducted by the Declaration of Helsinki. Approval for the study was granted by the Local Ethics Committee of Kocaeli University.*

Authorship contributions: *WA: conceptualization, formal analysis, investigation, methodology, supervision, writing -original draft, writing - review & editing. CYO: data curation, formal analysis, investigation, methodology, validation, visualization, and writing. GKM, ED, VK, BM, and BO: data curation, formal analysis, investigation, methodology, validation, and visualization. ZC, BC, and IT: data curation, formal analysis, investigation, methodology, validation, visualization. HK: data curation, formal analysis, investigation, methodology, validation, visualization, writing-review & editing.*

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Research Article

The Effect of Rehabilitation without Specific Cognitive Rehabilitation on the Improvement of Cognitive Functions in Stroke Patients: Evaluation with Risk Factors

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Abstract

Aim: This study aimed to evaluate if rehabilitation without specific cognitive rehabilitation improved cognitive functions in patients who had suffered a stroke more than 1 year ago, and to correlate this finding with risk factors.

Material and Methods: Thirty stroke patients were included in the study. A rehabilitation program was administered to the patients for a total of 30 sessions, 5 days a week. In addition, demographic data of the patients were collected, as well as several risk factors that may impair their cognitive function. The pre-and post-treatment cognitive function of the patients was evaluated using mini-mental state examination (MMSE) and functional independence measure (FIM)-cognitive. With the FIM cognitive evaluation, cognitive functions such as comprehension, expression, social interaction, problem solving, and memory were evaluated. With MMSE, from cognitive functions; orientation, registration, attention and calculation, recall, language, and praxis were evaluated. Pre- and post-treatment motor function was measured by the Brunnstrom motor recovery stage (BMRS). Pre- and post-treatment walking ability was assessed with Functional Ambulation Categories (FAC). Along with the general comparison of cognitive function pre- and post-treatment, additional pre- and post-treatment comparisons were made according to risk factors.

Results: According to MMSE and FIM-cognitive scores, improvement in cognitive function was detected following treatment ($p<0.001$, $p=0.001$, respectively). There was no statistical improvement in FAC and BMRS scores. According to MMSE, cognitive functions were more impaired before treatment in women, those with <5 years of education, and those with aphasia ($p=0.025$, $p=0.004$, $p=0.002$, respectively). According to FIM-cognitive, cognitive functions were lower in patients with aphasia, and those with left-sided brain damage ($p=0.002$, $p=0.045$, respectively). There was no difference in the magnitude of improvement between the risk factors.

Conclusion: This study showed that the rehabilitation program applied without a specific cognitive rehabilitation program in patients with chronic stroke can improve cognitive functions, although it does not cause a significant improvement compared to BMRS and FAC. Therefore, we believe that rehabilitation without specific cognitive rehabilitation will improve patients' daily activities and increase their participation in treatment.

Keywords: Stroke, cognitive impairment, rehabilitation

INTRODUCTION

Stroke is an important disease with a high mortality rate and long-term impairments in cases of survival (1). In addition, stroke results in not only physical disability but also post-stroke cognitive impairment (PSCI) in 1/3 of stroke survivors. The risk of developing cognitive impairment increases at least 5-8 times after stroke (2). Patients with PSCI are less likely to participate in rehabilitation programs. In addition to physical disability, people with cognitive disabilities show less improvement in physical functions. As a result, the patient's dependence on daily activities in life increases (3). Therefore, treatment of cognitive impairment is also essential.

Cognitive function is not restricted to a single domain. It encompasses a variety of domains such as attention, executive function, visuospatial ability, memory, and language (4). Cognitive rehabilitation includes compensatory, restorative, and educational interventions.

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Compensatory interventions aim to alleviate the patient's cognitive disability by facilitating their adaptation to the external environment through the use of aids and tools. Educational intervention entails informing family members about stroke and post-stroke cognitive impairment. This education provides information about the definition of a stroke, its management, and how the process will progress. Restorative interventions aim to directly restore impaired functions. These restorative interventions include domain-specific ones as well as interventions for generalized cognitive impairments. Aerobic exercises can be used to aid with generalized cognitive rehabilitation (5). However, domain-specific intervention is primarily performed by people who have received specialized education on this subject. Therefore, domain-specific interventions cannot be applied universally.

This study aims to determine whether cognitive functions improve in patients who have had a stroke over 1 year ago when only a neurologic rehabilitation program is applied without applying domain-specific cognitive rehabilitation and its relationship with certain risk factors.

MATERIAL AND METHOD

The prospective study was conducted at Giresun University Faculty of Medicine, Department of Physical Medicine and Rehabilitation between May 2019 and February 2020. The ethics approval for the study was obtained from the Ethics Committee of Giresun University with a decision number (2019/KAEK-55). All participants gave written informed consent and the study was performed following the 1964 Declaration of Helsinki.

G Power 3.1 software was used to calculate the required sample size. The effect size was calculated as 0.72 in the power analysis calculated according to the mean change in the FIM cognitive (6). Based on a power of 90% and a 5% level of significance, we calculated that the total sample size required was 23. Patients who had suffered a stroke more than one year ago were included in the study. The exclusion criteria were as follows: Systemic findings of conditions that may adversely affect the post-stroke rehabilitation program (such as cardiovascular, pulmonary) (n=4), psychiatric and cognitive problems before suffering a stroke (with patient records evaluated by psychiatrists and neurologists, and information obtained from patient relatives), severe communication difficulties (n=3), patients with severe communication-impairing aphasia (n=3), patients with neglect (n=0). A total of 40 patients were evaluated. Ten patients were excluded from the study due to the exclusion criteria. As a result, a total of 30 patients were assessed.

The demographic data (age, gender, duration of education), smoking status, presence of other diseases, duration from stroke onset to admission, type of stroke (hemorrhagic, ischemic), brain side affected, and presence of aphasia included in the study were recorded.

Upper extremity, hand, and lower extremity motor function measured by the Brunnstrom motor recovery stage (BMRS). Upper extremity BMRS includes 7 stages, hand, and lower extremity BMRS include 6 stages and higher stages demonstrate better recovery (7).

Walking ability was assessed with Functional Ambulation Categories (FAC). It assesses how much human support a participant requires when walking, with or without assistive devices, on a 6-point scale (0-5) (8).

The patient's cognitive functions were evaluated using a mini-mental state examination (MMSE). The MMSE has a maximum score of 30 points. With MMSE, from cognitive functions; orientation, registration, attention and calculation, recall, language, and praxis were evaluated (9).

Functional disability was assessed using the Functional Independence Measure (FIM). FIM consists of 2 parts, 13 motor items (FIM-motor) and 5 cognitive items (FIM-cognitive). Each item is assigned a point value between 1 and 7. A higher score indicates greater functional independence. With the FIM-cognitive evaluation, cognitive functions such as comprehension, expression, social interaction, problem solving, and memory were evaluated (10).

The rehabilitation program, prepared specifically for the patient by the physician, was administered by physiotherapists 5 days/per week under the physician's supervision. A total of 30 sessions of treatment were administered. The rehabilitation program includes a range of motion, neurophysiological, posture, balance-coordination, proprioceptive, stretching and relaxation, strengthening, breathing, and swallowing exercises, gait training, bladder and bowel training, and electrical stimulation. Apart from this, according to the necessity, occupational therapy was given by the occupational therapist, speech therapy was given by the speech therapist, and psychotherapy was given by the psychologists. Appropriate orthoses were prescribed to increase functionality, reduce spasticity, and maintain range of motion according to the needs of the patients.

After 30 sessions of treatment, the MMSE and FIM-cognitive tests for the patients were repeated.

Statistical Analysis

Statistical analysis was performed using SPSS version 23.0 (IBM Corporation). Continuous variables were expressed in mean ± standard deviation (SD) and median (interquartile range), while categorical variables were reported in terms of number and frequency. The assessment of normality was analyzed using the Shapiro-Wilk test. To compare quantitative data between the groups, the independent samples t-test or Mann-Whitney U test were employed according to the normality of data. To compare the pre and post-treatment data, the in-group paired sample t-test or Wilcoxon test was used according to the normality of data. The chi-square and Fisher's

exact tests were used to identify the significance of the relationships between categorical variables. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 30 patients who had suffered a stroke, 13 (43.3%) female and 17 (56.7%) male were included in the study. The patients' mean age was 63.80±12.14 years and the mean disease duration was 52.20±41.71 months. The other demographic and stroke-related data of the patients are given in Table 1.

The MMSE, FIM, BMRS, and FAC values of the patients before and after the treatment are shown in Table 2. These results showed statistically significant improvement after treatment in MMSE and FIM scores to pre-treatment. There was no statistical improvement in FAC and BMRS scores after treatment.

Pre- and post-treatment MMSE and FIM-cognitive scores according to several risk factors for PSCI are presented in Table 3.

According to these results, pre-treatment MMSE scores were lower in women, those with <5 years of education, and those with aphasia (p=0.025, p=0.004, p=0.002, respectively). However, there was no statistically significant difference between pre-treatment MMSE scores according to the presence of hypertension (HT), diabetes mellitus (DM), smoking, affected brain side, and stroke type. In addition, pre-treatment FIM-cognitive scores were lower in patients with aphasia and those with an impaired left hemisphere (p=0.002, p=0.045, respectively). However, while the pre-treatment FIM-cognitive scores were relatively lower in women and those with <5 years of education, this difference was not statistically significant (p=0.050, p=0.060). There was no statistically significant difference between pre-treatment MMSE scores according to the presence of HT, DM, smoking, and stroke type.

There was a statistically significant increase in MMSE scores after treatment in all risk factor groups (all p<0.05), except for patients without HT (p=0.118) (Table 3). In addition, FIM-cognitive scores were increased in all risk groups after treatment, except for those with >5 years of education, DM, and hemorrhagic SVO (p=0.060, p=0.071, p=0.072) (Table 3).

A comparison of treatment changes (Δ) of the MMSE and FIM-cognitive scores according to pre-treatment is presented in Table 4. There was no statistically significant difference between risk factors in terms of changes (Δ) in the MMSE and FIM-cognitive values after treatment (all p>0.05).

Table 1. Demographic and stroke-related data

		Stroke (n=30)		
		N (%)	Mean±SD	Median (IQR)
Age (years)			63.80±12.14	65.00 (11.00)
Gender	Female	13 (43.3)		
	Male	17 (56.7)		
BMI (kg/m²)			25.61±4.06	24.61 (4.84)
Marital status	Married	16 (53.3)		
	Single	14 (46.7)		
Duration of education	≤5 years	24 (80)		
	>5 years	6 (20)		
Smoker	Yes	6 (20)		
Diabetes mellitus	Yes	10 (33.3)		
Hypertension	Yes	24 (80)		
Aphasia	Yes	8 (26.7)		
Duration of stroke (months)			52.20±41.71	26.00 (58.00)
Type of stroke	Ischemic	24 (80)		
	Hemorrhagic	6 (20)		
Affected brain side	Left	17(56.7)		
	Right	13 (43.3)		
Lesion site	Frontal	23 (76.7)		
	Temporal	12 (40.0)		
	Parietal	26 (86.7)		
	Occipital	2 (6.7)		

BMI: Body mass index; SD: Standard deviation; IQR: Interquartile range, BMRS: Brunnstrom motor recovery stages

Table 2. Comparison of MMSE, FIM, BMRS, and FAC scores before and after treatment

	Pre-treatment		Post-treatment		P value[w]
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	
MMSE	17.17±6.38	18.00 (9.00)	19.40±6.48	21.50 (8.00)	<0.001
FIM-motor	61.73±24.87	64.00 (40.00)	65.47±24.59	69.00 (37.25)	<0.001
FIM-cognitive	27.03±6.22	29.00 (12.00)	28.27±5.44	30.00 (9.50)	0.001
FIM-total	88.67±28.70	92.00 (40.00)	93.97±27.08	96.50 (38.00)	<0.001
BMRS-upper extremity	4.30±1.82	4.00 (3.00)	4.37±1.81	4.50 (3.00)	0.157
BMRS-hand	4.07±1.64	5.00 (2.00)	4.13±1.66	5.00 (2.25)	0.157
BMRS-lower extremity	4.07±1.51	4.00 (2.25)	4.17±1.46	4.00 (2.25)	0.083
FAC	3.47±1.29	4.00 (1.25)	3.57±1.28	4.00 (2.00)	0.083

MMSE: Mini-mental state examination; FIM: Functional Independence Measure; SD: Standard deviation; IQR: Interquartile range; BMRS: Brunnstrom motor recovery stages; FAC: Functional Ambulation Classification [w]Wilcoxon test

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Table 3. Pre- and post-treatment MMSE and FIM-cognitive scores according to some risk factors for PSCI

	Δ MMSE					Δ FIM-Cognitive				
	Pre-Treatment		Post-Treatment		P_2 Value[m]	Pre-Treatment		Post-Treatment		P_2 Value[m]
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)		Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	
Gender										
Female (n=13)	14.23±7.14	13.00 (15.50)	16.77±7.89	18.00 (16.00)	***0.009***[p]	24.31±6.59	23.00 (11.50)	25.85±5.93	27.00 (11.00)	***0.006***[p]
Male (n=17)	19.41±4.81	20.00 (5.50)	21.41±4.42	22.00 (7.50)	***<0.00***[p]	29.12±5.18	31.00 (10.00)	30.12±4.34	31.00 (13.00)	***0.028***[w]
P_1 value	***0.025***[i]					*0.050*[m]				
Education										
≤5 years (n=24)	15.58±5.90	16.50 (7.00)	17.88±6.27	18.00 (9.00)	***<0.001***[w]	26.04±6.25	26.00 (11.00)	27.21±5.39	28.50 (10.50)	***0.004***[w]
>5 years (n=6)	23.50±3.99	23.00 (5.25)	25.50±2.59	25.00 (2.75)	***0.033***[p]	31.00±4.56	32.00 (7.50)	32.50±3.33	34.00 (5.75)	*0.060*[p]
P_1 value	***0.004***[i]					*0.060*[m]				
Smoker										
Yes (n=6)	19.33±6.41	19.00 (6.50)	21.67±5.75	22.00 (6.00)	***0.017***[p]	27.00±5.93	27.00 (12.00)	29.00±5.40	30.00 (10.00)	***0.042***[w]
No (n=24)	16.63±6.39	17.00 (9.00)	18.83±6.64	19.00 (8.00)	***<0.00***[w]	27.04±6.41	29.00 (11.75)	28.03±5.55	30.00 (10.50)	***0.006***[w]
P_1 value	*0.361*[i]					*0.938*[i]				
Hypertension										
Yes (n=24)	17.50±6.09	18.00 (8.25)	19.50±6.09	20.50 (7.50)	***<0.001***[p]	27.54±5.88	29.00 (11.50)	28.71±4.97	30.00 (8.25)	***0.004***[w]
No (n=6)	15.83±7.94	15.50 (15.50)	19.00±8.53	23.00 (15.00)	*0.118*[p]	25.00±7.69	25.00 (15.00)	26.50±7.29	27.00 (14.25)	***0.045***[w]
P_1 value	*0.536*[i]					*0.360*[m]				
Diabetes Mellitus										
Yes (n=10)	19.10±3.45	19.00 (5.25)	20.70±3.65	21.50 (5.50)	***0.006***[p]	29.50±5.25	31.50 (11.00)	30.70±3.62	32.00 (6.25)	*0.071*[w]
No (n=20)	16.20±7.32	16.50 (11.25)	18.75±7.51	20.00 (11.75)	***<0.001***[p]	25.80±6.41	26.00 (10.75)	27.05±5.85	28.50 (10.50)	***0.003***[p]
P_1 value	*0.151*[i]					*0.126*[i]				
Aphasia										
Yes (n=8)	11.50±6.44	11.50 (12.75)	13.25±7.03	14.50 (13.75)	***0.017***[p]	21.25±4.71	21.00 (4.50)	23.13±4.16	22.00 (6.00)	***0.011***[p]
No (n=22)	19.23±5.07	20.00 (6.00)	21.64±4.68	22.50 (7.00)	***<0.001***[p]	29.14±5.35	31.00 (10.25)	30.14±4.62	30.50 (6.25)	***0.011***[w]
P_1 value	***0.002***[i]					***0.002***[m]				
Affected brain side										
Left (n=17)	15.94±7.55	17.00 (12.00)	18.06±7.66	20.00 (12.50)	***0.004***[p]	24.94±6.58	23.00 (11.00)	26.24±5.76	27.00 (9.00)	***0.012***[p]
Right (n=13)	18.77±4.17	20.00 (7.50)	21.15±4.16	22.00 (7.50)	***<0.001***[p]	29.77±4.62	31.00 (7.50)	30.92±3.71	33.00 (5.00)	***0.010***[w]
P_1 value	*0.235*[i]					***0.045***[m]				
Stroke type										
Ischemic (n=24)	17.96±5.84	18.00 (8.25)	20.21±5.99	22.00 (6.75)	***<0.001***[w]	27.21±6.37	29.00 (11.50)	28.50±5.47	30.00 (8.25)	***0.004***[w]
Hemorrhagic (n=6)	15.00±8.00	13.00 (15.50)	16.17±7.94	14.50 (16.00)	***0.001***[p]	26.33±6.06	25.00 (11.50)	27.33±5.72	27.00 (11.50)	*0.072*[p]
P_1 value	*0.178*[i]					*0.620*[m]				

MMSE: Mini-mental state examination; FIM: Functional Independence Measure; SD: Standard deviation; IQR: Interquartile range; [i]independent samples t-test; [p]paired sample t test; [w]Wilcoxon test; [m]Mann Whitney U test; P_1Value=Pretreatment comparison between groups, P_2Value=Intra-group comparison of pre and post-treatment

Table 4. Comparison of treatment changes (Δ) of the MMSE and FIM-cognitive scores according to pre-treatment

	Δ MMSE		P Value[m]	Δ FIM-Cognitive		P Value[m]
	Δ MMSE	Δ MMSE		Mean±SD	Median (IQR)	
Gender						
Female (n=13)	2.54±2.96	2.00 (3.00)	0.932	1.54±1.66	1.00 (3.00)	0.490
Male (n=17)	2.00±1.50	2.00 (2.50)		1.00±1.70	1.00 (2.00)	
Education						
≤5 years (n=24)	2.29±2.37	2.00 (2.75)	0.979	1.17±1.74	1.00 (3.00)	0.650
>5 years (n=6)	2.00±1.67	2.50 (3.25)		1.50±1.52	1.50 (2.50)	
Smoker						
Yes (n=6)	2.33±1.63	2.50 (3.25)	0.541	2.00±1.41	2.00 (2.50)	0.165
No (n=24)	2.21±2.38	2.00 (3.00)		1.04±1.71	1.00 (2.50)	
Hypertension						
Yes (n=24)	2.00±1.50	2.00 (2.75)	0.915	1.17±1.76	1.00 (2.75)	0.689
No (n=6)	3.17±4.12	2.00 (5.75)		1.50±1.38	1.50 (3.00)	
Diabetes mellitus						
Yes (n=10)	1.60±1.43	1.50 (3.00)	0.311	1.20±1.81	0.50 (3.25)	0.602
No (n=20)	2.55±2.50	2.50 (2.75)		1.25±1.65	1.00 (2.75)	
Aphasia						
Yes (n=8)	1.75±1.58	2.00 (3.00)	0.581	1.88±1.55	2.00 (2.75)	0.246
No (n=22)	2.41±2.42	2.00 (2.25)		1.00±1.69	1.00 (2.00)	
Affected brain side						
Left (n=17)	2.12±2.64	2.00 (3.00)	0.230	1.29±1.90	1.00 (3.00)	0.730
Right (n=13)	2.39±1.61	3.00 (2.50)		1.15±1.41	1.00 (1.50)	
Stroke type						
Ischemic (n=24)	2.25±2.47	2.00 (3.00)	0.853	1.29±1.81	1.00 (3.00)	0.710
Hemorrhagic (n=6)	2.17±0.75	2.00 (1.25)		1.00±1.10	1.00 (1.50)	

MMSE: Mini-mental state examination; FIM: Functional Independence Measure; SD: Standard deviation; IQR: Interquartile range; [m]Mann-Whitney U test

DISCUSSION

This study showed that although no improvement according to BMRS and FAC staging, there was a significant improvement in cognitive functions as a result of a rehabilitation program without applying specific cognitive rehabilitation to chronic stroke patients. According to the MMSE and FIM-cognitive, cognitive impairment is higher in patients with aphasia. In addition, cognitive functions were more affected in those who had an education period of <5 years and females according to MMSE, and in those whose left side of the brain was affected according to FIM-cognitive.

Stroke is a risk factor for vascular dementia and Alzheimer's disease (11). Cognitive functions can be impacted at different levels following a stroke, and improvement of these functions is observed most rapidly during the first three months. However, studies have shown a greater improvement in these functions in patients with good cognitive functions following acute stroke (12). It is important to have good cognitive functions to improve patients' quality of life, motor levels, and functionality, and to reduce the risk of falling (13,14). In this direction, cognitive functions and their level of functioning should be incorporated into the treatment plan so that patients can return to normal life sooner.

In a meta-analysis of stroke patients who did and did not receive a cognitive rehabilitation program, it was shown that those who received a cognitive rehabilitation program experienced fewer mental problems immediately after treatment than those who did not. However, it has been reported that there is no long-term effect. This effect was found to be small to moderate in magnitude. However, there is no evidence that its impact is sustained long-term (15). Another meta-analysis published on attention, one of the cognitive functions, showed that cognitive rehabilitation did not affect subjective measures of attention in stroke patients, either in the short- or long term. Stroke patients who received cognitive rehabilitation demonstrated an improvement in measures of divided attention immediately after treatment as compared with control. However, it is not clear whether this effect persists to a long-term follow-up. Additionally, there is no

evidence that cognitive rehabilitation has an immediate or permanent impact on alertness, selective attention, and sustained attention (16). In our study, subgroups of cognitive functions in stroke patients were not evaluated in detail. As in previous studies, evaluation was made through the MMSE and FIM-cognitive, which assess the subgroups of cognitions generally. Accordingly, when a rehabilitation program is applied without specific cognitive rehabilitation, a statistical improvement was detected in the cognitive functions immediately following treatment.

A meta-analysis showed that physical exercise improved cognitive functions in people over 50 years of age independently from baseline cognitive function. In particular, it is suggested that aerobic and resistant moderate-intensity exercises should be performed several days a week (17). According to the results of this study, we think that the exercise (aerobic, balance-coordination, posture, neurophysiological, etc.) performed by stroke patients may improve their cognitive functions.

Numerous risk factors have been identified that may affect post-stroke cognitive impairment, including age, gender, education level, HT, DM, hyperlipidemia, atrial fibrillation, smoking, and the presence of aphasia (18-21).

Language is a critical component of cognition (22). In patients with aphasia, not only linguistic but also non-linguistic cognitive functions may be impaired (23-25). In our study, MMSE and FIM-cognitive scores were lower in patients with aphasia than those without. However, both the MMSE and FIM-cognitive scores improved significantly following the rehabilitation program in patients with and without aphasia. There was no difference between the two groups in terms of the level of improvement.

A study investigating risk factors for cognitive dysfunction in patients who had experienced a stroke showed that there is a decrease in global cognition as a result of both ischemic and hemorrhagic strokes. However, among ischemic strokes, thromboembolic strokes carry the highest risk in terms of global cognition disorder (18). In our study, no difference was found between patients who have suffered an ischemic or hemorrhagic stroke in terms of impaired cognitive function. However, ischemic strokes have not been compared according to their etiology.

There is evidence that people with left hemisphere involvement experience non-linguistic cognitive impairments to skills such as attention, working memory, and executive functions in addition to aphasia. In addition, those with right hemisphere involvement may exhibit a certain degree of attention and visual-spatial recognition (25). A study demonstrated that left hemisphere involvement has a greater effect on cognitive functions (26). On the other hand, our study found that FIM-cognitive scores were lower in patients with left hemisphere involvement. While MMSE scores were relatively lower in patients with left hemisphere involvement, there was no statistically significant difference. After treatment, improvement in cognitive functions was detected in both patients with left and right hemisphere involvement. However, there was no difference in the magnitude of improvement between the groups.

When other risk factors affecting cognitive functions were evaluated, the MMSE and FIM-cognitive scores were lower in female stroke patients in our study. Similar studies to ours indicate that cognitive functions are affected more in women (20,21). On the contrary, a study shows that cognitive function is more adversely affected in men (18). Considering patients' duration of education, in this study, MMSE scores were lower in those with ≤5 years of education. Although it was not statistically significant, the FIM-cognitive score was relatively lower in those with ≤5 years of education. Similar to our findings, it has been reported in the literature that cognitive functions are more affected in individuals with lower education levels (18,21,26,27). However, we believe that the test results evaluating the cognitive functions of people with low education levels before their stroke may also have been low. Some studies' data indicate that smoking (20,28), HT (18,20,28,29), and DM (21,27,29) may also be risk factors for the development of PSCI. However, our study revealed no difference between smokers and non-smokers, or between those with and without HT or DM. Although cognitive functions improved after 1 month of treatment in all groups, only the MMSE scores of those without HT and the FIM cognitive scores of those with DM and >5 years of education were statistically insignificant. There was no difference between the groups in terms of the level of improvement in any of the groups.

There were some limitations in our study. First of all, cognitive dysfunction manifests itself in various ways. We made evaluations using MMSE and FIM-cognitive, which are used frequently in other investigations. Although the patients included in the study were chronic patients for longer than 12 months, it would be better to compare this improvement with the patients who received a specific cognitive rehabilitation program to determine whether this improvement is due to the natural course of the disease or the treatment administered. However, we think that spontaneous neurological recovery is less after 12 months of ongoing chronic stroke.

CONCLUSION

As a result, domain-specific cognitive rehabilitation cannot be applied in every center, and we believe that a rehabilitation program without specific cognitive rehabilitation can improve the cognitive functions of individuals regardless of risk factors. Although there is no improvement according to BMRS and FAC staging, rehabilitation to be applied in patients with chronic stroke will improve the cognitive functions and functional independence of the patients. Improvements in these cognitive functions will encourage people to participate in treatment and carry out their daily activities.

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Research Article

Comparison of Feature Selection Methods in Breast Cancer Microarray Data

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Abstract

Aim: We aim to predict metastasis in breast cancer patients with tree-based conventional machine learning algorithms and to observe which feature selection methods is more effective in machine learning methods related to microarray breast cancer data reducing the number of features.

Material and Methods: Feature selection methods, least squares absolute shrinkage (LASSO), Boruta and maximum relevance-minimum redundancy (MRMR) and statistical preprocessing steps were first applied before the tree-based learning conventional machine learning methods like Decision-tree, Extremely randomized trees and Gradient Boosting Tree applied on the microarray breast cancer data.

Results: Microarray data with 54675 features (202 (101/101 breast cancer patients with/without metastases)) was first reduced to 235 features, then the feature selection algorithms were applied and the most important features were found with tree-based machine learning algorithms. It was observed that the highest recall and F-measure values were obtained from the XGBoost method and the highest precision value was received from the Extra-tree method. The 10 arrays out of 54675 with the highest variable importance were listed.

Conclusion: The most accurate results were obtained from the statistical preprocessed data for the XGBoost and Extra-trees machine learning algorithms. Statistical and microarray preprocessing steps would be enough in machine learning analysis of microarray data in breast cancer metastases predictions.

Keywords: Microarray, breast cancer, metastasis, machine learning, feature selection

INTRODUCTION

Cancer can be defined as a disease with uncontrolled cell growth, metastasizing and attacking other tissues (1). After non-melanoma skin cancer, breast cancer is the 2nd common cancer for women in worldwide (2) and it is known as the primary cancer among women (3). In addition to that this is also a big problem for patients who are diagnosed with cancer and recover, so Bahceli and Kucuk (4) showed that fear of cancer recurrence is high in women with breast cancer in Turkey. Metastasis is a process in which cancer cells disperse from the primary tumor site and spread from there to different parts of the body (5). The majority of breast cancer deaths are due to breast cancer metastases (6). Thereby, predicting whether metastasis would occur or not is important in terms of taking precautions.

DNA Microarray technology is an old but effective method and results obtained from microarray analysis are robust, since it has been possible to calculate the thousands of genes simultaneously with microarray technology (7). With DNA microarray technology, large microarray data of gene expression have begun to be produced and this data has been used for the discovery and classification of diseases (1). In addition, cancer studies with microarray data have been carried out for a long time; Dhanasekaran et al. (8) studied prostat cancer on microarray data and they successfully classified the metastatic prostate, normal prostate and localized prostate cancer. Chang et al.

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(9) demonstrated the using cDNA microarrays to identify arrays involved in transformation in oral cancer. van't Veer et al. correctly predicted the output of the breast cancer disease for 65 patients out of 75 patients. In addition to these studies, many machine learning (ML) researches with microarrays to determine breast cancer have been published recently. Paksoy and Yangin (23) predicted the colon cancer on microarray data. Pirooznia et al. (10) first applied feature selection (FS) algorithms, such as correlation FS, support vector machine recursive feature elimination and chi squared methods, after that, they run ML models on selected featured data and compared the results. Cho and Won (11) predict and diagnose cancer on microarray data with ML algorithms after applying signal to noise ratio FS algorithms, correlation coefficients, Euclidean distance and information gain. Alagukumar and Kathirvalavakumar (12) applied FS algorithms, like Welch test, ANOVA, Wilcoxon test, Kruskal–Wallis, LIMMA, and F-test to extract the microarray genes and proposed classifier. Lonith (13) proposed principal component analysis used to decrease the number of features on microarray data for breast and liver cancer and particle swarm optimization to increase the ML algorithms accuracy. Mod et al (14) proposed some hybrid FS algorithms whale optimization algorithm, grey wolf optimization, gravitational search algorithm, cuckoo search algorithm, firefly algorithm, artificial bee colony optimization and particle swarm optimization for the breast cancer microarray data.

As briefly explained in the literature review, microarray data includes huge number of genes with very small observations (n<<p), so FS methods gain importance in microarray data. FS is one of the key steps of the ML algorithm, because the dataset that best expresses the output will come from the best features. In this study, we try to improve the metastasis prediction accuracy with the latest FS algorithms and proposed best arrays for the future studies and compare the FS methods on breast cancer microarray data. For this reason, we applied three different FS algorithms such as LASSO (15), boruta (16) and MRMR (17,18) and statistical method as preprocessing microarray analysis.

MATERIAL AND METHOD

In this study, as represented in flowchart of Figure 1, two different data GSE102484 and GSE20685 were first downloaded.The datasets can be found in NCBI Geo Databank. After that, we combined the data by using R programming language 4.1.1. Both datasets includes breast cancer patients with metastasis (label-1) and non-metastasis (label-0). We excluded outliers (3 standard deviations away from the mean) and missing observations and normalized the variables in the Microarray Preprocessing step. After that, we have 54675 features and 202 observations/patients (101 metastasis and 101 non-metastasis). Since the number of features are less than the observations (n<<p), we applied statistical data preprocessing (Statistical FS) analysis to reduce the number of features. In this part, we selected the features that were well distinguished by classes and we call it histogram differences method. This method is briefly explained under the Histogram Differences part. We selected the 235 features after the statistical and microarray preprocessing steps and this is our original feature pool. We plotted the heatmap to see the correlation between the patient and gene expression sequences, as can be seen in Figure 2. According to heat map, there is an associations between patients and arrays, so the microarray data with 235 features is applicable to the ML classification problem. After Microarray and Statistical data analysis preprocessing, we had more feature than observation (n:202<p:235). Even this is applicable for the ML analysis, we can still apply feature selection methods in order to continue with the data set that is less and better describes the output. For this reason, finally, we executed the least absolute shrinkage and selection operator (LASSO), boruta and maximum relevance-minimum redundancy (MRMR) FS algorithms to minimize the dataset and compared the results.

Machine Learning Algorithms

Machine learning (ML) is a research topic in statistics and computer sciences that makes inferences from data by imitating the way human's learning. With the increasing amount of data and developments in computer science, the interest in ML has increased in recent years in health sciences. Tree-based algorithms are used in many ML related studies (19,20). In this study, we use tree-based conventional ML algorithms, Decision Trees, Extremely Randomized Trees and Gradient Boosting Trees, since they give the variable importance of the model. Decision Trees (DT) is the first and simplest version of tree-based models that make decisions using leaves and nodes. Extremely Randomized Trees (Extra-Trees) (21), also known as Extra-Trees is a kind of ensemble machine learning method that is similar to random forest model. Gradient Boosting Tree (XGBoost) (22) is used in both regression and classification algorithms like the other algorithms that used in this study and it is an advanced learning ensemble method that uses progressively improved predictions to obtain a final prediction result.

Feature Selection (FS) Algorithms

Feature selection algorithms can be divided into 3 categories as Filter, Wrapper and Embedded. We tried to select different feature selection algorithms from the different part of the category such as LASSO (embedded), boruta (wrapper) and MRMR (filter).

Histogram Differences

This method simply selects the features that is well distinguished by classes and we call this method histogram differences method in this study. To do that, we followed the following steps;

I) min and max values of the features were calculated in the entire data set. It was observed that the min-max

range of the features are between 1.9732-14.3942.

II) Range between 0 to 15 was chosen to keep the histograms in the standard range and 100 was selected as the number of bins.

III) Two histograms were extracted for each feature, one with a Y value of label-1 and the other with Y value of label-0.

IV) These two histograms for each feature were subtracted from each other, the absolute value was taken and divided by the number of samples.

V) The sum of the two histogram differences was converted into a score, and the distribution of scores for all features was examined. A certain threshold value was selected (3 in this study) and the features higher than 3 were selected.

Least absolute shrinkage and selection operator (LASSO)

LASSO (15) is a regression technic that can be used in both variable selection and regularization by using the following loss formula: (1)

$$\text{Loss}=\sum_{i=1}^{N}|y_i-\sum_j\beta_j x_{ij}|^2+\lambda\sum_j|\beta_j|$$

where λ is numerical value between 0 and 1, x is input, y is output. LASSO gives better results as feature selection method when there are few observations and too many variables. LASSO feature selection ensures that unrelated variables are removed from the model by making their coefficients zero (24,25).

Boruta

Boruta FS (16) is an ensemble feature selection algorithm which uses random forest classifier and it was first developed as a package of R-programming language. Boruta algorithm uses a Random Forest (26) classifier-based wrapper approach for robust feature selection method (27).

Maximum Relevance - Minimum Redundancy (MRMR)

MRMR was first defined by Peng et al. (17) as a embedded feature selection algorithm based on mutual informations with the following formula; (2)

$$max[\frac{1}{|S|}\sum_{x_i\in S}I(x_i;C)-\frac{1}{|S|^2}\sum_{x_i\in S}\sum_{x_j\in S}(I(x_i;x_j))]$$

where x_i is the mth feature in subset S. Since the MRMR method is fast and effective, it has been studied extensively. Zhao et al. (18) determined FCQ-MRMR method which uses the F-score to measure the relevance and calculate the correlation between features to measure redundancy as represented the formula below (3).

$$f_{FCQ}=F(X_i,y)/\frac{1}{|S|}\sum_{X_s\in S}\rho(X_s,X_i)$$

Where F is the F-score between ith feature and response variable and ρ is the correlation between the features. Ding and Peng (28) applied the MRMR method on microarray data and compared the feature selected results and baseline features. They showed that MRMR outperform then the baseline features for both continuous and discrete datasets.

Accuracy Measures

In binary classification problems, we can show the accuracy values of ML methods by various methods. Since our dataset is balanced and we want to specify the success of true metastasis prediction, we only used F1, recall and precision measures as formulated below (4-6).

$$\text{Precision}=\frac{TP}{TP+FP},$$
$$\text{Recall}=\frac{TP}{TP+FN},$$
$$\text{F1}-\text{Score}=\frac{2*TP}{2*TP+FP+FN},$$

Where FP (false positive) represents the wrong prediction classes when the actual class is positive (metastasis), TP (true positive) shows true predicted classes, and FN (false negative) is the wrong prediction when the actual one is negative.

RESULTS

In this study, feature selection (FS) methods on microarray breast cancer data were used and tree-based conventional ML methods were executed to see the model prediction accuracy and variable importance. As can be seen in the flowchart in Figure 1, 54675 features are very high compared to the observations for the ML applications.

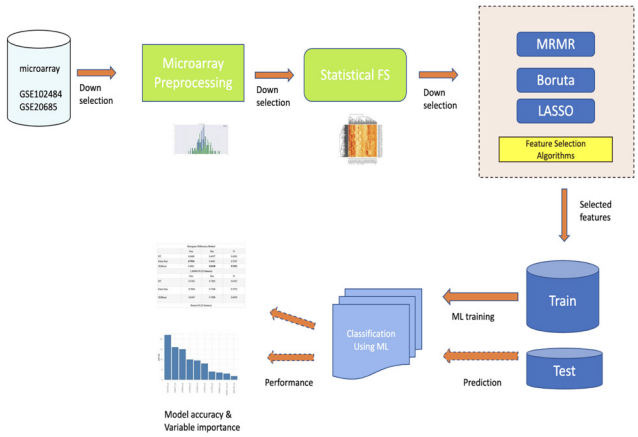

Figure 1. Flowchart of the methodology

Thereby, we applied microarray FS methods and statistical preprocessing method to the dataset and the number of features were reduced to 235 arrays. Afterwards, tree-based ML methods were applied to this dataset and it is observed that XGBoost is the best method with recall 0.8140 and F1-measure: 0.7423 on prediction metastasis, but we think that the accuracy values could be increased by selecting appropriate features from 235 datasets, since conventional ML algorithms work best with the less

Table 1. Cross validated accuracy measure results of tree-based machine learning algorithms on selected features

Histogram Differences Method			
	Prec	**Rec**	**F1**
DT	0.6069	0.6937	0.6505
Extra-Tree	**0.7934**	0.6941	0.7367
XGBoost	0.6801	**0.8140**	**0.7423**
LASSO FS (23 features)			
	Prec	**Rec**	**F1**
DT	0.5182	0.7482	0.6167
Extra-Tree	0.7054	0.7544	0.7332
XGBoost	0.6267	0.7498	0.6939
Boruta FS (22 features)			
	Prec	**Rec**	**F1**
DT	0.6198	0.6212	0.6156
Extra-Tree	0.6738	0.6170	0.6469
XGBoost	0.7531	0.5643	0.6416
MRMR FS (20 features)			
	Prec	**Rec**	**F1**
DT	0.4346	0.3810	0.4032
Extra-Tree	0.7509	0.5606	0.6428
XGBoost	0.6302	0.5608	0.617

Prec: precision; Rec: recall; F1:F1-score/measure

features. Therefore, we applied boruta, MRMR and LASSO feature selection methods to this dataset. Results are listed in Table 1 with precision, F1 and recall values, with the highest values shown in bold.

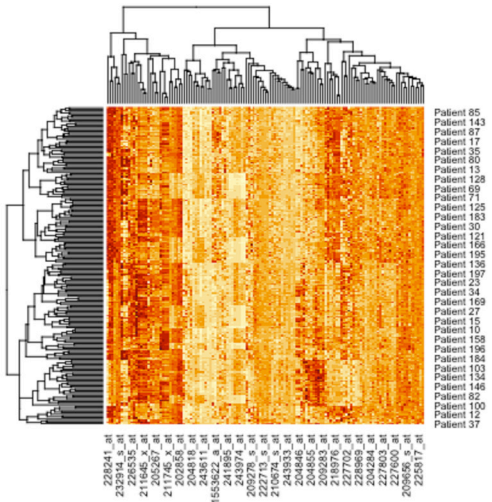

Figure 2. Heat map of the microarray data after preprocessing steps

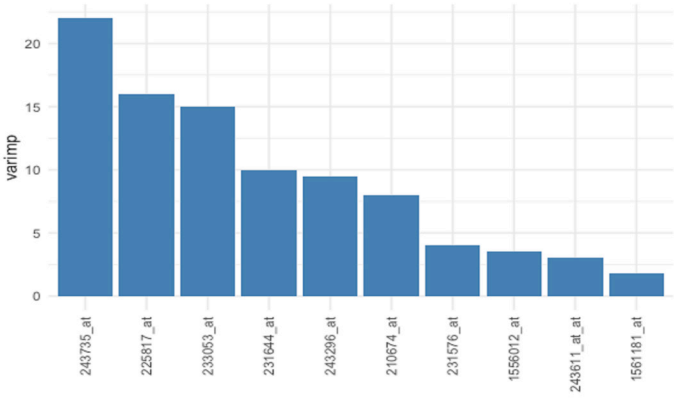

Figure 3. Variable importance of features selected from the Histogram Differences Method with XGBoost algoirthm

DISCUSSION

According to the Table 1, the highest value in precision was seen in Histogram Difference Method Extra-tree model as 0.7934, and the highest values in recall and F1 were obtained in XGBoost as 0.8140 and 0.7423, respectively. It is observed that the best results were coming from the

Histogram Difference method. So the top 10 arrays from this method is listed in Figure 3 by variable importance. Figure 3 lists the top 10 features provided by the XGBoost model. According to the Figure 3, the arrays of highest importance used in the model are listed; 243735_at, 225817_at, 233053_at, 231644_at, 243296_at, 210674_at, 231576_at, 1556012_at, 243611_at, 1561181_at as listed in Figure 3.

Limitations

This paper has some limitations. Performing the analysis with very few patients was not enough for us to use all machine learning methods. However, we were able to start analyzes with 202 patients, as the preprocessing such as merging and cleaning the data took too much time. Other limitation is, extracting missing data in microarray preprocessing step may have caused information loss, but there were too many missing observations for missing data imputation.

CONCLUSION

High number of features with few observations (n<<p) is a problem in microarray data. Thereby, statistical and microarray preprocessing steps can be used to reduce the dimension of feature, especially in ML studies. We can say that XGBoost is the most useful conventional ML algorithm in ML studies for metastasis prediction in breast cancer patients. For the future studies, cancer researchers can examine the arrays listed in Figure 3, in addition to that prediction can be done with Super Learner (19) which approaches the same level of accuracy as the best algorithm among the candidate learners in asymptotically and deep learning methods.

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MEDICAL RECORDS-International Medical Journal

Research Article

Comparison of the Interpretations of Brain Computed Tomography of Emergency Medicine Specialists and Neurosurgeons

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Abstract

Aim: We aimed to examine the similarity in the brain computed tomography (CT) reports of emergency medicine and neurosurgery specialists.

Material and Methods: Patients who applied to the emergency department and underwent cranial CT in traumatic and non-traumatic conditions were analyzed retrospectively. The reports of emergency medicine and neurosurgeon specialists were reviewed. The radiologist's report was set as the gold standard. All CT examinations were performed independently of the radiologist by physicians with at least 5 years of experience.

Results: Emergency medicine and neurosurgery specialists were found to be highly compatible in detecting pathologies in CT reports. There was significant similarity in the diagnosis of bone defects and maxillofacial trauma in the CT reporting of emergency medicine specialists and neurosurgeons.

Conclusion: It was observed that emergency medicine specialists were as successful as neurosurgeons in the interpretation of brain CT pathologies of traumas. Since their knowledge and experience in non-traumatic pathologies are insufficient, they should receive support from radiology in residency training.

Keywords: Emergency medicine, neurosurgery, radiology, computed tomography

INTRODUCTION

Fast and accurate diagnosis is critical for patients who have applied to the emergency department with cranial problems. For this, imaging methods; Computed tomography (CT) is often needed. The correct interpretation of CT images is very important for the correct intervention in patients (1). CT is the most preferred and gold standard radiological imaging method in patients with head trauma (2,3). With its increasing popularity, brain CT has become increasingly common for neurologists and neurosurgeons (4). However, there is not yet a standard application about who will interpret it and how it will be interpreted (5). Although most emergency medicine specialists (EMS) express their opinions in the first stage interpretations of emergency services, consultation from radiology specialists may be requested in some cases. EMSs, who have to manage critically ill patients on their own, can sometimes make mistakes in their CT interpretation.

A study showed that there are inconsistencies in the interpretation of brain CTs between emergency physicians and radiologists in approximately 11% of cases (6). Studies done so far were mostly about CT interpretations of two different branches (7). When the brain CT interpretations of the three departments were examined in our study, it was

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seen that the comments of neurosurgeons and RS were similar. Because of this similarity, brain CT interpretations of these two branches were not compared. In our study, the similarities and differences in the interpretations of EMS and neurosurgeons were examined. Among the patients who applied to the emergency department and had a brain CT scan, the comments of the cases evaluated by the EMS and then consulted with the neurosurgeon were examined.

MATERIAL AND METHOD

Ethical Approval

This study was carried out in the university hospital in Kars city. Patients included in the study were selected retrospectively during the one-year period between 2019-2020. The study was initiated after ethical approval was obtained from the ethics committee of the Faculty of Medicine of Kafkas University with the date of 06.05.2020 and number 80576354-050-99/136.

Study Plan

The examination notes of all these patients who applied to the emergency department were obtained from patient follow-up forms and computer epicrisis notes. The hospital PACS system was used to access the images of the patients. Brain CTs of the study were obtained using Siemens Somatom Emission 16-section tomography device. The records of EMS, neurosurgeons, and radiology specialists' physicians' comments on all brain CT images scanned in the emergency department during the study period were reviewed. As a reference for comparison, radiologist interpretation was considered the diagnostic gold standard in CT scanning. The comments made by the EMS and its neurosurgeons were compared with that of the radiology specialists. All CT examinations were performed independently of the radiologist by physicians with at least 5 years of experience.

Study Criterions

Two EMS and 2 neurosurgeons with at least 5 years of experience in the field took part in the study. Physicians performed CT examinations independently and unbeknownst to the radiologist. Doctors reported in their comments what they saw as important for their specialty. If there is pathology, they describe what it is and its localization. Brain CT interpretations of EMSs and neurosurgeons were accepted as compatible if they matched with the reports of radiologists, and inconsistent if they did not. The report of sinusitis was also considered insignificant, as CT was performed to look for evidence of intracranial lesions rather than sinuses. Any intracranial bleeding was considered significant if patients were using anticoagulants. In such studies, the report of the radiologist is accepted as the gold standard (7,8). Since neurosurgeon and radiologist reports were similar in this study, both departments were accepted as the gold standard with equal strength.

All traumatic and non-traumatic patients over the age of 18 who underwent brain CT and for whom neurosurgery consultation was requested were included in the study. As exclusion criteria; Patients under the age of 18, patients with no or incomplete radiologist comments, and patients who left the hospital without approval were determined. All cases considered normal by the RS and neurosurgeons after the examinations were excluded from the study.

Statistical Analysis

After compiling all data, statistical analyzes were performed using SPSS 22 (SPSS, Chicago) to calculate sensitivity and specificity. The obtained data were given as mean ± standard deviation (SD), number (n), mean, and percent (%). Chi-square and t-tests were used for comparisons between groups. Cohen's Kappa test was used to show the similar status of the groups. In all statistical analysis results, $p<0.005$ was considered significant.

RESULTS

After reviewing cranial CT scans of a total of 572 patients, we completed the study with 269 patients who met the inclusion criteria. Of these patients, 169 (57.8%) were male and 100 (42.2%) were female. The mean age of all patients was 47.06 years. The mean age of men was 44.21(±20.10) years. The mean age of the women was 51.88(±22.32) years.

In the CT reports of our study, it was observed that most of the pathological results were higher in the male gender, while the masses were more common in the female gender. Among all diagnoses, 14 (5.2%) of the intracranial masses belonged to the female gender (Table 1).

Table 1. Gender relationship with brain CT reports

CT Diagnoses	Gender		Total
	Man	Woman	
Epidural Bleeding	14 5.2%	5 1.9%	19 7.1%
Subarachnoid Bleeding	17 6.3%	10 3.7%	27 10.0%
Ischemia	17 6.3%	14 5.2%	31 11.5%
Cysts	16 5.9%	4 1.5%	20 7.4%
MFT	35 13.0%	14 5.2%	49 18.2%
Masses	8 3.0%	14 5.2%	22 8.2%
Bone Defects	17 6.3%	4 1.5%	21 7.8%
Parenchymal Bleeding	24 8.9%	21 7.8%	45 16.7%
Subdural Bleeding	21 7.8%	14 5.2%	35 13.0%
Total	169 62.8%	100 37.2%	269 100.0%

In CT reports, 18.2% (n=49) maxillofacial trauma (MFT) at most, followed by 16.7% (n=45) parenchymal bleeding, 13% (n=35) subdural bleeding, 11.5% (n=31) ischemia, respectively. 10% (n=27) subarachnoid bleeding, 8.2% (n=22) mass, 7.8% (n=21) bone defects, 7.4% (n=20) cyst, and 7.1% (n=19) epidural bleeding was detected. Considering the age distribution, ischemia and subdural hemorrhage occurred in the elderly group (Table 2). In CT reports, ischemia and subdural hemorrhages were seen in advanced ages. On the other hand, cysts, MFT, and epidural hemorrhages were observed at younger ages (Table 2).

Table 2. Age relationship with brain CT reports

CT Diagnoses	Mean	n	SD
Epidural Bleeding	35.47	19	14.88
Subarachnoid Bleeding	56.40	27	16.20
Ischemia	68.77	31	15.36
Cysts	27.10	20	7.39
MFT	34.10	49	15.63
Masses	49.27	22	23.62
Bone Defects	47.23	21	20.41
Parenchymal Bleeding	39.28	45	17.75
Subdural Bleeding	65.00	35	13.84
Total	47.06	269	21.24

In the results of brain CT reports in our study, it was observed that the age range was in a normal distribution (Figure 1).

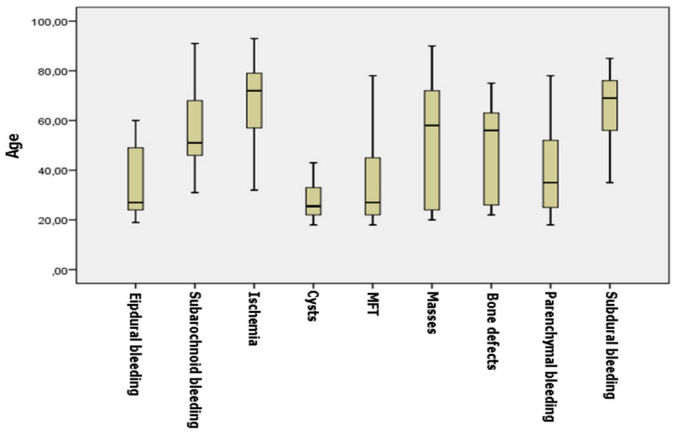

Figure 1. Age distribution graph with brain CT findings

As a result of the evaluation of EMS and neurosurgeons to detect pathologies in CT reports, it was seen that their evaluations were highly compatible with each other (k=.273, p=.000) according to Cohen's kappa test result applied to determine the compatibility between them (Table 3).

The similarity compatibility of EMS and neurosurgeons in CT reporting was found to be statistically significant, especially in bone defects and MFTs (p=0.002; 0.005). Although there were similarities in other diagnoses, there was no statistical significance (p>0.05) (Table 4).

Table 3. Compatibility of evaluations of neurosurgeons and emergency medicine specialists

		Neurosurgery Specialist Evaluation			
		Compatible	Incompatible	Total	Cohen's Kappa
Emergency Medicine Specialist Evaluation	Compatible	175	6	181	k=.273 p=.000
	Incompatible	68	20	88	
	Total	243	26	269	
Cohen's Kappa test					

Table 4. Compatibility of CT reports of emergency medicine and neurosurgeon specialists

CT Diagnoses	Specialist Doctors	Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Epidural Bleeding	EMS	.174	.157	1.345	.179
	Neurosurgeons	19			
Subarachnoid Bleeding	EMS	.158	.091	1.523	.128
	Neurosurgeons	27			
Ischemia	EMS	-.054	.045	-.391	.696
	Neurosurgeons	31			
Cysts	EMS	.091	.069	.976	.329
	Neurosurgeons	20			
MFT	EMS	.324	.102	2.835	.005
	Neurosurgeons	49			
Masses	EMS	-.078	.066	-.482	.629
	Neurosurgeons	22			
Bone Defetcs	EMS	.644	.325	3.158	.002
	Neurosurgeons	21			
Parenchymal Bleeding	EMS	-.068	.036	-.511	.609
	Neurosurgeons	45			
Subdural Bleeding	EMS	.146	.180	1.092	.275
	Neurosurgeons	35			
Total	EMS	.237	.055	5.055	.000
	Neurosurgeons	269			

DISCUSSION

The use of CT has become one of the most frequently preferred diagnostic methods in emergency services because it is fast, effective, and noninvasive. Unfortunately, there are many studies in the literature regarding the request for large amounts of unnecessary tests for out-of-indication patients (9). Diagnostic and clinical decision-making algorithms should be used for the use of diagnostic tests in the emergency room. In cases of head trauma, the CT request should be evaluated in the presence of clinical findings. However, doctors want more CT, especially due to malpractice cases in emergency services. The increase in the use of CT in the emergency department by more than 80% causes emergency departments to become

diagnostic centers (10).

The key element of our study is to demonstrate whether there is an agreement between the examiner and the consultant in the interpretation analysis of brain CTs and in determining normal or abnormal results. Many studies have been conducted comparing CT interpretations between radiologists and emergency physicians (11,6). However, studies comparing the compatibility of neurosurgeons and radiology specialists in interpreting CT imaging examinations are few (12). A gold standard is required to make observational research on radiological studies and to compare the compliance rate. To improve the quality of this type of research, it is recommended to use a panel of radiologist (13,14). In this study, we considered radiologists comments as a definitive diagnosis. In line with the data we obtained, there was a general agreement between the departments, except for a negligible rate.

In the study of Al-Reesi et al., acute findings were detected in 82 of them in brain CT examinations. While 17 of them were clinically significant, insignificant findings were observed in 65 of them (8). In our study, 269 of 572 patients had pathological findings in their reports, while the results of 303 were found to be normal.

The interpretations of EMSs in CT interpretation of the head, thorax, extremity, and abdomen in traumatized cases admitted to the emergency department were mostly consistent with the radiology specialists' reports. When abdominal CT interpretations made in non-traumatic cases between EMS and radiology specialists were compared, it was found that EMSs were insufficient in this regard, and their power to accurately define pathological findings was low (15). In our study, only brain CT interpretations were examined. Among the EMSs and neurosurgeons' assessments of detecting different pathologies in their reports, their assessments of MFT and bone defects were found to be highly concordant. On the other hand, EMSs in the detection of ischemia, cyst, parenchymal hemorrhage, and masses had a higher rate of discordance with radiologist reports. In some studies, it has been reported that EMSs have insufficient accuracy in brain CT evaluation (15,16). However, different studies have shown that the accuracy of EMSs in CT interpretation is similar to that of radiologists (17-19). Although every EMS working in the emergency department is experienced, it is not possible to measure their level of personal knowledge regarding IT assessments. It has been observed that EMS training is insufficient for CT interpretation training, especially brain CT (8). Pieces of trainings or organizations can be organized to increase the knowledge and skills of emergency physicians (20).

Limitations

We think that comparing CT images with the same radiologist's report after being evaluated by the same physician may yield more objective results. EMSs and neurosurgeons are more in control of patients' clinical knowledge. However, the other limitation is that radiologists have only as much information as the image on the computer screen. Not being able to reach the required radiologist, not being able to read the CT on time even when reaching the radiologist, and not documenting the reports officially can be listed. Changed or newly added CT findings during the follow-up of the patients were not included in the study.

CONCLUSION

In our study, it was seen that EMS and neurosurgeons were highly compatible with each other in the assessment of detecting pathologies in CT reports. It was concluded that their knowledge and experience in non-traumatic (such as mass, cyst, and ischemia) pathologies were insufficient. We can recommend that emergency medicine residents receive more support from radiology in their specialization training.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *This retrospective study was approved by the ethics committee of Kafkas University (approval date/ number: 06.05.2020-80576354-050-99/136).*

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Research Article

Relationship between Arterial Stiffness and CHA_2DS_2-VASc Score in AF-related Stroke Patients

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Abstract

Aim: Arterial stiffness is related with both atrial fibrillation (AF) and stroke. The CHA_2DS_2-VASc score is used to assess stroke risk in patients with AF. In this study, it was aimed to examine the relationship between arterial stiffness and CHA_2DS_2-VASc score in AF-related stroke patients.

Material and Methods: Thirty stroke patients with paroxysmal AF participated in this research. Calculations of the patients' pre-stroke CHA_2DS_2-VASc scores were made. The SphygmoCor device was used to assess the Carotid-Femoral Pulse Wave Velocity (cfPWV), which served as a surrogate for arterial stiffness. It was determined whether or not there was a statistical connection between the CHA_2DS_2-VASc score and arterial stiffness.

Results: The patients were seperated into groups based on their CHA_2DS_2-VASc scores prior to the stroke (group 1: score=0-1, group 2: score=≥2). The two groups' characteristics were comparable, except for age, BMI and systolic blood pressure. Patients with high CHA_2DS_2-VASc scores (group 2) demonstrated significantly greater cfPWV values than those with low scores (group 1). The CHA_2DS_2-VASc score and the cfPWV revealed a favourable association in the correlation study.

Conclusion: The CHA_2DS_2-VASc score and cfPWV were substantially and linearly associated. Calculation of CHA_2DS_2-VASc and monitoring of arterial stiffness in stroke-prone individuals may be stimulus for taking preventive measures from stroke in these patients.

Keywords: CHA_2DS_2-VASc, atrial fibrillation, pulse wave velocity

INTRODUCTION

Arterial stiffness is defined as the limitation of the artery to expand or contract against pressure, depending on the stiffness of the arterial wall (1). It can emerge both physiologically (aging) and pathologically. The significance of arterial stiffness, which is regarded as a marker of vascular illnesses, in the emergence of cardiovascular (CV) disorders has been the focus of intense investigation recently. In patients with hypertension (HT), diabetes mellitus (DM), and end-stage renal disease, arterial stiffness has been shown to predict CV morbidity and mortality (2). In one study, it was shown that each standard deviation increases in carotid-femoral pulse wave velocity (cfPWV) raised the risk of CV events and death by 30% (3). The main diagnostic method for measuring arterial stiffness is cfPWV (2). It is recommended to use cfPWV 10 m/s as the cut-off value for the estimation of CV events (4).

Stroke is still a serious public health issue due to the morbidity and mortality it causes. Stroke risk is increased by artery stiffness (5). It's also associated with vascular events after stroke (6). In the studies of Gasecki and Kwarciany et al., it was shown that short and long-term follow- up outcomes in patients with ischemic stroke

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were worse in the group with high PWV (7, 8).

According to research, there is a link between arterial stiffness and atrial fibrillation (AF), which causes stroke (9). The CHA_2DS_2-VASc scoring system is used to assess stroke risk and initiate anticoagulation in individuals with AF (10). This scoring method can also be used to assess stroke risk in patients who do not have AF (11). However, to our knowledge, there is lack of data to compare arterial stiffness between high and low CHA_2DS_2-VASc score groups. This study's objective was to investigate the relationship between arterial stiffness and the CHA_2DS_2-VASc score in stroke patients who had paroxysmal AF.

MATERIAL AND METHOD

Study Population

Patients who had been hospitalized for a stroke were enrolled in the study (n=104). Patients having a history of cardiac surgery, chronic AF, acute coronary syndrome, uncontrolled HT (≥140/90 mmHg) at the time of cfPWV measurement, and moderate to severe valvular disease were excluded (n=74). The demographic features of the stroke patients including age, sex, history of heart failure, HT, DM, coronary or peripheral artery disease (PAD) noted and pre-stroke CHA_2DS_2-VASc scores calculated according to the guideline (10). Patients with low score (0-1) were assigned to group 1, while those with high scores (≥ 2) were assigned to group 2. Resting blood pressure (systolic and diastolic) measured from right upper arm and the mean of the two measurements recorded. The kg/m^2 formula and the patients' recorded weights (kg) and heights (meters) were used to compute the BMI. This study received permission from the regional ethical committee (approval date/number: 07.11.2016/3). The rights of all participants were protected, and written informed consents were obtained prior to the procedures.

Echocardiographic evaluation

Echocardiographic evaluation was made with 2-D GE Vivid 7 ultrasound device (Horten, Norway) via transthoracic. Diameters, mass index and ejection fraction (used Simpson's method) of left ventricle (LV) and left atrium diameters were measured.

Arterial Stiffness Measurement

The cfPWV results measured with the SphygmoCor system were utilized to assess arterial stiffness. Patients were advised not to smoke or to consume caffeine-containing drinks or alcohol 12 hours before the assessment. After 10 to 15 minutes of supine resting, the patient's cfPWV was ascertained by successively recording the waveforms of arterial pressure across the femoral and carotid arteries using a micromanometer probe placed on the skin at the location of peak arterial pulsation. After 10 to 15 minutes of supine resting, the patient's cfPWV was ascertained by successively recording the waveforms of arterial pressure across the femoral and carotid arteries using a micromanometer probe placed on the surface at the location of peak artery pulse. Simultaneous electrocardiogram (ECG) was used to obtain the cfPWV measurements. The distances among the carotid and femoral sampling places to suprasternal notch were measured using an elastic tape measure (2). The cfPWV was calculated as the average of at three consecutive beats obtained over 10 seconds. Furthermore, the presence of cfPWV at speeds greater than 10 m/s has been linked to target organ damage (12). As a result, in both groups, we assessed the presence of cfPWV > 10 m / s as an indicator of target organ damage.

Statistical Analysis

Categorical variables were presented as numbers and percentages, while continuous variables were shown as a mean ± standard deviation. The distribution patterns of the continuous variables were analyzed by histogram and Kolmogorov-Smirnov test. While Mann-Whitney U test was used to evaluate continuous variables without normal distribution, independent t-test was utilized to analyze normally distributed variables. Using the chi-square test, categorical variables were assessed. Finally, the relationship between continuous variables was determined using Pearson correlation analysis. Statistical significance was defined as a two-sided p-value <0.05. SPSS (v.13.0) was utilized for statistics (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1. The study population's baseline characteristics

	Group 1 (CHA_2DS_2-VASc 0-1 [n = 10])	Group 2 (CHA_2DS_2-VASc ≥2 [n = 20])	p-value
Age (years)	51.9±10.5	73.1±5.8	0.001
Age 65-74, n, (%)	0	11 (55%)	0.004
Age >75, n, (%)	0	9 (45%)	0.013
Gender			
Female, n, (%)	3 (30%)	13 (65%)	0.122
HF n, (%)[a]	0	1 (5%)	1.0
HT n, (%)	6 (60%)	17 (85%)	0.18
DM n, (%)	0	5 (25%)	0.14
Blood pressure (mmHg)			
Systolic	117.5±10.9	127.5±11.6	0.03
Diastolic	75±5.3	76±9.9	0.72
BMI	25.2±2.5	28.7±5	0.04
Left ventricle			
ESD (mm)	29.7±6.3	28.6±5.5	0.58
EDD (mm)	48±6.1	44.7±5.1	0.09
Mass index	102.1±27	111.6±21	0.15
EF (%)	62.5±4.2	61.3±8.3	0.87
LA diameter (mm)	36.4±4.7	37.3±3.7	0.30
CF-PWV (m/s)	8.95±1.72	11.8±2.5	0.003
CF-PWV >10 (m/s)	2 (20%)	14 (70%)	0.02

BMI: body mass index, CF-PWV: carotid-femoral pulse wave velocity, DM: diabetes mellitus, EDD: end-diastolic diameter, EF: ejection fraction, ESD: end-systolic diameter, HF: Heart failure, HT: hypertension, LA: left atrium, LV; left ventricle (Values were presented as numbers (%), mean SD, or medians (inter quartile range, IQR)

Thirty patients hospitalized for stroke (Total 104 patients included between December 2016 and May 2017; according to exclusion criteria 74 patients were excluded) without exclusion criteria were participated in the study. The distribution of men and women was comparable (53% female). Group 1 (CHA_2DS_2-VASc 0-1) consisted of ten patients, while group 2 (CHA_2DS_2-VASc ≥2) consisted of twenty.

Table 1 shows the baseline demographic features, and those were similar in both groups except for age, BMI and systolic blood pressure. The age, BMI and systolic blood pressure was higher in group 2. Similar echocardiographic measurements were found in both groups, however group 2's cfPWV was greater (8.95±1.72 vs. 11.8±2.5; p=0.03, respectively). Additionally, patients in group 2 had greater aortic target organ damage than group 1 as measured by the presence of cfPWV > 10 m/s.

The CHA_2DS_2-VASc score and the cfPWV significantly correlated in Pearson correlation analysis (r=0.562, p=0.001).

DISCUSSION

We investigated the link between arterial stiffness and the CHA_2DS_2-VASc score in stroke patients with non-valvular AF in this study. The key findings were as follows: first, subjects with a high CHA_2DS_2-VASc score (group 2) had higher cfPWV than those with a low score (group 1); second, cfPWV was substantially associated to CHA_2DS_2-VASc score; and third, aortic target organ damage was higher in group 2.

Previous research has found that stroke patients have higher arterial stiffness and arterial stiffness can significantly predict stroke (5). The main determinants of PWV are age, blood pressure, diabetes status and race, while smoking, lipid levels and gender do not significantly affect PWV (9,13). In our study, age and blood pressure values were higher in the group 2, and cfPWV was also higher. This could be because the characteristics affecting stroke and arterial stiffness are also common parameters that determine a high CHA_2DS_2-VASc score. The group with high CHA_2DS_2-VAScscore also had a higher percentage of patients with a cfPWV of >10 m/sec. This shows that this group has a higher predicted risk of CV events.

According to study by Kowalczyk et al., the course of PWV after stroke changes over time and is higher in the acute stroke period (14). Kwarciany et al., demonstrated that hypertensive response in stroke is linked with increased arterial stiffness (15). Antihypertensive drugs reduce blood pressure and arterial stiffness. Since blood pressure is increased in some of the stroke patients (5), a decrease in arterial stiffness values can be achieved with antihypertensive treatment to be used in these patients. CHA_2DS_2-VASc calculation and arterial stiffness monitoring in stroke-prone individuals may serve as a catalyst for these patients to take stroke prevention strategies. In our study, stroke patients did not have long-term arterial stiffness measurements, so we do not know how arterial stiffness progresses in stroke patients with different CHA_2DS_2-VASc scores.

AF-related stroke accounts for 30% of all ischemic strokes (10). Various studies have found a connection among arterial stiffness and AF. Arterial stiffness has been shown to have adverse effects on both atrial diameters and strain (9). These factors are also predictive factors for AF and therefore stroke. Chen et al discovered that cfPWV was elevated in patients younger than 60 years of age with isolated AF who had no additional risk factors for AF. Changes in vascular structure, both structural and functional, may have a role in the etiology of AF (16). In a recent investigation, Chung et al. discovered that in people with intermediate or high CV risk, increased arterial stiffness significantly predicts the development of AF (17). All participants in our study were patients with AF-related stroke. However, patients with low CHA_2DS_2-VASc scores had low arterial stiffness measurements. This might be explained by the relative small number of patients in this group overall.

