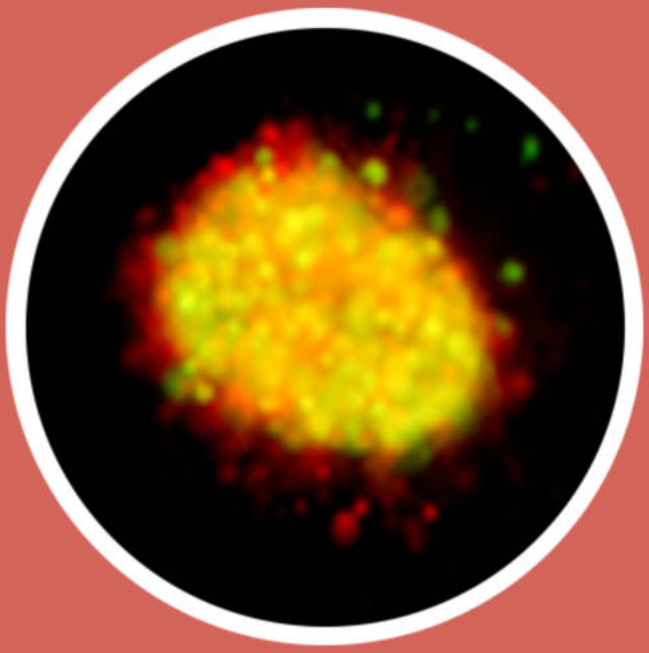
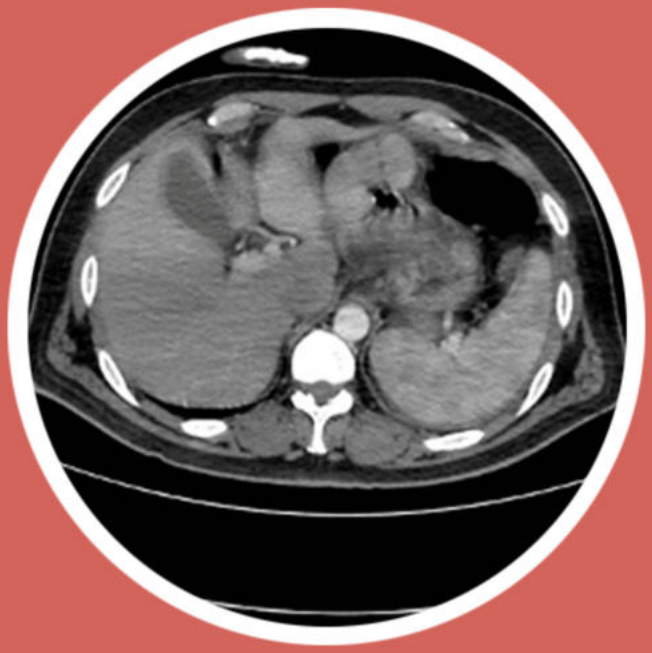
EuRJ

Volume 8 · Issue 6 · November 2022

e-ISSN: 2149-3189

The European Research Journal





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Abstracting and Indexing

The journal is abstracted and indexed with the following: ULAKBIM TR Index (ULAKBİM TR DİZİN), NLM Catalog (NLM ID: 101685727), Google Scholar (h-index: 9), Index Copernicus (ICV 2021: 100), EMBASE, ProQuest Central, ROAD, SciLit, MIAR (ICDS 2021: 3.8), J-Gate, SHERPA/RoMEO, BASE, EZB, CrossRef, JournalTOCs, WorldCat, TURK MEDLINE, Turkish Citation Index, EuroPub, OpenAIRE, ResearhGate, SOBIAD, Advanced Science Index, ScienceGate, OUCI, Publons, (Clarivate Web of Science)

Publisher

The European Research Journal (EuRJ)
Prusa Medical Publishing
Konak Mh. Kudret Sk. Şenyurt İş Mrk. Blok No:6 İç kapı no: 3
Nilüfer/BURSA-TURKEY

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e-ISSN: 2149-3189

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Original Articles

Case Report

The European Research Journal 2022;8(6):747-754 Original Article

DOI: 10.18621/eurj.872835 *Endocrinology and Metabolism*

Can non-functional adrenal incidentaloma be ranked among cardiovascular risk factors?

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ABSTRACT

Objectives: We aimed to evaluate the potential association of a nonfunctional adrenal incidentaloma (NFAI) with cardiovascular risk factors.

Methods: Forty-three patients over the age of 40 found to have NFAI and 28 healthy controls were included in this prospective study. The control group was selected from individuals who were similar in age and gender. Glucose, insulin, c-peptide, lipid profile, erythrocyte sedimentation rate, high sensitivity c-reactive protein, fibrinogen and 25-hydroxy cholecalciferol and carotid artery intima-media thickness (CIMT) were measured in both groups.

Results: Waist circumference, erythrocyte sedimentation rate, triglyceride and CIMT values were found higher in the patient group ($p = 0.002$, $p < 0.001$, $p = 0.001$, $p = 0.024$, respectively). It was observed that 10 (23.2%) of the patients had no suppression with 1 mg dexamethasone but suppression was provided with 2 mg dexamethasone for 2 days, and all of these patients with 'possible autonomous cortisol secretion' had at least one comorbidity. While there was no significant difference between the groups in terms of the presence of comorbidity, a significant difference was found in terms of diabetes mellitus (90% of the patients with autonomous cortisol secretion, 24.2% of those who were suppressed with 1 mg dexamethasone had diabetes mellitus; $p < 0.001$; Chi-square test).

Conclusions: Higher waist circumference, erythrocyte sedimentation rate, triglyceride and CIMT values in our patients with NFAI and increased diabetes mellitus frequency in patients with autonomous cortisol secretion suggest that NFAI may be one of the cardiovascular risk factors.

Keywords: Autonomous cortisol secretion, cardiovascular risk, carotid intima-media thickness, non-functional adrenal incidentaloma

The incidence of adrenal incidentalomas (AI) increases day by day. The most important reason for this is the development and use of high-tech diagnostic methods [1]. The basic approach in a patient in whom an adrenal mass is detected by coincidence is to differentiate benign/malignant mass and evaluate its hormonal status.

The presence of AI is thought to be associated with various cardiovascular disease (CVD) risk factors [2, 3]. Some studies have shown that the frequency of obesity, hypertension (HT), glucose intolerance, diabetes mellitus (DM), hyperuricemia, and hyperlipi-

Received: ***February 4, 2021;*** *Accepted:* ***April 25, 2021;*** *Published Online:* ***January 15, 2022***

How to cite this article: *Yağız B, Akalın A, Yorulmaz G, Macunluoğlu AC, Yağız O. Can non-functional adrenal incidentaloma be considered among cardiovascular risk factors? Eur Res J 2022;8(6):747-754. DOI: 10.18621/eurj.872835*

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e-ISSN: 2149-3189

demia (HPL) is increased in patients with AI [2, 4-8]. Although these pathologies are more common in subclinical Cushing's syndrome, it has been noticed that they are also common in non-functional adrenal masses, and therefore it has been suggested that the presence of AI may be the cause of the metabolic syndrome [2, 5, 6]. It is thought that the presence of increased cardiovascular and metabolic risk factors may be due to the increased cortisol production from these masses, albeit slightly [9]. However, unluckily, this moderate cortisol elevation cannot be demonstrated with sufficient sensitivity and specificity [4, 10-12]. Therefore, it is still unclear whether non-functional adrenal masses increase risk of the CVD and whether there is an autonomous cortisol function in this type of adrenal masses.

study aimed to demonstrate increased cardiovascular risk factors and endothelial dysfunction by studying inflammation markers, lipid profile, homocysteine, 25-hydroxy cholecalciferol (25-OH-D3) parameters that play a role in atherosclerosis in patients with nonfunctional adrenal incidentaloma (NFAI) and by measuring carotid artery intima-media thickness (CIMT), which is an indicator of subclinical atherosclerosis and to evaluate whether the presence of NFAI could have an impact on cardiovascular and metabolic parameters.

METHODS

The study was conducted prospectively in Eskişehir Osmangazi University Faculty of Medicine, Department of Endocrinology and Metabolic Diseases. Before the study, all patients were given an informed consent form containing the study's details, and the patients whose consents were obtained were included in the study. The study was approved with the decision of Eskişehir Osmangazi University Ethics Committee dated 27.01.2012 and numbered 14.

Forty-three patients over the age of 40 admitted due to incidental detection of an adrenal mass during imaging studies performed for different reasons were included in our study. The control group consisted of 14 healthy controls similar in age and gender, with a body mass index (BMI) between 19-25 and 14 obese controls with a BMI of ≥30. Both control groups were selected from individuals without adrenal mass and metabolic syndrome components such as coronary artery disease (CAD), DM, HT, and HPL.

Patients who were found to have incidentaloma were questioned about diseases such as DM, HT, HPL, CAD, and medication use history. General physical examinations and examinations regarding hypercortisolism's phenotypic features were performed, and systolic and diastolic blood pressures were measured. Height, weight, and waist circumference of all patients were recorded, and BMI was calculated using the formula weight/height² (kg/m²).

After 10 hours of fasting, the patients' morning glucose and insulin levels were measured, and insulin resistance was calculated using the Homeostasis Model Assessment (HOMA) formula. ESR, high sensitivity c-reactive protein (hsCRP), and fibrinogen levels as markers of inflammation and c-peptide and lipid profile [low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglyceride (TG)] as metabolic parameters were studied from blood samples taken during fasting. Serum samples were obtained after fasting for homocysteine measurement.

The patients were divided into two groups according to the 25-OH-D3 vitamin level (20 was taken as a cut-off) and CVD risk factors were compared between these two groups. Correlation analysis of 25-OH-D3 deficiency with other risk factors for CVD was also performed.

To determine the hormone activity of the adrenal mass detected before the study, adrenocorticotropic hormone (ACTH) and morning cortisol from the blood sample taken at 08:30 in the morning at the latest were measured. Night cortisol was taken at 23:00 to comply with diurnal rhythm. Urine-free cortisol was studied in the 24-hour urine sample. An overnight 1 mg dexamethasone suppression test (DST) was applied to the patients, and cortisol was studied at 08:30 in the morning at the latest from the patient who was given 1 mg DXM at 23:00. Patients who were not suppressed with 1 mg DXM were tested with a 2-day low dose. Suppression was considered sufficient if morning cortisol levels were less than 1.8 µ/dL. However post-dexamethasone serum cortisol levels between 1.9-5.0 µg/dL should be evaluated as evidence of 'possible autonomous cortisol secretion (ACS)' and post dexam-

ethasone cortisol levels 5.0 µg/dL should be considered as evidence of 'ACS' [13]. The values of Vanillylmandelic acid (VMA), metanephrine-normetanephrine, adrenaline-noradrenaline, serotonin, and dopamine were studied in 24-hour urine collected in storage containing 25% hydrochloric acid after a special diet devoid of phenolic acid-containing foods and beverages for five days. Biochemically proven non-functional patients were included in the study.

Adenoma and non-adenoma differentiation were made with dynamic adrenal computed tomography (CT) of adrenal masses detected in various imaging methods performed in our patients for different reasons. The size, localization, density, smoothness of the borders and the presence of invasion were determined, so only those considered adenomas radiologically were included in the study.

CIMT measurement was performed with the help of carotid doppler ultrasonography (USG) and was measured by the same person using the Toshiba Doppler USG device. Ideally, measurements were taken from the common carotid artery, approximately 1 cm before the carotid separation, at the artery's thickest part.

Glucose, insulin, C-peptide, erythrocyte sedimentation rate (ESR), high sensitivity c-reactive protein (hsCRP), fibrinogen, homocysteine, lipid profile, and 25-OH-D3 vitamin levels of the control group were also studied, and insulin resistance was calculated. Height, weight, and waist circumference were recorded, BMI was calculated. CIMT measurement was made.

Statistical Analysis

The compatibility of the parameters to normal distribution was examined using the Shapiro Wilk test. Continuous parameters were expressed with median (minimum: maximum) and mean±standard deviation values, and categorical variables with n (%). Independent sample t-test or Mann Whitney U test was used to compare two groups according to the normality test results. When the number of groups was more than two, the ANOVA test was used for comparisons and Tukey HSD test for the subgroup analysis, or Kruskal Wallis test was used and Dunn-Bonferroni approach for subgroup analysis. Chi-square test, Fisher's Exact test, or Fisher-Freeman-Halton test were performed for intergroup comparisons of categorical parameters. Relationships between continuous parameters were analyzed using correlation analysis, and the Pearson correlation coefficient or Spearman correlation coefficient was calculated according to the result of the normality test. Multiple linear regression analysis was performed to estimate CIMT. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) program was used for statistical analysis and a p-value of $p < 0.05$ was considered statistically significant.

RESULTS

A total of forty-three patients, thirty (69.77%) females and thirteen (30.23%) males over the age of 40 were included in our study (Table 1). It was observed that the mean age was 53 years (40-67 years) in the patient group and 49 years (44-69 years) in the control group ($p > 0.05$, Mann Whitney U test).

When the patient and the control group were compared a statistically significant difference was found between the patient and the control group in terms of waist circumference, ESR, TG and CIMT measurements. Waist circumference, ESR, TG and CIMT values were higher in the patient group compared to the control group ($p = 0.002$, $p < 0.001$, $p = 0.001$, $p = 0.024$, respectively). BMI, glucose, insülin, HOMA, hsCRP, fibrinogen, homocysteine and LDL levels were higher but statistically not significant in the patients than the control group.

Then the control group was divided according to BMI levels as normal BMI and obese groups. The patients' median ESR values were found to be significantly higher than the control group with normal BMI ($p < 0.001$). The patients' median TG values were found to be higher than both the normal weight and obese groups ($p = 0.004$). While there was a significant difference between the patient and the control groups in terms of CIMT, no difference was found when the three groups were compared.

Ten (23.2%) of the patients had no suppression with 1 mg dexamethasone (DXM) but suppression was provided with 2 mg DXM for 2 days, and all of these patients with 'possible ACS' had at least one co-

Table 1. Comparison of patients and control groups in terms of parameters

	Patient Group (n = 43)	Normal BMI Group (n = 14)	Obese Group (n = 14)	*p* value
Gender				
Female	30 (69.77%)	6 (42.86%)	11 (78.57%)	0.111[c]
Male	13 (30.23%)	8 (57.14%)	3(21.43%)	
Age	53 (40-67)	49 (44-69)	50 (44-68)	0.723[a]
Body mass index (kg/m^2)*	31.17 ± 6.14	25.18 (16.90-28.20)	31.70 (30-41.10)	**< 0.001[a]**
Waist circumference (cm)[€]	103.84 ± 14.34	85.93 ± 11.63	100.07 ± 8.86	**< 0.001[b]**
Glucose (mg/dL)	93 (63-363)	91 (79-123)	88.50 (81-100)	0.486[a]
Insulin (mlU/L)	9.95 (0.20-96)	7.53 (3.57-20.60)	9.63 (6.85-19.20)	0.441[a]
HOMA	2.25 (0.13-17.70)	1.68 (0.80-6.25)	1.98 (1.40-4.59)	0.393[a]
ESR (mm/h)[¥]	21 (1-59)	5 (2-17)	10.50 (3-36)	**< 0.001[a]**
hsCRP (mg/L)	3.40 (0.40-15.50)	3.08 (0.50-3.30)	3.19 (1.30-20.10)	0.103[a]
Fibrinogen (mg/dL)	355.65 ± 92.84	323.43 ± 66.77	339.64 ± 75.45	0.451[b]
Homocysteine (mcmol/L)	13.30 (5.80-50)	14.10 (7.15-22.30)	11.45 (9.15-16.60)	0.247[a]
LDL (mg/dL)	128.07 ± 29.99	120.36 ± 26.91	121.57 ± 28.20	0.601[b]
HDL (mg/dL)	45 (29-89)	51 (31-77)	55.50 (29-75)	0.152[a]
TG (mg/dL)[&]	132 (62-259)	89 (39-194)	97.58 (43-248)	**0.004[a]**
25-OH-D3 (ng/mL)	12.50 (3.96-39)	16.80 (9.33-29.63)	11.09 (4.90-6.30)	0.514[a]
CIMT (mm)	0.70 ± 0.24	0.30 (0.80-0.55)	0.55 (0.30-0.80)	0.105[a]

Data are expressed as n (%), median (minimum: maximum) and mean ± standard deviation. HOMA = Homeostasis Model Assessment, ESR = Erythrocyte sedimentation, hsCRP = high sensitivity c-reactive protein, LDL = low-density lipoprotein cholesterol, HDL = high-density lipoprotein cholesterol, TG = triglyceride, 25-OH-D3 = 25-hydroxy cholecalciferol, CIMT = carotid artery intima-media thickness.

[a]Kruskal-Wallis test, [b]ANOVA test, [c]Fisher-Freeman Halton test, *The median BMI values of the normal weight group were found to be lower than the patient and obese groups ($p < 0.001$ and $p < 0.001$, respectively). No statistically significant difference was found between the patient group and the obese group ($p = 0.689$). [€]The mean waist circumference values of the normal weight group were lower than the patient and obese groups ($p < 0.001$ and $p < 0.016$, respectively). [¥]Median ESR values of the patient group were found to be higher than the normal weight group ($p < 0.001$). No statistically significant difference was found between the patient group with the obese group, and the normal weight group with the obese group ($p = 0.202$ and $p = 0.104$, respectively). [&]Median TG values of the patient group were found to be higher than the normal weight and obese group ($p = 0.015$ and $p = 0.049$, respectively). No statistically significant difference was found in TG values between the normal weight group and the obese group ($p > 0.05$).

morbidity (DM, HT, HPL, CVD). Of the 33 patients suppressed with 1 mg of DXM, 72.3% had comorbidity. While there was no significant difference between the groups in terms of the presence of comorbidity, a significant difference was found in terms of DM when comorbidities were examined one by one (90% of the patients with ACS had DM, 24.2% of those who were suppressed with 1 mg DXM had DM; $p < 0.001$; Chi-square test).

Then these ten patients with ACS were removed and adrenal incidentaloma patients with suppressible cortisol secretion with 1 mg DXM were compared with control groups (Table-2). The patient group had more DM, HT, HPL, and macrovascular diseases than the control group ($p = 0.005$, $p < 0.001$, $p = 0.01$ and $p = 0.017$, respectively). In the group of patients, CIMT was also significantly higher ($p < 0.05$).

In the patient group, no significant relationship was found between right and left CIMT values and ACTH, morning and night cortisol, cortisol after DXM suppression, urinary cortisol, and adenoma size ($p > 0.05$). In the multiple linear regression analysis,

Table 2. Comparison of patients with suppressible cortisol secretion with 1 mg DXM and control groups in terms of parameters

	Patient Group* (n = 33)	Control Group (n = 28)	*p* value
Gender			0.629∞
Female	22 (66.6%)	17 (60.7%)	
Male	11 (33.3%)	11 (39.2%)	
Age (years)	52.24 ± 8.93	51.42 ± 7.44	0.704 α
Weight (kg)	80.97 ± 16.32	75.53 ± 13.68	0.168 α
Body mass index* (kg/m^2)	30.78 ± 6.34	28.73 ± 5.34	0.183 α
Waist circumference (cm)	103.78 ± 15.94	93.0 ± 12.43	**0.005** α
Diabetes mellitus	8 (24.2%)	0	**0.005**∞
Hypertension	16 (48.4%)	0	**< 0.001**∞
Hyperlipidemia	7 (21.2%)	0	**0.01**∞
Macrovascular disease	6 (18.1%)	0	**0.017**∞
Glucose (mg/dL)	104.51 ± 52.42	91.14 ± 9.22	0.159 α
Insulin (mlU/L)	14.01 ± 17.61	9.90 ± 4.55	0.205 α
HOMA	3.25 ± 3.5	2.31 ± 1.29	0.161 α
ESR (mm/h)	18.75 ± 10	12.76 ± 8.58	**0.002** α
Fibrinogen (mg/dL)	351.06 ± 82.10	331.53 ± 70.39	0.328 α
Homocysteine (mcmol/L)	14.36 ± 7.79	12.99 ± 3.22	0.363 α
LDL (mg/dL)	130.09 ± 30.27	120.96 ± 27.05	0.223 α
HDL (mg/dL)	47.45 ± 13.54	52.50 ± 12.77	0.142 α
TG (mg/dL)	141.27 ± 52.84	102.46 ± 52.93	**0.006** α
25-OH-D3 (ng/mL)	15.92 ± 9.31	17.92 ± 16.89	0.561 α
CIMT (mm)	0.68 ± 0.21	0.59 ± 0.15	**0.05** α

*10 patients who were not suppressed with 1 mg dxm were removed. Data are expressed as n (%), median (minimum-maximum) and mean±standard deviation. DXM = dexamethasone, HOMA = Homeostasis Model Assessment, ESR = Erythrocyte sedimentation, LDL = low-density lipoprotein cholesterol, HDL = high-density lipoprotein cholesterol, TG = triglyceride, 25-OH-D3 = 25-hydroxy cholecalciferol, CIMT = carotid artery intima-media thickness.

αIndependent sample t-test, ∞Chi-square test

it was found that the only variable that showed a positive correlation with CIMT independently was age.

DISCUSSION

Adrenal masses that are clinically asymptomatic and detected incidentally in diagnostic tests for unrelated diseases are called AI [14-16]. Developments in imaging methods have resulted in an increasing number of detections of AI [15]. Because of the increasing incidence of AI with age [17], it is crucial to determine whether AI is among the risk factors in this group, in whom the risk of CVD increases.

AI is mostly considered as benign, asymptomatic lesions. However, recent studies have shown that abnormalities in the ACS and hypothalamic-pituitary-adrenal axis are more common than thought. The prevalence of ACS, which is the most common hormonal change in AI, varies due to differences in diagnostic tests and different cut-offs in studies [18]. Bulow *et al*. [19] reported a prevalence of 2%, Libe *et*

al. [20] 18%, and Terzolo *et al*. [21] 5-20% (review of different series) [22, 23]. The possible ACS diagnosis was based on a 1 mg DXM in our study, and the rate was found to be 23.2%.

ESR is a nonspecific inflammation marker and is one of the known risk factors for CVD. In our study, the ESR value was higher in patients than in the control group ($p < 0.001$). However, there was no difference between the patients and the obese group and between the normal weight and obese groups. In a study conducted by López-Bermejo *et al.* [24], it was found that ESR is independently associated with obesity. Although obesity is associated with increased ESR, the lack of difference between obese and normal control groups in our study suggests the presence of subclinical inflammation in patients with AI.

It was reported that increased CIMT is an indicator of atherosclerosis and correlates with myocardial infarction, stroke, and peripheral artery diseases [25-28]. The first finding encountered in atherosclerosis is an increase in intima-media thickness [25, 29]. Similar to various previous studies [30-32] in our study, CIMT was statistically higher in patients with AI than in the control group.

Type2 DM is comorbidity associated with ACS, and it was present in 90% of our patients with ACS. Patients with ACS have a higher prevalence of Type 2 DM estimated in the range of 20-75%, depending on the diagnostic criteria used [22]. Also, ACS prevalence varies between 0-9.4% in type 2 DM [33-35], and the risk increases in those with poor metabolic control, microvascular complications, obesity, and HT [36]. Therefore, it is unclear whether AI increases the risk of metabolic syndrome or whether this type of adrenal tumor is more common in people with cardiometabolic risk factors such as type 2 DM.

In our study, the patient group with NFAI had more DM, HT, HPL, and macrovascular diseases than the control group. The presence of increased cardiovascular and metabolic risk factors is thought to be due to slightly increased cortisol production from these masses [9]. Unfortunately, due to a lack of sensitivity and specificity, this mild cortisol elevation cannot be demonstrated [9-12].

Limitations

Our main limitation is that we do not have a group that has AI but no DM, HT, CAD, PAD. Although the rate of other diseases other than HT is low and patients with concomitant diseases under control were selected, this situation may appear like a confounding factor. However, the fact that our study was conducted prospectively is a significant advantage as well as being its limitation.

CONCLUSION

In this study, we aimed to evaluate the relationship between cardiovascular diseases, one of the most important causes of death today, and AI. Increased ESR and increased CIMT were significant risk factors for atherosclerosis as well as traditional risk factors, and DM was more common in the ACS group. It was thought that subclinical inflammation and insidious cortisol autonomy, which is thought to have metabolic effects, may cause this situation.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):755-761 Original Article

DOI: 10.18621/eurj.1020958 *Urology*

The impact of the COVID-19 pandemic on the urology practice in a large tertiary hospital

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ABSTRACT

Objectives: We aimed to reveal how four different areas that are important in the functioning of the urology clinic (outpatient clinic, inpatient clinic, operating room, and consultations) were affected during the COVID-19 pandemic.

Methods: Patients admitted to the surgical branches between March 11, 2018 and March 10, 2021 were retrospectively evaluated in terms of their demographic data. The data between these dates were analyzed by dividing the patients into three groups as Groups A, B, and C for the pandemic period, the year before the pandemic, and two years before the pandemic, respectively.

Results: A total of 1,222,967 patients were included in the study. During the pandemic period, the number of urology outpatient clinic admissions decreased by more than half compared to the previous years (37,471, 93,582, and 89,031 for Groups A, B, and C, respectively). Admissions to the urology inpatient clinic decreased both numerically and proportionally when compared to the other surgical branches (1,301 [5.1%] for Group A, 3,884 [7.7%] for Group B, and 3,761 [7.7%] for Group C]). While the mortality rate did not change proportionally in the urology clinic in all groups (0.3%), it increased both numerically and proportionally in all surgical branches (339 [1.3%], 304 [0.6%], and 256 [0.5%]).

Conclusions: Admissions to the urology clinic were determined to have decreased during the pandemic compared to the pre-pandemic period, especially due to restriction measures taken by countries and concerns about the unknowns of the disease. As a result of this decrease, the number of operations and the number of hospitalized patients were also reduced. Although the mortality rate was not affected in the short-term follow-up of patients, long-term outcomes remain uncertain.