Our study had some limitations; it was a single-center study and arterial stiffness was measured in patients who had a recent stroke. This was not a follow-up study, so long-term follow-up data on arterial stiffness and CHA_2DS_2-VASc score findings and clinical outcomes were not available. Also, there may be errors in measurements and calculations made by the SphygmoCor device. For example, minor errors in measuring the distance among the the carotid and femoral sites can result in significant calculation errors, especially in people with a large chest and abdominal obesity. In addition, noisy ECG recordings and failure to obtain stable waveform recordings may lead to miscalculations. All arterial stiffness measurements were meticulously performed to prevent these possible mistakes and only high-quality recordings with a quality index of more than 80% were included. The association between arterial stiffness and CHA_2DS_2-VASc in patients with the same CHA_2DS_2-VASc score, who have similar age and blood pressure, can be demonstrated more accurately, but prospective, multicenter studies are needed for this.

CONCLUSION

There are common parameters between the constituents of the CHA_2DS_2-VASc score and the determinants of arterial stiffness. Both AF and stroke are possible outcomes of arterial stiffness. The adoption of arterial stiffness as a target value in stroke-prone patients has the potential to prevent a sizable population from stroke. Measurement of arterial stiffness in addition to the CHA_2DS_2-VASc score may contribute to a more accurate stroke risk assessment. We need further studies on this topic.

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Research Article

Effects of Smoking on Pattern Visual Evoked Potentials

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Abstract

Aim: The aim of the study is to get a better understanding of the side effects of smoking by evaluating the effect of recently elevated smoking rate on Visual Evoked Potentials (VEP) and to determine whether it is necessary to use different normals when evaluating the VEP measurements of smoking patients.

Material and Methods: The patients who have applied to our ophthalmology and neurology outpatient clinics during 2021-2022 are included to the study. Detailed ophthalmologic examination of the patients as well as their VEP test is completed followed by a dilated fundus examination assessment. The patients with normal results are included to the study. The smoking rate is calculated on pack/year basis. Pattern VEP (PVEP) recording is performed based on Keypoint (Dantec, Denmark) and International Society for Clinical Electrophysiology of Vision (ISCEV) criteria. Data obtained through the study are analyzed by SPSS 21.0 version software. Countable variables with normal distribution between two independent groups are analyzed with Independent Sample T test whereas variables without normal distribution are analyzed with Mann Whitney U test. Chi-square test is used for comparing categorical variables.

Results: 71 patients were included to the study where 33 of them were placed in smoking group and 38 in non-smoking group (control group). Smoking group had a yearly cigarette package consumption of 5.20±8.93 (0.2-40). VEP latency and amplitude changes were compared and according to the obtained results; there was P100 latency prolongation in between left and right eye of the patients in the control group and smoking group but it did not have any statistical significance (p=0.910 and p=0.697 respectively). There was no statistically significant difference in either left nor right eye in terms of smoking and P100 and N70 latencies (p=0.707, p=0.838, p=0.717 and p=0.621 respectively). Similarly, there was no significant correlation between yearly package consumption and P100 and N70 latencies and amplitudes of left and right eyes (p=0.503, p=0.410, p=0.776 and p=0.940 respectively).

Conclusion: No significant effect of smoking is found on VEP values thus leading us to believe that the same normal intervals can be used in the evaluation of VEP results of both smoking and non-smoking patients.

Keywords: Smoking, VEP (Visual Evoked Potential), nicotine

INTRODUCTION

The side effects of smoking, a habit which has become even more popular after the 20th century, have begun to be understood over the years. Cigarettes contain nicotine and carbon monoxide which cause not only cholinergic neurotransmitter effect due to cholinergic agonist but also changes in the electrical activity of peripheric and central nerve system due to demyelination in the body. Receptors in the eyes and on the visual pathways are affected by this neuropathy. However, the tests run in the ophthalmology and neurology clinics might be insufficient to diagnose the early changes of the neuropathy. This is where the Visual Evoked Potential (VEP) test plays an important role (1-4).

VEP test is an important ocular electrophysiological visual measurement which is based on the occipital field recording of electroencephalographic signals generated in the brain by visual stimulus received through the eye. VEP test enables us to obtain quantitate data on visual pathways from retina to brain by means of optic disc hence supporting the clinical diagnosis of unexplained vision loss, optic nerve damage and neurological diseases (5). VEP allows the assessment of all visual pathways especially starting from the field of vision obtained from the eye to the visual cortex placed in the occipital lobe.

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Furthermore, the fibers coming from central retina to visual cortex are located close to surface in the occipital cortex whereas fibers coming from peripheral retina are located much deeper in the calcarine sulcus, hence the effect of stimulus received from the peripheral retina is much less on the signals during the VEP measurements (6).

Clinic VEP use has somewhat decreased in the recent years due to advanced magnetic resonance imaging (MRI) technology. Although MRI has the advantage of showing all anatomic intracranial changes in detail, VEP still is better in terms of showing functional changes (5,6). VEP remains to be the superior method especially in terms of determining functional disorders in pre chiasma anterior vision nerve transmission or in other words, in the optic nerves (5). Recording of the visually stimulated potentials are done according to the protocol suggested and lately updated in 2016 by ISCEV (International Society for Clinical Electrophysiology of Vision) (7,8).

In our current study, we wanted to evaluate the effect of smoking on pattern VEP which is a very important electrophysiological test. Based on this information, we also aimed to understand whether different normal values should be used for smoking and non-smoking patients while evaluating VEP results.

MATERIAL AND METHOD

This study is conducted by the Ophthalmology and Neurology outpatient clinics of our hospital. Both smoking and non-smoking patients have been included to our study. The study was performed in accordance with the principles of Declaration of Helsinki and approved by the institutional (Ethics committee of Duzce University-181/2022). Furthermore, informed consent forms were signed and submitted by each patient prior to each procedure related to the protocols and goals of the study. Only the patients with no systemic disease nor alcohol consumption and are within the age of 18-65 are included to the study.

Based on the exclusion criteria; patients with neurological diseases or diseases that might affect the optic disc such as multiple sclerosis, papilledema, optic neuritis, etc. as well as patients with alcohol and addictive substance history, patients with high intraocular pressure and glaucoma results or systemic diseases such as diabetes or high blood pressure, patients who have undergone eye surgery for any given reason or have amblyopia, diplopia, cataract, myopia or hyperopia of over 3D, astigmatism of over 1D or anisocoria and pupil size of under 3 mm are not included to the study.

Best corrected visual acuity, eye movement, pupil reflexes, slit lamp biomicroscopy, intraocular pressure measurement with goldmann applanation tonometry and dilated fundus examinations were performed on the patients. VEP test was performed before the dilated fundus evaluation considering that there might be changes in the VEP test after the dilation. All VEP tests were performed by a single experienced neurologist and all measurements were taken in between the hours of 9:00-11:00 to avoid the effect of any diurnal change.

Two groups, namely smoking and non-smoking, were created for our study and smoking rate was calculated on pack/year basis.

VEP measurements were obtained by using Keypoint (Dantec, Denmark) device and 16-inch screen. In order to perform the Pattern VEP (PVEP) recording, active electrode was placed 2 cm above the protuberantia occipitalis externa of occipital bone whereas reference electrode was placed on vertex and ground electrode was placed on the hairline border of the forehead. Electrical potentials emerged in the bilateral occipital cortex of the patient were recorded while he/she was in a dark room, staring at the fixation point located in the middle of moving chessboardlike designs on the screen placed 1 meter away. The recording was done with one eye, while the other one is closed and the process was repeated similarly for both eyes. There were 12x16 number of 2-inch squares on the screen and all the small squares were of the same size. Contrast was 99% according to Michelson constant. Sweep rate was set to 30 ms/D and 5uV/D, sensitivity to 30 uV/D and filter to 1 Hz-200 Hz. 250 stimuli were given during recording for the averaging and the average of measurements were calculated automatically. The staring of the patient at the fixation point was supervised closely by an experienced electrophysiology technician. The measurements of the patients wearing glasses were evaluated together with their glasses. Detailed information and standardization criteria related to visual stimuli respond recording were published by ISCEV. We complied with the ISCEV criteria in recordings of our study (8).

Obtained data was evaluated with the SPSS 21.0 (IBM Corp.; Armonk, NY, USA) software. Categorical variables were indicated by numbers and percentages whereas countable variables were indicated by average±SD. Countable variables with normal distribution between two independent groups were analyzed with Independent Sample T test whereas variables without normal distribution were analyzed with Mann Whitney U test. Chi-square test was used when comparing categorical variables. When the correlation between two countable independent variable was analyzed, Pearson correlation analysis was used for data with normal distribution and Spearman correlation analysis for data without normal distribution. $p<0.05$ was taken to be significant.

RESULTS

Demographic data of the participants in the study are given in Table 1. 71 patients participated to the study where 33 of them were in smoking group and 38 in non-smoking group (control group). Furthermore, the age average of the groups was similar; 36.30±9.36 in smoking group and 36.39±6.78 in control group. Similarly, the gender distribution between two groups was similar; 13(39.4)/20(60.6) M/F in smoking group and 15(39.5)/23(60.5) M/F in control group. Smoking rate in the smoking group was found to be 5.20±8.93 (0.2-40) pack/year.

Table 1. Comparison of demographic data between groups

(n)	Smoking (33)	Nonsmoking (38)	P
Age (range)	36.30±9.36 (21-57)	36.39±6.78 (22-49)	0.962*
Gender M (%) / F (%)	13(39.4) /20(60.6)	15(39.5) /23(60.5)	0.995#
Pack/year (Range)	5.20±8.93 (0.2-40)	0	

M: Male, F: Female, *Independent Sample T test # Chi-square test

VEP latency and amplitude changes were compared throughout the study and the obtained data were evaluated as a table (Table 2). According to these findings; no significant difference was found between the smoking and control groups in terms of the P100 and N70 latencies of both right and left eye (p=0.697, p=0.419 right eye respectively and p=0.910, p=0.542 left eye respectively). Similarly, there was no statistically significant difference between the smoking and control groups in terms of N70 latency of both right and left eye (p=0.572 and p=0.419 respectively).

Table 2. Comparison of VEP measurements between groups

	Smoking (33)	Nonsmoking (38)	P
L P100 ms	107.23±5.38	107.07±6.12	0.910
R P100 ms	106.81±5.28	106.23±7.08	0.697
L N70 ms	76.45±5.49	77.34±7.44	0.572
R N70 ms	77.53±4.89	76.16±8.60	0.419
L P100 uV	-7.43±4.26	-6.95±3.34	0.600
R P100 uV	-7.97±3.85	-7.69±2.95	0.729
L N70 uV	1.56±2.11	2.16±2.70	0.304
R N70 uV	1.71±2.34	2.44±2.99	0.266

L: Left, R: Right

Although the N70 amplitudes of both right and left eyes of non-smoking patients were found to be high, there was no statistically significant difference (p=0.266 and p=0.304 respectively). There was no statistically significant difference between both groups in terms of P100 amplitude of both right and left eye (p=0.729 and p=0.600 respectively).

Based on the analysis of latency and amplitude differences in P100 and N70 values of right and left eyes, no statistically significant difference was found between two groups (Table 3).

Table 3. Comparison of VEP measurements between groups

	Smoking (33)	Nonsmoking (38)	P
L/R dif. P100 ms	1.84±2.07	2.21±1.81	0.420
L/R dif. N70 ms	5.18±4.27	3.63±3.72	0.106
L/R dif. N70 uV	0.99±0.77	1.24±1.02	0.254
L/R dif. P100 u V	1.28±1.06	1.20±0.93	0.756

L: Left, R: Right, Dif: Difference

The correlation analysis between the measured VEP parameters and smoking pack/year rates are shown in Table 4. Based on this analysis, there was no statistically significant correlation between the pack/year consumption and P100 and N70 latencies for both right and left eye (p=0.707, p=0.838, p=0.717 and p=0.621 respectively). Similarly, there was no statistically significant correlation between the pack/year consumption and P100 and N70 latencies for both right and left eye (p=0.503, p=0.410, p=776 and p=0.940 respectively).

Table 4. Comparison of the smoking amount and VEP values

	L P100 ms	R P100 ms	L N70 ms	R N70 ms	L/R dif. P100 ms	L/R dif. N70 ms
Pack / year	0.707	0.838	0.717	0.621	0.153	0.083
	L N70 uV	R N70 uV	L P100 uV	R P100 uV	L/R dif. N70 uV	L/R dif. P100 uV
Pack / year	0.503	0.410	0.776	0.940	0.122	0.659

L: Left, R: Right, Dif: Difference

DISCUSSION

As a result of this study, we found that there was no significant difference in the VEP tests of smoking and non-smoking healthy people.

It is a known fact that smoking causes vasoconstriction and the rate of vasoconstriction varies according to the smoking amount. Consequently, the veins in the brain also change, causing differences in the VEP values. It may lead to differences in both latencies and amplitudes of VEP (9,10).

Review of literature shows that there is no final consensus on the effects of smoking on VEP amplitudes and latencies. In two studies done by Friedman J et al. as well as other studies done by Hall RA et al. and Woodson PP et al., an increase in the VEP amplitudes was detected (11-14). Furthermore, Knott VJ et al. stated a decrease in amplitudes as a result of their study (15). In their study, Durukan AH et al. stated a decrease in amplitudes after an acute smoking period (1). In our study, the effect of acute smoking was not evaluated and no difference was found in amplitudes between chronic smokers and nonsmokers. In their studies, Pritchard et al., Woodson et al. and Conrin et al. found a decrease in P100 latencies (2,14,16). Hetzler et al. analyzed various latencies and found a general prolongation in latencies (4). Pandey et al. found no statistically significant difference in between latencies. Likewise, we did not find any significant difference between smoking and non-smoking groups in terms of P100 latency in our study. Smoking has various effects on the human body therefore we found diverse results in the literature related to VEP. Previous studies show that chronic smoking has effect on ocular blood flow. In their study, Robinson et al. reported an increase in the blood flow of macula after smoking (17). Furthermore, smoking is found to be decreasing the choroid blood flow in the study done by Kocak et al. and further found to be increasing the blood flow around the optic disc head in the study done by Tamaki et al (18,19).

However, this theory is not always sufficient to justify the

unexpected findings in the audio and visual modalities of some of the studies (1,15). Therefore, a more reliable approach might be considering other accompanying factors besides plain smoking, such as smoking history/ duration, life style, etc., when evaluating the effect of smoking on VEP altitudes or similar measurements (1,20-22). Combining these results would also enable us to get a better understanding about the smoking habit which might have various underlying reasons like psychological urge, stimuli requirement, concentration tool, stress management, mood stabilizer, even referring to Pomerleau hypothesis when heavy smoking is involved (1,12, 23,24).

In their study made with patients whose ocular blood flow in 3 retrobulbar veins were measured, Kurysheva et al. found statistically significant correlation between P100 amplitudes and ocular blood flow as a result of their VEP evaluations (25). There are studies which show that smoking causes not only demyelination in the optic disc retrobulbar area but also increase in reactive oxygen molecules due to decreased ocular blood flow and changes in pVEP due to generated free radicals and disrupted neuro transmitter balance. Therefore, it is possible to determine the changes with pVEP before neuropathy emerges.

The limiting factors of our study are relatively limited number of participating patients and being single-centered. Another limitation of our study is that only chronic smokers were included, and the effect of acute smoking was not evaluated. On the other hand, the previous studies related to effects of smoking on VEP have been mostly done 2-3 decades ago. Therefore, it gives our study the strength of being one of the recent studies done on the effects of smoking on VEP, especially after the current developments of VEP.

CONCLUSION

VEP test, with an ever-increasing importance, is a non-invasive measurement method which is being frequently used in neuro-ophthalmologic evaluations. On the other hand, the increase in smoking rates appears as a severe public health issues. When the multi-organ effects of smoking are considered; it is evident that it should be treated seriously in terms of its effects both on visual and brain activities. Furthermore, it is very important not only to acknowledge the normal values while evaluating the VEP results but factors effecting the normal values should be well known as well. The increasing importance of the VEP test and the fact that there is no conducted study related to the elevating smoking in the last decade despite the developments lead us to the idea of addressing this subject. There was no significant difference between the VEP parameters of smoking and non-smoking groups in our study which enables us to conclude that the same VEP normal values can be used for both smoking and non-smoking individuals. However, even though we have not found any significant results in our study, there are contrary findings in the limited literature reviews. We believe that additional studies should be conducted on a wider scale and multi-centered basis with a larger group of patients.

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MEDICAL RECORDS-International Medical Journal

Research Article

Clinical Significance of the Morphometric Structures of the Scapula with the Emphasis on the Glenoid Cavity

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Abstract

Aim: The aim of this study is to determine the average of the morphometric parameters of the scapula to accurate and successful analyzes in the clinic.

Material and Methods: A total of 24 dry bone scapula were included in the study. Parameters determined on the scapula; superio-inferior glenoid diameter (SIGD), anterio-posterior glenoid diameter 1 (APG1), anterio-posterior glenoid diameter 2 (APG2), anterio-posterior glenoid diameter 3 (APG3), scapula width (SW), scapula length (SL), basis-spina distance (BS), spina scapula width (SSW), acromiocoracoid width (AW), scapula margo lateralis length (SML), scapula margo medialis length 1 (SML 1), scapula margo medialis length 2 (SML 2), cavitas glenoidalis antero-posterior width (CGAPW), cavitas glenoidalis superio-inferior width (CGSIW), incisura scapula width (ISW), incisura scapula depth (ISD), the maximum length of proccessus coracoideus (MLPC), maximum proccessus coracoideus thickness (MPCK), the shortest distance between the lateral edge of the proccessus coracoideus tip and the anterior upper edge of the cavitas glenoidalis (PCL-CGK).

Results: Among the determined parameters, SSW, MSW, ISW, ISD, MPCU, MPCK, PCL-CGK were found to be in parallel with the analyzed literatures.

Conclusion: It is thought that the results of the analysis of the parameters determined in the study will add clinical depth to many surgical approaches such as glenohumeral arthrodesis, internal fixation, fracture stabilization, and rotator cuff tendinitis, in more accurate analysis of shoulder anomalies and fractures.

Keywords: Scapula, morphometry, cavitas glenoidalis

INTRODUCTION

The scapula articulating with the clavicle and humerus has a complex anatomical peculiarity due to its irregular shape and ossifies approximately seven different anatomical points, including the body and the processes (1). The lateral angle connecting the upper and outer lateral edges of the scapula is the thickest corner, possessing the glenoid cavity, which forms the concave articular surface of the shoulder joint (2,3). The superior margin is the shortest and thinnest edge comprising the scapular notch at the junction of the upper edge and the base of the coracoid process (3–5). The scapula reaches the shoulder girdle to the thorax and vertebral column via the musculotendinous structures medially (6).

The scapula plays a significant role clinically because the mobile parts of the upper extremity are connected to the trunk by this bone and clavicle. The scapula, clavicle, and humerus together form the joint with the widest range of motion in the body. The shoulder joint has approximately 170º anterior elevations, approximately 60º extensions, and approximately 120-180º rotations. The shoulder provides this range of motion with the simultaneous movement of five joints. These joints are the sternoclavicular, acromioclavicular, glenohumeral, scapulothoracic, and subacromial from medial to lateral (1,7–9). In addition, the scapula plays a major role in the strength, and energy of shoulder function. Muscles attached to the scapula play a role in scapular stabilization, and glenohumeral and scapulothoracic joint movements (10). With these in mind, revealing and analyzing the morphometric peculiarities of the scapula having such significance, with the emphasis on the glenoid cavity, will surely collocate a clinical perspective to many surgical approaches such as

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glenohumeral arthrodesis, internal fixation, and fracture stabilization, and rotator cuff tendinitis. It will also provide more accurate analysis in the field of physiotherapy and rehabilitation in anomalies and fractures of the shoulder. Therefore, this study aimed at documenting and analyzing the morphometric characteristics of the scapula, focusing primarily on the glenoid cavity.

MATERIAL AND METHOD

The study was conducted on 24 (16 right, 8 left) dried human scapula of unknown age and gender. Measurements were not taken from the partially broken, fragmented, and damaged parts of the dry bones. Permission was obtained from the Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee. Decision No:2022/144 Date:07.06.2022 The measurements were performed by using a digital caliper nearest 0.1 mm (Baker 0-150 mm) on the osteometry board. Descriptive statistics for continuous variables in the study mean±sd and categorical variables are expressed as percentages (%). The results were evaluated using the R Studio (R Tools Technology, Inc. 4.1.1 USA) program. The t-test was used for independent group comparisons. The significance level was taken as $p<0.05$. The reference points of the parameters are as follows (2,6–11):

•Superio-Inferior Glenoid Diameter (SIGD): The maximum distance from the lower point of the glenoid margin to the most prominent point of the supraglenoid tubercle.

•Anterio-Posterior Glenoid Diameter 1 (APG1): The maximum width of the cavitas glenoidalis joint is perpendicular to the height of the cavitas glenoidalis.

•Anterio-Posterior Glenoid Diameter 2 (APG2): Anterio-posterior diameter of the upper half of the midpoint between the upper edge and the midline.

•Anterio-Posterior Glenoid Diameter 3 (APG3): Anterio-posterior diameter at the incisura glenoidalis crest.

•Scapula Width (SW): The maximum distance between the base of the spina and the center of the glenoid cavity.

•Maximum Scapula Width (MSW): The maximum length measured along the center of the cavitas glenoidalis and the base of the spina scapulae.

•Scapula Length (SL): The maximum distance between the angulus superior and the angulus inferior.

•Basis-Spina Distance (BS): The maximum distance between the base of the spina and the innermost part of the glenoid cavity.

•Spina Scapula Width (SSW): The maximum distance between the base of the spina and the most prominent part of the acromion.

•Acromiocoracoid width (AW): The maximum distance between the ventral part of the coracoid process and the most dorsal part of the acromion.

•Scapula Margo Lateralis Length (SML): The maximum distance between the lower point of the cavitas glenoidalis and the angulus inferior.

•Scapula Margo Medialis Length 1 (SML1): Scapula corpus width is taken 2 cm above margo inferior.

•Scapula Margo Medialis Length 2 (SML2): Scapula corpus width is taken 4 cm above margo inferior.

•Scapula Margo Medialis Length 3 (SML3): Scapula corpus width is taken 6 cm above margo inferior.

•Cavitas Glenoidalis Antero-Posterior Width (CGAPW): Anterio-posterior maximum distance.

•Cavitas Glenoidalis Superior-Inferior Width (CGSIW): The maximum length between the lowest point of the cavitas glenoidalis and the tip of the tuberculum supraglenoidale.

•Incisura Scapula Width (ISW): The distance between the point where incisura scapulae start in margo superior and the beginning of proceccus coracoideus.

•Incisura Scapula Depth (ISD): The distance between the line tangent to the Margo superior and the deepest point of the notch.

•Maximum length of processus coracoideus (MLPC)

•Maximum processus coracoideus thickness (MTPC)

•The shortest distance between the lateral edge of the apex of the processus coracoideus and the anterior upper edge of the cavitas glenoidalis (PCL-CGK)

•Scapular index (SI): (SW)/(SL)*100

•Infraspinate index (ISI): (SW)/(SML2) *100

•Glenoid index (GI): (CGAPW)/(CGSIW)*100

•Shape of the Cavitas Glenoidalis (SCG): According to the shape made by the slightly raised edge of the glenoid space, it is divided into a pear, oval and inverted comma.

•Shape of the Coracoglenoid Area (CAS): It was divided into three groups according to the shape of the region between processus coracoideus and cavitas glenoidalis. Type I; round, Type II; square and Type III; hook.

•Shape of the Incisura Scapula (SIS): Incisura scapulas were divided into 5 groups according to their shape and depth. Type I; deep and U-shaped, Type II; shallow and U-shaped, Type III; deep and V-shaped, Type IV; shallow and V-shaped, Type V; its notch is grouped into a hole shape.

The reference points of the measurements are shown in figure 1 and figure 2.

RESULTS

The morphometric measurements of the 24 scapular bones (16 right, 8 left) are shown in table 1 and table 2.

•The index values are respectively, SI; 65.8±4.07, II; 103.86±0.18, GI; 69.74±5.8.

•SCG; 87.5% pear, 8.3% oval ve 4.16% inverted comma.

•CAS; 33.3% round, 57.14% square, 9.52% hook.

•SIS; 12.5% type 1, 16.6% type 2, 37.5% type 3, 33.3% type 4.

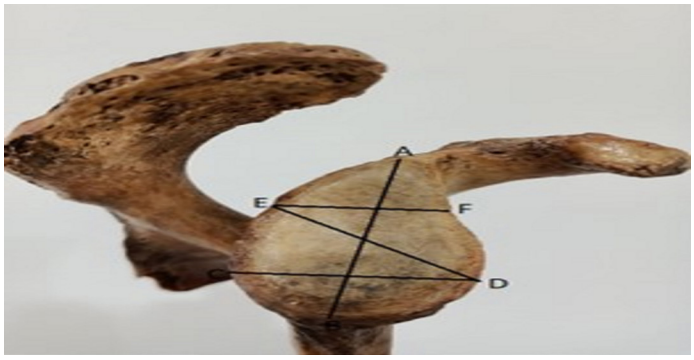

Figure 1. AB: Superio-Inferior Glenoid Diameter (SIGD), CD: Anterio-Posterior Glenoid Diameter 1 (APG1), EF: Anterio-Posterior Glenoid Diameter 2 (APG2), ED: Anterio-Posterior Glenoid Diameter 3 (APG3)

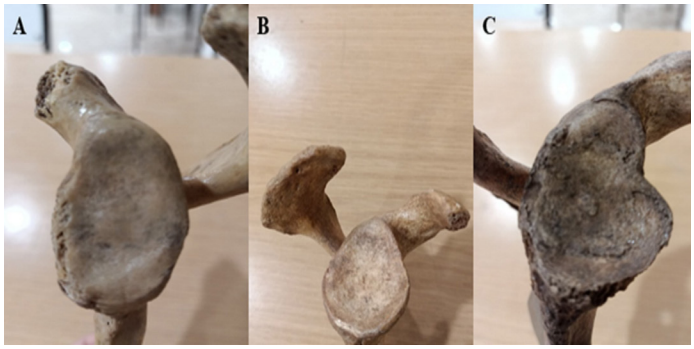

Figure 2. A: Oval, B: Inverted Comma, C: Pear

Figure 3.a. AD: Basis-spina distance (BS), BD: Scapula width (SW), CD: Spina scapula width (SSW), GM: Scapula margo medialis length 1 (SML1) FL: Scapula margo medialis length 2 (SML2), EH: Scapula margo medialis length 3 (SML3), NP: Scapula length (SL)

Figure 3.b. AB: Maximum scapula width (MSW), AC: Scapula margo lateralis length (SML)

Table 1. Morphometric measurements of the scapula

Parameters	Mean±SD (mm)	Mean±SD		p
	Total	Right	Left	
SIGD	37.9±0.31	37±0,3	38±0.34	0.9
APG1	24.7±0.27	24.1±0.2	25±0.32	0.5
APG2	20.1±0.31	19.3±0.28	21.8±0.29	0.6
APG3	29.4±0.55	28±0.2	30.6±0.93	0.9
SW	103.5±0.84	99.7±0.64	110.6±0.6	0.5
SL	150±1.17	149±0.72	161±1.05	0.4
BS	73±0.82	70±0.7	77.1±0.71	0.5
SS	144.5±1.05	139±0.9	150.6±1.24	0.9
SSW	144±1.2	150±1.2	139±0.9	0
AW	62±0.92	57.9±0.8	69.1±0.49	0.9
SML	134.3±0.93	134.2±0.6	134.4±1.33	1
SML1	50.1±0.51	49±0.4	51.4±0.52	0.6
SML2	110.6±1.1	108±0.6	114.1±1.53	0.5
SML3	101.5±0.71	99±0.51	106.7±0.82	0.5
MSW	154±1.05	149.6±0.72	161±1.05	0.8
CGAPG	26.2±0.26	26.1±0.26	26.5±0.26	1
CGSIG	37.7±0.3	38±0.29	37.1±0.3	0.5
ISW	658.9±4.07	85±2.31	91.6±1.93	0.3
ISD	2038.6±18.4	48.5±1.57	57.3±1.7	0.9
MPCU	697.4±5.8	443.4±2.7	451±3.8	0.6
MPCK	88.4±2.18	100±1.1	101.3±2.5	0.4
PCL-CGK	52.5±1.69	145±1.03	146±1.9	0.9

Table 2. Comparison of scapula width (SW) and scapula length (SL) by various authors

	SW	SL
Costa et al. (male-female)	102.43-90.81	151.143-132.63
Ülkir	101.5	150.4
Aydemir	105	147
El-Din et al.	107.22	151.16
Gosavi et al.	141.4	123.02
Chhabra et al.	103.65	98.69
Paraskevas et al.	101.9	147.6
Lingamdenne et al.	98.69±6.98	141.49±9.74
Piyawinijwong et al.	104±7.8	139.3±11.1
Kabakcı et al.	98.5	140.8
This study	104	150.2

Table 3. Comparison of the scapular index (SI), infraspinat index (ISI), and glenoid index (GI) by various authors (1,4,10,17,18,22–25)

	SI	ISI	GI
Ülkir	67.8	102.2	-
Kabakçı et al.	121.52	91.03	68.49
El-Din et al.	70.93	-	-
Chhabra et al.	73.32	99.60	-
Singhal et al.	68.5	94.6	-
Krishnaiah et al.	73.99	98.33	-
Parmar et al.	-	-	69.09
Polguj et al.	63.7	-	72.35
Dhindsa et al. (right-left)	-	-	70.37-68.59
This study	65.8	101.8	69.7

DISCUSSION

This study has documented and analyzed the morphometric characteristics of the scapula, particularly those of the glenoid cavity. Yet, various dimensions and incidences of the glenoid cavity have been measured and compared with the findings in the literature which contain various results (2,11-15). The average SIGD value has been documented as 37.9±3.1 (37.7±3 right, 38.5±3.4 left) mm in this study, indicating numerical differences. Similarly, research (11) has examined the right side of the 43 and left side of the 57 glenoid cavities of the scapula, finding these values as 34.76±3 mm and 34.43±3.21 mm, respectively, again showing a slight numerical difference. Another study (2) has reported those values on 202 dried scapula to be 33.67±2.82 mm on the right side and 33.92±2.87 mm on the left, revealing slightly lower values. Yet, three studies (12–14) have measured the same values in males and females as 36.08±2.05 mm, and 31.17±1.17 mm, respectively, the other studies' mean±std. Values as 36.3±3 mm, 35.9±3.6 mm. These values were lower than what was examined in the current study. The average SIGD of the male and female observed was 38.71±2.71 mm and 33.79±3.08 mm respectively (15). While the male value was higher than the female value was lower than the average SIGD value of the current study.

The average APG-1 value of the right scapula in this study was 24.1±2.2 mm while that of the left side was 25.8±3.2 mm, and the average of both sides was 24.7±2.7 mm in the current study. This suggests that the right glenoid was quite similar to the left glenoid. In two studies conducted in India; APG-1 on the right side was 23.31±3 mm and 23.35±2.04 mm respectively, and that on the left side was 22.92±2.80 mm and 23.02±2.30 mm (2,11), which were lower than what was examined in this study. The female and male APG-1 values were recorded in the East Anatolian population and Guatemalan contemporary rural and indigenous population; who were found to be 22.31±1.49 mm and 22.72±1.9 mm respectively female, that of the male values were 26.31±1.57 mm and 27.33±2.4 mm respectively (14,15). The APG-1 values were recorded in two studies examining glenoid cavity patterns and scapula pillars; 27.2±3 mm and 22.62+2.9 mm respectively. The average APG-1 of both sides was 24.7±2.7 mm in the current study and that value was between these values (11,13). In the current study, APG-2 and APG-3 values on the right side were 19.3±0.28 mm and 28.8±2.2 mm respectively, and on the left side values were 21.8±2.9 mm and 30.6±9.3 mm respectively. This suggests that the left glenoid cavity was broader than the right glenoid cavity. While the APG-2 and APG-3 values of the Indian population were 15.10±2.54 mm, 16.2±3.23 mm, respectively, on the right side, they were 13.83±2.45 and 15.24±2.04 mm, respectively, on the left side (11). This suggests that the right glenoid cavity was broader than the left glenoid cavity. unlike our study. Average values of the CGSIW and the CGAPW values were respectively. 37.7±3 mm and 26.2±2.6 mm in this study. A study conducted on the Turkish population stated that CGSIW and CGAPW are 25 and 35. respectively (6). These values were found to be quite close to the results of the study. In the current study, various SGC and the percentage of incidence were recorded at 88% of the pear, 8% of the oval, and 4% of the inverted comma-shaped.

In two studies investigating the SGC; in right and left sides respectively; firstly, the right side values are 35% and 34% of glenoids were inverted commas, 49% and 46% of glenoids were the pears and 16% and 20% of glenoids were oval-shaped, on the left side, 39% and 33% of glenoids were inverted commas, 46% and 43% of glenoids were the pears and 15% and 24% of glenoids were oval-shaped. This suggests that the large rate is pear-shaped in both studies (2,11).

The studies conducted by the Turkish adult population in 72% of the glenoid notches of the scapula were absent or oval-shaped, whereas in 28% the notch was well expressed and the glenoid cavity was pear-shaped (12).

These findings were higher than that of the current findings and on the contrary results, in terms of shape, which takes the largest rate. The literature data on the width and height of the scapula as mm are shown in table 3 (1,6,10,16–21).

When the data between the current study and the studies done by different authors in the literature are compared; there appear to be few similarities in scapula weight and height. The BS values in the Brazilian population (9) are 79.44 mm, and 69.6 mm respectively for males and females, for the same parameter in a study conducted on the Turkish population; it was found to be 81.9 mm (10). The result of this study, which did not discriminate between males and females of 72.6 mm.

In this study, the SSW values as 144 mm, which was very close to that reported in the Turkish population (6) 133 mm, and lower values in South India. Thais and Turkish population respectively as 123.35±7.8.124.9±9.3 and 81.9 mm (10,20,21). The AW values as 61.8 mm, which was lower than reported in the Egyptian population (17) 90.69 mm in males, and 77.9 mm in females. The SML values in the Turkish population (10) were 122.5 mm, which was very close to that reported in this study 143.3 mm.

These differences may be due to factors such as age, gender, race, and a variety of techniques like measurement methods. The literature data on the scapular index, infraspinat index, and glenoid index as % are shown in table 3.

The percentage values of SI, ISI, and GI are almost similar to literature data.

The number of bones in the laboratory where the study was conducted is limited. In addition, deformed bones were excluded from the study. Therefore, the number of bones used in the study and the age, gender and identity of these bones are not known.

Consequently, the parameters determined and analyzed in this study, and their interpretation with the literature, even though the number of scapula used hereby is limited to

a certain degree, will surely be helpful in the journey on particular physical therapy and rehabilitation of sports injuries. Yet, knowing the patterns of the glenoid cavity, in particular, will assist orthopedic surgeons in deciding on prosthesis design and application procedures in shoulder arthroplasty.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Permission was obtained from the Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee. Decision No: 2022/144 Date: 07.06.2022.*

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Research Article

Changes in the Anterior Segment and Dry Eye Parameters of People Using Oral Tetracycline

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Abstract

Aim: The purpose of the study is to evaluate the effect of anti-inflammatory efficiency of tetracycline on dry eye and anterior segment parameters.

Material and Methods: This prospective study was conducted with 61 people who had been using tetracycline for the past 2 months. Each participant went through a detailed ophthalmological examination where intraocular pressure (IOP), central cornea thickness (CCT), K1 and K2 from keratometry measurements, anterior chamber depth (ACD), axial length (AXL), tear breakup time test (BUT), lissamin green staining (LG), Schirmer's tear test-1 (ST) and ocular surface disease index (OSDI) score were evaluated. Measurements of the patients who would be using a daily dose of 100 mg doxycycline for 2 months were done prior to the medication as well as 1 month and 2 months after the medication. 3 data sets of the participants were compared at the end of 2 months.

Results: When results were compared, it was found that ST and LG parameters gradually increased in the first and second months and this increase was statistically significant ($p<0.001$ and $p=0.018$, respectively). On the other hand, BUT and OSDI scores decreased over time but it was not statistically significant. There were no statistically significant differences in terms of other parameters either ($p>0.05$). When female and male participants were compared in terms of BUT, it was found that the decrease in females at the second month was higher and this was statistically significant ($p=0.01$).

Conclusion: t was concluded that the young individuals using tetracycline displayed an early stage of improvement in dry eye parameters.

Keywords: Dry eye, tetracycline, anterior segment

INTRODUCTION

Chronic blepharitis is an ocular disease widely seen in the society and demands frequent use of doxycycline group in clinical conditions which require oral antibiotic treatment. Furthermore, variable ratios of 3-58% have been reported for the commonly observed ocular rosacea frequency. The symptoms of the disease cover eye lid, conjunctiva and cornea (1-3). The most frequent complaints of the patients who apply to daily clinic with non-specific symptoms are; itching, burning, stinging, watering, redness, feeling of a foreign object, photophobia, pain and blurred vision. The most frequent diagnosis, on the other hand are blepharitis, telangiectasia at the edge of the eye lid, meibomitis, repeated chalazion, hordeolum, superficial punctate keratitis (SPK) and conjunctival hyperemia. In advanced cases, episcleritis, scleritis, keratitis, iritis, corneal vascularization, corneal perforation and vision loss can be seen but with much less frequency. As far as this disease is concerned, it is believed that all findings are generated from eye lid involvement. Tetracycline and derivatives are used in ophthalmology not only for bacterial and chlamydial infections but also for ocular rosacea, causing the remission of symptoms in 2-3 weeks together with an improvement in the ocular findings. Tetracyclines, as a result of their both anti-inflammatory and anti-bacterial effect, are believed to have a major impact on the relevant treatment with enhanced improvement in the ongoing complaints. Apart from rosacea, tetracycline is a commonly used antibiotics in ophthalmology when

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diseases such as chalazion, intense blepharitis and hordeolum are concerned (4-7).

In general, the studies conducted with tetracycline are based on the improvement effect of the treatment on ocular findings and symptoms (8-13). Besides, in some studies the comparison of tear parameters of normal individuals verses patients with rosacea are analysed (4, 6,14). But this is the first study that analyses the effect of oral doxycycline treatment on both cornea and tear parameters of chronic blepharitis patients.

Inflammation is known to be the most frequently seen factor in dry eye etiology. Therefore, we conducted this study to determine whether anti-inflammatory efficiency of tetracycline causes any changes in the dry eye parameters, hence whether it could be used for the purpose of reducing dry eye symptoms.

MATERIAL AND METHOD

Patients who were prescribed to use doxycycline between the dates of June 2022-December 2022 due to systemic reasons were included to the study. This study was conducted by the eye policlinic of our hospital in accordance with the Declaration of Helsinki with a further approval from Institutional Ethics Committee (68/2022-Clinical Trail Protocol). All patients provided informed consent to the protocols and goals of the study prior to each procedure. Only patients without any systemic disease and aged between 18-31 were included. The study initially started with 82 patients but patients who have not arrived for treatment control or abandoned the treatment program were excluded. A total of 61 patients completed the study.

Pregnant or breastfeeding patients, patient with doxycycline, oxibuprokain, lissamin green or fluorescein hypersensitivity, patients who have undergone oral or topical treatment in the last 6 weeks, patients with a previous dry eye diagnosis, contact lens usage or ocular problems such as previous surgery or trauma, were not included to the study.

Patients were tested for corrected best visual acuity, eye movements, pupillary reflex, slit lamp biomicroscopy, fundus examination and intraocular pressure with Goldman applanation tonometry. Patients with corrected best visual acuity of 20/20, no corneal involvement and normal values for all tests are included to the study. Intraocular pressure (IOP), central cornea thickness (CCT), K1 and K2 from keratometry measurements, anterior chamber depth (ACD), axial length (AXL), tear breakup time test (BUT), Lissamin green staining (LG), Schirmer's tear test (ST) and ocular surface disease index (OSDI) scores are analyzed.

Examination started with keratometry measurements on non-contact basis (K1 and K2). It was followed by placing the special filter paper for ST in between the lower eye lid, 1/3 outer section of the eye. ST measurements were taken and recorded after 5 minutes (<10 mm was accepted as significant for the ST result). Eye was stained with fluorescent dye for the BUT and patient was asked to blink a couple of times before the cornea was examined under cobalt blue. Patient was asked not to blink during the examination. Formation of 2 black dots on the cornea are taken as base for BUT value (<10 sec was accepted as significant for BUT).

Sufficient time was taken for conjunctiva to be washed before the cornea was stained (liquid with 1% LG content) and examined for LG. LG results are recorded as 'degree 0' and 'degree 1' in this study (15). OSDI scoring (interval: 0-100), which is a subjective test, is used to determine symptoms related to drug-use and patient comfort (15). After the lissamine green stain is washed, IOP is measured using Goldman applanation tonometry and ACD, AXL and CCT are measured using contact method (NIDEK US-500 Echoscan, Tokyo, Japan) under topical anesthesia with 0.5 % proparacaine (Alcaine, Alcon Laboratories Inc., Fort Worth, TX, USA). All ACD (mm), AXL (mm), IOP (mmHg), K1, K2, ST (mm), BUT (sec), LG, OSDI and CCT (µm) parameters were measured under the same conditions by the same experienced ophthalmologist. All measurements were done during the 9:00-11:00 hour interval in order to avoid any possible diurnal discrepancies. All measurements followed exactly the same procedure and schedule. Measurements of the patients who have been scheduled to undertake oral 100 mg/day doxycycline treatment for 2 months were done before the medication and after the first and second month of medication.

Statistical Analysis

Findings of the study were analyzed by using SPSS 25.0 version software. The distribution of the data was displayed by descriptive analysis parameters (mean, standard deviation, minimum, maximum, frequency and percentage). Kolmogorov-Smirnov test was employed to analyze whether the data is consistent with the normal distribution. Correlation between the categorical variables is analyzed with chi-square test. The mean comparison between two independent groups where the data was non consistent with the normal distribution, was done with Mann Whitney U Test. As for the analysis of discrepancies between the repeated measurements; Cochran's Q Test was used for categorical variables and Friedman Test was used for constant variables. Spearman's Correlation was used for the correlation between constant variables.

RESULTS

Demographical specifications of the participants are shown in the table (Table 1).

Table 1. Demografic features

	X±SD	Min-Max
Age	21.7±3.6	18-31
Gender	**n**	**%**
Female	23	37.7
Male	38	62.3

Table 2. Change of parameters by gender over time

Variable	Time	Stage	Male	Female	Statistics	p
Lissamine Green [n(%)]	0.Month	0.Stage	19 (31.1)	32 (52.5)	0.027[a]	0.870
		1.Stage	4 (6.6)	6 (9.8)		
	1.Month	0.Stage	20 (32.8)	35 (57.4)	0.428[a]	0.513
		1.Stage	3 (4.9)	3 (4.9)		
	2.Month	0.Stage	20 (32.8)	35 (57.4)	0.428[a]	0.513
		1.Stage	3 (4.9)	3 (4.9)		
BUT (sec) [X±SD]	0.Month		21.7±7.7	31.4±16.3	-2.580[b]	**0.010***
	1.Month		24.9±10.1	27.4±11.5	-1.094[b]	0.274
	2.Month		23.2±12.8	27.9±14.1	-1.539[b]	0.124
Schirmer (mm) [X±SD]	0.Month		6.5±2.6	6.1±2.9	-1.085[b]	0.278
	1.Month		10.3±7.0	11.3±5.8	-0.897[b]	0.370
	2.Month		11.5±6.3	12.8±5.1	-1.220[b]	0.223
OSDI [X±SD]	0.Month		23.3±16.6	20.4±23.4	-1.209[b]	0.227
	1.Month		16.9±22.9	21.1±25.2	-1.371[b]	0.170
	2.Month		16.7±21.6	20.7±23.2	-1.551[b]	0.121
IOP (mmHg) [X±SD]	0.Month		16.7±2.3	15.5±1.4	-1.997[b]	**0.046***
	1.Month		15.5±1.6	15.6±1.5	-0.808[b]	0.419
	2.Month		15.8±1.5	15.7±1.4	-0.457[b]	0.647
Central corneal thickness (µm) [X±SD]	0.Month		547.6±16.4	545.5±20.3	-0.895[b]	0.173
	1.Month		539.9±16.9	557.6±22.6	-3.134[b]	**0.002***
	2.Month		544.3±9.5	550.7±18.0	-1.674[b]	0.094
K1 [X±SD]	0.Month		42.2±0.7	43.0±1.6	-1.761[b]	0.078
	1.Month		42.2±0.7	43.0±1.6	-1.467[b]	0.142
	2.Month		42.1±0.7	42.9±1.6	-1.093[b]	0.274
K2 [X±SD]	0.Month		43.1±0.8	43.5±1.8	-0.030[b]	0.976
	1.Month		43.0±0.9	43.4±1.7	-0.060[b]	0.952
	2.Month		43.0±0.9	43.5±1.8	-0.464[b]	0.642
ACD (mm) [X±SD]	0.Month		3.0±0.3	3.0±0.4	-1.070[b]	0.285
	1.Month		3.1±0.5	3.1±0.5	-0.464[b]	0.642
	2.Month		3.1±0.5	3.1±0.5	-1.088[b]	0.277
AXL (mm) [X±SD]	0.Month		22.7±0.5	22.6±0.6	-0.517[b]	0.605
	1.Month		22.6±0.7	22.7±0.8	-0.979[b]	0.328
	2.Month		22.7±0.8	22.8±0.7	-2.334[b]	**0.020**

a: Chi-Square Test; b: Mann Whitney U Test, *p<0.05
BUT: Break Up Time; IOP: Intraocular Pressure; ACD: Anterior Chamber Depth; AXL: Axial Length

There is a total of 63 participants in the group, of which 38 are male (62.3%) and 23 are female (37.7%). The age average of the group is 21.7±3.6.

The distribution of the changes in the parameters over time based on gender was examined in tabular form (Table 2). There was no statistically significant difference in the LG tests results over time based on gender (p=0.870, p=0.513 and p=0.513, respectively). BUT results, on the other hand, were higher and statistically significant for males compared to females for the pre-medication period whereas there was no statistically significant difference after the medication (p=0.010, p=0.274 and p=0.124, respectively). There were no statistically significant differences over time based on gender for ST and OSDI results (p=0.278, p=0.370 and p=0.223; p=0.227, p=0.170 and p=0.121, respectively). IOP results were higher and statistically significant for males compared to females for the pre-medication period however, there was no statistically significant difference after the medication (p=0.046, p=0.419 and p=0.647, respectively). CCT results were higher and statistically significant for females compared to males 1 month after the medication started however, there was no statistically significant difference in the other periods (p=0.002, p=0.173 and p=0.094, respectively). There was no statistically significant difference over time based on gender in terms of K1, K2 and ACD results (p=0.078, p=0.142 and p=0.274; p=0.976, p=0.952 and p=0.642; p=0.285, p=0.642 and p=0.277, respectively). AXL results were statistically higher and statistically significant for males compared to females at the end of the 2 months medication period however, there was no statistically significant difference among the other parameters (p=0.020, p=0.605 and p=0.328, respectively).

The distribution of the changes in the parameters over time are shown in the table (Table 3). LG test displayed a improvement in the number of patients in Stage 1 over time whereas the number of patients in Stage 0 improved, concluding that there is a statistically significant difference (p=0.018). It was found that ST and K2 values increase over time after the medication and this difference is statistically significant (p=0.018 and p=0.032, respectively). No statistically significant difference was found in terms of BUT, OSDI, IOP, CCT, K1, K2, ACD and AXL (p=0.172, p=0.051, p=0.600; p=0.124, p=0.061, p=0.694 and p=0.285, respectively).

Table 3. Change of parameters over time

	Stage	Time			Statistics	p
		0.Month	1.Month	2.Month		
Lissamine Green [n(%)]	0.Stage	51	55	55	8.000[a]	0.018*
	1.Stage	10	6	6		
BUT [X±SD]		27.7±14.4	26.4±11.0	26.1±13.7	3.520[b]	0.172
Schirmer (mm) [X±SD]		6.2±2.8	10.9±6.2	12.3±5.6	72.120[b]	<0.001*
OSDI [X±SD]		21.5±21.0	19.5±24.3	19.1±22.5	5.940[b]	0.051
IOP (mmHg) [X±SD]		15.9±1.9	15.6±1.5	15.8±1.4	1.022[b]	0.600
CCT (µm) [X±SD]		546.3±18.8	550.9±22.2	548.3±18.7	4.172[b]	0.124
K1 [X±SD]		42.7±1.4	42.7±1.3	42.6±1.4	5.583[b]	0.061
K2 [X±SD]		43.3±1.5	43.2±1.5	43.3±1.5	6.898[b]	0.032*
ACD (mm) [X±SD]		3.0±0.4	3.1±0.5	3.1±0.5	0.731[b]	0.694
AXL (mm) [X±SD]		22.7±0.5	22.6±0.7	22.8±0.7	2.510[b]	0.285

a: Cochran's O Test; b: Friedman Test, *p<0.001
BUT: Break Up Time; CCT: Central Corneal Thickness; IOP: Intraocular Pressure; ACD: Anterior Chamber Depth; AXL: Axial Length

DISCUSSION

We found that oral doxycycline use improves the dry eye parameters in individuals with chronic eye disease.

Blepharitis is an ocular disease widely seen in the society independent of patient age and is known as the inflammation of the eyelids. Blepharitis can be acute due to various reasons such as bacterial, viral or even parasitic or it can be chronic with a repeating or idle pattern. Chronic blepharitis, which is classified under seborrheic, staphylococcal and meibomian gland disfunction, can be treated with certain type and amount of antibiotics depending on the relevant clinical practice. As such, oral tetracycline and doxycycline might serve as an effective treatment in cases of not only posterior blepharitis but also when topical medications and eyelid sanitation are not sufficient, which might be the case with most meibomian gland disfunction patients (2).

Ocular rosacea progress with non-specific symptoms like itching, burning, stinging, watering, redness, feeling of a foreign object, photophobia, pain and blurred vision. Although ocular rosacea is a commonly seen condition, it can be easily overlooked due to the non-specificity of the symptoms and the limited diagnostic criteria (15).

Before the study, BUT was found to be high in females and IOP was found to be high for males. It is assumed that the found differences are generating form the relatively limited number of participating patients. Furthermore, CCT was found to be high in females in the first month. No further comparison was done since there was no previous literature related to the effect of tetracycline on eyes. However, it is a known fact that there is cornea involvement in acne rosacea and chronic blepharitis cases (6,8,11,16). The reason for this might be neutrophil chemotaxis, lymphocyte proliferation and matrix metalloproteinase activity (3,17-19).

As a result of previous literature review, it was seen that there was very limited amount of study related to the effect of tetracycline on dry eye and that there was no complete consensus among the existing studies. The doxycycline comparison study conducted by Arman A et al. and Bilgin B et al. showed a statistically significant increase in the ST score (8,20). Andrade FMX et al., did not find any statistically significant difference in their study with 39 participants (16). In our study, we found that ST increased in a statistically significant manner after the treatment. Kashkouli et al. found that there was no statistically significant difference in terms of BUT (11). Andrade FMX et al. found statistically significant increase in terms of BUT in their study (16). In the study conducted by Arman A et al. with patients using doxycycline, no statistically significant difference was found compared to pre-treatment in terms of OSDI (8). Similarly, in our study, we found no statistically significant difference in terms of BUT and OSDI scores. Additionally, as a result of LG staining results of our study, we found that there was a statistically significant decrease in the number of patients in stage 1 after tetracycline use. Further comparison studies were not done since there was no other study on LG analysis in the literature.

Previous studies suggested that in cases of meibomian gland inflammation, eyelid sanitation and topical medication have an improving effect but not completely sufficient for the ocular surface. Hence, these primary treatments are reinforced with short-term topical steroid, long-term topical cyclosporine and oral doxycycline providing a much more desired solution to patients' complaints. Doxycycline, with its antibacterial quality and anti-inflammatory effect is commonly used in rosacea treatment and is capable of not only inhibiting neutrophil chemotaxis, angiogenesis, lymphocyte proliferation but also of blocking matrix metalloproteinase activity and collagenase and lipase production. Therefore, it has been preferred to be used in ocular rosacea and chronic blepharitis cases together with exiting topical treatments (2,3,17-19).

The limiting aspect of our study is being mono-centered and having relatively small number of participating patients. On the other hand, we have seen that although

there were a few studies on acne rosacea patients, there was no study done on chronic blepharitis patients. Therefore, the strength of our study lies in the fact that this is the first study in literature on the effects of tetracycline on cornea and dry eye parameters when used by chronic blepharitis patients.

CONCLUSION

Chronic blepharitis is a commonly seen ocular condition in ophthalmology. Additionally, dry eye is another ocular condition considerably seen in patients. Various agents are being tested for chronic blepharitis treatment. As a result of our study, we have found an improvement in the dry eye parameters of chronic blepharitis patients. Therefore, we suggest that tetracycline will be more favorable with patients suffering from chronic blepharitis and dry eye. Considering that our study is one of the first studies done on this subject, it is our belief that it would serve as an example to many more to follow. We also believe that further studies should be conducted based on multi-centered and wide spread patient participation.

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Research Article

Development of the Orbit and Eyeball during the Fetal Period

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Abstract

Aim: We aimed to investigate the morphometric development of the orbit and eyeball in the fetal period.

Material and Methods: The present study was carried out on 136 fetal eyes (86 males, 50 females) obtained from 68 fetuses aged between 15-40 gestational weeks. In this study, the area, height, width, volume, depth, and circumference parameters of the orbit were measured, while the anteroposterior and transverse diameters, weight and volume parameters of the eyeball were measured. Also, the diameter at the place where the optic nerve enters the eyeball was measured.

Results: It was determined that all parameters increased during the trimesters and there was a statistically significant difference between the trimesters in all parameters. There was no statistically significant difference in all parameters in terms of gender. In the comparison of the right and left sides, orbital area ($p<0.011$), orbital circumference ($p<0.048$) and orbital width ($p<0.048$) were higher on the right side. There was no difference between the parties in any of the parameters related to the eyeball ($p>0.05$). However, the diameter of the optic nerve was higher on the left side ($p<0.001$).

Conclusion: The data we obtained will be very useful in evaluating the pregnancy follow-up by imaging methods such as ultrasonography or magnetic resonance, in the early diagnosis of malformations or diseases, and in planning the treatment.

Keywords: Optic nerve, imageJ, trimester, morphometry

INTRODUCTION

The cavity that contains and protects the eyeball and its auxiliary formations are called the orbit. The orbit is a prism-shaped structure that is formed by the joining various bones with the base at the front and top at the back to each other (1). The eyeball is positioned forward in the orbit to protect it and to provide good vision. Therefore, the eyeball occupies only 20% of the orbital volume. The space in the orbit not occupied by the eyeball is filled with veins and nerves supported by the orbital fat and connective tissue (2).

Ocular growth in fetuses can be determined by measuring different ocular structures at various stages of the fetal period. Data on the gestational ages of the eyeball and orbit are probably associated with fetal anthropometric growth. Therefore, knowledge of the normal anatomy of the eyeball and orbit can be helpful in the early diagnosis of fetal growth abnormalities and ophthalmic pathologies.

The wide usability and development of ultrasound technology have allowed for the prenatal measurements of the eyeball as well as the orbital parameters. Most of the studies in the literature have used prenatal ultrasonography (USG) for the measurement of the eyeball and orbital parameters (2-5). More recently, it has been used to obtain the normal growth parameters of fetal eyes with the magnetic resonance imaging (MRI) method (6). Data on ocular and orbital parameters from fetuses of

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different gestational ages are probably the most accurate morphometric and morphological assessment methods. Such morphometric studies may help understand the growth of the eyeball and orbit. Therefore, in the present study, we aimed to investigate the development of the orbit and eyeball in the fetal period.

MATERIAL AND METHOD

The present study was carried out on 136 fetal eyes (86 males, 50 females) obtained from 68 fetuses aged between 15-40 gestational weeks and not having external abnormality and pathology, which were provided by Maternity and Children Hospital by receiving permission from the families between 1996-2014 in the laboratory of Faculty of Medicine, Department of Anatomy. Approval for this study was obtained from the Ethics Committee of the Faculty of Medicine. The gestational week of the fetuses was determined according to the biparietal width, head circumference, femur length, and foot length. The fetuses in the fetal period were divided into the following three groups and evaluated: fetuses between 15-25 weeks were regarded to be in the 2nd trimester, fetuses between 26-37 weeks were regarded to be in the 3rd trimester, and fetuses between 38-40 weeks were regarded to be full term. 36 eyes from the 2nd trimester, 68 eyes from the 3rd trimester, and 32 eyes from the full term were included in the study.

The eyeball was removed from the orbit. The orbits were photographed with a ruler. The photographs were uploaded to the computer, and the orbital area, height, width, and circumference were measured in the ImageJ analytical software (National Institutes of Health, Bethesda, MD) (Figure 1).

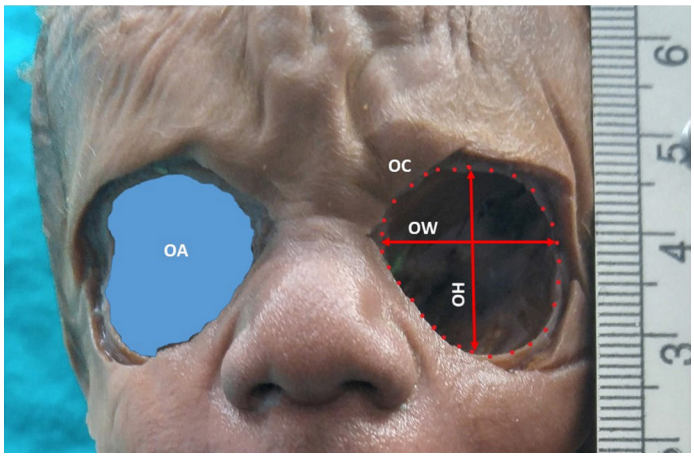

Figure 1. Measurement of orbital area, height, width and circumference. OA: Orbital area, OC: Orbital circumference, OW: Orbital width, OH: Orbital height

The mixture of Dentplus (organopolysiloxane dental impression material, EEC) mostly used by dentists and activator gel (EU) substances were applied to the orbital cavity to fill the orbital volume completely. This material was removed from the orbit after hardening and taking the exact shape of the orbit. The orbital depth (anteroposterior diameter) was measured using a digital caliper over the material that had taken the shape of the orbit (Figure 2). The orbital volume was determined by the water immersion method using the obtained orbital form.

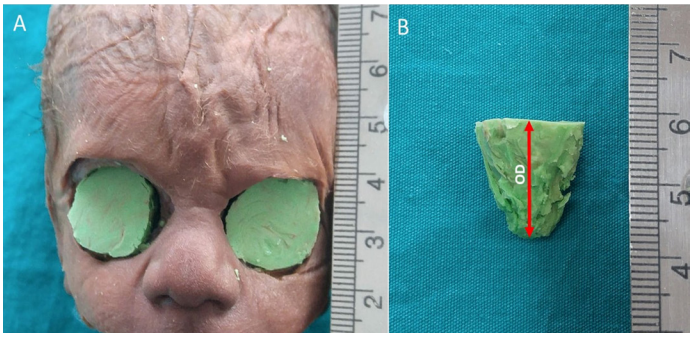

Figure 2. Orbital depth measurement. OD: Orbital depth

The tissues around the eyeball were cleaned. Physiological saline solution was injected into the eyeball with decreased intraocular fluid, and it was ensured that they reached a normal anatomical structure. The intraocular pressure was measured using a Schiotz Tonometer, and it was compared with normal values according to the scale. The intraocular pressure was measured at least three times, and the average value was calculated. Using the digital caliper, the diameter at the place where the optic nerve enters the eyeball, and the anteroposterior (AP) and transverse diameter of the eyeball (Figure 3) were measured. Using the Shimadzu AX 200 precision scales, the weight of the eyeball was measured, while the volume of the eyeball was measured by the water immersion method.

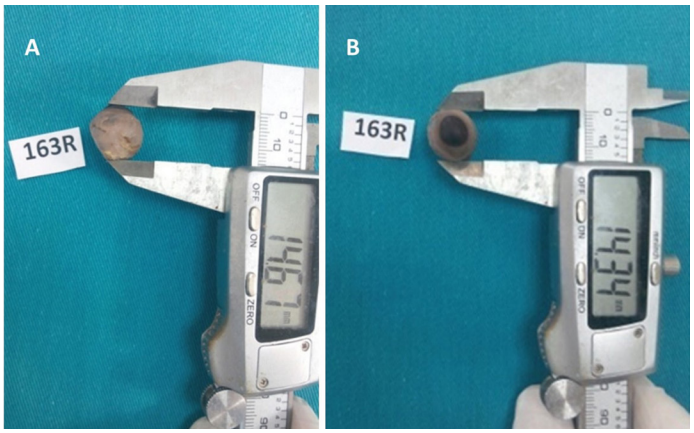

Figure 3. Diameters of the eyeball. A. Anteroposterior diameter of the eyeball B. Transverse diameter of the eyeball

Statistical Analysis

SPSS 17.0 statistical program was used in the analysis of the data. Since the data showed normal distribution, Independent Samples T-Test was used for pairwise comparisons (side and gender), and One-Way ANOVA test was used for trimester comparisons. In addition, the Pearson Correlation test was used in the correlation analysis. In the statistical analysis, the significance level of the data was taken as $p<0.05$.

RESULTS

Minimum, maximum, mean values and standard deviations

of all parameters evaluated in our study are presented in Table 1.

Table 1. Minimum, maximum, mean values and standard deviations of all parameters

	N	Minimum	Maximum	Mean	Standard Deviations
Orbital area (mm^2)	136	83.10	778.67	344.49	150.87
Orbital circumference (mm)	136	33.18	102.63	66.51	15.74
Orbital height (mm)	136	9.93	32.35	21.07	5.43
Orbital width (mm)	136	9.21	29.52	19.15	4.62
Orbital depth (mm)	136	12.17	31.41	20.57	4.08
Orbital volume (ml)	136	0.50	7.00	3.89	1.69
Eyeball antero-posterior diameter (mm)	136	6.14	21.00	14.38	2.95
Eyeball transverse diameter (mm)	136	6.92	19.30	13.92	2.37
Eyeball weight (gr)	136	0.28	3.15	1.58	0.67
Eyeball volume (ml)	136	0.10	3.00	1.48	0.71
Optic nerve diameter (mm)	136	0.94	4.31	2.57	0-.66

In the present study, the orbital area, orbital circumference, orbital width and height, orbital depth and volume were measured, and the mean and standard deviations of these parameters were calculated according to the trimesters, gender and sides (Table 2). It was determined that the orbit-related parameters increased during the trimesters and there was a statistically significant difference between the trimesters. There was no statistically significant difference in the orbit-related parameters in terms of gender. In the comparison of the right and left sides, there was no statistically significant difference in terms of the orbital height, orbital depth and orbital volume. However, it was determined that there was a statistically significant difference in the orbital area, orbital circumference and orbital width parameters between the right and left sides, and the right-side orbital values were higher (Table 2).

The AP diameter and the transverse diameter of the eyeball, the weight and volume of the eyeball, and the diameter at the point where the optic nerve enters the eyeball were measured, and the mean and standard deviations of these parameters were calculated according to the trimesters, gender and sides (Table 3). It was determined that the parameters related to the eyeball increased during the trimesters and there was a statistically significant difference between the trimesters. Upon evaluating the parameters related to the eyeball, no statistically significant difference was observed between the genders. When the right and left sides were compared, it was found that there was no statistically significant difference between the AP and transverse diameter of the eyeball, its weight and volume, but the diameter of the optic nerve was higher on the left side ($p<0.001$) (Table 3).

Table 2. Mean and standard deviation values of orbital parameters and their comparison by trimester, gender and sides

	N	Orbital Height (mm)	Orbital Width (mm)	Orbital Area (mm^2)	Orbital Circumference (mm)	Orbital Depth (mm)	Orbital Volume (ml)
^{2}nd trimester	36	15.37±4.21	14.04±3.42	192.72±122.64	49.52±13.72	16.95±3.25	2.08±1.31
^{3}rd trimester	68	21.98±4.03	19.74±3.04	353.21±107.54	68.25±10.51	20.44±2.75	4.01±1.01
Full term	32	25.57±3.59	23.66±2.77	496.70±79.50	81.94±6.33	24.92±3.08	5.64±1.14
P		<.001*	<.001*	<.001*	<.001*	<.001*	<.001*
Right	68	21.92±5.64	20.15±4.98	370.00±164.60	69.17±16.52	20.70±4.12	3.88±1.68
Left	68	20.23±5.11	18.15±4.02	318.98±132.09	63.85±14.56	20.44±4.07	3.90±1.71
P		0.069	0.011*	0.048*	0.048*	0.705	0.940
Male	86	21.14±5.64	18.99±4.88	341.94±157.32	65.99±16.68	20.25±4.11	3.81±1.68
Female	50	20.95±5.11	19.43±4.16	348.87±140.54	67.42±14.09	21.11±4.02	4.01±1.73
P		0.843	0.576	0.791	0.594	0.236	0.520

*p<0.05

Table 3. Mean and standard deviation values of eyeball parameters and their comparison by trimester, gender and sides

	N	Optic nerve Diameter (mm)	Eyeball Anteroposterior Diameter (mm)	Eyeball Transvers Diameter (mm)	Eyeball Weight (gr)	Eyeball Volume (ml)
[2]nd trimester	36	2.04±0.62	11.11±2.64	11.45±2.36	0.87±0.59	0.83±0.56
[3]rd trimester	68	2.61±0.53	15.06±1.95	14.49±1.48	1.65±0.40	1.51±0.48
Full term	32	3.09±0.51	16.60±1.78	15.49±1.77	2.22±0.47	2.14±0.61
P		<.001*	<.001*	<.001*	<.001*	<.001*
Right	68	2.37±.66	14.66±3.02	14.27±2.56	1.59±0.68	1.55±0.73
Left	68	2.77±.61	14.10±2.87	13.57±2.14	1.57±0.67	1.41±0.68
P		<.001*	0.267	0.083	0.840	0.242
Male	86	2.54±0.71	14.39±3.11	13.79±2.63	1.56±0.71	1.48±0.74
Female	50	2.63±0.59	14.36±2.67	14.13±1.86	1.62±0.62	1.47±0.65
P		0.433	0.941	0.382	0.588	0.937

*p<0.05

Table 4. The ratio of eyeball width to orbital width in trimester groups

	[2]nd trimester	[3]rd trimester	Full term
Eyeball transverse diameter (mm)	11.45±2.36	14.49±1.48	15.49±1.77
Orbital width (mm)	14.04±3.42	19.74±3.04	23.66±2.77
Eyeball transverse diameter (mm) / Orbital width (mm)	0.816	0.734	0.655

DISCUSSION

Investigating the development of the orbit, eyeball, and its attachments in the fetal period and revealing the standard parameters will guide the diagnosis and treatment of diseases in the studies to be conducted on this region. In previous studies on the orbit, the orbital depth, volume, circumference, area, width and height parameters have been evaluated in the fetal and adult periods.

In the study carried out on 70 fetuses aged 13-42 weeks, Haas et al. (7) measured the orbital depth and reported that the orbital depth increased linearly during the gestational age and there was no statistically significant difference in the parameters between the right and left sides. In the study conducted on 18 fetuses aged between 17-28 weeks, Tomasik et al. (8) indicated that the orbital depth increased together with gestational age. In our study, in parallel to previous studies, it was determined that the orbital depth increased during the gestational age (rweek=0.761, rtrimester=0.690) and there was no statistically significant difference between the right and left sides. Fitzhugh et al. (9) carried out a study on the orbital depth on 41 orbits of 21 adult skulls and determined no statistically significant difference in terms of gender. In our study, no statistically significant difference was also found in terms of the orbital depth between the genders. Hypotelorism, hypertelorism, masses, anophthalmia, microphthalmia, and cataract are some of the fetal orbital abnormalities (10). It is important to know the normal values of the orbit and to compare them with each other to identify malformations, to guide the diagnosis and treatment (11).

In order to calculate the orbital volume, Haas et al. (7) injected a silicone-like substance into the orbit and removed the substance after hardening and calculated the orbital volume. They used the weight formula while calculating the orbital volume. In the study, they found out that the orbital volume increased during the trimesters and there was no difference between the right and left sides. In a study carried out by Ji et al. (12), the orbital volume was determined by CT in 64 adults aged 18-50 years. Upon evaluating the orbital volume in terms of gender, the volume values were determined to be higher in males compared to females, but no difference was observed between the right and left sides. In our study, it was determined that the orbital volume increased during gestational age (rweek=0.818, rtrimester=0.747) and there was no statistically significant difference between the right and left sides and genders.

Goldstein et al. (4) measured the orbital circumference in the fetal period by USG in 14-36 week-old fetuses. Accordingly, they found a linear correlation between the orbital circumference and gestational week. Dilmen et al. (5) examined the relationship between bi-parietal diameter (BPD) and orbital diameters by USG in 15-40-week-old fetuses and accordingly found a linear growth between BPD and orbital diameters. Tomasik et al. (8) examined the orbital diameters in the fetal period and determined that the orbital diameters increased together with gestational age. Sukonpan et al. (13) measured the fetal orbital diameter, orbital circumference, and BPD by USG in 15-40 week-old fetuses during pregnancy and found

a strong linear correlation between the gestational week and the orbital diameter and circumference, and also they determined a strong growth relationship between the orbital diameter and BPD.

Fitzhugh et al. (9) measured the circumference of 41 orbits in 21 adult skulls and determined that the orbital circumference was wider in males. Ji et al. (12) measured the orbital circumference by CT in 64 adult individuals aged 18-50 years. Accordingly, the orbital circumference was higher in males compared to females, but no statistically significant difference was determined between the right and left sides. Seiji et al. (14) measured the orbital width, height, and circumference to investigate orbital asymmetry in 127 heads. The cases were examined by being divided into the following 4 groups: the 1st group was the intrauterine period, the 2nd group was the period between 0-2 years, the 3rd group was the period between 3-20 years, and the 4th group was the period between 21-76 years. Although the asymmetry ratios on the right and left sides were found to be higher in females, statistical significance was determined only in the 2nd group. Furthermore, these values were determined to be higher on the right side. In our study, it was determined that the orbital circumference increased during the trimesters (r=0.734) and there was a statistically significant difference between the trimesters. There was no statistically significant difference between the genders. However, a statistically significant difference was determined between the right and left sides and these values were found to be higher on the right side.

In a radiological study on the orbital area carried out on 30 fetuses aged between 18-41 weeks, Denis et al. (11) showed that there was a correlation between the orbital diameter and BPD. In the study on the orbital areas and diameter, Goldstein et al. (4) determined a linear correlation between gestational age and orbital areas and diameter. In a study conducted by USG, Dilmen et al. (5) indicated that there was a linear growth between BPD and orbital diameters. Tomasik et al. (8) demonstrated that the orbital diameter increased together with age. Sukonpan et al. (13) performed fetal orbital measurements by USG and determined a linear correlation between gestational age and the orbital area and diameter and between BPD and the orbital diameter. In the literature reviews we performed, no study on the orbital area in adults was encountered. In our study, it was determined that the orbital area increased during the trimesters (r=0.716) and there was a statistically significant difference between the trimesters. There was no statistically significant difference between the genders. A statistically significant difference was determined between the right and left sides and the orbital area on the right side was found to be larger (p=0.048).

Haas et al. (7) evaluated the orbital width and orbital height in fetuses. Accordingly, it was stated that the orbital width and height showed a linear increase with fetal age and there was no statistically significant difference between the right and left sides. Tomasik et al. (8) examined the orbital width and height in 17-28-week-old fetuses and demonstrated that both values increased with fetal age. Pommier (15) evaluated the orbital width and height in the fetal period by computed tomography (CT) and indicated that these distances could be used to determine fetal age. In a study conducted during the fetal period, Denis et al. (11) reported that there was no statistically significant difference between the genders in terms of orbital height. Fitzhugh et al. (9) measured the orbital width and height in adults and found these values to be higher in males compared to females. Ji et al. (12) measured the orbital width and height by CT. Accordingly, while there was no statistically significant difference in both parameters between the right and left sides, upon evaluating in terms of gender, the orbital width was determined to be higher in males compared to females and no statistically significant difference was found in the orbital height between genders.

According to our study, it was determined that the orbital width (r=0.743) and height (r=0.672) increased during the trimesters and there was a statistically significant difference between the trimesters. In our study, when the orbital width in the fetal period was compared between the right and left sides, it was found statistically significantly higher on the right side. However, no statistically significant difference was observed in the orbital height between the right and left sides.

In the MR study conducted on 17-39- week-old fetal eyes, Paquette et al. (16) demonstrated that the eyeball size plateaued at week 42. They determined no statistically significant difference in the eyeball width between the right and left sides. They also found that the size of all parameters in the fetuses doubled from the 17th week to the 22nd week. In a study carried out on 21-37 week-old fetuses, Ying et al. (17) determined the eyeball AP lengths and cranial AP lengths and found the ratios of these lengths. Accordingly, they found out the eyeball and cranial AP lengths increased during gestational age, but the cranial AP length was higher than the eyeball AP length from week 28 to full term. In our study, it was determined that the AP diameter (r=0.670), transverse diameter (r=0.613), weight (r=0.714) and volume (r=0.657) of the eyeball increased during the trimesters and there was no statistically significant difference between the trimesters. There was no statistically significant difference between the genders and the right and left sides. Moreover, when the width of the eyeball was proportioned to the orbital width in our study, this ratio was determined to decrease during gestational age, in other words, the orbit grew proportionally faster than the eyeball (Table 4).

In the literature reviews we performed, no study on the optic nerve diameter in the fetal period was encountered. Songur et al. (18) conducted research on 40 optic nerves in 20 adult cadavers and determined the width, height, and length of the optic nerve. In their study, the width and height of the optic nerve were measured from the midpoint of the nerve and no statistically significant difference was found between the right and left sides. In our study, the diameter

at the point where the optic nerve enters the eyeball was measured, and it was determined that the optic nerve diameter increased during the trimesters (r=0.562) and there was a statistically significant difference between the trimesters. There was no statistically significant difference in terms of gender. In the comparison between the right and left sides, a statistically significant difference was determined and the left optic nerve diameter was found to be greater.

We believe that the data we obtained will be very useful in evaluating the pregnancy follow-up by imaging methods such as USG or MR, in the early diagnosis of malformations or diseases, and planning the treatment. Our study is a basic study conducted in a large series on the orbit and eyeball during the fetal period, and the obtained data will shed light on clinical studies and studies to be conducted in the future on the eyeball and orbit.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Approval was obtained from the Süleyman Demirel University Faculty of Medicine Ethics Committee for this study (Date: 11.19.2014, Decision Number: 185).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Comparison of Cardiovascular Disease Risk Indicators in Bipolar Disorder Patients with Healthy Controls

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Abstract

Aim: It is important to assess the likelihood of cardiovascular disease in patients with bipolar disorder (BD). In this study, indicators of increased cardiovascular disease risk on electrocardiogram (ECG) and laboratory were evaluated.

Material and Methods: In the present investigation, we studied the demographic details, ECG variables, and blood test results of 90 healthy controls (HC) and 97 patients we followed for BD diagnosis.

Results: Age and gender trends were similar between the BD and HC groups (p=0.844 and p=0.664). BD had a higher mean number of fragmented QRS (fQRS) than the HC group, and fQRS was more frequent (p=0.002 and p=0.007). The frontal QRS-T angle was wider in the BD group than it was in the HC group (p=0.038). Monocyte-to-lymphocyte ratio (MLR), monocytes to high-density lipoprotein cholesterol (HDL-C) ratio (MHR), and atherogenic index of plasma (AIP) were statistically greater in BD patients (p=0.021, p<0.001, and p<0.001).

Conclusion: In brief, the report indicates that impaired ventricular repolarization is related to an elevation in the frontal QRS-T angle in BD. As a result, BD patients have a greater risk of cardiovascular mortality and ventricular arrhythmias. As a result, clinicians ought to have a greater understanding of the frontal QRS-T angle and conduct an ECG examination on regular controls.

Keywords: Bipolar disorder, frontal QRS-T angle, fragmented QRS

INTRODUCTION

Manic and depressive episodes characterize bipolar disorder (BD), a serious mental illness that lasts a lifetime. During a manic episode, symptoms such as an increase in the amount of speech, flight of ideas, exuberance, grandiose delusions, risky behaviors, increased libido, insomnia, increased energy, and agitation are observed. In a depressive episode, the patient experiences anhedonia, feelings of unworthiness and guilt, suicidal ideas, introversion, and low energy (1). The lifelong frequency of BD is predicted to be between 5% and 5%, with an average prevalence of 1.3 percent in the community.

Average life expectancy in BD decreases due to cardiovascular diseases (3). Atypical antipsychotics and lithium used in the treatment of BD are thought to elevate the incidence of cardiovascular disease. Cardiovascular mortality is thought to increase 1.5 to 2.5 times in BD (4). Hypertension can be seen in 33%, hyperlipidemia in 27%, and diabetes in 15% of BD patients (5). Using atypical antipsychotics in the therapy can cause insulin resistance, weight gain, and obesity (6).

Myocardial depolarization and repolarization heterogeneity are thought to be reflected in the frontal QRS-T angle. The frontal QRS-T angle is the actual difference between the T axis, which shows myocardial repolarization, and the QRS axis, which shows myocardial depolarization. The electrocardiogram (ECG) easily measures this brand-new

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measure. The available evidence suggests that frontal QRS-T might be a cardiovascular disease marker (7). The ECG sign of a scar in the myocardium is the fragmented QRS (fQRS). The QRS complex identifies fQRS as a notching. It is generally accepted that fQRS can predict cardiovascular diseases (8).

Kalelioğlu et al. demonstrated that BD depressive state is accompanied by increased AIP. The significant correlations between AIP and other conventional cardiovascular risk factors indicate that AIP may be more useful than absolute lipid parameters to identify BD individuals at high risk for cardiovascular disorders (9). Nunes et al. reported that AIP was increased in BD patients when compared with healthy controls (10).

Due to the deterioration of BD patients' functionality over time, changes in treatment compliance, and the treatments they receive, it is essential to assess the likelihood of cardiovascular disease. Monocyte to HDL ratio (MHR), AIP and lipid panel are used in cardiovascular disease risk assessment. In addition, there is information in the literature that Neutrophil lymphocyte ratio (NLR) is increased in cardiovascular diseases. ECG is a quick, inexpensive, simple, and quick-to-access test that can be evaluated quickly. In the present investigation, we studied the demographic details, ECG variables, and blood test results of 90 healthy controls (HC) and 97 patients we followed for BD diagnosis. We could not find any study in the literature evaluating frontal QRS-T angle and fQRS in BD patients. In this sense, the data to be obtained from this study can evaluate the risk of cardiovascular disease in the chronic period in BD patients. In addition, the evaluation of laboratory data related to cardiovascular disease risk will help to explain the differences in the mentioned ECG parameters. We wanted to use ECG parameters to figure out the chances of heart conditions in BD.

MATERIAL AND METHOD

Study Design

The current study is interpretive and comparative. The Local Ethics Committee accepted the research protocol (Approval date: 2021-09-21; IRB Number: 2021/7-1). Permission was secured from all subjects before the project. The examination was implemented following the Declaration of Helsinki. This study was carried out in Adıyaman Training and Research Hospital Psychiatry Clinic. The patients in this study were being followed up and treated in our psychiatry clinic with the diagnosis of BD. The ECGs of the patients were evaluated by a cardiology specialist with 10 years of experience.

Study Group

The sample size was calculated as 103 using G*Power (3.1 Version, Dusseldorf, Germany) (The power of test: 0.8, alpha significance level: 0.05, Cohen's d effect size: 0.71). The examination included 113 BD patients as classified by The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). This research did not include people who had hypertension (3), coronary artery disease (1), valve disease (1), arrhythmia (1), or were not between the ages of 18 and 65 (2). In addition, 8 BD patients with poor quality ECG recording were not included in the study. The study included 90 HC without psychiatric or organic disease. The participants' age, gender, smoking status, hemogram, biochemistry, blood pressure, and ECG parameters were used. The squared ratio of a person's height to their weight was used to calculate their BMI. BD patients were diagnosed by psychiatry specialists with the structured clinical interview for DSM-5 (SCID-V) (11). These BD patients did not have any additional psychiatric disorders. BD patients were in the euthymic period during our study.

Electrocardiogram Examination

Each subject was recorded while lying in a supine position using a 12-lead ECG with a value of 10 mm/mV, a velocity of 25 mm/s, and a frequency range of 0.16–100 Hz. The parameters for the QRS and QT interval were created methodically. By setting a constant heart rate of 60, the QT interval is corrected (QTc). Bazett's method adjusts the QT interval to account for heart rate (12). The ECG machine's documentation included the QRS and T axes, which were easily accessible. The report checked them, and the exact difference between the QRS and T axes was used to calculate the frontal QRS-T angle (Figure 1). Using this difference, if the angle is greater than 180°, the angle is calculated by subtracting 360° from it. Notching in the QRS complex is the definition of fQRS (Figure 2).

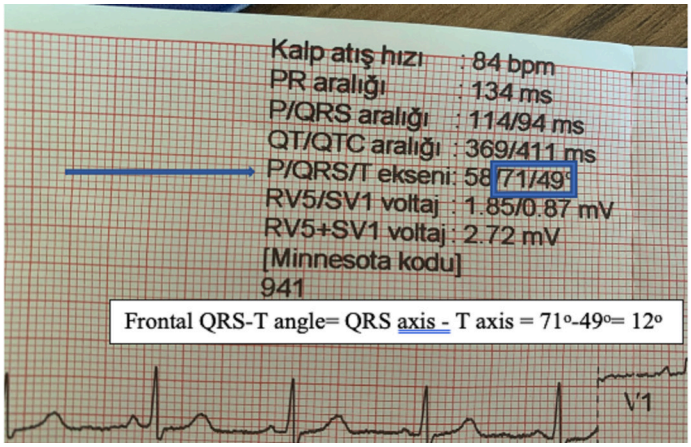

Figure 1. Calculation of frontal QRS-T angle in electrocardiogram

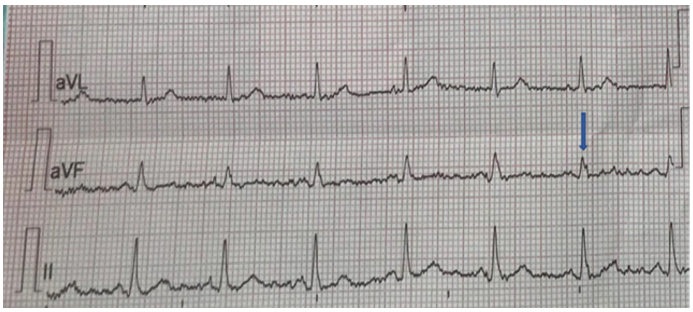

Figure 2. Fragmented QRS in electrocardiogram

Laboratory Analyses

On admission to the hospital, a venous blood sample was taken. Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (Total-C), and fasting triglyceride levels were measured. White blood cells (WBC), total blood cell counts, and biochemistry results were assessed. NLR, monocyte lymphocyte ratio (MLR), and platelet lymphocyte ratio (PLR) are computed. Additionally, the MHR and the neutrophils to albumin ratio (NAR) were evaluated. The logarithm (fasting triglyceride/HDL-C) is used to AIP.

Statistical Analysis

The SPSS application 26.0 (SPSS Inc., Chicago, IL, USA) was implemented for the statistics processing. The numerical measures were represented by mean values and standard deviations, whereas the qualitative measures were represented by percentages. The Kolmogorov-Smirnov test was carried out to look at patterns in the data. Independent samples t test was used for normally distributed data, and Mann-Whitney U test was used for data not normally distributed. Chi-square tests were performed between study groups to compare qualitative measures.

RESULTS

Sociodemographic and clinical parameters of BD patients are shown in Table 1. Accordingly, the mean disease duration of the BD patients was 11.89±8.08 and the mean number of hospitalizations was 3.01±3.91. The comparison of sociodemographic and ECG parameters of BD patients and HCs is shown in Table 2. The mean age of BD patients was 37.4±7.12, and the mean age of HCs 37.88±10.6. There were 46 females and 51 males in the BD group. There were 42 females and 48 males in the HC group. In the BD and HC groups, age and gender did not vary statistically (p=0.844 and p=0.664). There was notable dissimilarity in the BMI between the BD and the HC groups (p<0.001). In the BD group, smoking rates were remarkably greater than those in the HC group (p<0.001).

Table 1. Sociodemographic and clinical characteristics of bipolar disorder patients

Marital Status	Single=48 (49.5) Married=41 (42.3) Divorced=8 (8.2)
Education Level	Primary school graduate=39 (40.2) High school graduate=26 (26.8) University graduate=25 (25.8) Illiterate=7 (7.2)
Working Status	Not working=64 (66) Working=33 (34)
Family History of Psychiatric Disease	25 (25.8)
Alcohol and Substance Use	Alcohol use=5 (5.2) Substance use=0 (0)
Duration of Ilness, Years	11.89±8.08
Number of Hospitalizations	3.01±3.91
Treatment (Mood Stabilizers)	Valproic acid=48 (49.5) Lithium=22 (22.7) Lamotrigine=5 (5.2) Carbamazepine=4 (4.1)
Treatment (Antipsychotics)	Quetiapine=42 (43.3) Olanzapine=21 (21.6) Aripiprazole=12 (12.4) Risperidone=12 (12.4) Clozapine=5 (5.2) Paliperidone (Depot)=3 (3.1) Chlorpromazine=2 (2.1) Haloperidol=2 (2.1) Amisulpride=2 (2.1) Zuclopenthixol decanoate=2 (2.1) Trifluoperazine=1 (1.0) Paliperidone (Oral)=1 (1.0)
Treatment (Others)	SSRI and SNRI=26 (26.8) Tricyclic antidepressant=6 (6.2) Benzodiazepines=3 (3.1) Methylphenidate=1 (1.0) Modafinil=1 (1.0)

n (%) and m±sd was used to present variables. SSRI, Selective serotonin reuptake inhibitor; SNRI, Serotonin and norepinephrine reuptake inhibitor

Table 2. Comparison of sociodemographic features and electrocardiographic parameters of bipolar disorder patients and healthy controls

	BD (n=97)	HC (n=90)	*p*
Age	37.4±7.12	37.88±10.6	0.844[1]
Gender			0.664[2]
Female	46 (47.4)	42 (46.6)	
Male	51 (52.6)	48 (53.4)	
Smoking	50 (51.5)	29 (32.2)	***<0.001***[2]
BMI, kg/m²	28.45±4.7	26.8±4.8	***<0.001***[1]
Systolic blood pressure mmHg	124.3±12.3	115.4±16.4	0.352[1]
Diastolic blood pressure mmHg	78.3±6.9	72.4±7.9	0.336[1]
Heart rate, bpm	81.09±16.23	78.49±12.84	0.304[3]
QRS, msec	89.2±8.78	89.86±8.14	0.464[1]
QT, msec	364.31±28.04	364.02±34.26	0.950[1]
QTc, msec	420.07±34.64	404.66±26.07	***0.001***[3]
Frontal QRS-T angle	27.98±16.94	24.27±19.59	***0.038***[3]
Having fQRS	73 (75.3)	48 (53.3)	***0.002***[2]
fQRS count	1.35±1.15	0.99±1.24	***0.007***[3]

n (%) and m±sd was used to present variables. [1]Independent t test was used. [2]Chi-square test was used. [3]Mann-Whitney U test was used. p<0.05 was accepted as statistically significant.
BD, bipolar disorder; HC, healthy control; BMI, body mass index; fQRS, fragmented QRS; QTc, corrected QT interval

Table 3. Comparison of laboratory parameters of bipolar disorder patients and healthy controls

	BD (n=97)	HC (n=90)	*p*
Hemoglobin, mg/dL	14.52±1.52	14.34±2.07	0.493[1]
Albumin, mg/dL	4.07±0.33	4.23±0.28	***0.001***[1]
WBC, 10^3/µL	8.03±1.99	8.16±2.23	0.836[2]
Neutrophil, 10^6/µL	4.57±1.59	4.85±1.73	0.382[2]
Lymphocyte, 10^3/µL	2.59±0.92	2.64±1.06	0.692[2]
Monocyte, 10^3/µL	0.64±0.26	0.54±1.19	***0.017***[2]
Eosinophil, 10^3/µL	0.16±0.12	0.16±0.16	0.332[2]
Basophil, 10^3/µL	0.03±0.03	0.10±0.10	***<0.001***[2]
Platelet, 10^3/µL	248.98±70.75	245.08±54.47	0.749[2]
Total-C, mg/dL	179±42.16	170.11±38.47	***0.041***[2]
LDL-C, mg/dL	95.63±32.35	77.51±29.34	***<0.001***[1]
HDL-C, mg/dL	45.82±14.17	67.48±16.32	***<0.001***[2]
Fasting Triglyceride, mg/dL	192.14±159.58	125.53±99.02	***<0.001***[2]
NLR	1.97±0.93	2.09±1.11	0.658[2]
MLR	0.27±0.20	0.22±0.12	***0.021***[2]
PLR	105.31±41.47	105.46±47.60	0.674[2]
MHR	0.014±0.006	0.008±0.003	***<0.001***[2]
NAR	1.13±0.42	1.15±0.42	0.863[2]
AIP	0.54±0.35	0.19±0.32	***<0.001***[1]

n (%) and m±sd was used to present variables. [1]Independent t test was used. [2]Mann-Whitney U test was used. p <0.05 was accepted as statistically significant.
BD, bipolar disorder; HC, healthy control; WBC, white blood cell; Total-C, total cholesterol; LDL-C, low-density cholesterol; HDL-C, high-density cholesterol; NLR; neutrophil lymphocyte ratio; MLR, monocyte lymphocyte ratio; PLR, platelet lymphocyte ratio; MHR, monocyte HDL-C ratio; NAR, neutrophil albumin ratio; AIP, atherogenic index of plasma

ECG-related Parameters

The BD and HC groups had similar QT intervals, QRS, and heart rates. The BD group had a considerably longer QTc than the HC group (p=0.001). BD had a higher mean number of fQRS than the HC group, and fQRS was more frequent (p=0.002 and p=0.007). The frontal QRS-T angle was wider in the BD group than it was in the HC group (p=0.038).

Laboratory Parameters

Comparison of laboratory parameters of BD patients and HCs is shown in Table 3. Albumin levels were low in BD patients (p=0.001), but hemoglobin concentrations were similar in both groups. In summary, the numbers of WBC, neutrophils, lymphocytes, and eosinophils were similar between the groups. The monocyte number was numerically greater (p=0.017) and the basophil number was substantially less in patients with BD (p=0.001).

Fasting triglycerides, Total-C, and LDL-C were statistically greater in BD patients (p=0.041, p<0.001, and p<0.001). BD patients had statistically lower HDL-C concentrations (p<0.001). MLR, MHR, and AIP were statistically higher in BD patients (p=0.021, p<0.001, and p<0.001).

DISCUSSION

These are the primary implications of this investigation: 1) The ECG QTc interval in the BD patient group was longer than in the HC patient group, 2) fQRS number was increased in BD patients than in HC patients, and iii.) There was a wider frontal QRS-T angle in the BD group than in the HC group.

As reported in previous studies, prolongation in the QT interval is associated with syncope, sudden death, and ventricular arrhythmias. The most important arrhythmias associated with QT interval elongation are torsades de pointes and polymorphic ventricular tachycardia. QT interval elongation can cause ventricular fibrillation due to ventricular repolarization delay. Thus, prolonging the QT interval might result in cardiac arrest (13).

High frontal QRS-T angle and increased fQRS number on ECG indicate ventricular depolarization and repolarization abnormalities. The high rate of these ECG abnormalities in the bipolar disease group in our study may explain why the probability of ventricular arrhythmia and cardiovascular mortality increased 1.5-2.5 times in the BD group compared to healthy individuals (4).

Several researches have documented that severe mental illnesses are associated with cardiovascular mortality. It has been demonstrated that patients with BD and schizophrenia have elevated cardiovascular mortality scores than the general population (14). Also, it has been established that BD patients are more prone to metabolic syndrome (15). The higher BMI, smoking rate, increased LDL-C and reduced HDL-C in the BD group could all be predictors of higher cardiovascular mortality.

Inflammatory processes, neurotrophic factors, oxidative stress, and microvascular events have been associated with cardiovascular disease risk in BD patients (16). High blood C-reactive protein, tumor necrosis factor-a, and interleukin-6 concentrations have been related to the presence of atherosclerosis in BD (17,18).

Bortolasci et al. documented that in BD patients there is an increase in fasting triglyceride level and a lower HDL-C (19). Increased oxidative stress, decreased endogenous antioxidants and anti-inflammatory agents, increased AIP were found be useful to delineate BD patients at risk for comorbid cardiovascular disorders (20).

Systemic inflammation has been shown to play a significant role in cardiac arrhythmias and conduction disturbances. The possible reason of cardiac arrhythmias and conduction disturbances seems to be related to myocardial inflammation, focal fibrosis, and ischemia within the conduction system (21). In a recent study, Kadi et al. showed that fQRS increased even in patients with rheumatoid arthritis without cardiovascular disease, in which it is speculated that inflammatory processes may play a pivotal role to produce fragmentations on ECG (22).

In 2012 Çetin et al. found that fQRS was related to increased C-reactive protein. fQRS that may result as an end effect of inflammation at cellular level can represent increased cardiac risk by different causative mechanisms in patients with acute coronary syndrome (23).

In the literature review, large-scale studies investigate repolarization abnormalities in psychiatric disorders and examine their relationship with QTc, QT interval, QT dispersion, and frontal QRS-T angle. In a study evaluating the frontal QRS-T angle with schizophrenia in May 2022, the frontal QRS-T angle was wider in schizophrenia patients than in the control group, as in our study (24). However, no studies have been published in the literature examining the possible link between frontal QRS-T angle and BD. Hence, our study is essential in terms of its contribution to the literature.

This study has some limitations. Since the drugs used by BD patients may affect the ECG parameters, it is a limitation that this information was not provided in our study. In the literature, it has been observed that antidepressants, lithium, and antipsychotic drug combinations may exacerbate the possibility of cardiovascular disease by altering the QT interval and ventricular repolarization. For this reason, it is also important whether the patient group included in our study received combined drugs or not (25). In addition, since patients' blood electrolyte and thyroid hormone concentrations will change the ventricular repolarization parameters, not examining them is another limitation of our study. More extensive laboratory and detailed studies, such as blood drug levels, are needed for the relation between BD and frontal QRS-T angle.

CONCLUSION

The findings of the current research reveal that the frontal QRS-T angle increases with impaired ventricular

repolarization in BD. Therefore, the probability of ventricular arrhythmias and cardiovascular mortality is elevated in BD patients. Therefore, clinicians should have more information about the frontal QRS-T angle and perform ECG evaluation in normal controls. Through this way, cardiovascular mortality, and morbidity can be diminished in BD patients..

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MEDICAL RECORDS-International Medical Journal

Research Article

Diagnostic Accuracy of MRI Evaluation of Patellar Position According to the Physeal Line in Pediatric Patients

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Abstract

Aim: Abnormal positioning of the patella, of which the superior position is defined as patella alta (PA), whereas the inferior position is defined as patella baja (PB). Most of the measurements of patellar position evaluations are time-consuming. In this study, we aimed to examine the diagnostic accuracy of visual evaluation of the patellar position according to the physeal line and to determine the inter- and intraobserver agreement of this evaluation in MRI examinations.

Material and Methods: Knee MRI examinations performed between 2019-2021 with different knee symptoms and prediagnoses were retrospectively analyzed in this study. As a reference test, Insall-Salvati Ratio was calculated by the following formula: Tendon length/patellar length. Two visual evaluation methods were used; physis line to the patella (PLP) and physis line to patellar joint cartilage (PLC).

Results: Three hundred and sixty consecutive children aged 60-215 months were included in the study. There was excellent an agreement of both intra- and interobserver on PLP and PLC for two observers (κ > 0.800, for all). When we evaluated intra- and interobserver agreements according to groups, almost perfect agreements were detected (κ > 0.750, for all). Diagnostic accuracy for both two observers on the visual evaluation of PLP was almost perfect (Sensitivity 95.5%, specificity 87.2% for observer 1, and Sensitivity 94.7%, specificity 87.2% for observer 2), and on the PLC evaluation was also good (Sensitivity 89.3%, specificity 82.9% for observer 1, and Sensitivity 88.1%, specificity 85.5% for observer 2).

Conclusion: Even though direct radiography is used in the diagnosis of PA and PB, it has been shown that MRI can also be used in pediatric patients in daily practice. Instead of the time-consuming measurements used in MRI, it may be kept in mind to use these methods in our study for practical and accurate diagnosis.

Keywords: MRI, insall-salvati ratio, patella alta, patella baja, agreement, pediatric patients

INTRODUCTION

The vertical position of the patella, of which the superior position is defined as patella alta (PA), whereas the inferior position is defined as patella baja (PB), is clinically important. Abnormal patella positioning leads to anterior knee pain and patellar dislocation (1,2). Patellar dislocation is associated with patellar chondromalacia and joint effusion (3–5).

Several methods are used in defining the patellar position; however, the Insall-Salvati ratio (ISR) is the most commonly used method. ISR is the ratio of patellar tendon length to patella length and determines the position of the patella in lateral knee radiography with the knee in 30° flexion (6). The use of ISR in other imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography (US) in determining patellar position in children and adults has been reported in the literature (7-10).

MRI enables and eases the measurements of non-ossified cartilage components in children, compared to radiographs in which ossification is necessary to measure patellar tendon length and patellar height (1). ISR is the most accepted technique since it is not dependent on the

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degree of knee flexion and applicability in MRI (1,11). So, ISR can be applied to MRI despite position differences (11). In most age groups, PA is defined as a ratio bigger than 1.2, and PB is defined as lower than 0.8 (12).

MRI is an imaging method that can be used for various reasons in children, and the abnormal patellar position, which is one of the causes of knee pain, has been shown to be evaluated with MRI. Most of the measurements of patellar position evaluations are also time-consuming. For these reasons, we aimed to examine the usability of visual evaluation of the patellar position according to the physeal line instead of ISR measurement in MRI evaluations and to test the intra- and interobserver agreement of this evaluation.

MATERIAL AND METHOD

Knee MRI examinations performed between 2019-2021 with different knee symptoms and clinical history were retrospectively analyzed in this study. Three hundred and sixty consecutive children aged 60-215 months were included in the study. Five years old was determined as the lower age threshold for inclusion because patellar ossification starts at this age (15). Patients with closed physeal line but not discernible, patients with bipartite patella, patella fracture, prominent suprapatellar fluid, patellar dislocation, and anterior cruciate ligament rupture were excluded from the study. Malatya Turgut Özal University Ethical Commitee, Decision ID:2022/129)

Examinations were performed with MRI, 1.5-T system (SIEMENS Magnetom Amira, Germany), and slice thickness was 3 mm. Visual evaluation and measurements were done on T1-weighted sagittal sequences. The location of the patella was assessed visually according to the femoral distal epiphysis line. Two different visual assessment methods were used; the line from the physis to the patella (PLP) and the line from the physis to the articular cartilage (PLC). In the PLP method, an imaginary line is drawn from the central apex of the physeal line to the patella in the section where the longest axis of the patella is observed in sagittal MRI. This imaginary line was considered "normal" if it corresponded to the middle 1/3 of the patella length, "PB" if it corresponded to the upper 1/3, and "PA" if it corresponded to the lower 1/3 (Figure 1). In the PLC method, an imaginary line is drawn from the central apex of the physeal line to the patellar cartilage in the section where the longest axis of the patella is observed in sagittal MRI. This imaginary line was considered "normal" if it corresponded to the middle 1/3 of the patellar cartilage length, "PB" if it corresponded to the upper 1/3, and "PA" if it corresponded to the lower 1/3 (Figure 2). MRI evaluations were performed blind to the symptoms of patients.

Visual evaluations were performed by two pediatric radiologists two times within four weeks period. Each researcher was blinded to the previous self-evaluation results and the other researcher's results. Measurements of ISR were performed by one pediatric radiologist and used for comparison with visual assessments.

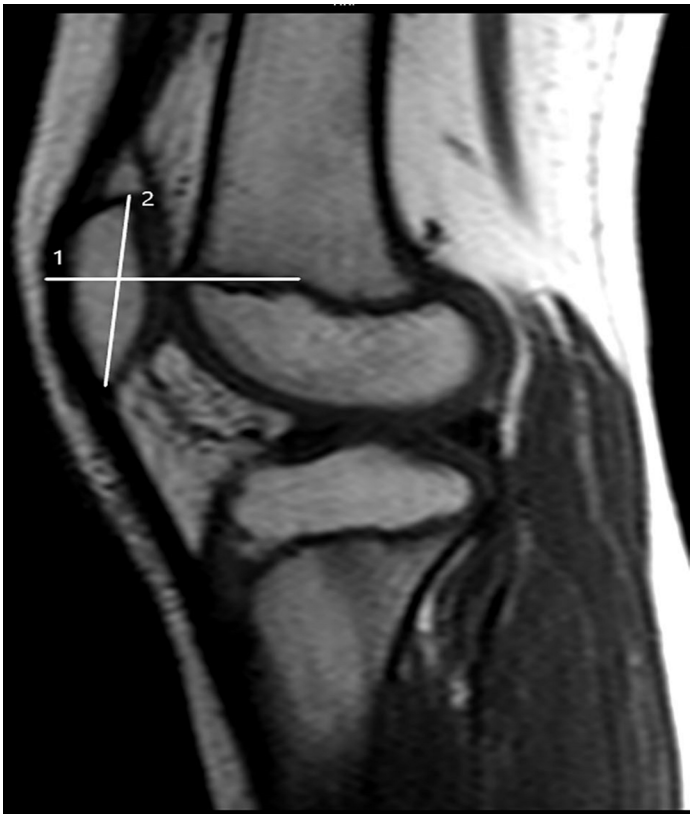

Figure 1. PLP method a normal patellar position, imaginary physeal line to the patella (1) and imaginary patellar length (2)

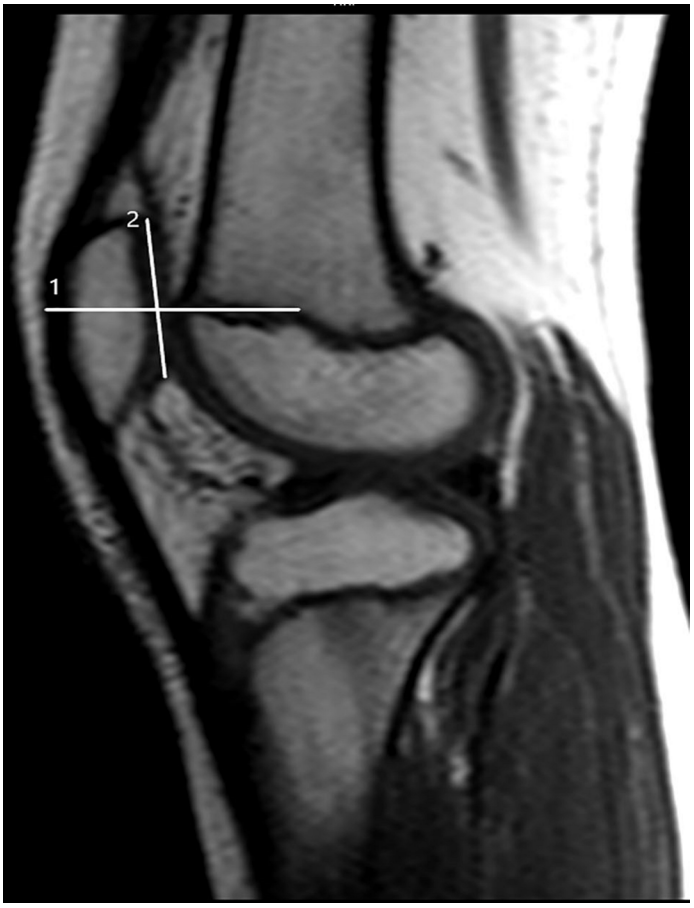

Figure 2. PLC method a normal patellar position, imaginary physeal line to the patella (1) and imaginary patellar cartilage length (2)

For ISR calculation, patella length (PL), the longest craniocaudal length of the patella, and patellar tendon length (TL) from the lower patellar pole to insertion on the tibial tuberosity from the dorsal side were measured in MRI (Figure 3). If the patellar tendon origin on the lower patellar pole or insertion on tibial tubercle was in a different sagittal sequence, the patellar tendon origin was marked, then the sequence was changed to distal insertion, and measurement was terminated on this sequence. ISR was computed by the following formula: TL/PL. If ISR was between 0.8 and 1.2 defined as "normal"; if smaller than 0.8 defined as "PB"; and if larger than 1.2 defined as "PA" (12). The inter-observer and intra-observer variability and agreement of ISR measurements and observers' visual evaluation were statistically evaluated.

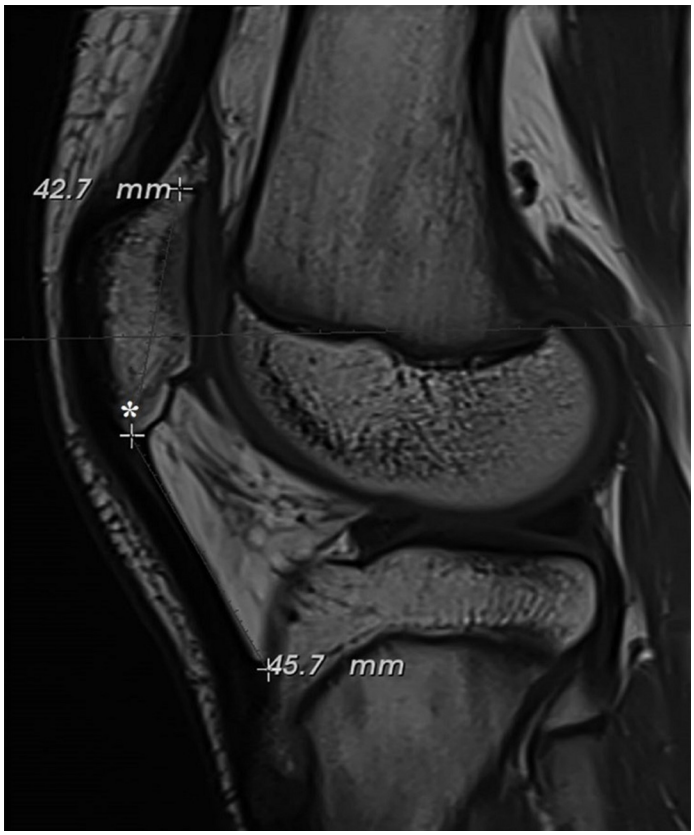

Figure 3. ISR calculation; the longest craniocaudal length of patella length (PL), patellar tendon length (TL), imaginary physeal line. Astral = Tongue-like extension on the inferior patellar without articular surface

Statistical Analysis

Statistical analysis was performed with SPSS Statistics (version 24.0; IBM Corp., USA) and Jamovi (version 1.6.23.0). The normality of data was assessed with Shapiro Wilk Test. ANOVA test was used in the analysis of continuous variables between groups. The Bonferroni test was used for post-hoc analysis. Kruskal-Wallis test and Chi-Square test were used in the analysis of categorical variables. Pearson and Spearman correlation tests were used in correlation analysis. Cohen's kappa test was used in intra- and interobserver reliability analysis. The power of interobserver reliability was defined as follows: slight if $\kappa \leq 0.20$; fair if $\kappa = 0.21$ to 0.40; moderate if $\kappa = 0.41$ to 0.60; substantial if $\kappa = 0.61$ to 0.80; and almost perfect if $\kappa = 0.81$ to 1.00 (13). Specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) were assessed for diagnostic accuracy, validity, and reproducibility. $p<0.05$ was accepted as statistically significant.

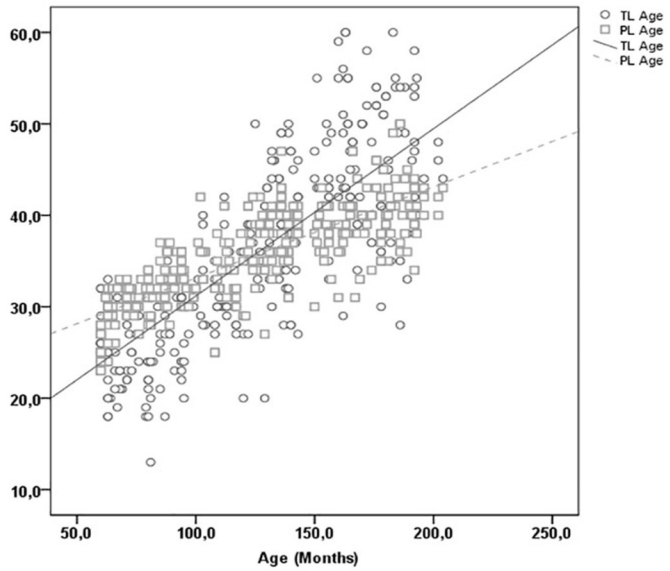

Figure 4. Relationship between patella and tendon length and age

RESULTS

Children were divided into three groups according to age: group 1: 60-107 months, group 2: 108-143 months, and group 3: 144-215 months. Age, gender, PL, TL, ISR, PA, and PB data are given in Table 1. TL, PL, ISR, and PA were found to increase with age, while PB decreased with age.

Table 1. Demographic parameters of the study population

	Group 1 (n = 120)	Group 2 (n =120)	Group 3 (n =120)	*p* value
Age, mounth	79.1±13.6	128.6±10.6	173.6±13.8	<0.001
Gender, male (n, %)	67 (55.6%)	63 (53.4%)	64 (54.2%)	0.941
Tendon length (mm)	26.8±4.9	36.7±6.2	44.9±7.2	<0.001
Patellar length (mm)	31.0±3.4	36.6±3.8	39.9±3.5	<0.001
ISR	0.87±0.16	1.01±0.16	1.13±0.21	<0.001
PA (n, %)	3 (2.4%)	19 (16.4%)	42 (35.0%)	<0.001
PB (n, %)	42 (33.9%)	8 (6.9%)	3 (2.5%)	<0.001

ISR = Insall-Salvati ratio, PA=Patella alta, PB=Patella baja, TL= Tendon length, PL=Patellar length

A positive correlation was found between age with PL and TL (r=0.795, p<0.001 and r=0.800, p<0.001, respectively).

Table 2. Intra- and interobserver variability of values according to the patella and cartilage measurements

Factor	Intraobserver aggreement (Kappa)	Interobserver aggreement (Kappa)
PLP		
Observer#1	0.965	0.931
Observer #2	0.930	
PLC		
Observer#1	0.944	0.880
Observer #2	0.891	

PLP = Physis line to patella, PLC = physis line to patellar joint cartilage

Intra- and interobserver agreements of values PLP and PLC are shown in Table 2. There was an almost perfect agreement of both intra- and interobserver on PLP and PLC for two observers.

Excellent agreement was detected when we evaluated intra- and interobserver agreements according to groups. With increasing age, intra- and interobserver agreements in PLC were lower than in PLP; however still well and acceptable (Table 3).

When sensitivity, specificity, and positive and negative predictive values of PLP and PLC were evaluated (Table 4), high values were detected for validity and reproducibility.

Table 3. Intra- and interobserver variability of the groups of values according to the patella and cartilage measurements

Factor	Intraobserver aggreement (Kappa)	Interobserver aggreement (Kappa)
Group 1		
PLP		
Observer#1	0.930	0.947
Observer #2	0.982	
PLC		
Observer#1	0.981	0.893
Observer #2	0.965	
Group 2		
PLP		
Observer#1	0.978	0.933
Observer #2	0.909	
PLC		
Observer#1	0.863	0.786
Observer #2	0.758	
Group 3		
PLP		
Observer#1	0.982	0.897
Observer #2	0.879	
PLC		
Observer#1	0.951	0.901
Observer #2	0.885	

PLP = Physis line to patella, PLC = Physis line to patellar joint cartilage

Table 4. Sensitivity, specifity, positive and negative predctive values of the patella and cartilage measurements

	Patella				Cartilage			
	Sensitivity	**Specifity**	**PPV**	**NPV**	**Sensitivity**	**Specifity**	**PPV**	**NPV**
Observer #1	95.5%	87.2%	93.9%	90.3%	89.3%	82.9%	91.6%	78.9%
Observer #2	94.7%	87.2%	93.9%	88.7%	88.1%	85.5%	92.6%	77.5%

PPV = Positive predictive value, NPV = Negative predictive value

DISCUSSION

The main findings of our study are as follows: i) TL, PL, ISR, and PA increased with increasing age while PB decreased, ii) Both intra- and interobserver agreements of values according to the patella and cartilage measurements had excellent results, (iii) when this agreement was assessed according to groups we found perfect agreement, highly usable in terms of validity and reproducibility.

Multiple time-consuming measurements and proportional calculations are required for methods used to evaluate the patellar position in daily practice. ISR is the most sensitive and reproducible method to rule out PA, and the most widely used method in clinical settings for all age groups (14,15). Verhulst et al. (11) showed that, although radiography and CT have high reliability, only ISR has an acceptable agreement between radiography and MRI. It has been demonstrated that the ISR value calculated on MRI and radiographs can be used interchangeably, regardless of the threshold value used (6,7). Kurowecki et al., in their comparative study of 49 pediatric patients with unfused growth plates, demonstrated a strong association between ISR and PA derived from MRI and radiographs in children ages 7.5 years and older (7). In this observational study, when compared with ISR, visual inspection, according to the physis line for detection of abnormal patellar localization, may be used as a sensitive and specific method with high validity and reproducibility.

It is known that MRI is safer than radiography imaging, because direct X-ray contains ionizing radiation and stochastic effects of X-ray especially in children. It has been also stated that MRI may be more accurate in determining the ISR due to the inclusion of non-ossified cartilage in the true patellar length measurement, but

the ratio could potentially be kept higher (7). However, in the present study, ISR was found to be lower in younger patients, contrary to the previous studies (7,10). In the comparison study of Kurowecki et al., the age of inclusion of the patients was 7.5 years, while in our study, this value was five years. Including a smaller age group in the present study and including non-ossified components in measurements may result in higher PL measurements. Smaller TL in small age groups may lead to lower ISR values (7). In our study, the youngest age of the patients was five years old. We think that the inclusion of the younger age group in the study, and the longer PL measurement due to the inclusion of components that will ossify with age in the measurement, and the shorter TL due to younger age may be possible reasons for these small ISR values. Among the possible reasons for this, we think that the non-ossifying cartilage segments are included due to the evaluation of patellar height with MRI in ISR, and that the denominator is kept partially constant in this proportional calculation. At the same time, the numerator (TL) increases with age (Figure 4). However, large-scale randomized studies are needed to support our results.

It has been stated that another important factor in determining the patella height is the position of the articular surface of the patella relative to the trochlear cartilage (16,17). Biedert et al. aimed to describe a new method of measuring patellar height using the true articular cartilage on MRI and found that measurements of the articular cartilage congruence can help define an underlying pathology of patellar height (16). In our study, we found that with our visual method, which can detect patellar pathology faster and more accurately in patients undergoing MRI scans, both inter- and intraobserver agreement are almost perfect, and this method has high validity and reproducibility with high specificity and sensitivity rates. It has been stated that since the relationship between ISR and cartilage surface change may also differ due to changes in patella type, this may underestimate the incidence of PA or overestimate PB (18). Although we had high rates for both in our study, we think that the specificity and sensitivity values of cartilage assessments are relatively lower than patellar assessments, and this is due to the shape difference of the patella (Figure 3).

There were some limitations of our study. First, in this retrospective study, the entire extended knee position in MRI could not be fixed, where 30° flexion is a standard position in knee radiography. Another possible confounding factor in determining patellar tendon length on MRI is that not all patellar tendons are straight and appear retractile. The other is that our study was done without knowing the patient's clinical history.

CONCLUSION

To the best of our knowledge, our study is the first to visually evaluate patella localization relative to the physeal line with MRI. Although direct radiography is used in the diagnosing PA and PB, which is the subject of this study, it has been shown that MRI can also be used in pediatric patients in daily practice. Instead of the time-consuming measurements used in MRI, it may be kept in mind to use these methods in our study for practical and accurate diagnosis. Large-scale randomized studies are needed to support our results.

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MEDICAL RECORDS-International Medical Journal

Research Article

Investigation of the Sox-9 and Cited-1 Immune Activity in Placentas of Women with Placenta Accreta

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Abstract

Aim: In this study, we investigated the immune activity of the Sox-9 and Cited-1 in women with placenta accreta.
Material and Methods: 20 healthy and 20 placenta accreta were processed for routine histological tissue processing. Placentals samples were dissected and fixed in 10% formaldehyde solution. Samples were embedded in paraffin blocks. Clinical and biochemical parameters were recorded. Placental sections were cut from paraffin blocks and stained with Sox-9 and Cited-1 immunostaining.
Results: In our study, control group showed negative Cited-1 expression in decidual cells, root villi and connective tissue areas in general. Placenta accreta group showed increased Cited-1 expression in degenerated decidual cells, fibroblastic cells and endothelium. In control group, Sox-9 expression was negative in the syncytial knots, in the vascular endothelial cells. In placenta accreta group, Sox-9 reaction was positive in the root villi, in the blood vessels, in the connective tissue.
Conclusion: It was observed that the Sox-9 reaction was increased and inflammation was induced, depending on the differences in decidual cells, in the syncytial area and in the vascular endothelium in in placentas of women with placenta accreta. It is thought that Sox-9 signaling processes are being determined and Cited-1 may be stimulants that affect cell proliferation and angiogenesis regulation and affect placental development.

Keywords: Placenta accreta, Sox-9, Cited-1, immunostaining

INTRODUCTION

Placenta is a temporary organ which regulates many activities between fetus and mother. Placenta supply nourishment to fetus, secretes hormone for the continuation of pregnancy (1). Placenta is implanted in uterus but the correct placement is important. In such cases, placement of placenta prevents delivery of baby it placenta is low lying. This clinical condition can also cause unusual bleeding during pregnancy or delivery (2). These abnormal placental implantation causes abnormal placental development and prevent the nourishment of fetus by fetal and maternal blood circulation. Structural changes such as thickening of the basement membrane of fetal capillaries, increased fibrous tissue in the villous stroma and fibrinoid accumulation in chorionic plate and on root villi in the junction may be observed (3,4).

Placenta accreta spectrum (PAS) is a syndrome characterized by the abnormal implantation of the placenta to the uterus, where it is invasive or adherent. Its other name is morbidly adherent placenta (5). In this spectrum, trophoblast invasion occurs abnormally into the myometrium. This invasion can sometimes cross the tunica serosa. In PAS, if the placental villi are attached to the myometrium, it is defined as placenta accreta. The incidence of placenta accreta in PAS is 80% (6). The etiology of placenta accreta is unknown, but abnormal

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decidualization or absence of decidualization is the most accepted theory. Reasons such as a history of cesarean section, surgical interventions, uterine curettage, and advanced maternal age are also risk factors for placenta accreta. Another theory is excessive extra-villous trophoblastic invasion (7).

Immunohistochemistry is a molecular technique to localize the specific protein of interest in the tissue. It is commonly used for pathological diagnosis of many diseases such as cancer. In this study, we aimed to investigate expression level of Sox-9 and Cited-1 in placentas of women diagnosed with placenta accreta by immunohistochemical techniques.

MATERIAL AND METHOD

Ethical approval was taken from Dicle University Clinical Research Ethics Committee. In our study, 20 healthy women and 20 women with placenta accreta were included. Placentas were obtained from Gynecology and Obstetrics Clinics. All patients signed informed patient consent form. Biochemical and clinical parameters for each patient were recorded. Placental tissues were processed for routine paraffine wax embedding protocol.

Histological tissue processing

Placental tissues were excised and taken into 10% formaldehyde solution for 2 days. Then samples were washed in tap water for 24 hours. Samples were put into alcohols to remove the water. Tissues samples were soaked in xylol solution for 20 minutes two times. Tissues samples were taken into 58°C incubator to incubate in paraffin wax. Tissue parts were put into paraffin blocks. 5 µm thick sections were cut with rotary microtome. Sections were put into xylene to remove excess paraffine for 15 minutes two times. Sections were dipped into alcohols and washed in distilled water. Sections were stained with routine Hematoxylin and Eosin and Sox-9 and Cited-1 immunohistochemical staining (8).

Immunohistochemical Analysis

Formaldehyde-fixed tissue was embedded in paraffin wax for further immunohistochemical examination. Sections were deparaffinized in absolute alcohol. Antigen retrieval process was performed twice in citrate buffer solution (pH:6.0), first for 7 minutes, and second for 5 minutes, boiled in a microwave oven at 700 W. They were allowed to cool to room temperature for 30 minutes and washed twice in distilled water for 5 minutes. Endogenous peroxidase activity was blocked in 0.1% hydrogen peroxide for 20 minutes. Ultra V block (Cat. No:85-9043, Invitrogen, Carlsbad, CA, USA) was applied for 10 minutes prior to the application of primary antibodies Sox-9 and Cited-1 (AFG scientific, US, 1:100) Secondary antibody (Cat. No:85- 9043, Invitrogen, Carlsbad, CA, USA) was applied for 20 minutes. Slides were then exposed to streptavidin–peroxidase for 20 minutes. Chromogen diaminobenzidine (DAB Invitrogen, Cat. No:34002 Carlsbad, CA, USA) was used. Control slides were prepared as mentioned above but omitting the primary antibodies. After counterstaining with hematoxylin and washing in tap water for 8 minutes and in distilled water for 10 minutes, the slides were mounted with Entellan (9).

Statistical Analysis

The data were recorded as median (minimum – maximum). Statistical analysis was done using the IBM SPSS 25.0 software (IBM, Armonk, New York, US).

RESULTS

Age, gravida, parity, systolic BP, diastolic BP, hemoglobin, platelet, glucose, urea, creatinine, ALT, AST-urine protein was recorded in healthy and placenta accreta women. Data were shown in Table I.

Table 1. Patients characteristic and their blood test values were listed

Parameter	Healthy (N=20)	Accreta (N=20)
Age	20 (42-26)	21 (45-28)
Gravida	0 (5-2)	1 (8-3)
Parity	0 (3-0)	0 (7-2)
Systolic blood pressure	90 (144-110)	136 (200-148)
Diastolic blood pressure	64 (82-69)	92 (118-96)
Hemoglobin	9.8 (14.3-12)	9.45 (14.7-10.4)
Platelet	110 (358-231)	168 (412-269)
Glucose	65 (102-76)	64 (110-78)
Urea	11 (21-15)	13.5 (35.8-16)
Creatinine	0.52 (0.72-0.61)	0.51 (0.8-0.57)
ALT	7 (25-12)	8 (51-12)
AST	12 (55-18)	11 (45-22)
2h-urine protein	102 (179-142)	300 (920-541)

Histopathological staining

Figure 1 shows Cited-1 and Sox-9 immune staining. In the Cited-1 immunohistochemical staining of the control group, it was determined that the Cited-1 expression was negative in decidual cells, root villi and connective tissue areas in general, while the Cited-1 reaction was found to be positive in the sections with syncytial nodes (Figure 1a). Placenta accreta group Cited-1 immunohistochemical staining showed a positive reaction in maternal areas, especially due to the degeneration of decidual cells. It was observed that the cited-1 reaction was also positive in fibroblastic activities with collagenized degenerations where hyalinized areas were found. Positive vascular endothelium was also observed (Figure 1b). In the examination of the Sox-9 immunohistochemical staining section of the control group, it was observed that Sox-9 expression was negative especially in the syncytial regions and in the maternal area. Again, negative Sox-9 expression was found in the vascular endothelial cells in general. Positive Sox-9 expression was detected in some syncytial

nodes (Figure 1c). In the Sox-9 immunohistochemical staining of the Placenta Accreta group, it was observed that the Sox-9 reaction of the syncytial cells from the root villi in the maternal region was positive in the histopathological examination. Again, it was determined that Sox-9 was positive in the endothelial cells in the blood vessels, in the connective tissue cells in between, and in areas where leukocyte cell infiltration is intense. It was thought that Sox-9 could play an important role in trophoblastic regulation (Figure 1d).

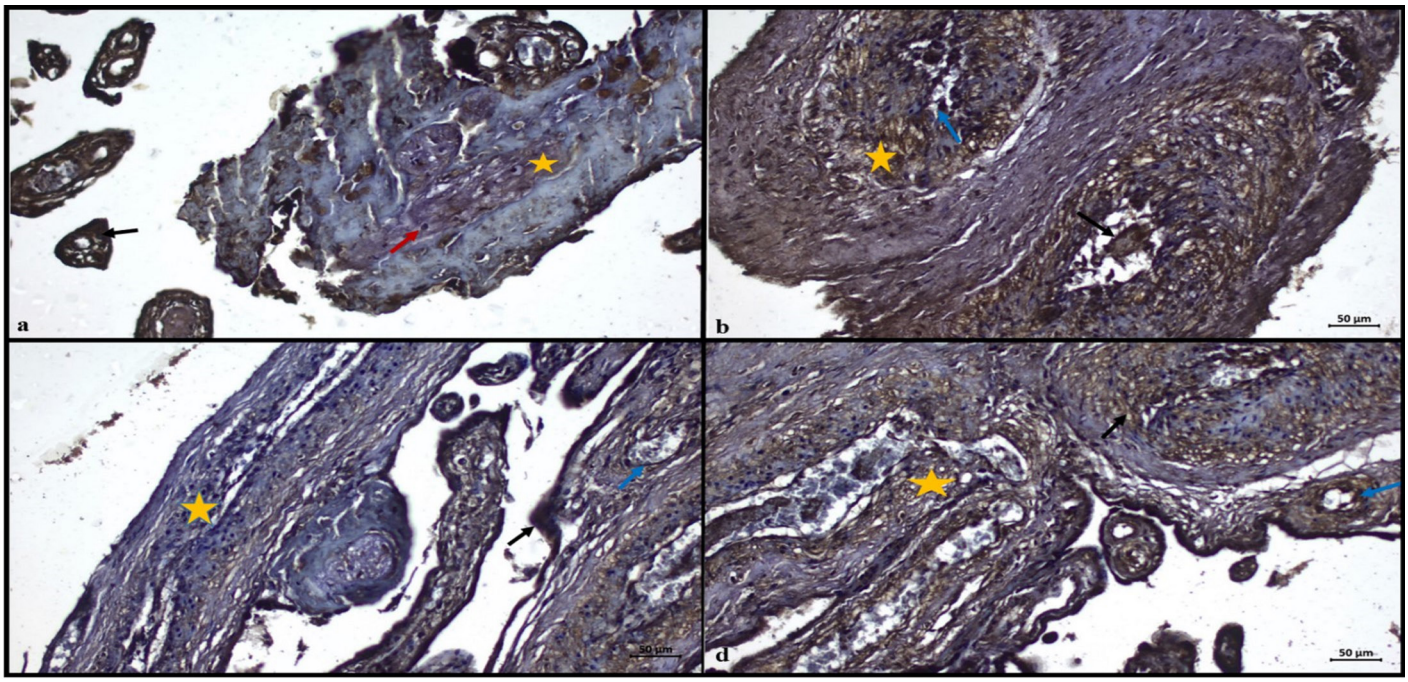

Figure 1. a) In the control group Cited-1 immunohistochemical staining, negative Cited-1 expression in decidual cells (red arrow) and connective tissue areas (yellow star), positive Cited-1 expression in syncytial nodes (black arrow); b) Placenta Acreata group Cited-1 immunohistochemical staining showed positive cited-1 reaction in degenerated decidual cells (blue arrow), connective tissue areas (yellow star), vascular endothelium (black arrow); c) Negative Sox-9 expression in maternal region (yellow star) and vessel endothelium (blue arrow) in Sox-9 immunohistochemical staining of control group, positive Sox-9 expression in syncytial nodes (black arrow); d) In the Sox-9 immunohistochemical staining of Placenta Acreata group, positive Sox-9 expression in syncytial cells (yellow star), vascular endothelium (blue arrow), and connective tissue cells in between (black arrow) from root villi

DISCUSSION

Placenta is an organ that develops within uterine wall and provide metabolic exchanges between fetus and mother (10). Placental abnormalities depend on the anatomical location of implantation. Placenta previa is a placental abnormality where placenta lie on the lower segment of the uterus, completely or partially covering cervix (11). Placenta previa is still a leading reason of maternal, fetal and neonatal morbidity and mortality characterized by third trimester bleeding. Pathophysiology of placenta previa is still not fully understood however many factors such as maternal age≥35, multiparity, multiple pregnancy, previous cesarean history and smoking increases risk of placenta previa (12,13,14,15). Otçu et al. examined the placenta of previa patients and revealed that increased syncytial knots, intervillous hemorrhage, fibrin accumulation, and hyalinization (16). Studies on histopathology of placenta previa revealed fibrinoid necrosis, polymorphonuclear cell infiltration, abnormal vasculatures, dilated vessels. Biswas et al. recorded increased trophoblastic giant cells, hemorrhage, absence of chorionic villi in the myometrium and inflammation in placenta previa tissues (17). Silver et al. also reported increased villous infarction with fibrinoid and congested vessels in pathological examination of placenta previa (18). Jung et al. studied 93 patients with placenta previa in terms of histological perspectives. They found that maternal under perfusion, villous infarction, increased intervillous fibrin deposition in their histopathological findings (19).

Cited is a coactivator in transcription and possibly responsible for melanocytes pigmentation. It mediates events in transcription regulated by estrogen (20). Cited consists of four nuclear proteins as Cited-1, Cited-2, Cited-3, and Cited-4. Since Cited protein has no DNA binding site, its role is mainly transcriptional regulator (21). Sriraman et al. studied progesterone receptor in cultured granulosa cells and found that progesterone receptor induced many genes that regulated granulosa cells activity (22). one of the genes was Cited-1 that is affected by progesterone receptor during ovulation. Hatzirodos et al. investigated the transcriptome profile of granulosa cells in bovine ovarian follicles. author found that as follicle develops larger, transcriptional regulators was high in number (23). One of the regulators was Cited-1. In our study, control group showed negative cited-1 expression in decidual cells, root villi and connective tissue areas

in general (Figure 1a). Placenta accreta group showed increased Cited-1 expression in degenerated decidual cells, fibroblastic cells and endothelium (Figure 1b).

SRY-box transcription factor 9 or Sox-9 is a transcription factor that is required for testicular development, organogenesis of liver and pancreas, cytoskeleton and chondrocytes. Mutations in Sox-9 gene can lead to autosomal sex reversal, skeletal formation and testis development (24,25). Sekido et al. studied two genes in Sertoli cell by investigating SRY expression. They found that upregulation of Sox-9 gene in supporting cells determine their fate as Sertoli cells, which shows importance of sox-9 gene in testis (26). Zhao et al. studied endothelial to mesenchymal transition in murine endovascular progenitors. They found that endothelial to mesenchymal transition was dependent on relative expression of Sox-9 along with Notch signaling, affecting their plasticity which may be a therapy tool for fibrotic diseases (27). Xian et al. studied showed that stimulation of Sox-9 can induce cellular differentiation gene and this can be a mechanism in transformation of extra-villous trophoblast to endovascular trophoblasts during placentation (28). In control group, Sox-9 expression was negative in the syncytial knots, in the vascular endothelial cells (Figure 1c). In placenta accreta group, Sox-9 reaction was positive in the root villi, in the blood vessels, in the connective tissue (Figure 1d).

CONCLUSION

It was observed that the Sox-9 reaction was increased and inflammation was induced, depending on the differences in decidual cells, in the syncytial area and in the vascular endothelium in placentas of women with placenta accreta. It is thought that Sox-9 signaling processes are being determined and Cited-1 may be stimulants that affect cell proliferation and angiogenesis regulation and affect placental development.

Strength and Limitation

The strengths: The study strongly stated the histopathology of placenta accreta with two potential markers (Sox-9 and cited-1).

Limitations: The study only focuses on histological perspective. The result could be supported with other techniques such as TUNEL assay, western blot and flow cytometry.

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MEDICAL RECORDS-International Medical Journal

Research Article

Expression of VEGF in Fallopian Tubes in Ovarian Ischemia-Reperfusion

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Abstract

Aim: Our aim was to investigate expression level of VEGF in tuba uterine in ovarian ischemia-reperfusion (I/R) by immunohistochemical techniques.

Material and Methods: 30 Sprague Dawley female rats were categorized into three groups. Sham group: The abdomen was opened and closed without any treatment. Ischemia (I) group: 1-hour ischemia was allowed to create ischemic injury. Ischemia-reperfusion (I/R) group: 1-hour ischemia and then 3-hour reperfusion was allowed to create I/R injury.

Results: MDA and MPO levels were increased after ischemia and IR while GSH content was decreased. Histological scores of follicular degeneration, inflammation, hemorrhage were high in I and IR groups. Normal histology of tuba uterine was observed in sham group. In I and IR group, degenerated cilia, desquamative epithelial cells impaired basement membrane leukocytes infiltration, apoptotic nuclei, vascular dilatation, thrombosis and inflammation and adenoma were observed. VEGF expression was mainly in sham group. In I and IR group, endothelial cells, adenoma structures, vessels, macrophage and inflammatory leukocyte cells and fibroblast cells showed positive VEGF expression.

Conclusion: IR damage affected inflammation and angiogenesis, changes in implantation.