Keywords: COVID-19, hospitalization, pandemic, surgery, urology

In December 2019, cases of pneumonia of unknown origin began to be reported in the People's Republic of China, and soon the virus causing the new coronavirus disease (COVID-19) was isolated [1]. The first individual who tested positive for COVID-19 in Turkey was reported in the capital Ankara on March 11, 2020. The COVID-19 pandemic has led to the need to take radical decisions that have had many important effects and results in Turkey, primarily in health, followed by social, economic, political, economic, administrative, legal, military, religious, and cultural areas [2].

University of Health Sciences Bursa Yuksek Ihtisas Training and Research Hospital is the largest and most equipped hospital in the southern Marmara region of the country, with 1,520 registered beds. The

Received: **November 9, 2021;** *Accepted:* **May 9, 2022;** *Published Online:* **August 4, 2022**

How to cite this article: *Dündar G, Erkan A. The impact of the COVID-19 pandemic on the urology practice in a large tertiary hospital. Eur Res J 2022;8(6):755-761. DOI: 10.18621/eurj.1020958*

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e-ISSN: 2149-3189

urology clinics of our hospital serve both a large and relatively urgent patient population, including those with oncological diseases, surgical priority diseases, life-threatening diseases, and diseases affecting the quality of life. Despite this, the observation that there has been a decrease in the number of admissions has made it essential to investigate how much urology clinics have been affected by the ongoing pandemic [3, 4].

In this study, the aim was to reveal how the urology clinic at a tertiary training and research hospital was affected by the pandemic in the light of concrete data. By analyzing the data obtained retrospectively, we aimed to have an idea about possible future pandemic waves and the effects of current or new pandemics on the urology clinic.

METHODS

For this study, permission was obtained from the Clinical Research Ethics Committee of Health Sciences University Bursa Yuksek Ihtisas Training and Research Hospital with the protocol number 2011-KAEK-25 2021/01-21. Data were obtained retrospectively from the hospital information management system following the first anniversary of the first reported case in Turkey. In this observational study, data were analyzed by dividing patients admissions into three groups: Group A, from March 11, 2020, to March 10, 2021 (one year after the first case was reported in Turkey); Group B, from March 11, 2019, to March 10, 2020 (the year before the first reported case in Turkey); and Group C, from March 11, 2018, to March 10, 2019 (two years before the first case in Turkey). These groups were further divided into 12 equal periods for monthly evaluations using graphs.

The number of admissions to outpatient clinics, mean age of patients, number of repeated admissions, number of appointments to outpatient clinics, time of arrival at outpatient clinics, time spent in outpatient clinics, number of hospitalized patients, mean age of hospitalized patients, mean number of hospitalization days, death status of hospitalized patients, consultations requested from the emergency department, mean age of the consulted patients, and number of patients who underwent surgery were evaluated. The surgery clinics included in the study were urology, orthopedics and traumatology, neurosurgery, general surgery, cardiovascular surgery, thoracic surgery, ear-nose-throat diseases, pediatric surgery, gynecology, and obstetrics, plastic and reconstructive surgery, ophthalmology, and pediatric urology.

Although the forms and scopes of restrictions vary in Turkey, they first started on March 21, 2020, and ended on June 1, 2020. Due to the increase in the number of cases in autumn, restrictions were started to be implemented for the second time starting from November 17, 2020. As of March 10, 2021, when the last data were included in the study, the second restriction period continued.

Statistical Analysis

The data were analyzed with the Shapiro-Wilk test to determine whether they showed a normal distribution. The results were presented as mean ± standard deviation or frequency and percentage values. Normally distributed data were compared with the independent-samples t-test or one-way analysis of variance. The Bonferroni test was used as a multiple comparison method. Categorical variables were compared between groups using Pearson's chi-square test and the Fisher-Freeman-Halton test. The level of statistical significance was set at $p < 0.05$. Statistical analyses were made using IBM SPSS ver. 23.0 (IBM Corp. Release 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

RESULTS

The data on outpatient clinics, hospitalization, consultations, mortality, and surgery are summarized in Table 1 according to the groups. The data in each row are divided into two parts for the urology clinic and total data for all surgical clinics (except row E).

Outpatient Clinic Admissions

The number of admissions to urology outpatient clinics decreased with the beginning of the pandemic, but the ratio of urology admissions to all outpatient clinic admissions was higher in Group A than in the remaining groups (Table 1.A). The relationship between the number of outpatient clinic visits of the groups for the same periods are shown in Fig. 1. Due to the effect of the pandemic, repeated admissions to

Table 1. Data overview

		Group A	Group B	Group C
A	Urology	19,250 (12.9)[a]	44,094 (11.8)[b]	43,302 (11.4)[c]
	Total	149,534 (100.0)	374,730 (100.0)	378,808 (100.0)
B	Urology	3.10 ± 2.23 (2-35)[a]	3.26 ± 2.24 (2-34)[b]	3.21 ± 2.21 (2-33)[c]
	Total	3.36 ± 2.47 (2-40)[a]	3.32 ± 2.32 (2-39)[b]	3.32 ± 2.29 (2-45)[b]
C	Urology	46.83 ± 17.86[a]	50.08 ± 19.00[b]	50.02 ± 19.27[b]
	Total	39.51 ± 19.48[a]	41.70 ± 20.52[b]	41.37 ± 20.69[c]
D	Urology	16,772 (14.6)[a]	56,796 (14.7)[a]	57,654 (14.3)[b]
	Total	114,934 (100.0)	386,856 (100.0)	403,893 (100.0)
E	Urology	-0:11:47 ± 1:03:30[a]	-0:20:14 ± 1:24:43[b]	-0:15:39 ± 1:29:19[c]
F	Urology	0:20 ± 0:55[a]	0:18 ± 1:11[b]	0:13 ± 0:47[c]
	Total	0:30 ± 0:56[a]	0:24 ± 1:02[b]	0:19 ± 0:49[c]
G	Urology	1,301 (5.1)[a]	3,884(7.7)[b]	3,761(7.7)[b]
	Total	25,714 (100.0)	50,719 (100.0)	49,004 (100.0)
H	Urology	57.45 ± 17.45[a]	56.14 ± 19.13[b]	56.59 ± 19.18[b]
	Total	39.59 ± 20.99[a]	43.46 ± 21.19[b]	41.47 ± 20.75[c]
I	Urology	4.21 ± 4.6 (0-62)[a]	3.91 ± 4.20 (0-62)[b]	3.81 ± 3.57 (0-43)[b]
	Total	3.12 ± 4.66[a]	3.11 ± 5.05[b]	3.25 ± 4.94[c]
J	Urology	4 (0.3)[a]	11 (0.3)[a]	12 (0.3)[a]
	Total	339 (1.3)[a]	304 (0.6)[b]	256 (0.5)[b]
K	Urology	1,061 (3.9)[a]	1,658 (4.1)[a]	1,575 (3.5)[b]
	Total	27,058 (100.0)	40,613 (100.0)	44,455 (100.0)
L	Urology	52.72 ± 22.79[a]	52.70 ± 22.73[a]	50.18 ± 23.81[b]
	Total	35.09 ± 24.49[a]	35.21 ± 24.10[a,b]	34.74 ± 23.20[a,c]
M	Urology	998 (6.6)[a]	3,030 (9.3)[b]	2,965 (9.6)[b]
	Total	15,181 (100.0)	32,500 (100.0)	30,798 (100.0)

A. Number of outpatient clinic admissions (repeated applications not included), n (%) **B**. Repeated admissions to the outpatient clinic, mean ± SD (min-max) **C**. Age of patients admitted to the outpatient clinic, mean ± SD **D**. Number of appointments made for the outpatient clinic, n (%) **E.** Patients' arrival times at outpatient clinics with an appointment, mean ± SD (hh:mm:ss) **F.** Outpatient clinic procedure times, mean ± SD, **G.** Number of patients admitted to the inpatient clinic, n (%) **H.** Age of hospitalized patients, mean ± SD **I.** Number of days of hospitalization, mean ± SD (min-max) **J.** Mortality status of patients admitted to the inpatient clinic, n (%) **K.** Consultations requested from emergency services to the branches, n (%) **L.** Age of patients for whom consultation was received, mean±SD **M.** Number of patients who underwent surgery, n (%)

[a, b, c] : There is no statistically significant difference between the groups marked with the same letters in each row. In other words, different letters indicate that the difference between the groups in that row is statistically significant ($p < 0.05$)

the urology outpatient clinics decreased in Group A (Table 1.B). It was observed that the mean age was significantly lower in Group A than in the remaining groups (Table 1.C). Fig. 2 presents the age weights of the patients who were admitted to the outpatient clinics according to the monthly periods.

Admissions to outpatient clinics were in two forms: with and without an appointment. The ratio of appointments did not significantly change in the first year of the pandemic compared to the year before the

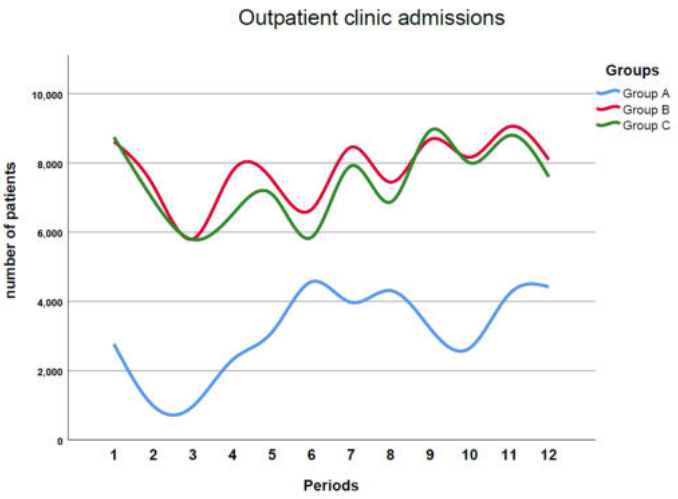

Fig. 1. Relationship between the number of outpatient clinic visits of the groups according to the monthly evaluation.

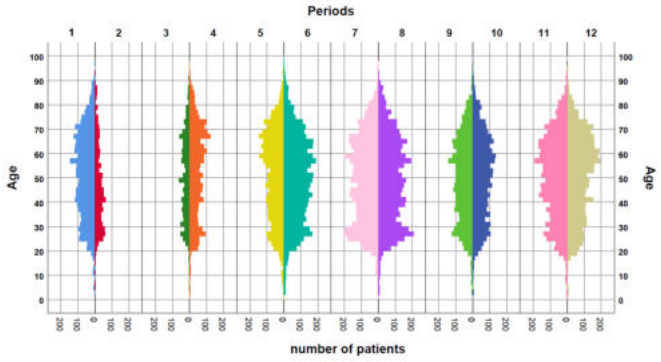

Fig. 2. Age weights of patients admitted to outpatient clinics according to the monthly evaluation.

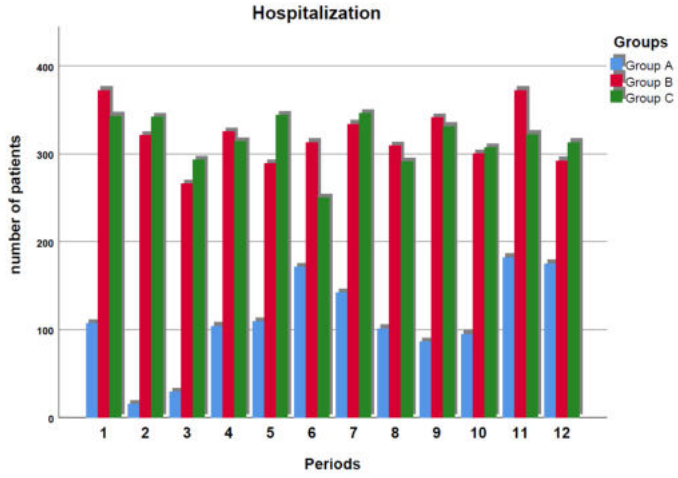

Fig. 3. Comparison of the number of hospitalized patients between the groups according to the monthly evaluation.

pandemic (Table 1.D). It was observed that the patients mostly presented to outpatient clinics before the appointment time, and during the pandemic period, they arrived at the hospital closer to their appointment times for urology outpatient clinics (Table 1.E). It was seen that the duration of procedures in urology outpatient clinics increased during the pandemic (Table 1.F).

Hospitalization

With the effect of the pandemic, as in all surgical clinics, there was a decrease in the number of patients admitted to the inpatient urology clinic (Table 1.G). The comparison of the number of hospitalized patients between the groups for the same periods are shown in Fig. 3. It was observed that the mean age of the patients who were hospitalized and their hospitalization time were significantly higher during the pandemic year (Table 1.H, 1.I).

Inpatient Clinic Mortality

No statistically significant difference was found between the three groups in relation to the mortality rate among patients admitted to inpatient clinics ($p = 0.959$) (Table 1.J). Similarly, the in-hospital mortality rate of urology patients was not affected by the pandemic.

Consultation from Emergency Department

In terms of emergency department consultations with the urology clinic, the difference between Groups A and B was not statistically significant while there was a statistically significant difference between Groups A and C (Table 1.K). The mean age of the consulted patients did not statistically significantly differ between Groups A and B ($p = 1$) but it was significantly higher during the pandemic compared to second years before the pandemic (Table 1.L).

Number of Operations

With the effect of the pandemic, the number and rate of patients that underwent surgery in the urology clinic significantly decreased (Table 1.M).

DISCUSSION

At the time of writing this paper, the COVID-19 pan-

demic had become the third largest pandemic causing the highest number of deaths after the Spanish flu and human immunodeficiency virus pandemics seen in the 20th and 21st centuries, respectively [5,6]. Due to the sudden increase in the number of patients, the health system in many countries has had difficulties coping with the pandemic and has been forced to compensate for the rapidly increasing need for personal protective equipment, inpatient clinics, and intensive care beds, and ventilator devices, in addition to providing standard healthcare services [7]. The usual service delivery of the healthcare system has had to be transformed in order to meet different demands. This has caused radical changes in standard health parameters.

With the demonstration that the virus causing COVID-19 can spread from person to person through droplets, countries have taken strict measures, such as closing schools, working at home, and home quarantine [8, 9]. This situation has limited people's access to health services. On the other hand, at the beginning of the pandemic, people tended to strictly implement all social and personal precautions and respected restrictions, as they were afraid of contracting an incurable disease they had not encountered before [10]. Therefore, the number of outpatient clinic admissions decreased by more than half, especially during the pandemic period. This decrease was particularly evident in the first quarter of the pandemic. At the second and third months period after the beginning of the pandemic, the number of outpatient clinic admissions decreased by approximately 90% compared to the previous years (Fig. 1). Quarantine conditions and people's fear of getting seriously ill play an important role in this decrease. After June, with the relaxation of measures and decrease in the number of COVID-19 patients, there was an increase in outpatient clinic admissions. In the literature, various articles have reported a decrease of up to 50% in outpatient clinic admissions, especially in the first months of the pandemic [11-13]. In these publications, at six months after the onset of the pandemic, outpatient clinic admissions increased to similar numbers as observed in years before the pandemic. In our study, we determined that even during the calmest periods of the pandemic, outpatient clinic applications were approximately 30% less compared to previous years. This decrease reached 85% in the most severe periods of the pandemic. However, there was a greater decrease in other surgical branches. We consider that the consequences of this reduction in admissions will be demonstrated by future long-term studies.

The mean age of the patients who were admitted to urology clinic during the pandemic period was found to be lower (46.83 ± 17.86 years) when compared to the previous years. When the subgroup analysis was performed, the lowest mean age was found in the second period of the pandemic year (45.95 ± 17.29) years. Fig. 2 shows the age distribution of the patients who were admitted to outpatient clinics according to the periods. It was thought that the curfew imposed on citizens aged over 65 years in Turkey may have been effective in this finding. Similarly, due to of the pandemic, the number of repeated admissions to outpatient clinics decreased compared to the previous years. When the patients who presented to the hospital with an appointment were evaluated, it was observed that those in Group A arrived at the hospital closer to their appointment hours compared to the previous years, possibly to comply with the social isolation rules. The reason for the statistically significant increase in the duration of outpatient clinic procedures during the pandemic may be the obligatory hospitalization of patients with higher morbidity due to isolation measures and prolonged anamnesis and examination times.

With the spread of COVID-19, there has been a serious decrease in the number of admissions to ED. Even in life-threatening diseases, such as acute myocardial infarction and stroke, reductions of up to 40% are observed, while admissions for urological reasons, such as urinary tract infections were reported to have decreased by 50% [11]. In a previous study, it was shown that consultations from ED to the urology clinic decreased to one-third [14]. In our study, consultations showed more than a 30% decrease during the pandemic compared to the previous years, there was a similar decrease in other surgical branches. When compared with the literature, we consider that consultation services were less affected in our hospital. Perhaps the most important question here is how much the health of patients will be affected by this situation in future.

With the onset of the pandemic, hospitalizations dropped dramatically due to COVID-19 patients being prioritized, health delivery, and the easy spread of the disease. In addition, many elective operations and hos-

pitalizations were postponed due to the fear of infecting the environment by aerosol emitted during surgery. Shortly after the onset of the pandemic, the European Association of Urology (EAU) divided urological diseases and conditions into four main priority groups [15]. Similarly, in their respective studies, Ficarra *et al*. and Stensland *et al*. categorized urological cancer cases and set a priority order [16, 17]. Many different clinics have managed patients in line with these categories and provided similar recommendations [18, 19]. We also postponed elective operations and hospitalizations in our clinic by accepting only surgical patients in the high priority group. Considering the pandemic period, 1,301 patients were admitted to inpatient urology clinics (Fig. 3). Compared with the first and second years before the pandemic, the number of hospitalized patients during the pandemic decreased by 66% and 65%, respectively. However, this decrease was also seen in other clinics, although not to the extent observed in the urology clinic. Considering the demographic data of the hospitalized patients, the patients that were hospitalized during the pandemic were older compared to the previous years. The mean hospitalization duration of the patients admitted to the inpatient urology clinic during the pandemic was calculated as 4.21 ± 4.6 days, and the length of hospitalization was longer compared to the one and two years before the pandemic. Many low-priority operations were postponed in line with the EAU guidelines [15]. During the pandemic year, 998 (76%) of the 1,301 hospitalized patients underwent surgery. Compared to the previous years, the number of operations decreased by one-third. A lesser reduction was observed in other surgical branches than in urology.

One of the most important problems caused by the pandemic is the increase in the mortality rate. In most publications, it has been reported that this increase is due to patients' late admission to health institutions, as well as interventions being undertaken at more advanced stages of diseases [11, 13, 20]. During the pandemic, we determined that the number of deaths increased to 339 from 256 in previous years. In addition, when mortality was compared proportionally to the number of hospitalized patients, it was seen that there was a more than twofold increase in Group A compared to Groups B and C (1.3%, 0.6% and 0.5%, respectively). This was attributed to difficulties in accessing health services as a result of restrictions or individuals refraining from visiting health institutions due to their fear of contracting COVID-19. However, when the mortality rates of the urology clinic were examined, there was no change during the pandemic compared to the previous years, unlike the situation in other branches (0.3%). The reason why the mortality rate of the patients treated in the urology clinic did not change may be that urological operations were continued to be performed by taking precautions and following algorithms. However, long-term mortality rates may increase.

Limitations

One of the limitations of our study is that the results obtained cannot be generalized to all other countries due to the differences in health systems.

CONCLUSION

The COVID-19 pandemic has taken its place as one of the most important health problems that the whole world has had to face in the last century. While countries have had to react rapidly to provide healthcare services to tackle the disease, the management of other diseases has also been affected. As seen in our study, the number of outpatient clinic admissions, hospitalizations, and operations significantly decreased, especially in the early stages of the pandemic. During the pandemic period evaluated, although the spread of the disease was partially brought under control through measures and its impact decreased at certain times, these parameters did not return to their pre-pandemic levels. As a result of the decreased number of admissions, patients may have started to receive health services at more advanced stages, and the number of deaths may have increased secondary to this. In terms of urology, the number of hospitalized patients and the number of operations decreased to a greater extent, while the number of mortality and outpatient clinic admissions were less affected, compared to the other surgical branches. Although this study was conducted with a large number of patients presenting to a large hospital, there is still a need for multicenter studies with longer follow-ups.

Authors' Contribution

Study Conception: GD; Study Design: GD; Super-

vision: GD; Funding: GD; Materials: N/A; Data Collection and/or Processing: GD; Statistical Analysis and/or Data Interpretation: GD; Literature Review: GD, AE; Manuscript Preparation: GD, AE and Critical Review: GD.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgement

We thank Ass. Prof. Guven Ozkaya for his contribution to the statistical analysis and Kerem Onur Ilbasi (IT staff) for his support in data collection. The first author also thanks Prof. Cengiz Alyılmaz for his academic guidance.

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The European Research Journal 2022;8(6):762-770 Original Article

DOI: 10.18621/eurj.1125754 *Psychiatry*

Evaluation of psychiatric symptoms in patients diagnosed with fibromyalgia during COVID-19 pandemic: a cross-sectional study

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ABSTRACT

Objectives: Our aim was to assess depression, anxiety, trauma symptoms and sleep problems in patients with fibromyalgia during the COVID-19 pandemic

Methods: This study was conducted face-to-face with 62 SARS-CoV-2 negative fibromyalgia patients. Sociodemographic and Clinical Data Form, Hospital Anxiety and Depression Scale (HADS), Pittsburgh Sleep Quality Index (PSQI), Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5), Coronavirus Anxiety Scale (CAS), Obsession with COVID-19 Scale (OCS), the American College of Rheumatology (ACR) Preliminary Diagnostic Criteria for Fibromyalgia 2010 were applied to the subjects.

Results: Patient mean scores on the HAD Depression and HAD Anxiety scales were 8.42 ± 3.73 and 9.58 ± 3.86, respectively. The patients' PSQI and PCL-5 scores were 8.10 ± 3.49 and 33.90 ± 16.91, respectively. While the CAS score average was found to be 1.13 ± 2.79, the OCS score average was found to be 1.81 ± 2.27. The mean scores for the Widespread Pain Index (WPI), Symptom Severity Scale (SSS), and Visual Analog Scale (VAS) were 9.90 ± 3.03, 7.84 ± 2.07 and 7.37 ± 1.81, respectively. Poor sleep quality was identified in 75.8% and a risk of post-traumatic stress symptoms in 16.1%. SSS scores were found to be higher in those at risk of depression than those without (U=265, $p = 0.003$). OCS (U=256, $p = 0.007$), SSS (U=203.5, $p = 0.001$), VAS (U=263.5, $p = 0.012$), PCL-5 (U=144, $p = 0.001$) scores were found to be higher in those at risk of anxiety than those without.

Conclusions: It was observed that depression was a predictor of fibromyalgia symptom severity and anxiety was a predictor of obsession with coronavirus, fibromyalgia symptom severity, VAS, and post-traumatic stress symptoms.

Keywords: Fibromyalgia, depression, anxiety, COVID-19

The world is struggling with the COVID-19 pandemic and its aftermath caused by the Sars-CoV-2 coronavirus, which first emerged in Wuhan, China in 2019. Post-traumatic stress disorder, depression and anxiety disorders have been known to have been detected in infected individuals during the SARS-Cov-1 epidemic. A mental health review during the SARS-CoV-2 pandemic found that post-traumatic stress disorder and depressive symptoms were high in COVID-19 patients and symptoms have worsened in those with psychiatric symptoms; It has been shown that in the general population, mental well-being was

Received: *June 3, 2022;* ***Accepted:*** *August 25, 2022;* ***Published Online:*** *August 29, 2022*

How to cite this article: *Aşçıbaşı K, Özçete ZA. Evaluation of psychiatric symptoms in patients diagnosed with fibromyalgia during COVID-19 pandemic: a cross-sectional study. Eur Res J 2022;8(6):762-770. DOI: 10.18621/eurj.1125754*

e-ISSN: 2149-3189

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low and the severity of anxiety and depression symptoms was high (1). Post-traumatic stress symptoms are positively associated with female gender and subjective sleep quality during the COVID-19 pandemic [1].

Fibromyalgia (FM) is one of the most common causes of widespread chronic pain and its prevalence reaches 2-3% worldwide. Although pain is one of its main distinguishing features, it is a multi-symptomatic syndrome that includes fatigue, sleep disorders, autonomic disorders, cognitive dysfunction, hypersensitivity to external stimuli, somatic symptoms, and psychiatric disorders. Stress is very important to the onset or worsening of the syndrome in many people with FM. Psychologically, patients with FM mostly have negative emotions characterized by a general state of stress. As a result of this negative mental state, patients with fibromyalgia can develop depression and anxiety disorders. While the lifetime prevalence of anxiety disorders is 60%, the prevalence of depression is between 14% and 36%, which is significantly higher than in the rest of the population [2].

Sleep problems can occur in FM syndrome, characterized by all kinds of insomnia and frequent awakenings. Non-restorative sleep predominates. Even with normal sleep duration and quality, patients complain that they do not get enough rest from sleep. The presence of chronic widespread pain affects sleep quality in patients with FM. There is also a bidirectional relationship between sleep disorders and depression and anxiety disorders [3].

The relationship between FM and trauma has been demonstrated in many patients [4]. Patients with FM may have reduced psychological resilience and coping skills. A low level of resilience is a risk factor for the development of post-traumatic stress disorder, anxiety, and mood disorders [5].

Salaffi *et al*. [6] studied patients with FM who had or did not have COVID-19 infection. In this study, FM-associated symptoms (distant pain, sleep disturbances, fatigue, functional symptoms) and quality of life of the patients were evaluated. The scores of FM patients with COVID-19 infection were statistically higher on all scales [6].