Keywords: VEGF, ischemia-reperfusion, fallopian tubes, histopathology

INTRODUCTION

Insufficient oxygenation of tissues is called ischemia and delivery of oxygen to ischemic tissue called reperfusion. During the IR injury, cell damage occurs irreversibly. Generally, IR cause more damage than ischemia itself in tissues because of imbalance in oxidant and antioxidant system of cells. IR causes elevation of reactive oxygen species and nitrogen species, release of cytokines, induces inflammatory pathway. To overcome this situation, cells scavenge free radicals and produce antioxidant enzymes. This system is not always successful, so medicinal plants could be an alternative to help the restoration of tissue homeostasis (1,2). Ovarian IR is a common clinical emergency with 2.7% incidence. It mainly affects the women of reproductive age. Early diagnosis and treatment are critical in preventing infertility (3).

Tuba uterine consists of a pair of thin tubes 10-12 cm long that extend from the upper part of the uterus to the surface of the ovary. Its role is to provide a suitable environment for fertilization and to transport the ovum from the ovary to the uterus. Anatomically, it consists of 4 parts: infundibulum, ampulla, isthmus, intramural (4,5). Histologically, it consists of three layers: tunica mucosa, tunica muscularis, and tunica serosa. Occasionally, ovarian IR damage is seen in cases involving the tuba uterine. Therefore, IR has effects on tuba uterine and its associated structures (6,7).

In this study, we aimed to investigate VEGF immune expression on fallopian tubes tissue in ovarian ischemia-reperfusion.

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MATERIAL AND METHOD

Experimental Design

All experimental protocol was approved by the Dicle University Local Animal Ethics Committee (2022/02). Sprague Dawley female rats (weighing 200-250 g) were bought and housed in separate cages at 23±2°C, 12 hours light/12 hours dark period at 45-55% humidity, and were fed with standard pellet and water. Vaginal smear was analyzed under microscope to observed estrous cycles of rats at once in every 6-12 hour. 30 female rats were selected in estrus cycle. All experimental procedure was conducted under general anesthesia with injection of ketamine and xylazine. Rats were divided into three groups (10 rats per group) and the following procedures was applied to the groups.

Biochemical Analysis

Tuba uterine tissues were process for malondialdehyde (MDA) levels and glutathione peroxidase (GSH-Px) activities. Tissue samples were homogenized in physiologic saline solution at 10% ratio. All protocols were conducted on ice using homogenizer. Samples were spinned at 2000 rpm for 10 min in a centrifugation vehicle. Supernatant was collected for further analysis. 340 nm absorbance value were selected in spectrophotometry. MDA were expressed nmol/g and performed by Draper et al (9). GSH-Px were done by Paglia et al (10). GSH-Px were expressed as U/g protein. MPO level in tuba uterine tissues were calculated by Hillegas et al (11). MPO is expressed as U/g tissue.

Histological tissue processing

At the end of experiment, animals were sacrificed and fallopian tubes were dissected. The tuba uterine tissues were taken into formalin solution, dehydrated in increasing alcohol series, soaked in xylol solution and incubated in paraffin wax at 58°C. samples were put into paraffin blocks and 4 µm sections were cut and stored for hematoxylin eosin staining.

Immunohistochemical examination

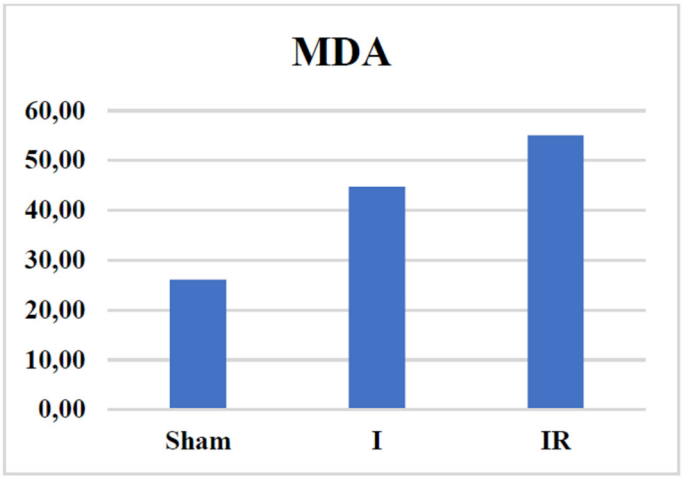
Uterine sections were cleared in xylol solution, dehydrated in alcohol and cleared in distilled water. Epitope retrieval was inducted by EDTA (ethyl diamine tetra acetic acid) solution (pH: 8.0) for 15 minutes in a microwave oven at 90°C. After sections were cooled down, they were rinsed in phosphate buffered saline (PBS) three times for 5 minutes. 3% hydrogen peroxide (H_2O_2) was dropped on slides to block endogen peroxidase activity. After washing in PBS, sections were incubated with rabbit polyclonal VEGF antibody (catalog no: A43269, AFG Bioscience, US) overnight at + 4°C. Sections were dipped into PBS and biotinylated antibody solution () was dropped onto slides for 14 minutes. Sections were reacted with streptavidin peroxidase solution was (ThermoFischer, US) for 15 minutes. After PBS washing, diaminobenzidine (DAB) chromogen was used to observe color change for maximum 10 minutes. Reaction were stopped with PBS solution and sections were stained with hematoxylin dye. Slides were analyzed under light microscope.

Statistical Analysis

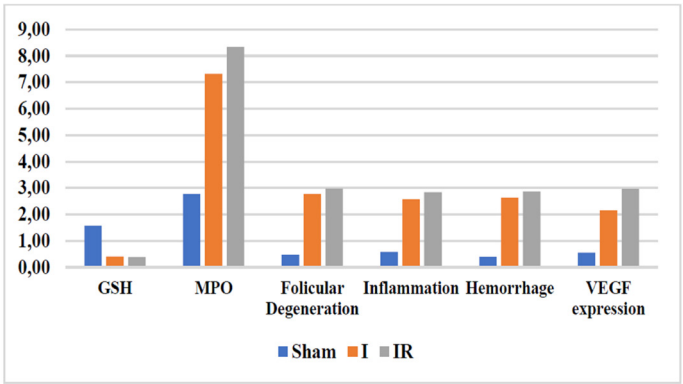
For statistical analysis, IBM SPSS Statistics 25.0 (IBM Inc, Chicago, IL, USA) will be used with a computer program. First, normality tests will be applied to the data and it will be checked whether the data are normally distributed. Kruskal Wallis test (non-parametric) will be used for comparison between independent groups, and if there is a difference, Mann Whitney U test will be used for paired comparisons. The data of this study will be given as mean ± standard error. A value of P <0.05 in all tests will be considered statistically significant.

RESULTS

Statistical analysis of biochemical and histochemical parameters was shown in Table I. MDA and MPO levels were higher in I and IR group than sham group. Histological scores of follicular degeneration, inflammation, hemorrhage were significantly higher in I and IR group than sham group. GSH content was the significantly lower in I and IR group compared to sham group. Table 1 was shown in Figures 1a and 1b.



1-a



1-b

Figures 1a-1b. Graphics of biochemical and histochemical parameters

Table 1. Biochemical and histological parameters of sham, I and IR groups

Parameter	Groups	n	Median (Min-Max)	P value
MDA	Sham	10	26.12 (10.33–42.84)	*p<0.001 **p<0.001
	I	10	44.69 (35.34–54.25)	
	IR	10	55.05 (40.68–68.58)	
GSH	Sham	10	1.57 (1.15-1.98)	*p<0.001 **p<0.001
	I	10	0.40 (0.23-0.72)	
	IR	10	0.38 (0.28–0.70.)	
MPO	Sham	10	2.78 (1.75–3.57)	*p<0.001 **p<0.001
	I	10	7.32 (4.45–9.46)	
	IR	10	8.34 (6.48–10.63)	
Follicular degeneration	Sham	10	0.50 (0.00-1.00)	*p<0.001 *p<0.001
	I	10	3.00 (1.00-3.00)	
	IR	10	3.00 (1.00-3.00)	
Inflammation	Sham	10	0.00 (0.00-1.00)	**p<0.001 *p<0.001
	I	10	3.00 (1.00-3.00)	
	IR	10	3.00 (1.00-3.00)	
Hemorrhage	Sham	10	0.00 (0.00-1.00)	*p<0.001 *p<0.001
	I	10	3.00 (1.00-3.00)	
	IR	10	3.00 (1.00-3.00)	
VEGF expression	Sham	10	0.50 (0.00-1.00)	**p<0.001 *p<0.001
	I	10	2.00 (1.00-3.00)	
	IR	10	3.00 (1.00-3.00)	

* sham vs I; **vs IR

In the control group, the epithelial structure and basement membrane were preserved in the transversal section of the tuba uterine. Connective tissue cells are solitarily distributed. Cells and fibers were detected in regular structure. The circular muscle fibers in the lamina propria were regular. The submucosal area was seen regularly (Figure 2a).

In the ischemia group, it was found that the cilia structure was completely lost in the transversal section of the tuba uterine, and desquamative epithelial cells were shed towards the lumen. The basement membrane structure was impaired, and leukocytes were present in the lamina propria. Crypt structures showed desquamative degenerative changes. Apoptotic nuclei are present. Crypts resembling adenoma were detected in some areas. Inflammation was markedly increased and the number of neutrophils and eosinophils was high (Figure 2b).

In the ischemia-reperfusion group, complete disappearance of the epithelial structure was observed. Vascular dilatations and thrombosis are evident in the connective tissue areas, and there is a dense solitary distribution of inflammatory cells around the vessel. Muscle structure is hyperplastic (Figure 2c).

In the control group section, it was observed that the epithelial structure was clearly preserved, the cryptic structures were regular, and the lamina propria, muscular and vascular structures were smooth. Positive VEGF expression was observed in the crypts, but negative VEGF was detected in endothelial cells (Figure 2d). In the section of the tuba uterine belonging to the ischemia group, vessel dilatation and degenerated basement membrane structure were observed in the lamina propria and muscular layer. Expression of VEGF in endothelial cells was positive. VEGF positive expression was detected in some adenoma structures, macrophage and inflammatory leukocyte cells and fibroblastic structures (Figure 2e). Thrombosis in dilated vessels and loss of integrity in the basement membrane were observed in ischemia-reperfusion sections. VEGF expression was positive in endothelial cells, crypts, inflammatory cells and macrophage cells, vascular structures (Figure 2f).

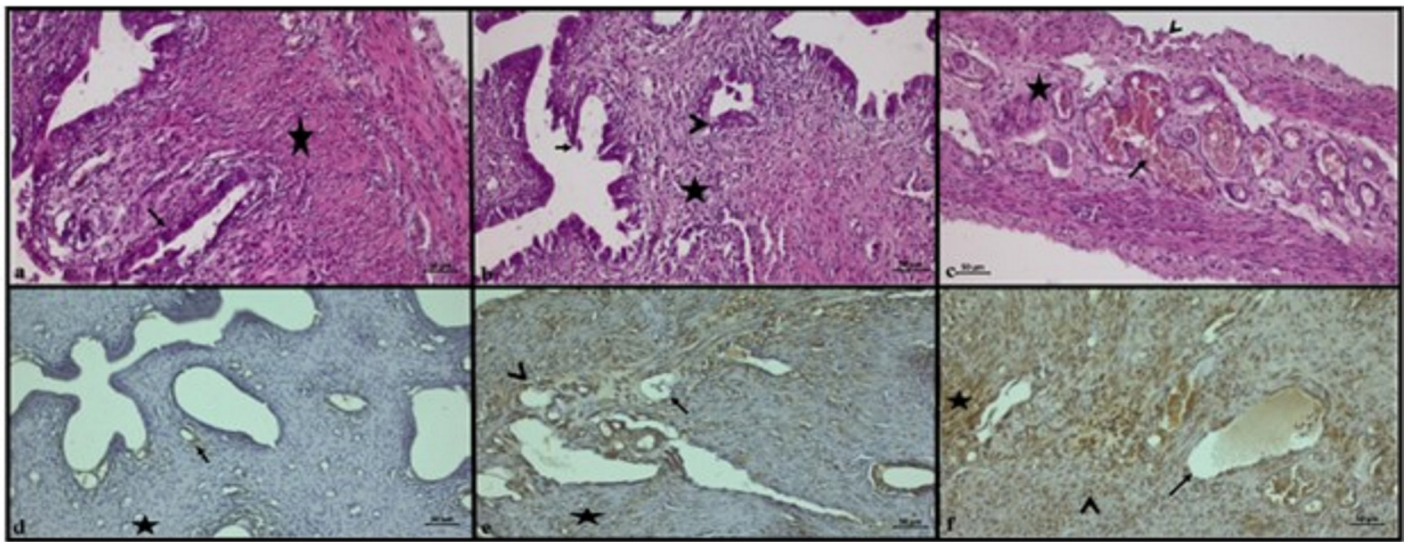


Figure 2. Sham group: Regular basement membrane (black arrow), solitary connective tissue cells, regular muscle fibers (star) (Figure 2a); Ischemia group: degenerated cilia and (arrow) basement membrane, leukocytes (star), adenoma (arrowhead) (Figure 2b); IR group: Degenerated epithelium (arrowhead), thrombosis (arrow), solitary inflammatory cells (asterisk) (Figure 2c); Sham group: positive VEGF expression in crypts (arrow), negative VEGF expression (asterisk) in lamina propria and endothelial cells (Figure 2d); Ischemia group: positive VEGF in dilated vessels (arrow), adenomas (arrowhead), macrophage and fibroblast cells (star) (Figure 2e); IR: positive expression of VEGF in endothelial cells (arrow), macrophage cells (star) and plasma cells (arrowhead) of dilated vessels (Figure 2f)

DISCUSSION

Ovarian ischemia reperfusion causes many pathologies in ovarian tissues, as well as remote organs such fallopian tubes, uterine and cervix. Aktaş et al studied ovarian IR and found that intense fibrosis, vascular dilatation and congestion, stromal inflammation in ovarian tissues after IR (12). Peker et al investigated IR injury in ovarian tissues and revealed that IR caused edema, inflammation, congestion, degenerated follicles, and cells with pyknotic nuclei (13). Eser et al graded the ovarian tissues histologically after IR injury and recorded that the histological scores of ovarian tissues with IR was lower than sham group (14). In our study, tuba uterine histology was normal with regular epithelial structure and basement membrane. Connective tissue and muscle fibers were normal (Figure 2a). In the ischemia group, degenerated cilia structure and desquamative epithelial cells were observed. Increased leukocytes were seen in the lamina propria. Many apoptotic bodies were present. Crypts resembling adenoma were detected in some areas. Inflammation was high (Figure 2b). Severe histopathology was observed in IR group with complete disappearance of the epithelial cells, dilatated vessels and intense inflammatory cells (Figure 2c).

Vascular endothelial growth factor, acronym for VEGF, involves in angiogenesis and promote the angiogenic activities. It is a growth factor having mitogenic and anti-apoptotic effect on endothelial cells (15). There are several members of VEGF family such as VEGFA, VEGFB, VEFGFC, VEGFD etc. A study conducted by Ersoy et al investigated the expression of VEGF in ovarian and uterine tissues. The authors found that VEGF expression was increased compared to sham group in both ovarian and uterine tissues after IR (16). Deger et al studied rat ovarian torsion detorsion model to investigate the VEGF expression in ovarian tissues. They recorded that VEGF immune activities were increased in ovarian degenerated follicles (17). Parlakgumus et al investigated expression level of VEGF in rat ovarian torsion model. The authors found that VEGF expression was significantly lower in IR group than in sham group with lower vascularization (18-20). In our study, in the control group section, VEGF expression was mainly negative (Figure 2d). In the ischemia group, VEGF expression was positive in endothelial cells, adenoma structures, macrophage and inflammatory leukocyte cells and fibroblastic structures (Figure 2e). In IR group. VEGF expression was positive in endothelial cells, crypts, inflammatory cells and macrophage cells, vascular structures (Figure 2f).

CONCLUSION

It has been thought that IR damage caused cilia loss and endothelial dysfunction occur in the tubal epithelium, leading to both an increase in inflammation and a change in angiogenesis, eventually affecting implantation.

Limitations and future perspectives

The animal number is small and may be led to a bias in the results. The conclusion can be supported with other molecular techniques quantitatively. IR injury is a crucial emergency and may lead to infertility. A therapeutic approach to VEGF may be an alternative for treatment. More large-scale experimental study should be further conducted.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *All experimental protocol was approved by the Dicle University Local Animal Ethics Committee (2022/02).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Evaluation of the Temperature Values in the Use of Different Types of Burs

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Abstract

Aim: In our study, we aimed to measure the amount of released head by recording it with a thermal camera during the osteotomies made utilizing round, fissure and lindemann burs to the synthetic bone blocks to simulate the mandible ramus region which is often preferred when obtaining autogenous bone from the mouth.

Material and Methods: The burs in our study were used at rotational speeds of 10000 rpm and 15000 rpm and feed rates of 60 mm/min and 90 mm/min, and each osteotomy was made with a CNC milling machine in order to standardize the applied force.

Results: According to the results of our study, the highest temperatures were observed in the fissure bur groups, and the round bur and lindemann bur groups gave similar results. In addition, when the feed rate is increased from 60 mm/min to 90 mm/min in all groups at constant rotational speed, the heat released increases significantly. When the groups are evaluated within themselves; the temperature values observed at 15000 rpm and 60 mm/min feed rate in the groups using round bur were found to be significantly lower than the group observed at 10000 rpm and 60 mm/min feed rates (p=0.028), in fissure bur groups, the temperature values observed at 10000 rpm and 60 mm/min feed rate were significantly lower than the values observed at 15000 rpm and 60 mm/min feed rates (p=0.028). No statistically significant difference was observed between the heat exchange averages of the 10000 rpm and 15000 rpm groups at a Lindemann bur 60 mm/min feed rate (p=0.182).

Conclusion: This study has shown that while the generated heat in the bone is thought to increase when the bur speeds are increased, the head generated according to bur designs can decrease and it is necessary to operate according to the characteristic features of the preferred bur.

Keywords: Burs, heat exchange, osteotomy

INTRODUCTION

Osteotomies performed using a bur are often preferred in surgical procedures such as dental implant surgery, obtaining autogenous graft and jaw fracture repair (1). Especially osteotomies are performed directly to the cortical bone during autogenous graft intake from the mouth. Usually, maxillofacial surgeons use round bur, lindemann bur and fissure bur during these autogenous graft operations. During these operations, grafts can often be taken by completely cutting the cortical layer in the bone. Bone is a structure with low thermal conductivity (2). Its low conductivity increases the bone temperature, especially in cases where fast drilling is performed or irrigation cannot reach the end of the bur (3). Osteotomy procedures involve cutting the bone and removing the debris accumulated around the bur. The bone is subjected to cutting and torque forces throughout performance of these actions, and most of the mechanical energy generated is converted into heat energy (4). The heat generated as a result of the forces must be kept below the values that will cause osteonecrosis in the bone tissue (5).

In a study by Eriksson et al., they applied 50°C heated implants to rabbit tibias and observed up to 30% bone resorption. It was reported in this study that temperatures above 47-50°C decreased the callus volume around the implant (6). Other investigations have revealed that exposing bone to 43°C for an hour, 47°C for a minute, and 55°C for 30 seconds resulted in the development of necrosis in each case (7-9).

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Several areas of the mouth can be used to harvest autogenous bone. When selecting the donor area, the characteristics and physiology of the bone to be used, as well as the width of the area to be reconstructed, are among the factors that should be evaluated (10,11). The ascending ramus region is the most frequently chosen in maxillofacial surgery due to the donor region's breadth and the surgeon's ease of access. The ramus region is also the most cortical and bone-dense region of the mandible. Up to this date, there is no study in the literature to determine the temperatures to which the bone is exposed according to the technique and speed used while harvesting the autogenous ramus graft. In this study, we employ an infrared thermal imager to measure the heat produced on the artificial bone model at different speeds and feed rates by round, fissure, and lindemann burs, which are frequently used to obtain autogenous bone.

MATERIAL AND METHOD

In our research, we evaluated the heat produced on the bone by three distinct burs, namely the round, fissure, and lindemann burs. The rotational speeds of the burs used in the study were determined as 10000 rpm (revolutions per minute) and 15000 rpm, and the feed rates were determined as 60 mm/minute and 90 mm/minute for all burs. A standard force of 35N was applied to the bone blocks for each bur. Each osteotomy was made 20 mm long and 2 mm deep. The experiments were carried out using a CNC milling machine under constant pressure of 2 kg and at room temperature in the range of 24°C.

The technical properties of the burs, applied force and torque amounts used in our study are given in Table 1.

Table 1. The technical features of the burs employed in our study, the amount of force and torque applied

	TIP ANGLE	HELİX ANGLE	NUMBER OF CUTTERS	LATERAL CUTTING FORCE	FEED RATE	CUTTING TIP DIAMETER	DEPTH OF CUTTING	TORQUE	RPM
ROUND BUR	110-180	66	8	35 NEWTON	60MM/MIN 90MM/MIN	2MM	2MM	14-18N/MM	10K-15K
FISSURE BUR	0	60	4	35 NEWTON	60MM/MIN 90MM/MIN	2MM	2MM	18-22N/MM	10K-15K
LINDEMANN BUR	0	30	3	35 NEWTON	60MM/MIN 90MM/MIN	1.8MM	2MM	26-30N/MM	10K-15K

All burs in our study were used at two different rotational speeds and two different feed rates.

For each group, 11 osteotomies with a length of 2 cm and a depth of 2 mm were carried out, and a total of 132 osteotomies were performed. Accordingly, there are 12 groups in total. The study's groups are listed in Table 2 below.

Table 2. Groups in our study

GROUPS	BUR TYPE	ROTATIONAL SPEED	FEED RATE
GROUP1A	ROUND BUR	10000 rpm	F60
GROUP 1B	ROUND BUR	10000 rpm	F90
GROUP 2A	ROUND BUR	15000 rpm	F60
GROUP 2B	ROUND BUR	15000 rpm	F90
GROUP 3A	FİISSURE BUR	10000 rpm	F60
GROUP 3B	FİISSURE BUR	10000 rpm	F90
GROUP 4A	FİISSURE BUR	15000 rpm	F60
GROUP 4B	FİISSURE BUR	15000 rpm	F90
GROUP 5A	LINDEMANN BUR	10000 rpm	F60
GROUP 5B	LINDEMANN BUR	10000 rpm	F90
GROUP 6A	LINDEMANN BUR	15000 rpm	F60
GROUP 6B	LINDEMANN BUR	15000 rpm	F90

Standardized Synthetic Bone Blocks

In the research, universally-representative synthetic bone blocks of solid-rigid-polyurethane Sawbones (Malmo, Sweden) with dimensions of 130 mm x 180 mm x 40 mm and a density of D1 (D1=0.48 g/cc) were utilized (Figure 1). These blocks have been successfully used in different implant studies and have been approved by the American Society for Testing and Materials (ASTM). Sawbones bone blocks are recognized as a standard material for testing orthopedic devices and instruments, therefore they are also ideal for comparative testing of screws and implants inserted into the bone.

Temperature Measurement

Thermal image series were captured during the osteotomy processes utilizing a 14-bit digital infrared thermal imager (FLIR E6xt, FLIR Systems OU, Estonia). Thermal image acquisition parameters set as: 240×180 (43.200 pixels) focal plane array; 7.5–13 µm spectral range; <0.06°C (0.11°F) / <60 mK thermal sensitivity (NETD); 9 Hz display frequency; 45°×34° field of view. The camera was positioned 30 cm distant from the test block for maximum spatial resolution and FLIR MSX imaging (Multi-Spectral Dynamic Imaging). The collected pictures were utilized to measure temperature changes in artificial bone blocks during implant site preparation. The maximum temperature reached in the bone was measured during each implant osteotomy (Figure 2).

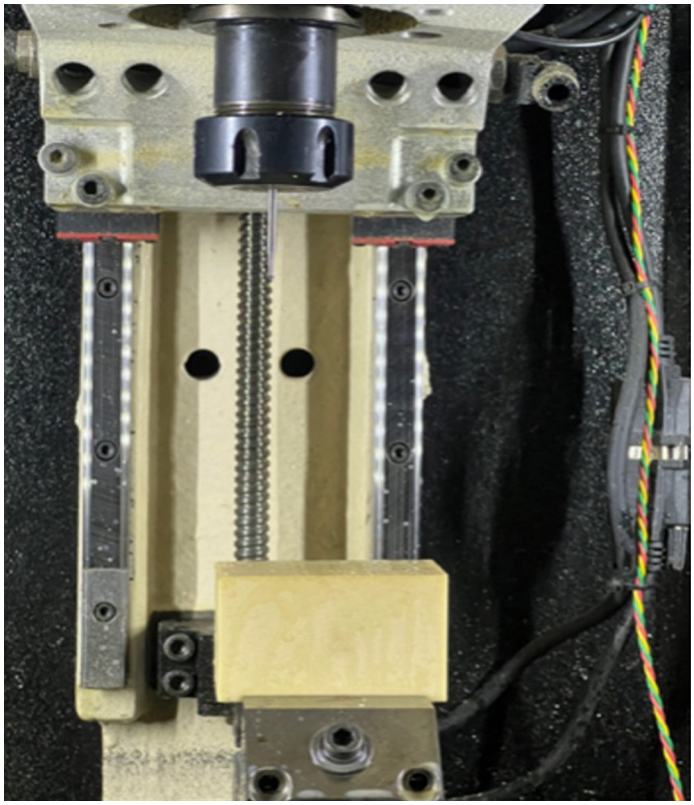

Figure 1. D1 density synthetic bone block used in our study

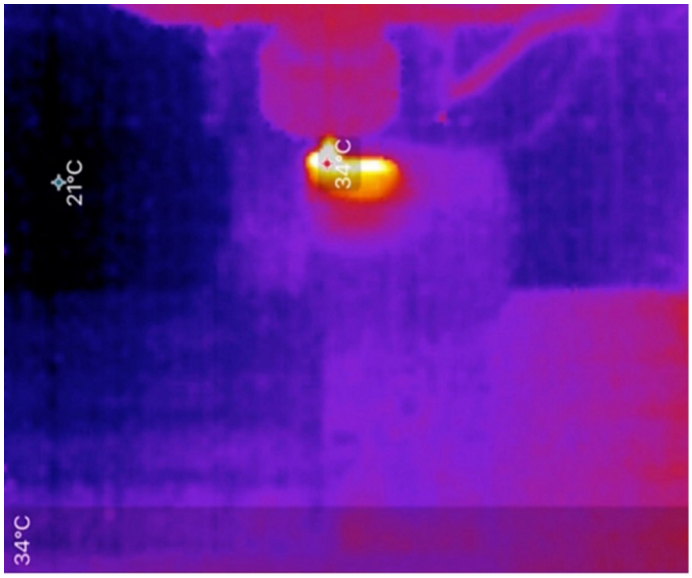

Figure 2. Digital image of infrared thermal camera

Experimental Procedure

To simulate the physical characteristics of a surgeon's osteotomy movement during the process, the application setup has been placed on a computerized numerical control milling tool (KCNM-3050, Kale CNC Istanbul, Turkey) using the parameters listed in Table 1 (Figure 3). Direct current and voltage controlled servo motors and square slide bearings were employed to prepare the experimental setup. The friction coefficients of the square slides and ball bearings were calculated to ensure that the lateral force was 35 N during machining.

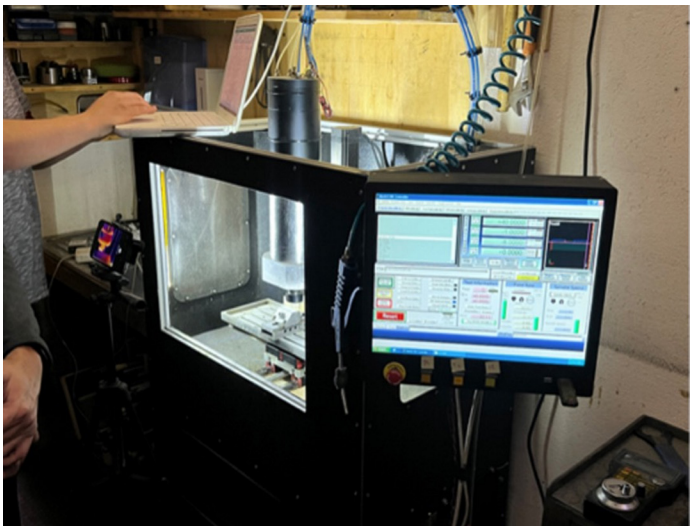

Figure 3. CNC milling machine

After the bone block had been secured to the appropriate area of the CNC milling tool, the experiment's preset tip had been attached, and the codes for the milling tool's rotational speed, feed rate, and osteotomy number had been set. All of the burs that were employed made right-angle contact with the bone blocks. In our investigation, we waited 3 seconds for the bone to return to its initial temperature between successive procedures in order to prevent the accumulated heat that will occur throughout sequential procedures.

The size of each executed osteotomy was 20 mm long and 2 mm deep. The temperature readings were instantly captured with the thermal camera, which was previously set up at a distance of 30 cm. The highest temperature value observed in each osteotomy was determined (Figure 4-5).

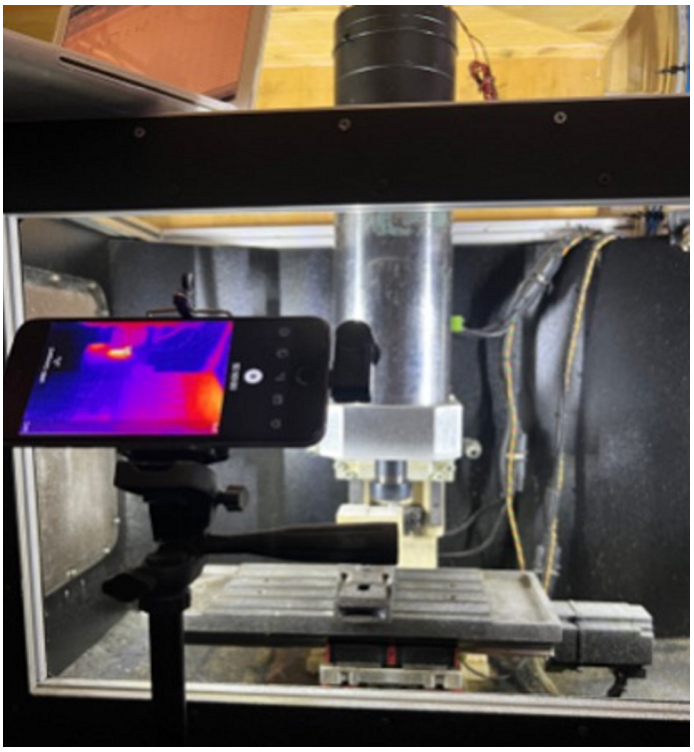

Figure 4. Thermal camera recording during cutting

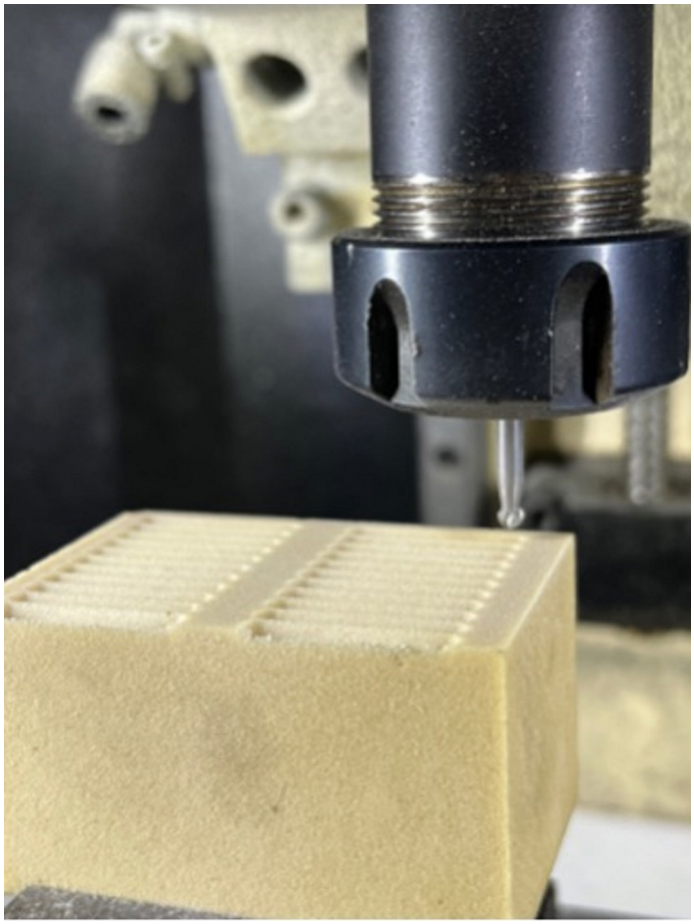

Figure 5. Image of the block after the osteotomy is completed

Statistical Analysis

The NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program was used to perform the statistical analyses for this study.

In the evaluation of the data, descriptive statistical methods (mean, standard deviation) as well as Shapiro–Wilk normality test and distribution of variables were examined, Three-Way ANOVA test was used for the comparisons between Bur, Rotational Speed and Feed Rate of normally distributed variables, and Tukey multiple comparison test was used for subgroup comparisons. The results were evaluated at the significance level of $p<0.05$.

Three-Way ANOVA				
Bur	Rotational Speed	F60	F90	Total
Ruond Bur	1000 rpm	36.00±1.34	38.73±1.10	37.36±1.84
	1500 rpm	34.27±2.01	37.55±0.69	35.91±2.22
	Total	35.14±1.89	38.14±1.08	36.64±2.15
Fissure Bur	1000 rpm	41.00±1.73	51.82±2.75	46.41±5.97
	1500 rpm	46.00±2.00	53.82±1.99	49.91±4.45
	Total	43.50±3.14	52.82±2.56	48.16±5.50
Lindemann Bur	1000 rpm	35.55±0.93	37.73±0.65	36.64±1.36
	1500 rpm	34.82±1.47	37.45±0.82	36.14±1.78
	Total	35.18±1.26	37.59±0.73	36.39±1.59
Total	1000 rpm	37.52±2.84	42.76±6.74	40.14±5.77
	1500 rpm	38.36±5.77	42.94±7.91	40.65±7.25
	Total	37.94±4.53	42.85±7.29	40.39±6.53

RESULTS

An infrared camera was utilized to assess the heat levels of the three distinct burs that were used in our investigation on the D1 bone model without irrigation at two different rotational speeds and two different feed rates and the highest temperature in each osteotomy has been recorded. A total of 132 osteotomies were made in 12 different groups in total. The raw version of the data obtained in the study is given in Table 3.

Table 3. Temperature values observed during the osteotomy

GROUPS	1	2	3	4	5	6	7	8	9	10	11
1A	33	37	38	37	36	35	36	35	36	36	37
1B	37	41	39	38	39	40	39	38	38	38	39
2A	30	38	36	35	34	34	33	35	33	35	34
2B	37	36	37	38	38	38	38	38	38	38	37
3A	44	41	40	42	41	41	39	44	39	40	40
3B	45	50	54	54	51	53	51	53	55	51	53
4A	49	46	45	45	44	44	44	46	47	46	50
4B	51	52	56	57	56	55	54	52	52	53	54
5A	38	35	35	36	36	35	35	35	35	35	36
5B	38	37	38	38	39	37	38	38	38	37	37
6A	39	35	35	35	35	34	34	34	34	34	34
6B	39	36	38	38	37	38	37	38	37	37	37

At 10000 rpm F60 Feed Rate, a statistically significant difference was observed between the mean temperature changes of Round Bur (Group 1A), Fissure Bur (Group 3A) and Lindemann Bur (Group 5A) groups (p=0.0001). The heat exchange averages of the Fissure Bur group were found to be statistically significantly higher than the temperature change averages of the Round Bur and Lindemann Bur groups (p=0.0001), and no statistically significant difference was observed between the mean temperature changes of the Round Bur and Lindemann Bur groups (p=0.721) (Table 4).

At a 10000 rpm F90 Feed Rate, there was a statistically significant difference between the mean temperature changes of the Round Bur (Group 1B), Fissure Bur (Group 3B), and Lindemann Bur (Group 5B) groups (p=0.0001). In comparison to the temperature change averages, the heat exchange averages of the Fissure Bur group were found to be statistically considerably greater than the Round Bur and Lindemann Bur groups (p=0.0001), while there was no statistically significant difference between the mean temperature changes of the Round Bur and Lindemann Bur groups (p=0.385) (Table 4).

At 15000 rpm F60 Feed Rate, there was a statistically significant difference between the mean temperature changes of the Round Bur (Group 2A), Fissure Bur (Group 4A), and Lindemann Bur (Group 6A) groups (p=0.0001). The heat exchange averages of the Fissure Bur group were found to be statistically significantly higher than the temperature change averages of the Round Bur and Lindemann Bur groups (p=0.0001), there was no statistically significant difference between the mean temperature changes of the Round Bur and Lindemann Bur groups (p=0.769) (Table 4).

At 15000 rpm F90 Feed Rate, there was a statistically significant difference between the mean temperature changes of the Round Bur (Group 2B), Fissure Bur (Group 4B), and Lindemann Bur (Group 6B) groups (p=0.0001). The heat exchange averages of the Fissure Bur group were found to be statistically significantly higher than the temperature change averages of the Round Bur and Lindemann Bur groups (p=0.0001), while there was no statistically significant difference between the mean temperature changes of the Round Bur and Lindemann Bur groups (p=0.985) (Table 4).

With Round Bur at 10000 rpm and 15000 rpm, the heat exchange averages of the F90 group were found to be statistically considerably greater than those of the F60 group (p=0.0001) (Table 4).

In the Round Bur F60 Feed Rate, the temperature change averages of the 10000 rpm group were found to be statistically significantly higher than the 15000 rpm group (p=0.028) (Table 4).

In the Round Bur F90 Feed Rate, the temperature change averages of the 10000 rpm group were found to be statistically significantly higher than the 15000 rpm group (p=0.007) (Table 4).

With Fissure Bur at 10000 rpm and 15000 rpm, the heat exchange averages of the F90 group were found to be statistically considerably greater than those of the F60 group (p=0.0001) (Table 4).

In the Fissure Bur F60 Feed Rate, the temperature change averages of the 10000 rpm group were found to be statistically significantly lower than the 15000 rpm group (p=0.028) (Table 4).

In the Fissure Bur F90 Feed Rate, there was no statistically significant difference between the mean heat exchange rates of the 10000 rpm and 1500 rpm groups (p=0.065) (Table 4).

With Lindemann Bur at 10000 rpm and 15000 rpm, the heat exchange averages of the F90 group were found to be statistically considerably greater than those of the F60 group (p=0.0001) (Table 4).

At the Lindemann Bur F60 Feed Rate, there was no statistically significant difference between the mean heat exchange rates of the 10000 rpm and 15000 rpm groups (p=0.182) (Table 4).

At the Lindemann Bur F90 Feed Rate, there was no statistically significant difference between the mean heat exchange rates of the 10000 rpm and 15000 rpm groups (p=0.397) (Table 4).

Table 4. Results of a three-way anova test

	Type III Sum of Squares	Df	Mean Square	F	p
Bur	3981.02	2	1990.51	791.88	0.0001
Rotational Speed	8.76	1	8.76	3.48	0.064
Feed Rate	795.27	1	795.27	316.38	0.0001
Bur * Rotational Speed	152.02	2	76.01	30.24	0.0001
Bur * Feed Rate	322.68	2	161.34	64.19	0.0001
Rotational Speed * Feed Rate	3.67	1	3.67	1.46	0.230
Bur * Rotational Speed * Feed Rate	22.47	2	11.24	4.47	0.013

DISCUSSION

The temperature rise in the bone during rotational system osteotomies is influenced by a number of variables. Some of these variables include the osteotomy site, specifically the amount of cortical and cancellous bone, and the potential influence of bone density. The quantity of the heat produced is directly influenced by elements including the feed rate, rotational speed, and bur geometry. Regardless of the source, this temperature rise can cause bone damage or impaired healing. As was previously stated, the acknowledged threshold for thermal damage to bone is 47°C for a duration of 1 minute (12-13).

The round, fissure, and lindemann burs, which are frequently used during the harvesting of autogenous

grafts in the clinic (14), were chosen in our investigation, and unlike previous studies, osteotomies were not carried out by the surgeon. The reason for this is that the amount of force transmitted to the bone cannot be standardized in studies performed using the human hand. This is why, in our study, a CNC milling equipment that can apply constant force, feed rate, and torque in every osteotomy has been constructed, and this device has been employed while performing osteotomies, in order to resolve this issue.

There are no studies in the literature in the field of maxillofacial surgery and dentistry evaluating autogenous graft harvesting and its thermal effect without the use of irrigation. In our investigation, osteotomies were performed without irrigation in order to demonstrate how much the burs heated up regardless of irrigation. Due to physical impossibilities, irrigation sometimes does not reach the bur completely during clinical usage, and more than expected amount occurs in the bur.

In a study, temperature increases and osteotomy times were investigated in different bone materials (15). The bone model created from bovine bone is not different from human bone or other simulation models examined, and it can replicate human ribs, as shown in this study. Bovine and polyurethane-based bone models are also shown to be similar to human bone. An additional study found that polyurethane-based artificial bone blocks were very beneficial due to their repeatability and ability to simulate human bone (16). Parallel to these trials, bone blocks made of polyurethane were used in our study. We preferred to use an infrared thermal imager because it can measure heat indirectly instantaneously and its reliability has been proven scientifically. These cameras create a thermal profile of the bur area and the surrounding tissue by detecting it on the surface through a color scale and in this way, they can easily detect make temperature from a certain distance (17).

According to the data from our investigation, an increase in the feed rate raises bone temperature when the rotation rate is maintained constant in practically all groups.

The sawdust produced by the cutting edge wants to progress toward the channels that have not yet been emptied as the feed rate rises, compressing the chip that was made in the previous rotation but has not yet been discharged and releasing heat in the process. In this instance, when the feed rate rises, eventually the cutting tip will be unable to make contact with the material, and the force of the feed will result in deformation of the shaft of the bur's end. Our research indicates that while round burs and lindemann burs produce less heat than fissure burs, their use is potentially safer because the lowest temperature values in our study were observed in the groups where these burs were used at F60 feed rate and 15000 rpm. In cases where the speed is lower in round bur and lindemann bur (10000 rpm), each individual cutting tip comes into contact with the surface for a longer time depending on the cutting speed. Accordingly, the temperature values formed in the bone are higher than the groups used at 15000 rpm.

In cases where the fissure bur is used, a speed of 10000 rpm and a feed rate of F60 will be preferred, which will produce the lowest possible heat. Fissure burs release more heat than round and lindemann burs, so it's important to remember to maintain excellent irrigation while using them. The angle of the cutting tip of the fissure bur and the outermost point of the cutting tip and the tooth bottom diameter are less than other burs, which makes it difficult to remove the cutting material. The sooner the surface relationship of the removed chip and the bur is cut off, the less the heat is released due to friction. The fissure bur cannot successfully remove the sawdust without irrigation at the feed rates used in the experiment and the feed force simulating a surgeon's hand strength. This leads to heat generation.

The size and volume of chips that are ultimately removed from the surface depend on the tip geometry, the number of rotations, and the cutting speed. The volume and size of the removed sawdust should correspond to the design of the cutting tip. When this harmony is disturbed, the removed sawdust accumulates instead of being removed from the cutting surface and causes friction. Large exit gaps are very effective in removing chips (e.g., round burs), as the chip exit angles between the cutting edges increase (lindemann burs), it becomes easier to remove chips. In comparison to other burs, the fissure bur has a shorter distance between the cutting edge's tip and the tooth's bottom diameter. This causes less chips to be thrown in each round (18).

The cutting tip similarly requires a certain amount of force to lift material from the surface. When the cutting end does not have enough force due to insufficient cutting speed, rotations, or feed rate, heat is produced as the end surface rubs against the surface rather than cutting. For this reason, in general, the volume of chips removed per unit time in multi-cutting tip (round bur) or small cutting tip (lindemann bur) burs can be used at higher speed numbers than will occur with chips of very small sizes. As the diameter of the bur or cutting tip increases (fissure bur), it is also worked at lower speeds. Overly much friction and heat are generated if the working speed is lower or higher than it should be (19).

The limits of our research can be demonstrated by the absence of actual bone tissue and irrigation in any of the study groups for the reasons already mentioned. It is necessary to conduct studies utilizing actual bone in the same experimental design.

CONCLUSION

In spite of the fact that increasing bur speeds is assumed to increase the heat created in the bone, this study demonstrated that it is important to act in accordance with the distinctive qualities of the selected bur since the heat produced according to bur designs can actually decrease.

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Research Article

Association of *Helicobacter Pylori* Infection with Neutrophil-to-Lymphocyte Ratio, Platelet-to-Lymphocyte Ratio, and Neutrophil-to-Monocyte Ratio

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Abstract

Aim: In our study, we aimed to show the relationship between neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, neutrophil-monocyte ratio and mean platelet volume, and *Helicobacter pylori* positivity and severity.

Material and Methods: In this study, we included 596 patients without active and/or chronic disease who underwent upper gastrointestinal system endoscopy due to dyspeptic complaints in a state hospital between July 2021 and July 2022. Demographic and laboratory data were obtained retrospectively from electronic patient records. The patients were divided into two groups as positive and negative for *Helicobacter pylori* according to the pathology report. *Helicobacter pylori* presence was defined as none, mild, moderate and severe. Hemogram parameters were compared between the groups.

Results: Mean age of the patients (n=596) was 41.8±13.57 years, 374 (62.8%) were female, and 331 (55.5%) were H*elicobacter pylori* positive. There was no statistically significant difference between *Helicobacter pylori* positive and negative patient groups in terms of age, gender, leukocytes, lymphocytes, monocytes, platelets, hemoglobin, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, neutrophil-monocyte ratio and mean platelet volume values. When the hemogram parameters were evaluated according to the severity of *Helicobacter pylori*, a statistically significant difference was found between the groups only in terms of lymphocyte levels (p=0.028). However, this difference was not considered clinically significant.

Conclusion: Neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, neutrophil-monocyte ratio and mean platelet volume are thought to be popular recently and easy, accessible and inexpensive parameters for diagnosis and degree of inflammation in many systemic diseases, but in our study, no statistically significant relationship was found between *Helicobacter pylori* positivity and severity and hemogram parameters. Prospective studies with larger numbers of cases are needed to accept these parameters as predictors for *Helicobacter pylori* infection.

Keywords: Helicobacter pylori, neutrophil-lymphocyte ratio, thrombocyte-lymphocyte ratio, neutrophil-monocyte ratio

INTRODUCTION

Helicobacter pylori (HP) is a gram-negative, spiral, flagellated, and microaerophilic bacterium (1). More than half the world's population is infected by HP. Person-to-person transmission usually occurs by either the oral–oral or fecal–oral route (2). After colonizing the gastric mucosa, HP plays a role in the development of chronic active gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and gastric adenocarcinoma (3). Furthermore, some epidemiological studies have associated HP infection with extragastric diseases such as idiopathic thrombocytopenic purpura, iron deficiency anemia, cardiovascular diseases, and non-alcoholic fatty liver disease. Given that HP infection affects a vast proportion of the world's population, the diagnosis and treatment have become increasingly important (4). HP induces local inflammation in the stomach and systemic humoral

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immune responses. Most patients have an asymptomatic but chronic inflammation (5). Previous studies have investigated several parameters such as leukocytes, C-reactive protein, and procalcitonin as markers of inflammation in HP-associated gastritis (6). Components of complete blood count (CBC), a simple and inexpensive test, such as leukocytes, neutrophils, lymphocytes, platelets, mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) can help differentiate the causes of hematological diseases and predict some inflammatory events. NLR and PLR are two of the most important parameters among all these components (7-10).

This study sought to investigate whether the presence and severity of HP infection are associated with parameters of CBC in patients undergoing upper gastrointestinal (GI) endoscopy for dyspeptic complaints.

MATERIAL AND METHOD

In this study, the authors scanned the results of upper GI endoscopy procedures performed on 3865 patients in a state hospital's endoscopy unit between July 2021 and July 2022. Among all these patients, 596 patients who underwent endoscopy only for dyspeptic complaints, who received a CBC test, and who had no active and/or chronic diseases were retrospectively analyzed. Upper GI endoscopy data of the patients included in the study were obtained from the hospital's endoscopy unit database. Age, sex, and reason for endoscopy were recorded from upper GI endoscopy reports. Data on the presence, severity, and site of HP infection according to the Sydney System for Classification were obtained from the pathology reports in the hospital's electronic database. Electronic health records were reviewed for the presence of chronic kidney disease, diabetes mellitus, ischemic heart disease, pregnancy, chronic liver disease, celiac disease, and active infection. Furthermore, components of CBC including leukocytes, neutrophils, lymphocytes, monocytes, platelets, hemoglobin, and MPV tested within the last one month were accessed and used to calculate NLR, PLR, and neutrophil-to-monocyte (NMR) ratio.

All patients scheduled for upper GI endoscopy were advised to fast for 8 hours before the procedure. Written informed consent was obtained from all patients. Endoscopic examinations were performed by a gastroenterologist and an endoscopy nurse using EG 530WR; Fujinon device (Tokyo, Japan). During gastroscopy, at least two biopsy specimens were collected from the gastric antrum and corpus to investigate HP. The presence of HP was determined by using light microscopic examination of gastric mucosal biopsy slides stained with hemotoxylin eosin and giemsa. The Sydney System for Classification was routinely used. HP infection was classified into absent, mild, moderate, and severe.

Exclusion criteria: age under 18 years, having chronic kidney disease, diabetes mellitus, hypertension, ischemic heart disease, heart failure, pregnancy, chronic liver disease, celiac disease, gastric adenocarcinoma, gastric or bulbar ulcer, active infection, and using proton pump inhibitors.

The study was approved by the Medical Research Ethics Committee (Ethics committee decision date/no: 06.10.22/22-10T/26). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Statistical analysis and calculations were performed using SPSS Statistics software Ver. 22.0. Variables were checked for normal distribution using visual (histogram) and analytical methods (Kolmogorov–Smirnov test). Continuous data was expressed in mean and standard deviation, whereas categorical data was expressed in rate and percentage. Analyses for HP infection status of the patients were performed using Mann–Whitney U test for the comparison of continuous variables between groups and Chi-squared test for the comparison of categorical variables. HP severity and CBC parameters between groups were compared using the Kruskal–Wallis test, and post-hoc analyses were performed using Mann–Whitney U test and Bonferroni correction. Statistical significance was set at $p<0.05$.

RESULTS

The 596 patients included in the study were assessed retrospectively. The mean age of the patients was 41.8±13.57 years. Positive HP test was observed for 331 (55.5%) patients. There was no statistically significant difference between HP-positive and HP-negative patients in terms of age, sex distribution, leukocyte, lymphocyte, monocyte, platelet, hemoglobin, NLR, PLR, NMR and MPV values (Table 1).

Of 331 HP-positive patients, infection was severe in 134 (40.5%), moderate in 117 (35.3%), and mild in 80 (24.2%). Based on biopsy specimens, the most common site of HP infection in the stomach was in the antrum and corpus in 297 (89.7%) patients, only in the corpus in 23 (6.9%) patients and only in the antrum in 11 (3.4%) patients.

Analysis of CBC parameters for HP severity showed a statistically significant difference between the groups in terms of lymphocyte counts (p=0.028) (Table 2). The difference between the groups arose from the difference between patients with moderate and severe HP. The lymphocyte count was 2266.8±733.3 µL in patients with moderate HP and 2538.5±776.6 µL in patients with severe HP.

Table 1. Comparison of demographic characteristics and CBC parameters of patients by HP status

	HP (+)	HP (-)	Total	P
Age (years)[a]	40.35±13.25	41.98±13.94	41.8±13.57	0.164
Sex, n (%)				
Female	203 (61.3)	171 (64.5)	374 (62.8)	0.422
Male	128 (38.7)	94 (35.5)	222 (37.2)	
CBC Parameters[a]				
Leukocytes (/µL)	7581.8±1850.7	7517±1804.2	7553±1828.9	0.888
Neutrophils (/µL)	4358.9±1442.6	4336.4±1485.7	4348.9±1460.7	0.970
Lymphocytes (/µL)	2412.6±764.7	2366.9±709.1	2392.3±740.2	0.367
Monocytes (/µL)	546.1±159.5	557.5±162.2	551.2±160.7	0.305
Platelets (/µL)	251418.1±56744.9	256097.7±65719	253498.8±60890.2	0.708
Hb (g/dL)	14.5±1.9	14.5±1.75	14.5±1.83	0.866
NLR	1.98±1.1	1.99±1.1	1.98±1.1	0.792
PLR	114.1±46.6	116.3±43.2	115.1±45.1	0.208
NMR	8.39±3.3	8.1±2.73	8.25±3.1	0.309
MPV (fL)	8.12±1.56	8.02±1.4	8.1±1.5	0.621

[a]Values are given as mean ± standard deviation. Hb, hemoglobin; MPV, mean platelet volume; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; NMR, neutrophil to monocyte ratio

Table 2. Association between HP severity and CBC parameters

CBC Parameters[a]	HP Severity			
	Mild	Moderate	Severe	P
Leukocytes (/µL)	7502 ±2008	7343.6±1730.6	7837.6±1836.3	0.086
Neutrophils (/µL)	4293.5±1512.9	4278.9±1440.1	4467.9±1405.3	0.411
Lymphocytes (/µL)	2414.9±761.3	2266.8±733.3	2538.5±776.6	0.028*
Monocytes (/µL)	514.5±155.7	544.7±153.4	566.1±164.9	0.068
Platelets (/µL)	249476.3±52665.3	249299±57440.4	254427.6±58727.7	0.762
Hb (g/dL)	14.5±1.84	14.57±1.99	14.45±1.87	0.811
NLR	1.9±0.94	2.14±1.37	1.89±0.81	0.432
PLR	112.92±43.6	121.26±52.39	108.59±42.24	0.102
NMR	8.9±4.55	8.26±3.01	8.2±2.66	0.439
MPV (fL)	8.3±1.62	8.13±1.52	8±1.56	0.252

[a]Values are given as mean ± standard deviation. *p < 0,05
Hb, hemoglobin; MPV, mean platelet volume; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; NMR, neutrophil to monocyte ratio

DISCUSSION

Considering that HP infects more than half the world's population, this study investigated the effect of HP and its severity on components of CBC, NLR, PLR, and NMR, which are simple, inexpensive, and easily accessible tests. Literature offers conflicting data on NLR and PLR. Of the 596 patients included in our study, 331 (55.5%) had a positive HP test. HP-positive and HP-negative groups had no significant difference in terms of CBC parameters. The strengths of our study were the exclusion of patients with chronic inflammatory conditions, chronic disease, and active infection that may affect CBC parameters and ratios and the inclusion of patients with dyspeptic complaints.

Previous studies around the world, on patients with dyspeptic complaints, reported different HP infection rates, including rates between 11%–48.8% for European

countries and 7.1%–30% for the United States (11,12). However, infection rates have been reported to be between 31.2% and 95% in developing countries and Asian countries (13-20). The HP Prevalence study conducted in Turkey in 2003 (TURHEP) reported the prevalence of HP to be 83% based on urea breath test. The TURHEP study classified the country into five regions; HP infection was found to be higher in those living in Central and Eastern Anatolia regions than in those living in Western and Southern regions (21). Recent studies conducted in Turkey reported HP infection rates between 36.6% and 66% (22-26). Peculiarly, patients who underwent endoscopy due to dyspeptic complaints were found to have HP infection rates between 28.8% and 62.6% (27-30). In our study, the rate of HP infection in patients with dyspeptic complaints was found to be 55.5%, which is in line with previous studies from Turkey. This supports the fact that Turkey, a country of transition between Asia and Europe, has a prevalence rate between that of developing and developed countries.

In this study, the rate of HP infection in both the antrum and corpus was found in 89.7% of the patients and in antrum or corpus only was found in 10.3% of the patients, which supports the recommendation that biopsy specimens should be collected from both the antrum and corpus and examined in two separate dishes (according to Sydney or Kimura protocols) as stated in the Maastricht VI/Florence consensus report (31).

Leukocytes and their subgroups and NLR have been shown to be markers of systemic inflammation (7-9,32). Recently, a limited number of studies have investigated the association between HP and CBC parameters, including NLR and PLR and have yielded conflicting results. Yalın et al. (2022) found no statistically significant difference between HP-positive and HP-negative groups in terms of leukocytes, neutrophils, lymphocytes, platelets, hemoglobin, NLR, and PLR (10). Similarly, Koç et al. (2022) also reported no statistically significant difference between HP-positive and HP-negative groups in terms of leukocytes, neutrophils, platelets, NLR, and PLR (25). In contrast to these two studies, Farah et al. reported HP-positive patients to have significantly higher levels of leukocytes, neutrophils, lymphocytes, and NLR (33). Ferhatoğlu et al. reported no significant difference between HP-positive and HP-negative patients in terms of leukocytes, neutrophils and lymphocytes, but found NLR to be significantly higher in HP-positive patients (34). Nalbant et al., on the other hand, found no statistically significant difference between the groups in terms of lymphocytes, monocytes, hemoglobin, NLR, and PLR, but unlike other studies, they found neutrophil count to be significantly lower in HP-positive patients (28). Such higher levels of leukocytes, neutrophils, and NLR reported in some studies may be attributed to subclinical microinflammatory reactions caused by HP. However, our study found no statistically significant difference between HP-positive and HP-negative groups in any component of CBC.

MPV, which has recently been shown to be an inflammatory marker for some local and systemic diseases, has been associated with disease severity and prognosis. Previous studies have reported that acute and chronic inflammation activates platelets, resulting in increased MPV (35-39). Chronic gastritis is an example of local chronic inflammation, and HP is one of the most common causes. Out of the studies that have investigated the association between HP and MPV, one has found a statistically significant difference between HP-positive and HP-negative groups in terms of MPV (40), while others have found no statistically significant difference between the groups in terms of MPV (10,27,30). Our study found no statistically significant difference between HP-positive and HP-negative groups in terms of MPV. A possible explanation of this result is that HP infection does not cause as much inflammation as in other systemic diseases, resulting in inadequate secretion of cytokines and immunomodulators and inadequate activation of platelets.

Two studies reported that as HP infection increased in severity, NLR and PLR decreased significantly (25,33). Our study, on the other hand, found that HP severity had a statistically significant correlation with only lymphocyte count. However, this association was not considered to be clinically significant since lymphocyte count did not increase with increasing severity of HP infection.

The limitations of this study include its retrospective design and lack of data about smoking and alcohol use among patients.

CONCLUSION

NLR, PLR, NMR, and MPV have recently become increasingly popular and are considered to be easy, accessible, and inexpensive markers for diagnosing numerous systemic diseases and for predicting the severity of inflammation. However, further prospective studies with a larger sample size are needed in order for these parameters to be recognized as markers of HP infection.

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MEDICAL RECORDS-International Medical Journal

Research Article

MDA-MB-231 Human Breast Cancer Cell Line Treated with Ginseng (Panax Quinquefolius): Evaluation by Annexin V and AgNOR Staining

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Abstract

Aim: In this study, it was aimed to examine the time and dose dependent effects of Ginseng (Panax quinquefolius) on MDA-MB-231 cell lines.

Material and Methods: MDA-MB-231 breast cancer cell line was used in the study. MDA-MB-231 cells were exposed to ginseng at 37°C and 5% CO_2 for varying durations (24 and 48 hours) and doses (1 and 2 µg/ml ginseng). At the end of the incubation period, viability, apoptosis, cell cycle and Argyrophilic nucleolar organizing region (AgNOR) protein status of MDA-MB-231 cells were examined in the Muse Cell Analyzer.

Results: It was observed that the dose inducing apoptosis was 1 µg/ml ginseng for 24 and 48 hours, and 2 µg/ml ginseng for 48 hours in the group that stopped the cell cycle in the G0/G1 phase. When comparing the two groups; while no difference was determined between the control and 1 µg/ml ginseng groups, the significant differences were detected between the control and 2 µg/ml ginseng groups for mean AgNOR number in 48 hours incubation. However, there was no significant difference for the TAA/NA ratio, in the groups for 48 hours.

Conclusion: The current study showed that ginseng had a crucial function against cancer development. Also, both AgNOR values might be used as biomarkers for detection of the most reliable therapeutic dose selection for cancer and it has been shown that correct consumption of Ginseng can be effective in preventing cancer formation and slowing its progression.

Keywords: Ginseng, MDA-MB-231, AgNOR

INTRODUCTION

Among the cancer types, breast cancer is the most common type of cancer in women, and it is the most common cause cancer death in all societies after developed western countries. It is a serious disease that affects morbidity and mortality all over the world. For this reason, components that are effective in cancer treatment or prevention have begun to be investigated. Bioactive substances obtained from different parts of plants have been studied in research on cancer. The effects of these substances are supported by epidemiological data. The ginseng plant is a large, tap-rooted, non-seasonal fringed herb. The diameter of the roots of 6-8 year-old ginseng is 10 centimeters, and they are red or white in color. Since the aged, plump, and branched fringes of the plant are used in the preparation of the active substance, it is cultivated in countries such as America, Japan, Korea, and China, as well as growing wild in nature. In vivo and in vitro studies have shown that ginseng affects many steps by stimulating apoptosis in cancer cells and stopping the cell cycle (1,2).

CITATION

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Traditional herbal medicines, which have been used for years, provide an advantage in maintaining a balanced state of health (3). In addition, the protective effect of ginseng (Panax quinquefolius) in cancer chemoprevention has been demonstrated by preclinical studies and extensive laboratory works. Ginseng is chemoprophylactic and generally acts on molecular and cellular targets through various signaling pathways, thus inhibiting tumors through cell cycle regulation, apoptosis induction, angiogenesis and inhibition of proliferation. The anticancer effects of ginseng are thought to be effective in the modulation of various signaling pathways, including the regulation of growth factors, cell proliferation mediators and tumor suppressors. AgNOR is the nucleolus organized on acrocentric chromosomes. It is the name given to proteins that interact with NORs and are stained with silver because of their argyrophilic (silver-loving) properties. NOR regions within the nucleolus of human cells to which AgNOR proteins are bound during interphase represent transcriptionally active ribosomal RNA synthesis regions, also called rDNA. Therefore, cell proliferation rate can be indirectly evaluated by determining AgNOR parameters (4,5).

In recent years, a number of epidemiological studies have indicated that ginseng consumption affects the incidence of cancer (6,7). In this study we showed to represent any possible effects of ginseng treatment on the NOR protein synthesis and apopitotic effect on the MDA-MB-231 cancer cell line.

MATERIAL AND METHOD

Preparation of Ginseng (Panax quinquefolius)

Ginseng (Panax quinquefolius: Gnc Herbal Plus®) used in the study was obtained from a certified compant. The aqueous extract was obtained by infusing 3 g in 100 ml of water at 85° C for 5 minutes, then centrifuged and the supernatant was sterilized with a 0.22 µm filter and taken into falcons to be used in cells.

Cell Culture

MDA-MB-231 cells were derived from American Type Culture Collection (Manassas, VA, USA). The MDA-MB-231 human breast cancer cells were cultured Dulbecco's modified Eagles medium (DMEM Capricorn Scientific, CP21-4310) including streptomycin/penicillin (100U/ml; Sigma Life Science,046M4846V) and 15% fetal calf serum (FCS) (Biowest, S181G-500) in a humidified (Sanyo, MCO-19 A/C(UV)) atmosphere of 5% CO_2 air at 37°C.Then healthy MDA-MB-231cells were divided into control and experimental groups (1 and 2 µm/ml). 24 well culture plate including 1000 µL of fresh medium with 1×105 MDA-MB-231 cells were used to find optimum Ginseng dose. After the medium was removed and MDA-MB-231 cells were rinsed with 500 µl phosphate-buffered saline (PBS) 3 times. The experimental groups were generated as log concentrations of Ginseng (1 and 2 µm/ml) on breast cancer cells.

Cell Viability Assay and Proliferation

The number of cells per ml of cell suspension was determined with Trypan Blue cell counting method. For cell counting an amount of the cell suspension was taken into the eppendorf tube and Trypan Blue solution was added in the same proportion as the amount taken from the cell. After 5 minutes of incubation, the coverslip was transferred to both sides of the closed Thoma slide (Marienfeld-Superior). Stained and unstained cells were noted by counting with a microscope (Nikon Eclipse TS100).

Experimental Design

Annexin V for 24 and 48 hour incubations and control, 1 and 2 µg/ml Ginseng groups for cell cycle testing were formed.

Annexin V Assay

Muse Cell Analyzer device and compatible Muse Annexin V kit and dead cell assay reagent were used for apoptosis analysis (Millipore; MCH100115).

MDA-MB-231 cell line was cultivated in 24-well plates with $1x10^5$ cells per well and left to incubate for 24 and 48 hours. Then, cells were treated with trypsin and stained with dead cell reagent and Annexin V according to the manufacturer's protocols and analyzed using Muse Cell Analyzer (Millipore Corporation).

Cell Cycle Assay

MuseR Cell Cycle Kit were used for detect the cell cycle stage of cells (Millipore; MCH100106). MDA-MB-231 cells were cultivated in 24-well plates with $1x10^6$ cells per well and left to incubate for 24 and 48 hours. Later, cells were treated by removing with trypsin. After, the cells were stained with MuseR Cell Cycle Kit according to the manufacturer's protocols (Millipore Corporation) and analyzed using Muse Cell Analyzer (Millipore Corporation, MCH100106).

AgNOR Staining

As a result of the experiment for AgNOR staining, MDA-MB-231 cells cultured with 1 and 2 µg/ml ginseng were spread on a slide and dried at room temperature for approximately 30 minutes. The silver staining solution obtained from 50% $AgNO_3$ and gelatinous formic acid mixture was dripped 3-4 drops on the preparations with a staining pipette and covered with a coverslip. Then, the lid of the petri dish was quickly closed, wrapped with aluminum foil in such a way that it would not get any light, and left in an oven at 37°C for 15 minutes. At the end of the 15th minute, the preparations that were removed from the oven were washed with distilled water until the coverslips fell off. Photographs of the preparations covered with Entellan were taken under a light microscope (Leica DM 3000) at a magnification of 100 (Imaging Color 12 BIT, Made in Canada). Analyzes were performed in the Image J program (ImageJ version 1.47t, National Institutes of Health, Bethesda, Maryland, USA). By evaluating cell

nuclei, both the total AgNOR area (TAA/NA) and the average AgNOR number per nuclear area were calculated using the "freehand selections" tool.

Statistical Analysis

The distribution of the data in this study was evaluated with ShapiroWilk's test statistics, histogram, and q-q graph. One-way analysis of variance was used for comparison between groups. The homogeneity of variance was evaluated with Levene's test. Tukey was used as a multiple comparison test. For each group, the differences between the 24-hour and 48-hour measurements were performed using a paired t-test. Analysis of the data was analyzed by statistical programme (Turcosa Analytics Ltd Co, Turkey, www.turcosa.com.tr) data software. P<0.05 was considered significant.

RESULTS

Annexin V findings

The percentages of total apoptotic cells after 24 and 48 hours of incubation of MDA-MB-231 cells are demonstrated in Figure 1 and Table 1.

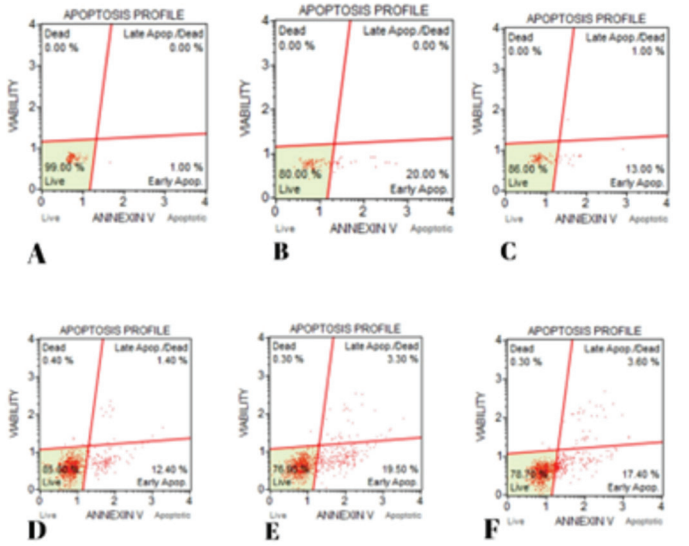

Figure 1. Annexin V test results (A) 24-hour control. (B) 1 mg/ml ginseng for 24 hours. (C) 2 mg/ml ginseng for 24 hours. (D) 48 hour control. (E) 1 µg/ml ginseng for 48 hours. (F) 48 hours 2 µg/ml ginseng

Table 1. Total apoptotic cell results after 24 and 48 hours in Annexin V & Dead cell test

Groups	Total Apoptotic Cell 24 hours	Total Apoptotic Cell 48 hours
Control	1.0±0.10[a]	13.8±2.8[a]
1 µg/ml ginseng	20.0±5.0[b]	22.8±2.5[b]
2 µg/ml ginseng	14.0±1.20[b]	21.0±1.8[b]
P*	0.001	0.008

P*; One-way variant analysis, P*; According to the Paired t test, Multiple comparison test (Tukey), the fact that the alphabetic superscripts contain the same letter indicates that the difference between the groups is not significant, while the difference indicates statistical significance. Data were expressed as arithmetic mean and standard deviation

A statistically significant difference was found between the 24 and 48 hour measurements of total apoptotic cells in the control, 1 and 2 µg/ml ginseng groups (p<0.050). According to the multiple comparison test, the control group was found to be significantly lower than the 1 and 2 µg/ml ginseng groups in the measurement of total apoptotic cells at 24 and 48 hours (p<0.050) (Table 1).

While there was a statistically significant difference between live cell measurements at 24 and 48 hours in the control group (p<0.012), no significant difference was found in the 1 and 2 µg/ml ginseng groups (p>0.05). While there was a statistically significant difference between the live cell measurements of the control, 1 and 2 µg/ml ginseng groups at 24 hours (p<0.001), there was no significant difference in the 48 hour measurement (p=0.354). According to the multiple comparison test, the control group was found to be significantly higher than the 1 and 2 µg/ml ginseng groups in the 24-hour measurement of live cells (p<0.05).

Cell Cycle Findings

With this test, cell cycle findings after 24 and 48 hours of incubation of MDA-MB-231 cells are demonstrated in Figures 2 and 3 and Table 2-3.

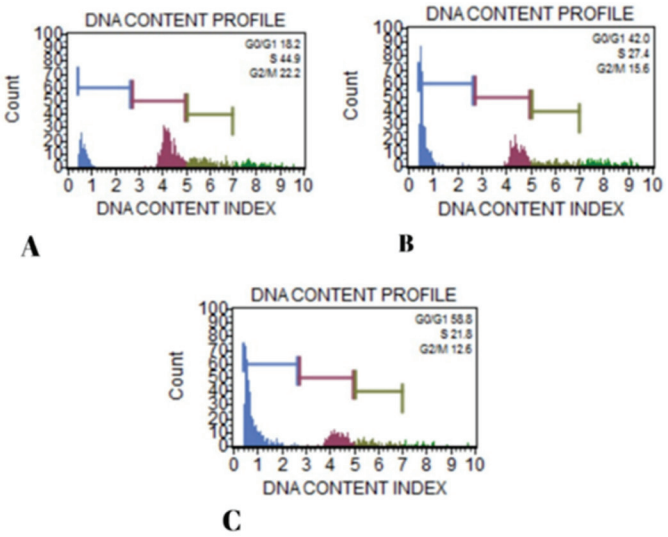

Figure 2. Cell Cycle test results (A) 24-hour control. (B) 1 µg/ml ginseng for 24 hours (C) 2 µg/ml ginseng for 24 hours

Table 2. G0/G1 phase change results at the end of 24 and 48 hours in the cell cycle test

Groups	G0/G1 24 hours	G0/G1 48 hours
Control	18.27±0.70[a]	76.7±12.4
1 µg/ml ginseng	42.0±6.0[b]	90.8±3.50
2 µg/ml ginseng	58.8±1.40[c]	92.2±6.40
P*	<0.001	0.113

P*; One-way analysis of variance, P*; According to the Paired t test, Multiple comparison test (Tukey), the fact that the alphabetic superscripts contain the same letter indicates that the difference between the groups is not significant, while the difference indicates statistical significance. Data were expressed as arithmetic mean and standard deviation

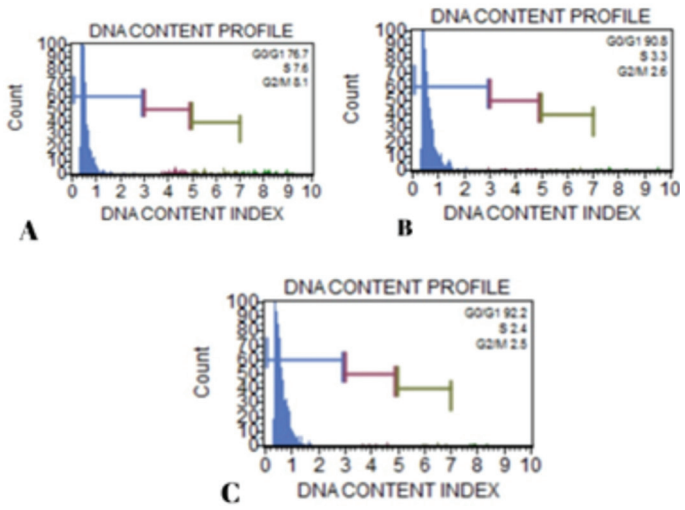

Figure 3. Cell Cycle test results (A) 48 hour control. (B) 48 hours 1 µg/ml ginseng (C) 48 hours 2 µg/ml ginseng

A statistically significant difference between the 24-hour measurements of the G0/G1 measurement of the control, 1 and 2 µg/ml ginseng groups ($p<0.001$), there was no significant difference in the 48-hour measurement ($p=0.113$). According to the multiple comparison test, G0/G1 measurement in 24-hour measurement Control group; It was found to be significantly lower than the 1 and 2 µg/ml ginseng groups ($p<0.05$) (Table 2).

While there was a statistically significant difference between the 24 hour measurements of the S measurement in the control and 1 µg/ml ginseng groups ($p<0.05$), there was no significant difference in control and 2 µg/ml ginseng groups ($p>0.05$) (Table 3). While there was a statistically significant difference between the 48 hour measurements of the S measurement of the 1 and 2 µg/ml ginseng groups ($p<0.01$). There was no significant difference was in the 24-hour measurement ($p=0.067$). According to the multiple comparison test, S measurement was found to be significantly higher than the control group, 1 and 2 µg/ml ginseng groups in 48 hours measurement ($p<0.05$) (Table 3).

Table 3. The results of the change in S phase at the end of 24 and 48 hours in the cell cycle test

Groups	S 24 hours	S 48 hours
Control	44.9±10.4	7.6±2.2[a]
1 µg/ml ginseng	27.4±5.2	3.3±1.2[b]
2 µg/ml ginseng	21.8±12.8	2.4±0.4[b]
P*	0.067	0.010

P*; One-way analysis of variance, P*; According to the Paired t test, Multiple comparison test (Tukey), the fact that the alphabetic superscripts contain the same letter indicates that the difference between the groups is not significant, while the difference indicates statistical significance. Data were expressed as arithmetic mean and standard deviation

AgNOR Staining Results

TAA/NA values and the mean AgNOR number are demonstrated in the Table 4 and 5. 24 hours incubation, the value was statistically significant in all ginseng groups compared to the control group ($p<0.05$). The mean 48 hours incubation AgNOR number was significantly in the 2 µg/ml ginseng groups compared to the control group ($p<0.05$). Additionally, 1 and 2 µg/ml ginseng groups statistically significant.

Table 4. Mean AgNOR number after 24 and 48 hours of incubation

Hours/Groups	Control	1 µg/ml	2 µg/ml	*p*
24 hours	4.18±1.17[a]	3.56±1.01[b]	3.36±0.94[bc]	<0.001
48 hours	3.96±1.26[ac]	3.46±0.97[c]	2.86±1.14[b]	<0.001

$p<0.05$ was considered statistically significant. Data are expressed as mean±SD (Standard deviation). There is no statistically significant difference between the groups containing the same letter ($p>0.05$). 1 and 2 µg/ml: ginseng groups. AgNOR: Argyrophilic nucleolar organizer region

Table 5. TAA/NA value at the end of 24 and 48 hours of incubation

Hours/Groups	Control	1 µg/ml	2 µg/ml	*p*
24 hours	0.06±0.02[a]	0.08±0.03[b]	0.06±0.02[a]	<0.001
48 hours	0.06±0.03	0.06±0.03	0.07±0.03	>0.001

$p<0.05$ was considered statistically significant. Data are expressed as mean±SD (Standard deviation). There is no statistically significant difference between the groups containing the same letter ($p> 0.05$); 1 and 2 µg/ml ginseng groups. TAA/NA: Total AgNOR area (TAA)/Total nuclear area (NA) ratio

When the data was evaluated for 24-hours incubation, TAA/NA value was statistically significant ($p<0.05$). In this data belonging to 48 hours incubation, these values were not statistically significant in ginseng groups compared to the control group ($p>0.05$).

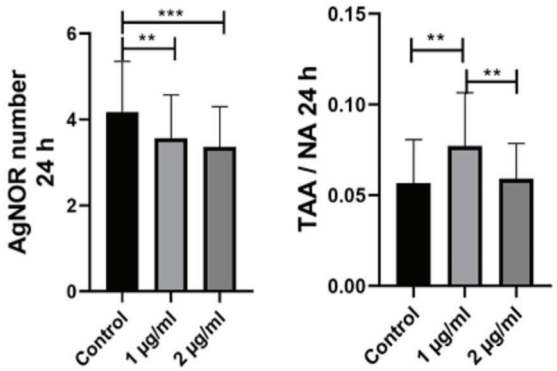

Figure 4. Comparison of AgNOR number and TAA/NA ratio between groups after 24 hours of incubation

DISCUSSION

Studies have shown that ginseng reduces the viability of the breast cancer cell line MDA-MB-231 and inhibits cell proliferation of MCF-7 cells in the dose range of 1 to 2.5 mg/mL (8). It was stated that prostate cells lost their adhesion ability after incubation with 250 μM ginsenoside for 48 hours, suppressing the expression of androgen receptor, prostate specific antigen, proliferating cell nuclear antigen expression and 5α-reductase biomarker genes. Ginsenoside has been shown by fluorescence microscopy to cause a change in the morphology of the cell undergoing apoptosis. As a result, it has been suggested that it arrests prostate cells in the G1 phase and then inhibits cell growth through the caspase-3-mediated apoptosis mechanism (9). It has been reported that ginseng reduces cell viability when administered alone in hepatocellular carcinoma cells depending on a dose of 0.64±0.02 mg/mL (10). In addition, when MCF-7 and MDA-MB-231 human breast cancer cells were treated with 0.5 mg/ml ginseng (Panax quinquefolius), it increased antiproliferative activity, decreased viable cell count, and kept cancer cells in the G phase of the cell cycle (11). In another study, they reported that 1.0 mg/ml ginseng inhibited proliferation in HCT116 human colon carcinoma cells and stopped the cell cycle in the G0/G1 phase. (12). Another study showed that components of Panax ginseng roots inhibited human renal cell carcinoma cells in the transition from G1 to S phase by blocking cell cycle progression (13). In a study in leukemia cells (K562), 400 mg/l ginseng polysaccharide was administered to K562 cells for 48 hours. It was determined that the G0/G1 phase increased significantly, and the G2+M and S phases of the cell cycle were significantly decreased. It has been reported that various concentrations (0-25-50 and 100 μM) of Rg5 (the active ingredient of ginseng) induce cell cycle stop in the G0/G1 phase through the regulation of cell cycle-related proteins in breast cancer cells (14). It was stated that GE treatment significantly inhibited the viability of each MCF-7 cells in a concentration-dependent manner in the presence of 5 μM Rh2, and also caused cell cycle stopping and inhibition of G1-S phase-specific enzyme activities with ginseng (15).

These results in the literature support the results that we showed in our study. In addition, it was observed that MDA-MB-231 human cancer cells treated with ginseng caused apoptosis and stop it in the G0/G1 phase of the cell cycle. Phytochemicals obtained from medicinal plants have been used in the treatment of human diseases from past to present. Identification of new phytochemical agents and determination of the most reliable dose for cancer therapy are crucial for improving the diagnostic accuracy and management of diseases. NORs are associated with most regulatory proteins and have roles as functional subunits of the nucleolus. Changes in AgNOR protein amounts reflect the metabolic activities and protein synthesis capacities of cells. There are different studies on malignant and benign lesions (5).

In our study, results were obtained with 1 and 2 μg/ml doses of ginseng and over two times as 24, 48 hours, the effective dose that kills breast cancer cells and leads to apoptosis is 1 μg/ml for 24 and 48 hours, also, in the group that stopped in the G0/G1 phase, there was 2 μg/ml ginseng group for 48 hours. Having common points with other studies, it has been seen that Ginseng plant, which has not yet been fully clarified in the literature, can lead to early apoptosis of breast cancer cells and increase total apoptosis. In this study, it was seen that ginseng has a very important function against the development of cancer. However, both AgNOR values can be used as biomarkers in determining the most reliable therapeutic dose selection for cancer, and it has been shown that the correct consumption of Ginseng can be effective in preventing cancer formation and slowing its progression.

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MEDICAL RECORDS-International Medical Journal

Research Article

Correlation of Fine Needle Aspiration Cytology and Histopathological Evaluation in Salivary Gland Masses: A Single Center Retrospective Study

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Abstract

Aim: Fine-needle aspiration cytology (FNAC) in the preoperative diagnosis of salivary gland (SG) masses is a very fast, inexpensive, and reliable diagnostic method. In our study, the correlation of cytological-histopathological diagnosis in cases diagnosed with fine needle aspiration cytology in our clinic was investigated, and possible causes of diagnostic entrapment in discordant cases were discussed.

Material and Methods: Salivary gland FNAC cases with histopathological diagnosis between 2008 and 2019 were retrospectively analyzed.The age, gender, localization of the lesion, preoperative cytology, and postoperative histopathological diagnosis of the patients were recorded. Cytology results were analyzed in 5 categories: unsatisfactory, uncategorized, benign, suspected malignancy, and malignant. Histopathology results were recorded in 2 groups benign-nonneoplastic and malignant. Statistically significant difference level was accepted as $p<0.05$. The validity of the cytology result according to the biopsy result was evaluated by sensitivity, specificity, positive predictive value, and negative predictive value.

Results: 316 cases of salivary gland fine needle aspiration were detected. 156 (49.4%) of 316 cases had histopathological diagnosis. When calculating the cytological-histopathological diagnosis, the cases that were found to be inadequate and uncategorized by cytology were not taken into consideration. The suspected malignancy group was evaluated within the malignant category. Therefore, diagnostic agreement was calculated in 124 cases. Of these 124 cases, 116 (93.6%) cytology-histopathology diagnosis were compatible, and 8 (6.4%) were not.

In our series, the overall sensitivity and specificity were 83.3% and 97.7%, respectively. The positive predictive value was 93.7% and the negative predictive value was 93.4%. The accuracy rate was calculated as 93.5%.

Conclusion: In our study, high sensitivity and specificity values were determined by FNAC in accordance with the literature. It should be kept in mind that there may rarely be differences between preoperative cytological and histopathological diagnoses, possibly due to experience, method, and lesion-related limitations and pitfalls.

Keywords: Salivary gland, fine needle aspiration cytology, histopathology, sensitivity, specificity

INTRODUCTION

Fine-needle aspiration cytology in salivary gland (SG) masses is a reliable, fast, and inexpensive preoperative diagnosis method for centers with sufficient clinical experience. It is a minimally invasive application that can be performed in outpatient settings. In today's practice, the treatment approach to a detected SG mass is decided after evaluating the clinical-radiological-cytological data. Therefore, routine use of fine-needle aspiration cytology (FNAC) is recommended for preoperative diagnosis on an SG mass (2-11). In recent years, the 'Milan Reporting System' has been defined to ensure that cytological findings are reported using a common language in certain diagnostic categories and to analyze the malignancy risk for each diagnostic category and to develop a clinical approach algorithm (8).

CITATION

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Many non-neoplastic processes in SG can cause mass lesions. For example, intra-gland lymph node pathologies that do not require surgery can be confused with a primary tumor. Primary SG tumors have an extensive classification list with rare subtypes added daily (1). Even in a single tumor type, histomorphological heterogeneity may be evident. These features create difficulties in cytological and histopathological diagnosis. When the literature is examined, it has been reported that the diagnostic value of FNAC in SG masses is variable and its accuracy rate is relatively low compared to tumors of the other head and neck region (2-5). In recent years, the accuracy rate of FNAC in major SG masses is over 90% in studies based on large series of experience (6-11).

In our study, the correlation of cytological-histopathological diagnosis in cases diagnosed with fine needle aspiration cytology in our clinic was investigated, and possible causes of diagnostic entrapment in discordant cases were discussed.

MATERIAL AND METHOD

Our study was approved by the Ondkouz Mayıs University Clinical Research Ethics Committee with the decision dated 30.12.2021 and numbered B.30.2.ODM:0.20.08/843. A total of 316 salivary gland FNAC cases were diagnosed in Ondokuz Mayıs University, Pathology Department between 2008 and 2019 and the histopathological diagnoses of these cases were evaluated retrospectively.

Patient age, gender, and localization of the lesion were recorded. FNAC and histopathological diagnoses were compared. FNAC in our clinic; There were four main diagnostic categories: "Inadequate/Non-diagnostic", "Malignancy negative" (nonneoplastic or benign neoplasia), "suspicious malignancy" and "Malignant". In addition, there is a fifth separate diagnostic group for the cases that cannot be classified and reported as "not categorizable" in cases where cytological findings are not guiding. The histopathological diagnosis, which is accepted as the gold standard, was determined in 156 (49.4%) cases. The histopathological diagnoses given in the surgical materials were examined in two main diagnosis groups as "nonneoplastic or benign" and "malignant". The categories of "unsatisfactory" and "not categorizable" were not included in the statistical evaluation. The "suspicious malignancy" group was evaluated within the malignant diagnosis group. In the remaining 124 cases, cytological-histopathological agreement was calculated.

The research data were analyzed using the SPSS version 22.0 statistical program. The conformity of the data to the normal distribution was evaluated with the Kolmogorov-Smirnov test. Since the continuous variables do not follow the normal distribution while expressing the data, the median (1. Quarterly: Q1 - 3rd Quarter: Q3) and categorical variables were presented with frequency and percentage distributions. Mann Whitney U test was used to compare the age variable between the groups. Statistically significant difference level was accepted as $p<0.05$. The validity of the FNAC result according to the biopsy result was evaluated by calculating the sensitivity, specificity, positive predictive value, and negative predictive value.

RESULTS

Of 316 patients with salivary gland fine needle aspiration biopsy, 178 (56.3%) were male and 138 (43.7%) were female. The median age of the patients was 56 (Q1:42.25 - Q3:66.0). While the median age was 56.5 (46.0-66.0) in men, it was 54 (39.0-66.0) in women, and there was no age difference between the sexes (p:0.23). The number of children (18 years and younger) was 16 (5.06%). Of the FNACs, 274 (86.7%) belonged to the parotid gland, and 42 (13.3%) belonged to the submandibular gland. The distribution of the number of cases by year is given in Figure 1.

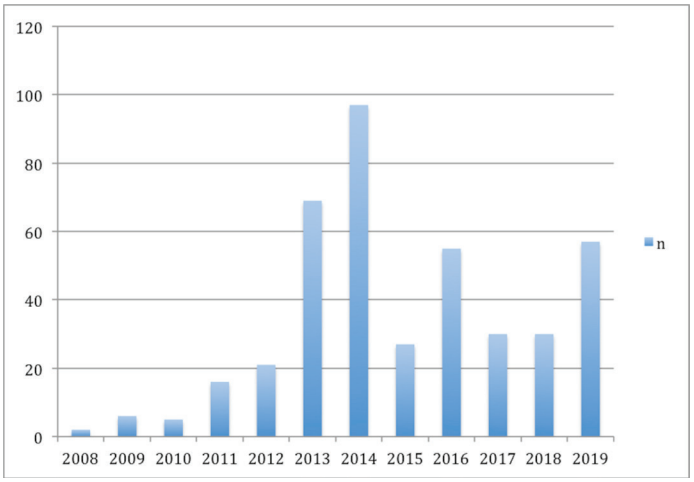

Figure 1. Number of salivary gland fine needle aspiration cytology material by years

Of 316 FNACs, 44 (13.9%) were categorized as "inadequate/non-diagnostic", 180 (57.0%) as "malignancy negative" (nonneoplastic-benign), 37 (11.7%) "categorized" undetectable", 20 (6.3%) "suspicious malignancy" and 35 (11.1%) "malignant" categories. 156 (49.4%) of 316 cases had histopathological diagnosis. FNACs of the patients in the inadequate and uncategorized group were not repeated. Repeated FNACs were not included in the calculation.When calculating the cytological-histopathological diagnosis, the cases that were found to be inadequate and uncategorized by cytology were not taken into consideration. The suspected malignancy group was evaluated within the malignant category. Therefore, diagnostic agreement was calculated in 124 cases.

While cytology-histopathology agreement was found in 116 (93.6%) of 124 cases included in the study, the diagnosis was inconsistent in 8 (6.4%). The cytological and histopathological diagnosis distributions of the cases are summarized in Table 1. It was interpreted as benign neoplasia in 79 (85.8%) and nonneoplastic processes in 13 (14.2%) of 92 cases in the "malignancy negative" group. 6 cases that we interpreted as benign neoplasia were diagnosed as malignant neoplasia, and 2 cases that we interpreted as malignant were diagnosed as benign neoplasia.

Table 1. Histopathological diagnoses of fine needle aspiration cytology evaluated for diagnostic compliance

Histopathological diagnosis distribution	n:124	%
Neoplastic (malignant)	**n:35**	**28.3%**
Squamous cell carcinoma	18	14.5%
Adenoid cystic carcinoma	6	4.8%
Malignant lymphoma	3	2.4%
Asinic cell carcinoma	2	1.6%
Carcinoma Ex pleomorphic adenoma	1	0.8%
Mucoepidermoid carcinoma	1	0.8%
Malignant melanoma	1	0.8%
Low graded salivary gland carcinoma	1	0.8%
High graded salivary gland carcinoma	1	0.8%
Adenocarcinoma, not otherwise specified	1	0.8%
Neoplastic (benign)	**n:77**	**62%**
Pleomorphic adenoma	48	38.7%
Warthin tumor	27	21.7%
Basal cell adenoma	2	1.6%
Nonneoplastic	**n:12**	**9.7%**
Sialadenitis	6	4.8%
Cyst	3	2.4%
Intraparotidal lymph node	2	1.6%
Vascular lesion	1	0.8%

In our series, the overall sensitivity and specificity were 83.3% and 97.7%, respectively. The positive predictive value was 93.7%, the negative predictive value was 93.4%, and our accuracy rate was 93.5% (Table 2). The sensitivity was 88.2%, the specificity was 97.4%, and the accuracy rate was 94.6% when calculated only for the cases for which we made a benign interpretation. When we made a malignant interpretation, the sensitivity was 76.9%, the specificity was 97.7%, and the accuracy rate was 92.9%.

Table 2. Cytological and histopathological diagnostic compatibility

	n
Number of cases included in statistical evaluation	124
True negative	86 (69.3%)
True positive	30 (24.1%)
False negative	6 (4.8%)
False positive	2(1.6%)
Sensitivity	83.3%
Specificity	97.7%
Positive predictive value	93.7%
Negative predictive value	93.4%
Accuracy rate	93.5%

The cytology of six cases with false negativity in FNAC were reported as cellular pleomorphic adenoma (PA) (n:4), reactive intraparotid lymph node (n:2). Three of the patients with PA in FNAC were diagnosed as adenoid cystic carcinoma (ACC) and one of them was mucoepidermoid carcinoma histopathologically after surgery. One of the 2 cases that we interpreted as intraparotidal lymph node in FNAC was diagnosed as follicular lymphoma and the other was diagnosed as diffuse large B-cell lymphoma (Figure 2-3).

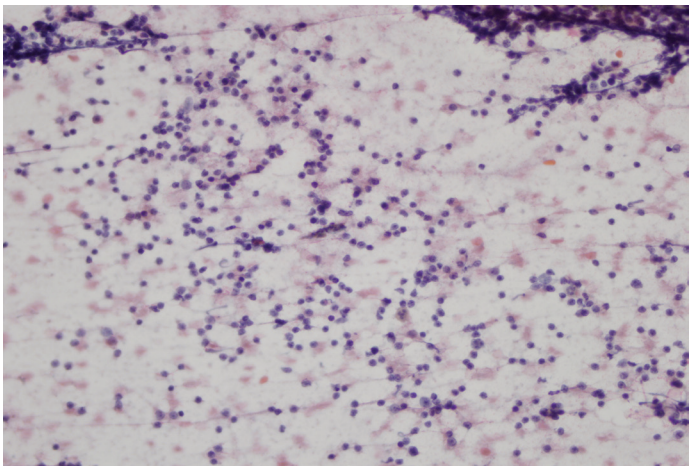

Figure 2. Lymphoid cells in salivary gland cytology diagnosed as malignancy negative, (PAPX400)

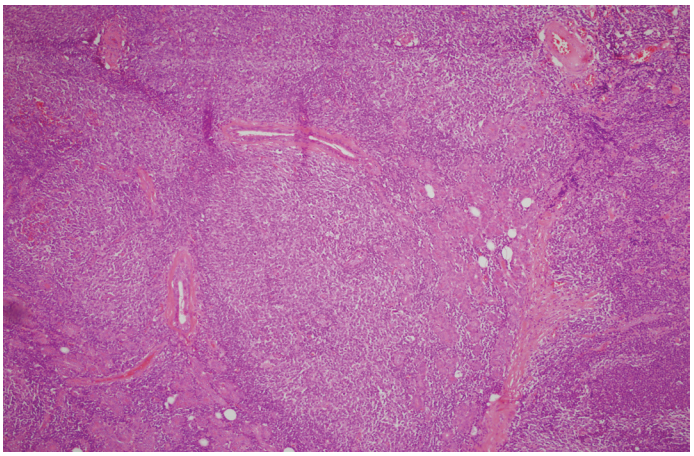

Figure 3. Histopathological section of cytology diagnosed as negative for malignancy. Follicular lymphoma (HEX200)

False positivity was detected in two cases. The tissue diagnosis of the case whose cytological diagnosis was ACC was basal cell adenoma (BCA)(Figure 4-5), and the tissue diagnosis of the case we interpreted as papillary thyroid carcinoma was PA (Table 3).

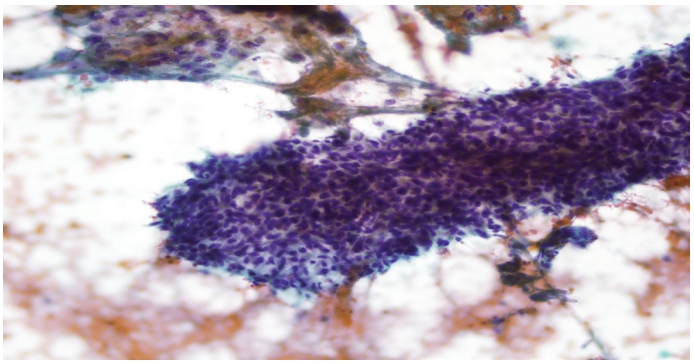

Figure 4. Crowded hyperchromatic basal cells diagnosed as malignancy positive (PAPx200)

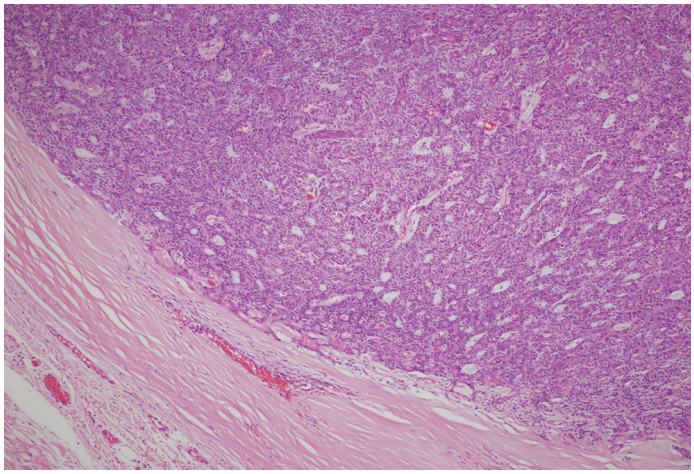

Figure 5. Basal cell adenoma (HEX100)

Table 3. The false negative and positive diagnoses given cytology cases

	n	Cytological diagnosis (n)	Histopathological diagnosis (n)
False negativity	6	Pleomorphic adenoma (4)	Adenoid cystic carcinoma (3)
			Mucoepidermoid carcinoma (1)
		Intraparotidal lymph node (2)	Follicular lymphoma (1)
			Diffuse large B -cell lymphoma (1)
False positivity	2	Adenoid cystic carcinoma (1)	Basal cell adenoma (1)
		Papillary thyroid carcinoma (1)	Pleomorphic adenoma (1)

DISCUSSION

Primary SG tumors constitute 3% of head and neck tumors (1,12-13). Most of these histologically complex tumors are benign. Especially well-differentiated malignant SG tumors have pathological findings overlapping with benign tumors. The primary treatment of salivary gland tumors is surgery and adjuvant radiotherapy and/or chemotherapy can be applied depending on the histopathological type, grade and stage of the tumor. Especially in the preoperative diagnosis of major SG primary tumors, FNAC is the most important diagnostic method. Its diagnostic accuracy is between 80-95% and it is superior to physical examination and imaging methods in the diagnosis of SG lesions (2-11). With FNAC, it is tried to answer whether the mass is inflammatory, neoplastic, benign or malignant. If a malignancy decision is made, it should be reported whether it is a primary salivary gland tumor or a metastatic tumor. If a primary salivary gland tumor is diagnosed, its grade (low/high) should be specified. Thus, the distinction between masses that require surgery and those that do not, or the type of surgery is partially determined, and complications related to the treatment of the patient are partially avoided (11,14-21).

The sensitivity, specificity, and accuracy of FNAC in the preoperative evaluation of primary SG tumors is over 90% (6,7,22). Sensitivity and specificity rates for FNAC in benign lesions have been reported as 64-100% and 75-100%, respectively, and the accuracy rate as 69-100% by various authors (6,7,23-27). Alphs et al. found the accuracy of FNAC to be 90-95%, and Al Salamah found 89% in their study (28,29). In a study conducted in our country, Yıldız et al. reported the sensitivity of preoperative FNAC as 59.09%, specificity as 97.85%, accuracy as 93.75%, positive predictive value as 76.47%, and negative predictive value as 95.2% for the diagnosis of malignancy (30).

Our results also showed that benign and malignant masses could be detected with a high accuracy rate (93.5%) with FNAC, similar to previous studies. Again, similar to the literature, 83.3% sensitivity and 97.7% specificity rates were obtained in benign and malignant SG masses, respectively.

In general, the factors that most affect the diagnostic value of FNAC are the adequacy of the cytological material, its preparation with a good technique, and the experience of the pathologist. Another important factor is the difficulties arising from salivary gland tumors having different cytological/histopathological features within the same tumor. Examples of reactive inflammatory conditions may be indistinguishable from low-grade lymphoma. Similarly, cases of ACC may be indistinguishable from cellular pleomorphic adenoma or cases of low-grade mucoepidermoid carcinoma from Warthin tumor or from non-neoplastic processes such as chronic sialadenitis and retention cysts (15-21). In the literature and in our study, it was seen that the diagnostic difficulties experienced during the cytological evaluation were concentrated in certain entities. It is difficult to distinguish reactive inflammatory conditions such as nonspecific or obstructive sialoadenitis and Mikulicz syndrome from primary SG low-grade lymphomas such as extranodal marginal zone lymphoma at the cytological level (31-34). Cohen et al. found that half of the false-negative results were low-grade lymphomas (31). Zurrida et al. They reported that only 2 of 7 parotid lymphoma cases were diagnosed correctly with FNAC and these were high grade (32). In our series, no lymphoma case was found in FNAC. However, two false-negative cases in our series were interpreted as reactive intraparotid lymph nodes. Follicular lymphoma and diffuse large B-cell lymphoma were diagnosed in the histopathological evaluation. Therefore, it should not be forgotten that flow cytometric examination should be added simultaneously with cytomorphological sampling in cases with clinical pre-diagnosis of lymphoma (35). It is also known that FNAC is very useful in differentiating lymphomas from SG carcinomas (33).

In the review of the American College of Pathologists, it was stated that approximately half (53%) of monomorphic adenomas were interpreted as "false positive" (36). ACC, which is usually rich in monotonous small blue cells with narrow cytoplasm in cytology smears, lacks the classical nuclear features of malignancy. Therefore, it can be diagnosed as cellular PA or BCA and vice versa. The cytological features of the tumor stroma and the cell-stroma interface may help differentiate benign and malignant entities but may be insufficient (3,36). Darvishian et al. stated that the presence of pleomorphism,

coarse chromatin, prominent nucleoli, mitotic figures, and necrosis was only observed in malignant myoepithelial lesions, and they suggested that the presence of any of these features may require wide excision and lymph node dissection with a more aggressive surgical approach (37). For these cases with overlapping cytomorphological findings, the term basaloid neoplasms and the diagnostic category "neoplasm with uncertain malignant potential" can be used (8). One of the false positive cases in our series is an example. It should be remembered that another primary SG malignancy with cytological features with an innocent appearance is ACC (5,23-27). If there is a suspicion of neoplasm, a consultation request from a pathologist/cytopathologist familiar with head and neck pathology will be the most practical and quick solution.

Mucoepidermoid carcinoma is another entity with which we have a diagnostic mismatch, as it contains heterogeneous cell populations, different grade foci, and cystic/solid areas (3,5,36). When the series in the literature are examined, the critical reason for the diagnosis mismatch is sampling errors (6,7,35).

In one case in our series, a false positive was caused by incorrectly filling out the pathology request form. Namely, the cytology sample taken from the submandibular gland was sent as "thyroid" and it was reported as papillary thyroid carcinoma instead of PA diagnosis by our colleague dealing with endocrine pathology because of overlapping cytomorphological findings.

Cytological sampling is insufficient in cystic, small, mobile or prominent fibrotic masses. It may be reported in the "non-diagnostic" category (3,25). Insufficient material ratios have been reported in many studies with variable values such as 1.1% and 12% (2-6,23-27,38). The inadequate rate in our study, which was slightly higher than in the literature, can be resolved by performing FNAC by an experienced radiologist/clinician and a rapid on-site evaluation until experience is gained. Attempting to make a diagnostic interpretation of unsatisfactory samples may indicate a diagnostic inconsistency, often in the form of false negatives; we did not encounter this situation in our series. The pathologist should be comfortable with having adequate data when making diagnostic interpretations. In our series, clinicians did not prefer re-aspiration from cases diagnosed as inadequate and uncategorized, and planned the treatment according to clinical- radiological findings.

The most common malignant tumors that metastasize to the salivary gland are squamous cell carcinoma (SCC), malignant melanoma, and less commonly kidney, breast, and thyroid carcinomas. Therefore, the possibility of metastasis should always be kept in mind, especially in the differential diagnosis of high-grade primary SG neoplasms (39). In our series, the diagnosis of SCC was high (14.5%). Primary SCC of SG is rare and cutaneous or mucosal regions, including the scalp, should be examined for a possible primary focus in the head and neck region (40). Adequate clinical information, radiological imaging findings and knowing the sampling site will prevent misinterpretation of cytological findings.In our study, the number of cases increased significantly over the years. In this process, the cases were reported by the cytopathologist, in-clinic consultation, and surgical pathologists, and only by the head and neck pathology team. Our clinic uses a conventional reporting system in FNAC, and the weak point of our study is the need to integrate our diagnostic categories with the Milan system. After accumulating retrospective analyses similar to our study and increasing cytology applications, the second stage will be the routine use of the Milan system introduced to clinicians.

Our study has shown that we have reached similar rates to the high sensitivity and specificity values reported in the literature regarding the diagnostic value of FNAC. In cases where a clear diagnostic categorization cannot be made, it may be useful to use the diagnostic approach of "Uncategorized; please read the comment", which can guide the clinician. Despite the false-negative and false-positive values, with limitations and pitfalls always in mind, our results showed that the application of FNAC is rapidly increasing and advantageous for clinicians and patients in preoperative diagnosis.

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MEDICAL RECORDS-International Medical Journal

Research Article

Nerve Conduction Studies and Measurement of Median Nerve Cross-Sectional Area in Patients Newly Diagnosed with Hypothyroidism

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Abstract

Aim: This study aimed to investigate the rate of carpal tunnel syndrome (CTS) and polyneuropathy in patients with new-diagnosed hypothyroidism and the relationship between median nerve conduction and the cross-sectional area of the median nerve by ultrasonography.

Material and Methods: It was a prospective, cross-sectional and case-control study. This study included thirty-five new-diagnosed hypothyroidism cases and thirty-five healthy controls. Bilateral sensory and motor nerve potentials were noted in the lower and upper extremities. The cross-sectional area of the median nerve was examined at the entrance of the CT with the axial plan by ultrasonography. The relationship between the cross-sectional areas of the median nerve and nerve conduction parameters was investigated.

Results: CTS was determined electrophysiologically in 8 (22.9%) patients. The control group had no CTS. There was no significant electrophysiological finding to support polyneuropathy in the patients. The cross-sectional areas of the median nerve were higher in the patient group but did not reach statistically significant ($p>0.05$). There was a positive correlation between the right and left cross-sectional area of median nerves and body mass index (BMI) ($p<0.05$).

Conclusion: The rate of CTS is high in patients with newly diagnosed hypothyroidism. There is a positive correlation between cross-sectional nerve areas and BMI. There was no significant correlation between nerve conductions and median nerve ultrasonographic cross-sectional areas.

Keywords: Nerve conduction, hypothyroidism, neuropathy, ultrasonography

INTRODUCTION

Hypothyroidism can cause a variety of neurological signs and symptoms. Hypothyroidism can involve both the central and peripheral nervous systems. In hypothyroid patients, proximal muscle weakness and peripheral neuropathy (numbness, paresthesia, and hypoesthesia) may develop. Some studies report carpal tunnel syndrome as the most common neuropathy associated with hypothyroidism (1). Again, some other studies suggest that primarily sensorimotor axonal polyneuropathy is the primary pathology (2-4). However, there are also studies speculating that there is a demyelinating affection rather than axonal neuropathy (5,6).

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Ultrasonography (US) can be used as a supportive method in diagnosing entrapment neuropathies, detecting the level of entrapment, and determining the etiology of entrapment. It may also contribute to the planning and follow-up of treatment (7).

Our study aimed to evaluate the degree of entrapment neuropathy or polyneuropathy (PNP) with nerve conduction studies (NCS) in newly diagnosed hypothyroid patients and to measure the median nerve cross-sectional areas by US in the carpal tunnel and to examine the correlation with electrophysiological parameters.

MATERIAL AND METHOD

This study was approved by the ethics committee of Selcuk University (No: 2019/04)

Participants and ethical procedure

Our study included 35 patients of both sexes, aged between 18-60, who applied to the endocrinology outpatient clinic of the Selcuk University Medical Faculty Hospital and were diagnosed with clinical hypothyroidism and had not yet been treated. As the control group, 35 healthy individuals who were similar in age and gender without any medical disease or drug use were included. Serum-free T4 (fT4) and thyroid-stimulating hormone (TSH) levels of the patients and control group were measured. Normal serum concentration ranges of TSH and fT4 were taken as 0.27–4.2 mU/L and 0.93–1.7 ng/dL, respectively. Healthy individuals with TSH and fT4 in this range were included in the control group. Patients with increased TSH and low fT4 levels were considered to have clinical hypothyroidism.

Sociodemographic data of the patients before the study were recorded in patient evaluation form as the weight, height, and body mass index (BMI). The backgrounds of the participants were questioned in detail, and the drugs they used, their history of operation, and known diseases were learned.

Pregnant women, those with vitamin B12 deficiency, kidney and liver disease, diabetes mellitus, alcoholism, use of drugs known to cause neuropathy or myopathy, malignancy, heart failure, and a personal or family history of neuropathy or neuromuscular disease were excluded from the study.

Nerve conduction studies and evaluations

Electrophysiological studies were performed by the same person with the Nihon Kohden Corporation Model MEB-9200K (Japan, 2005) EMG device according to the guidelines of the American Society for Electrodiagnostic Medicine (8). Motor-sensory nerve conduction and delayed responses of the cases and controls were recorded in the electrophysiology laboratory with a standard protocol. NCS bilateral median, ulnar sensory, and motor in the upper extremities; bilateral sural sensory, tibial, and peroneal nerve motor conduction studies were performed in the lower extremities. Recorded parameters: motor nerve-associated compound muscle action potential (CMAP) amplitude and areas, terminal and proximal latencies, and conduction velocities; sensory nerve action potential (SNAP) onset latency, amplitude, area, and conduction velocities were determined. Reflex responses: At least ten consecutive stimulations were given, and the right median F responses in the upper extremity and the left Tibialis F responses in the lower extremities were recorded. F responses minimum (Fmin) latencies were evaluated. Normative data were created prospectively from the lower and upper extremities of 35 healthy, age- and gender-matched individuals without any neurological or medical disease or drug use, using the same protocol, or adapted from similar literature3. Patients diagnosed with CTS electrophysiologically were grouped as mild, moderate, and severe according to their findings. Mild CTS: Reduced median sensory distal conduction velocity and/or decrease the sensory potential amplitude below normal. Moderate CTS: Extension of the distal latency of the median motor nerve in addition to the findings mentioned above. Severe CTS: It was often determined as the absence of sensory potential and a decrease in the normative values of motor response amplitude below 2 SD or prolongation of its latency (8-10).

The conduction velocity slows down more than 30% from the lower limit of the normal value in more than two motor and sensory nerves, except for the entrapment regions, distal latencies being above 150% of the normal upper limits, and acquired in significant conduction blocks was evaluated as demyelinating polyneuropathy; when there was a slight slowdown in conduction velocities, a slight prolongation of latencies, and a decrease in amplitudes in the foreground, it was evaluated as axonal polyneuropathy (11).

Ultrasonography examination

Nerve diameters were determined by US in terms of median nerve entrapment in both upper extremities by a person blinded to the results of NCS. The cross-sectional areas of the median nerve in the carpal tunnel were measured by the US and it was evaluated whether there was an increase in nerve thickness. Cross-sectional area measurement with median nerve US was performed as mentioned; while the patient was in the sitting position, the upper extremity arm on the measuring side was at semiflexion on the elbow, the forearm was positioned in supination, the fingers were semi-flexed, the wrist was on a flat surface, the probe was in the axial position at the entrance of the carpal tunnel. In order to increase the accuracy of the measurement, the hypoechoic inner border was measured by leaving the hyperechoic epineurium outside. During the measurement, care was taken not to apply pressure to the skin as much as possible with the probe, thus, not to measure the diameter of the nerve shorter (12). EsaoteMyLab Twice device 12 MHz linear probe was used for measurements, and area measurements were calculated by the device.

Statistical Analysis

The research data were analyzed using the SPSS (Statistical Package for Social Sciences v 22.0) software. Number, percentage, mean, and standard deviation were used as descriptive statistical methods. An independent sample t-test was conducted to compare continuous quantitative data between two independent groups. Categorical data were compared with Chi-square or Fisher's exact test. Relationship analyses between parameters were tested with Pearson correlation. $p<0.05$ was significant.

RESULTS

There were 30 (85.7%) females and 5 (14.3%) males in the patient and control groups. The mean age and height of the groups were similar ($p>0.05$). Although weight and BMI values were slightly higher in hypothyroid patients, this difference was not statistically significant ($p>0.05$) (Table 1).

Table 1. Patient and control group demographics, body mass index, and thyroid function tests mean and standard deviations

	Hypothyroid group (n = 35)	Control group (n = 35)	t	p
Age	37.40±10.88	37.80±10.35	0.16	0.88
Height	1.64±0.09	1.66±0.07	1.37	0.18
Weight	77.97±16.30	76.83±10.36	-0.35	0.73
BMI	29.11±6.04	27.75±3.46	-1.16	0.25
TSH	8.42±5.30	2.13±1.06	-6.89	**0.001**
T4	0.98±0.24	1.24±0.19	5.18	**0.001**
Female	30 (85.7%)	30 (85.7%)	X^2=0.00	p=0.63
Male	5 (14.3%)	5 (14.3%)		

TSH = Thyroid stimulating hormone, BMI=Body mass index, n=number, %=percent

Eight (22.9%) of 35 patients had CTS electrophysiologically, and 4 of them were bilateral. One of these patients had moderate CTS, and 7 had mild CTS. Severe CTS was not determined.

No significant electrophysiological abnormality was noted to support polyneuropathy. In general, the evaluation did not reach the level to confirm the definitive diagnosis of polyneuropathy, but there were isolated mild abnormalities in the subgroup analyses.

The cross-sectional area of the median nerve was higher in the hypothyroid group. Still, it did not reach a statistically significant difference ($p=0.06$ for the right median nerve, $p=0.26$ for the left median nerve) (Table 2).

Table 2. Comparison of ultrasonographic cross-sectional areas in the patient and control groups

	Control group (n = 35)	Hypothyroid group (n = 35)	t	p
Right median	7.66±1.60	8.33±1.34	-1.90	0.06
Left median	7.76±1.79	8.21±1.56	-1.14	0.26

A significant positive correlation was found between BMI and nerve cross-sectional areas. There was a significant positive correlation between BMI and right median ultrasonographic nerve cross-sectional area (UNCA) ($p=0.001$, $r=0.378$) and between BMI and left median UNCA ($p=0.002$, $r=0.363$).

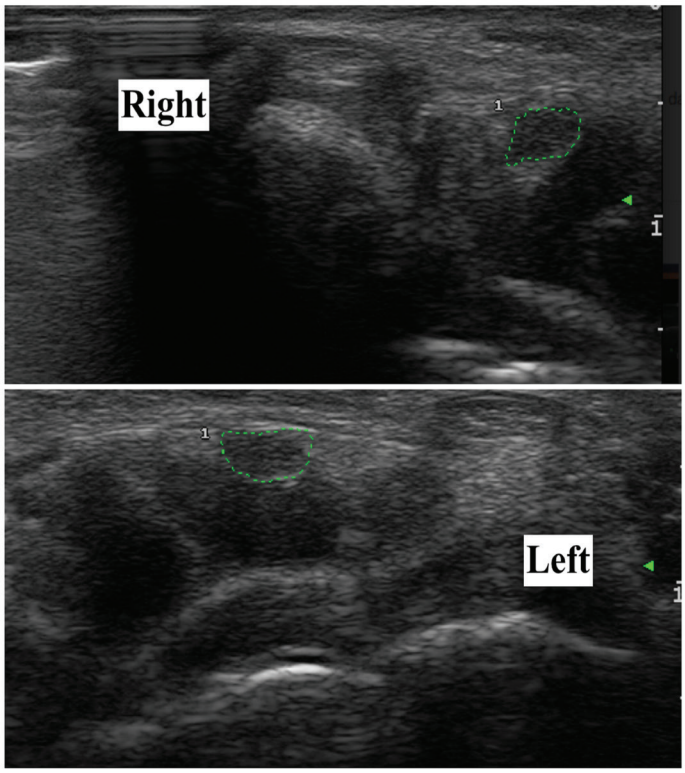

Figure 1. It shows a cross-section area of the right and left median nerve with wrist level gray scale ultrasound (dot lines indicate median nerve)

A slightly significant positive correlation was determined between left median sensory latency and left median UNCA ($p=0.008$, $r=0.314$) and between right median sensory latency and right median UNCA ($p=0.007$, $r=0.321$). There was also a slightly significant negative correlation between right median sensory conduction velocity and right median UNCA ($p=0.001$, $r=-0.380$) (Table 3).

Table 3. Correlation analyzes of nerve conduction and ultrasonographic cross-sectional areas

		Right median nerve	Left median nerve
sMLleft	r	0.335	0.314
	p	0.005	**0.008**
sMLright	r	0.321	0.290
	p	**0.007**	0.015
sMIHright	r	-0.380	-0.292
	p	**0.001**	0.014

sML = median sensory latency, sMIH = median sensory conduction velocity

No significant correlation was determined between TSH and T4 and NCS and other parameters.

DISCUSSION

The main result of our study is the presence of CTS with a high rate in newly diagnosed hypothyroid cases, but it supports that no significant PNP has developed, electrophysiologically. In studies on newly diagnosed hypothyroidism, CTS has been reported with a frequency of 16-55% (3,13). In the study of Asia et al., CTS was detected in 5 (19%) of 26 patients newly diagnosed with hypothyroidism. They suggested hypothyroidism predisposes to CTS via segmental demyelination by affecting Schwann cells (14). In our study, CTS was determined electrophysiologically in 8 (22.9%) of the patients. In 50% of these, the findings were compatible with bilateral CTS. The incidence of CTS in patients newly diagnosed with hypothyroidism varies. This may be related to the variable time elapsed between the onset of hypothyroidism and diagnosis. In addition, it may be caused by the differences in demographic characteristics such as age, gender, occupation and BMI of the cases in the study population. Study populations are small samples, large-scale studies are needed for clearer results. Moreover, CTS is a common medical condition, and its incidence increases in advanced age, independent of hypothyroidism. One of the factors limiting our study is the low number of cases.

US examination can be used as a supportive method in diagnosing entrapment neuropathies, determining the level of entrapment and the etiology of entrapment. It can contribute to the planning and follow-up of the treatment. Swelling of the nerve just proximal to the entrapment site can be demonstrated by US. The most commonly used ultrasonographic measurement method in carpal tunnel syndrome is nerve cross-sectional area measurement. Various studies have shown that the cross-sectional area of the median nerve is significantly higher in CTS patients than in the normal population (7). In our study, the cross-sectional area of the median nerve was higher in the hypothyroid group, but it did not reach a statistically significant level of difference. Slightly significant positive correlation between median sensory latencies and median UNCA in the correlation analyses of US and NCS; a slightly significant negative correlation was found with sensory conduction velocity. As Hamdy et al. reported (15), there was no high level of correlation. These findings suggest that US examination may contribute to the diagnosis of CTS in suspected cases of NCS.

USG evaluation in CTS may have some advantages over NCS. Other lesions, such as tenosynovitis, mass lesions and anatomical defects, which show similar symptoms to CTS, can be excluded by USG. Moreover, USG is inexpensive and includes a shorter examination time. There are authors recommending the use of USG as a first-line method in the diagnosis of CTS (16). Holovacova et al. showed that the increase in median nerve cross-sectional area caused by hypothyroidism and the clinical symptoms of CTS were completely reversible after TSH and T4 target levels were reached with thyroxine treatment. They speculated that monitoring the cross-sectional area of the median nerve with USG might prevent unnecessary surgery (7). There was a significant positive correlation between BMI and the right median ultrasonographic nerve cross-sectional area (UNCA) (p=0.001) and between BMI and the left median UNCA (p=0.002). The significant positive correlation between cross-sectional nerve areas and BMI is a distinguishing result of our study because there is no sufficient study in the literature. Our results can be speculated that the increase in BMI due to hypothyroidism may increase cross-sectional nerve areas, thus contributing to the development of entrapment neuropathy.

In studies of newly diagnosed hypothyroidism, electrophysiological findings of polyneuropathy were reported at a rate of 9-72% (3,13,17-19). Garget al. (18) detected electrophysiologically sensory-motor polyneuropathy in 10 (25%) of 40 patients with newly diagnosed hypothyroidism. Akarsu et al. (20) found no electrophysiological abnormality to support polyneuropathy in 31 patients with overt hypothyroidism and 139 patients with subclinical hypothyroidism. Similarly, no significant electrophysiological abnormality was found to support polyneuropathy in our study. Although a definitive diagnosis of polyneuropathy could not be carried out in the general evaluation, there were isolated mild abnormalities in the subgroup analyzes.

CONCLUSION

While our study reveals the presence of CTS with a high electrophysiological rate in newly diagnosed hypothyroid cases, it supports that no significant PNP has developed. The significant positive correlation between cross-sectional nerve areas and BMI can be speculated that the increase in BMI due to hypothyroidism may increase cross-sectional nerve areas, thus contributing to the development of entrapment neuropathy. In our study, the correlation between US cross-sectional areas and NCS suggests that it may contribute to and support the diagnosis of entrapment neuropathies.

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Research Article

Is Dexmedetomidine Toxic on Kidney Cells (Hek-293)? Effects on Cytotoxicity, Reactive Oxygen Species (ROS) and Apoptosis

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Abstract

Aim: Dexmedetomidine; it is widely used in anesthesia and intensive care. We aimed to examine and compare the cytotoxic, reactive oxygen species (ROS) and apoptotic effects of dexmedetomidine on kidney cells (Hek-293) in vitro at two different high and cumulative doses.

Material and Methods: The half-maximum inhibitory concentration (IC50) dose of dexmedetomidine on Hek-293 cells was determined using the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) method. Then at two different doses of the drug; apoptotic effects were determined by Annexin-V Method, morphological examinations were determined by Acridine Orange Ethidium Bromide Method and intracellular ROS levels were determined by flow cytometry.

Results: The IC50 value of dexmedetomidine for Hek-293 cells was determined as 64.6559 µg/mL. Compared with the control group, doses of 50 and 100 µg/mL of dexmedetomidine tended to show cytotoxicity ($p<0.05$). dexmedetomidine was found to have a lower cytotoxic effect at a dose of 50 µg / mL than at a dose of 100 µg / mL ($p<0.05$).

Conclusion: In the study, it was determined that dexmedetomidine increased intracellular ROS more than clinical doses at two different concentrations on Hek-293 cells, cytotoxic doses caused an increase in ROS in cells and induced apoptosis. We think that the toxic effects of dexmedetomidine can be prevented with the data obtained from this study and further studies.

Keywords: Anesthesia, apoptosis, cytotoxicity, dexmedetomidine, human kidney cells (Hek-293), reactive oxygen species (ROS)

INTRODUCTION

Dexmedetomidine; it is frequently used in anesthesia practice and especially in intensive care. It is an effective and highly specific agonist of α-2 adrenoceptors. It has been described as a unique sedative with analgesic, sympatholytic and pulmonary functions protective properties (1,2). Dexmedetomidine may exert hypnotic effects depending on the application. If high enough dose is administered, it can produce deep sedation and even general anesthesia like ptopofol etc. This indicates that dexmedetomidine has the potential to be a general anesthetic such as propofol or thiopental (2).

It has been approved by the US Food and Drug Administration (FED) for use in intensive care units for sedation for less than 24 hours in patients with or without the need for mechanical ventilation (2). Dexmedetomidine is widely used in the intensive care unit and operating room (3) for the above indications. However, with the emergence of many positive physiological effects, clinical application methods have increased greatly in recent years (4). Dexmedetomidine is a selective α2-adrenoceptor agonist with properties such as hemodynamic stabilization, anti-inflammation, diuresis and inhibition of central sympathetic outflow. And it has been shown in many studies to have an organ-protective effect on the heart, brain, kidneys, and lungs (5–7). Dexmedetomidine has both somatic and visceral pain-reducing effects when applied as an adjuvant in central and peripheral blocks (8). A meta-analysis (reviewing 16 randomized controlled trials) showed that administration of dexmedetomidine significantly reduced postoperative pain and need for

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analgesic medication but increased the risk of bradycardia (8).

The effects of dexmedetomidine on renal function are complex. It may exert a diuretic effect by inhibiting the antidiuretic effect of vasopressin (AVP) in the collecting duct (9). There are studies expressing that this effect may be independent of AVP, by increasing osmolality and protecting the renal cortical membrane (2,9). It reduces the blood flow of the kidney by decreasing the release of norepinephrine from the renal cortex (10). There is also evidence from animal studies that it reduces ischemia-reperfusion injury (10). In a recent study, it was reported that perioperative infusion reduced the frequency and severity of acute kidney injury in the postoperative period in patients undergoing heart valve surgery (11).

Despite their widespread use, the specific mechanism of action of anesthetic drugs remains unclear (12), and in some cases, these drugs lead to undesirable side effects and cause various complications. Since there is no alternative to these drugs, they continue to be used frequently despite possible complications (13). Today, studies are carried out to elucidate the mechanism of action of such complications.

The mechanism by which the kidney metabolizes and excretes various drugs and toxins significantly influences drug nephrotoxicity. High renal blood flow, equivalent to approximately 25% of cardiac output, and high drug and toxin delivery expose the kidney to significant drug concentrations (14). In addition to hepatic metabolism, some drugs are biotransformed by the renal enzyme systems, including CYP450 and flavin-containing monooxygenases (15,16). This may result in the potential generation of nephrotoxic metabolites and reactive oxygen species (17). These byproducts of biotransformation can tip the balance in favor of oxidative stress, which outpaces natural antioxidants and increases kidney damage. DNA strand breaks, nucleic acid alkylation or oxidation, lipid peroxidation, and protein damage may develop (14). The development of drug-induced nephrotoxicity is best understood by examining the factors contributing to nephrotoxicity (14).

The kidneys are the main junction in the excretion of drugs. And it is also involved in the metabolism of drugs to some extent (18). Clinical, in vivo and in vitro studies are carried out on the kidneys. Human kidney epithelial (Hek-293) cells are a good choice for an in vitro model as they allow researchers to study the physiological functions of kidney cells (19). Therefore, we used Hek-293 cells to examine the clinical and high-dose effects of Dexmedetomidine.

Although there is no toxic effect at clinical doses, it has been determined that in long-term use, if clinical doses are exceeded or repeated doses of these drugs are used for sedation, a cumulative effect may occur and have a cytotoxic effect on many cells. Apoptosis is involved in the emergence and development of various renal pathological injuries. When an organism receives a strong stimulus, it encourages excessive reactive oxygen species (ROS) production (20). Excessive ROS may activate oxidative stress by disrupting the balance between oxidative and antioxidant systems (20).

There are studies claiming that dexmedetomidine can prevent isoflurane-induced apoptosis in the brain and some other organs, or vice versa (12,21–24). The cardiac side effects of dexmedetomidine may limit its use, especially in some patients (25). In the SPICE III study in which dexmedetomidine was compared with alternative sedatives, it was associated with an increased risk of mortality in the group of patients aged 65 years and younger (25). It has also been stated that toxicity may develop in cases where single doses of drugs or continuous infusions such as anesthetic drugs are used (14,23,24,26–29). Dexmedetomidine is thought to have unknown effects at present.

Lavon et al. (30) found that moderate and high doses of dexmedetomidine increased tumor cell involvement and growth of secondary tumors in animal models. There are studies showing that dexmedetomidine may increase neuronal apoptosis at high and cumulative doses (24,28). The specific mechanism of action of dexmedetomidine is not fully known. However, it has been reported that it exerts its effect through α-2 adrenergic receptors (30). These negative findings from laboratory experiments do not necessarily corroborate similar results in human studies. Therefore, further laboratory and clinical studies are required to understand the mechanism of action of dexmedetomidine and to improve its clinical use. As a result of all these, kidney damage may develop due to drugs.

Dexmedetomidine is a useful and attractive drug that has great potential in many clinical situations. However, some extended applications of dexmedetomidine require further evaluation. In order to ensure the safe use of dexmedetomidine, it is necessary to choose the patient carefully and to determine the appropriate dose (31). There are studies showing that dexmedetomidine may have protective or toxic effects on cells. However, the mechanisms of action still remain unclear.

The most distinctive feature of this study is the in-vitro comparison of the effects of dexmedetomidine, whose clinical effects have been investigated many times, in HEK-293 cell lines for the first time, at two different high doses. Our aim is to compare the possible mechanisms of action of this drug by examining the effects of dexmedetomidine on cytotoxicity, apoptosis, and intracellular free oxygen radical (ROS) levels on Hek-293 cells in-vitro at two different high doses.

MATERIAL AND METHOD

Cells and Culture Conditions

In the study, we obtained and stocked from the American Type Culture Collection (ATCC); Hek-293 cells (Human kidney epithelial cell) (19,32) were used Cell lines were

grown in Dulbecco's Modified Eagle Medium:F12(DMEM) medium containing 1% P/S, 10% FBS and 1% glutamine. All cells were incubated at 37°C in an atmosphere of 5% CO. Cells were removed with a mixture of 0.25% trypsin, 0.03% Ethylenediamine tetraacetic acid (EDTA) and passaged at a ratio of 1:2 or 1:3 as recommended by ATCC. Unused cells were stored in a cell freezing solution prepared with 95% medium and 5% dimethylsulfoxide (DMSO) in a deep freezer at -80°C for a short or long term in liquid nitrogen.

Administration of Drugs to Cells

Cytotoxicity Analysis with MTT

The medium was refreshed 24 hours after Hek-293 cells were seeded in 96 sterile plates as 104 cells per well. Seven doses (0-2,5-5-10-25-50-100- µg/ml) were determined for the drug concentration to be applied to the cells. Dexmedetomidine was administered in 7 doses with 3 repetitions. No drug was administered to the control group. The cell lines were incubated in the incubator for 24 hours after the drug was applied. After drug administration, the cell medium was removed from the medium. The cytotoxic effect of dexmedetomidine was evaluated using the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide method. Measurements were read at absorbance at 570-690 nm using a plate reader (Thermo Multiskan Go, USA). Graphics have been created. The IC50 dose was then calculated. Other tests were examined at doses of 50 and 100 µg/ml, which are one lower and one upper dose of the IC50 value found in cytotoxicity tests.

Cell Morphology and AO/EB Analysis

Cell morphology images were taken with an inverted microscope (Olympus CKX). According to cell nucleus morphology, apoptosis fluorescence in cells was examined by Acridin orange / ethidium bromide staining method. The medium was removed 24 hours after the administration of the drugs, 50 µL of AO/EB dye (Sigma Aldrich) was added and images were taken with a fluorescent microscope (Olympus CKX 51, DP73).

Determination of Apoptosis by Annexin V/PI Method

This analysis was performed using the commercially available FITC Annexin-V Apoptosis Detection Kit I (Cat No./ID:556.547, BD, New Jersey, USA) method. According to the kit protocol, shortly after administration of drugs, cells were harvested and stained immediately according to the kit protocol. The stained cells were analyzed by flow cytometry (FACS VIA, BD). Annexin V is displayed in green and PI in red. Viable cells [(FITC-)/(PI-)] were differentiated as early and moderately apoptotic [(FITC+) /(PI-)], late apoptotic and necrotic cells [(FITC+) /(PI+)].

Intracellular ROS Determination

Intracellular free radical exchange was carried out according to the protocol of the commercially available kit (MHC100111, Millipore-Merck). The Muse® Oxidative Stress Kit provides quantitative (cell count and percentage) measurements of Reactive Oxygen Species (ROS), ie superoxide radicals, in cells exposed to oxidative stress. After administration of drugs, cells were harvested using trypsin enzymes and washed with cold phosphate buffered saline (PBS). After adding 100 µl of ROS working solution, cells were incubated at 37°C for 30 minutes. After incubation the cells were analyzed by flow cytometry (FACS VIA, BD).

Statistical Analysis

The data in the study were analyzed using the SPSS Version 25.0 package program. Data were presented as mean±standard deviation (SD). After the data were checked for normality tests, pairwise comparisons were made with Student-t test. P-values less than 0.05 were considered statistically significant.

RESULTS

Cytotoxic effect of Dexmedetomidine on Hek-293

As a result of the analysis, it was determined that the cytotoxic effects of drug increased depending on the dose increase (Figure 1). The IC50 value of dexmedetomidine for Hek-293 cells was detected as 64.6559 µg/mL. Compared to the control group, dexmedetomidine tended to show cytotoxicity at doses of 50 µg/mL and 100 µg/mL (*p<0.001).

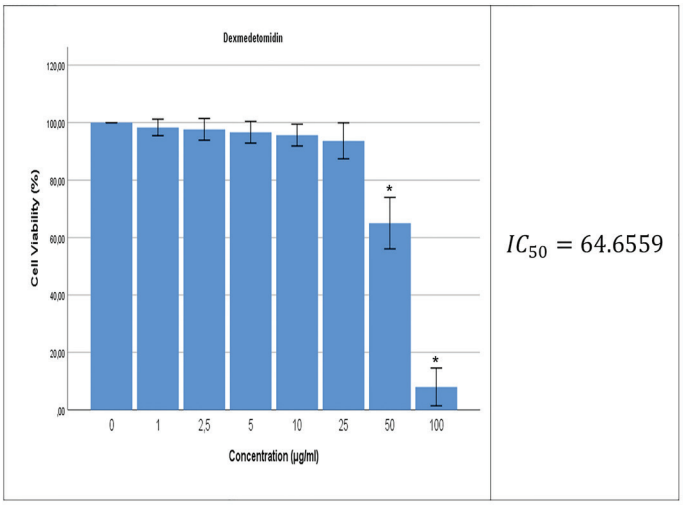

Figure 1. % Changes in viability of Hek-293 cells treated with different concentrations of dexmedetomidin for 24 hours. The data obtained are shown as mean ± standard deviation. (*p <0.001 vs control group for Dexmedetomidin. *#p<0.001 vs 50 and 100 µg/ml dose group.)

Effects of Dexmedetomidine on Hek-293 Cell Morphology and Apoptosis

The effects of 50 and 100 µg/ml doses of Dexmedetomidine on Hek-293 cell morphology were examined (Figure 2a). When the effects on cell morphology were compared with the control group, it was observed that the number of cells decreased and the number of apoptotic cells increased depending on the dose increase (Figure 2a).