In this study, we aimed to determine depression symptoms, anxiety symptoms, sleep quality, trauma symptoms, anxiety, and obsession levels for the COVID-19 pandemic in patients with FM during the COVID-19 pandemic. In addition, it was also examined whether there was a significant difference between patients diagnosed with FM with and without depression and anxiety disorders in relation to the variables used in the study.

METHODS

Study Design and Panels

This study was conducted face-to-face involving 62 SARS-CoV-2 negative FM patients treated and followed-up in the Department of Physiotherapy and Rehabilitation in a third-level hospital. The research team consisted of a psychiatrist and a specialist in physiotherapy and rehabilitation. All participants were at least 18 years old and gave written informed consent. The study protocol was approved by the ethics committee. (Date: 07/12/2019, No.: 2020/10-12).

Sociodemographic and Clinical Data Form, Hospital Anxiety and Depression Scale (HADS), Pittsburgh Sleep Quality Index (PSQI), Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5), Coronavirus Anxiety Scale (CAS), Obsession with COVID-19 Scale (OCS), the American College of Rheumatology (ACR) Preliminary Diagnostic Criteria for Fibromyalgia 2010 were applied to the subjects.

Study Assessments

The socio-demographic and clinical data form collects information on the following areas: gender, age, marital status, level of education, work status, monthly income, smoking status, and alcohol consumption. The Hospital Anxiety and Depression Scale is used to determine the risk of anxiety and depression, as well as to measure the severity and change in severity of anxiety and depression in a given patient [7]. It has two subscales, one for depression and one for anxiety. The reliability and validity study of the Turkish version has been completed [8]. The cut-off scores for the Anxiety and Depression subscales of the Turkish version were set at 10 and 7 points, respectively. In this study, Cronbach's alpha value for the 14 items was 0.790.

The Pittsburgh Sleep Quality Index (PSQI), developed by Buysse *et al*. [9]) assesses sleep quality through a standardized questionnaire that is easy to understand and answer, distinguishing between good and bad sleepers [9]. It evaluates sleep quality over 1

month. The PSQI components are as follows: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of these 7 components forms the global PSQI score. A global PSQI score of 5 is consistent with poor sleep quality. The reliability and validity study of the Turkish version has been completed [10].

PCL-DSM-5 (PCL-5): It is a self-report scale consisting of 20 items rated from 0 to 4, out of a maximum of 80 [11]. PCL-5 assesses PTSD symptoms explained in 4 clusters (reexperiencing, avoidance, negative changes in cognition and mood, hyperarousal) in The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The PCL-5 can be used to monitor symptom changes, screen for PTSD, or provide a preliminary PTSD diagnosis. The reliability and validity study of the Turkish version was completed and the cut-off score of 47 points for the PTSD diagnosis was established [12].

CAS is a 5-point scale with scores between 0 and 4. The cut-off score of 9 distinguishes dysfunctional anxiety and non-anxiety in people with a COVID-19 infection [13]. The Turkish validity and reliability study of the scale was conducted but a cut-off score was not determined. The higher the score, the greater the anxiety associated with COVID-19 [14]. In this study, Cronbach's alpha value for the 5 items was 0.914.

OCS is a 4-point scale ranging from 0 to 4. The cut-off score of 7 distinguishes non-functional COVID-19 thought patterns from those without such patterns [15]. The Turkish validity and reliability study of the scale was conducted but a cut-off score was not determined. The higher the score, the greater the obsession related to COVID-19 [14]. In this study, Cronbach's alpha value for the 5 items was 0.640.

The ACR 2010 consists of 2 scales, the Widespread Pain Index (WPI) and the Symptom Severity (SS) scale. The widely used Pain Index Questionnaire is designed to determine if you experience pain or tenderness in the shoulder girdle, hip, jaw, upper back, lower back, upper arm, thigh, chest, neck, abdomen, forearm and in the lower leg had last week. The purpose of the Symptom Scale Questionnaire is to identify the severity of fatigue, difficulty thinking or remembering, and waking up tired (unrefreshed) in the previous week by a rating from 0 to 3 [16]. Patients with symptoms for at least 3 months with a WPI 7 and SS score 5 or WPI 3-6 and SS scale score 9 and without a disorder that would otherwise explain the pain were considered to have met the ACR 2010 criteria [17].

Statistical Analysis

The data obtained in the study was entered into the database created in the program SPSS (Statistical Package For Social Sciences) 18.0 and statistical evaluations were carried out with the same program. The agreement of continuous variables with the normal distribution was examined. As a result of both sample size, graphical examination, and normality tests, it was decided that the conditions for normality agreement were not met for these variables, and nonparametric methods were preferred for the comparisons of independent groups. Independent group comparisons were performed using the Mann-Whitney U test. Median

Table 1. FM Patients' demographics and clinical characteristics

	n	%
Gender		
Male	6	9.7
Female	56	90.3
Marital status		
Single	14	22.6
Married	48	77.4
Education level		
No Education	12	19.4
Primary school	17	27.4
High school	10	16.1
Bachelor's degree	23	37.1
Working status		
Not working	32	51.6
Working	30	48.4
Income status		
Low	6	9.7
Middle	38	61.3
High	18	29.0
Smoking status		
Yes	12	19.4
No	40	81.6
Alcohol use		
Yes	2	3.2
No	60	96.8
Past psychiatric treatment		
Yes	47	75.8
No	15	24.2

FM = Fibromyalgia

minimum and maximum values were presented as descriptive statistics. Categorical variables were crosstabulated as frequencies and percentages and their distributions compared using chi-square test methods. The relationship between numerical variables was examined using the correlation method. In all statistical comparison tests, the margin of error for the first type was determined to be = 0.05 and it was tested with two tails. If the p-value was less than 0.05, the difference between the groups was considered statistically significant.

RESULTS

FM Patients' Demographics and Clinical Characteristics

Fifty-six (90.3%) participants were female patients. The mean age was 42.44 ± 9.66 years. Forty-seven (75.8%) patients stated that they had received psychiatric treatment for depression and anxiety disorders in the past. Additional sociodemographic and clinical data are summarized in Table 1.

The Scores of the Patients from the Scales

Patient mean scores on the HAD Depression and HAD Anxiety scales were 8.42 ± 3.73 and 9.58 ± 3.86, respectively (Table 2). The patients' PSQI and PCL-5 scores were 8.10 ± 3.49 and 33.90 ± 16.91, respectively. While the CAS score average was found to be 1.13 ± 2.79, the OCS score average was found to be 1.81 ± 2.27. WPI, SSS, and VAS score averages were found to be 9.90 ± 3.03, 7.84 ± 2.07 and 7.37 ± 1.81, respectively (see Table 2).

Evaluation of FM Patients According to the Cut-off Values of the Scales

According to HADS, patients with and without risk of depression and anxiety were assessed for poor sleep quality and PTSD using the chi-square method. A significant difference was only found with regard to an anxiety disorder ($X^2 = 5.846$; $p = 0.03$) (Table 3). According to the HAD Anxiety Scale, patients with or without risk of an anxiety disorder were assessed for depression, poor sleep quality, and PTSD using the chi-square method. A significant difference was only found for anxiety PTSD ($X^2=11.327$; $p = 0.002$) (see Table 3).

No significant difference was found when the sociodemographic and clinical characteristics of the pa-

Table 2. The scores of the patients from the scales

	Valid (n)	Mean	Standard Deviation	Minimum	Maximum		Frequency (n)	Percent (%)
HAD Depression	62	8.42	3.73	2.00	18.00	> 7	34	54.8
						≤ 7	28	45.2
HAD Anxiety	62	9.58	3.86	0.00	19.00	> 10	21	33.9
						≤ 10	41	66.1
PSQI Score	62	8.10	3.49	2.00	15.00	> 5	47	75.8
						≤ 5	15	24.2
PCL-5	62	33.90	16.91	7.00	7.00	> 47	10	16.1
						≤ 47	52	83.9
CAS Score	62	1.13	2.79	0.00	13.00			
OCS Score	62	1.81	2.27	0.00	9.00			
WPI	62	9.90	3.03	5.00	19.00			
SSS	62	7.84	2.07	5.00	12.00			
VAS	62	7.37	1.81	3.00	10.00			

HADS = Hospital Anxiety and Depression Scale, PSQI = Pittsburgh Sleep Quality Index, PCL-5 = Posttraumatic Stress Disorder Checklist for DSM-5, CAS = Coronavirus Anxiety Scale, OCS = Obsession with COVID-19 Scale, WPI = Widespread pain index, SSS = Symptom severity score, VAS = Visual analog scale

Table 3. Evaluation of FM patients according to the cut-off valuesof the scales

		HAD Depression			
		> 7	≤ 7	X^2	*p* value
HAD Anxiety	> 10	16 (76.2%)	5 (23.8%)	5,846	**0.030**
	≤ 10	18 (43.9%)	23 (56.1%)		
PSQI	> 5	29 (61.7%)	18 (38.3%)	2,638	0.104
	≤ 5	5 (33.3%)	10 (66.7%)		
PCL-5	> 47	8 (80.0%)	2 (20.0%)	3,048	0.097
	≤ 47	26 (50.0%)	26 (50.0%)		
		HAD Anxiety			
		> 10	≤ 10	X^2	*p* value
PCL-5	> 47	8 (80.0%)	2 (20.0%)	11.327	**0.002**
	≤ 47	13 (25.0%)	39 (75.0%)		
PSQI	> 5	18 (38.3%)	29 (61.7%)	1.700	0.322
	≤ 5	3 (20.0%)	12 (80.0%)		

FM = Fibromyalgia, HADS = Hospital Anxiety and Depression Scale, PSQI = Pittsburgh Sleep Quality Index, PCL-5 = Posttraumatic Stress Disorder Checklist for DSM-5

tients were examined using the chi-square statistical method in relation to HAD depression, HAD anxiety and PSQI cut-off scores.

Comparison of Patients with FM in Terms of Depression, Anxiety, and Sleep Quality

In this study, patients were divided into groups according to the cutoff score of HADS and PSQI scales related to risk of depressive disorder, risk of anxiety disorder, and poor sleep quality, and these groups were compared according to CAS, OCS, WPI, SSS, VAS, PCL-5, and age variables (Table 4). The Mann-Whitney U test was used to compare group means because the group data were not normally distributed. As a result between the group with and without risk of depression and SSS (u = 268; p = 0.03); between the group with and without risk of anxiety and OCS (u = 256; p = 0.007), SSS (u = 203.5; p = 0.001), VAS (u = 263.5; p = 0.012), PCL-5 (u = 144; p = 0.001) a statistically significant difference was found between the group with and without poor sleep quality and SSS (u = 197.5; p = 0.01) (see Table 4).

Correlations between Variables of FM Patients

As a result of the correlation between the numerical values obtained from the scales applied to the patients, many statistically significant values were obtained (Table 5. Positive significant correlations were found between risk of depressive disorder and risk of anxiety disorder (r = 0.469; p = 0.001), poor sleep quality (r = 0.414; p = 0.001), FM symptom severity (r = 0.406; p = 0.001), VAS (r = 0.321; p = 0.011), between anxiety disorder risk and poor sleep quality (r = 0.349; p = 0.005), PTSD risk (r = 0.634; p = 0.001), CAS (r = 307; p = 0.015), OCS (r = 0.292; p = 0.021), FM symptom severity (r = 0.408; p = 0.001), VAS (r = 0.374; p = 0.003) and between poor sleep quality, FM symptom severity (r = 0.485; p = 0.001) and VAS (r = 0.274; p = 0.031) (see Table 5).

DISCUSSION

In this study, the risk of depression was found in 54.8% of FM patients and the risk of anxiety in 33.9%. In addition, the majority of FM patients (75.8%) were found to have poor sleep quality and 16.1% were at risk of PTSD.

The studies conducted found that the prevalence of lifelong depression in FM patients ranged from 13% to 48% (18, 19) and the prevalence of anxiety disorders ranged from 27% to 60% [20]. In addition,

Table 4. Comparison of FM patients according to HADS and PSQI scale cut-off scores

	HAD Depression								
	> 7				≤ 7				
	n	Med.	Min.	Max.	n	Med.	Min.	Max.	*p* value
CAS	34	0.00	0.00	13.00	28	0.00	0.00	8.00	0.943
OCS	34	1.00	0.00	9.00	28	1.00	0.00	8.00	0.736
WPI	34	10.00	5.00	19.00	28	9.00	7.00	18.00	0.328
SSS	34	8.00	5.00	12.00	28	7.00	5.00	11.00	**0.003**
VAS	34	8.00	4.00	10.00	28	7.00	3.00	10.00	0.056
PCL-5	34	35.50	9.00	70.00	28	26.50	7.00	76.00	0.063
Age	34	44.50	26.00	65.00	28	42.00	21.00	58.00	0.474
	HAD Anxiety								
	> 10				≤ 10				
	n	Med.	Min.	Max.	n	Med.	Min.	Max.	*p* value
CAS	21	0.00	0.00	13.00	41	0.00	0.00	8.00	0.114
OCS	21	2.00	0.00	9.00	41	1.00	0.00	8.00	**0.007**
WPI	21	9.00	5.00	19.00	41	9.00	7.00	18.00	0.210
SSS	21	9.00	6.00	12.00	41	7.00	5.00	11.00	**0.001**
VAS	21	8.00	4.00	10.00	41	7.00	3.00	10.00	**0.012**
PCL-5	21	46.00	22.00	76.00	41	25.00	7.00	62.00	**< 0.001**
Age	21	41.00	21.00	65.00	41	45.00	26.00	64.00	0,208
	PSQI								
	> 5				≤ 5				
	n	Med.	Min.	Max.	n	Med.	Min.	Max.	*p* value
CAS	47	0.00	0.00	13.00	15	0.00	0.00	10.00	0.408
OCS	47	1.00	0.00	9.00	15	1.00	0.00	9.00	0.418
WPI	47	9.00	5.00	19.00	15	11.00	6.00	18.00	0.210
SSS	47	8.00	5.00	12.00	15	6.00	5.00	11.00	**0.010**
VAS	47	8.00	3.00	10.00	15	7.00	4.00	10.00	0.12
PCL-5	47	33.00	7.00	76.00	15	36.00	8.00	70.00	0.616
Age	47	44.00	25.00	65.00	15	40.00	21.00	50.00	0.199

n = number of participants, med = median, max = maximum, min = minimum, FM = Fibromyalgia, HADS = Hospital Anxiety and Depression Scale, PSQI = Pittsburgh Sleep Quality Index, PCL-5 = Posttraumatic Stress Disorder Checklist for DSM-5, CAS = Coronavirus Anxiety Scale, OCS = Obsession with COVID-19 Scale, WPI = Widespread pain index, SSS = Symptom severity score, VAS = Visual analog scale

one study has shown that most FM patients (70-90%) have poor sleep quality [21]. PTSD is one of the most important causes of anxiety in FM patients. In the studies conducted, it was found that the rate of patients who fully met the PTSD criteria varied between 3-57% [20, 22].

A review assessing the prevalence of depression and anxiety during the COVID-19 pandemic found a depression prevalence of 33.7% and an anxiety prevalence of 31.9% in the general population [23]. In a study evaluating the impact of the COVID-19 pandemic on sleep disorders, sleep disorders were found

Table 5. Correlations between variables of FM patients

	1	2	3	4	5	6	7	8	9	10
1. Age	1									
2. HAD-D	.157	1								
3. HAD-A	.020	.469**	1							
4. PSQI	.204	.414**	.349**	1						
5. PCL-5	-.066	.376**	.634**	.203	1					
6. CAS	-.045	.129	.307*	.078	.250*	1				
7. OCS	-.091	.056	.292*	.083	.325**	.654**	1			
8. WPI	-.200	.098	-.096	.004	.045	.009	-.160	1		
9. SSS	-.053	.406**	.408**	.485**	.419**	.222	.248	.175	1	
10. VAS	.020	.321*	.374**	.274*	.324*	.075	.117	.117	.600**	1

FM = Fibromyalgia, HADS = Hospital Anxiety and Depression Scale, PSQI = Pittsburgh Sleep Quality Index, PCL-5 = Posttraumatic Stress Disorder Checklist for DSM-5, CAS = Coronavirus Anxiety Scale, OCS = Obsession with COVID-19 Scale, WPI = Widespread pain index, SSS = Symptom severity score, VAS = Visual analog scale

*$p < 0.05$, **$p < 0.01$

in 42.2% of participants [24].

During the COVID-19 pandemic, Alaosh *et al*. [25] found that 22.1% moderate-severe generalized anxiety disorder, 29.5% severe generalized anxiety disorder, 28% moderate-severe depressive disorder, 23.8% severe depressive disorder, 42.8% moderate-severe insomnia, 29.4 % severe insomnia in the patients with FM diagnosis.

When interpreting these obtained rates, it was found that the depression rate in FM patients during the COVID-19 pandemic was higher than the depression rate in FM patients, with the exception of the Covid-19 pandemic and the depression rate in the general population during the COVID-19 pandemic period. This result shows that the negative psychological effects of the COVID-19 pandemic increase the rate of depression in patients with FM. The anxiety rate was within expected limits. As expected, the sleep disturbance rate is higher than in the general population and similar to the rate in patients with FM. The PTSD rate was also within the expected range.

When compared to other studies conducted with FM patients during the COVID-19 pandemic, it was observed that the mean values of the WPI scores were similar and there was a relative difference in the mean values of the SSS and VAS scores. The reason for this difference in SSS and VAS may be the impact of existing psychological problems such as depression and anxiety in FM patients.

In this study, a significant relationship was found between the presence of depression and the presence of anxiety in FM patients. Recent data shows that FM, depressive disorders, and anxiety disorders tend to be comorbid conditions. They share a common neurochemical dysfunction, such as changes in the reactivity of the hypothalamic-pituitary-adrenal axis and the hypofunctional serotonergic system [26]. Therefore, this result was realized as expected.

A significant association was found between the presence of an anxiety disorder and PTSD. Johnson *et al*. [27] found an association between anxiety and PTSD symptoms in their study during the COVID-19 pandemic. It is known that FM patients' existing symptoms worsen with stress and that anxiety and post-traumatic stress disorder are in their etiology. It is an expected finding that the symptoms of anxiety and post-traumatic stress caused by the COVID-19 pandemic are related.

There was a significant difference in SSS between FM patients who were at risk of depression and those who were not. There is no other study examining FM patients at and without risk of depression for SSS in the COVID-19 pandemic. But in the study by Aloush *et al*. [25] in which they examined FM patients in the Covid-19 pandemic, they found a significant correlation between depression and SSS.

A significant difference was found between FM patients with and without anxiety risk in terms of obsession, symptom severity, VAS and post-traumatic symptom severity for COVID-19. Kharko *et al*. [28] showed that there is a positive correlation between pain and anxiety in FM patients during the COVID-19 period in their study. Studies have shown that people with more post-traumatic stress symptoms also have more anxiety symptoms [27, 29]. Taking into account the correlation scores, there is a positive correlation between depression and anxiety and SSS deterioration, sleep quality, and VAS deterioration; A positive correlation was found between deterioration in sleep quality and SSS. In the study by Aloush *et al*. [25], as expected, a positive correlation was found between COVID-19 anxiety, COVID-19 obsession and both general anxiety and post-traumatic stress symptoms.

Limitations

It can be noted that this is a study conducted from a single center and the number of samples is relatively small. We believe that conducting new prospective studies with control groups to determine the change in symptoms in FM patients over time will further expand our knowledge in this area.

CONCLUSION

In this study, SARS-CoV-2 negative FM patients with and without risks of depression and anxiety were examined. It was observed that depression was a predictor of FM symptom severity and anxiety was a predictor of obsession with coronavirus, FM symptom severity, VAS, and post-traumatic stress symptoms. In addition, it was found that there was a significant difference in symptom severity between those with good sleep quality and those without good sleep quality. A positive correlation was found between worsening sleep quality and the risk of depression and anxiety. Another positive correlation was found between the severity of trauma and the severity of depression and anxiety. Finally, a positive correlation was found between obsessions regarding COVID-19 and trauma symptoms. All of this data suggests that patients diagnosed with FM may have been affected by the psychological burden of COVID-19 during the COVID-19 pandemic and FM patients should be followed up more frequently from a psychiatric perspective.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):771-776 Original Article

DOI: 10.18621/eurj.1090251 *Pediatric Dentistry*

Prevalence of asymptomatic SARS-CoV-2 infection in children in Sivas province, Central Anatolia

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ABSTRACT

Objectives: Transmission from asymptomatic patients is one of the biggest challenges in controlling the Coronavirus disease 2019 (COVID-19) outbreak because these cases are a potential source for disease spread. Based on this situation, the aim of our study is to determine the prevalence of COVID-19 in asymptomatic pediatric dental patients representing Sivas and surrounding provinces.

Methods: The population of the study consists of pediatric patients between the ages of 0-14 who applied to Sivas Oral and Dental Health Hospital General Operating Room for dental treatments between July 2020 and August 2021.

Results: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was detected in only 5 patients (approximately 1.80%) out of 278 asymptomatic pediatric patients.

Conclusions: It is thought that the COVID-19 infection, which threatens the whole world, can progress asymptomatically in children, and therefore it may be a risk factor for the spread of the infection. To tackle the COVID-19 pandemic, it is recommended to maintain a high level of infection control measures in schools and day-care and to implement widespread testing on a global scale targeting the pediatric population.

Keywords: COVID-19, SARS-CoV-2, asymptomatic infection, children

Coronavirus disease 2019 (COVID-19) is an infectious caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), affecting 234 million people worldwide as of October 2021 [1]. SARS-CoV-2 is transmitted primarily by salivary droplets or nasal discharge that occurs when and patient sneezes or coughs [2]. The most common findings of the disease are cough, fever, myalgia, fatigue, dyspnea, anosmia and loss of taste. Also, some patients have headache, hemoptysis, diarrhea, myalgia and increased sputum production [3]. However, none of the aforementioned disease findings are sufficient to diagnose COVID-19 alone without microbiological tests. In addition, the overall estimate of the proportion of people infected with SARS-CoV-2 and remaining asymptomatic throughout the infection has been reported to be 20% [4].

Transmission from asymptomatic patients is one of the biggest challenges in controlling the COVID-19 outbreak because these cases are a potential source for disease spread [5]. The replication of SARS-CoV-2 in the upper respiratory tract is a factor for the high infectivity of the virus, and unlike SARS, SARS-CoV-2 can be communicated by asymptomatic individuals [6, 7]. Although asymptomatic SARS-CoV-2 infections have been reported worldwide in recent months,

Received: ***March 19, 2022;*** *Accepted:* ***May 14, 2022;*** *Published Online:* ***September 5, 2022***

How to cite this article: *Candan M, Yıldırım S. Prevalence of asymptomatic SARS-CoV-2 infection in children in Sivas province, central Anatolia. Eur Res J 2022;8(6):771-776. DOI: 10.18621/eurj.1090251*

e-ISSN: 2149-3189

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little data are available on the infectivity, epidemiological significance, virological characteristics and mechanism of infections in people with asymptomatic COVID-19 [4, 7, 8]. It is thought that people with asymptomatic COVID-19 may be less contagious than symptomatic patients [5, 8]. However, transmission from asymptomatic cases is estimated to account for > 50% of all transmissions [9]. In a study conducted in Turkey, it was reported that 22.7% of pediatric patients diagnosed with COVID-19 were asymptomatic [10]. In order to be able to effectively control the spread of the infection; In addition to identifying and isolating people with symptomatic COVID-19, it also requires reducing the risk of transmission from asymptomatic patients [9].

COVID-19 infection is a disease that can be seen in all age groups [11]. Although children are thought to be less affected by COVID-19 than adults, their rates of being infected may be similar to adults [12, 13]. This inconsistency in data was attributed to the fact that children may be asymptomatic during the disease or have symptoms so mild that they do not require medical attention [13]. Also, a meta-analysis conducted in 2021 reported that children tent to have mild SARS-CoV-2 infection with a good prognosis compared to adults [11]. In conclusion, the prevalence of the infection among undiagnosed children is also not fully known, since the symptoms are usually less severe [14] or absent [15] in pediatric patients. Also, the susceptibility levels of children or the transmission rates of COVID-19 are still important research topics for researchers.

SARS-CoV-2 seroprevalence may differ between various variables such as geographical regions and ethnic groups [16]. Therefore, it is also important to detect regional seroprevalences as well as the global seroprevalence of the infection.

Consequently, the aim of the this study is to determine the prevalence of asymptomatic SARS-CoV-2 infection in healthy children without lower or upper respiratory tract symptoms and signs of infection who applied for dental treatment under general anesthesia from Sivas and surrounding provinces.

METHODS

Sample Size and Selection

The population of the study consists of pediatric patients between the ages of 0-14 who applied to Sivas Oral and Dental Health Hospital General Operating Room for dental treatments between July 2020 and August 2021. The preoperative evaluation record of the related cases were reviewed retrospectively. Demographic data (age, gender) obtained from patient records, presence-absence of systemic disease or any syndrome, consultations requested for patients, preoperative assesments records including the signs of infection and lower-upper respiratory tract symptoms of the relevant patients, the record of whether the relevant patient is in contact or not with a COVID-19 patient and polymerase chain reaction (PCR) test to detect SARS-CoV-2 results will be evaluated.

Ethical Commitee Approval

The present study was carried out with the the permission of the Republic of Turkey Ministry of Health, General Directorate of Health Services (Date: July 28,2021, Decision No: 2021-07-28T14_02_57) and approval of the Ethics Committee of Sivas Cumhuriyet University Non-Interventional Clinical Research (Approval number: 2021-08/31).

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 22.0 (IBM Corporation, USA). In the present descriptive study, the results were given as numbers and percentages for categorical variables, and as mean and standard deviation for numerical variables.

RESULTS

Since Sivas Oral and Dental Health Hospital is one of the few dental hospitals that has a general operating room, Anesthesia and Reamination and Pediatric dental specialists in its own staff; It is an institution where patients from Sivas and surrounding provinces apply for dental treatments under general anesthesia.

When the patient's preoperative assesment records of 278 asymtomatic patients who applied to the Sivas Oral and Dental Health Hospital General Operating Room for dental treatments under general anesthesia within the specified date range were evaluated; it was detected that only 5 patients (approximately 1.80%)

Table 1. Age, gender, systemic disease, date of diagnosis and the variation status of the SARS-CoV-2 virus of children diagnosed with COVID-19

Patient	Age (years)	Gender	Systemic disease	Date of diagnosis (dd.mm.yyyy)	Presence of SARS-CoV-2 delta variant
No. 1	4	Girl	None	13.07.2020	-
No. 2	13	Girl	Cerebral palsy	30.10.2020	-
No. 3	4	Girl	None	10.04.2021	-
No. 4	6	Boy	None	23.08.2021	+
No. 5	7	Boy	None	31.08.2021	+

with a mean age of 6.8 ± 3.7 years were diagnosed with COVID-19.

The age, gender, systemic diseases, date of diagnosis and the variation status of SARS-CoV-2 were shown in Table 1.

DISCUSSION

Although operative and anaesthetic strategies should be personalized for each case, in clinical practice many pre-operative strategies and routine tests are used generally. These tests and protocols, whether or not guided by the case's clinical and medical history, have been part of pre-operative clinical practice for many years [17]. The anesthesiologist reviews the child's respiratory, cardiological and medical conditions and, when deemed necessary, requests the consultations based on the child's relevant medical anamnesis. In order to minimize the complications of anesthesia; It is necessary to provide optimal conditions for dental treatment, which is an elective surgery. Therefore, it is not desired for the patient to have active lower and upper respiratory tract symptoms, cardiological symptoms, fever and fatigue etc. findings. Symptoms such as fever, cough, pulmonary auscultation findings or oxygen saturation are the most&first easily accessible diagnostic data in COVID-19 infection [18]. However, diagnostic tests play an important role in the diagnosis of diseases, since there are no specific clinical signs that can reliably distinguish COVID-19 from other viral respiratory infections and the possibility of the infection being asymptomatic [5, 18]. Serological testing can have an important role in detecting cases with milder disease that may not have been noticed by other surveillance tests, or in diagnosing convalescents [19]. Therefore, these tests are applied to most people who have potential for infection to determine the probable duration of SARS-CoV-2 infection, to contribute to the epidemiological studies and to prevent the spread of the infection [5, 19]. In the present study, the prevalence of COVID-19 in asymptomatic children was investigated, albeit indirectly, with the results of PCR tests for COVID-19 requested from asymptomatic children without any respiratory tract infection for the administration of general anesthesia.

The Infectious Diseases Society of America (IDSA) panel agrees that pre-surgery COVID-19 diagnostic testing could be applied to asymptomatic individuals based on available evidence supporting that asymptomatic cases may have similar transmission potential and viral loads as symptomatic patients. Therefore, IDSA recommends SARS-CoV-2 RNA testing in asymptomatic population who will undergo a time-sensitive aerosol generation procedure and who have no known exposure to virus [20]. Similarly, during the pandemic period, retrospective screening was possible because PCR test for SARS-CoV-2 RNA was requested from patients before aerosol-generating surgical and dental procedures to be performed under general anesthesia in Sivas Oral and Dental Health Hospital.

COVID-19 patients who are not in a severe systemic conditions and individuals who have been in contact with any COVID-19 patients are quarantined at home to prevent the spread of the SARS-CoV-2 [21]. The follow-ups of the patients who are quarantined during the quarantine period regarding their systemic diseases or any ailments are observed through telemedicine applications, home health care services or emergency health services. All of the children who

applied to Sivas Oral and Dental Health Hospital with the request of general anesthesia at the time intervals specified in the present study were outpatients. Therefore; It may be possible to say that the asymptomatic COVID-19 cases detected in this study did not have any known contact with COVID-19 patients before.

When the literature on COVID-19 infection is evaluated, the literature on pediatric patients is not as numerous as in adult patients. In a study conducted in 2021, the frequency of PCR positivity for COVID-19 in Fortaleza/Brazil was found to be 3.5% and 3.6% in children and adolescents, respectively [22]. However the incidence of COVID-19 in asymptomatic children is unknown due to the lack of widespread testing and priority given to testing adults and severely ill cases [23]. In other words, the detection of the prevalence of especially asymptomatic children is important for public health because of its effects on transmission and control measures. As a result of the assessment of studies evaluating the prevalence of SARS-CoV-2 among asymptomatic individuals [24-26], the IDPA stated that the prevalence of infection in asymptomatic patients in the general population would be between < 1 and 10% [20]. In the present study; the prevalence of asymptomatic pediatric patients was found 1.8%, similar to the reported prevalence range [20]. Similar to the results of the present study, Patel *et al*. [27] reported the prevalence of SARS-CoV-2 infection among asymptomatic children to be 1-2% in Chicago.

COVID-19 seroprevalence is related to climate or/and geographic latitudes, human development indices, income levels and varies significantly between geographic regions [16]. Longitudinal studies to continuously monitor seroprevalence worldwide are critical to control efforts and support infection prevention and can demonstrate levels of endemic stability or instability in certain regions/countries [16]. When the literature was reviewed, it was reported that the number of SARS-CoV-2 cases detected in children was rarely documented in the daily reports of most countries outside of Asia [11]. In this context, the present study only represents the pediatric dental patient population from Sivas and its surrounding provinces, namely the central Anatolia region.

It has been reported that most young children have been infected with COVID-19 by family members [28]. However, the Center of Diaseas Control and Prevention reported that community mitigation measures and school closures may have reduced COVID-19 transmission among children and so opinions about SARS-CoV-2 infection in children may change after schools open [23]. In this context, we would like to state that the schools were closed at the time the data of the present retrospective study was obtained.

The infection risk of COVID-19 can be affected by various parameters such as the age, gender and underlying comorbidities of the patient concerned, or the variants of SARS-Cov-2 [29-31]. It has been reported that the Delta variant of SARS-CoV-2 may be more transmissible than the Alpha variant and pose the highest risk among any currently available SARS-CoV-2 variants [30]. In addition, the Delta variant was associated with higher viral RNA loads and greater infectivity in both fully vaccinated and unvaccinated people [32]. It is catchy that two patients detected in the same month in this study were infected with the Delta variant and were male. However, since the number of cases in the present study population is limited; There is a need for long-term clinical studies with larger population, especially in the pediatric population.

Detecting the asymptomatic COVID-19 children may be possible by a universal screening policy that tests all hospital admissions. Without a hospital-wide screening policy, these children may pose a risk to hospital staff and other patients [28]. If we consider this risk in terms of households; Children with asymptomatic COVID-19 can be a source of SARS-CoV-2 infection, which infects their parents, caregivers and social circles. On the other hand, socializing among children with the opening of schools can be a major concern for the spread of SARS-CoV-2 [33]. For this reason, it is important to establish social screening policies in terms of detecting asymptomatic cases, especially in pediatric patients.

Limitations

The limitation of the present study is the limited number of asymptomatic patients who participated in this retrospective study, as PCR tests for SARS-CoV-2 were requested from pediatric patients who applied to Sivas Oral and Dental Health Hospital only for general anesthesia. In addition, the study only represents the pediatric patient population from Sivas and surrounding provinces. Therefore, studies in larger populations are needed to determine the universal prevalence of asymptomatic pediatric patients.

CONCLUSION

It is thought that the COVID-19 infection, which threatens the whole world, can progress asymptomatically in children, and therefore it may be a risk factor for the spread of the infection. In this context, taking into account that children can never be completely free from the risk of contagious diseases, it is necessary to take the maximum possible precautions for their care. To tackle the COVID-19 pandemic, it is recommended to maintain a high level of infection control measures in schools and day-care and to implement widespread testing on a global scale targeting the pediatric population. However, more robust and well-designed studies are still needed to have a relevant public health intervention.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

The authors would like to thank the Republic of Turkey Ministry of Health, General Directorate of Health Services and Sivas Provincial Health Directorate for permission to carry out this study.

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The European Research Journal 2022;8(6):777-782 Original Article

DOI: 10.18621/eurj.1090380 *Rheumatology*

C-reactive protein to albumin ratio in Behçet's disease

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ABSTRACT

Objectives: This study aims to evaluate the relationship between C-reactive protein (CRP)/albumin ratio (CAR) and other hematological parameters in Behçet's disease (BD).

Methods: A total of 200 participants (100 BD patients and 100 healthy control) were recruited from the rheumatology outpatient clinic in this cross-sectional study. Laboratory tests were conducted to measure complete blood count, erythrocyte sedimentation rate (ESR), CRP, albumin, CAR, neutrophil to lymphocyte ratio (NLR), and platelet to lymphocyte ratio (PLR). Laboratory findings of BD patients and healthy controls were compared and evaluated. BD Activity scores (Behçet's Disease Current Activity Form [BDCAF]) were calculated.

Results: In the BD group, there were 42 male and 58 female participants with a mean age of 42.49 ± 13.15 years and in the healthy control group, 44 male and 56 female participants with a mean age of 44.90 ± 10.98 years. NLR, CRP, ESR and CAR values were significantly higher in patient group than in the healthy controls ($p < 0.05$). BDCAF score varied between 0 and 4 with a mean of 1.55 ± 0.64. A statistically significant correlation was observed between BDCAF and CRP, ESR and CAR ($p < 0.0001$).

Conclusions: In this study, a significant correlation was found between CAR and BDCAF, NLR, CRP, albumin and ESR. CAR can be useful in the diagnosis and following of BD patients.

Keywords: Albumin, Behçet's disease, C-reactive protein, C-reactive protein/albumin ratio

Behçet's disease (BD) is a systemic vasculitis with mucocutaneous, articular, ocular, vascular, neurological and gastrointestinal involvements, which may be seen alone or in combination in patients [1]. Etiology of the disease, which is also known as Behcet syndrome is not fully known [2].

The diagnosis of the disease and response to treatment are evaluated with clinical symptoms. There is no specific laboratory test for the diagnosis and follow up of BD. Although the specificity values of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are low, they are the most frequently used tests [3]. Previous studies have examined the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in different rheumatological diseases [3-8]. High CRP and low albumin are associated with inflammation. CAR has been shown to be associated with inflammation in some studies. Recent studies of the CRP/albumin ratio (CAR) have reported that it could be a marker of disease activity in rheumatological diseases [9-16]. Only one study could be found in literature related to CAR in BD patients. In this study, patients with Behçet's uveitis were evaluated [15].

The aim of this study was to investigate the relationship between CAR and other laboratory and clinical parameters in patients with BD.

Received: *March 19, 2022;* ***Accepted:*** *August 4, 2022;* ***Published Online:*** *September 8, 2022*

How to cite this article: *Ünal Enginar A. C-reactive protein to albumin ratio in Behçet's disease. Eur Res J 2022;8(6):777-782. DOI: 10.18621/eurj.1090380*

e-ISSN: 2149-3189

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METHODS

This cross-sectional study included BD patients who presented at the Rheumatology Clinic between October 2021 and December 2021, and healthy individuals as a control group. The clinical and laboratory data were obtained retrospectively from the hospital database.

The study included 100 patients diagnosed with BD according to the Behcet Disease International Working Group Diagnostic Criteria [17] and 100 age and gender-matched individuals. Exclusion criteria were defined as age < 18 years or > 65 years, pregnancy or breast-feeding, the presence of a malignant disease, other chronic inflammatory disease, active hepatitis, or chronic liver or kidney disease.

A record was made for all the study subjects of age, gender, neutrophil, lymphocyte, platelets, albumin, CRP, ESR, NLR, PLR, and CAR values. NLR is calculated as the ratio of neutrophil count to lymphocyte count, PLR as the ratio of platelet count to lymphocyte count, and CAR as the ratio of CRP to albumin.

The drugs used by the patients in the BD group and the BD involvements were recorded. To evaluate disease activity, the Behçet's Disease Current Activity Form (BDCAF) was completed. This form is scored in 12 clinical categories (headache, oral ulceration, genital ulceration, erythema nodosum, skin pustules, arthralgia, arthritis, nausea or vomiting or abdominal pain, diarrhea or hematochezia, eye, nervous system and major vessel involvement). According to this scale, values of ≥ 2 show disease activity [18]. A Turkish version of BDCAF has been tested and validated [19].

The normal ranges were accepted as 0–5 mg/L for CRP, 35–52 g/L for albümin and 0-20 mm/h for ESR. Neutrophil, lymphocyte, and platelet counts were recorded as 10^3 u/L.

The study protocol was approved by the Ethics Committee of Necmettin Erbakan University (decision no: 2022/3573).

Statistical Analysis

The SAS version 9.4 was used for statistical analysis. While evaluating the study data, descriptive statistics, including the mean, standard deviation, median, frequency, ratio, minimum and maximum values were obtained. The Kolmogorov-Smirnov test was conducted to check the normality distribution of independent data. Since the data were found to be normally distributed, the independent t-test was used for the comparative analysis. The chisquare test was used for the analysis of qualitative independent data. Pearson correlation coefficient (rs) was used for correlations between parametric data. Statistical significance was accepted as $p < .05$.

RESULTS

Evaluation was made of 100 BD patients, comprising 58 (58%) females and 42 (42%) males with a mean age of 42.49 ± 13.15 years, and 100 healthy control group subjects, comprising 56 (56%) females and 44 (44%) males with a mean age of 44.90 ± 10.98 years. No statistically significant difference was determined between the groups in respect of age and gender (p = 0.16 and p = 0.78, respectively). Involvements in the BD patients were determined as mucocutaneous in 94 (94%) patients, arthritis in 12 (12%), ocular in 26 (26%), vascular in 5 (5%), and neurological in 1 (1%).

Drugs being taken by the patients in the BD group were recorded as colchicine (89.58%), azathioprine (27.08%), low-dose steroids (6.25%), sulphasalazine (4.17%), and tumor necrosis factor (TNF) inhibitors (6.25%). In 4 patients who had previously used colchicine, the drugs were terminated on patient request. None of these patients had any complaints. The disease activity index of the BD patients evaluated with the BDCAF was determined to be mean 1.55 ± 0.64. The NLR, CRP, CAR and ESR values were significantly higher in the BD patient group than in the healthy control group (p = 0.022, p = 0.013, p = 0.011 and $p < 0.001$, respectively) (Table 1).

A statistically significant correlation was determined between CAR and the BCDAF score, neutrophil count, NLR, CRP, albumin, and ESR ($p < 0.0001$). The correlation between CAR and lymphocyte count and PLR was statistically significant but weaker ($p < 0.05$) (Table 2).

A statistically significant correlation was observed between BDCAF and CRP, ESR, CAR ($p < 0.0001$). The correlation between BDCAF and neutrophil and lymphocyte count was statistically significant but weaker ($p < 0.05$) (Table 3).

Table 1. Demographic, clinical and laboratory parameters of the BD patients and healthy subjects

n = 200	Behçet disease (n = 100)	Control (n = 100)	*p* value
Age (years)	42.49 ± 13.15	44.90 ± 10.98	0.16
Sex			
Female	58 (58.00%)	56 (56.00%)	0.78
Male	42 (42.00%)	44 (44.00%)	
BDCAF	1.55 ± 0.64		
Neutrophil (×10^3 µ/L)	4.49 ± 1.81	4.08 ± 1.26	0.06
Lymphocyte (×10^3 µ/L)	2.24 ± 0.74	2.32 ± 0.66	0.41
NLR	2.20 ± 1.22	1.87 ± 0.73	**0.022****
Platelet (×10^3 µ/L)	270.27 ± 67.55	273.69 ± 65.12	0.72
PLR	132.19 ± 51.26	125.24 ± 39.61	0.28
CRP (mg/L)	4.08 ± 6.70	2.34 ± 1.77	**0.013****
Albumin (g/L)	45.35 ± 2.83	46.10 ± 2.62	0.05
CAR	0.09 ± 0.16	0.05 ± 0.04	**0.011****
ESR (mm/h)	17.80 (15.24)	9.92 (8.40)	**< 0.001*****
Mucocutanous, n (%)			
+	94 (94.00)		
-	06 (6.00%)		
Arthritis, n (%)			
+	12 (12.00%)		
-	88 (88.00%)		
Ocular, n (%)			
+	26 (26.00%)		
-	74 (74.00%)		
Vascular, n (%)			
+	05 (5.00%)		
-	95 (95.00%)		
Neurological, n (%)			
+	01 (1.00%)		
-	99 (99.00%)		
Colchicine, n (%)			
+	86 (89.58%)		
-	10 (10.42%)		
Azathioprine, n (%)			
+	26 (27.08%)		
-	70 (72.92%)		
Steroid, n (%)			
+	06 (6.25)		
-	90 (93.75)		
Sulphasalazinei, n (%)			
+	04 (4.17)		
-	92 (95.83)		
TNF inhibitors, n (%)			
+	06 (6.25)		
-	90 (93.75)		

Data are shown as mean±standard deviation or n (%). BDCAF = Behçet's Disease Current Activity Form, NLR = neutrophil to lymphocyte ratio, PLR = platelet to lymphocyte ratio, CRP = C-reactive protein, CAR = C-reactive protein to albumin ratio, ESR = erythrocyte sedimentation rate, TNF = tumor necrosis factor

Table 2. C-reactive protein/albumin ratio and its correlation with other clinical parameters

		CAR
BDCAF	r	0.40998
	p value	**< 0.0001**
Neutrophil	r	0.31384
	p value	**< 0.0001**
Lymphocyte	r	-0.16240
	p value	**0.0216**
NLR	r	0.45205
	p value	**< 0.0001**
Platelet	r	-0.03510
	p value	0.6217
PLR	r	0.15288
	p value	**0.0307**
CRP	r	0.99782
	p value	**< 0.0001**
Albumin	r	-0.28275
	p value	**< 0.0001**
ESR	r	0.52936
	p value	**< 0.0001**

BDCAF = Behçet's Disease Current Activity Form, NLR = neutrophil to lymphocyte ratio, PLR = platelet to lymphocyte ratio, CRP = C-reactive protein, CAR = C-reactive protein to albumin ratio, ESR = erythrocyte sedimentation rate

Table 3. BDCAF and its correlation with other clinical parameters

		BDCAF
Neutrophil	r	0.22197
	p value	**0.0264**
Lymphocyte	r	0.04289
	p value	0.6718
NLR	r	0.14016
	p value	0.1643
Platelet	r	0.20258
	p value	**0.0432**
PLR	r	0.14788
	p value	0.1420
CRP	r	0.41355
	p value	**< 0.0001**
Albumin	r	-0.10776
	p value	0.2859
CAR	r	0.40998
	p value	**< 0.0001**
ESR	r	0.53736
	p value	**< 0.0001**

BDCAF = Behçet's Disease Current Activity Form, NLR = neutrophil to lymphocyte ratio, PLR = platelet to lymphocyte ratio, CRP = C-reactive protein, CAR = C-reactive protein to albumin ratio, ESR = erythrocyte sedimentation rate

DISCUSSION

BD may involve all types and sizes of vessels, and very different clinical tables may develop. There is no specific laboratory test for BD either in diagnosis or follow up. ESR and CRP are the most frequently used tests [20]. In a previous study of BD patients, a relationship was reported between elevated ESR and CRP and newly developed erythema nodosum, superficial thrombophlebitis or joint involvement. In the same study, a correlation was also determined between BDCAF score and ESR and CRP values [21]. However, there may be false positives and false negatives in these tests [20]. Therefore, clinicians continue to seek a practical, inexpensive and easy-to-apply test. There are studies in literature related to NLR and PLR in BD, just as in different rheumatological diseases [5-8]. In the current study, NLR was determined to be higher in the BD patients than in the control group. Hammad *et al*. [5] reported that NLR was higher in active BD patients than in non-active BD patients, and that both NLR and PLR were correlated with the BDCAF score. In a meta-analysis and another study, NLR was determined to be higher in active BD patients [6, 7], and PLR was found to be higher in active BD patients in another previous study [8]. Hou and Guan [3] reported that disease duration (≤ 60 months), NLR (≥ 2), CRP (≥ 10 mg/L), ESR (≥ 20mm/h), and albumin globulin ratio (< 1.5) were risk factors in BD patients independently of disease activity.

Recent studies have been conducted related to CAR in rheumatological diseases such as axial spondyloarthropathy, ANCA-related vasculitis, rheumatoid arthritis, Behcet uveitis, relapsing poly-

chondritis, and Kawasaki disease [9-16]. To the best of our knowledge, there is only one study in the literature related to CAR and BD. In that study, Kim *et al* examined 50 patients with BD-related uveitis and 52 patients with HLA-B27-related uveitis. A correlation was determined between CAR and the severity of ocular inflammation, and it was reported that CAR could be useful in respect of acute flare-ups in patients with chronic uveitis [15]. In a study by Bozkurt *et al*. [16], the CAR values were determined to be higher in 35 non-infective uveitis patients than in the healthy control group. CAR and CRP were reported to increase with activation and the severity of uveitis. Only 7 BD patients were included in that study [16]. In the current study, the NLR, CRP, CAR, and ESR values were determined to be higher in the BD patients than in the healthy control group. Moreover, a statistically significant correlation was determined between CAR and the BDCAF score, neutrophil count, lymphocyte count, NLR, PLR, CRP, albumin, and ESR. Thus, it can be said that CAR may be a more effective marker in BD patients than the evaluation of CRP alone as a positive acute phase protein, or albumin alone as a negative acute phase protein.

Limitations

There were some limitations to this study, primarily the retrospective design and relatively low number of patients. As the majority of BD patients in the study had low disease activity, no comparisons could be made with BD patients with high disease activity. That comorbidities and other factors such as diet were not evaluated could also be considered a limitation.

CONCLUSION

In conclusion, it was observed from the results of this study that CAR is a parameter that could be used in the diagnosis and follow up of BD patients. Nevertheless, there is a need for further studies to support these findings.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):783-789 Original Article

DOI: 10.18621/eurj.1071588 *Intensive Care*

Relationships between vaccination, age, and mortality in the COVID-19 intensive care patients

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ABSTRACT

Objectives: To investigate the effects of vaccination on age, mortality, and healthcare workers among patients followed in the COVID-19 Intensive Care Unit.

Methods: We examined age, gender, occupation, demographic characteristics, comorbid diseases, hemogram, biochemistry parameters, coagulation tests, morbidity–mortality characteristics of 548 patients in Bozyaka Training and Research Hospital COVID-19 intensive care unit admitted between March and October 2021. In addition, the vaccination status of the patients and the type of vaccination were recorded via the Ministry of Health Vaccine Tracking System (VTS). Within the vaccine follow-up system, patients who received at least 2 doses of vaccine 4 weeks prior to study were considered vaccinated.

Results: The data of 548 patients in the COVID-19 intensive care unit between March 2021 and October 2021 were analyzed. The mortality rate was 50.7% (n = 278). It was determined that 428 (78.1%) of the patients followed in the COVID-19 intensive care unit were not vaccinated. In terms of age distribution, the number of patients under the age of 65 was 357 (65.1%), while the number of patients aged 65 and over was 191 (34.9%). When mortality rates were compared based on vaccination status, the mortality rate in the unvaccinated group was found to be statistically significantly higher than in the vaccinated group ($p < 0.05$). Mortality rate in the vaccinated group was 12.5% whereas it was 61.4% in the unvaccinated group.

Conclusions: Vaccination to protect against SARS-CoV-2 infection reduces intensive care unit admission and reduces mortality rates. Being unvaccinated increases hospitalization and mortality in intensive care units in addition to carrying risks for all age groups.

Keywords: COVID-19 intensive care, vaccination, mortality, age, healthcare workers

The new SARS-CoV-2 infection emerged in Wuhan, China in December 2019, and spread to countries in a short time, becoming a pandemic health problem that concerns the whole world on March 11, 2020. This pandemic infection has killed more than five million people by October 2021. It has caused many organ and function losses that cannot be detected on individuals in the society. In addition, it has been observed that this pandemic has very negative effects on the countries health system and economies [1].

SARS-CoV-2 virus affects every segment of society. Healthcare workers have been heavily affected due to exposure to infected cases, along with the most

Received: ***February 11, 2022;*** *Accepted:* ***April 11, 2022;*** *Published Online:* ***September 30, 2022***

How to cite this article: *Demir İ, Yılmaz RS, Köse B, Özkarakaş H, Çalık Ş. Relationships between vaccination, age, and mortality in the COVID-19 intensive care patients. Eur Res J 2022;8(6):783-789. DOI: 10.18621/eurj.1071588*

e-ISSN: 2149-3189

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affected elderly group in the society. There have been more losses of lives in the elderly and healthcare workers than in any other period of time [2]. In Turkey, only 11% of COVID-19 cases were from group of patients over 65 years old while majority of deaths (71%) were from this group. While the elderly are 21% of the cases in the United States, 80% of the deaths are over the age of 65 years [3]. SARS-CoV-2 infection requires a need for intensive care and results in higher mortality rate with patients who have malignancy and immunosuppression or aging associated diseases such as hypertension, obesity, lung disease, cardiovascular disease, renal failure [4].

According to the data reported by the World Health Organization (WHO), while healthcare workers are 3% of the entire population, they constitute at least 14% of all COVID-19 infected cases. With these data, it has been observed that healthcare workers constitute one out of every seven COVID-19 patients in the world [5]. In Turkey, it was reported that 10.9% of the total COVID-19 cases were healthcare workers and 52 healthcare workers lost their lives until September 2, 2020 [6]. In England, 16.9% of the 235,685 participants who had COVID-19 infections were health workers and 1.4% of these were found to be severe disease cases. It has been stated that the possibility of a more severe course of COVID-19 disease in healthcare workers increases due to the excess viral load [7].