The apoptotic effect of drugs on Hek-293 cells was examined using the AO/ET fluorescent staining method (Figure 2b). In the picture obtained, viable cells are seen in green, apoptotic cells in orange, and necrotic cells in

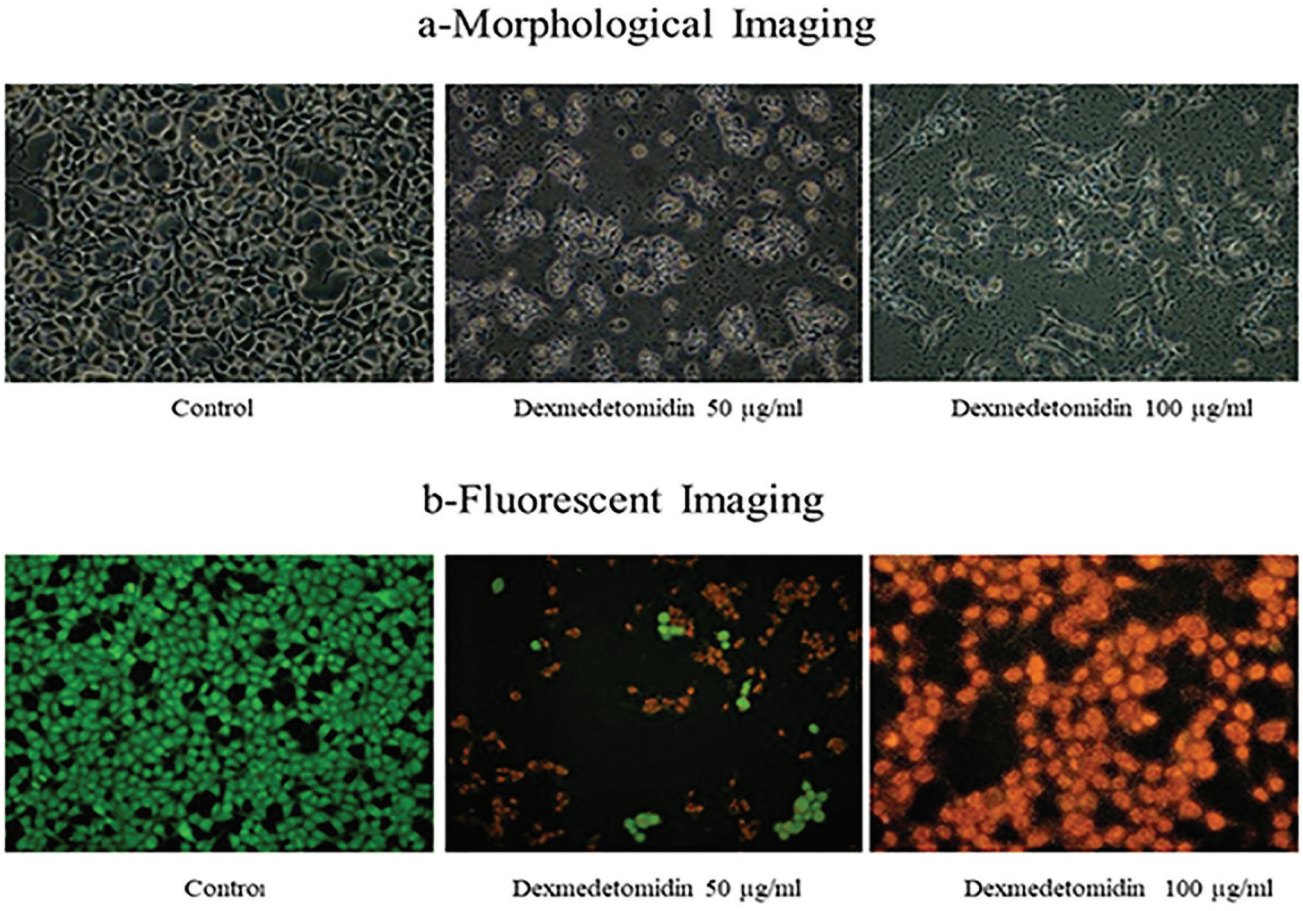

Figure 2. The image of the morphological and apoptotic effects of dexmedetomidine on Hek-293 cells

red. When the apoptotic effects of the drug are compared among themselves according to the doses; It was observed that 50 µg/ml > 100 µg/ml, while the apoptotic and cytotoxic effect was higher at 100 µg/ml dose.

Flow Cytometric Annexin-V Analysis of Hek-293 Cell Apoptotic Effect of Drugs

While 31.50% and 31.30% of dexmedetomidine-treated Hek-293 cells were viable at 50 and 100 µg/ml doses, 57.73% and 65.16% of them were observed to have necrosis (Table 1, Figure 3). These results were found to be significant compared to the control group ($p<0.000$).

When the two doses were compared, there was a statistically significant difference in viability, late apoptosis, and necrosis at a dose of 100 µg/ml ($p<0.05$).

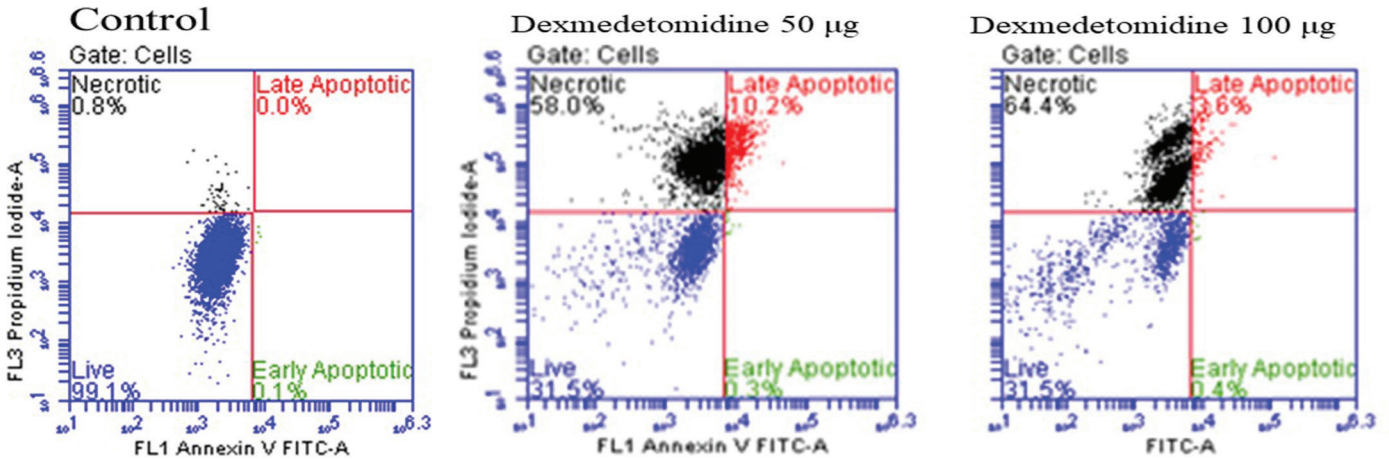

Figure 3. Flow cytometric annexin-V analysis of the Hek-293 cell apoptotic effect of Dexmedetomidine

Table 1. Flow cytometric annexin-V analysis of Hek-293 cell apoptotic effect of dexmedetomidine

Viability	Control	Doses (µg/ml)	Dexmedetomidine	*p*-value
Live	98.26±0.90	50 µg/ml	31.50±1.60	*p*=0.920 *
		100 µg/ml	31.30±2.80	*p*=0.920 *
Early Apoptotic	0.23±0.15	50 µg/ml	0.33±0.05	*p*=0.643 #
		100 µg/ml	0.30±0.10	*p*=0.643 #
Late Apoptotic	0.13±0.15	50 µg/ml	10.43±0.97	*p*=0.000 ¥
		100 µg/ml	3.20±0.36	*p*=0.000 ¥
Necrotic	1.36±0.73	50 µg/ml	57.73±2.51	*p*=0.031 α
		100 µg/ml	65.16±3.02	*p*=0.031 α

Values are given as mean±standard deviation.
p=0.920 indicates comparison of dexmedetomidine for 50 and 100 µg/ml dose groups.
*,#,¥,α indicates p values <0.05 lower for comparison of Dexmedetomidine and Control groups for all doses

Flow Cytometric Investigation of the Effects of Dexmedetomidine on the Formation of Intracellular Free Radicals (ROS) in Hek-293 Cells

It was determined that the amount of intracellular free radicals increased depending on the dose (Table 2, Figure 4). The drugs we use include intracellular free radical levels at doses of 50 and 100 µg/ml; It was determined as 57.36% and 78.30%. Significant change was observed compared to the control group (p=0.001).

Table 2. ROS effect of different doses of drugs on Hek-293 cells

	Doses	Control	Dexmedetomidin	*p*-value
ROS (-)	50 µg/ml	98.30±1.25	42.90±1.83	*p*=0.000 *
	100 µg/ml	98.30±1.25	21.26±2.63	*p*=0.000 *
ROS (+)	50 µg/ml	1.60±1.41	57.36±2.05	*p*=0.000 #
	100 µg/ml	4.03±1.89	78.30±2.40	*p*=0.000 #

Values are given as mean±standard deviation.
p=0.000 indicates comparison of dexmedetomidine for 50 and 100 µg/ml dose groups.
*,# indicate p-values less than <0.05 for comparison of groups, Dexmedetomidin and Control for all doses.

In conclusion; it was determined that with the increase in dose of dexmedetomidine, the viability decreased and accordingly the ROS formation increased. A statistically significant difference was found between the control group and the doses used in the comparisons (p<0.001).

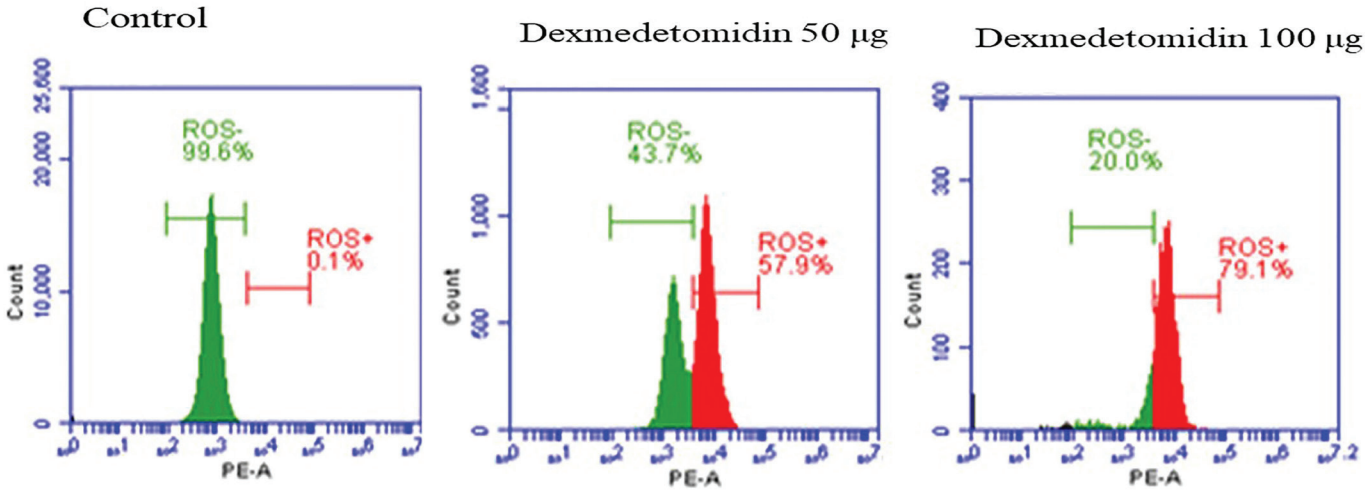

Figure 4. Flow cytometric examination of the effects of dexmedetomidine on Hek-293 cell intracellular free radical (ROS) formation

DISCUSSION

When we compared the effects of Dexmedetomidine on Hek-293 cells at two different high doses; We found that it undergoes apoptosis due to increased intracellular ROS. Dexmedetomidine IC50 dose on Hek-293 cells; We found it to be 64.65 µgr/ml. We found that it showed more toxicity at two different high doses cumulative than clinical doses. However, it did not show any cytotoxic effect at clinical doses.

Dexmedetomidine-induced cell death has been reported (24,26–28). It does not have any toxic effects at clinical doses. Dexmedetomidine has been used intraperitoneally at a dose of 25-100 µg /kg in previous studies (12,24,28,29). In our study, we found the IC50 dose in Hek-293 cells as 64.67 µg/ml and we used two different doses as 50 and 100 µg/ml according to this dose. We found that the doses we applied had a more severe cytotoxic effect compared to the control group (p<0.001).

The protective roles of dexmedetomidine during acute stress-induced kidney injury are unknown. Dexmedetomidine may be an effective drug to prevent kidney damage caused by acute stress. Dexmedetomidine also has antioxidative stress effects (33–36). Clinical and laboratory studies have reported that dexmedetomidine has a protective effect on many organs. It exerts this effect by reducing the oxidant response in organs and

inactivating apoptosis signaling pathways that protect cells from damage. Çanakçı E. et al. (2) reported that dexmedetomidine had a protective effect on the kidney in colistin-induced kidney damage, and therefore it was a very valuable sedation agent for clinicians in intensive care units. Dexmedetomidine; In long-term use, a cumulative and toxic effect may occur if clinical doses are exceeded or repeated doses of these drugs are used for sedation (14,24,27,28). Wang Z at al. (29) showed that dexmedetomidine at 10-30-50 μM doses inhibited LPS-induced ROS production and apoptosis in tubular epithelial cells of mice, but reversed the protective effects of dexmedetomidine in sepsis-associated AKI at 50 μM. They showed that the corrective effects of sepsis-associated acute kidney injury observed after treatment with dexmedetomidine are due to attenuation of oxidative stress (29). In our study, which we used above clinical doses, we found that 50 and 100 μg/ml dexmedetomidine caused cytosolic ROS formation and apoptosis, consistent with Wang Z et al's study ($p<0.05$, Table 1,2).

Hanci V et al. (37) found that 100 μmol/L dexmedetomidine increased nitric oxide synthase (NOS) and nitric expression and had a toxic effect with an increase in inflammatory cytokines. Lai et al. (38) reported that the protective effect of dexmedetomidine was related to its concentration. In in vitro experiments, they treated the increased inflammatory cytokine release by LPS with different concentrations of dexmedetomidine. In their study, they found that 0.01 μmol/L dexmedetomidine had no effect, the addition of 1 μmol/L dexmedetomidine was effectively protective, 100 μmol/L dexmedetomidine stimulated nitric oxide synthase and nitric expression and had a toxic effect. Consistent with these studies, in our study, we found that cytosolic ROS production, apoptosis and necrosis increased statistically significantly at high doses in Hek-293 cells compared to the control group ($p<0.05$, Table 1,2).

Recently, it is thought that dexmedetomidine exerts a protective effect on many vital organs, which is thought to be associated with anti-inflammation, anti-oxidative damage and inhibition of apoptosis (39). However, long-term infusions and cumulative doses are also thought to cause toxic effects. The antioxidant effect of dexmedetomidine is provided by different mechanisms in pathological conditions. However, most of the available dexmedetomidine studies are based on preclinical studies and the mechanism remains unclear. More research and clinical studies are needed. In addition, although dexmedetomidine is thought to have an organ protective effect, there are conflicting results in the literature about whether α2AR agonism is protective or toxic. However, while evaluating the positive effects of dexmedetomidine, the use of this substance at different times, effective doses and drug profile should be investigated (31). To confirm the potential of using dexmedetomidine to preserve organs in the future, it needs to be supported by realistic, important clinical observations and studies.

Although dexmedetomidine is widely used in anesthesia practice, its specific mechanism of action remains unclear (12,31). In vitro studies are frequently used to determine the effects of drugs on organisms. In these studies, researchers often select the type of cell in which drugs are metabolized or in which they show activity. The kidney is the major junction in the excretion and to some extent metabolism (18) of most drugs, especially anesthetic drugs Therefore, Hek-293 cells are a good choice for an in vitro model as they allow researchers to examine the physiological functions of kidney cells (19,32). In our study, we used Hek-293 cells to examine the clinical and high-dose effects of dexmedetomidine.

Considering the increase in anesthesia methods applied, it is of great importance that anesthesia be given safely. Patients who will be given anesthesia may be exposed to oxidant or antioxidant stress before, during and after surgery (40). Anesthesiologists are in a position to improve the postoperative outcome by taking measures against oxidative stress. Therefore, there is a need for a better understanding of the effect of anesthetic agents on oxidative stress and clinical outcomes and more studies on this subject to improve the treatment of patients.

Our study has some limitations. We tested the toxicity of dexmedetomidine using cultured cells. We primarily used established cell lines derived from primary cultured cells, not from various tissue origins. Although we performed our tests using 10% FBS, the free fraction of the drug could not be determined. It can be done in an in vivo study using experimental animals to confirm our findings. One of the limitations of our study; results from a cell model cannot simply be translated/transported into a clinical setting.

CONCLUSION

In conclusion, dexmedetomidine on Hek-293 cells in our study; It has been found to cause toxic effects by increasing intracellular ROS at two different concentrations higher than clinical doses. It was observed that this toxic effect was due to the increase in intracellular ROS that triggered apoptosis. With the examination of the data obtained as a result of this study and the new studies to be done; We believe that the benefits and complications associated with the use of this drug can be clarified and avoided.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *This article is an experimental study. Ethics committee approval is not required as it is performed on cultured cells.*

Authors' contributions: *Study concepts: B.P., V.F.P., E.D. Study design: B.P., V.F.P., E.D. Literature search: B.P., V.F.P., İ.K. Experimental studies: B.P., V.F.P., E.D., İ.K. Data analysis: B.P., V.F.P., I.K. Statistical analysis: V.F.P. Article preparation: B.P., V.F.P. All authors contributed to the final manuscript.*

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MEDICAL RECORDS-International Medical Journal

Research Article

An Appraisal of Clinical and Hematological Parameters Linked to Recurrence in Surgically Drained Primary Psoas Abscesses: A Retrospective Comparative Study

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Abstract

Aim: Psoas abscess is rare infectious condition with frequent complications in the diagnosis and treatment process. Unfortunately, there is limited information in the literature about the prognostic factors that determine the prognosis of psoas abscess. Therefore, the aim of this study was to evaluate the clinical and laboratory parameters associated with recurrence in primary psoas abscess.

Material and Methods: Fifty-two patients who were diagnosed with psoas abscess and treated with surgical drainage in a university hospital between 1998 and 2018 were included in our study. The patients were seperated into two groups as those who recovered after surgical drainage (Group A) and those who developed recurrence (Group B). Clinical and laboratory data of the patients from the beginning of the preoperative period to the postoperative period were compared.

Results: The mean age was 47.42±14.12 years in Group A and 53.81±15.83 years in Group B. The mean follow-up time was 43.96±14.29 months. The neutrophil to lymphocyte ratio was 11.38±1.69 in Group A and 18.75±2.31 in Group B (p=0.001). The platelet to lymphocyte ratio was 114.96±30.31 in Group A and 139.70±42.25 in Group B (p=0.016). The Acute Physiology and Chronic Health Evaluation (APACHE II) score was higher in Group B (p=0.001).

Conclusion: According to the results of the current study, the neutrophil to lymphocyte ratio, the platelet to lymphocyte ratio, the APACHE II score, and the delayed diagnosis are all important prognostic indicators linked to recurrence in instances with primary psoas abscess.

Keywords: Primary psoas abscess, recurrence, surgical drainage, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, APACHE II score

INTRODUCTION

Psoas abscess (PA) is a potentially very dangerous infective process that results from the accumulation of pus throughout the iliopsoas muscle due to various condition. The iliopsoas muscle is the strongest of the hip flexors and is primarily responsible for hip flexion. It originates from the twelfth thoracic vertebra and extends distally, and attaches on the lesser trochanter after traveling within the pelvic ring and passing the hip joint anteriorly. Although the worldwide prevalence is unknown, it is known that it is extremely low, with up to 12 instances recorded annually in centers that address this issue (1-3). Psoas abscesses are etiologically classified as primary and secondary. The pathogenesis of primary psoas (pPA) abscesses is still unclear. The mechanism generally considered is that the infection spreads from a distant part of the body by lymphatic or hematogenous route (4,5). The etiology of pPAs typically include intravenous drug abuse, diabetes, acquired immunodeficiency syndrome (AIDS), alcoholism, malnutrition, or malignancy. They are more common under the age of 20 and in men at a ratio of 3:1.3-5 (4,6). Secondary psoas abscess (sPA) occurs through direct spread from the focus of infection

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in organs adjacent to the psoas muscle, such as kidney, bladder, ureter, sigmoid colon, jejunum, and vertebral column (2,7). While pPA is more common in younger ages with a prevalence ranging from 30% to 61%, the prevalence of sPA is 70% and the most common cause is reported to be Chron's disease (5). The followings should be considered in the differential diagnosis of PA: vascular patologies, pyelonephritis, appendicitis, urolithiasis, vertebral osteomyelitis, avascular necrosis of the femoral head, aortic or iliac artery aneurysm, gastrointestinal tract pathologies, Chron's disease, and malignancies. For the differential diagnosis of PA, the medical history of the patient should be taken carefully and physical, laboratory, and radiological examinations should also be performed carefully (8).

There is limited information in the literature regarding prognostic factors that determine the prognosis of PA. Delayed diagnosis, comorbidities, positive blood culture, elevated serum C-reactive protein (CRP) and creatinine levels, and advanced age are the known poor prognostic factors for PA (9-11). The goal of treatment is to drain the purulent fluid collected in the psoas compartment through surgical or percutaneous drainage and to use appropriate antibiotic therapy (4,12).

The factors of recurrence of psoas abscess has not been revealed in the previous published reports. Our aim in this study was to evaluate and compare the prognostic factors in the diagnosis and treatment process of pPA, which are rare and may have catastrophic consequences, and to provide the literature with a broader perspective.

MATERIAL AND METHOD

Study Design and Participants

We undertook a single-center, retrospective study. The data of the patients during the diagnosis and treatment process were retrospectively analyzed from archive records. The study group consisted of patients, who were admitted to the emergency department or orthopaedic outpatient clinic of a university hospital center between December 1998 and January 2018, and treated by surgical drainage due to PA diagnosis.

Patients were divided into two groups: Group A consisting of patients who were recovered after surgical drainage and Group B consisting of patients requiring repeated surgery due to recurrence. The data of 52 patients who were followed for at least 24 months were analyzed retrospectively.

The study was conducted in accordance with the Declaration of Helsinki. This research did not receive support from any national or international institution or organization.

Methods of Measurement

Clinical conditions and laboratory data, length stay in hospital, time until diagnosis, abscess morphology, intraoperative pus drained, drain removal time (days), blood culture, pus culture, smoking habit, intravenous drug abuse, comorbidities, and microbiological data of the patients were analyzed and compared to determine the factors associated with recurrence. The comorbidities of the patients were evaluated according to the age-adjusted Charlson comorbidity index. The Charlson Comorbidity Index predicts patients' survival with several comorbidities. It is comprised of 17 items linked to a variety of health issues connected with mortality (13). The Charlson index can predict both short-term and long-term outcomes, such as function, hospitalization duration, and death rates (Table 1). When calculating the age adjusted charlson comorbidity index, 1 more point is added for each decad after the age of 40. The Acute Physiology and Chronic Health Evaluation (APACHE II) scoring system, which incorporates many clinical and laboratory parameters, was used to evaluate the severity of the disease and its relationship with recurrence (14). Physiological variables evaluated in APACHE II include body temperature, mean arterial pressure, heart rate, respiratory rate, oxygenation, arterial PH, venous HCO3-, sodium, potassium, serum creatine, hematocrit, leukocytes, and Glasgow Coma Score. Computed tomography (CT) and magnetic resonance imaging (MRI) were used for radiological diagnosis in all patients. In the post-treatment period, MRI and ultrasonography (USG) were used for follow-up. Presence of an infectious process in the adjacent organs was also evaluated with imaging methods.

Table 1. Charlson comotbidity index items

Comorbidity	Score
Myocardial infarction	1
Congestive heart failure	1
Peripheral vascular disease	1
Cerebrovascular disease	1
Dementia	1
Chronic pulmonary disease	1
Rheumatologic disease	1
Peptic ulcer disease	1
Liver disease	1 if mild, 3 if moderate/severe
Diabetes	1 if controlled, 2 if uncontrolled
Hemiplegia or paraplegia	2
Renal disease	2
Malignancy	2 if localized, 6 if metastatic tumor
Leukemia	2
Lymphoma	2
AIDS	6

All patients were operated under general anesthesia. The performed surgical method was retroperitoneal surgical drainage with a mini-incision, debridement, and washing with saline solution. Regardless of the amount of pus that came intraoperatively and the character of the abscess, all patients were washed with 10,000 cc of saline.

Postoperative Follow-up

Postoperative surgical material culture was tested for

bacteria, fungi, and tuberculosis and antibiotic therapy specific to the growing microorganism was administered IV. Hemovac drain was removed when drainage was below 10 ccs and this was maintained for at least 12 hours, patients' erythrocyte sedimentation rate (ESR), white blood cell (WBC), and C reactive protein (CRP) values decreased, and there was no visualizable pus on USG. Patients were discharged if no exacerbations were observed during the postoperative period; WBC, CRP, and ESR values returned to normal; and healing was confirmed by USG. In the postoperative period; clinical, radiological, and laboratory parameters of the patients were evaluated at one-month intervals for the first three months and at three-month intervals thereafter. After 24 months, patients were called for control on an annual basis.

Statistical Analysis

Analyses for the demographic characteristics of the data obtained from the study were carried out using descriptive statistical analysis methods (frequency and percentage analysis, mean and standard deviation values). While categorical variables were tested with chi-square, t-test was used to test numerical variables. The data that were significant in the univariate analysis (P value < 0.05) were detected. Multivariate logistic regression analysis was applied to identify independent predictors that were associated with recurrence. Statistical analysis was performed with SPSS for Windows version 25.0 and a P value < 0.05 was accepted as statistically significant.

RESULTS

Characteristics of Study Subjects

This retrospective single center comparative study analyzed a total of 65 patients who were treated by surgical drainage due to the diagnosis of pPA in our clinic. Four patients died during the follow-up, due to sepsis. Although nine patients recovered after surgical drainage, they were excluded from the study due to insufficient data. The mortality rate was 6.1% (n=4), and the recurrence rate was 24.6% (n=16) in pPA cases presenting to our clinic. The mean age of the patients was 47.42±14.12 years in Group A and 53.81±15.83 years in Group B. While the ratio of males to females was 2:1 in Group A, it was 1.6:1 in Group B. One patient in Group B had bilateral PA. The mean follow-up time was 43.96±14.29 months and the mean length of surgery was 43.29±10.28 minutes. Before receiving the diagnosis, 44 (84.6%) patients had applied to the emergency department or orthopedic outpatient clinic at least once and 35 (67.3%) of these patients had applied to the hospital more than once. The most common accompaying comorbidities were diabetes mellitus (28.8%), followed by hypertension (25%) and coronary artery disease (17.3%), respectively. The most common clinical symptom in both groups was fever. The triad of fever, flank pain, and hip movement limitation were present in 36% of the patients in Group A and 50% of the patients in Group B (Table 2).

Table 2. Descriptive and demographic variables of patients

		Group A (n=36)		Group B (n=16)		P value
Age (years), mean ±SD		47.42±14.12		53.81±15.83		0.153
Sex	Male	24 (66.7%)		Male	10 (62.5%)	0.771
	Female	12 (%33.3)		Female	6 (37.5%)	
Length stay in hospital (day), mean±SD		24.36±4.33		25.31±4.44		0.471
Time to recurrence (month), mean ±SD		-		5.19±1.76		-
Age-adjusted Charlsoncomorbidity index		2.02±1.47		2.09±1.69		0.426
Clinical presentation (Since there is more than one symptom in the same patient, the total number may exceed the number of patients)	Fever	18 (50%)		Fever	10 (62.5%)	-
	Flank pain	16 (44.4%)		Flank pain	8 (50%)	
	Restricted hip mobility	13 (36.1%)		Restricted hip mobility	8 (50%)	
	Anorexia	12 (33.3%)		Weight loss	9 (56.2%)	
	Nausea and vomiting	10 (27.8%)		Anorexia	9 (56.2%)	
	Weight loss	10 (27.8%)		Nausea and vomiting	6 (37.5%)	
	Thigh pain	9 (25%)		Thigh pain	6 (37.5%)	
	Flank mass	6 (16.6%)		Flank mass	4 (25%)	
	Abdominal pain	6 (16.6%)		Abdominal pain	4 (25%)	
	Local redness	4 (11.1%)		Local redness	3 (18.7%)	

SD; Standart deviation

Abdominal and thoracic CT was combined with MRI in all patients. Diagnosis was established using CT alone in 49 (94.2%) patients. In three (5.7%) patients, the diagnosis was confirmed by MRI and a diffuse sPA was ruled out. Thanks to the radiological examinations, exclusion was achieved for sPA.

Main Results

Preoperatively measured hematological parameters were analyzed and compared between the groups (Table 3). The neutrophil to lymphocyte ratio (NLR) was 11.38±1.69 in Group A and 18.75±2.31 in Group B (p=0.001). The platelet to lymphocyte ratio (PLR) was 114.96±30.31 in Group A and 139.70±42.25 in Group B (p=0.006). The amount of intraoperatively drained pus was 140.28±58.63 cc in Group A and 170.63±52.97 cc in Group B (Table 4). Patients in Group B were observed to have higher APACHE II scores (p=0.001).

Multiple logistic regression identified the following risk factors for recurrence in pPA: time until diagnosis (odds ratio (OR):1.038; 95% confidence interval (95% CI):1.008–1.070) (p value:0.013), NLR (OR: 1.077; 95% CI:1.034–1.122; p<0.001), PLR (OR:1.043;95% CI:1.012–1.083; p=0.016) and APACHE 2 score (OR:1.168; 95% CI:1.108-1.230; p<0.001). BMI was insignificant in multiple logistic regression analysis (OR:1.069; 95% CI:0.808–1.416; p= 0.639).

Although 32 (61.5%) patients had blood culture positivity, there was no significant relationship between the groups (p=0.924). Forty-three bacterial pathogens were isolated from 38 patients with bacterial growth. Polymicrobial flora was present in one patient in Group A and two patients in Group B. Staphylococcus Aureus was the most common microorganism isolated from the culture of surgical material in both groups (n=32, 61.5%), (Table 5). In the present study, microbiological pathogens were

Table 3. Hematological parameters associated with recurrence

Variables	Group A (n=36)	Group B (n=16)	P value
Preoperative albumin level (g/L), mean±SD	2.42±0.67	2.31±0.81	0.347
Preoperative platelet to lymphocyte ratio	114.96±30.31	139.70±42.25	**0.001**
Preoperative neutrophil to lymphocyte ratio	11.38±1.69	18.75±2.31	**0.001**
Preoperative red blood cell distribution width (%), mean±SD	16.69±2.04	16.88±2.47	0.773

SD; Standart deviation

Table 4. Clinical and microbiological features associated with recurrence

Variables	Group A (n=36)		Group B (n=16)		P value
Intraoperative pus drained (cc), mean±SD	140.28±58.63		170.63±52.97		0.082
Abscess morphology	Multiloculated	24 (66.7%)	Multiloculated	10 (62.5%)	0.771
	Monoloculated	12 (33.3%)	Monoloculated	6 (37.5%)	
Drain removal (days), mean±SD	7.82±0.84		7.88±0.92		0.588
Time until diagnosis (weeks), mean±SD	8.36±2.42		19.31±4.17		**0.001**
Side	Left	21 (58.3%)	Left	6 (37.5%)	0.076
	Right	15 (41.7%)	Right	10 (62.5%)	
Body mass index (kg/m²), mean±SD	26.5±2.98		21.21±3.01		0.007
APACHE II score, mean±SD	11.22±1.99		14.19±2.86		**0.001**
Age-adjusted Charlson comorbidity index	2.02±1.47		2.09±1.69		0.426
Blood culture	Positive	22 (61.1%)	Positive	10 (62.5%)	0.924
	Negative	14 (38.9%)	Negative	6 (37.5%)	
Pus culture	Positive	26 (72.2%)	Positive	12 (75.0%)	0.835
	Negative	10 (27.8%)	Negative	4 (25.0%)	
Smoking habit	Smoker	21 (58.3%)	Smoker	13 (81.2%)	0.109
	Nonsmoker	15 (41.7%)	Nonsmoker	3 (18.8%)	
Intravenous drug abuse	User	10 (27.7%)	User	6 (37.5%)	0.573
	Nonuser	26 (72.3%)	Nonuser	10 (62.5%)	

detected in pus culture in 38 (73%) patients. There was bacterial growth in the blood culture of two patients who did not have any growth in the pus culture. Exact pathogen was determined in 40 (76.9%) patients using the combination of blood and pus culture. Methicillin-resistant Staphylococcus aureus (MRSA) was observed in two patients in both groups. In Group B, growth of Candida albicans was observed in a 62-year-old male patient with diabetes mellitus and chronic kidney disease. Average duration of IV antibiotherapy in hospital was 24.36±4.33 days in Group A and 25.31±4.44 days in Group B (p=0.471). The mean recurrence time after discharge was 5.19±1.76 months. In the postoperative period; clinical, radiological, and laboratory parameters of the patients were evaluated at one-month intervals for the first three months and at three-month intervals thereafter. After 24 months, patients were called for control on an annual basis.

Table 5. Microbiological analysis of pus culture

Microorganisms (n=43)	Group A (n=28)	Group B (n=15)
Staphylococcus aureus	21	11
Escherichia coli	2	1
Streptococcus viridans	2	1
Group B Streptococcus	1	1
Bacteroides	1	0
Acinetobacter baumannii	1	0
Candida albicans	0	1
Culture-negative	10	4

n= Total number of microorganisms from pus culture

DISCUSSION

According to the literature review and our knowledge, this is the largest series in the literature evaluating pPAs. Yacoub et al. reported seven cases per year in a regional study conducted in the United States. The annual incidence in our clinic was eight cases per year (15). In the literature, the mortality rate is known to be 2.3% in pPAs while it can reach 18.9% in sPAs (16,17). Recurrence rate is reported to range from 14% to 37.5% (10,17,18). In the present study, the mortality and recurrence rates were 6.1% and 24.6%, respectively.

The pPAs are more common in patients younger than 20 years of age (2,20). Recent studies have reported that the prevalence of pPA has increased to 38.3% in advanced age (21,22). In contrast to the old literature, age distribution concentrated on the fourth to the fifth decades of life in Group A whereas the fifth and sixth decades of life in Group B in the present study, compatible with the current literature. In this study, age was not a predictive factor for recurrence. Lai et al. reported the ratio of males to females as 1.62:1 (23). Compatible with the literature, the number of male patients was higher in our study. The rate of intravenous drug abuse is reported to be up to 86% in pPA cases (24).

The rates of intravenous drug abuse and smoking in the present study were 30.7% and 65.3%, respectively and neither of them were found to be associated with recurrence. Additional pathology may be present in 50% of patients with PA, regardless of being primary or secondary (25).

Diabetes mellitus was the most common comorbid condition (28.8%) in the present study. The time from the onset of symptoms to diagnosis ranged from 11 to 120 days (10,26). The time until diagnosis was significantly shorter in Group A than in Group B. The present study shows that the longer the diagnosis time, the higher the likelihood of recurrence.

The literature review has shown that there is no relationship between the volume of abscess drained and prognosis (11). Although the volume of abscess drained in Group B was larger in the present study, it was not statistically significant. There are studies reporting that the drainage time ranged from 2.4 to 45 days in psoas abscesses (18,27). No association was detected in our study between prolonged drainage time and recurrence. We recommend that if the amount of drainage is below 10 ccs for the last 24 hours after sufficient surgical drainage, the drain should be removed following a USG control, and thus, complications that may result from prolonged drain time can be avoided.

In a study by Baier et al., while APACHE II score was found to be associated with mortality, there was no relationship between recurrence and APACHE II score (9). In contrast to their study, the APACHE II score was found to be associated with recurrence in the present study. Thus, we believe that it can be used as a predictive factor for pPA. The reason why this score is significant in our study may be, we only included patients who underwent surgical drainage in our study. Different factors may be associated with recurrence in patients receiving conservative treatment or undergoing percutaneous drainage. Lopez et al. reported weight loss in 37.1% of patients with PA (10). In the present study, the body mass index (BMI) was found to be lower in Group B than Group A but this is not significant. This may be explained by the fact that patients potentially experience loss of appetite and the time to diagnosis in those who developed recurrence is longer.

Elevated NLR levels have been used as a guide in the prognosis of various diseases in many previous studies, such as pneumonia, sepsis, and cancer; however, its relationship with prognosis in PAs has not been investigated in any studies (28). Elevated PLR is an easily accessible, effective, and novel inflammatory marker used in many diseases to determine the severity of inflammation and mortality (29). The present findings suggest that NLR and PLR are safe and easily applicable markers to predict the likelihood of recurrence in pPAs. Besides the studies reporting that high red cell distribution width (RDW) predicts increased mortality in infective processes, such as sepsis and septic shock, there are also studies indicating that it does not have any predictive

feature (30,31). Although the increased level of RDW was observed to be within normal limits in both groups in the present study, it was found to have no predictive value for recurrence in PAs.

Positive blood culture is reported to be associated with increased mortality in some studies whereas there are studies reporting no relationship between the presence of positive blood culture and increased mortality (10,11). We found that high bacterial growth rate in blood or pus culture was not a prognostic marker in pPAs. In the present study, MRSA growth was observed in the pus cultures of four (7.6%) patients. The incidence of MRSA in PAs is variable, and it is difficult to estimate the accurate incidence rates (10). Patients with MRSA should be isolated and given an appropriate treatment based on the antibiogram results

Limitations

Our study had several limitations. Firstly, we conducted our study retrospectively. Although we made a good clinical evaluation and used effective imaging methods, we had to classify patients whose foci of infection were unidentified as primary bacteremia. We only included patients who underwent surgical drainage in our study. Different factors may be associated with unsatisfactory clinical outcomes in patients receiving conservative treatment or undergoing percutaneous drainage. There is a need for randomized controlled trials involving larger patient populations. The present study has notable strengths. This study provided novel findings relevant to the recurrence in pPA. The other strength of this study is that it is more innovative and has a larger sample size than all previous studies.

CONCLUSION

The present study has analyzed and updated easy-to asses, safe, and valid hematological and clinical parameters used for the prediction of recurrence in pPAs. The following conclusions can be drawn from this study: Elevated NLR and PLR levels are prognostic hematological parameters for recurrence in pPA cases. APACHE II score, delayed diagnosis can be used as clinical markers associated with recurrence. In conclusion, predicting the development of recurrence in this rare disease, whose diagnosis and treatment process is challenging, will ensure that treatment and follow-up plans are made according to the possibility of recurrence.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *The study protocol was approved by the Scientific Research Ethics Committee of Gaziantep University, (Noninterventional Clinical Studies Institutional Review Board 24.06.2020, 2020/211).*

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Research Article

Anxiety and Coping Attitudes in Medical School Students During the COVID-19 Pandemic

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Abstract

Aim: The coronavirus (COVID-19) pandemic process has affected individuals of all ages, from children to the elderly, both physically and mentally. This study aimed to determine the anxiety levels and coping attitudes of Medical Faculty students during the COVID-19 pandemic period.

Material and Methods: A personal information form (containing questions about COVID-19), the State Trait Anxiety Inventory and the Coping Strategies Scale were applied online to the students (n=186) who volunteered to participate in the study, studying at the Faculty of Medicine. The scores of the scales were evaluated statistically.

Results: The study included 186 participants consisting of 57% (n=106) females and 43% (n=80) males. A statistically significant positive correlation was found between trait anxiety scale score and emotion focused scale score (r=0.151 p=0.040). A statistically significant positive correlation was found between trait anxiety scale score and dysfunctional coping scale score (r=0.455 p<0.001). According to the simple linear regression analysis, a 1-unit increase in the trait anxiety scale score increased the dysfunctional coping scale score by 0.35 units.

Conclusion: As seen in our study, an increase in anxiety levels leads to dysfunctional coping attitudes. Dysfunctional coping attitudes are among the reasons that lead people to mental illnesses. Therefore, goals should be determined to reduce the anxiety levels of future physicians and to increase their functional coping attitudes.

Keywords: COVID-19, coronavirus, pandemic, medical students, anxiety, coping strategies

INTRODUCTION

The new type of coronavirus disease (COVID-19), which the World Health Organization determined as a public health emergency of international concern on January 30, 2020, started in China and quickly spread all over the world and was accepted as a pandemic (1). The low predictability and little awareness of COVID-19 affects the mental health of individuals in terms of cognitive and emotional as well as physical health (2). In the studies, anxiety and depression levels were found to be higher in healthcare workers compared to non-health workers during the pandemic period (3,4).

Stress is a physiopsychological reaction that occurs when the physical and mental health of the person is threatened and these limits are pushed (5). It can be said that the basis of stress lies in the evaluation of human perception and experiences, and giving meaning to, evaluating and directing their experiences is the main factor in reducing or increasing stress (6). In this context, it can be predicted that the individual's gaining preventive health behavior is related to the development of coping skills with stress (5). Coping attitudes are considered as the adaptation process that is at a conscious and voluntary level and that the individual carries out to re-establish the internal and external balance that is disrupted in stressful situations (7).

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The way a person solves a problem in the face of a stressor can affect the normal course of stress and complicate the solution of the problem. Therefore, knowing the coping attitudes that the person uses in the face of a stressful situation; It will help in determining the treatment targets and monitoring the therapeutic effectiveness of the mental problems that may occur (8). The multiplicity of classes, long working hours, exams, peer competition, insomnia and other similar factors put medical students more stressed and difficult to cope with, which gradually puts them into mental problems. Over the Covid-19 pandemic and mandatory curfew in Turkey, medical school students faced economic ambiguity, occupational pressure, threat of infection, difficulties of distance education, lack of protective equipment at work, etc. In the literature, very few studies have been found on the anxiety levels of medical faculty students during the pandemic process. Apart from individual risks, medical school students are exposed to many stress factors arising from both medical education and the difficulties in the pandemic process. As far as we know, there is no study examining anxiety and coping attitudes together in this group. This study goaled to investigate students' attitudes, anxiety and coping strategies during the COVID-19 pandemic.

MATERIAL AND METHOD

Hitit University Medicine Faculty students were asked to fill in the State Trait Anxiety Scale (STAI) and Coping Attitudes Scale (COPE) online via google questionnaire for individuals aged 18-65 who volunteered to participate in the study. The study was carried out in October-December 2021. The scales were administered online to minimize face-to-face interactions and facilitate participation. At the start of the survey, each participant indicated their electronic informed consent to engage in the study by answering a yes-or-no question. All participants were made aware that the information they provided was coded and kept private. The study was approved by the Hitit University Non-Interventional Ethics Committee with protocol number 2021/78 and was completed at Hitit University Faculty of Medicine. The study was conducted in compliance with the Helsinki Declaration and publishing ethics.

The State-Trait Anxiety Inventory (STAI): The scale includes two subscales consisting of 20 items, namely State Anxiety (STAI-I) and Trait Anxiety (STAI-II) (9). Each subscale consists of 20 items and includes a total of 40 items. The answers given to the items are scored between 1 and 4. The scores obtained from the scale range from the lowest 20 to the highest 80. A high score indicates a high level of anxiety. It was developed by Spielberger in 1970, and its Turkish adaptation was made by Öner N and Le Compte A (1983) (10).

The Coping Strategies Scale (COPE): Coping Strategies brief form consists of 28 items and 14 sub-dimensions. There are two items in each sub-factor. The COPE scale consists of 3 main groups.

(a) Emotion-focused:

1.Humor is making jokes or making fun of a stress situation.

2. Acceptance is acknowledging the existence of the stressful situation.

3. Positive reinterpretation is recreating the stress situation in a positive way.

4. Religion is creating a source of emotional support through a positive reinterpretation of the situation.

5.The seeking for emotional social support is to provide moral support, sympathy and decency.

(b) Problem-focused:

1. Active coping is the process of taking steps to eliminate stress or its effects.

2. Planning is thinking about how to deal with stress.

3. Seeking social support for instrumental reasons is a referral, a help, a search for information.

(c) Dysfunctional coping:

1. Focus on and venting of emotions the tendency to focus on the stress experienced by a person and to express their emotions.

2. Behavioral distancing is a decrease in one's efforts to cope with stress, or even a lack of effort to reach a goal.

3. Mental disengagement diverting one's attention away from the stress situation.

4. Denial is the refusal to believe that the stressful situation exists.

5. Substance use includes the use of alcohol and other substances.

6. Self-blame is a tendency to criticize oneself (11,12).

Participants were asked to fill in the strategy defined in each item, taking into account the COVID-19 process, and how often they used it. The Biref COPE was used to evaluate coping strategies during previous epidemics of infectious disease, like the SARS epidemic (13).

Statistical Analysis

Statistical analysis of the data obtained by using questionnaires and scales in our study was performed with SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA, Undergraduate: University) package program. The normal distribution of data was tested with the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Descriptive statistics of continuous data obtained from questionnaires and scales were reported together with mean±standard deviation (SD) and median (min-max) depending on data distribution. Descriptive statistics of categorical variables obtained from socio-demographic questions were presented with frequency and percentage (%). When comparing the scale scores according to socio-demographic characteristics, Student's t-test was used

for data showing normal distribution and Mann-Whitney U test was used for data not showing normal distribution between two independent groups. When more than two independent groups were compared, normally distributed data were calculated with one-way analysis of variance (ANOVA), and data that were not normally distributed were calculated with the Kruskal Wallis test. Correlations between the numerical scores to be obtained from the scales were investigated with the Pearson or Spearman correlation coefficient, depending on the normal distribution of the data. Simple linear regression analysis was used to identify the cause-effect relationship between two scale scores with significant correlations. Statistical significance level was evaluated as $p<0.05$.

RESULTS

A total of 186 medical students participated in the study. 57% (n=106) of the participants were female and 43% (n=80) were male. 61.3% (n=114) of the participants were receiving preclinical and 38.7% (n=72) clinical class education. Other descriptive statistics regarding the participants are presented in Table 1.

Table 1. Descriptive statistics of the socio-demographic characteristics of the participants

		N	%
Gender	Female	106	57
	Male	80	43
Class	Pre-clinic	114	61.3
	Clinic	72	38.7
Accommodation	Family home	23	12.4
	Student house	51	27.4
	Dormitory	112	60.2
Chronic disease status	Yes	15	8.1
	No	171	91.9
Status of being diagnosed with COVID-19	Yes	45	24.2
	No	141	75.8
Ongoing pre-diagnosed psychiatric disease state	Yes	29	15.6
	No	157	84.4
Psychotropic drug use status	Yes	23	12.4
	No	163	87.6
Total		186	100

The comparison of the STAI and the subscale scores of the coping scale (COPE) between the research groups (preclinical and clinical) are given in Table 2. The scores of the STAI and the COPE subscale scores (emotion-focused, problem-focused and dysfunctional coping) were not statistically different between the research groups ($p>0.05$; Table 2).

Table 2. Comparison of STAI subscale scores (STAI-I and STAI-II) and COPE subscale scores (emotion-focused, problem-focused and dysfunctional coping) among research groups

	Pre-clinic (n=114)	Clinic (n=72)	p value
STAI			
STAI-I	40 (29-58) (40.65±5.80)	41 (23-61) (41.69±7.02)	0.204[b]
STAI-II	47 (35-63) (47.42±6.54)	47 (27-65) (47.11±7.29)	0.886[b]
COPE			
Emotion-focused	27 (17-39) (26.98±4.52)	27 (11-40) (27.06±5.49)	0.600[b]
Problem-focused	(16.93±3.30) 17 (8-24)	(16.20±3.98) 16 (6-24)	0.178[a]
Dysfunctional coping	24 (14-47) (24.60±5.12)	25 (15-48) (25.20±5.09)	0.430[b]

STAI State and trait anxiety scale, STAI-I State Anxiety, STAI-II Trait anxiety, COPE Coping Strategies Scale, [a] Students' t-test with Mean±SD, [b] Mann-Whitney U test with Median (min-max), SD Standard deviation

The STAI scores among the socio-demographic characteristics of the participants is presented in Table 3. The STAI scores were statistically significantly different between the genders and places of residence of the participants (respectively; $p=0.025$; $p=0.033$; Table 3). The STAI scores of the participants were not statistically different between the presence of a chronic disease, the status of being diagnosed with COVID-19, whether or not they had a history of ongoing psychiatric illness, and their use of psychiatric drugs ($p>0.005$; Table 3).

The comparison of the COPE subscale scores (emotion-focused, problem-focused, and dysfunctional coping) among the socio-demographic characteristics of the participants is presented in Table 4. Emotion-focused, problem-focused, and dysfunctional coping scale scores were not statistically different among the socio-demographic characteristics of the participants ($p>0.005$; Table 4).

The results of the correlation analysis between the STAI-I, STAI-II and the COPE subscale scores are presented in Table 5. The STAI-I score and the scores on the emotion-focused, problem-focused, and dysfunctional coping scales did not show any statistically significant link ($p>0.05$; Table 5). There was a very low statistically significant positive correlation between STAI-II score and emotion focused scale score ($r=0.151$ $p=0.040$; Table 5). The STAI-II score and the dysfunctional coping scale score were shown to have a statistically significant low level positive correlation ($r=0.455$ $p<0.001$; Table 5). The STAI-II scores and the problem focused scale scores did not show any statistically significant link ($p>0.05$; Table 5). According to the simple linear regression analysis, a 1-unit increase in the STAI-II score increased the dysfunctional coping scale score by 0.35 units (Figure 1).

Table 3. Comparison of STAI scores among the socio-demographic characteristics of the participants

		STAI-I	p value	STAI-II	p value
Gender	Female	40 (25-55) 40.06±5.87	**0.025[b]**	47.5 (27-63) 48.28±6.60	**0.016[b]**
	Male	41 (23-61) 42.37±6.64		45.5 (34-65) 46±6.93	
Accommodation	Family home	43 (29-54) 42.39±5.64	**0.033[d]**	46.52±6.69 46 (36-60)	0.607[c]
	Student house	42 (23-61) 42.21±7.36		46.76±7.83 46 (27-65)	
	Dormitory	40 (29-58) 40.25±5.82		47.70±6.37 47 (35-63)	
Chronic disease status	Yes	41 (28-58) 41.13±7.73	0.968[b]	48 (35-61) 47.13±7.8	0.914[b]
	No	41 (23-61) 41.05±6.19		47 (27-65) 47.31±6.75	
Status of being diagnosed with COVID-19	Yes	41 (29-58) 42.71±7.03	0.093[b]	49 (34-61) 48.82±6.79	0.053[b]
	No	41 (23-61) 40.53±5.98		46 (27-65) 46.81±6.78	
Ongoing pre-diagnosed psychiatric disease state	Yes	41 (30-61) 40.55±6.28	0.586[b]	49 (35-58) 48.75±6.22	0.147[b]
	No	41 (23-58) 41.15±6.32		46 (27-65) 47.03±6.91	
Psychotropic drug use status	Yes	40 (31-61) 40.21±6.45	0.339[b]	48 (35-55) 47.47±5.81	0.729[b]
	No	41 (23-58) 41.17±6.29		47 (27-65) 47.27±6.97	

[a] Students' t-test (Mean±SD), [b] Mann-Whitney U test (Medyan (min-max)), [c] ANOVA (Mean ±SD),[d] Kruskal Wallis with (Median (min-max)), SD Standart Deviation

Table 4. Comparison of the subscale scores of the COPE (emotion-focused, problem-focused and dysfunctional coping) among the socio-demographic characteristics of the participants

		Emotion-focused	p value	Problem-focused	p value	Dysfunctional coping	p value
Gender	Female	28 (11-39) 27.33±4.78	0.249[b]	16.68±3.35 17 (6-24)	0.887[a]	**25 (15-47)** **25.55±4.71**	**0.006[b]**
	Male	27 (13-40) 26.58±5.07		16.61±3.90 16.5 (6-24)		**24 (14-48)** **23.88±5.47**	
Accommodation	Family home	28.43±4.83 28 (20-40)	0.186[c]	17 (12-24) 18±3.86	0.204[d]	24 (18-48) 25.08±6.38	0.831[d]
	Student house	26.19±5.51 26 (11-38)		16 (6-24) 16.05±3.66		25 (14-34) 24.64±4.97	
	Dormitory	27.09±4.60 27 (13-39)		17 (6-24) 16.65±3.45		24 (15-47) 24.87±4.92	
Chronic disease status	Yes	27 (20-33) 26.33±4.13	0.574[b]	17 (12-23) 17±2.75	0.666[b]	24 (16-33) 25.33±5.12	0.621[b]
	No	27 (11-40) 27.07±4.97		17 (6-24) 16.62±3.66		24 (14-48) 24.79±5.12	
Status of being diagnosed with COVID-19	Yes	28 (20-36) 27.42± 4.04	0.435[b]	17 (8-24) 16.73±3.96	0.907[b]	25 (15-40) 25.22±4.53	0.419[b]
	No	27 (11-40) 26.88±5.16		17 (6-24) 16.63±3.48		24 (14-48) 24.71±5.28	
Ongoing pre-diagnosed psychiatric disease state	Yes	26 (17-33) 25.72±4.78	0.172[b]	16 (10-24) 16.82±3.77	0.959[b]	26 (14-40) 25.89±5.26	0.099[b]
	No	27 (11-40) 27.25±4.91		17 (6-24) 16.62±3.57		24 (15-48) 24.64±5.07	
Psychotropic drug use status	Yes	26 (17-33) 25.73±4.87	0.280[b]	17 (12-24) 17.17±3.49	0.658[b]	25 (14-40) 25.65±5.21	0.350[b]
	No	27 (11-40) 27.25±4.91		17 (6-24) 16.58±3.61		24 (15-48) 24.72±5.10	

[a] Students' t-test (Mean±SD), [b] Mann-Whitney U test (Medyan (min-max)), [c] ANOVA (Mean ±SD),[d] Kruskal Wallis with (Median (min-max)), SD Standart Deviation

Table 5. Correlation analysis results between STAI subscale scores and COPE subscale scores (emotion-focused, problem-focused, and dysfunctional coping) (n=186)

		Emotion-focused	Problem-focused	Dysfunctional coping
STAI-I	r	0.075	0.081	-0.043
	P	0.308	0.271	0.563
STAI-II	r	**0.151**	0.133	**0.455**
	P	**0.040**	0.071	**<0.001**

r: Spearman correlation coefficient, STAI-I State Anxiety, STAI-II Trait anxiety

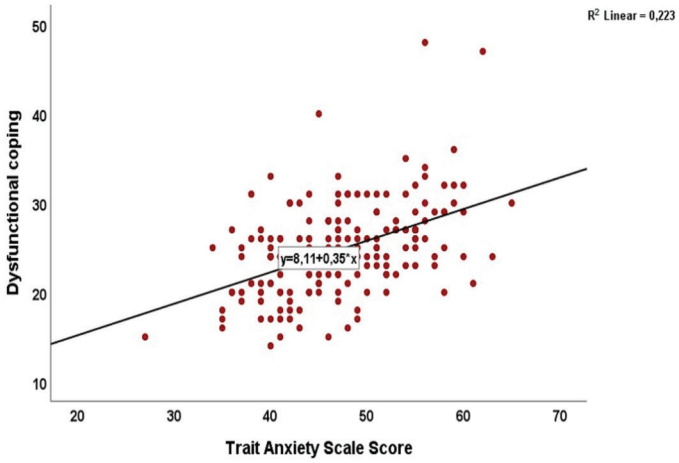

Figure 1. Scatterplot and regression curve showing the relationship between trait anxiety scale score and dysfunctional coping scale score (n=186)

DISCUSSION

The current study examines medical school students' pandemic coping mechanisms and how they relate to anxiety symptoms. Pre-pandemic study on medical students' anxiety levels in the literature revealed that they had higher levels of anxiety than their peers in other faculties (14,15). Also, it was observed that there was a similarity in anxiety prevalence in medical school students before and after the pandemic (16,17). During the pandemic, while the anxiety levels of medical students remained stable, it was shown to increase in their non-medical peers and general population. This situation has been interpreted as increasing the knowledge and cognition about the transmission, treatment, prognosis and prevention of COVID-19, as the anxiety levels are negatively proportional (18). On the other hand, there are also studies that emphasize the increase in anxiety rates during the COVID-19 process (18,19). There are studies showing that preclinical students have more anxiety symptoms than clinical students (19,20). Although there was no significant difference in the state and trait anxiety levels between preclinical and clinical students, the mean scores were found to be higher than the stated mean score of the STAI, scale in both groups in our study (Table 2) (21,22). The presence or absence of a diagnosis of COVID-19 infection did not make a significant difference in the anxiety levels of the students. The anxiety levels of female students were found to be substantially greater than those of male students, which is consistent with the literature (16,23,24).

Some occupations are more stressful than others. The profession of medicine is among these stressful professions in terms of working conditions and requires effective coping attitudes, especially in difficult processes such as pandemics. Developing the ability to cope with stress effectively in medical school students will not only protect their mental and physical health and their relationship with the environment, but will also help maintain their professional motivation and directly affect their approach to patients. In the literature, coping strategies of medical school students during the pandemic have not been investigated yet. In our study, coping attitudes were examined in 3 groups (emotion-focused, problem-solving-focused, and dysfunctional coping attitudes). No difference was found between preclinical and clinical medical students in terms of coping attitudes, but female students' use of dysfunctional coping attitudes was found to be significantly higher than male students in our study (Table 2,4). This may lead to the interpretation that female students use dysfunctional coping attitudes more because of their high level of anxiety. This is also supported by our research, which found a significant positive correlation between trait anxiety scale scores and dysfunctional coping attitudes. Furthermore, simple linear regression analysis revealed that for every unit increase in trait anxiety scale scores, the dysfunctional coping scale score increased by 0.35 units (Figure 1). Similarly to our study, it was reported that there is a positive significant correlation between desperate and the submissive approaches which can be evaluated in the dysfunctional coping attitudes group and anxiety level (25). From another perspective, using emotion-focused or problem focused functional coping styles suggests that it may be a protective factor for anxiety. The more functional coping attitudes, the lower the stress severity. These findings were similar to studies that found functional coping attitudes as the method with significant impact on reducing stress (26,27).

Limitations

The limitations of our study are that it did not include a clinical interview, was a cross-sectional study, used self-reported screening scales, could not establish a cause-effect relationship, and had a heterogeneous sample. At the same time, it should be noted that the results of our study conducted at our university may not be representative of the whole population. Despite these limitations, our study offers some common implications for the management of medical students' distress in exceptional circumstances such as the COVID-19 outbreak.

CONCLUSION

Our research may serve as a roadmap for therapeutic interventions designed to enhance and safeguard medical students' mental health throughout the ongoing

COVID-19 pandemic. As seen in our study, an increase in anxiety levels leads to dysfunctional coping attitudes. Dysfunctional coping attitudes are among the reasons that lead people to mental illnesses. Therefore, goals should be determined to diminish the anxiety levels of future physicians and to increase their functional coping attitudes.

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Conflict of Interest: *The author declare that they have no competing interest.*

Ethical approval: *The study was approved by the Hitit University Non-Interventional Ethics Committee (reference number:2021-78).*

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Research Article

The Effect of Vaginal Bleeding in Early Pregnancy on First Trimester Screening Test, Uterine Artery Doppler Indices and Perinatal Outcomes

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Abstract

Aim: We aimed to prospectively investigate the effect of first trimester vaginal bleeding on first trimester screening test, uterine artery (UtA) Doppler results and perinatal outcomes.

Material and Methods: Fifty cases that presented with vaginal bleeding in the first trimester between 2019 and 2020 constituted the early vaginal bleeding (abortus imminens-threatened abortion) group and fifty cases without a history of vaginal bleeding in pregnancy constituted the control group. Demographic datas were noted at the first visit. Both groups were followed up until birth. First trimester screening test (double screening test) between 11-14 weeks and UtA Doppler examination between 20-24 weeks of gestation was performed. Perinatal outcomes and values of nuchal translucency (NT), free β human chorionic gonadotropin (f β-hCG), pregnancy-associated plasma protein A (PAPP-A), and UtA Doppler were compared between the two groups.

Results: There was no statistically significant difference was found between the two groups in terms of NT and PAPP-A among the first trimester screening test results (p=0.741 and p=0.937, respectively). In the group with threatened miscarriage, f β-hCG value was numerically higher, but there was no statistically significant difference (1.24±0.59 vs. 1.1±0.93, p=0.057). In the Doppler examination of the UtA, there was no statistically significant difference between the groups in terms of systolic/diastolic ratio, pulsatility index, resistive index and the presence of a notch (p=0.713, p=0.528, p=424, p=0.538, respectively). Perinatal complication rate was statistically significantly higher in the study group (p=0.013; Odds Ratio:3.2, 95% CI 1.2-8.3).

Conclusion: Contrary to some different studies, we believe that first trimester screening test parameters or uterine artery Doppler flow indices do not have a place in predicting perinatal outcomes of pregnant women with a history of vaginal bleeding in the first trimester. In addition, early vaginal bleeding does not significantly affect screening parameters. Perinatal complication rate was found to be statistically significantly higher in the group with a history of threatened miscarriage.

Keywords: Doppler, uterine artery, abortus imminens, threatened abortion, vaginal bleeding, first trimester

INTRODUCTION

Chromosomal major diseases cause serious health, sociological and economic problems. Early screening methods have been developed for these diseases, which do not have a specific treatment. The two main methods used today are first trimester screening test (double screening test) and fetal anomaly screening ultrasound. The first trimester screening test is performed in the first trimester between 11-14 weeks. In fetal trisomy screening in the first trimester, the rate of trisomy 21 detection reaches around 90% with 5% false positivity, with the double screening test performed by adding nuchal translucency (NT) measurement to maternal serum pregnancy-associated plasma protein A (PAPP-A) and free β human chorionic gonadotropin (f β-hCG) values (1–3). In our tertiary center, the first trimester screening test is performed in accordance with the recommendations of

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the American College of Obstetricians and Gynecologists (ACOG) (3).

Fetal anomaly screening ultrasound is the detailed examination of the organs, systems and tissue structures of the fetus. Fetal anomaly screening ultrasound is performed between 18-24 weeks (4). Fetal anomaly screening ultrasound is also performed in our tertiary care center in line with The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) recommendations (4). One component of this screening ultrasound is uterine artery (UtA) Doppler indices. Compliance decreases with trophoblastic invasion to the spiral artery, which is a branch of the uterine artery. Placental insufficiency prevents the decrease in compliance and changes that would give findings in UtA Doppler measurement cause adverse perinatal outcomes (5). The specificity of the test (86-96%) in diagnosing perinatal complications (fetal growth restriction, preeclampsia, and prenatal death) is higher than its sensitivity (24-89%) (6).

Threatened abortion (abortus imminens) is vaginal bleeding in which the cervix is closed before 20 weeks of gestation (7). Early vaginal bleeding occurs in about a quarter of pregnant women (8). The etiology of vaginal bleeding has not been fully elucidated, but one of the most important causes is the separation of decidual vessels on the maternal-fetal face (9). It is well known that vaginal bleeding in early pregnancy affects the serum alpha-fetoprotein value (10,11), but its effect on other parameters is controversial. Some studies argue that early pregnancy bleeding affects serum PAPP-A and f β-hCG values (12,13), while some studies argue that there is no significant effect (14,15). Similarly, some studies have associated uterine artery resistance with early pregnancy bleeding, but all these studies were conducted at the time of bleeding in early pregnancy (16,17), long-term follow-up was not investigated. There are no suitable randomized prospective cohort studies on these parameters. Therefore, we aimed to prospectively investigate the effects of first trimester vaginal bleeding on NT, f β-hCG and PAPP-A values measured in first trimester screening, and UtA Doppler indices measured in second trimester fetal anomaly screening. We also evaluated the perinatal outcomes of these patients.

MATERIAL AND METHOD

This prospective cohort study was conducted with pregnant women selected between January 2019 and April 2019 in a tertiary referral center. Power analysis was performed with G-power for the number of samples. Accordingly, a minimum limit of 34 people was found for each group. A total of 100 pregnant women, 50 pregnant women who applied to the emergency service and pregnancy outpatient clinics with first trimester (before the 12th gestational week) vaginal bleeding (threatened abortion group), and 50 pregnant women who did not experience any vaginal bleeding during pregnancy (control group), were included in this study. Exclusion criteria were being younger than 18 and older than 40 years of age, previous diagnosis of habitual abortion and/or cervical insufficiency, and a history of conization. The cases were followed up until January 2020, when all of them already gave birth (Figure 1).

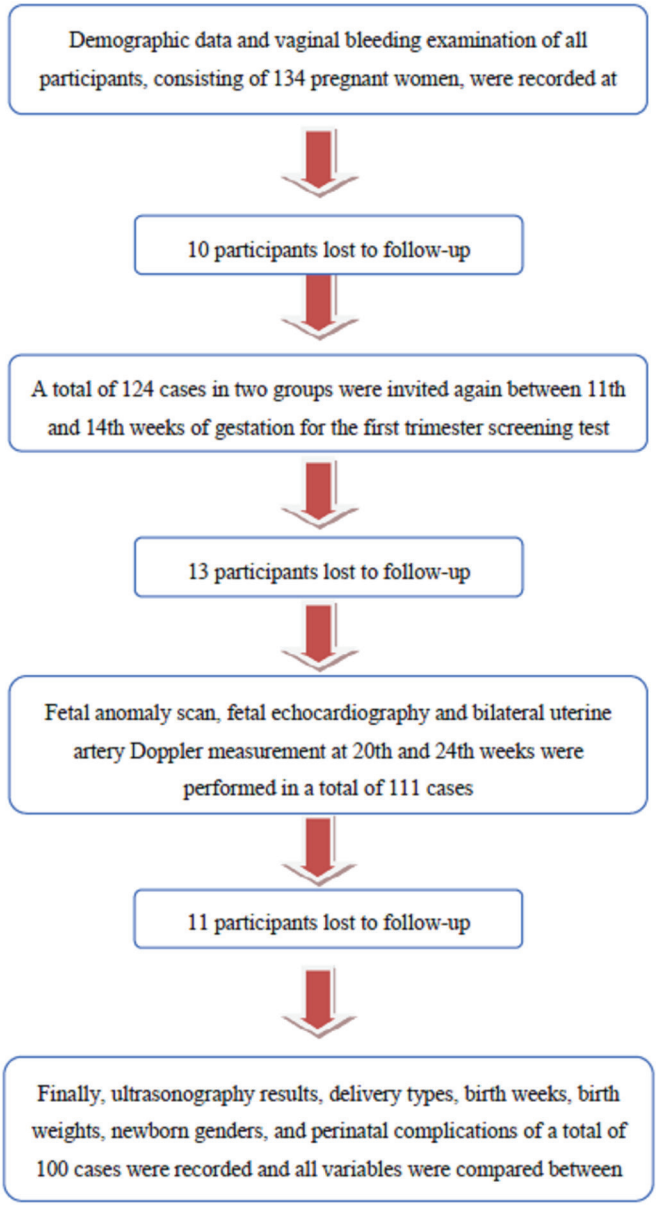

Figure 1. Flow chart of participants

This study was conducted following the Helsinki Declaration Ethical Standards. The ethics committee approval for this study was obtained from the University of Health Sciences Tepecik Training and Research Hospital Local Ethics Committee (approval number: 2018/ 16-9). The nature and aims of the study were fully explained to all the participants, and consent forms were signed by all participants.