In SARS-CoV-2 infection, drug treatments such as antiviral drugs, nutritional supplements, intensive antibiotic, hydroxychloroquine, in addition to plasma, immunomodulators, anticoagulants are applied. All of these methods are individual treatment methods, and it has been seen that they cannot prevent a pandemic widespread infection. In the light of the scientific knowledge gained from previous experiences, it is thought that the most important and effective method in the fight against epidemic diseases is vaccination [8]. Therefore, there are 48 vaccine studies in which phase 2 and phase 3 studies are ongoing in various countries around the world. To protect against pandemic COVID-19 infection, inactive [Sinovac] and mRNA [BioNTech] vaccines have been started to be administered quickly, even though it is in the clinical research phase in Turkey. First, health workers were vaccinated due to high exposure and being a risky group, then it was widely used in the elderly population and in all age groups within a short time [9].

COVID-19 vaccination in Turkey has started on January 2021 with CoronaVac (Sinovac). Sinovac is an inactivated vaccine containing attenuated virus that does not cause disease but produces an immune response. The second vaccine application is an mRNA vaccine, which is a state-of-the-art technology using genetically engineered RNA particles to produce the viral antigenic protein that induces an immune response [10]. Active mRNA and inactivated vaccine are available to public use while the domestic vaccine TurcoVac is only administered to volunteers. It is recommended that a two-dose application regimen at least 4-6 weeks apart is required for developing protection against COVID-19. The protection of the vaccine was found to be between 90.7-100.0% with the phase II studies of Sinovac in Turkey, and it was between 50-84.0% in Phase III studies [11]. The efficacy of the Pfizer/BioNTech vaccine, which is the second type of vaccine used in Turkey, was evaluated in 43538 volunteers aged 16 years and older in a phase III randomized and controlled trial conducted in most countries, including Turkey. Two doses of vaccines administered 4-6 weeks apart and placebo groups were compared. When all age groups were evaluated, the effectiveness of the vaccine in protection against COVID-19 infection was found to be 95%. It was observed that only local adverse events occurred in 84% of the vaccinated patients, who were found to have similar adverse effects as side effects in both vaccine administrations, and most of them were mild to moderate. Fatigue, headache, and fever arthralgia were to be the most common reported as systemic vaccine adverse events [12, 13]. There are many epidemiological studies in the community that support the effectiveness of both vaccines, but there are not many comprehensive studies on intensive care. In our study, we aimed to investigate the relationship between age profiles, occupations, vaccination status, and mortality of the patients followed in the COVID-19 intensive care unit of our hospital in the post-vaccination period.

METHODS

We obtained ethical approval for the study from the Ethics Committee of Bozyaka Training and Research Hospital on October 27, 2021.

We investigated the age, gender, occupation, demographic characteristics, comorbid diseases, hemogram, biochemistry parameters, coagulation tests, morbidity-mortality of 548 patients diagnosed with COVID-19 in the COVID-19 intensive care unit of Bozyaka Training and Research Hospital between March 2021 and October 2021. In addition, the vaccination status of the patients and the type of vaccination were recorded through the Ministry of Health Vaccine Tracking System (VTS). Patients who received at least 2 doses of vaccine 4 weeks prior to study were considered vaccinated.

Statistical Analysis

Statistical analyzes on clinical data collected were performed with SPSS version 25.0. The conformity of the variables to the normal distribution was examined using the Kolmogorov-Smirnov /Shapiro Wilk tests. In the analysis of continuous data, t-test was used for independent groups in normal distribution, and Mann-Whitney U test was used in case of non-normal distribution. Fisher's Exact and Pearson's Chi Square tests were used to compare categorical variables. A p value below 0.05 was considered statistically significant.

RESULTS

The data of 548 patients in our hospital's COVID-19 intensive care unit between March 2021 and October 2021 were examined. Of the patients, 301 (55%) were males and 247 (45%) were females (Table 1, Fig. 1). The mortality rate was found to be 50.7% (n = 278). The mortality rate was found to be 64.9% (n = 180) for males and 35.1% (n = 98) for females. It was determined that 428 (78.1%) of the patients followed in the COVID-19 intensive care unit were not vaccinated. In terms of age distribution, the number of patients under the age of 65 was 357 (65.1%), while the number of patients aged 65 and over was 191 (34.9%).

When mortality rates were compared according to vaccination and non-vaccination status in Table 2. The mortality rate in the unvaccinated group was found to be statistically significantly higher than in the vaccinated group ($p < 0.05$). While mortality was 12.5% in the vaccinated group, it was 61.4% in the unvaccinated group.

It was found that mortality rates decreased in patients followed up in the COVID-19 intensive care unit with vaccination, and very significant mortality rates were detected in the unvaccinated patient group. Being unvaccinated carries a risk for all age groups (Table 3).

The biochemical parameters of the patients hospitalized in the COVID-19 intensive care unit such as hemoglobin, leukocyte, thrombocyte, D-dimer, fibrinogen, lactate dehydrogenase (LDH), urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), creatine kinase (CK), total-bilirubin, C-reactive protein (CRP), procalcitonin (PCT), and intubation parameters were examined de-

Table 1. Demographic features (n = 548)

Variables	Frequency (n)	Percentage (%)
Gender		
Male	301	55.0
Female	247	45.0
Mortality		
Death	278	50.7
Live	270	49.3
Gender/Mortality Rate		
Male	180	64.9
Female	98	35.1
Vaccination		
Not Vaccinated	428	78.1
Vaccinated	120	21.9
Intubation		
Not Vaccinated	280	88.0
Vaccinated	38	12.0
Age Groups		
> = 65	191	34.9
< 65	357	65.1
Mortality/Age Groups		
> = 65	132	47.3
< 65	146	52.7
Profession		
Healthworkers	0	0
Non-healthworkers	548	100

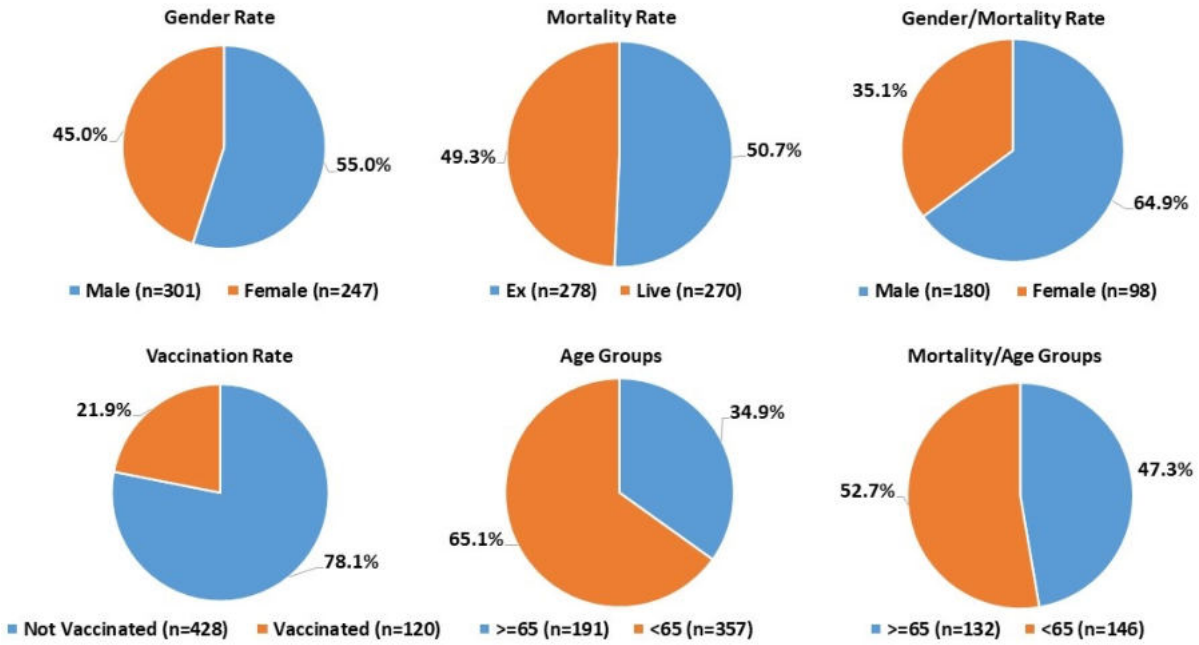

Fig. 1. Distrubition of demographic features.

pending on their status of mortality and found to carry statistically significant differences between the comparison groups (Table 4). However, glucose parameter alone was found to have no statistically significant difference between live and death groups.

DISCUSSION

In our study, one of the most prominent findings from the 548 patients we followed in the COVID-19 intensive care unit was that 78.1% (n = 428) of the patients followed-up were unvaccinated. On the other hand, only 21.9% (n = 120) of the patients were vaccinated. In Turkey, 85% of the population over the age of 18 are vaccinated for two doses [14]. If the vaccinations had no effect, we would have expected that 85% ICU patients to be vaccinated. However, we encountered the opposite results in our findings. In other words, 78.1% of the patients followed in the intensive care units were unvaccinated as opposed to 21.9% vaccinated. Therefore, this observation shows that the vaccine is protective in the community and the need for intensive care in the vaccinated group is low.

Another finding of our study in the patients we followed up in the intensive care unit was that the mortality rates were lower in the vaccinated group than in the unvaccinated group. In our study, we found a mortality rate of 12.5% (n = 15) in the vaccinated group, while it was 61.4% (n = 263) in the unvaccinated group. Vaccination has reduced the need for intubation, intensive care unit stay, and follow-up in patients. While the intubation rate was 12% (n = 38) in the vaccinated patient group, it was 88% (n = 280) in the unvaccinated group. Vaccination results in a decrease in

Table 2. The effect of vaccination status on mortality

Mortality n (%)	Vaccination status n (%)		Total n (%)	*p* value	χ^2
	Yes	No			
Yes	15 (12.5)	263 (61.4)	278 (50.7)	**< 0.0001**	***89.8***
No	105 (87.5)	165 (38.6)	270 (49.3)		
Total	120 (21.9)	428 (78.1)	548		

Pearson's Chi-Square analysis was used and $p < 0.05$ was considered significant.

Table 3. The effect of vaccination status on mortality in patients under 65 years of age and over

Age Groups	Mortality n (%)	Vaccination Status n (%)		Total n (%)	*p* value	χ^2
		Yes	No			
< 65	**Yes**	9 (5.1)	162 (92.1)	171 (48.1)	**< 0.0001**	**271.1**
	No	172 (94.9)	14 (7.9)	186 (51.9)		
	Total	181 (50.7)	176 (49.4)	357		
> = 65	**Yes**	8 (12.0)	108 (88.6)	116 (60.9)	**< 0.0001**	**109.4**
	No	61 (88.0)	14 (11.4)	75 (39.1)		
	Total	69 (36.2)	122 (63.8)	191		

Pearson's Chi-Square analysis was used and $p < 0.05$ was considered significant.

mortality in patients, shorter hospital stays, and less intensive care needs.

Another finding in our study is the change in the age profile of the patients we followed up in the intensive care unit in the post-vaccination period. When the age data of the patients followed in the intensive care unit are compared with the pre-vaccine periods, we observed that the age profile was reversed. Among our findings, 66.9% (n = 357) of the 548 patients we followed up in the intensive care unit were under the age of 65, and 23.1% (n = 191) of the patients were over the age of 65. Based on the results we previously reported as a subgroup analysis of d-dimer-fibrinogen mortality relationship before vaccination [15], 69% of the patient group in our intensive care unit serving in the same region was over the age of 65. In the post-vaccination period, we find that this rate is 23.1% of the patients over 65 years of age. This significant change in the post-vaccination period clearly demonstrates the effectiveness of vaccination.

Another finding in our study is the changing age profile in intensive care units as well as the change in total mortality rates over 65 years of age. In our study, we found a mortality rate over 65 years of age 47.3% (n = 146), while a mortality rate under 65 years old was 52.7% (n = 171). Previously, we reported a mortality rate of 64.7% over the age of 65 during pre-vaccination period. With these findings, we see that it also changes the mortality risk that occurs with aging due to vaccination in the geriatric age group. In the study of Guilon *et al*. [16], the demographic characteristics of the patients they followed in the intensive care unit were 72% in the geriatric age group, and they found mortality over 65 years old as 62.5% in the intensive care unit and 72.1% for the age group over 80 years old. They found a significantly higher mortality in the elderly group compared to the younger patient group [16]. Similar findings were reported from Italy. They found that 74% of all deaths in COVID-19 cases were in the geriatric age group [17]. In the United States, 67% of the cases were found to be ≥ 45 years old, with the same characteristics as in China, with 80% of the deaths being over the age of 65 [18]. These observations show that, with vaccination, COVID-19 infection is no longer an important risk in the elderly, and that the vaccine is effective.

Our final determination in our study is that active healthcare workers do not need intensive care as patients. Before the vaccination period, 2% of our intensive care patients were health workers. There are many studies report that health workers are affected all over the world. It was determined that 16.9% of COVID-19 cases in England were healthcare workers and 1.4% of these cases were severe disease cases. They stated that due to the excess viral load, the likelihood of a more severe course of COVID-19 disease in healthcare workers increased [19]. It was determined that 6.4% of the actively working hospital personnel from the Netherlands were positive for COVID-19 and the deaths of active and retired healthcare workers were 14% of all deaths [20]. In a study from Russia, %5 of those Who died in the pandemic were found to be healthcare workers [21]. In Turkey, the need for follow-up in intensive care units has decreased with regular widespread vaccination (as almost all of our healthcare workers are vaccinated). There was no vac-

Table 4. Comparison of biochemistry parameters according to mortality status in COVID-19 patients (n = 548)

Parameters	Death (n = 278)	Live (n = 270)	*p* value
Hemoglobin (g/dL)	10.1 ± 2.1	11.6 ± 1.8	**< 0.0001**
Leukocyte	9600 ± 6700	8800 ± 3700	**< 0.0001**
Thrombocyte	236000 ± 192000	212000 ± 160000	**< 0.0001**
D-dimer (mg/dL)*	1525.5 ± 658.2	618.4 ± 651.2	**< 0.0001**
Fibrinogen (mg/dL)	893.0 ± 307.3	605.4 ± 213.7	**< 0.0001**
LDH (U/L)	132.3 ± 239.2	109.2 ± 63.1	**< 0.0001**
Glucose (mg/dL)	107.2 ± 79.2	82.2 ± 33.6	0.339
Urea (mg/dL)*	82.4 ± 53.9	33.3 ± 14.1	**< 0.0001**
Kreatinin (mg/dL)*	3.8 ± 0.8	1.5 ± 0.9	**< 0.0001**
ALT (U/ml)	165.6 ± 436.4	42.2 ± 25.5	**< 0.0001**
AST (U/mL)*	213.4 ± 560.5	67.7 ± 11.0	**< 0.0001**
PT (seconds)	15.6 ± 21.4	10.1 ± 12.1	**0.0002**
aPTT (seconds)	38.6 ± 15.2	29.7 ± 4.3	**< 0.0001**
INR	2.4 ± 2.7	1.6 ± 3.2	**0.048**
CK (mg/dL)	641.0 ± 766.1	185.5 ± 32.1	**< 0.0001**
Bilirubin-total (mg/dL)	4.0 ± 3.9	1.6 ± 1.2	**< 0.0001**
CRP (mg/dL)**	410.2 ± 111.3	291.1 ± 12.3	**< 0.0001**
Ferritin (mg/dL)*	446.5 ± 389.7	212.4 ± 297.7	**< 0.0001**
PCT (mg/dL)*	40.5 ± 10.2	33.3 ± 5.8	**< 0.0001**
SOFA Score	12.5 ± 2.5	9.0 ± 1.8	**< 0.0001**
APACHE II Score	22.7 ± 4.3	18.1 ± 3.3	**< 0.0001**
Time of stay (day)*	9.4 ± 8.0	6.7 ± 5.9	**< 0.0001**
Intubation, n (%) **	250 (90)	68 (25)	**< 0.0001**

Data are shown as mean ± standard deviation or n (%). LDH = lactate dehydrogenase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, PT = prothrombin time, aPTT = activated partial thromboplastin time, INR = international normalized ratio, CK = creatine kinase, CRP = C-reactive protein, PCT = procalcitonin, SOFA = sequential organ failure assessment, APACEHE-II = acute physiology and chronic health evaluation II

Independent t-test was used and $p < 0.05$ was considered significant.

*Mann-Whitney U test was used. ** Pearson Chi-Square test was used.

cinated health worker that we followed in the intensive care units of our hospital where we conducted our study. The fact that there is no healthcare worker with vaccination who needs intensive care is clearly the result of the vaccine protection.

CONCLUSION

Vaccination to protect against SARS-CoV-2 infection reduces the COVID-19 intensive care unit stay and reduces mortality rates. Being unvaccinated increases the intensive care unit admission and mortality. Being unvaccinated carries risks for all age groups. As a result, COVID-19 vaccine studies continue rapidly in many countries around the world. However, more studies are needed on the efficacy and safety of these vaccines. We believe that one of the COVID-19 vaccines, which has high efficacy and safety, will positively affect the course of the pandemic and end it.

Authors' Contribution

Study Conception: İD; Study Design: İD; Supervision: ŞÇ; Funding: ŞÇ; Materials: BK; Data Collection and/or Processing: RSY; Statistical Analysis and/or Data Interpretation: RSY, HÖ; Literature Review: İD; Manuscript Preparation: İD and Critical Review: İD.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):790-799 Original Article

DOI: 10.18621/eurj.1033999 *Radiology*

Correlation of morphologic findings and apparent diffusion coefficient values with Ki-67 proliferation index in patients with glioblastoma

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ABSTRACT

Objectives: Glioblastoma is the most common primary neoplasm of the central nervous system (CNS) and has a very poor prognosis. Ki-67 proliferative index is a value that reflects the mitotic index of the tumor and is associated with poor prognosis. The radiological features of the tumors can predict the course of the disease. The aim of this study is to evaluate the relationship between the morphology and the apparent diffusion coefficient (ADC) values of the tumor with the Ki-67 index on preoperative magnetic resonance imaging (MRI).

Methods: Preoperative MRI images of 52 patients with pathological diagnosis of glioblastoma were evaluated retrospectively. A score ranging from 1 to 3 was assigned to each of the morphological features of the tumors (peritumoral edema, necrosis, contrasting pattern, heterogeneity, hemorrhage, mass effect, tumor contour irregularity), and was added up to obtain the total score. In addition, the ADC values of each tumor were measured at the workstation. ADC value and total score of each tumor, and Ki-67 values obtained histopathologically were compared.

Results: There was a negative correlation between Ki-67 index of tumors and ADC values (r = -0.895, p = 0.0001). A significant positive correlation was found between the morphological features of the tumors and their total scores (r = 0.772, p = 0.0001). A statistically significant negative correlation was found between total score and ADC values (r = -0.780, p = 0.0001). Heterogeneity and necrosis were the features most closely associated with Ki-67. These were followed by mass effect, hemorrhage and contour irregularity, respectively.

Conclusions: The morphological findings and ADC values obtained from preoperative MRI are related to the Ki-67 value, and thus can be used to predict prognosis and guide treatment in the early period.

Keywords: Glioblastoma, Ki-67, magnetic resonance imaging, prognosis

Glioblastoma is the most common and aggressive malignant brain tumor, classified as high grade according to the World Health Organization (WHO) classification in adults. It constitutes approximately 12-15% of all intracranial tumors. It is the most malignant form of astrocytomas with poor prognosis [1-

Received: December 8, 2021; Accepted: February 15, 2022; Published Online: June 8, 2022

How to cite this article: Öncü S, Şerifoglu I, Arslan FZ, Karagülle M, Şimşek S, Kaya GG, Cimilli AT. Correlation of morphologic findings and apparent diffusion coefficient values with Ki-67 proliferation index in patients with glioblastoma. Eur Res J 2022;8(6):790-799. DOI: 10.18621/eurj.1033999

e-ISSN: 2149-3189

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3]. It is very important to know the histological features and grade of astrocytic tumors in order to determine the treatment approach and predict the prognosis [4]. Ki-67 protein is a cellular marker associated with ribosomal RNA transcription and cell proliferation. Ki-67 proliferation index; it can objectively reflect the proliferation and malignancy of tumor cells [5]. It has been shown in previous studies that increased Ki-67 expression is positively correlated with an increased degree of malignancy and poor prognosis in glioblastoma patients [6]. However, defects in tumor sampling, particularly inadequate stereotactic biopsies and heterogeneity of astrocytomas, may lead to an underestimation of the degree of malignancy [7]. Also, histological examination cannot show tumor effects on adjacent brain tissue.

Magnetic resonance imaging (MRI) can accurately assess the macroscopic features of the tumor associated with clinical symptoms. Morphological findings such as peritumoral edema, necrosis, enhancement pattern, heterogeneity, hemorrhage, mass effect, tumor contour irregularity, and apparent diffusion coefficient (ADC) values obtained in diffusion-weighted imaging (DWI) represent the biological behavior of the tumor. Therefore, the integration of histological data with MRI findings may provide a better evaluation of the clinical outcomes of patients with astrocytoma [8]. Recently, many study evaluated advanced MRI or morphological imaging. However, as in this study, there are only few study in the literature assesing both advanced MRI or morphological imaging [9-11].

In this study, we compared the quantitative values obtained by scoring the morphological findings according to their severity and ADC values with the Ki-67 proliferative index. Thus, we wanted to reveal the prognostic importance of morphological findings and ADC values.

METHODS

Patients

The study included 52 patients who were diagnosed with glioblastoma histopathologically and had preoperative MRI (34 males and 18 females; mean age: 52.4 years; and age range: 5-81 years). Patients with a known history of malignancy and the solid component of the tumors smaller than 1 cm were excluded from the study. The patients included in the study had not undergone any previous tumor-related operation and did not receive chemotherapy or radiotherapy. The ethical approval was obtained from a local commitee (Decision No: 2019.12.2.08.098, Date: 27.12.2019).

MRI Protocol

All examinations were performed with a 1.5 T (Achieva, Philips, Netherlands) MRI device with an 8-channel head coil. The MRI protocol consists of the following sequences: Axial, coronal, and sagittal T2-weighted turbo spin-echo sequence (TR/TE=4800/100; FA:90; slice thickness: 5.5 mm; interslice gap, 1 mm; FOV 23 cm, NEX: 1), axial T1 weighted spin-echo sequence (TR/TE=450/15; FA:69; slice thickness: 5.5 mm; interslice gap, 1 mm; FOV 23 cm; NEX: 1), axial FLAIR sequence (TR/TE =7000 /97; FA:90; section thickness: 5.5 mm; interslice gap, 1 mm; FOV 23 cm; NEX: 1), sagittal T1-weighted gradient echo sequence after intravenous injection of 0.1 mmol/kg of contrast medium (TR/TE: 25/4 ms; FA: 30; slice thickness 1 mm; interslice gap, 1 mm; FOV 23 cm; NEX: 1). DWIs were obtained with single-shot SE echo planar imaging sequence (EPI) by applying gradient in three directions in the axial plane (TR/TE=2800/72; FA: 90; slice thickness 5 mm; interslice gap, 1 mm; FOV 23 cm) ; NEX: 2; b-values; 0 and 1000 s/mm2). The ADC map is automatically generated by the device.

Evaluation of Images

Images were evaluated by a radiologist with 13 years of neuroradiology experience (İ.Ş.). Ki-67 proliferation index values were not known during the evaluation. The following MRI morphological findings were scored between 1-3 according to their severity, and a total score was obtained by summing these scores for each tumor (Table 1).

DWI images were evaluated on the workstation of the MRI device company (IntelliSpace Portal, version 7.0.1, Philips Healthcare, Best, The Netherlands). While ADC mapping, which was created automatically by the MRI device, 3 ROIs (region of interest) were drawn manually within the non-cystic or non-necrotic, non-hemorrhagic and enhanced solid components of the tumors. The average of the values of

Table 1. Scores given to MRI characteristics of tumors

Edema	1: no or little edema
	2: there is edema, smaller than the tumor volume
	3: there is edema, more than tumor volume
Necrosis	1: no necrosis
	2: located less than half of the tumor
	3: located more than half of the tumor
Hemorrhage	1: no
	2: yes
Enhancement	1: no enhancement
	2: poor or nodular
	3: pronounced and heterogeneous
Heterogeneity	1: homogeneous tumor
	2: tumor is heterogeneous on T2WI
	3: the tumor is heterogeneous in both T1WI and T2WI
Mass effect	1: lost of subarachnoid space
	2: compression to the ventricular system
	3: midline shift
Irregular contour	1: well-circumscribed
	2: Irregular contour

MRI = magnetic resonance imaging, T1WI and T2WI = T1-and T2-weighted axial imaging

these 3 measurement was recorded as the final ADC value. On ADC mapping, ROIs drawn manually to the lowest ADC areas. Then automatically minimum, maximum and mean ADC values were obtained from measured area within ROI.

Ki-67 Expression Analysis

Ki-67 was assessed by immunohistochemistry using paraffin-embedded tissue samples which were cut into 5 μm thickslices. Briefly, all sections were deparaffinized and rehydrated, then antigen retrieval wasperformed. Non-specific binding sites were blocked by serum at 37 °C for 15 min (Beijing Zhongshan Golden Bridge Biotechnology Company, Beijing, China).The sections were stained with monoclonal-mouse anti-human Ki-67 antibody (Beijing Zhongshan Golden Bridge Biotechnology Company) was incubated in a humidified chamber at 37°C for 120 min. Then the specimens were incubated with secondary goat anti-mouse antibody, respectively (Beijing Zhongshan Golden Bridge Biotechnology Company, China) at 37 °C for 30 min. Ki-67 expression were visualized using 3,3′-diaminobenzidine (DAB)followed by counterstaining with hematoxylin. The expression of Ki-67 was determined in an area with the highest frequency of immunohistochemically stained nuclei on the assumption that it represent best the proliferation potential within a tumor. At least, 1000 cells were 400×counted at amagnification. Ki-67 was considered positive when the cell nuclei were stained brown-yellow. Ki-67 index was calculated as the percentage of positive cells.

Statistical Analysis

The statistical analyses were performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. In the evaluation of the data, besides the descriptive statistical methods (mean, standard deviation), the distribution of the variables was examined with the Shapiro-Wilk normality test. In the intergroup comparisons of the normally distributed variables; one-way analysis of variance, in comparison of paired groups; independent t test, in determining the relations

Table 2. Demographic distribution of the cases included in the study, minimum-maximum values and averages of Ki-67 and ADC values and total score

		n	Mean ± SD	Minimum	Maximum
Age (years)	**Man**	34	51.44 ± 16.96	5	81
	Woman	18	54.17 ± 13.07	32	79
	Total	52	52.38 ± 15.65	5	81
Ki-67		52	38.13 ± 17.64	8	90
ADC (× 10^{-3} mm^2/s)		52	0.8 ± 0.21	0.36	1.2
Total Score		52	15.56 ± 2.25	10	19

ADC = apparent diffusion coefficient

of variables with each other; Pearson correlation test was used. P value less than 0.05 was considered as significant.

RESULTS

Of the patients included in the study, 34 (65.38%) were male and 18 (34.62%) were female. The mean age of males was 51.44 ± 16.96 years, and the mean age of females was 54.17 ± 13.07 years. The mean age of all patients was 52.38 ± 15.65) years.

The lowest ki-67 value of tumors with pathological diagnosis of glioblastoma was 8, while the highest value was 90. The mean ki-67 value was found to be 38.13 ± 17.648). The lowest ADC value was 0.36 × 10-3 mm^2/s and the highest was 1.2 × 10-3 mm^2/s. The mean ADC value was 0.8 ± 0.21 × 10-3 mm^2/s. The total score obtained by scoring the morphological features of the tumors was found to be 10 as the lowest

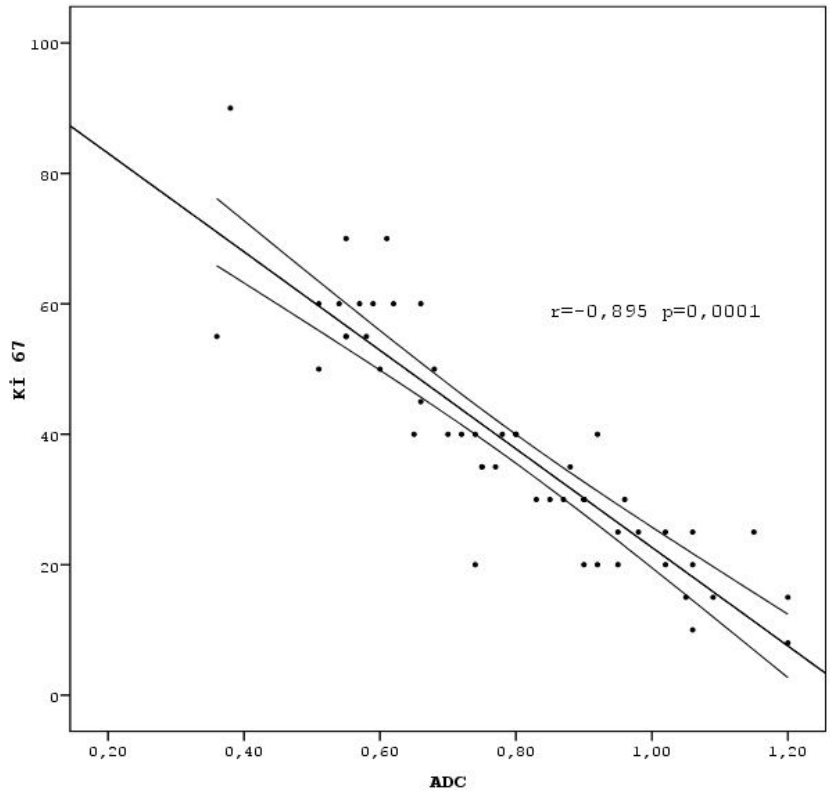

Fig. 1. Negative correlation graph between Ki-67 and ADC values. ADC = apparent diffusion coefficient

Table 3. Relationship of Ki-67, ADC and total score values in tumors with each other and patient age

		Ki-67	ADC	Total Score
Ki-67	**r**		-0.895	0.772
	***p* value**		**0.0001**	**0.0001**
ADC	**r**	-0.895		-0.780
	***p* value**	**0.0001**		**0.0001**
Total Score	**r**	0.772	-0.780	
	***p* value**	**0.0001**	**0.0001**	

ADC = apparent diffusion coefficient, Pearson Correlation Test

and 19 as the highest. The mean total score value was found to be 15.56 ± 2.25. (Table 2).

Pearson correlation test was used to determine the relationships between Ki-67 proliferation index, ADC and morphological total score values. There was a negative correlation between Ki-67 index values and ADC values (Fig. 1), and a positive correlation between Morphological Total Score values (r=-0.895, p = 0.0001), (r = 0.772, p = 0.0001). A statistically significant negative correlation was observed between ADC values and morphological total Score values (r = -0.780, p = 0.0001) (Table 3 and Figs. 2 and 3).

Morphological Features and Ki-67 Index

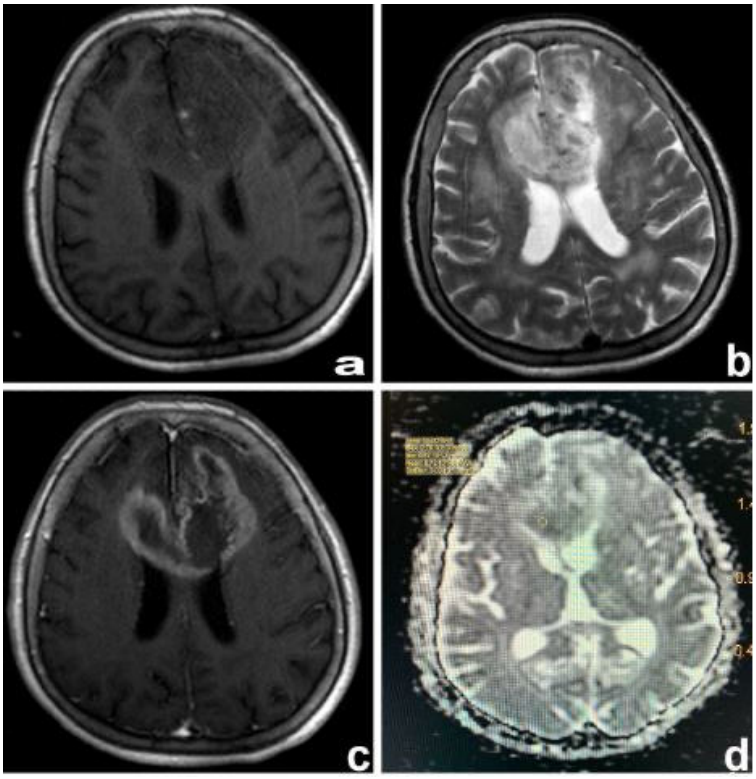

Fig. 2. A 73-year-old female patient has a lesion with a diameter of 62 mm located at the frontal region. Hyperintense foci determined as hemorrhage are seen in T1WI (a). It is observed that the lesion is shifted on contrast-enhanced T2W and T1W images, extends from the midline to the opposite hemisphere, contains large areas of necrosis, has irregular contours, shows intense contrast enhancement, has a small amount of peritumoral edema, and is heterogeneous only on T2W (b, c). A total of score was 17 obtained from their morphological features. ADC value of 0.72 × 10-3 mm2/sec was obtained (d) from the areas showing tumoral enhancement. T1WI and T2WI = T1-and T2-weighted axial imaging, ADC = apparent diffusion coefficient.

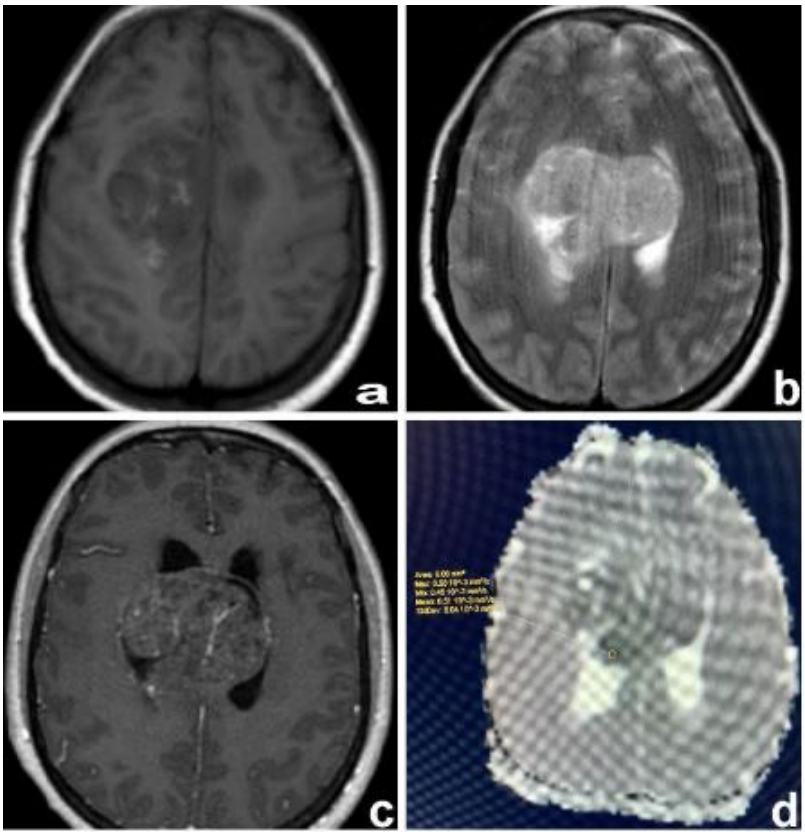

Fig. 3. A 37-year-old female patient has a lesion located at the cingulate gyrus- corpus callosum region with a 57 mm diameter. On T1W images, hyperintense foci evaluated as hemorrhage are seen (a). It is observed that the lesion shifts and extends to the opposite hemisphere on contrast-enhanced T2W and T1W images, contains necrosis less than half of the tumor volume, has regular contours, shows little contrast, has little peritumoral edema, and is heterogeneous in both T1W and T2W (a, b, c). A total of score was 15 obtained from their morphological features. ADC value of 0.51 × 10-3 mm2/sec was obtained (d) from the areas showing tumoral enhancement. T1WI and T2WI = T1-and T2-weighted axial imaging, ADC = apparent diffusion coefficient.

Heterogeneity and necrosis were the features that were most closely associated with Ki-67 proliferation index values ($p = 0.0001$). Tumors heterogeneous on both T1 and T2W had a higher Ki-67 mean than tumors heterogeneous on T2W only ($p = 0.0001$). In addition, mean Ki-67 values of tumors where more than half of the tumor volume is necrosis; were higher compared to tumors with less necrosis ($p = 0.0001$). These were followed by mass effect, hemorrhage and contour irregularity, respectively. There was no statistically significant correlation between the amount of edema and Ki-67 ($p = 0.058$) (Table 4).

DISCUSSION

Ki-67 protein is a cellular marker associated with cell proliferation and can objectively reflect tumor aggression [12, 13]. It has been shown in many studies that increased Ki-67 expression shows poor prognosis in glioblastoma patients [14, 15]. Tissue sampling and histopathological examination used in glioblastoma grading and selection of treatment algorithms have some limitations. Inadequate tissue sampling, especially during stereotactic needle biopsy and after tumor resection, causes inaccurate evaluations in some patients due to the inability to examine every section of the tumor and the dependency of the practitioner [16, 17].

MRI, on the other hand, provides information about the macroscopic features and degree of diffusion that histological examination can not evaluate. Therefore, MRI findings of tumors can be used to contribute to classification and predict prognosis. In previous studies, it was shown that there may be a correlation between the morphological features of gliomas (pe-

Table 4. The relationship of morphological features with the Ki-67 index

		n	Ki-67	*p* value
Edema	1: No or little edema	3	33.33 ± 20.21	0.058‡
	2: There is edema, smaller than the tumor volume	20	31.40 ± 15.47	
	3: There is edema, more than tumor volume	29	43.28 ± 17.69	
Necrosis	1: No necrosis	1	25	**0.0001***
	2: Located less than half of the tumor	26	28.19 ± 11.70	
	3: Located more than half of the tumor	25	49.00 ± 16.77	
Hemorrhage	1: No	30	31.93 ± 14.59	**0.002***
	2: Yes	22	46.59 ± 18.22	
Enhancement	1: No enhancement	1	10	**0.011***
	2: Poor or nodular	11	27.09 ± 13.19	
	3: Pronounced and heterogeneous	40	41.88 ± 17.12	
Heterogeneity	1: Homogeneous tumor	4	19.50 ± 11.45	**0.0001‡**
	2: Tumor is heterogeneous on T2WI	30	33.67 ± 16.55	
	3: The tumor is heterogeneous in both T1WI and T2WI	18	49.72 ± 13.56	
Mass effect	1: Lost of subarachnoid space	8	23.75 ± 8.35	**0.002‡**
	2: Compression to the ventricular system	14	32.00 ± 16.00	
	3: Midline shift	30	44.83 ± 17.15	
Irregular contour	1: Well-circumscribed	14	27.14 ± 6.99	**0.005***
	2: Irregular contour	38	42.18 ± 18.69	

‡ One-Way Analysis of Variance
* Independent t test

ripheral edema, mass effect, degree of enhancement due to blood-brain barrier destruction, degree of intratumoral necrosis, heterogeneity, hemorrhage) and the grade of tumor in these features [18-20]. The role of MRI findings and ADC values in predicting prognosis in glioblastoma patients can be investigated by revealing their relationship with the Ki-67 proliferation index, which is another important prognostic marker. Studies have shown that ADC values are associated with tumor grade, proliferation status and prognosis. Shahmohammadi *et al*. [21] found a negative correlation between ADC values and Ki-67 values in glioma patients (r = −0.634, p = 0.027). Calvar *et al*. [22] showed an inverse relationship between ki-67 and ADC values in glioblastoma patients. Higano *et al*. [23] found a negative correlation between Ki-67 and ADC values among malignant astrocytic (anaplastic astrocytoma and glioblastoma) patients, but no significant association was found in the glioblastoma group. Oh *et al*. [24] investigated the relationship between ADC values and survival by examining the images before surgery and chemoradiotherapy in glioblastoma patients. They showed that patients with low ADC values have a shorter average life expectancy (11.2 and 21.7 months, respectively) [24]. In our study, we found a negative correlation between ADC values and Ki-67 values of glioblastoma patients, which is consistent with the literature (r = -0.895 p = 0.0001). Our results parallel to the literature findings that there is a significant relationship between ADC values and Ki-67 and thus life expectancy in glioblastoma patients.

Highly malignant cerebral tumors are usually associated with significant amounts of necrosis [25]. Detection of necrosis on MRI scanning is a necessary criterion to include glioblastoma in the differential diagnosis. Hasse *et al*. [26] showed that patients with high Ki-67 values had a higher rate of necrosis on MRI examinations. Hammoud *et al*. [27] found that as the amount of necrosis increased in glioblastomas, the mean life expectancy decreased. Lacroix *et al*. [28]

showed that as the amount of necrosis increased in glioblastoma patients, median survival decreased. Among the 52 patients included in our study, we found that the mean of Ki-67 of the patients with necrosis volume more than half of the tumor volume was higher than the other patients, and the mean ADC was lower ($p = 0.0001$ and $p = 0.001$). In addition, in our study, we found that necrosis and heterogeneity were MRI findings that showed the best correlation with the Ki-67 proliferation index ($p = 0.0001$). In the light of these data, detection of necrosis and lesion heterogeneity in preoperative MRI evaluations can provide information about histological grade and prognosis.

It is known that the mass effect formed by the tumors adversely affects the prognosis. Steed *et al*. [29] showed that lateral ventricular compression adversely affects the prognosis. Park *et al*. [30] showed that patients with glioblastoma with tumor volume greater than 50 cm3 had poor postoperative survival. Gamburg *et al*. [31] showed that the life expectancy of shifting tumors was shorter. In another study, the Ki-67 values of the midline shifting group were higher than the Ki-67 values of the tumors causing ventricular compression and causing lost in the subarachnoid space [32]. We think that tumor volume and mass effect should be evaluated and clearly stated in MRI reports, since they affect the treatment method in glioblastoma patients and provide valuable information about prognosis. On MRI, glioblastomas are generally seen as necrotic centrally and enhanced as peripheral rims [33-35]. The contrast may be minimal, nodular, or prominent. In our study, the mean Ki-67 of the significantly enhanced group was statistically significantly higher compared to the minimally enhanced group ($p = 0.011$). Tervonen *et al*. [36] was found that significant contrast enhancement was associated with advanced tumor grade and high cellularity. Although neglected compared to other morphological features of glioblastoma, tumor surface features can also provide important information about tumor progression. Surface irregularity is an important prognostic marker [37, 38]. In our study, we found that the ki-67 values of tumors with irregular borders were significantly higher ($p = 0.005$).

A large amount of edema around the glioma suggests a more malignant phenotype [39, 40]. Levin *et al*. [41] showed that the presence of a large peritumoral low-density area (edema) on CT scan was associated with favorable tumor progression. In contrast to these, Taillandier *et al*. [42] showed that the amount of peritumoral edema had no effect on clinical outcome. In our study, when we classified the patients according to the amount of peritumoral edema, we could not find a statistically significant difference between the ki-67 values ($p = 0.058$).

Hemorrhage represents bleeding within the tumor matrix. Hypertension, anticoagulation, surgical intervention, angiogenesis and invasion of blood vessels by malignant cells may cause hemorrhage [43]. McGahan *et al*. [44] showed that the presence of hemorrhage in glioblastomas had no effect on prognosis. Contrary to this result, we found that the mean Ki-67 values of hemorrhagic tumors is higher than the non-hemorrhagic ($p = 0.002$).

We created a total score by summing the scores we obtained from each MRI finding. When we compared the total scores with the Ki-67 values, there was a positive correlation. Tumors with a high total score also had high Ki-67 values ($r = 0.772$, $p = 0.0001$). Semiquantitative evaluation of morphology findings in MRI examinations of glioblastomas can give us very important clues about the course and prognosis of the disease, which may support histopathological classification and help choose the right treatment methods.

Limitations

Our study had some limitations. The patient age was not included as contributing parameter in the multivariate analysis is not included. We did not measure the morphological findings with computerized systems in order to be fast and practical. Naturally, this may cause subjective results. In addition, we used only Ki-67 values histopathologically to determine the prognostic value of the scores we obtained from the morphological findings. For more accurate results, future studies should compare MRI morphological findings with other histopathological markers that have been shown to have an effect on prognosis. The measurements were made by a single reader, which reduces the reliability of the study.

CONCLUSION

In conclusion; despite the advances in diagnosis and

treatment methods in glioblastomas in recent years, delayed diagnosis and treatment, inadequate histopathological examination may occur. Preoperative MRI findings can be used to prevent delayed diagnosis and to contribute to the histopathological examination. With MRI scoring, radiologists can easily determine the possible pathological grade of the tumor. Thanks to the scoring, where we can get information about almost every patient non-invasively, we can predict the prognosis in the early period and shape the treatment process in glioblastoma patients.

Authors' Contribution

Study Conception: SÖ; Study Design: FZA; Supervision: FZA; Funding: SÖ; Materials: SÖ; Data Collection and/or Processing: İŞ, FZA, MK; Statistical Analysis and/or Data Interpretation: SŞ, GGK, ATC; Literature Review: FZA; Manuscript Preparation: FZA and Critical Review: FZA.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):800-809 Original Article

DOI: 10.18621/eurj.968032 *Biostatistics*

Investigation of cause-specific mortality rates of European Union member and candidate countries by World Health Organization global health estimate categories

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ABSTRACT

Objectives: It is aimed to examine the position of our country and the European Union member and candidate countries in terms of mortality rates according to the motality causes defined in global health estimate categories determined by the World Health Organization and to reveal the similarities or differences.

Methods: According to the World Health Organization global health estimate categories given in the Global Burden of Disease 2019 study of World Health Organization, age-standardized mortality rates per 100,000 population were obtained for a total of 31 European Union member and candidate countries, and the muldimensional scaling analysis performed groups of the countries according to their dimensions obtained from multidimensional scale were determined and among these groups comparisons have been made.

Results: As a result of applying multidimensional scaling analysis, it was seen that countries can be represented in two-dimensional space according to the variables of interest.

Conclusions: It has been observed that our country differs from countries with cardiovascular diseases in the first dimension from the World Health Organization categories, while in the second dimension, infectious and parasitic diseases differ from countries with high standardized mortality rates.

Keywords: Multidimensional scaling, World Health Organization, global health estimate categories, Euclidean distance

Global, regional and country statistics on population and health indicators are critical for assessing development and health progress and guiding resource allocation [1]. Knowing the causes of death is important for the continuity of both preventive and curative services [2]. Understanding the causes of death of people enabling an effective response to changing epidemiological conditions, reducing preventable deaths and adapting health systems to respond effectively; will help improve health services in every country. This is as in the health policy of countries; it will guide the policies and resource allocations to be followed in different sectors such as transportation, food and agriculture.

The main cause of death is defined as the disease or injury that directly initiates the process resulting in death [3, 4]. The World Health Organization (WHO) is grouped the causes of death and disability into three large categories: communicable (infectious diseases, along with maternal, perinatal and nutritional conditions), noncommunicable (chronic diseases) and injuries. The sub-categories also have been given [1].

Received: ***July 9, 2021;*** *Accepted:* ***September 13, 2021;*** *Published Online:* ***September 14, 2021***

How to cite this article: *Sığırlı D, Kılıçarslan S. Investigation of cause-specific mortality rates of European Union member and candidate countries by World Health Organization global health estimate categories. Eur Res J 2022;8(6):800-809. DOI: 10.18621/eurj.968032*

e-ISSN: 2149-3189

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Statistics of deaths by cause are reported annually to WHO by country, year, age and sex. These statistics are available from the WHO Mortality Database [5]. Societies may differ in terms of various characteristics that may affect mortality. Age distribution is one of the most important features [6]. For this reason, it is important to use standardized criteria, especially according to age and gender, when comparing countries, regions, etc. When Turkey's death statistics are analyzed according to their causes, circulatory system diseases took the first place with 36.8% in 2019. This cause of death was followed by benign and malignant tumors with 18.4% and respiratory system diseases with 12.9% [7].

In this study, with multidimensional scaling analysis, it is investigated how the European Union member and candidate countries are grouped according to different dimensions of cause-related mortality rates and from which variables the differences between these groups arise. In this direction, in this study, it is aimed to evaluate the current situation of Turkey among other countries in terms of cause-specific mortality rates.

METHODS

Multidimensional scaling (MDS) is a statistical analysis method used to classify objects or units by identifying similarities and differences with the help of various distance measures [8, 9]. MDS analysis is a graphical method that helps to obtain the representation of objects or units in a space consisting of an appropriate number of dimensions, using distances calculated by various techniques depending on the variables included in the analysis. Thus, it helps to determine the relationships between both units and variables [10]. It is a method that can be applied in many fields such as health sciences, social sciences, educational sciences, marketing research [11-13]. For example, MDS analysis was used to group patients with similar diagnoses in psychiatry according to their similarities and evaluate the course of the disease according to the disease groups determined [14]. Rouzier *et al*. [15] used multidimensional scaling analysis in their study where they suggested that the molecular classification of breast cancer be made based on the gene structures of human tumors.

Depending on the type of data, metric and non-metric scaling techniques are used in MDS analysis. While the metric MDS technique is used for data measured with the least interval scale, the non-metric MDS technique should be applied for data measured with an ordinal scale [16-19]. Observational differences and compatibility of distances are evaluated with Shepard diagram and R^2 value. The Shepard diagram shows the relationship between the observed distances and the configuration distances obtained by the MDS analysis [11, 16-18]. A R^2 value of ≥ 0.60 indicates a good fit [20].

In the MDS analysis, the stress value (i.e. the measure of correspondence between the original distances and the display distances), which is an expression of the difference between the multidimensional (p-dimensional) real shape and the predicted shape in reduced (k-dimensional) space, is calculated. The stress value is given in Equation (1) [20]. It is desirable that the stress value be close to zero [20-22].

$$\text{Stress}=\sqrt{\frac{\sum\sum(d_{ij}-\hat{d}_{ij})^2}{\sum(d_{ij})^2}} \quad (1)$$

d_{ij}: i. and j. configuration distance between individuals

$\hat{d}_{ij}$: i. and j. defined as the data distance between individuals.

A low stress rate indicates that the MDS solution is appropriate. A high value indicates a bad fit. The fitness values corresponding to the stress value presented by Kruskal in 1964 are given in Table 1 [14, 20, 23, 24].

Generally, two or at most three dimensions are preferred for dimension selection in MDS analysis. The number of dimensions is decided according to the stress value, R^2 value, Shepard diagram [25-28].
There are various measures of distance and similarity that are used in calculating distances in MDS. These

Table 1. Interpretation of the stress value

Stress value	Compatibility
≤ 0.20	Incompatible display
0.10- < 0.20	Low fit
0.05 - < 0.10	Good fit
0.025- < 0.05	Perfect fit
0.00- < 0.025	Complete fit

can be listed as follows;

a) Euclidean (Euclidean) Distance Measure

In a p-variable structure i. and j. The euclidean distance between the observations is as follows.

It is one of the most commonly used distance measures.

$$d_{ij=}\sqrt{\sum_{k=1}^{p}\left(x_{ik-}x_{jk}\right)^2} \quad (2)$$

x_{ik}: i. observation k. variable value,
x_{jk}: j. observation k. variable value,
p: the number of variables.