Age, body mass index (BMI), parity, blood pressure and heart rate values of the cases were recorded at the first visit. The threatened abortion group was given 400 mg natural micronized progesterone (Progestan ®, Kocak Pharma, Tekirdag, Turkey) daily treatment during the period

of bleeding. The bleeding was classified as mild/severe according to its amount. Spotting and bleedings up to 1 pad/day were considered mild and bleedings of 2 pads/day and above were considered severe. Control group cases were enrolled consecutively in similar pregnancy weeks. A total of 100 cases in two groups were invited again between 11-14 weeks of gestation (within the range of 45mm≤ crown-rump length (CRL) <84mm) for the first trimester screening test. Fetal imaging was performed abdominally with Toshiba Aplio 500 (Toshiba Medical Systems, Tokyo, Japan) ultrasound device. Following the CRL and NT MoM measurements in the first trimester ultrasound examination, f β-hCG MoM and PAPP-A MoM values in maternal blood detected in the laboratory were recorded. The results obtained were combined with maternal age and weight, diabetes and smoking history, as well as a history of having a baby with Down Syndrome and evaluated with Prisca 5.0 Software (Prenatal Risk Calculator, Typolog Software GmBH, Hamburg, Germany) in terms of trisomy 21, 13, and 18, and risk scores were established.

UtA Doppler measurements were made between 20-24 weeks of pregnancy in all these cases. All UtA Doppler measurements were performed by the same individual to prevent inter-practitioner variability. The practitioner (M.B.) with the Doppler certificate of The Fetal Medicine Foundation made an abdominal evaluation in the supine position by using Toshiba Aplio 500 (Toshiba Medical Systems, Tokyo, Japan). First, the uterine artery was defined with color Doppler by slightly bending the probe in the sagittal plane at the same level as internal cervical os and a regular flow wave form was created with pulsed wave Doppler. UtA systolic/diastolic ratio (S/D), resistive index (RI), pulsatility index (PI) and the presence of notch were evaluated bilaterally in the wave form image. Presence/absence of bilateral notch, presence/absence of notch on any side, presence/absence of an increase in bilateral RI resistance, presence/absence of an increase in RI resistance on any side, presence/absence of an increase in resistance in the mean RI value of the two uterine arteries and the mean RI, PI, S/D values of two UtA were compared between the two groups. It was considered that an increase was present when RI was >0.58.

Finally, ultrasonography results, delivery type, delivery weeks, birth weights, newborn genders, and perinatal complications of for all cases were recorded. Preterm birth threat, preterm labor, minor fetal anomaly, premature rupture of membranes, placenta accreta, fetal growth restriction, gestational hypertension, preeclampsia, oligohydramnios, gestational diabetes, cholestasis, postpartum atony, placenta abruption were defined as perinatal complications.

Statistical Analysis

Before starting the statistical analysis, all parametric variables were evaluated in terms of normality and homogeneity assumptions. Independent variables conforming to the assumption were evaluated with the Independent Samples T Test, and those not conforming to the assumption with Mann Whitney U test. Categorical variables were evaluated by using the Chi Square and Fisher's Exact Tests. Descriptive statistics were given as mean ± SD, median (minimum-maximum) and n (%). p <0.05 was considered significant. SPSS 22.0 (SPSS Inc., Chicago, Illinois) software was used in statistical analysis.

RESULTS

Our prospective cohort study was completed with the data of 100 patients including a case group that experienced threatened miscarriage (n=50) and a control group (n=50). In the study group, 40 (80%) of the patients had mild vaginal bleeding and 10 (20%) had severe vaginal bleeding. Demographic and clinical data of participants are summarized in Table 1. Both groups were similar in terms of maternal age, BMI, and parity. The pulses (79±8 vs. 77±8, p=0.546), systolic blood pressures (110±13 vs. 110±13, p=0.866), and diastolic blood pressures (64±19 vs. 67±11, p=0.536) of both groups were statistically similar.

Table 1. Demographic and clinical data of participants

	Threatened miscarriage n=50	Control n=50	P Value
Age (mean±SD)	29.1±5.8	27.7±5.4	0.195*
Body mass index (mean±SD)	26.1±5.0	26.5±5.1	0.712*
Parity (n,%)			0.689[a]
Nulliparous	23 (46%)	25 (50%)	
Multiparous	27 (54%)	25 (50%)	
Pulse (bpm) (mean±SD)	79±8	77±8	0.546*
Blood pressure (mmHg) (mean±SD)			
Systolic	110±13	110±13	0.866*
Diastolic	64±19	67±11	0.536*

*Independent Sample T Test, [a]Pearson Chi Square Test

First trimester screening test results are summarized in Table 2. No statistically significant difference was found between the threatened miscarriage and control groups in terms of CRL sizes on ultrasound (61.66±8.30 vs. 61.83±9.25, p=0.922), NT MoM values (0.90±0.36 vs. 0.88±0.21, p=0.741) and maternal serum PAPP-A MoM values (0.99±0.51 vs. 0.97±0.52, p=0.937). Although the f β-hCG MoM values were numerically higher in the threatened miscarriage group, the result could not reach a significant level (1.24±0.59 vs. 1.10±0.93, p=0.057).

Table 2. First trimester screening test results

	Threatened miscarriage n=50	Control n=50	P Value
CRL (mm) (mean±SD)	61.66±8.30	61.83±9.25	0.922*
NT (MoM) (mean±SD)	0.90±0.36	0.88±0.21	0.741*
f β-hCG (MoM) (mean±SD)	1.24±0.59	1.10±0.93	0.057*
PAPP-A (MoM) (mean±SD)	0.99±0.51	0.97±0.52	0.937*

Abbrevations: CRL: Crown-rump length, NT: Nuchal translucency, f β-hCG: Free beta human chorionic gonadotropin, PAPP-A: Pregnancy-associated plasma protein A, MoM: Multiple of the median
*Independent Sample T Test

Comparison of uterine artery Doppler measurement results is summarized in Table 3. Accordingly, bilateral (6% vs. 6%, N/A) and any side (11.1% vs. 14%, p=0.538) uterine notch was observed at a statistically similar rate in both groups. Bilateral S/D (0.62±0.09 vs. 0.62±0.10, p=0.713), RI (1.25±0.43 vs. 1.27±0.35, p=0.432) and PI (3.02±1.18 vs. 3.05±1.09, p=0.528) arithmetic mean was similar between the groups. Bilateral (20% vs. 14%, p=0.424), any side (58% vs. 46%, p=0.230) and arithmetic mean (68% vs. 76%, p=0.373) RI increases were observed to be similar in both groups.

Table 3. Comparison of uterine artery doppler measurement results

	Threatened miscarriage n=50	Control n=50	P Value
Bilateral Notch (n,%)			N/A
Yes	3 (6%)	3 (6%)	
No	47 (94%)	47 (94%)	
Notch (Any Side) (n,%)			0.538[α]
With	5 (11.1%)	7 (14%)	
Without	45 (88.9%)	43 (86%)	
Bilateral S/D Arithmetic Mean (mean±SD)	0.62±0.09	0.62±0.10	0.713[*]
Bilateral RI Arithmetic Mean (mean±SD)	1.25±0.43	1.27±0.35	0.432[*]
Bilateral PI Arithmetic Mean (mean±SD)	3.02±1.18	3.05±1.09	0.528[*]
Bilateral RI Increased Resistance (n,%)			0.424[α]
With	10 (20%)	7 (14%)	
Without	40 (80%)	43 (86%)	
RI Increased Resistance (Any Side) (n,%)			0.230[α]
With	29 (58%)	23 (46%)	
Without	21 (42%)	27 (54%)	
RI Arithmetic Mean Increased (n,%)			0.373[α]
With	34 (68%)	38 (76%)	
Without	16 (32%)	12 (24%)	

Abbrevations: S/D: systolic/diastolic ratio, RI: resistance index, PI: pulsatility index
[*]Independent Sample T Test, [α]Pearson Chi-Square Test

Perinatal outcomes are showed in Table 4. In the light of all data obtained, the perinatal complication rate was found to be statistically significantly higher in the group with a history of threatened miscarriage (p=0.013; Odds Ratio: 3.2, 95% CI 1.2 - 8.3). These complications were threatened preterm labor in 5 (26.3%) cases, preterm labor in 3 (15.8%) cases, minor fetal anomaly in 3 (15.8%) cases, premature rupture of membranes in 1 (5.3%) case, placental invasion abnormality in 1 (5.3%) case, fetal growth restriction in 1 (5.3%) case, gestational hypertension in 1 (5.3%) case, preeclampsia in 1 (5.3%) case, gestational diabetes in 1 (5.3%) case, cholestasis in 1 (5.3%) case, and postpartum atony in 1 (5.3%) case, respectively in the cohort group. Uneventful pregnancy rate in threatened miscarriage group was 42% (n=31), control group was 84% (n=42). Delivery week and birth weight are statistically similar between the groups (p=0.108 and p=0.495, respectively). In addition, there was no statistically significant difference in the mode of delivery and primary cesarean rates between the two groups (p=0.422 and p=0.389, respectively).

Table 4. Perinatal outcomes

	Threatened miscarriage n=50	Control n=50	P Value
Perinatal complication (n,%)			0.013[α]
With	19 (38%)	8 (16%)	
Preterm Birth Threat	5 (26.3%)	1 (12.5%)	
Preterm Labor	3 (15.8%)	0	
Minor Fetal Anomaly	3 (15.8%)	1 (12.5%)	
Premature rupture of membranes	1 (5.3%)	0	
Placenta Accreta	1 (5.3%)	0	
Fetal Growth Restriction	1 (5.3%)	1 (12.5%)	
Gestational Hypertension	1 (5.3%)	1 (12.5%)	
Preeclampsia	1 (5.3%)	1 (12.5%)	
Oligohydramnios	0	1 (12.5%)	
Gestational Diabetes	1 (5.3%)	1 (12.5%)	
Cholestasis	1 (5.3%)	0	
Postpartum Atony	1 (5.3%)	0	
Placental Abruption	0	1 (12.5%)	
Without	31 (42%)	42 (84%)	
Birth type (n,%)			0.422[β]
Vaginal	21 (42%)	25 (50%)	
C- Section	29 (58%)	25 (50%)	
C- Section type (n,%)			0.389[α]
Primer	14 (48.3%)	15 (60%)	
Seconder	15 (51.7%)	10 (40%)	
Birth time (week) median (min,max)	38 (23-41)	39 (26-41)	0.108[x]
Birth weight (g) (mean±SD)	2980±704	3140±591	0.495*
Newborn gender (n,%)			0.420[α]
Female	30 (60%)	26 (52%)	
Male	20 (40%)	24 (48%)	

[*]Independent Sample T Test, [x]Mann Whitney U Test [α]Pearson Chi-Square Test, [β]Fisher's Exact Test

DISCUSSION

In this study, it was found that vaginal bleeding in early pregnancy did not significantly affect first trimester screening test (double screening) parameters as NT, f β-hCG and PAPP-A. Also, contrary to expectations, UtA Doppler indices and RI resistance were not affected by first trimester bleeding. However, threatened miscarriage was associated with 3.2 times more perinatal complications.

In the literature, the effect of vaginal bleeding in early pregnancy on especially f β-hCG MoM, which is one of the first trimester tests, is controversial. Spencer et al. investigated the first trimester screening test results of 42183 pregnant women with a history of threatened miscarriage and 7470 pregnant women with a normal pregnancy in two groups. As in the present study, there was no statistically significant difference between the f β-hCG MoM and PAPP-A MoM values of the patients who had vaginal bleeding in the early gestational weeks (14). In another study, 253 pregnant women with vaginal bleeding in early gestational weeks were compared with 2077 pregnant women with a normal pregnancy in terms of first trimester screening test parameters, and there was a statistically significant increase in the f β-hCG MoM value, contrary to our study result; however, no difference was found in PAPP-A MoM and NT values (12). In a similar smaller-scale study, although there was a statistically significant increase in f β-hCG MoM and PAPP-A MoM values, no increase was observed in the risk of Down Syndrome of above 1/250 in the first trimester screening test (18). However, all these studies are retrospective. Our results are based on prospective, long-term follow-up.

Heinig et al. reported that first trimester screening test parameters would not be affected by bleedings in the form of spotting. However, when there was an increased amount of bleeding they found a statistically significant increase in f β-hCG MoM (13). In the present study, in the analyses of subgroups according to the amount of bleeding, there was no statistically significant difference in β-hCG, PAPP-A, NT, and UtA Doppler values. We did not find a significant difference in these values according to the amount of bleeding. This may be due to the relatively different evaluation of the amount of bleeding. Heinig et al. classified the amount of bleeding according to the amount in the menstrual cycle of women. On the other hand, we tried to classify more objectively by performing prospective pad follow-up.

Histological examinations showed that insufficient trophoblastic invasion into the spiral arteries caused the miscarriages (19). Therefore, we considered that early evaluation of placentation with Doppler measurement could give us information about pregnancy outcomes in cases with threatened miscarriage. In our study, the limit value for the increased resistance in UtA Doppler measurement was determined as RI=0.58, which is used to predict preeclampsia, fetal growth restriction, and fetal death (20). In the statistical analysis, based on the results of the unilateral and bilateral variations of the increased UtA resistance and notch presence, maternal-placental bleeding in the first trimester was not significantly associated with placental vascularization, contrary to the expectations. In this study, although there was no statistically significant difference in first trimester screening test parameters and second trimester UtA Doppler flow results of patients with a history of threat of miscarriage, a statistically significant increase was found in perinatal complication rates.

In a prospective study by Alcázar et al. in 49 patients with a history of threatened miscarriage and 129 patients with a normal pregnancy, pregnant woment with vaginal bleeding between gestational weeks 6 and 12 were examined with Doppler for UtA and spiral artery by transvaginal ultrasound and compared with the pregnant women in the control group who were in a similar gestational week. Similar to our study, no statistically significant difference was found between the two groups in terms of peak systolic velocity and PI. In the same study, cases that developed spontaneous abortion were compared with normal pregnancies, but once again, no statistically significant difference was found (17). Stabile et al. compared 38 cases of threatened miscarriage with 73 uncomplicated pregnancies and did not observe a statistical difference in RI values in the Doppler measurement performed on sub-placental vessels (21). In a similar study conducted by Kurjak et al., although the control groups were not homogeneously distributed, the UtA Doppler results of 20 cases with a history of threatened miscarriage and 130 pregnant women with a normal pregnancy were compared. There was a statistically significant difference between radial artery and spiral artery Doppler results. However, when the results were examined, the radial artery PI was high in the group with threatened miscarriage, while the spiral artery PI was found to be significantly lower in the group with threatened miscarriage (22). In another larger-scale study of the author, no statistically significant difference was found in the Doppler indices (23). In that study, in 9 cases with retroplacental hematoma on ultrasound, the UtA RI value was statistically significantly lower in the contra-lateral of the hematoma side, and this difference was attributed to the possible pressure of the hematoma (23). In a study by Pellizzari et al., pregnancies complicated by vaginal bleeding during gestational week 6-12 were compared with unproblematic pregnancies, and no difference was observed in the Doppler parameters between incomplete abortion, missed abortion, imminent abortion, and normal pregnancies (16). Behery et al. conducted a study with 90 cases diagnosed with threatened miscarriage in the first trimester and 50 controls and found that patients who developed missed abortion during their follow-up showed a statistically significant increase in unilateral PI in their UtA Doppler results (24). However, all these studies were generally done in the first trimester, when there is vaginal bleeding. We wanted to see the long-term consequences of bleeding and performed the UtA Doppler examination between 20-24 weeks of gestation and did not observe any interaction between UtA and threat of miscarriage.

Another reason for the insufficiency of uterine artery Doppler results in predicting perinatal complications is that it varies with the gestational week. During the first trimester, the blood flow rate in the uteroplacental arteries increases and pulsatility index values decrease as the pregnancy progresses (25). In a study conducted to predict the results of preeclampsia, fetal growth restriction, intrauterine death, and placental abruption,

specificity and sensitivity results were determined by referencing RI> 0.58 or RI> 0.7 and the presence of bilateral notch or notch on either side. All reference values were evaluated separately for each disease. Sensitivity and specificity were not observed to be above 80% at the same time based on a single reference value for any of these diseases (26). Since the current diagnostic value of Doppler examination is as mentioned, it is clear that it cannot be a guide alone in forecasting perinatal outcomes in patients with threatened miscarriage. In the literature, uterine artery Doppler examination has usually been performed in the first trimester in case of pregnancies complicated by vaginal bleeding, and no study has been found investigating its effect on Doppler findings with abdominal USG between gestational weeks 20 and 24 as in the present study. According to the results obtained from our current findings, it has been determined that UtA Doppler use is not statistically significant in predicting adverse perinatal outcomes in pregnant women with bleeding in early gestational weeks.

In the literature, the probability of occurrence of adverse perinatal outcomes in patients with threatened miscarriage is high, similar to the present study. Preterm delivery, premature rupture of membranes, and placenta abruption have been observed more commonly in these cases (27). In a large-scale review by Tuuli et al., the incidence of placenta abruption was increased by 5.7 times, preterm birth by 1.4 times, and premature rupture of membranes by 1.6 times (28). In a multi-center large-scale prospective study, the cases were divided into three groups as those with no bleeding, those with mild bleeding, and those with severe bleeding, and their perinatal outcomes were examined (29). It was observed that the probability of miscarriage before the 24th week of pregnancy increased by 2-4 times in pregnant women with vaginal bleeding, but no statistically significant increase was observed in the cesarean rates. Mild vaginal bleeding was mostly associated with preeclampsia, preterm birth, and abruptio placentae; on the other hand, severe vaginal bleeding was associated with preterm birth, abruptio placentae, fetal growth restriction, and preterm rupture of membranes. According to these results, we believe that it would be appropriate to follow up the cases with threatened miscarriage more frequently in especially 3rd trimester due to the increased rate of perinatal complications.

The strength of our study is that it is prospective and ensures the continuity of the patients in the study. We followed patients long-term until delivery and increased the reliability of our results. Our limitation was that our follow-up was long, but we could not get continuous measurements, so we may not have been able to analyze the change graphs of the parameters.

CONCLUSION

In conclusion; contrary to some different studies, we believe that first trimester screening test parameters or uterine artery Doppler flow indices do not have a place in predicting perinatal outcomes of pregnant women with a history of vaginal bleeding in the first trimester. In addition, bleeding does not significantly affect screening parameters. Perinatal complication rate was found to be statistically significantly higher in the group with a history of threatened miscarriage.

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Research Article

Rosmarinic Acid Ameliorates Deltamethrin Induced Hepatotoxicity and Nephrotoxicity

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Abstract

Aim: Deltamethrin (DM) is an insecticide and is widely used around the world. Rosmarinic acid (RA) is found in herbs and spices in the Lamiaceae (mint) family and has antioxidant, antiinflammatory and antiapoptotic effects. We objective to examine the protecting efficacy of Rosmarinic acid in preventing the toxic effects of Deltamethrin.

Material and Methods: In ours study we used 28 male rats. Group 1: Control group, Group 2: RA group, RA was given 20 mg/kg peroral (p.o.) for 7 days, Group 3: DM group, 35 mg/kg/dose of DM was given 24 hours before sacrification as a single dose by gavage, Group 4: RA+ DM group.

Results: BUN, creatinine, AST and ALT values of the RA+DM group were lower than the DM group. TAS and TOS grades were higher in the DM group matched to the RA+DM group. The damage scores of the DM group were higher aacording as those of the RA+DM group.

Conclusion: RA has been shown to have predicative influence in the therapy of deltamethrin-induced nephrotoxicity and hepatotoxicity.

Keywords: Deltamethrin, rosmarinic acid, hepatotoxicity, nephrotoxicity

INTRODUCTION

Deltamethrin (DM) is a widely used insecticide for protecting plants against ants, insect, etc. Additionally, it is topically used as an ectoparasiticide to control vector-borne diseases in farm animals (1). DM is generally preferred in the first place among insects because it has rapid metabolism and high efficiency on harmful insects (2). Consuming water or food contaminated with DM can cause serious harm (3). In their studies, Tuzmen et al and Tos-Luty et al have revealed that DM administration to the rats affects hepatic lipid peroxidation and antioxidant defense system and this causes generation of free oxygen radicals, and the destructive effects in liver and kidney textures were occured owing to these free oxygen radicals (4,5).

RA was first isolated and purified in 1958 from Rosmarinus officinalis by two Italian chemists, Scarpati and Oriente, who then named it according to the plant that they isolated it from (6). RA is soluble in water and found in herbs and spices like Rosmarinus officinalis L. (rosemary), Thymus vulgaris L. (thyme) etc. (7). RA has multiple effects, such as antioxidant (8), antiinflammatory (9) and antiapoptotic (10) effects. RA was found to be effective in preventing liver damage due to diabetes (11), tertbutyl hydroperoxide (12), and carbon tetrachloride (13). Lee et al. revealed that Rosmarinic acid has protective effects against hydrogen peroxide-induced neurotoxicity, with Bcl-2 downregulated but Bax upregulated (14).

In this study, we examined to RA intake in the results of DM that occur on the liver and kidney tissue damage.

CITATION
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MATERIAL AND METHOD

Chemicals

Deltamethrin (Butox® 50 mg/ml) was puechased from Intervet Co. (France), and RA was obtained from Sigma Aldrich Inc.

Animals and Experiment Procedure

Twenty-eight male Wistar Albino rats were used. Rats were permitted to eat standard chow ad libitum during the experiment. The rats were protected in steel cages, at 21 C, with alternating 12 hr light/dark cycles, subsequently all rats were placed under general anesthesia by intraperitoneal (i.p.) administration of Ketamine HCl (90 mg/kg, Pfizer Inc, USA) + Xylazine HCl (10 mg/kg, Bayer Health Care AG, Germany).

The animals were randomly divided into 4 equal groups:

Group 1 (n=7): Control Group; no experimental procedure was applied.

Group 2 (n=7): RA group; rats were administered 20 mg/kg/day of RA by gavage for 7 Days (15).

Group 3 (n=7): DM group; on the 7th day of the experiment, the rats were administered 35 mg/kg DM by gavage once, and 24 hours later the rats were sacrificed (16).

Group 4 (n=7): RA + DM group; the rats were administered RA for 7 days, 1 hour later a single dose of DM was given and 24 hours later the rats were sacrificed.

Collection and Staining of Tissue and Blood Samples

Liver tissues, kidney tissues were taken for pathological examination. We took blood samples from the all rats. The liver and kidney tissues taken were fixed in containers containing 10% buffered formol. Tissue samples washed in runing water after fixation were examined with routine histological examinations. routine histological examined with dyes Hematoxylin & Eosin (H&E) protocols for histological evaluation and Periodic Acid Schiff (PAS). Tumor Necrotizing Factor-α (TNF-α) (Santa Cruz, Cat no:sc-52746) antibodies were used to show inflammatory changes in immunohistochemical studies, and Apoptotic protease activating factor-1 (APAF-1) (Santa Cruz, Cat no:sc-65891) antibodies were used for proapoptotic changes.

Biochemical Analysis

Total Antioxidant (TAS) and Total Oxidant (TOS) Levels

Kits were used from Rel Assay Diagnostics for both TAS and TOS levels measurement with Erel's method (17,18).

Liver and Kidney Function Tests

On account of demonstrate liver functions, alanine aminotransferase (ALT) enzyme and aspartate aminotransferase (AST) enzyme levels were evaluated. Blood urea nitrogen (BUN) and Creatinine (Cre) levels were evaluated to evaluate kidney functions.

Histopathological analysis

Liver damage was classified as follows:

Hepatic damage was assessed using a grading system as follows:

• Grade 0: The tissues were normal.

•Grade 1: The injury was mild with pycnosis and cytoplasmic vacuoles.

•Grade 2: Moderate damage without cytoplasmic vacuoles, hepatocytes swelling, sinusoidal dilatation and congestion, and no obvious necrosis.

•Grade 3: Moderate damage with signs of extensive sinusoidal dilatation and congestion, with coagulation necrosis.

•Grade 4: There was severe damage with loss of tissue integrity (19).

Renal damage was classified as follows:

•Grade 0: The tissues were normal.

•Grade 1: Nuclear loss due to swelling in 1/3 of the cells in the tubules.

•Grade 2: Nuclear loss due to swelling in 2/3 of the cells in the tubules.

• Grade 3: Greater nuclear loss due to swelling in more than 2/3 of the cells in the tubules (20).

Immunohistochemical Analysis

After routine histological tissue follow-up, 5 µm thick sections of liver tissues was taken on positively charged slides. Sections were heated in ethylenediamine tetraacetic acid (EDTA) solution for 1 minute in a microwave oven for antigen retrieval treatment and then cooled at room temperature for 15 minutes. The sections were washed in buffered phosphate saline (PBS) solution. Then, the tissues in the sections were drawn with a hydrophobic pen and lined up on the bar. Endogenous peroxide blockade was performed for 20 minutes by dripping 3% H2O2 prepared in methanol on the sections. Sections were washed in PBS for 3x5 minutes.

The sections were incubated for 7 minutes by dripping in Ultra V Block solution (Thermo). The Blocking solution was then removed from the sections. The samples were incubated for a night at +4 °C with primary antibodies APAF-1 (Santa Cruz, Cat no:sc-65891) and TNF-α (Santa Cruz, Cat no:sc-52746) diluted 1/250 with antibody diluent (Thermo) without washing the samples.

In immunohistochemical analysis;

Immunoreactivity prevelance of the tissues were scored as; 0.1 (<25%), 0.4 (26-50%),

0.6 (51-75%), 0.9 (76-100%)

Immunoreactivity severity was leveled as; 0: none, +0.5: very little, +1: little, +2: moderate, +3: severe. The histoscore was created by using the formula:

Histoscore=PrevalencexSeverity (21).

Statistical Analysis

We used SPSS program for statistical analyzes (Chicago, IL, USA) . Normally distributed measurements was done by One-Way Analysis of Variance (ANOVA), and non-normally distributed measurements by Mann Whitney-U test. It was considered significant in case of p<0.05 value.

RESULTS

AST, ALT, BUN and Creatine Values

AST, ALT, BUN and Creatine values of the RA+DM group was lower than the DM

group's (p<0.05) (Table 1).

TAS and TOS Values

We found that TAS and TOS levels were higher in DM group then the RA+DM group (p<0.05) (Table 1).

Histopathological Evaluation

Histopathological evaluation of kidney tissues revealed fibrosis in the glomerular area, impairment in the tubules, pycnosis in the endothelial cells, and inflammatory cell infiltration and congestion in the DM group. In the RA+DM group; the glomeruli appeared near normal, and the proximal and distal tubules were normal. However, inflammatory cell infiltration continued at a mild score. We tracked that the kidney damage measuremed of the DM group was higher than the RA+DM group (p<0.05) (Figure 1). In liver tissues; Pycnotic cells were observed in the DM group. Inflammatory cell infiltration, dilatation and congestion were observed around the vena centralis, where the radial alignment of the cell cords was disrupted. In the RA+DM group, it was tracked that the radial alignment of the cell cords improved, while the congestion continued. Endothelial cells around the sinusoid were normal (Figure

Table 1. Mean±standard deviation values of AST, ALT, BUN, Creatine, TAS and TOS values

GROUPS	BUN (mg/dl)	Cre (mg/dl)	AST (u/l)	ALT (u/l)	TAS (µmol H2O2 equivalent/L)	TOS (mmol Trolox equivalent/L)
Control	54.42±2.37^{c,d}	0.58±0.02^{c}	168.85±12.81^{c,d}	61.14±9.42^{c}	1.29±0.07^{c,d}	19.86±9.67^{c,d}
RA	56.42±2.82^{c,d}	0.56±0.03^{c}	165.00±7.65^{c,d}	61.42±6.05^{c}	1.36±0.10^{c}	18.28±3.78^{c}
DM	95.28±18.14^{a,b,d}	0.85±0.09^{a,b,d}	524.71±81.16^{a,b,d}	91.57±6.80^{a,b,d}	1.90±0.08^{a,b,d}	82.66±18.27^{a,b,d}
RA+DM	67.85±4.52^{a,b,c}	0.60±0.04^{a,b,c}	225.85±32.57^{a,b,c}	70.00±5.68^{c}	1.48±0.10^{a,c}	46.44±12.26^{a,b,c}

AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, Cre; Creatinine, BUN, TAS; total antioxidant capacity (µmol H2O2 equivalent/L), TOS; total oxidant capacity (mmol Trolox equivalent/L) results are expressed as Mean ±SD. a: There is a difference with the control group, b: There is a difference with the RA group, c: There is a difference with the DM group, d: There is a difference with the RA+DM group

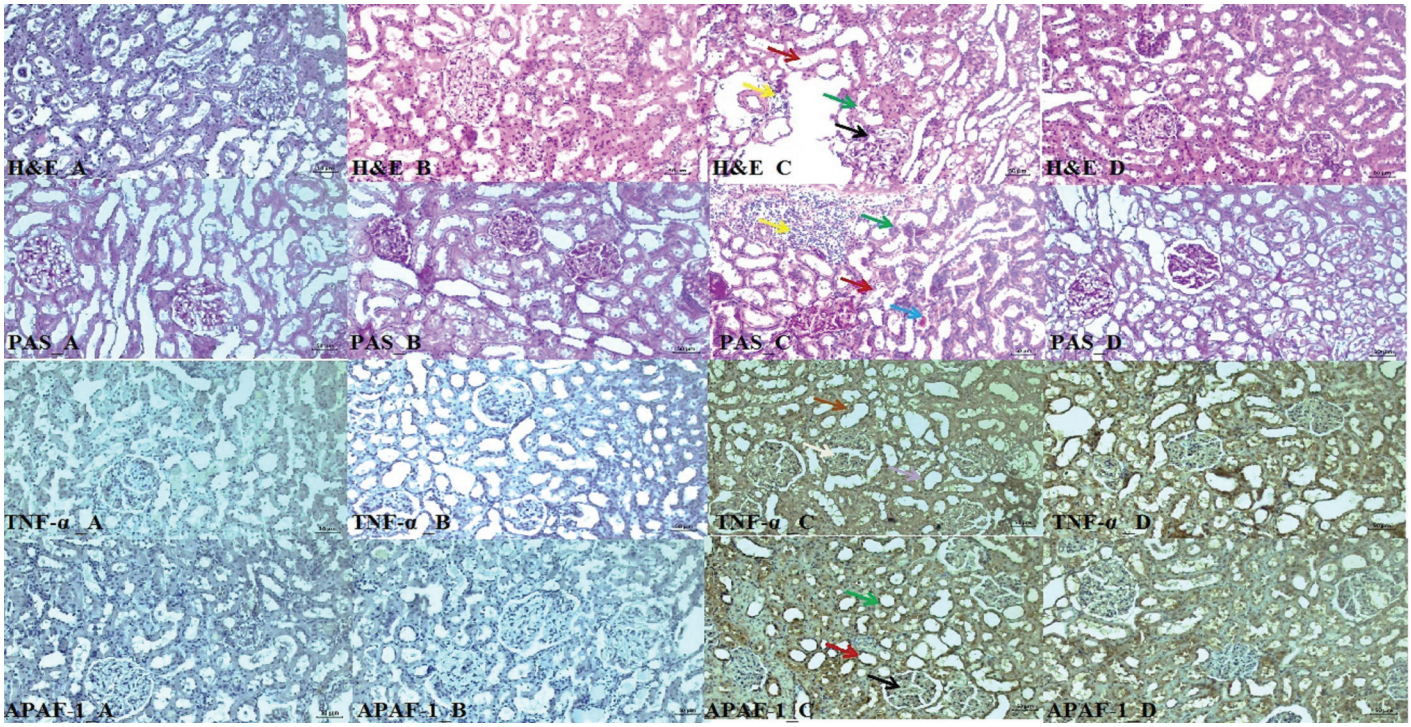

Figure 1. Light microscopic images of kidney tissues. First row: H&E, Second row: PAS, Third row: TNF-α, Fourth row: APAF-1. A: Control Group, B: RA Group, C: DM Group, D: RA+DM Group. Fibrosis in the glomerular area (black arrow), degeneration of the proximal tubules (red arrow), degeneration of the distal tubules (green arrow), inflammatory cell infiltration (yellow arrow), congestion (blue arrow). Intense expression in the glomerular area (white arrow), intense expression in the proximal tubules (Brown arrow), intense expression in the distal tubules (purple arrow)

2). The liver damage grade of the DM group was higher than that of the RA+DM group ($p<0.05$).

Examination of the Periodic acid Schiff (PAS) defilementing of kidney tissues under a light microscope revealed a narrowing in the Bowman's space in the DM group, while this narrowing improved in the RA+DM group (Figure 1). In the liver tissues, there was degeneration and deterioration in the basement membrane structure of the DM group, and the glycogen density decreased. It was observed that basement membrane integrity and glycogen density started to develop in the RA+DM group (Figure 2).

Immunohistochemical Evaluation

Immunohistochemical examinations of kidney tissues revealed that; while intense positive TNF-α expressions were observed in the cytoplasm of glomerular cells in the DM group, similarly intense positive TNF-α expressions were present in the tubules. The expressions were milder in the RA+DM group, than that in the DM group. When TNF-α and APAF-1 histoscoping was carried out, it was monitored that the expressions in the RA+DM group were lower than those in the DM group ($p<0.05$) (Figure 3). In immunohistochemical examinations of liver tissues; intense TNF-α and APAF-1 expressions were observed in hepatocyte nuclei and cytoplasm, while expressions were milder in the RA+DM group compared to the DM group (Figure 3).

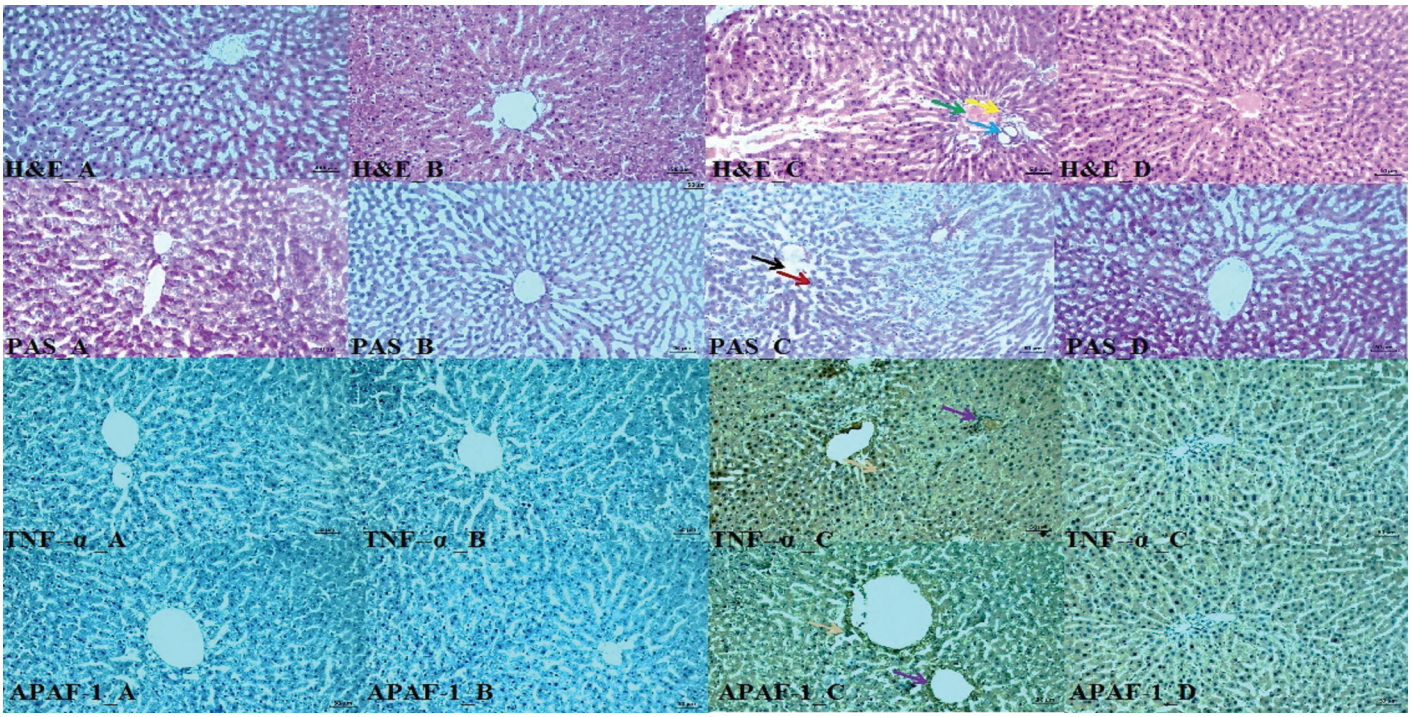

Figure 2. Light microscopic images of liver tissues. First row: H&E, Second row: PAS, Third row: TNF-α, Fourth row: APAF-1. A: Control Group, B: RA Group, C: DM Group, D: RA+DM Group. Congestion (green arrow), dilatation (blue arrow), mononuclear cell infiltration (yellow arrow), disruption of basement membrane integrity and degeneration (black arrow), decrease in glycogen density (red arrow). Intense expression in hepatocyte nuclei and cytoplasm (gray arrow), intense expression in endothelial cells (purple arrow)

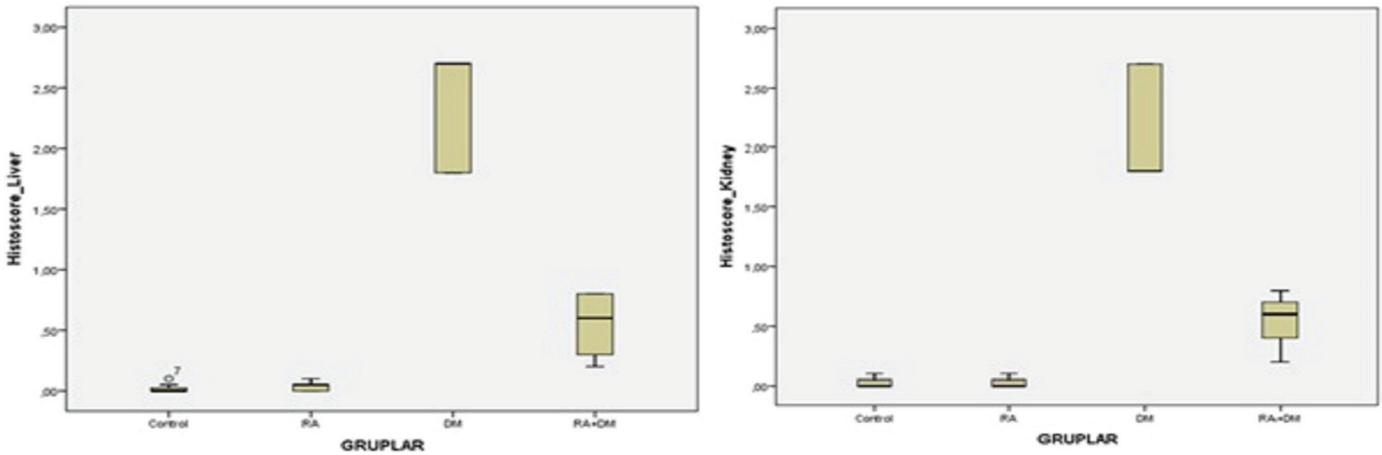

Figure 3. Average analysis graph of liver and kidney tissues with histoscoring

DISCUSSION

Rosmarinic acid (RA) is isolated from herbal balm mint plants, such as Rosmarinus officinalis, Melissa officinalis, and Prunella vulgaris L. (22, 23). It is a naturally occurring hydroxylated compound with anti-inflammatory, anticancer, antimutagenic, antibacterial, and antiviral activity (24, 25). Deltamethrin (DM) is a synthetic insecticide with a wide range of uses. Contaminated water and food are the leading sources of exposure to this pesticide (3). The main organ in which DM is metabolized is the liver, and the kidneys provide the excretion of its metabolites (26). We noted that DM showed toxic effects on either liver or kidney tissues after administering a dose of 35 mg/kg.

In their study, Yousef et al. and El-Demerdash et al observed that DM toxicity increased serum AST and ALT values (27, 28). In our study serum AST and ALT levels were seen to be higher in the DM group compared to the other groups. Domitrovic R et al. revealed that the serum ALT level of the RA group was close to normal (13). In our study, the serum AST and ALT levels of the RA+DM group were lower than the DM group.

In their study Aydin et al. a single acute thiacloprid dose of 112.5 mg/kg (aT) a subacute thiacloprid dose of 22.5 mg/kg (sT), a single acute deltamethrin dose of 15 mg/kg (aD), a subacute deltamethrin dose of 3 mg/kg (sD), or combined dose of these pesticides at the same rates by gavage to rats. They monitored that there was an improvement in serum BUN and creatine values when rats were subjected to DM at a dose of 3mg/kg for 30 days (29). These findings were compatible with our study. Ozturk et al. reported that RA reduced oxidative damage on kidney tissue and normalized serum creatinine and BUN levels of rats with RA compared to other groups in their kidney ischemia and reperfusion (I/R) study (30). The serum BUN and creatinine values in the RA group were close to normal in our study, and they were lower in the RA+DM group than that in the DM group.

Ramalho et al reported that RA protected the liver against I/R damage by showing a strong anti-inflammatory and antioxidant attachment in liver parenchymal cells (31). We observed that RA protects the liver endothelial cells against DM-induced liver damage in rats, reduces the number of pycnotic cells, and the radial arrangement in the cell cords begins to reappear. In addition, in the immunohistochemical examinations, we observed that the intense TNF-α and APAF-1 expressions in the hepatocyte nuclei and cytoplasm and endothelial cells were decreased in the RA+DM group compared to the DM group.

Abdel-Daim et al. found that DM administration increased lipid peroxidation by increasing hepatic and renal malondialdehyde (MDA) levels, and decreased hepatic and renal antioxidants (superoxide dismutase, catalase, GSH) (32). Similarly, we observed that the serum TAS level decreased and the serum TOS level increased in the DM group, and this balance changed in the opposite direction after RA application.

Al-Gerbed et al. observed that glomerular congestion, tubular degeneration, necrosis, swelling of the tubules and vacuolization in various regions along the renal cortex developed in the kidney tissues of rats administered DM (33). In our study, we observed that histopathological changes such as fibrosis in the glomerular area, degeneration in the tubules, inflammatory cell infiltration and congestion occurred in the kidney tissues of rats treated with DM. Our study showed consistent results with other studies in line with these findings.

Abdel- Daim et al. noticed an increase in lipid peroxidation, oxidative stress and proinflammatory cytokin levels (IL-1β, IL-6, TNF-α) in kidney tissues of rats given DM (32). Similarly, we observed intense positive TNF-α and APAF-1 expressions in the kidneys of the rats in DM group, and these expressions were decreased in the RA+DM group.

CONCLUSION

Our study revealed that Rosmarinic acid has positive effects in preventing deltamethrin induced nephrotoxicity and hepatotoxicity in terms of biochemical, histopathological and immunohistochemical examinations.

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

Ethical approval: *The ethics committee approval for this study was obtained from the Dicle University Local Ethics Committee (decision number: 2020/40).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Misophonia and its Relationship with Other Psychiatric Disorders

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Abstract

Aim: Research show that misophonia accompanies many psychiatric disorders and should be considered a mental disorder. Although there are suggested diagnostic criteria, no clear ones have been defined yet. This study aims to investigate the relationship of misophonia with other mental disorders and to determine its possible category in diagnostic classification systems.

Material and Methods: We included the patients who applied to the outpatient clinics of the XX University Faculty of Medicine, Department of Psychiatry for the first time and healthy volunteers without a history of psychiatric disorder. A sociodemographic data form, Misophonia Interview Scale, Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Barratt Impulsivity Scale (BIS), and Yale-Brown Obsession Compulsion Rating Scale (YBOCS) were administered to the participants.

Results: 60.1% of the participants (n=158) did not have misophonia, 21.3% (n=56) had disorder-level misophonia, and 18.6% (n=49) had symptom-level misophonia. Except for the YBOCS-total and obsession/compulsion scale scores of the group with misophonia, all other mean scale scores were significantly higher than those without misophonia ($p<0.05$ for each). The participants with misophonia were mostly in the group diagnosed with anxiety disorders. There was a moderately positive correlation between the Misophonia Symptom List total score and the BAI score in participants with an anxiety disorder ($p=0.001$).

Conclusion: The higher scale scores of individuals with misophonia support that it may be a mental disorder. The results that misophonia most frequently accompanies anxiety disorders and is associated with the severity of anxiety suggest that it can be classified as an anxiety disorder in the diagnostic classification. Recognition of misophonia by clinicians and the development treatment algorithms will increase patients' quality of life.

Keywords: Misophonia, mental disorders, anxiety disorder, misophonia symptom list

INTRODUCTION

Misophonia is a term derived from the Latin words 'misos,' meaning 'dislike,' and 'phonia,' meaning 'sound,' meaning dislike, aversion to sound (1). It is a pronounced discomfort from various sounds, leading to negative feelings such as irritability, overwhelm, and disgust, and significantly affecting the person's occupational functions, social life, and relationships, leading to impaired functioning. The most commonly disturbing sounds are gum chewing, mouth smacking, breathing, and foot rubbing (2).

Although there is no dysfunction in the pathways related to hearing, it is postulated that misophonia occurs due to heightened or strong connections in the limbic and sympathetic nervous systems, which cause abnormal processes triggered by sound (3-5). In misophonia, there is inappropriate and severe stimulation of the limbic and autonomic nervous systems due to the association of a harmless sound with a negative or unpleasant situation (6). Although there is still insufficient data on the prevalence of misophonia, studies suggest that it is not uncommon (7). In a study researching the prevalence of misophonia in the healthy population, approximately 80% of the sample had misophonia symptoms, and 10% of the group with misophonia symptoms were diagnosed with misophonia (8). There is no clear information in the literature about the age of onset of misophonia. In most studies, symptoms of misophonic individuals were reported to start before adolescence (9).

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Misophonia is not included in the current diagnostic classification systems (10). For this reason, several diagnostic criteria have been proposed for diagnosing misophonia, and studies have been conducted accordingly. Schröder et al. (11) proposed diagnostic criteria for misophonia and developed the Amsterdam Misophonia Scale using the Yale-Brown Obsession Compulsion Scale (12). Similarly, Öz et al. (8) and Dozier et al. (7,13) developed diagnostic criteria for misophonia.

Various sources have proposed that misophonia may be comorbid with other psychiatric disorders such as Obsessive Compulsive Disorder (OCD), Major Depressive Disorder, Anxiety Disorders, Obsessive Compulsive Personality Disorder (OCPD) and should be included in the spectrum of Obsessive Compulsive and Related Disorders (8,11,14). In previous studies, Post Traumatic Stress Disorder (PTSD) is one of the most common psychiatric disorders diagnosed in patients with misophonia (15). In addition, Attention Deficit Hyperactivity Disorder (ADHD) (14), tic disorders (16), and eating disorders (17) are among the psychiatric disorders that have been presented to be associated with misophonia.

Studies related to misophonia, which has a history of about twenty years, have become widespread today. These studies will help determine the place and importance of misophonia among mental disorders soon. Our study aims to explore the relationship between misophonia and other mental disorders and to contribute to determining its possible place in diagnostic classification systems.

MATERIAL AND METHOD

Sample

Power analysis of the study was performed with G Power 3.1 program. With a medium effect size (Cohen's d=0.50), a power of 0.95, and a margin of error of 0.05 (p=0.05), the minimum sample size required to be in a single group was calculated as 105, totaling 210 people. We concluded that the sample of 263 people had sufficient power.

The study included 213 literate patients between the ages of 18 and 65 years, who were admitted to Çukurova University Faculty of Medicine, Department of Mental Health and Disorders for the first time between 15.10.2020 and 15.04.2021, and 50 healthy volunteers from hospital staff and their relatives who agreed to participate in the study, had no history of psychiatric disorders and were not receiving treatment. We did not include the patients with an anatomical defect in the external auditory canal as a finding of physical examination, who reported hearing defects, and who used hearing aids. To prevent possible confounding effects of auditory hallucinations, patients with schizophrenia, bipolar disorder, schizoaffective disorder, major depressive disorder with psychotic features, and schizotypal personality disorder were excluded from the study. In addition, we did not include individuals with neurocognitive disorders and mental retardation, as they could not complete the self-report scales.

Procedure and measures

The Non-Interventional Clinical Research Ethics Committee of Çukurova University Faculty of Medicine approved the study on 02.10.2020 (meeting number 104). The study was conducted by the Principles of the Declaration of Helsinki, and all participants signed an informed consent form.

The mental disorders were diagnosed with the Structured Clinical Interview for DSM-5 Disorders-Clinician version (SCID-5/CV), and the diagnosis of misophonia was determined with the Misophonia Interview Scale. We administered to the participants the sociodemographic/clinical data form developed by us, Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Yale-Brown Obsession Compulsion Rating Scale (YBOCS) and Barratt Impulsiveness Scale (BIS). The first author accompanied the participants who had difficulty completing the forms and scales and explained the points where they had difficulty.

Structured Clinical Interview for DSM-5 Disorders - Clinician version (SCID-5/CV): First et al. developed the Structured Clinical Interview for DSM-5 Disorders (SCID-5) (18). The Turkish validity and reliability study of the SCID-5 was conducted. There are ten modules in the SCID-5: 1) psychotic symptoms, 2) disorders with psychosis, 3) mood disorder, 4) substance use disorder, 5) anxiety disorder, 6) OCD and related disorders, 7) PTSD, 8) ADHD 9) questions about screening for other disorders 10) adjustment disorder (18,19).

Sociodemographic/Clinical Data Form: With this form, data such as age, gender, duration of education, marital status, occupation, place of residence, history of physical illness, and family history of mental disorders were questioned. The patient's or relatives' statements and hospital or national health system records were utilized when questioning additional physical illnesses. This form was completed jointly by the participant and the clinician.

Misophonia Interview Scale: Öz et al. developed the misophonia interview scale (8,20). In our study, the diagnostic criteria recommended by Öz et al. were used, and the form of "Sound Disturbance Problems" was added to the misophonia interview scale.

The Sound Disturbance Problems Form was used to differentiate between hyperacusis, misophonia, phonophobia, and tinnitus. This form, which includes explanations and examples, was applied to the participants with sound sensitivity. The participants who answered hyperacusis, phonophobia, or tinnitus were included in the non-misophonia group.

The Misophonia Symptom List (MSL), another step of the misophonia interview scale, was administered to the participants who gave appropriate answers to the Sound Disturbance Problems Form. The MSL is a form that allows 50 different voices to be questioned and a four-point Likert-type (1=none, 2=somewhat, 3=moderately, 4=very

much) indicating the severity of misophonia. The total score for the severity of the misophonia varies between 50-200. With this form, it is determined how severely the participants are disturbed by which sound. In order to make the distinction between "disorder-level misophonia" and "symptom-level misophonia," participants who answered 'moderate or very' on the MSL were asked to fill out a form that included questions about their physical/emotional reactions and functionality.

Participants who responded "moderate or very" to at least one of the emotional/physical responses to sound (fear, disgust, anger, overwhelm/depression, blurred vision, blood pressure, sweating, shortness of breath, dry mouth), in addition to marking "moderate or very" in the question: "How much does your discomfort (or avoidance) with sound affect your life?", answering " yes" to one of these questions: "Are there things you cannot do because of sound?", "Are there any places you cannot enter because of the voices?", "Have these voices caused any deterioration in your relationships?" or answering "more than one hour" to the question: "How much of your day is affected by problems related to this condition?" were defined as having ***"disorder-level misophonia."*** Participants who did not meet this condition at any level (physical/emotional response or functionality questions) and who answered "moderate or very" to at least one sound in the MSL were defined as having ***"symptom-level misophonia."*** (8,20).

Beck Anxiety Inventory (BAI): It is a scale consisting of 21 questions to measure the frequency of anxiety symptoms. BAI consists of four Likert-type questions scored from 0 to 3. The total score ranges from 0 to 63. An increase in the total score indicates an increase in the frequency of anxiety. In the Turkish validity and reliability study, Cronbach's alpha value was 0.93 (21,22).

Beck Depression Inventory (BDI): BDI is a self-report scale comprising 21 questions developed to measure the emotion, cognition, behavior, and somatic components of depression. The scale consists of four Likert-type questions scored from 0 to 3, and the scale's total score varies between 0 and 63. In the Turkish validity and reliability study, Cronbach's alpha value was 0.80 (23,24).

Barratt Impulsiveness Scale (BIS): It is a self-report scale consisting of 30 items designed to assess impulsivity, and each item provides a four-point Likert-type measurement (1=never/rarely, 2=sometimes, 3=often, 4=almost always). BIS includes three components: attentional impulsivity, motor impulsivity, and non-planning. An increase in the scale's total score means a higher level of impulsivity. In the Turkish reliability and validity study, Cronbach's alpha value was 0.81 (25,26).

Yale-Brown Obsession Compulsion Rating Scale (YBOCS): YBOCS is developed to measure the severity of obsessions and compulsions and is evaluated by the interviewer according to the patient's symptoms. Although there are 19 items on the scale, the obsessions and compulsions scores (5 items each) are used to calculate the total score. Each item is scored between 0 and 4 points, and the scale's total score varies between 0 and 40 points. In the Turkish validity and reliability study, Cronbach's alpha value was 0.81 (12,27).

Statistical Analysis

IBM SPSS 25 program was used for data analysis. When the skewness and kurtosis values of the variables were between -1.5 and +1.5, they were considered normally distributed, and histogram graphs were analyzed (28). Whether the variables with normal distribution differed between groups in terms of their means was examined by independent groups t-test and shown as mean and standard deviation (mean±SD). The Mann-Whitney U test was used to examine whether the variables that did not show normal distribution differed between groups regarding their medians and were shown as medians and quartiles. Pearson Correlation analysis was used to examine the correlation between numerical variables since they were normally distributed. In the analysis of categorical variables, the Fisher Exact test was used if the expected number of observations was less than 5, the Yates statistic was used if the expected number of observations was between 5 and 25, and the Chi-square test was used in other possibilities. A value of $p<0.05$ was accepted as significant in the analyses.

RESULTS

According to the Sound Disturbance Problems form, 48.7% of the participants (n=128) stated that they had no sound sensitivity. 39.9% (n=105) stated that they were disturbed by sound in line with misophonia. 5.3% (n=14) stated that they had hyperacusis, 4.6% (n=12) had tinnitus, and 1.5% (n=4) had phonophobia.

According to the Sound Disturbance Problems form, no significant difference was found between the groups with (n=105) and without (n=158) misophonia in terms of mean age, duration of education, gender distribution, marital status, employment status, and place of residence (p=0.78, p=0.17, p=0.14, p=0.67, p=0.15, p=0.22, respectively). 39% (n=41) of the participants reported that their relatives also had misophonia symptoms. The sociodemographic characteristics of the participants are presented in Table 1.

In the group with misophonia, 53.3% (n=56) had an anxiety disorder, 16.2% (n=17) had major depressive disorder, 11.4% (n=12) had OCD, 5.7% (n=6) had ADHD, 1.9% (n=2) were diagnosed with somatic symptom disorder, 1.9% (n=2) with tic disorder, 1% (n=1) with PTSD and 8.6% (n=9) had no diagnosis of mental disorder (healthy individuals). In the group without misophonia, 41.1% (n=65) had an anxiety disorder, 15.2% (n=24) had major depressive disorder, 10.8% (n=17) had OCD, 2.5% (n=4) had ADHD, 1.3% (n=2) had PTSD, 1.3% (n=2) were diagnosed with eating disorders, 1.3% (n=2) with sleep disorders, 0.6% (n=1) with somatic symptom disorder and 25.9% (n=41) had no diagnosis of mental disorder (Table 2).

Compared to the participants' illness duration, excluding healthy individuals, the median duration of illness of the group with misophonia was significantly higher than that of the group without misophonia (p=0.004). Compared to the presence of comorbid physical illness, the rate of physical illness diagnosis was significantly higher in the group with misophonia than in the group without (42.9% & 30.4%, respectively, p=0.04). The frequency of a family history of mental disorder was significantly higher in the group with misophonia than in the group without (51.4% & 31.8%, respectively, p=0.002). There was no significant difference in the rates of suicide attempts between the groups with and without misophonia.

When the diagnosis of comorbid personality disorder (PD) was evaluated, 85.7% of the group with misophonia had no PD diagnosis. 4.8%(n=5) had OCPD, 3.8% (n=4) had borderline PD, 3.8% (n=4) had antisocial PD, 1.9% (n=2) had narcissistic BP. Whereas 94.9% of the group without misophonia had no PD diagnosis, 2.5% (n=4) had antisocial PD, 1.3% (n=2) had borderline PD, 0.6% (n=1) had OCPD, and 0.6% (n=1) had narcissistic PD. Table 2 presents the comparison of the participants according to clinical variables.

When the participants with and without misophonia were compared in terms of BDI, BAI, BIS, and YBOCS scores, all mean scale scores of the group with misophonia were significantly higher than the group without misophonia except for the YBOCS-total and obsession/compulsion scale scores (p<0.05, for each). There was no significant difference between the groups regarding YBOCS-compulsion/obsession and total scores. Scale scores of the groups with and without misophonia are shown in Table 3.

When the diagnostic criteria by the Sound Disturbance Problems Form and MSL were evaluated, 60.1% (n=158) of the participants did not have any misophonia. 21.3% (n=56) of the participants had disorder-level misophonia, and 18.6% (n=49) had symptom-level misophonia.

Among the participants with anxiety disorder, 25.6% (n=31) had disorder-level misophonia, and 20.7% (n=25) had symptom-level misophonia. 9.8% (n=4) of the individuals diagnosed with major depressive disorder had disorder-level misophonia, and 31.7% (n=13) had symptom-level misophonia. 27.6% (n=8) of individuals diagnosed with OCD had disorder-level misophonia, and 13.8% (n=4) had symptom-level misophonia. 16% (n=8) of healthy individuals were diagnosed with disorder-level misophonia, and 2% (n=1) with symptom-level misophonia. Due to insufficient sample size, significant data for ADHD, PTSD, eating disorders, sleep disorders, somatic symptom disorder, and tic disorder could not be obtained. According to the data, disorder, and symptom-level misophonia were mainly accompanied by the diagnosis of anxiety disorder. When examined separately, symptom-level misophonia is mainly seen in participants diagnosed with depressive disorder, while disorder-level misophonia is primarily seen in participants diagnosed with OCD.

According to the Yates statistic, there was a significant difference between the misophonia and diagnosis groups (p<0.001). In individuals not diagnosed with misophonia, the difference was between healthy individuals and individuals diagnosed with anxiety disorder. Also, in individuals with symptom-level misophonia, the difference was between healthy individuals and individuals diagnosed with anxiety disorder and between healthy individuals and individuals diagnosed with depressive disorder (Table 4).

When the correlations between the MSL total score and the BDI, BAI, BIS, and subscale scores were evaluated in participants diagnosed with anxiety disorder, there was a moderate positive relationship between the MSL total score and BAI (p=0.001). In participants diagnosed with depressive disorder, there was no correlation between MSL total score and BDI, BAI, BIS, and subscale scores. In participants diagnosed with OCD, there was no correlation between MSL total score and BDI, BAI, BIS, subscale scores, and YBOCS and subscale scores. Table 5 presents the correlations between MSL total score and other scale scores in participants diagnosed with anxiety disorder, depressive disorder, and OCD.

Table 1. Sociodemographic features of the participants

	Misophonia groups					
	Misophonia (+) (n=105)		Misophonia (-) (n=158)			
	Mean	SD	Mean	SD		
Age, years	35.66	11.48	36.05	11.29	t=-0.28	p=0.78
Education period, years	12.29	4.22	11.56	4.08	t=1.39	p=0.17
	n	%	n	%		
Gender					x^2 =2.23	p=0.14a
Female	72	68.6	94	59.5		
Male	33	31.4	64	40.5		
Marital status					x^2 =0.18	p=0.67a
Single	50	47.6	71	44.9		
Married	55	52.4	87	55.1		
Occupational status					x^2 =2.09	p=0.15a
Unemployed	64	61	82	51.9		
Employed	41	39	76	48.1		
Place of residence					x^2 =1.50	p=0.22a
Urban	84	80	116	73.4		
Rural	21	20	42	26.6		

a. Chi-square test, SD. Standart deviation

Table 2. Clinical characteristics of the participants

	Misophonia groups					
	Misophonia (+) (n=105)		Misophonia (-) (n=158)			
Diagnoses	**n**	**%**	**n**	**%**		
Anxiety Disorders	56	53.3	65	41.1		
Depressive Disorder	17	16.2	24	15.2		
OCD	12	11.4	17	10.8		
Healthy	9	8.6	41	25.9		
ADHD	6	5.7	4	2.5		
Somatic Symptom Disorders	2	1.9	1	0.6		
Tic Disorders	2	1.9	0	0		
PTSD	1	1	2	1.3		
Eating Disorders	0	0	2	1.3		
Sleep Disorders	0	0	2	1.3		
Disorder duration, year (median)	3 (1-8.5)		1 (0-5)		U=6588.5 Z=-2.87	p=0.004
Physical illness						
No	60	57.1	110	69.6		
Yes	45	42.9	48	30.4		
Family history of mental disorders					x^2=10.08	p=0.002a
No	51	48.6	107	68.2		
Yes	54	51.4	50	31.8		
Suicide attempts						
No	101	96.2	152	96.2		
Yes	4	3.8	6	3.8		
Personality disorders						
No	90	85.7	150	94.9		
Borderline PD	4	3.8	2	1.3		
Antisocial PD	4	3.8	4	2.5		
Narcissistic PD	2	1.9	1	0.6		
OCPD	5	4.8	1	0.6		

a. Chi-square test, OCD: Obsessive-compulsive disorder; ADHD: Attention Deficit Hyperactivity Disorder; PTSD: Post Traumatic Stress Disorder; PD: Personality Disorder; OCPD: Obsessive-compulsive Personality Disorder

Table 3. Scale scores of the participants

	Misophonia groups			
	Misophonia (+)	Misophonia (-)		
	mean ±SD	mean ±SD		
BDI	23.02±14.23	17.93±12.65	t=3.04	p=0.003
BAI	25.31±15.58	17.59±13.78	t=4.23	p<0.001
BIS-attentional	17.97±5.03	15.17±4.49	t=4.72	p<0.001
BIS-motor	20.54±5.64	17.22±4.75	t=5.15	p<0.001
BIS-non-planning	26.37±5.96	22.85±7.26	t=4.30	p<0.001
BIS-total	64.89±14.23	55.24±14.82	t=5.25	p<0.001
YBOCS-compulsion	12.75±3.31	11,88±3.37	t=0.69	p=0.50
	Median	**Median**		
YBOCS-obsession	14.5(12-16.75)	14(11.5-15)	U=88 Z=-0.62	p=0.56
YBOCS-total	26.5(22.5-31.5)	26(21.5-28.5)	U=86 Z=-0.71	p=0.50

BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; BIS: Barratt Impulsiveness Scale; YBOCS: Yale-Brown Obsession Compulsion Rating Scale

Table 4. Mental disorder diagnoses of the misophonia groups

		Misophonia			
		Misophonia (-)	Disorder-level misophonia	Symptom-level misophonia	x^2=25.70 p<0.001
		n (%)	n (%)	n (%)	
Diagnoses	Healthy	41(27.9)	8(15.7)	1(2.3)	
	Anxiety Disorders	65(44.2)	31(60.8)	25(58.2)	
	Depressive Disorder	24(16.3)	4(7.8)	13(30.2)	
	Obsessive- compulsive Disorder	17(11.6)	8(15.7)	4(9.3)	

DISCUSSION

Misophonia is a condition of being disturbed by certain sounds that have become increasingly important in the last 20 years. One of the reasons for its becoming increasingly important is that it is not rare in society, contrary to popular belief. For this reason, the number of studies on misophonia is increasing rapidly. Although it was initially considered a physical disorder, recent studies have increased evidence that it may be a mental disorder. Although many diagnostic criteria have been proposed for misophonia, no clear decision has yet been reached on its classification and diagnostic criteria. In our study, most of the participants who had misophonia symptoms were diagnosed with an anxiety disorder, and as the total score of the BAI increased, the total score of the MSL also increased. Anxiety disorders and anxiety severity are related to misophonia, suggesting that misophonia could be classified as an anxiety disorder.

In Schröder et al.'s study (11), 48% of 42 misophonic participants and 73.9% of 69 misophonic participants in Öz et al.'s (20) were women. In Erfanian et al.'s study (17), 57.7% of 52 misophonic participants, whereas in Vitoratou et al.'s study (29), 78.2% of 613 misophonic participants were women. Studies also have revealed that misophonia is more common in women and that the gender distribution is equal (30). Our result that there was no relationship between misophonia and gender may be related to the fact that different disorder groups were evaluated together and the prevalence of mental disorders varies according to gender.

The fact that the current psychiatric disorder duration was longer in individuals with misophonia than in individuals without may indicate that the predisposition to misophonia increases as the psychiatric disorder becomes chronic. There is no enough data in the literature on this issue. In contrast, more than half of the individuals without misophonia stated that they did not have a mental disorder history in their family. More than half of the individuals with misophonia stated that they had a mental disorder history in their family. These data may indicate that those with a family history of mental disorders may be more prone to misophonia and that there is a relationship in terms of genetic predisposition. In the literature, studies suggest that misophonia may also have a genetic origin. These studies have shown that 50-85% of the family members also have misophonia symptoms (9,31-33). In our study, 39% of the individuals with misophonia reported that their families also had misophonia. The presence of misophonia in the family suggests that there may be a genetic origin of misophonia or that this condition can be learned from the family by modeling this condition.

We found that 21.3% of the participants had disorder-level misophonia, and 18.6% had symptom-level misophonia. Jastreboff et al. (34) stated that 3% of the general population might have misophonia. Wu et al. (2) reported that 19.9% of the participants had clinically significant misophonia, and Zhou et al. (35) reported this rate as 6% in their study. This difference may be since the majority of the participants in our study had a psychiatric disorder, and this psychiatric disorder might lead to a predisposition to misophonia. Norris et al. identified two potential subgroups in misophonia: one with a more "pure form" of misophonia, defined by severe misophonia symptoms but with few concurrent conditions, and one with an increasing number of concurrent conditions, which may represent misophonia as an epiphenomenon of increased risk for neuropsychiatric conditions. These data suggest that misophonia has an etiology that is multidimensionally complicated and related to a variety of neuropsychiatric disorders (36).