Square Euclidean (Square Euclidean) measure of distance

$$d_{ij} = \sum_{k=1}^{p}\left(x_{ik-}x_{jk}\right)^2 \quad (3)$$

a) Chebychev distance measure

$$d_{ij} = max_k^p \, |x_{ik}\text{-}\, x_{jk}| \, (4)$$

b) Manhattan City-Block distance measure

$$d_{ij=} \sum_{k=1}^{p} |x_{ik} - x_{jk}| \, (5)$$

c) Minkowski distance measure

m=1 için Manhattan City-Block measure of distance, m=2 için returns the measure of Euclidean distance. As m increases, the distance approaches the Chebychev distance measure.

$$d_{ij} = \left[\sum_{k=1}^{p} |x_{ik} - x_{jk}|^m\right]^{\frac{1}{m}} \quad (6)$$

a) Karl Pearson distance measure/ Standardized Measure of Euclidean Distance is in the form [11].

$$d_{ij} = \sqrt{\sum_{k=1}^{p} \frac{1}{s_k^2}\left(x_{ik} - x_{jk}\right)^2} \quad (7)$$

In this study, age-standardized cause-related mortality rates were taken. Causes of death categories were made according to the Global Health Estimates (GHE) classification in the 2019 World Health Report and are given in Table 2 [1, 2]. In case of missing data for one or more countries in the sub-categories, the parent category to which that sub-category belongs was taken into account (Table 2). Euclidean distance was used in the determination of the distance matrix in the MDS. These data are has been obtained from 31 countries European Union members Germany, Austria, Belgium, Bulgaria, France, France, Netherlands, Finland, Ireland, France, Sweden, Italy, Cyprus, Let, Lithuania, Luxembourg, Hungary, Malta, Poland, Portugal, Romania, Slovakia, Slovenia, Greece and candidate countries Albania, Macedonia, Serbia and Turkey. Candidate country Montenegro was not in-

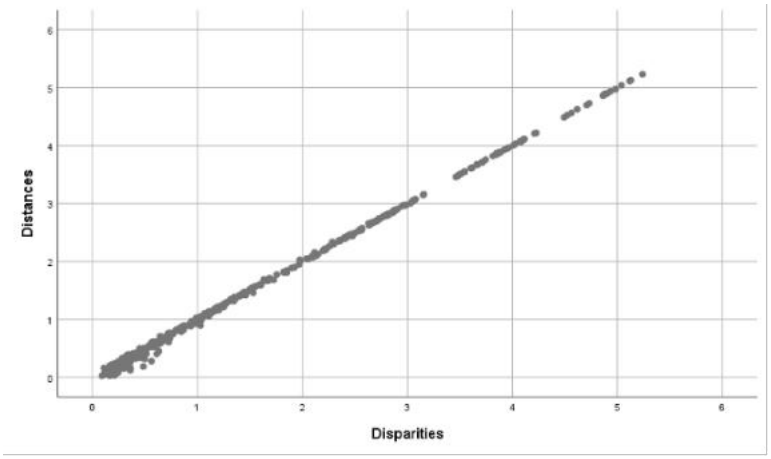

Fig. 1. Shepard diagram for observed distances and configuration distances

Table 2. Global health estimate cause categories and ICD-10 codes

GHE code	GHE cause	ICD-10 code
0	**All causes**	
10	**I. Communicable, maternal, perinatal and nutritional conditions[#]**	
20	I.A Infectious and parasitic diseases	A00-B99, G00-G04, G14, N70-N73, P37.3, P37.4
380	I.B. Respiratory Infectious	H65-H66, J00-J22, P23, U04
420	I.C. Maternal conditions	O00-O99
490	I.D. Neonatal conditions	P00-P96 (minus P23, P37.3, P37.4)
540	I.E. Nutritional deficiencies	D50-D53, D64.9, E00-E02, E40-E46, E50-E64
600	**II. Noncommunicable diseases[#]**	
610	II.A. Malignant neoplasms[#]	C00-C97
620	1. Mouth and oropharynx cancers	C00-C14
630	2. Oesophagus cancer	C15
640	3. Stomach cancer	C16
650	4. Colon and rectum cancers	C18-C21
660	5. Liver cancer	C22
670	6. Pancreas cancer	C25
680	7. Trachea, bronchus, lung cancers	C33-C34
690	8. Melanoma and other skin cancers	C43-C44
700	9. Breast cancer	C50
710	10. Cervix uteri cancer	C53
720	11. Corpus uteri cancer	C54
730	12. Ovary cancer	C56
740	13. Prostate cancer	C61
742	14. Testicular cancer*	C62
745	15. Kidney cancer	C64-C66
750	16. Bladder cancer	C67
751	17. Brain and nervous system cancers	C70-C72
752	18. Gallbladder and biliary tract cancer	C23-C24
753	19. Larynx cancer	C32
754	20. Thyroid cancer	C73
755	21. Mesothelioma	C45
760	22. Lymphomas, multiple myeloma	C81-C90, C96
770	23. Leukaemia	C91-C95
780	24. Other malignant neoplasms*	C17, C26-C31, C37-C41, C46-C49, C51, C52, C57-C60, C63, C68, C69, C74-C75, C77-C79
790	II.B. Other neoplasms*	D00-D48
800	II.C. Diabetes mellitus	E10-E14 (minus E10.2, E11.2, E12.2, E13.2, E14.2)
810	II.D. Endocrine, blood, immune disorders	D55-D64 (minus D64.9), D65-D89, E03-E07, E15-E34, E65-E88
820	II.E. Mental and substance use disorders	F04-F99, G72.1, Q86.0, X41-X42, X44, X45
940	II.F. Neurological conditions	F01-F03, G06-G98 (minus G14, G72.1)
1020	II.G. Sense organ diseases*	H00-H61, H68-H93
1100	II.H. Cardiovascular diseases	I00-I99
1170	II.I. Respiratory diseases	J30-J98
1210	II.J. Digestive diseases	K20-K92
1260	II.K. Genitourinary diseases	E10.2-E10.29,E11.2-E11.29,E12.2,E13.2-E13.29,E14.2, N00-N64, N75-N76, N80-N98
1330	II.L. Skin diseases	L00-L98
1340	II.M. Musculoskeletal diseases	M00-M99
1400	II.N. Congenital anomalies	Q00-Q99 (minus Q86.0)
1470	II.O. Oral conditions *	K00-K14
1505	II.P. Sudden infant death syndrome	R95
1510	**III. Injuries [#]**	
1520	III.A. Unintentional injuries	V01-X40, X43, X46-59, Y40-Y86, Y88, Y89
1600	III.B. Intentional injuries	X60-Y09, Y35-Y36, Y870, Y871

[#] Subcategories included, [*] Could not include due to lack of data.
GHE = Global health estimate cause categories defined in "WHO methods and data sources for country-level causes of death 2000-2019. (Global Health Estimates Technical Paper WHO/DDI/DNA/GHE/2020.2). Geneva, World Health Organization; 2020." [1]

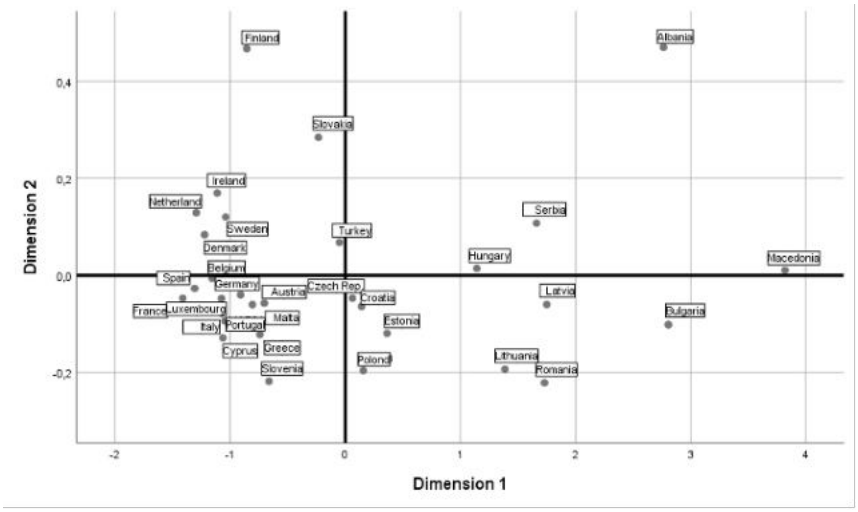

Fig. 2. **Graph of the Euclidean distance model**

cluded in the study due to lack of data.

Statistical Analysis

IBM Statistics SPSS for Windows v. 25.0 package program was used. The significance level was accepted as $\alpha = 0.05$. According to the analysis results obtained, the Kruskall stress statistic was found to be 0.022 for the r = 2 dimension. Accordingly, we can say that 2 dimensional scaling adequately reflects the data set we have. The Shepard graph showing the observed distances and the distribution of the configuration distances was found as in Fig. 1. The R^2 value is 0.99. Accordingly, it has been determined that there is a linear relationship between two different distance values and that a suitable solution can be presented with a linear model to the data. The positions of each country relative to each other in terms of cause-specific mortality rates selected by WHO are given in the two-dimensional graph of the Euclidean distance model in Fig. 2. Germany, Austria, Belgium, Czechia, Den-

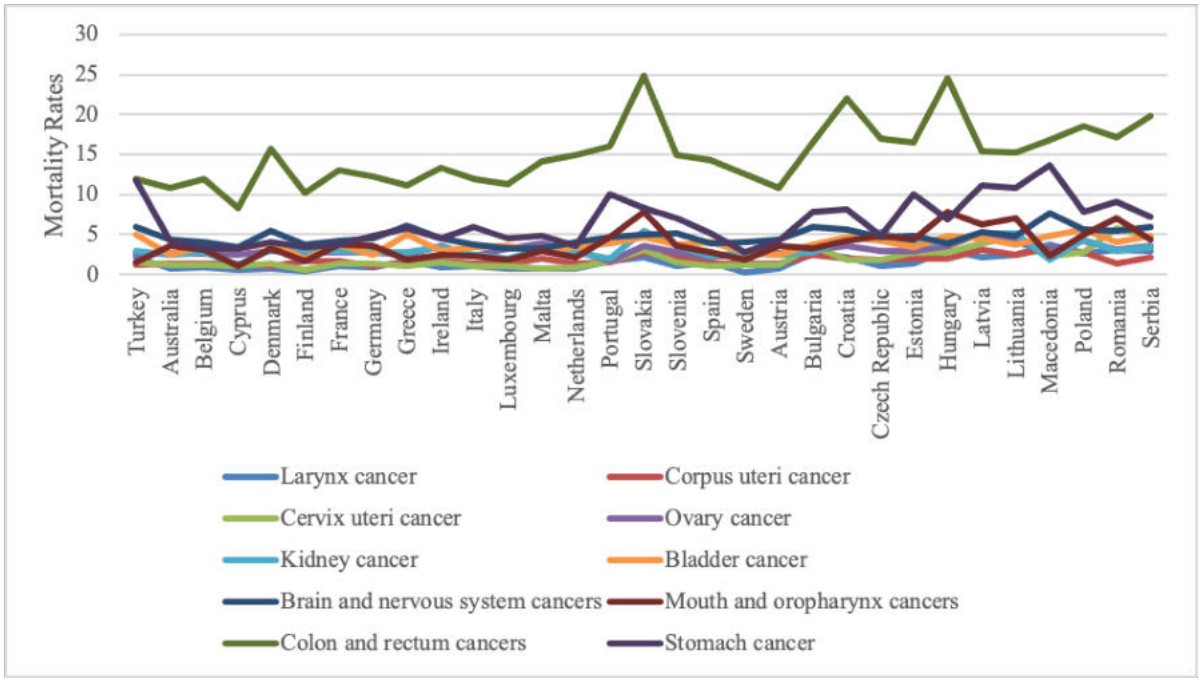

Fig. 3. **Distribution of mortality rates of countries according to cancer types which are found significant according fo first dimension**

Table 3. Comparison of variables according to the first dimension

Cause of death categories	x < 0	x > 0	*p* - value
Infectious and parasitic diseases	6.40 (1.90-17.50)	6.35 (2.70-12.70)	0.968
Respiratory infectious	8.40 (2.80-20.30)	10.85 (5.60-26.30)	0.311
Maternal conditions	0.10 (0.00-0.10)	0.10 (0.00-0.30)	0.115
Neonatal conditions	2.40 (1.30-6.20)	2.60 (1.30-6.60)	0.556
Nutritional deficiencies	0.70 (0.00-1.90)	0.35 (0.10-1.30)	0.061
Mouth and oropharynx cancers	2.70 (1.10-7.90)	4.60 (1.90-7.90)	**0.004**
Oesophagus cancer	2.50 (0.50-5.80)	2.45 (1.00-4.20)	0.871
Stomach cancer	4.50 (2.70-11.70)	8.65 (4.80-13.70)	**0.001**
Colon and rectum cancers	12.50 (8.30-24.90)	16.90 (4.30-24.50)	**0.001**
Liver cancer	4.50 (3.20-6.70)	4.35 (3.10-9.00)	0.382
Pancreas cancer	7.80 (5.50-9.50)	8.10 (4.80-10.40)	0.291
Trachea, bronchus, lung cancers	24.80 (16.10-39.70)	26.15 (21.50-43.50)	0.156
Melanoma and other skin cancers	2.10 (1.50-3.90)	2.80 (1.40-4.00)	0.059
Breast cancer	9.90 (6.40-11.00)	10.75 (5.50-14.20)	**0.024**
Cervix uteri cancer	1.20 (0.60-3.10)	2.80 (1.10-5.80)	**< 0.001**
Corpus uteri cancer	1.40 (1.10-2.80)	2.10 (0.90-3.30)	**0.004**
Ovary cancer	2.70 (1.70-3.90)	3.30 (0.90-5.20)	**0.009**
Prostate cancer	6.00 (3.90-9.50)	7.10 (5.40-8.90)	0.155
Kidney cancer	2.80 (1.20-5.50)	4.00 (1.90-5.30)	**0.033**
Bladder cancer	3.50 (2.00-5.00)	4.55 (3.40-6.60)	**0.003**
Brain and nervous system cancers	4.20 (3.20-6.10)	5.55 (3.90-7.70)	**0.004**
Gallbladder and biliary tract cancer	1.30 (0.60-3.80)	2.15 (0.90-3.30)	0.053
Larynx cancer	0.90 (0.30-2.20)	2.55 (1.10-3.70)	**< 0.001**
Thyroid cancer	0.40 (0.30-2.20)	0.40 (0.30-0.60)	0.180
Mesothelioma	0.70 (0.20-1.50)	0.20 (0.00-0.90)	**< 0.001**
Lymphomas, multiple myeloma	5.60 (4.70-9.00)	5.30 (1.60-6.80)	**0.046**
Leukaemia	4.20 (2.80-5.60)	4.50 (3.30-4.90)	0.556
Diabetes mellitus	6.50 (3.50-20.10)	10.80 (4.20-32.60)	0.081
Endocrine, blood, immune disorders	3.90 (1.90-5.90)	1.65 (0.50-3.50)	**< 0.001**
Mental and substance use disorders	4.60 (1.30-14.60)	4.60 (0.80-13.80)	0.823
Neurological conditions	28.20 (14.80-66.10)	17.95 (6.50-48.10)	**0.001**
Cardiovascular diseases	96.50 (68.90-173.50)	295.25 (182.30-470.60)	**< 0.001**
Respiratory diseases	22.90 (11.90-35)	21.85 (9.40-37.70)	0.968
Digestive Diseases	16.10 (11.10-33.80)	28.50 (14.10-45.00)	**< 0.001**
Genitourinary Diseases	8.80 (2.40-20.50)	9.50 (5.80-23.00)	0.516
Skin diseases	0.60 (0.10-1.80)	0.60 (0.00-1.50)	0.553
Musculoskeletal Diseases	1.80 (0.90-3.00)	1.20 (0.20-2.40)	**0.047**
Congenital anomalies	2.80 (1.40-6.00)	2.85 (1.80-4.40)	0.984
Sudden infant death syndrome	0.20 (0.00-0.40)	0.15 (0.10-0.40)	0.847
Unintentional injuries	16.60 (10.40-29.70)	21.55 (13.40-35.30)	**0.002**
Intentional injuries	9.10 (4.30-15.10)	10.00 (7.00-24.40)	0.282

Table 4. Comparison of variables according to the second dimension

Cause of death categories	y < 0	y > 0	*p* - value
Infectious and parasitic diseases	7.00 (1.90-17.50)	4.00 (2.20-7.90)	**0.022**
Respiratory infectious	10.60 (4.30-26.30)	10.50 (2.80-20.30)	0.726
Maternal conditions	0.10 (0.00-0.30)	0.10 (0.00-0.20)	0.783
Neonatal conditions	2.30 (1.30-4.70)	3.00 (1.30-6.60)	0.103
Nutritional deficiencies	0.50 (0.00-1.90)	0.40 (0.10-1.00)	0.507
Mouth and oropharynx cancers	3.55 (1.10-7.10)	2.30 (1.50-7.90)	0.173
Oesophagus cancer	2.45 (0.50-4.20)	2.50 (1.00-5.80)	0.695
Stomach cancer	5.90 (3.30-11.20)	6.80 (2.70-13.70)	0.918
Colon and rectum cancers	14.60 (8.30-22.10)	15.00 (4.30-24.90)	0.710
Liver cancer	4.50 (3.10-8.30)	4.20 (3.20-9.00)	0.804
Pancreas cancer	7.80 (5.50-10.30)	8.00 (4.80-10.40)	0.741
Trachea, bronchus, lung cancers	23.80 (16.60-34.90)	28.80 (16.10-43.50)	0.215
Melanoma and other skin cancers	2.20 (1.50-3.90)	2.90 (1.40-4.00)	0.086
Breast cancer	9.90 (6.90-11.50)	10.10 (5.50-14.20)	0.820
Cervix uteri cancer	1.45 (0.70-5.80)	1.50 (0.60-4.60)	0.918
Corpus uteri cancer	1.55 (1.10-3.10)	1.60 (0.90-3.30)	0.663
Ovary cancer	2.90 (1.70-5.20)	2.90 (0.90-3.60)	0.852
Prostate cancer	5.80 (3.90-8.90)	6.80 (5.70-9.50)	**0.039**
Kidney cancer	2.85 (1.20-5.30)	3.00 (1.90-5.50)	0.710
Bladder cancer	3.75 (2.50-5.60)	4.60 (2.00-6.60)	0.374
Brain and nervous system cancers	4.70 (3.20-6.10)	5.00 (3.80-7.70)	0.374
Gallbladder and biliary tract cancer	1.30 (0.60-3.30)	1.40 (0.70-3.80)	0.320
Larynx cancer	1.20 (0.50-3.10)	2.00 (0.30-3.70)	0.984
Thyroid cancer	0.40 (0.30-0.60)	0.40 (0.30-0.90)	0.692
Mesothelioma	0.55 (0.10-1.10)	0.40 (0.00-1.50)	0.559
Lymphomas, multiple myeloma	5.60 (3.80-9.00)	5.30 (1.60-7.40)	0.663
Leukaemia	4.35 (3.10-5.00)	4.10 (2.80-5.60)	0.508
Diabetes mellitus	7.95 (4.20-16.50)	7.40 (3.50-32.60)	0.853
Endocrine, blood, immune disorders	3.05 (0.60-5.90)	3.00 (0.50-4.40)	0.353
Mental and substance use disorders	4.90 (1.40-14.60)	4.10 (0.80-11.00)	0.509
Neurological conditions	23.05 (6.50-34.50)	40.40 (9.60-66.10)	**0.003**
Cardiovascular diseases	121.85 (68.90-393.80)	160.20 (79.10-470.60)	0.741
Respiratory diseases	20.35 (9.40-37.70)	30.10 (11.90-35.60)	0.052
Digestive Diseases	19.15 (11.10-45.00)	16.20 (11.70-34.60)	0.364
Genitourinary Diseases	9.65(3.80-15.40)	8.40(2.40-23.00)	0.901
Skin diseases	0.60 (0.10-1.80)	0.60 (0.00-1.60)	0.289
Musculoskeletal Diseases	1.75 (0.20-3.00)	1.50 (0.20-2.70)	0.725
Congenital anomalies	2.60 (1.40-5.00)	3.20 (2.00-6.00)	0.148
Sudden infant death syndrome	0.20 (0.00-0.40)	0.20 (0.00-0.40)	0.646
Unintentional injuries	19.05 (11.00-35.30)	15.60 (10.40-29.70)	0.098
Intentional injuries	9.55 (4.30-24.40)	9.70 (7.00-14.50)	0.934

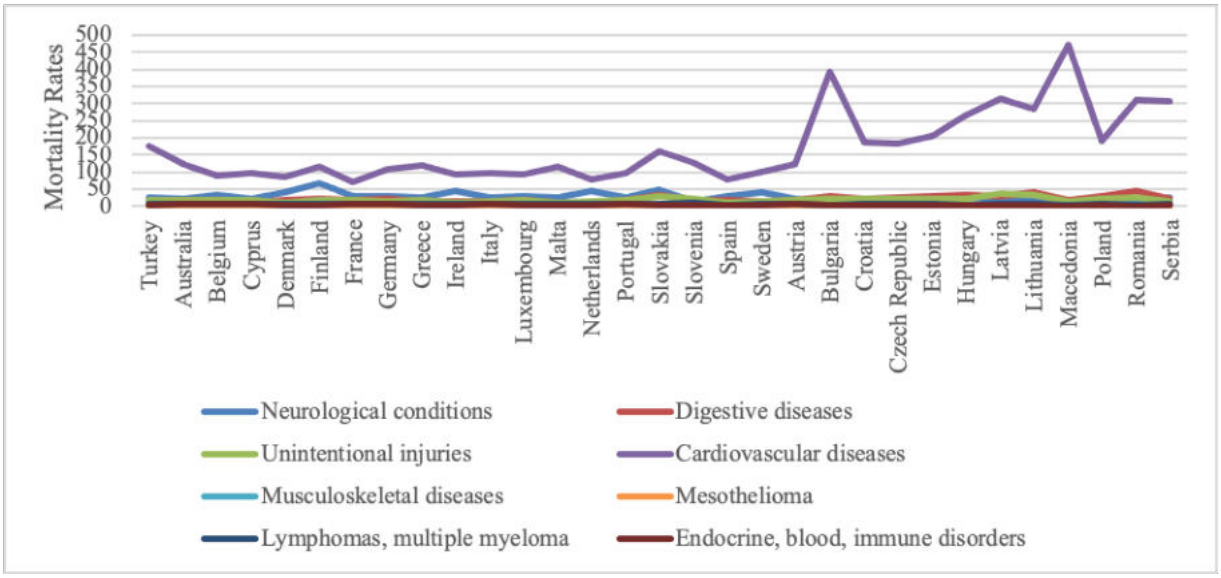

Fig. 4. Distribution of mortality rates of countries according to other causes of death which are found significant according to first dimension.

mark, France, Netherlands, Ireland, Spain, Sweden, Italy, Cyprus, Luxembourg, Malta, Portugal and Greece, which are close to each other in both dimensions, form a group; it has been observed that Turkey is close to these countries in terms of both dimensions.

RESULTS

According to the first dimension, the countries were divided into two groups according to the 0 abscissa, and the differences between the groups were analyzed according to the causes of death examined. Accordingly, the groups in the first dimension include cancers of the mouth and oropharynx, stomach, colon and rectum, breast, cervix, uterus, ovary, kidney, bladder, brain and nervous system, laryngeal cancers, cardiovascular diseases, digestive diseases, mesothelioma, lymphoma and multiple myelomas, endocrine, blood and immunity diseases, neurological disorders, musculoskeletal system diseases and unintentional injuries statistically significant difference

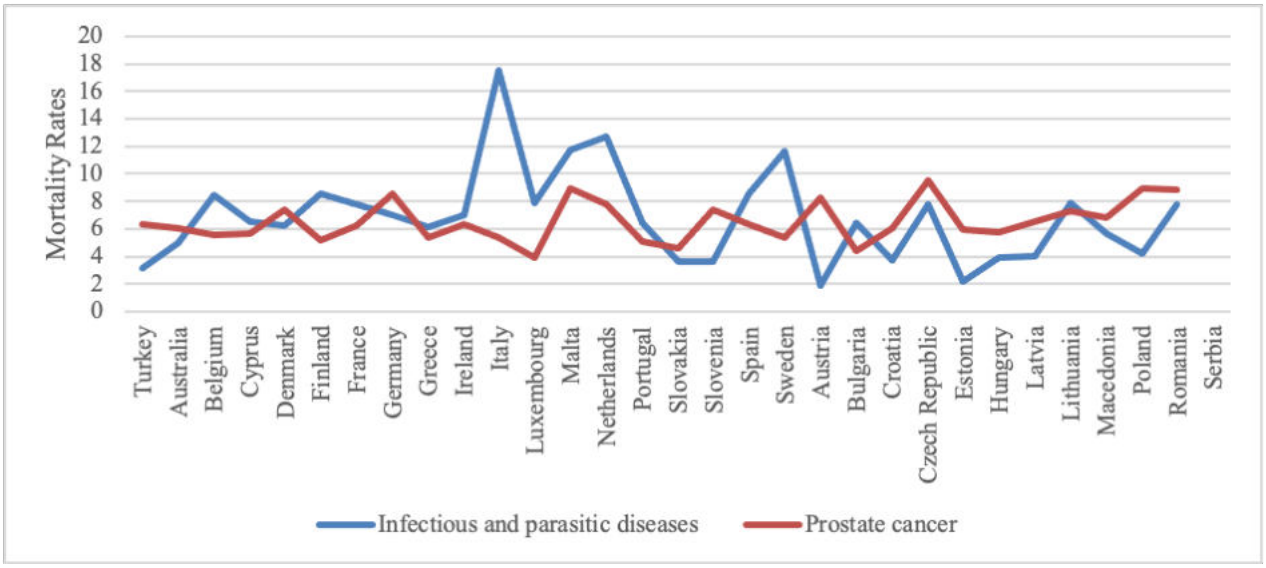

Fig. 5. Distribution of mortality rates of countries according to causes of death which are found significant according to second dimension.

was found in terms of mortality rates due to (Table 3, Fig. 3).