In our study, the levels of depression, anxiety, and impulsivity were higher in individuals with misophonia than in those without misophonia. Similarly, previous studies revealed that the severity of anxiety, depression, and impulsivity increased as the misophonia score increased (15,37). Our study is consistent with the data in the literature, and the data suggest that misophonia is associated with psychiatric symptoms and should be considered a psychiatric disorder.

We found that individuals diagnosed with anxiety disorders and OCD had a higher prevalence of misophonia than healthy individuals. Individuals diagnosed with anxiety disorders and depressive disorder were diagnosed with more symptom-level misophonia than healthy individuals. In individuals with anxiety disorders, the symptom level and the disorder level-misophonia were higher than in healthy individuals. These results suggest that anxiety disorders and misophonia are more

closely related than other mental disorders and should be considered in diagnostic classification. In a study in which 18 misophonic patients were evaluated, the fact that a diagnosis of anxiety disorder accompanied ten individuals with misophonia supports the relationship between anxiety disorders and misophonia, as seen in our study (38). Especially the fact that anxiety leads to anger reaction in misophonia suggests the prominence of anxiety in misophonia (2,35,39).

We found a significant relationship between MSL total score and anxiety severity in participants diagnosed with anxiety disorder. No significant relationship was between other diagnoses and symptom severity. Studies have shown a correlation between increased anxiety levels, misophonia severity, and emotional response (40), and there has been a strong relationship between anxiety sensitivity and anxiety disorders (41). Increased anxiety sensitivity also increases the severity of misophonia symptoms (42). It can be interpreted that an increased anxiety level causes the person to become more sensitive to the sounds in the environment, and intolerance to sounds increases.

In our study, 2.4% (n=6) of all participants and 4.8% (n=5) of the individuals with misophonia were diagnosed with OCPD. In other words, 83.3% of the individuals with OCPD have been diagnosed with misophonia. In a study by Jager et al. (14) with 575 participants diagnosed with misophonia, 26% had traits of OCPD. In Schröder et al.'s (11) study, 22 of the 42 misophonic participants were diagnosed with OCPD. In a study investigating the relationship between misophonia and personality disorders, three misophonic individuals were also diagnosed with OCPD (43). Jager et al. determined the rate of OCPD diagnosis to be only 2.4% but demonstrated that individuals with misophonia have clinical perfectionism (14). The underlying cause of the discomfort or intolerance to noise may be that the individual with OCPD creates one's truths due to a perfectionist personality. Perfectionism is a personality trait that was found to be associated with misophonia (44). In line with the study by Jager et al. (14), our result of low rates of OCPD diagnosis compared to previous studies suggests that perfectionism in individuals with misophonia should not be evaluated only based on OCPD. According to our results, although misophonia is not associated with the severity of obsessions-compulsions, it is associated with obsessive-compulsive personality traits and impulsivity. Future studies in which more personality dimensions, especially perfectionism, are evaluated are needed to confirm the relationship between misophonia and personality traits.

Our findings support the notion that misophonia is not uncommon in psychiatric outpatient clinics, implying that clinicians should be more aware of misophonia. Previous research has revealed that misophonia reduces a patient's quality of life, and some researchers have emphasized the importance of standardizing misophonia criteria using validated scales and the DSM-5 (45). Assume that misophonia research expands; the information gathered will lead to misophonia classification and the development of diagnostic criteria. Setting diagnostic criteria and identifying comorbid mental disorders may aid in identifying, treating, and improving life quality in people suffering from misophonia.

The strength of our study is that misophonia was investigated in both psychiatric disorders and healthy individuals in a relatively large sample. Our research has some limitations. We used the diagnostic criteria proposed by Oz et al. (8,20) as misophonia diagnostic criteria. Although many diagnostic criteria have been proposed in the literature, the lack of a standardized diagnostic method may have resulted in some participants being misdiagnosed or missed. The absence of a hearing test is the second limitation. Misophonia has been associated with diseases such as hyperacusis and tinnitus (1). In our study, we considered the statements of the participants and relatives, and we directly included the participants with these diagnoses in the group without misophonia. This may have resulted in overlooking participants with hyperacusis or tinnitus who also had misophonia. Although our study's sample was large, the low number of participants with diagnoses such as tic disorder, ADHD, PTSD, and eating disorders may have affected the results. Researchers have suggested that misophonia-related symptoms occur in populations such as autism spectrum disorder (ASD) (46). Therefore, not evaluating ASD and similar neurodevelopmental disorders is the last limitation of our study. Future studies to be conducted with a large sample in which the types of anxiety disorders are evaluated separately and the diagnoses of mental disorders are distributed balanced would clarify the relationship between misophonia and mental disorders.

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Conflict of Interest: *The author declare that they have no competing interest.*

Ethical approval: *The study was approved by the Çukurova University Non-Interventional Clinical Research Ethics Committee (meeting number:104).*

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Research Article

Awareness of Facial Exercises/Facial Yoga for Facial Rejuvenation: A Survey Study

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Abstract

Aim: There is evidence that exercises that work the facial and neck muscles that create facial expressions and facilitate lymph circulation with their movements are effective in facial rejuvenation and keeping facial youthfulness. This study aims to determine the awareness of facial aging and the methods affecting this process, especially facial exercises/facial yoga.

Material and Methods: A 15-question survey was created to question awareness and preferences for facial aging, protecting facial youth and facial rejuvenation methods. The survey was shared online on social media apps. Statistical analyzes were performed.

Results: The majority of volunteers were female (85.1%), between 25-34 years of age (32%), university graduates (44%), with income equal to expenditure (77%), and healthcare workers (26%). Individuals were most disturbed by the changes around the eyes (34%). Most of the participants had heard of facial rejuvenation (82%) and facial exercises (86%) before, but very few (23%) had applied them.

Conclusion: It was determined that individuals were aware that facial exercises were effective in facial rejuvenation, but they did not apply them. Making a habit of facial exercises at a young age and adding them to other non-invasive methods can delay the aging of the face and the transition to some costly invasive procedure.

Keywords: Face aging, facial rejuvenation, face yoga, facial exercise

INTRODUCTION

Socio-economic developments, education, technology and biomedical developments have enabled humanity to successfully adapt to life. As a result, the fact that the population is aging has emerged. Aging is the life stage that follows the maturity period, in which organ changes occur with a decrease in body functions and results in death. The aging of people is a process that differs according to chronological, biological, social and psychological areas (1).

The region that includes the face and neck is a part of the body that determines a person's identity and is used to distinguish one person from others. Structural changes occur in the face with chronological and biological (physiological, photo) aging. Genetic factors, hormones, lifestyle, habits and environmental factors accelerate or decelerate the aging process. Volumetric changes in facial structures, redistribution of subcutaneous adipose tissue, progressive bone resorption and decrease in tissue elasticity cause facial aging. Each face ages differently and gradually according to the duration and amount of these changes (2-5). Through impaired morphological and pathophysiological mechanisms, an increase in age-related fine lines, wrinkles and skin laxity is observed in addition to pigmentary and textural changes. With aging,

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the performance of the mimetic muscles, mainly in the midline of the face, decreases. Recurrent contractions and tone changes of facial muscles can make aging more pronounced. Although chronological aging cannot be prevented, the biological aging process can be delayed with a healthy diet, regular sleep, avoidance of harmful habits, and appropriate physical activities (2, 3, 5, 6).

The desire to preserve beauty and youth which is one of the oldest concepts in human history has emerged facial rejuvenation practices to address the signs of aging on the face (7). Procedures such as moisturizing, chemical peeling, abobotulinumtoxinA [BTX], injected fillers, thread facelift, and eyelid lifting are applied to eliminate the signs of photo and chronological aging on the face. Today, applications to protect youth such as facial acupuncture, facial acupressure, and facial exercises are also becoming popular (6, 7-11).

This survey study was performed to investigate the awareness of facial exercise/facial yoga by determining the methods that individuals apply both in delaying facial aging and in facial rejuvenation.

MATERIAL AND METHOD

Procedures

This study was approved by the non-interventional clinical research ethics committee (approval number 3359/2021). An electronic questionnaire was created about the demographic information of individuals, the awareness and preference of facial youth protection and rejuvenation methods. The questionnaire was first applied to a limited number of participants, and after this application, existing questions were edited and new questions were added. The new questionnaire was shared online through various applications of social media from July 2021 to August 2021.

The first part of the questionnaire was aimed at determining the socio-demographic characteristics of the participants (gender, age, education level, income level, occupation). Age was defined in six groups (15-24, 25-34, 35-44, 45-54, 55-64, 65 and over). Education levels were evaluated in four groups (primary education, high school, university and graduate graduation). Income was questioned at three levels: low (income less than expense), middle (income equal to expense) and high (income higher than expense). Five options (student, housewife, retired, health, education, other) were presented to determine the occupation.

The second part of the questionnaire consisted of 15 questions to determine the awareness of the participants about the age-related changes in the face and neck region and the applications to protect or rejuvenate them.

Statistical Analysis

Number, percentage, mean and standard deviations were used in the evaluation of descriptive statistics. The conformity of the data to the normal distribution was checked with the Shapiro–Wilk and Kolmogorov–Smirnov tests. Categorical data were analyzed with the Chi-Square test. Quantitative data were analyzed using an independent t-test for paired groups, and one-way ANOVA (post-hoc Tukey test), Mann-Whitney U and Kruskal-Wallis for three or more groups.

RESULTS

571 (15% male, 85% female) people who viewed the questionnaire electronically answered the questions. The sociodemographic characteristics of the participants are shown in Table 1. The majority of the participants are 25-34 years old (32%), university graduates (44%), income equal to the expense (77%) and health workers (26%).

Table 1. Socio-demographical characteristics of the participants

	n	%
Age		
15-24	120	21
25-34	184	32
35-44	128	22
45-54	107	19
55-64	29	5
65 age and over	3	1
Education level		
Elementary school	34	6
High school	46	13
University	249	44
Postgraduate	212	37
Income level		
Lower	58	10
Middle	438	77
Upper	73	13
Occupation		
Student	115	20
Housewife	73	13
Educator	99	17
Health worker	151	26
Other	107	19
Retired	26	5

Participants (n: 568) gave themselves an average score of 6.04 ± 2.06 out of 10 when they looked at the face and neck region in a photograph taken without a filter. They were dissatisfied with the appearance of the eye area (34%), cheek area (27%), nose area (20%), neck (17%), forehead (17%) and lip area (16%). The participants listed the causes of wrinkles and sagging on the face and neck as decreased or loss of skin elasticity (63%), weakening or tension of the muscles (56%), and excessive contraction of the muscles (20%). The rate of those who are aware of facial rejuvenation was 82%. The rate of those who stated

that they would allocate a budget from their income to feel better and have healthy skin when they looked in the mirror was 77% (n:438). The number of women (n:385, 88%) who stated that they would allocate a budget was statistically significantly higher than men (n:53, 12%) (p<0.001). While the preference for "budgeting" did not differ statistically according to income (p=0.169), statistically significant differences were found according to education and occupation (p<0.001) (Figure 1).

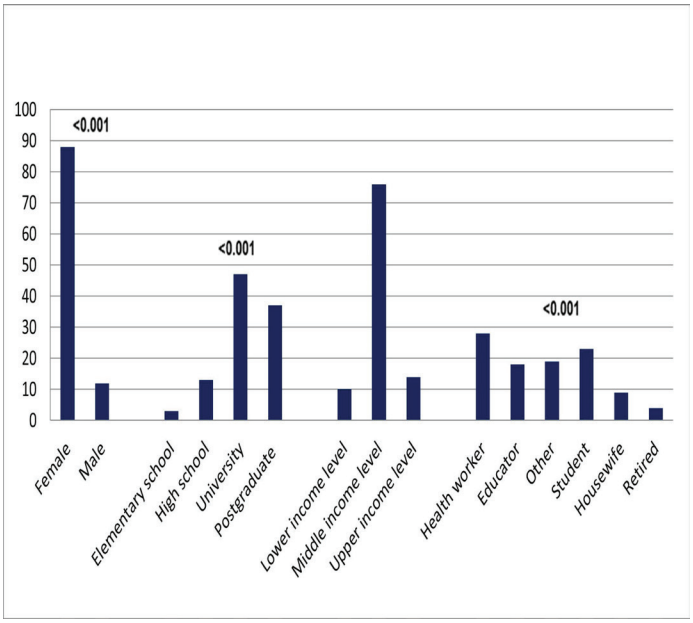

Figure 1. "When you look in the mirror, do you budget your income to feel better and have healthy skin?" Sociodemographic characteristics of the participants who answered "yes" to the question

It was questioned to keep the youth of the face and neck which methods (regular sun protection, balanced diet, daily water consumption of two liters or more, regular sleep, medical and/or cosmetic applications, and exercises) the participants used. The most effective socio-demographic factor in the preference of the applied methods is occupation (p<0.001, p<0.05) (Table 2). The application of all these methods is 18-32% in the 15-54 age group, and 5-7% in the 55 and over age group. Employees and students used these methods 24-70%, and housewives and retirees 18-58%.

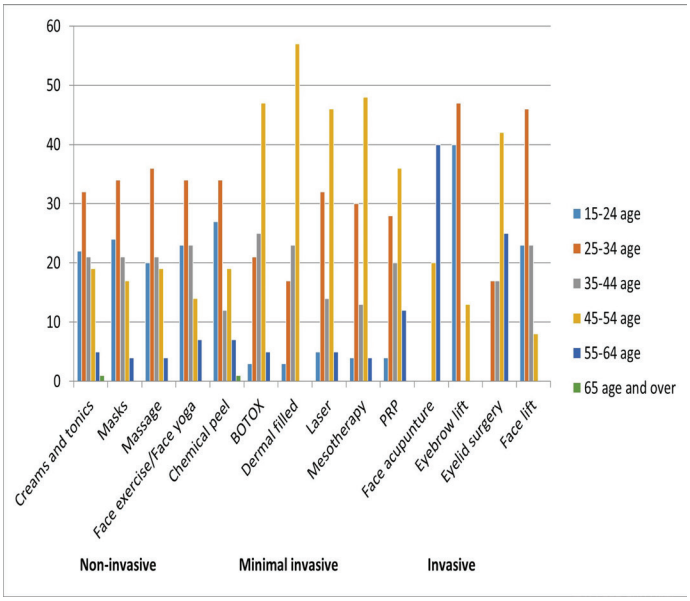

Figure 2. Preference rates of methods by age group

Table 2. The effect of socio-demographic characteristics on the selection of face and neck youth protection method

	Sex x^2	Age x^2	Education x^2	Income x^2	Occupation x^2
Regular sun protection	12.407‡	7.034	4.607	7.532†	20.010†
Balanced diet	1.963	18.510†	19.642‡	2.449	43.830‡
Daily water consumption	3.418	9.394	15.165†	1.188	15.827†
Regular sleep	3.790	8.949	8.737	5.081	22.251†
Medical /Cosmetic applications	31.691‡	5.648	18.123‡	3.289	21.950‡
Exercises	0.538	6.088	1.996	0.964	12.132†

x^2, Chi-Square test; †, p<0.05; ‡, p<0.001

All of the participants (n: 571) answered the question about the procedures to eliminate or reduce wrinkles, loss of tension and sagging on the face and neck. The awareness rates of the listed methods were 69-81% for the cream-tonic, mask, facelift, facial exercises/facial yoga, 59-64% for dermal fillers, massage, BTX, brow lift, eyelid surgery, and 37-50% for chemical peels, facial acupuncture, laser therapy, mesotherapy, PRP. The ratio of answers given to the question of which structures (skin and/or muscle) the mentioned methods affect is shown in Table 3. Most of the participants (79%) commented on the structures affected by facial exercises/facial yoga. 67% of these individuals stated that the skin and muscles were affected together (Table 3).

The distribution of method preferences according to the socio-demographic characteristics of the participants is shown in graphics (Figure 3-5). The comparison of the preference rates of the listed methods according to the socio-demographic characteristics is presented in Table 4. It was observed that all socio-demographic characteristics affected the preference of BTX, and the preference of dermal filler and mesotherapy was statistically affected by factors other than gender (p<0.05, p<0.001). Gender is the most potent factor in the preference for non-invasive methods, income and occupation in the preference for minimally invasive procedures, and age in the preference for invasive methods (p<0.05, p<0.001)(Table 4).

Table 3. Distribution of participant responses regarding which tissues are affected in face and neck rejuvenation methods

Applications	Skin %	Muscle %	Skin and Muscle %
Creams and tonics	92	1	9
Masks	96	1	4
Chemical peel	82	3	16
BTX	12	41	51
Dermal filled	36	18	50
Face acupunture	19	28	58
Face lift	43	15	46
Eyebrow lift	42	17	46
Face exercise/ Face yoga	12	30	67
Eyelid surgery	47	14	45
Laser	61	9	34
Mesotherapy	51	12	41
PRP	41	16	49
Massage	22	27	63

Table 4. The effect of socio-demographic characteristics on the selection of face and neck rejuvenation methods

		Sex x^2	Age x^2	Education x^2	Income x^2	Job x^2
Non-invasive	Creams and tonics	54.93‡	6.02	17.55‡	1.54	23.92‡
	Masks	16.93‡	22.08†	3.94	0.70	12.83†
	Massage	0.67	2.65	2.74	5.87	7.45
	Face exercise/Face yoga	8.42†	4.67	5.37	5.25	14.43
Minimal invasive	Chemical peel	2.13	8.66	2.28	0.83	5.39
	BOTOX	10.61†	55.95‡	36.81‡	13.56†	52.65‡
	Dermal filled	3.34	36.36‡	21.53‡	18.020‡	25.64‡
	Laser	1.93	12.60	7.39	16.27‡	11.44†
	Mesotherapy	2.10	19.20†	11.77†	16.17‡	20.21†
	PRP	2.45	10.59	9.54	7.44	18.31†
	Face acupunture	0.10	16.05	11.14	10.15†	3.89
Invasive	Eyebrow lift	0.32	11.55	2.51	2.20	2.40
	Eyelid surgery	0.42	16.75†	4.11	1.78	3.95
	Face lift	2.65	8.13	6.464	0.14	7.51

x^2, Chi-Square test; †, $p<0.05$; ‡, $p<0.001$

Most of the participants (86%) were aware of facial exercises/facial yoga for facial rejuvenation. Facial exercises/facial yoga were frequently heard on social media (71%), followed by television shows (31%), friends (25%), educational processes (8%), dermatologists (7%), and family physicians (1%). "Are there any other uses of facial exercises/facial yoga other than facial rejuvenation?" to the question, these participants answered "yes" (33%), "no" (2%), and "don't know" (75%).

When asked directly "Do you do facial exercises/facial yoga?", 35% of the participants stated that they applied it. The rate was 32% when questioned within the scope of protection practices, and 23% when questioned about the processes for the elimination of changes. While gender was effective (p, 0.004) in the preference for facial exercises/face yoga practices, age, education, income and occupation were not effective ($p>0.05$). Only 8% of individuals were doing these exercises regularly every day, and 63% did it whenever they thought of it, 19% when they felt bad, 10% when they felt good. The effect of facial exercises/face yoga on facial rejuvenation was scored as 7±2.06 out of 10 for all participants. The views of those who did and did not do facial exercise/face yoga on the effect of these practices on facial rejuvenation were close to each other (7.11±1.74, 6.23±2.14, respectively).

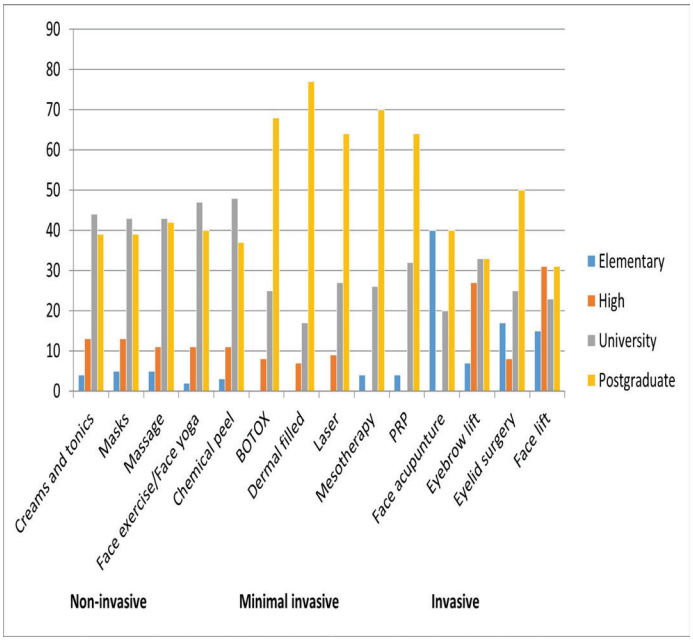

Figure 3. Preference rates of methods by education level

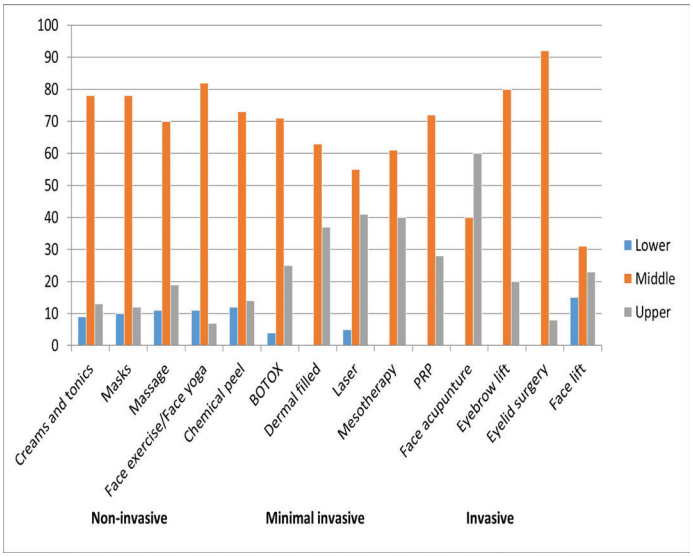

Figure 4. Preference rates of methods by income

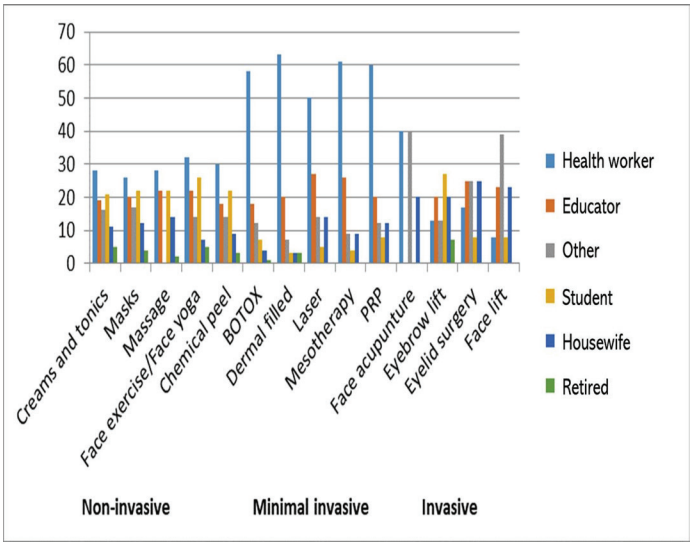

Figure 5. Preference rates of methods by occupation

DISCUSSION

The World Health Organization classifies adults as "non-elderly" (under 65 years old) and "elderly" (over 65 years old) (12). According to this classification, only 3 of the 571 people who agreed to participate in our study are elderly. The electronic questionnaire is a factor that negatively affects the participation of individuals over the age of 65. In addition to the difficulty of using technology in this population, age-related hearing, vision and movement losses and dependency on care are also in question.

The change in appearance that occurs with age on the human face is usually consistent and reveals the age of the person. Gradual changes reflecting the natural aging processes in the skin appear from about the third decade of life and become very evident over the age of 60. Facial soft tissues are organized into four concentrated tissue layers (1st skin; 2nd subcutaneous fibroadipose layer; 3rd superficial musculo-aponeurotic system (SMAS); 4th parotid-masseteric fascia). Biological aging of the face is the result of the complex interaction of these layers and structural changes in bone and displacement caused by the effect of gravity. Age-related changes vary according to functional regions and processes, and there is no uniformity. These changes are more prominent in the anterior part of the face than in the lateral part due to the structural and functional properties of the tissues (13). It has also been proven by MRI studies that dramatic changes in the soft tissue of the face occur between the ages of 30 and 60 in the temporal, infraorbital, lateral cheek and medial cheek regions (2, 14). In our study, which included individuals aged 15 to 65 years, 99% of the participants were young individuals (under 65 years of age). Participants were average (6.04±2.06/10) satisfied with their facial and neck appearance. They were not satisfied with the appearance of the eye area, cheek area, nose area, neck, forehead and lip area, respectively. This ranking is concordant with the structural features of the face, as well as the opinion of most people around the world that the eye area is the most important component of facial beauty (15).

Theories of facial aging largely focus on changes in the skin and subcutaneous fat and bone, while the role of muscles in aging has often been neglected (16). Facial aging occur at all soft tissue levels. Loss of elasticity in the skin is accompanied by a decrease in dermal thickness and vascularization. Fat redistribution contributes to the physical properties of loose and sagging skin (17). The superficial muscles in the face's soft tissue are located in the third layer (SMAS). SMAS, associated with the skin through connections in the subcutaneous layer, is muscular only where movement occurs (13). Wrinkles, which are signs of aging on the skin, are defined in two main types' dynamic (mechanical wrinkling of the skin by uncontrolled contraction of the underlying skeletal muscles) and static (wrinkles that occur secondary to volumetric tissue loss) (18, 19). In dynamic skin wrinkling, it appears as expression lines, the shape and depth of

the wrinkle are related to the repetitive contraction of the muscles, and the wrinkle disappears when the muscles relax. It is a static or permanent skin wrinkle that occurs in the early 30s regardless of muscle relaxation and increases in severity with aging. The transition from dynamic to static skin wrinkles may result from frequent muscle contractions and permanent wrinkles may progress with the aging process (18, 19). In this study, 63% of the participants thought that the signs of aging on the face and neck were caused by the skin, and 76% (weakness, 56%; overtraining, 20%) were of the opinion that the muscles were caused.

There are internal and external factors that affect the aging of the face. Inevitable intrinsic factors are heredity and various cellular and molecular processes programmed into the individual. External factors that also affect internal aging are individual habits, diet and environmental factors (4). In this study, it was determined that daily water consumption was frequently (63%) paid attention to, but practices that were directly related to income (regular sun protection, balanced diet, and medical and/or cosmetics) were less preferred (49%, 41%, 46% respectively). Among the habits aimed at delaying facial aging, the least preferred exercises were (32%). Although the aging process of female and male faces is similar with some exceptions, female participants preferred all applications at higher rates (female, 83-94%; male, 6-17%). Those who were active in business life and students had these habits more than housewives and retirees. The reason for the decrease in these habits, especially in participants aged 55 and over, may be economic inadequacy, increased health problems, difficulty in using technology and reduced social communication. These preferences were affected by occupation, education, gender, age and income level, respectively.

There are many non-invasive, minimally invasive and invasive approaches based on maintaining the natural and harmonious balance of all components of the facial structure for optimal facial rejuvenation (4, 6, 8-11, 15, 20). Our study population was highly aware of facial rejuvenation expression (82%) and was willing to budget their income (77%) to have healthy youthful skin. Regardless of income, this preference of females, university graduates and health workers was statistically significant ($p<0.001$).

Skin cleansing, moisturizing, facial massage, facial exercises and facial yoga are non-invasive facial rejuvenation methods (8). Skin resurfacing peels, injected neurotoxins, dermal fillers, lasers, facelifts, facial acupuncture are frequently used minimally invasive aesthetic procedures. These methods are treatments with minimal downtime, unlike surgery for fine lines, sun damage, uneven pigmentation or tissue problems on the face (8, 20). Invasive methods can be listed as brow lift, eyelid surgery, face tightening, and rhinoplasty (15). According to our study results, the awareness of the practices was as follows; 69-81% from cream-tonic, mask, facelift, facial exercises/facial yoga, 59-64% from dermal fillers, massage, BTX, brow lift, eyelid surgery, and 37-50% from chemical peels, facial acupuncture, laser therapy, mesotherapy, PRP application. Females highly preferred all methods. Creams and tonics, masks and facial exercise/facial yoga and BTX were chosen statistically significantly higher by women ($p<0.05$). Significant differences were found in the preference of creams and tonics, masks, BTX, dermal filler, mesotherapy, PRP and facial acupuncture according to education ($p<0.05$). It is reported in the literature that people with low education mostly prefer invasive procedures (21), while people with high education prefer minimally invasive procedures (22). In our population, minimally invasive procedures were found to be preferred at higher education. There was no significant difference in the preference for eyebrow lift and eyelid surgery, which are invasive methods. From the answers given to the facelift application, which is one of the invasive methods, it was concluded that the problem was not fully understood.

Mimic muscles are the component of facial SMAS. This system is a single tissue plane that is not directly connected to the bone, consisting of muscle fibers and connective tissue depending on the region. It is continuous with some mimetic muscles (including the periorbital fibers of the zygomaticus major, frontalis, and orbicularis oculi) anteromedially of the face and is indistinct on the lateral side of the face about 1 cm below the level of the zygomatic arch. Just lateral to the buccal angle on both sides of the face is the modiolus, a dense, compact, mobile, fibromuscular mass formed by the fusion of at least nine muscles. Major modiolar movements involve most, if not all, of the associated muscles (13). The origins of mimetic muscles are usually found in bone, except for sphincteric muscles, and, unlike skeletal muscles, they insert into the skin and between fibers of other muscles without any tendons (16). Although these muscles are independent in terms of innervation and function, they form a continuous layer thanks to their connections with facial muscle and ligament components. Both the continuity of the SMAS and the modiolar movements may suggest that it is of little value to consider the movements of individual muscles separately. Biting, chewing, drinking, sucking, swallowing, speaking, shouting, screaming, modulation of musical tones, crying, and the control of modioles in all permutations of facial expression integrate the activities of the cheeks, lips, oral opening and jaw (13).

In clinical practice guidelines for the face, especially eyebrow-raising and lowering muscles are included (16). However, it has been reported that functional muscles contribute to the appearance of the entire face, not just the eyebrows, and shape the jawline in particular (2, 8). Alam et al. (2018) found that individuals who participated in the facial exercise program they applied looked approximately 2.5 years younger than the baseline. It is known that long-term massage application improves the blood flow to the skin with vasodilation in the veins, thus increasing collagen production and providing a taut and bright appearance on

the skin (23). It is becoming more and more important for young individuals to have a symmetrical face and to reflect their emotions with a correct facial expression, and to raise awareness that the lost facial balance can be regained for older individuals (5). With this study, the population that is aware of the age-related changes in the human face and neck and is interested in delaying these changes and/or facial rejuvenation applications was reached, and the awareness of this population about facial exercises was investigated among other applications. Although 86% of the participants had heard of facial exercises/facial yoga for facial rejuvenation before, only 23-35% stated that they applied it. When the preference for facial exercises and face yoga was questioned by listing it among other methods, a statistically significant effect of gender (p, 0.004) was detected, while when questioned with a separate question, no significant effect of socio-demographic characteristics was found. Especially between the ages of 25-34, university graduates and middle-income females were using these methods. The rate of regular practice was quite low (8%). 71% of the participants had learned about these practices from social media. The effect of facial exercises/face yoga on facial rejuvenation was scored as 7±2.06 out of 10.

Most of the participants (75%) were unaware of the benefits of facial exercise and facial yoga other than facial rejuvenation. A young, beautiful and attractive appearance has a positive effect not only on the self-confidence of individuals but also on social behaviors and communication (3-5). In addition, these exercises help vocalists control their voices and make music at the pitch they want, and instrumentalists make a healthier and more appropriate sound from the instrument (24). Smile aesthetics and/or smile design are popular dental procedures today. In determining the smiling behavior, perfect upper incisors, young and pleasantly curved lips and lip commissures, as well as the contractility of the perioral muscles and their morphological characteristics are very important (25). Therefore, although evidence is needed, facial exercises can complement smile design procedures.

Limitations

Since this survey study was planned during the active period of the Covid-19 pandemic, it could not be applied face-to-face. Despite the pre-test, some of the applications were not fully understood by the participants. The elderly population could not be reached in sufficient numbers.

CONCLUSION

The Covid-19 pandemic, which has affected the whole world, has also shown that it is very valuable to gain habits independent of income and others in order to protect the health of the body and face. Unlike cost and time-consuming cosmetic procedures to preserve and restore facial youthfulness, facial exercises are free and almost certainly not harmful. However, there is not enough evidence and randomized controlled studies about the benefit of facial exercises for facial rejuvenation. According to the results of this study, all people, especially males (who are exposed to facial aging as much as females), individuals out of active working life, and individuals with low education, should be informed by health professionals about face exercises/face yoga. Facial exercises, which reduce the need for invasive methods and decelerate facial aging when applied correctly and regularly together with other non-invasive methods, should be applied in the presence of trained and experienced trainers and should be made a habit early.

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MEDICAL RECORDS-International Medical Journal

Case Report

Distal Renal Tubular Acidosis can be the Cause of Hypokalemia in Graves' Disease: A Rare Association

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Abstract

Distal renal tubular acidosis (dRTA) may rarely occur in the course of autoimmune diseases. We present a patient who was followed up with Graves' disease and vitiligo and who was diagnosed with dRTA upon detection of hypopotasemia. A 9.2-year-old girl presented with complaints of sweating, palpitations, and hand tremors. The patient had vitiligo on examination and was diagnosed with Graves' disease per clinical and laboratory findings. The patient, who received methimazole and was followed up as a euthyroid, was found to have hypokalemia in biochemical examinations performed at the age of 13 years. While investigating the etiology of hypokalemia, the patient was diagnosed with dRTA. Since she had two autoimmune pathologies, it was thought that the dRTA might be of autoimmune origin. Checking serum potassium levels in the follow-up of patients with Graves' disease may allow early diagnosis and treatment of accompanying dRTA.

Keywords: Distal renal tubular acidosis, Graves' disease, vitiligo, autoimmune disorder

INTRODUCTION

Distal renal tubular acidosis (dRTA) is a rare disease caused by the impaired acid-secretory function of the distal kidney tubules. Since the hydrogen (H+) ion cannot be secreted, metabolic acidosis develops, and urinary pH gets inappropriately alkaline (pH>5.5) (1). In most pediatric cases, dRTA is primarily caused by genetic defects (ATP6V1B1, ATP6V0A4, SLC4A1, KCC4) in the channels or enzymes involved in H+ secretion in the distal renal tubules (2). The autosomal recessive form is often diagnosed in infancy and has a more severe clinical course. On the other hand, the diagnosis of the autosomal dominant disease is usually made at a later stage (1,3). Hypopotassemia, hyperchloremia, metabolic acidosis, normal plasma anion deficit, positive urinary anion deficit, hypercalciuria, and nephrocalcinosis are important and diagnostic findings suggesting distal RTA (1-3). Secondary dRTA often occurs after damage to the distal or collecting ducts due to drugs, renal diseases, calcium disorders, hypergammaglobulinemia, or autoimmune diseases (systemic lupus erythematosus, primary biliary cirrhosis, Sjogren's syndrome, autoimmune thyroiditis, rheumatoid arthritis, etc.) (2-4). DRTA in autoimmune thyroiditis (Graves' disease, Hashimoto's thyroiditis) has been reported in a small number of cases to date (5,6).

This article presents a case detected to have Graves' disease and vitiligo in the prepubertal period and diagnosed during puberty with dRTA as due to incidental hypopotassemia at follow-up.

CASE REPORT

A 9-years and 2-months-old girl presented with sweating, palpitations, and hand tremors. She reported an inability to gain weight and a state of nervousness despite the increase in appetite. On physical examination, her body weight was 22 kg (-1.83 SDS) and her height was 122 cm (-1.94 SDS). She was prepubertal and had stage 1 diffuse goiter, moderate exophthalmos, hyperthermia

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(body temperature 38.0 °C), hypertension (135/85 mmHg), and tachycardia (pulse 118/min). Vitiligo was present on the trunk, arms, legs, dorsal hand, and genital area. Her complete blood count, biochemistry profile, and complete urine tests were normal and the laboratory findings were as follows: free serum thyroxine (fT4): 3.2 ng/mL (N: 0.93-1.7), free triiodothyronine (fT3): 12.04 pg/mL (N: 1.86-4.6), and thyroid-stimulating hormone (TSH): 0.005 mIU/L (N: 0.35-4.94 mIU/L). In thyroid ultrasonography, the right thyroid lobe was 10x10x29 mm, the left lobe was 10x10x28 mm, and AP isthmus thickness was 1.7 mm. The contours of both lobes and isthmus were smooth, and the parenchyma echoes were heterogeneous. Thyroid peroxidase antibody (anti-TPO) requested to study autoimmune thyroid diseases was positive, and Thyroglobulin antibody (Anti-TG) was negative. Her thyroid-stimulating hormone receptor antibodies (TRAB) were measured as 2.14 IU/L (0-1.75). She was diagnosed with Graves' disease, and oral treatment with Methimazole 0.3 mg/kg/day was started. Propranolol 1mg/kg/day in 2 doses was initiated for her tachycardia. The tachycardia improved, and propranolol was discontinued at follow-up. The patient stopped Methimazole treatment after 6 months and applied to us 6 months after discontinuing methimazole. At this admission, she was euthyroid, but TRAB was positive.

The patient was followed up for 21 months without medication as euthyroid. When she was 11.5 years old, subclinical hyperthyroidism emerged, and the TRAB positivity continued. Hence, 5mg/day methimazole was started again. During the follow-up, 25 mcg/day L-thyroxine was added to the treatment because the patient developed hypothyroidism with low-dose antithyroid therapy. Upon progression of exophthalmos to moderate-severe levels (eyelid retraction and prominent exophthalmos) with the onset of puberty in the follow-up, the patient received 250 mg/dose methylprednisolone (MPZ) IV for 6 weeks, then 125 mg/dose weekly IV MPZ for another 6 weeks. After this treatment, a slight regression was observed in her exophthalmos.

The patient was euthyroid when she came to control at the age of 13, under Methimazole and Levothyroxine treatments. Incidentally, hypopotassemia (K: 2.9 mEq/L) was detected in the biochemical analysis without active complaints. As the control biochemistry analysis revealed hypopotassemia three days later (K: 2.5 mEq/L), the etiology was investigated. Her serum biochemistry analyses returned the following results: glucose 82 mg/dl, urea 13.6 mg/dl, creatinine 0.67 mg/dl, calcium 9.5 mg/dl, phosphorus 4.2 mg/dl, sodium 139 mEq/L (136-145), potassium 2.5 mEq/L (3.4 -4.7), chlorine 116 mEq/L (98-107). While urine pH was 7 and density was 1001, potassium excretion in spot urine was 34 mEq/L. Blood gas analysis revealed the following: pH: 7.23, PCO2: 38.8, HCO3: 15.7, and BE: - 9.6. The 24-hour urinary calcium level was 5.36 mg/kg/day (>4mg/kg/day), which was high. The plasma anion gap was normal at 10.3 mmol/l. The urine anion gap was 30 mmol/l. Bilateral grade 2 medullary nephrocalcinosis was observed in the abdominal ultrasonography. Plasma renin activity and plasma aldosterone levels were normal. As a result, the patient was diagnosed with hypokalemic hyperchloremic metabolic acidosis and nephrocalcinosis with dRTA. The hearing test was normal, and celiac antibodies tested for additional autoimmune pathologies were negative. There were no clinical signs of systemic lupus erythematosus in the patient. However, she had positive antinuclear antibody and anti-dsDNA results. The patient started oral potassium citrate 1 mEq/kg/day and sodium bicarbonate 1 mEq/kg/day orally. In the follow-up, the patient's serum electrolyte and blood gas values returned to the normal range within 2 weeks. The patient is still being followed up with Methimazole, Levothyroxine, potassium citrate, and sodium bicarbonate treatments.

DISCUSSION

The etiology of hypopotassemia detected during the follow-up in an adolescent patient who was followed up with Graves' disease and vitiligo was investigated, resulting in the diagnosis of dRTA. One of the causes of hypokalemia in hyperthyroid patients is thyrotoxic hypokalemic periodic paralysis (THPP), a rare complication of hyperthyroidism. THPP is characterized by reversible muscle weakness and paralysis attacks due to the intracellular blockade of K+ by excessive thyroid hormones. The situation often disappears with the recovery of hyperthyroidism (7). In our case, unlike THPP, when hypokalemia was detected, there were no clinical and laboratory findings of hyperthyroidism, and there was no acute muscle weakness or paralysis. Therefore, THPP was not considered in this patient. In addition to hypokalemia, a diagnosis of dRTA was made with the findings of hyperchloremia, metabolic acidosis, normal plasma anion deficit, positive urinary anion deficit, alkaline urine, hypercalciuria, and nephrocalcinosis.

The association of hyperthyroidism and RTA was first described in 1959. After the diagnosis of hyperthyroidism in a 40-year-old female patient, hypercalcemia, nephrocalcinosis, and RTA were detected in the follow-up. Since the x-ray evidence shows that nephrocalcinosis develops in the hyperthyroid process, it has been suggested that the distal RTA in the patient arose due to the hypercalciuria and tubular damage secondary to nephrolithiasis caused by hyperthyroidism (8). It should be kept in mind that nephrocalcinosis due to hypercalciuria can also be seen in the course of primary dRTA. Wu et al. detected hyperthyroidism due to hypokalemia, RTA, and autoimmune lymphocytic thyroiditis in a 34-year-old patient who presented with proximal muscle weakness. Since hypercalcemia and nephrocalcinosis were not found in the patient, hyperthyroidism was improved with antithyroid and radioactive iodine treatment but dRTA continued, they proposed that RTA was not caused by metabolic mechanisms but by immunological mechanisms (9). A 20-year-old female patient was reported from

Korea, followed up with Graves' disease for three months. She was diagnosed with hypokalemic periodic paralysis and dRTA in the period of recurrent hyperthyroidism after discontinuing the treatment (5).

Furthermore, Guerra-Hernandez et al. reported dRTA in two children with acquired autoimmune hypothyroidism and suggested that dRTA may have developed due to hypothyroidism or autoantibodies (6). In our adolescent girl who was followed up with a diagnosis of Graves' disease and vitiligo, dRTA was detected while she was euthyroid. It was thought that the dRTA in our patient could be of autoimmune origin since she did not currently have hyperthyroidism but had autoimmune diseases. However, a histopathological evaluation was not performed.

The pathophysiological explanation of dRTA associated with autoimmunity is not fully resolved. The mechanism in dRTA seen with Graves' disease has not been fully explained, and the exact target of autoimmunity in the kidney has not been determined. It has been suggested that antibodies against TSH receptors may cross-react against the epithelial Na+ channel, carbonic anhydrase II enzyme, acid-base transporters, intercalated cells, or specific antibodies against these structures may exist in an autoimmune background (4,5,9,10). No antibody against renal tubules could be shown in Graves' disease.

In conclusion, it should be kept in mind that although distal RTA is generally a hereditary condition in children, it may also develop in the course of autoimmune diseases. Screening for dRTA at regular intervals (serum potassium levels, blood gas analysis, renal USG) will be appropriate in the follow-up of patients with Graves' disease.

What is new?

-Distal renal tubular acidosis (dRTA) can be seen in children during the course of autoimmune thyroid disease.

-In the course of Graves' disease in children, dRTA is the first case to develop.

- Screening for dRTA at regular intervals (serum potassium levels, blood gas analysis, renal USG) will be appropriate in the follow-up of patients with Graves' disease.

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Author contributions: *All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.*

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MEDICAL RECORDS-International Medical Journal

Erratum

In this article titled "The Relationship Between AKR1B1 rs759853 (C-106T) Polymorphism and the Diabetic Retinopathy Severity in Turkish Type 2 Diabetes Mellitus Patients" published in Medical Records Journal 2023;5(1):146-52,
-The institution number of the fifth author (Murat Atabey Ozer) was written incorrectly. This author's institution number has been corrected to 4.
-The conclusion part has been corrected.

Research Article

The Relationship Between AKR1B1 rs759853 (C-106T) Polymorphism and the Diabetic Retinopathy Severity in Turkish Type 2 Diabetes Mellitus Patients

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Abstract

Aim: Diabetes mellitus (DM) is an important health problem with an increasing incidence worldwide and causes many complications. Diabetic retinopathy (DR) is one of the most serious complications of DM. Polymorphisms of the AKR1B1 gene, which encodes an aldose reductase enzyme, have been associated with development of DM and DR in some studies. The current study aims to investigate the relationship of AKR1B1 rs759853 polymorphism with type 2 DM (T2DM), DR and DR severity in the Turkish population.
Materials and Methods: A total of 437 individuals, including 141 T2DM patients without DR, 125 T2DM patients with DR, and 171 healthy controls, were included in the study. Genotyping was performed using PCR-RFLP method.
Results: An association between T allele / TT genotype and increased risk of proliferative diabetic retinopathy (PDR) was detected. In the logistic regression analysis in which other risk factors were included, rs759853 polymorphism and diabetes duration were found to be associated with the PDR development. There was no significant relationship between the AKR1B1 rs759853 variation and the development of T2DM and DR.
Conclusion: Obtained data showed that AKR1B1 rs759853 polymorphism is not associated with the development of T2DM and DR in the Turkish patients, but TT genotype and diabetes duration are independent risk factors for the development of PDR.

Keywords: Type 2 diabetes mellitus, diabetic retinopathy, AKR1B1, aldose reductase, rs759853, polymorphism

INTRODUCTION

The prevalence of Diabetes mellitus (DM) is rising worldwide. Turkey has the highest DM prevalence with a rate of 11.1% among European countries according to the International Diabetes Federation 2019 data (1). Diabetic retinopathy (DR), an important microvascular complication of both type 2 DM (T2DM) and type 1 DM (T1DM), is a neurovascular disease characterized by progressive structural and functional disorders in the retina. It causes health problems such as vision loss and blindness in approximately 75% of patients who have had diabetes for at least 15 years (2,3). The early phase of DR is called nonproliferative DR (NPDR) when the late phase is called proliferative DR (PDR). While progressive microvascular changes are observed in the retina in NPDR, these changes are also observed outside the retina in PDR. PDR is also characterized by the growth of newly formed

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vessels in the retina and optic disc. Diabetic maculopathy (DMP), which can be seen in both NPDR and PDR causes decreased vision in patients with DM. Diabetic macular edema (DME), the most common form of DMP, is a thickening of the posterior pole of the retina (4). Diabetes duration, uncontrolled glucose level of blood and high blood pressure have influence on the development and progression of DR. Other risk factors include dyslipidemia, smoking and high body mass index (BMI). However, there are fundamental differences between individuals in terms of DR risk and disease severity, and these differences cannot be explained only by the risk factors listed above (5). Studies have shown that variations in many different genes are among the reasons for this difference between individuals. Vascular endothelial growth factor-A (VEGF-A), erythropoietin (EPO), protein kinase C-β (PKC-β), methylenetetrahydrofolatereductase (MTHFR), angiotensin-converting enzyme 1 (ACE-1), intercellular adhesion molecule 1 (ICAM-1) and aldo-keto reductase family 1, member B1 (AKR1B1) are counted among the genes associated with susceptibility to DR development (2,6).

AKR1B1, a NADPH-dependent aldo-keto reductase, is expressed in many tissues, such as retinal capillary pericytes (6). It is one of the best known enzymes of the polyol pathway and catalyzes the reduction of glucose to sorbitol using NADPH as a cofactor (7). Under normal glycemic conditions, AKR1B1 has low affinity for glucose and less than 3% of glucose is converted to sorbitol by AKR1B1. In the case of chronic hyperglycemia, glucose affinity of AKR1B1 increases (about 30% of glucose is converted to sorbitol) and this results with sorbitol accumulation as well as increased NADPH use. Sorbitol accumulation leads changes in cell membrane osmotic pressure, diffusion of water into the cell, electrolyte imbalance, and ultimately cell damage and oxidative stress. On the other hand, the increased use of NADPH by AKR1B1 causes a decrease in the amount of NADPH to be used for other metabolic processes such as nitric oxide synthesis. Decreased nitric oxide synthesis leads to vasoconstriction, ischemia, and slowing of nerve conduction. NADPH also acts as a cofactor of antioxidant enzymes such as glutathione reductase, and a decrease in its amount again causes increased oxidative stress. Oxidative stress is one of the main causes of DM and DM complications such as DR (7,8).

The AKR1B1 gene is on the 7q35 locus and contains 10 exons. The rs759853 (C-106T) polymorphism is in the AKR1B1 promoter region (6,7). In the literature, various polymorphisms of AKR1B1, including rs759853, have been associated with micro and macro complications of diabetes, such as DR, diabetic neuropathy, diabetic nephropathy, and stroke in some populations (6,7,9–12). To the best of our knowledge, the association between AKR1B1 polymorphisms and susceptibility to T2DM and DR has not been explored in the Turkish population. The present study aims to explore the relationship between the AKR1B1 rs759853 polymorphism and the susceptibility to T2DM and DR and the clinical features of the disease.

MATERIAL AND METHOD

Patients

The approval of the current study was provided by Giresun University's Faculty of Medicine Clinical Trials Ethics Committee (Approval No:23.12.2021-08). All participants gave their signed informed consent before recruitment. Power analysis revealed that a total of 421 patients should be included in the study for an analysis with an effect size of 0.3 (medium) and a power of 90%. A total of 437 individuals (141 T2DM cases without DR, 125 T2DM cases with DR, and 171 healthy subjects) were included in the current study. The patient group was between the ages of 44-84, and the control group was between the ages of 38-84. American Diabetes Association (ADA) criteria were used for the diagnosis of T2DM. All patients underwent a comprehensive eye examination with evaluation of visual acuity, fundoscopic examination, and fundus photography for the diagnosis of DR. Afterwards, staging was performed. Patients with one of the signs of hard exude, cotton wool spot, hemorrhage and intraretinal microvascular anomalies or venous segmentation in addition to microaneurysms but without PDR findings were staged as NPDR. Cases with at least one of the signs of neovascularization, preretinal hemorrhage, vitreous hemorrhage, or fibrovascular proliferation in the disc or elsewhere were staged as PDR. Individuals with other types of diabetes, malignant or inflammatory diseases did not include the study. Healthy individuals without diabetes and any eye disease were selected for the control group. Fasting venous blood samples were taken from all participants for genomic DNA extraction and biochemical analysis. In addition to the age, gender, height, weight and hypertension status of all participants, the duration of diabetes, insulin use, DME and DMP presence in the patient group were recorded. Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), glycated hemoglobin (HbA1c) and fasting plasma glucose (FPG) were measured using blood samples. BMI was calculated as weight/height2 (kg/m2).

DNA extraction and genotyping

DNA isolation was carried out by the High pure PCR template preparation kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol. Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) method was used for genotyping. PCR reaction was conducted using following primers; F 5' TTCGCTTTCCCACCAGATAC 3'; R 5' CGC CGT TGT TGA GCA GGA GAC 3'. PCR protocol was 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 65°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 5 min. The size of the PCR amplification product was 326 bp and all PCR amplicons are run on a 2% agarose gel. The BfaI enzyme was used to digest the PCR amplicons and the resulted amplicons were run on 3% agarose gel electrophoresis. Two bands of 234bp and 92bp for the CC

genotype, 4 bands of 234bp, 175bp, 92bp and 59bp for the CT genotype, 3 bands of 175bp, 92bp and 59bp for the TT genotype were observed.

Statistical analysis

A statistical software package (SPSS, Windows version release 15.0; SPSS Inc.; Chicago, IL, USA) was used to perform statistical analyses. Shapiro-Wilk Normality test was used to evaluate whether the data were normally distributed. Continuous variables were expressed as mean ± SD. Categorical data were presented as numbers and percentages. The genotype distribution of rs759853 among groups was tested for the Hardy–Weinberg equilibrium (HWE) using the $\chi 2$ test. Genotypes and allele frequencies among the groups were compared using $\chi 2$ tests. Mann–Whitney U and Kruskal Wallis tests were used to determine difference between the groups in terms of continuous variables. Binary logistic regression analysis was used to test the factors affecting the development of PDR. P values below 0.05 were considered as statistically significant.

RESULTS

Demographic and clinical characteristics of the patient and control groups are summarized in Table 1. As expected, BMI (p=0.046), presence of hypertension (p<0.001), lipid profiles (p<0.001 for total cholesterol and LDL, p=0.002 for HDL and triglyceride), HbA1c (p<0.001) and fasting blood glucose (p<0.001) were significantly different between DM (with and without DR) and control groups (Table 1). In addition, a difference was observed in terms of gender distribution (p=0.046). The frequency of male gender was found to be lower in the T2DM group compared to control group (Table 1). There was a significant difference between the patients with and without DR in case of BMI (p=0.013), duration of diabetes (p<0.001), total cholesterol (p=0.001), LDL (p=0.022), HbA1c (p<0.001), fasting blood glucose (p=0.027), presence of DME (p<0.001) and DMP (p<0.001) (Table 1). Genotype frequency of AKR1B1 rs759853 polymorphism was found to be compatible with HWE in all three groups (Tables 2 and 3). Genotype and allele frequencies did not differ between the T2DM group and the control group (Table 2), and between T2DM patients with and without DR (Table 3). As shown in Table 4, the total T2DM group and the T2DM group with DR were divided into groups according to rs759853 genotypes and it was evaluated whether there was a difference in terms of clinical parameters. Accordingly, no significant difference was found between individuals with different genotypes in case of BMI, duration of diabetes, presence of hypertension, total cholesterol, fasting blood sugar, LDL, triglyceride, HDL, DME and DMP presence in both the total T2DM group and the T2DM group with DR (Table 4). On the other hand, a significant relationship was found between the presence of PDR and genotype (p=0.017) and allele frequencies (p=0.003) in the T2DM group with DR (Table 5). According to logistic regression analysis including AKR1B1 rs759853 polymorphism, BMI, duration of diabetes, presence of hypertension, total cholesterol, LDL, HDL, triglyceride, HbA1c, and fasting blood glucose, TT genotype (p=0.019, OR=5.204, 95% CI=1.307- 20.718) and duration of diabetes (p=0.038, OR=1.108, 95% CI=1.006-1.222) were associated with the development of

Table 1. Demographic and clinical characteristics of study groups

Variables	T2DM (without DR)[a] N=141	T2DM (with DR)[b] N=125	Controls [c] N=171	P [a+b/ c] value	P [a / b] value
Sex (% male)	41.8	44	52.6	0.046	0.723
Age at study (years)	62.9±8.4	62.7±7.5	62.4±11.8	0.783	0.980
BMI (kg/m^2)	29.8±3.6	31.3±4.8	29.5±4.5	0.045	0.013
Diabetes duration (years)	8.4±3.7	15.6±5.1	-		<0.001
Presence of hypertension (%)	37.6	47.2	11.7	<0.001	0.113
Total cholesterol (mg/dl)	192.9±34.5	213.7±56.5	154±30.5	<0.001	0.001
LDL (mg/dL)	109.2±29.4	119±36.2	102.9±25.4	<0.001	0.022
HDL (mg/dL)	50.3±12.4	47.1±10.6	50.6±8	0.002	0.070
Triglyceride (mg/dL)	181.2±132.9	171.4±131.3	131.3±18.6	0.002	0.656
HbA1c (%)	7.3±1.2	8.1±1.5	4.7±0.6	<0.001	<0.001
Fasting glucose level (mg/dL)	163±51	187.3±87.8	85.6±9.9	<0.001	0.027
DME (%)	3.5	72.8	-		<0.001
DMP (%)	2.1	88	-		<0.001
PDR (%)	-	32	-		

T2DM: Type II Diabetes Mellitus, DR: Diabetic retinopathy, BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, DME: Diabetic macular edema, DMP: Diabetic maculopathy, PDR: Proliferative diabetic retinopathy

Table 2. Comparison of AKR1B1 genotype and allele frequencies between total T2DM patients and controls

AKR1B1 rs759853 Genotypes and alleles		Control N (%)	T2DM patients N (%)	OR (95% CI)	p value
Multiplicaptive	CC	84 (49.1)	127 (47.7)	Ref	-
	CT	69 (40.4)	109 (41)	1.045 (0.694-1.572)	0.833
	TT	18 (10.5)	30 (11.3)	1.102 (0.578-2.103)	0.767
Dominant	CC	84 (49.1)	127 (47.7)	Ref	0.778
	CT+TT	87 (50.9)	139 (52.3)	1.057 (0.719-1.552)	
Resesive	CT+CC	153 (89.5)	236 (88.7)	Ref	0.806
	TT	18 (10.5)	30 (11.3)	1.081 (0.582-2.006)	
Over dominant	TT+CC	102 (59.6)	157 (59)	Ref	0.896
	CT	69 (40.4)	109 (41)	1.026 (0.694-1.518)	
Alleles	C	237 (69.3)	363 (68.2)	Ref	0.740
	T	105 (30.7)	169 (31.8)	1.051 (0.784-1.409)	
		HWE: 0.489	HWE: 0.372		

T2DM: Type II Diabetes Mellitus, OR: Odds ratio, CI: Confidence interval, HWE: Hardy-Weinberg equilibrium

Table 3. Comparison of AKR1B1 genotype and allele frequencies between T2DM patients with and without DR

AKR1B1 rs759853 Genotypes and alleles		Control N (%)	T2DM patients N (%)	OR (95% CI)	p value
Multiplicaptive	CC	66 (46.8)	61 (48.8)	Ref	-
	CT	59 (41.8)	50 (40)	0.917 (0.549-1.531)	0.740
	TT	16 (11.3)	14 (11.2)	0.947 (0.427-2.101)	0.893
Dominant	CC	66 (46.8)	61 (48.8)	Ref	0.746
	CT+TT	75 (53.2)	64 (51.2)	0.923 (0.570-1.495)	
Resesive	CT+CC	125 (88.7)	111 (88.8)	Ref	0.970
	TT	16 (11.3)	14 (11.2)	0.985 (0.460-2.110)	
Over dominant	TT+CC	82 (58.2)	75 (60)	Ref	0.760
	CT	59 (41.8)	50 (40)	0.927 (0.568-1.512)	
Alleles	C	191 (67.7)	172 (68.8)	Ref	0.791
	T	91 (32.3)	78 (31.2)	0.952 (0.660-1.372)	
		HWE:0.612	HWE:0.445		

T2DM: Type II Diabetes Mellitus, DR: Diabetic retinopathy, OR: Odds ratio, CI: Confidence interval, HWE: Hardy-Weinberg equilibrium

Table 4. Comparison of clinical characteristics stratified by genotypes of AKR1B1 rs759853 polymorphism among total T2DM patients and T2DM patients with DR

Parameters	Total T2DM patients				T2DM patients (with DR)			
	CC	CT	TT	p	CC	CT	TT	*P* value
BMI (kg/m²)	30.3±4.3	30.4±4.2	31.6±4.2	0.117	31.8±4.9	31.8±4.9	32.2±4.8	0.262
Diabetes duration (years)	11.4±5.3	12±5.7	12.9±7	0.638	16.2±5	16.2±5	16.8±5.8	0.502
Hypertension N(%)								
No	68 (44.2)	66 (42.9)	20 (13)	0.325	29 (43.9)	29 (43.9)	7 (10.6)	0.635
Yes	59 (52.7)	43 (38.4)	10 (8.9)		21 (35.6)	21 (35.6)	7 (11.9)	
Total cholesterol (mg/dl)	200.2±46.2	204.6±47.7	206.4±51	0.652	216.6±54.8	216.6±54.8	221.1±63.3	0.276
LDL (mg/dL)	115±36.1	111.9±28.7	115.8±35.2	0.844	117.1±28.4	117.1±28.4	122.4±37	0.899
HDL (mg/dL)	48.5 ± 11.2	48.9 ± 12.8	49.8 ± 9.4	0.532	47.4±12.7	47.4±12.7	47.4±9.3	0.800
Triglyceride (mg/dL)	176.7 ±136.4	170.7 ±103.2	193.2±195.7	0.990	156.8±65	156.8±65	244.8±275.3	0.691
HbA1c (%)	7.7±1.4	7.7±1.5	7.7±1.1	0.625	8±1.7	8±1.7	8.2±1.2	0.358
Fasting blood glucose level (mg/dL)	172.6±83.1	175.3±59.4	178.8±61.1	0.149	179.9±66	179.9±66	195.9±60.9	0.308
DME N (%)								
No	83 (48.8)	69 (40.6)	18 (10.6)	0.847	13 (38.2)	13 (38.2)	3 (8.8)	0.804
Yes	44 (45.8)	40 (41.7)	12 (12.5)		37 (40.7)	37 (40.7)	11 (12.1)	
DMP N (%)								
No	74 (48.4)	62 (40.5)	17 (11.1)	0.972	5 (33.3)	5 (33.3)	1 (6.7)	0.625
Yes	53 (46.9)	47 (41.6)	13 (11.5)		45 (40.9)	45 (40.9)	13 (11.8)	

T2DM: Type II Diabetes Mellitus, DR: Diabetic retinopathy, BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, DME: Diabetic macular edema, DMP: Diabetic maculopathy

Table 5. Comparison of AKR1B1 genotype and allele frequencies between PDR and NPDR subgroups

Groups	Genotypes			*p* value	Alleles		*p* value
	CC	CT	TT		C	T	
NPDR N(%)	48 (56.5)	31 (36.5)	6 (7.1)	**0.017**	127 (73.8)	45 (26.2)	**0.003**
PDR N(%)	13 (32.5)	19 (47.5)	8 (20)		43 (55.1)	35 (44.9)	

PDR: Proliferative diabetic retinopathy, NPDR: Nonproliferative diabetic retinopathy

Table 6. Risk factors for PDR using logistic regression analysis

Variables	Odds ratio	95% CI	P value
BMI	1.063	0.969 - 1.167	0.194
Diabetes duration	1.108	1.006 - 1.222	**0.038**
Presence of hypertension	1.429	0.590 - 3.465	0.429
Total cholesterol	1.001	0.987 - 1.015	0.933
LDL	1.004	0.983 - 1.025	0.734
HDL	0.993	0.951 - 1.037	0.756
Triglyceride	0.999	0.995 - 1.003	0.628
HbA1c	0.788	0.535 - 1.161	0.228
Fasting glucose level	1.006	0.999 - 1.012	0.075
AKR1B1 CT genotype	2.281	0.900 - 5.780	0.082
AKR1B1 TT genotype	5.204	1.307 - 20.718	**0.019**

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein

PDR (Table 6).

DISCUSSION

Even if the blood glucose level is effectively controlled, some DM patients develop DR, while others do not. The data obtained from the studies showed that in addition to other risk factors such as long-term exposure to hyperglycemia and the presence of hypertension, genetic factors also involved in the development of DR (6,13). In the past, the relationship of AKR1B1 polymorphisms with the development and severity of DR in DM patients has been investigated, in various populations (12–17). To the best of our knowledge, present study is the first study exploring the association between AKR1B1 variations and the development of T2DM and DR in the Turkish population. In our study, there were three groups: T2DM patients with and without DR, and the control group. A relationship was detected between the AKR1B1 rs759853 variation and the severity of DR. When DR patients were divided into two groups as NPDR and PDR, it was observed that frequencies of TT genotype and T allele was statistically significantly higher in the PDR group. As a result of the logistic regression analysis, which included other risk factors, it was determined that diabetes duration and rs759853 TT genotype were risk factors for PDR. Our findings seem to be compatible with the data of a study conducted in Han Chinese patients (18) that reports patients with CT+TT genotype to have an increased risk for PDR. On the other hand, in a study performed in Brazilian patients, contrary to our study, it was reported that CC carriers had an increased risk of PDR development (19). Another study showed that, there was no relationship between DR severity and AKR1B1 rs759853 variation in Jordanian population (20).

In our study, no significant association was found between AKR1B1 rs759853 polymorphism and T2DM and DR susceptibility. There was also no relationship between rs759853 polymorphism and other clinical features except the presence of PDR. Similar to our study, no relationship was found between rs759853 polymorphism and T2DM susceptibility in studies performed by Abu Hassan et al. and Watari et al. in Jordanian and Japanese populations (10,20). On the other hand, Shawki et al. (21) found TT genotype to be associated with increased T2DM risk in the Egyptians. Opposite of this study, Sivenius et al. reported a relationship between C allele and T2DM susceptibility in the Finnish population (22).

In the literature, studies investigating the rs759853 polymorphism and DR susceptibility have also reported different results in different populations. For example, in 3 different studies conducted in Chinese and Brazilian populations, it was revealed that there is no significant relationship between rs759853 polymorphism and DR susceptibility, similar to our results (19,23,24). In addition, two different meta-analyses reported no association between rs759853 polymorphism and DR susceptibility (6,25). However, Cao et al. performed a subgroup analysis depending on the type of diabetes and they found that this polymorphism has a protective effect on the development of DR in T1DM patients (6,25). In another meta-analysis conducted in recent years, including 4313 DR and 5218 diabetes patients from 23 different studies, the T allele and CT+TT genotype were associated with a lower risk of DR (26). Conversely, studies performed in Jordanian, Chinese

and Indian populations showed that TT+CT genotypes or T allele were associated with increased DR risk (12,18,20).

In one of the studies evaluating the effects of the rs759853 polymorphism on AKR1B1 expression, it was reported that the AKR1B1 protein content in erythrocytes was higher in TT and CT carriers compared to the CC carriers (10). High expression of AKR1B1 causes an increase in conversion from glucose to sorbitol and sorbitol accumulation. It is known that the sorbitol accumulation in the retina can cause osmotic stress and ultimately retinopathy (18). Moreover, it has been reported that inhibition of AKR1B1 suppresses the expression of genes involved in angiogenesis and reduces neovascularization, which is one of the characteristic features of PDR (27). The higher frequency of TT genotype and T allele in patients with PDR compared to NPDR patients in our study may partially result in high AKR1B1 expression and higher sorbitol accumulation in these individuals. However, in our study, similar to many studies in the literature, no relationship was found between AKR1B1 genotype and allele frequency and the development of DR. Therefore, we think that the effect of AKR1B1 rs759853 in Turkish T2DM patients may not be very important at the onset of DR but may be effective in the increase of retinal vessel anomalies and the development of PDR once the disease has started. In another study on the effect of rs759853 on AKR1B1, it was claimed that the C allele showed higher transcriptional activity (28). Such a scenario suggests that different mechanisms may be dominant in the regulation of glucose conversion and sorbitol accumulation in Turkish patients.

Different results in various populations regarding the relationship between the AKR1B1 rs759853 variation and the development of T2DM, DR and PDR may be due to different ethnicity, the number of individuals included in the study, differences in environmental factors, and differences or errors associated with the statistical methods used.

CONCLUSION

To conclude, in this study, we revealed that there is no relationship between the AKR1B1 rs759853 variation and the development of T2DM and DR in Turkish individuals, but that the rs759853 variation and diabetes duration are independent risk factors for the PDR development. The relatively small number of patients and the inability to study other polymorphisms of AKR1B1 can be counted among the limitations of the current study. Therefore, future studies with more patients in the Turkish population are important to support our data.

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