According to the second dimension, when the countries are divided into two groups according to the 0 ordinate; A statistically significant difference was found between the two groups in terms of mortality rates due to infectious and parasitic diseases, prostate cancer and neurological diseases (Table 4, Fig. 4).

DISCUSSION

MDS, which is used to visually reveal the relationships between objects or units in a less dimensional space; it can be applied on various data types measured with ordinal, evenly spaced, proportional scale and is widely used [18, 19].

This study was carried out by taking into account the age-standardized mortality rates per 100,000 population according to the GHE categories of the European Union member and candidate countries. In this study, a MDS analysis was carried out in order to understand which variables caused the distinction between European Union member countries and candidate countries. As a result of examining the literature on the subject among 31 European Union member and candidate countries (Montenegro could not be included in the study due to lack of data), 41 variables were included in the analysis by considering a standard classification criterion for disease-specific mortality rates.

In the first dimension, countries, especially in terms of other cancer types except prostate cancer, in the second dimension, it is seen that it differs especially in terms of moratality rates due to infectious and parasitic diseases and prostate cancer. For both dimensions, the countries of Germany, Austria, Belgium, Czechia, Denmark, France, Croatia, Netherlands, Ireland, Spain, Sweden, Italy, Cyprus, Luxembourg, Malta, Portugal, Slovenia, Greece formed a close group and Turkey's were found to be close to these countries in both groups. In the first group, especially Albania, Macedonia and Bulgaria, Serbia, Latvia, Romania, Lithuania and Hungary differ from other countries, in the second group, it is seen that especially Finland, Albania and Slovakia differ from other countries.

As can be seen from Fig. 3 in the first dimension of the GSE categories, our country differed from countries with high rates of colon rectum cancer, especially Slovakia and Hungary, and it was observed that it got closer to countries with low mortality rates. As can be seen from Fig. 4 in the first dimension of the GSE categories, our country differs from countries such as Bulgaria and Macedonia with high rates of cardiovascular diseases. As can be seen from Fig. 5 in the second dimension of the GSE categories, our country differs from countries with high rates of infectious and parasitic diseases, such as Greece and Romania, and converges with countries with low mortality rates.

CONCLUSION

The solution of health problems has been one of the important and determining factors in every age, the goal of people to live a modern, contemporary and prosperous life. It would be beneficial to determine the standardized mortality rates according to GHE by comparing them with European Union member states and to carry out studies to solve them by taking into account the negative aspects.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):810-820 Original Article

DOI: 10.18621/eurj.1011372 *Medical Biology*

The in silico interaction analysis of CARMIL1 protein-containing leucine-rich repeat (LRR) regions with interleukin-1 receptor-associated kinase 1 (IRAK1) protein and LLR peptide

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ABSTRACT

Objectives: Capping protein Arp2/3 and myosin-I linker protein 1 (CARMIL1) encoded by the CARMIL, is a major, multidomain, membrane-linked protein regulating actin assembly; however, its function in inflammatory signaling is not fully elucidated. The leucine-rich repeat (LRR) region of CARMIL1 has been associated with interleukin (IL)-1 receptor-associated kinase (IRAK) in fibroblasts by many methods including tandem mass tag mass spectrometry, immunoprecipitation, and CRISPR-Cas9. This study, therefore, set out to assess the interaction of CARMIL1 with each IRAK1 protein and a novel LRR peptide.

Methods: The molecular docking techniques were employed to compare the binding modes and affinities of the 3D structure of CARMIL1 each of LRR peptides and IRAK1 protein. 3D structure model of CARMIL1 protein and LRR peptide was predicted through Robetta tool considering the structures and function of these proteins.

Results: As an overall conclusion of docking, the LRR peptide was observed to contact the residues in the LRR 1-2 of the human CARMIL1, whereas the IRAK1 protein was to interact with the residues in the LRR 1, 2, and 10 regions of the human CARMIL1.

Conclusions: Our computational results suggest that LRRs in CARMIL1 are involved in the formation of protein-peptide binding interfaces with its structural conformation.

Keywords: CARMIL1, IRAK1, LRR peptide, molecular docking simulation

Interleukin-1 (IL-1), a critical intermediate for inflammation, assists the degradation and remodeling of extracellular matrices (ECMs) by enhancing the expression of matrix metalloproteinases (MMPs), thus, plays a central role in various inflammatory diseases including rheumatoid arthritis, chronic periodontitis, and severe lung injury [1]. The binding of IL-1 to its signal receptor [IL-1 receptor type 1 (IL-1R1)], allows the IL1R assistant proteins to be recruited. Activation of the IL-1R complex is followed by recruitment of MyD88 (myeloid differentiation factor 88). This newly formed adapter protein complex then binds to IRAK1 and IRAK2 (Interleukin-1 receptor-associated kinase 1 and 2) [2]. IRAK is quickly phosphorylated, subsequently released from MyD88, and initiates signal transduction pathways, like the mitogen-activated

Received: *October 18, 2021;* ***Accepted:*** *June 21, 2022;* ***Published Online:*** *August 3, 2022*

How to cite this article: *Beşli N, Yenmiş G. The in silico interaction analysis of CARMIL1 protein-containing leucine-rich repeat (LRR) regions with interleukin-1 receptor-associated kinase 1 (IRAK1) protein and LLR peptide. Eur Res J 2022;8(6):810-820. DOI: 10.18621/eurj.1011372*

e-ISSN: 2149-3189

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protein kinase (MAPK) family members ERK [3].

Fibroblasts are a major group of cells in the connective tissues, directly included in the matrix degradation in widespread inflammatory diseases such as periodontitis and rheumatoid arthritis. Concerning IL-1 signal transmission in fibroblasts, IL-1R1 needs to be recruited to focal adhesions (Fas) [4]. Following this recruitment, FA kinase is activated, Ca2+ is released from the endoplasmic reticulum [5], and ERK is activated eventually [3]. Such mechanisms in the central parts of the signaling system increase the level of MMPs expression [6]. Despite the comprehensive examinations, the control of IL-1 signaling in anchorage-dependent stromal cells is not fully elucidated.

Human CARMIL1 (Capping Protein Arp2/3 myosin I linker) from CARMIL-family is a large multidomain protein, profoundly conserved. CARMIL proteins have several membrane-associated functions associated with actin assembly and signaling owing to their structural characteristics. CARMIL1 is expressed in plenty by fibroblasts, which is intensely linked to abnormal inflammatory processes [7].

CARMIL1 is approximately 1370-aa length protein with 16 leucine-rich repeats (LRRs), which has a non-canonical pleckstrin homology (PH) domain, a long helical domain (HD), and a C-terminus responsible for the interaction with F-actin-capping protein subunit alpha-2 (CAPZA2) [8, 9].

In a recent study, Wang *et al*. [10] have investigated the function of CARMIL1, its LRR region, and CPI-CSI motifs (CP-binding domain) in controlling the production of IL-1 signaling in circulating fibroblasts. They have concluded that the LRR of CARMIL1 would mediate IL-1-induced collagen degradation in the fibroblasts and might be a goal for the anti-inflammatory drug improvement. In the current study, inspired by the experimental findings from this recent investigation, we computed the interaction of LLR regions in a model of human CARMIL1 protein with IRAK1 protein and the designed LRR peptide by drawing on the tested/trusted bioinformatics tools and databases to find the polar contacts. The cellular and biological role of a protein is broadly direct linked to its 3D structure [11]. In this context, since Carmil1 contains the leucine-rich repeat as a protein recognition motif whose structural characterization of this

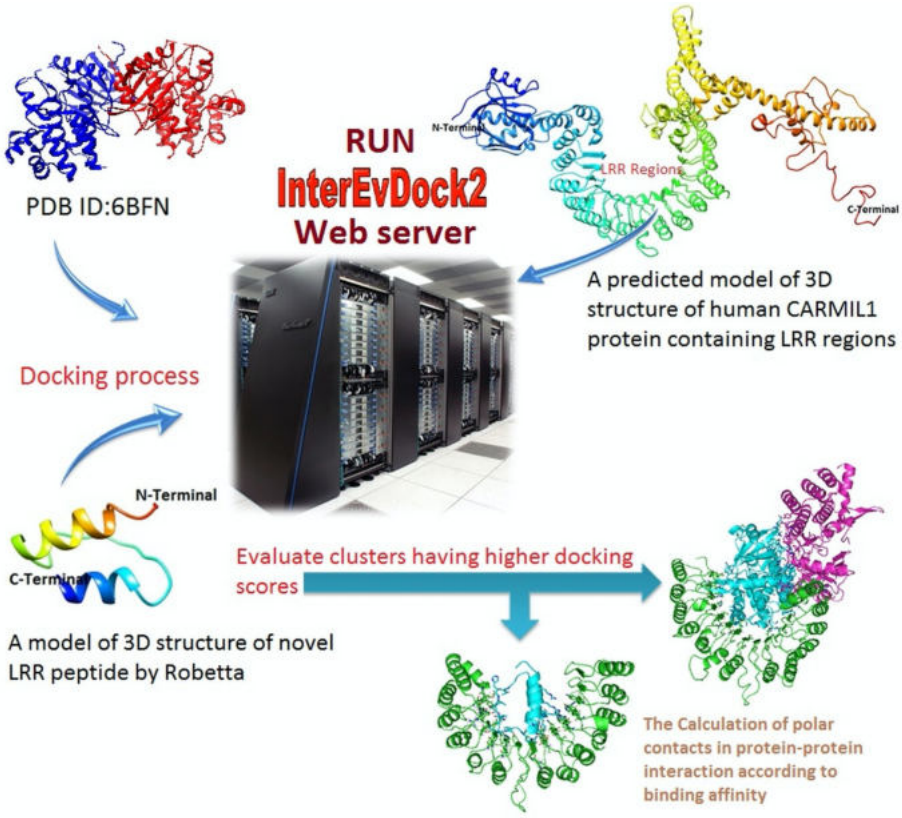

Fig. 1. A shortened work-schema of molecular docking of CARMIL1 with each of IRAK1 and LRR peptide.

motif has been implicated as a critical for the development of targeted drugs. Hence, the three-dimensional structure of the CARMIL1 protein and its conformation and binding interfaces at the atomic level with the novel LRR peptide was investigated. Through these findings, we examined what molecular features of LRR regions are responsible for interaction with the IRAK1 (Fig. 1).

METHODS

The Obtaining of the 3D Structure of Protein and Peptide

First, the protein sequence encoded by human CARMIL1 (UniProtKB/Swiss-Prot: Q5VZK9.1) in FASTA format was obtained from NCBI. LRR peptide sequence (GRKKRRQRRRPQTLVHLDLSGNVLRGDDLSHMYNFLAQPNK) was fetched from the paper of Wang *et al*. [10]. Then, each of the amino acid sequences were then subjected to the Robetta [12, 13], a common tool for homology-based approaches to possess a three-dimensional structure of CARMIL1 and LRR peptide as a model (see Fig. 2). The crystal structure of human IRAK1 (PDB ID: 6BFN) from PDB (Protein Data Bank) at http://www.rcsb.org/ was downloaded in PDB format. In Rosetta server job submission, it was used comparative modeling and all settings left as default and generated five 3D-structure models. It was selected the most accurate one from model proteins according to confidence scores, which indicates the accuracy of model protein in terms of predicted GDT (1.0 good, 0.0 bad).

Pre-Preparation for Molecular Docking

Energy Minimization and Quality Control of Model Protein Structures

The energy minimization of 3D model protein structures was subjected to the minimization method in chimera 1.14 [13]. The default steepest descent was set to 100 with 0.02 step sizes, without fixing any atoms, and was followed by 10 steps of conjugate gradient steps with 0.02 step size (Å) minimization. To control the quality of the model proteins, we evaluated both the structural quality using Qualitative Model Energy Analysis (QMEANDisCo) [15], as well as Ramachandran plots that were drawn to assign key secondary structures to specific regions in the plot.

Sequence Analysis

The protein sequence encoded by Human CARMIL1 (Reference Sequence: NP_060110.4) was retrieved from NCBI in FASTA format and run via PSI-BLAST (Position-Specific Iterated BLAST) [16]. As search setting, it was selected Protein Data Bank (PDB) proteins, as all general algorithm parameters including MATRIX: BLOSUM62, Existence:11 Extension:1, the threshold value (0.05), world size: 6, and

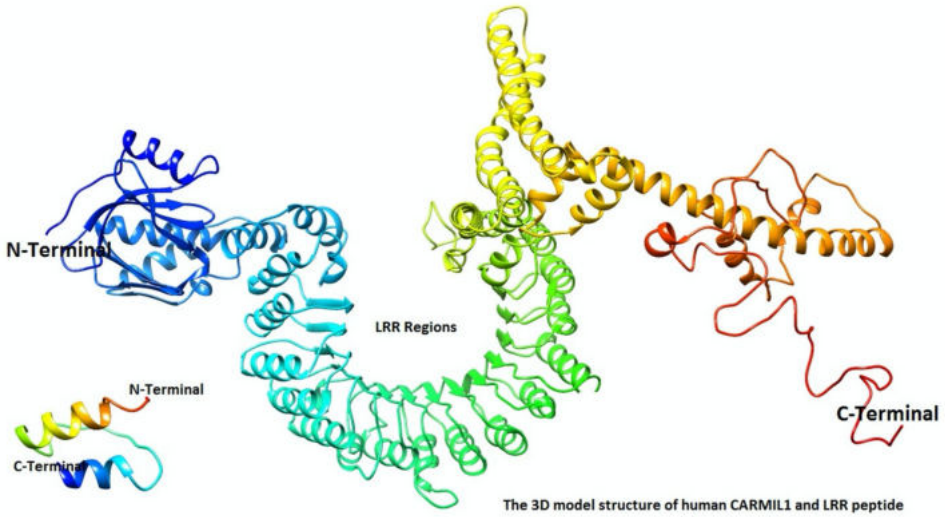

Fig. 2. The 3D structure of predicted CARMIL1 and LRR peptide. Robetta server predicted the 3D model structure of CARMIL1 protein (1-1000aa) and LRR peptide (1-41aa) by the homology-based model. The model was subjected to a mode of protein-protein and peptide docking. The cartoon model representation and image were produced with Chimera 1.14. Structures are symbolized as interactive colored ribbons to view the strand and helix forms.

Compositional adjustments: conditional compositional score matrix were left as the default settings.

Visualization of Molecular Docking Simulations

In this study, it was employed the method that one of the computer-aided drug design (CADD) approaches is structure-based drug design (SBDD) [17]. SBDD methods analyze macromolecular targeting 3D structural details, commonly of proteins or RNAs, to determine fundamental parts and interactions that are significant for their regarding biological procedures. In the docking process, there is no restrictions were placed between surface-exposed residues of protein-peptide and protein-protein but conversely performed blind docking. In InterEvDock2 docking server, as demonstration Mode for docking procedure, standard usage (easy level) was opted. Two protein sequences or structures and their respective multiple sequence alignments are employed to estimated binding modes via a free docking process at the web server.

The primary structure of CARMIL1 was colored by drawing on Jalview 2.11.1.3 [18]. PyMOL software [19] was used to illustrate the tertiary structure proteins-peptides and analyze the molecular modeling re-

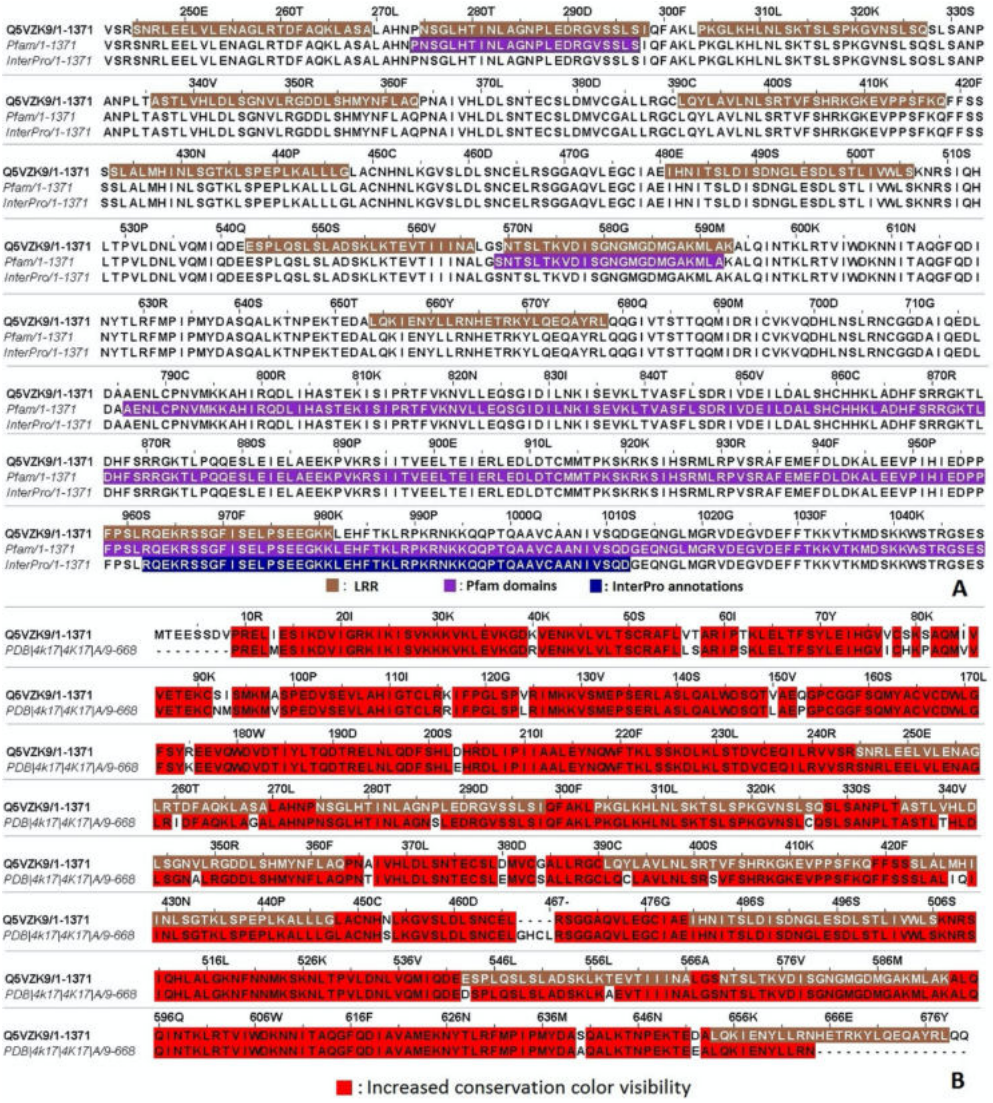

Fig. 3. (A) The visualization of the Pfam domain (purple), InterPro annotations (navy blue), and LRR regions (brown) in the CARMIL1 aa sequences. LRR_6 (274-297aa, 569-592aa), CARMIL_C (786-1081aa) from Pfam domains, Leucine-rich repeat-containing protein 16A (962-1012aa) from InterPro domains, There are 11 parts of known LRR regions from Uniprot. All feature sequences were retrieved from Uniprot and PDBeKB. (B) Representation of the primary structure of Human CARMIL1 protein with Mouse CARMIL1 in similar sequences after alignment. This figure solely provides the LRR region sequences in the human CARMIL1.

sults at the 3D atomic level. All complexes of protein-protein interactions were performed by a fully automatized InterEvDock2 docking server [20-22] through utilizing the SOAP_PP [23] and FRODOCK2 [24]. In the docking process, there is restrictions were placed between surface-exposed residues of protein-peptide and protein-protein but conversely performed blind docking. In InterEvDock2 docking server, as demonstration Mode for docking procedure, standard usage (easy level) was opted. Two protein sequences or structures and their respective multiple sequence alignments are employed to estimated binding modes via a free docking process at the web server.

RESULTS

The Alignments and Sequence Analysis

According to alignment results obtained through the PSI-BLAST tool, the CARMIL1 sequence demonstrated a 91.45% per identity and a query cover 48% with Chain A, Leucine-rich Repeat-containing Protein 16a [Mus musculus Accession: 4K17_A] as a top homolog protein. Thus, this protein was selected as a template for the homology modeling by the Robetta server. Following the alignments of the sequence of CARMIL1 and the template protein, we detected similar sequences in LRR regions (Fig. 3, section B). This finding boosts the accuracy of the prediction of the three-dimensional structure of the LRR regions of the CARMIL model protein.

Docking Consequences of CARMIL1 with Each of LRR Peptide and IRAK1

The docking scores and the residues of polar contacts between CARMIL1 and LRR peptides are listed in Table 1-2. As can be seen from Table 1, the top 10 consensus complexes include IES1 and FRODOCK2 with higher energy scores and SOAP_PP4 with lower energy scores. According to the online prediction tools FRODOCK2 and InterEvDock2, in all top docking complexes, E254 (the LRR1 in CARMIL1), K3, and R2 (LRR peptide), which are the top 5 residues (on each chain) on the consensus of the top 10 models, were predicted to be involved in contacts (see Fig. 4 sec. 3.1, and 4.1, Fig. 5 sec. 7.1 and 10.1). As an overall conclusion of docking, the LRR peptide contacted the residues in the LRR regions (one, seven) of the human CARMIL1.

FRODOCK1 and IES1, and SOAP_PP1 are the top consensus complexes by higher docking scores within the representative models from the 10 best clusters (see Table 2). According to the online prediction tools FRODOCK2 and InterEvDock2, in all top dock-

Table 1. The results obtained from the analysis of the docking CARMIL1 and LRR peptide

Docking complexes	LRR peptide	CARMIL1 (Residue number)	LRR region	Docking score
SOAP_PP9	R5, S20, S30, Q38	**H428**, **S432**, G456, **T485**, R630	LRR6, 7	-16724.74
FRODOCK6	R25	**D488**, **D577**	LRR7, 9	1909.43
SOAP_PP10	R2, R6, R10, K41	**E254**, **S313**, **S345**, D460, N463, **S490**, **S550**	LRR1, 3, 4, 7	-16539.82
FRODOCK7	K3, R25	**E254**, **D343**	LRR1, 4	1888.22
IES1	Q7, R8, H16	E475, G476, **T500**	LRR7	33.71
IES3	Y33, G26, D27	I478, **D497**, **S499**	LRR7	31.7
SOAP_PP1	R2, H31, N40	**E254**, **N255**, **N311**, **S313**, D371, **D488**	LRR1, 3, 7	-16991.95
SOAP_PP2	K41, S30	**E254**, **K265**	LRR1	-16925.85
FRODOCK2	R6	**E254**, D460, K608	LRR1	2060.55
SOAP_PP4	K3, R10, Y33, P39, N40	**E254**, **N255**, S373, **S490**, **K575**	LRR1, 7, 9	-16773.15

The table provides the residues of polar contacts between the model CARMIL1 and LRR peptide. The residue numbers of LRR regions in CARMIL1 are shown in bold font. The lower score is better in the docked complexes of SOAP_PP whilst the higher score is better in the docked complexes of FRODOCK and IES

Table 2. The results obtained from the analysis of the docking CARMIL1 and IRAK1 are presented.

Docking complexes	IRAK1	CARMIL1 (Residue number)	LRR region	Docking score
1.FRODOCK5	K242, N211	**E249**, **E254**, R630	LRR1	2335.05
2.IES1	Q254, T258, Y277, R194	**V252, A256, D261, E289**	LRR1, 2	820.97
3.IES2	Q254, Y277	**E250, R259**	LRR1	697.85
4.IES3	T383, T431	**E254, D261**	LRR1	658.16
5.IES4	R363, Q254, N211	**E254, N255, Q655**	LRR1,10	635.63
6.SOAP_PP1	S331, R194	K644, **E651**, **E658**	LRR10	-34824.7
7.SOAP_PP2	R194, K253, S333, S335	**N255**, N374, Y637, S640	LRR1	-34575.19
8.SOAP_PP3	Y277, S193, R366	**A256**, **G257**, Y637	LRR1	-34535.14
9.FRODOCK1	E248, S333	**T280**, **N282**, R630	LRR2	2548.03
10.FRODOCK2	R366, K253, Y284	**T280**, H452, **L661**, **L662**, **R663**	LRR2, 10	2431.33

The table provides the residues of polar contacts between the model CARMIL1 and IRAK1 protein. The residue numbers of LRR regions in CARMIL1 are shown in bont font. The lower score is better in the docked complexes of SOAP_PP whilst the higher score is better in the docked complexes of FRODOCK and IES.

ing complexes, E250, V252, E254 (the LRR1 in CARMIL1) and R194, Q254, Y277 (IRAK1 protein), which are the top 5 residues (on each chain) on the consensus of the top 10 models, were predicted to be involved in contacts (see Fig. 6 sec. 2.1, 3.1, 3.2, and 5.1). As a general result of the docking process, IRAK1 protein contacted the residues in the LRR regions (one, two, and ten) of the human CARMIL1.

DISCUSSION

This study explores to address the in silico analysis of human CARMIL1 protein, which recently was associated with IL-1-induced ERK activation and MPPs expression and whose 3D structure is not fully uncovered yet. CARMIL1, which has an N-terminal domain-containing LRRs that provide broad contacts with other regulatory proteins, is an actin regulator with diverse functions [7]. Until recently, there has been no reliable evidence concerning the role of CARMIL1 in inflammation through the degradation of ECMs by elevating the expression of MMPs. Given the strong molecular evidence, this protein assures a therapeutic target associated with inflammatory diseases such as rheumatoid arthritis, acute lung injury, and chronic periodontitis [10]. However, much uncertainty still exists about the relation between CARMIL1 and inflammatory disorders regarding targeted therapeutic drug development.

In this study, we aimed to predict the model interactions of CARMIL1 with the IRAK1 that displays a functional feature through interacting with CARMIL1 for ERK activation and MMPs expression, and with a novel LRR peptide.

Robetta is a protein structure prediction service continuously evaluated with CAMEO (Continuous Automated Model Evaluation), which constantly assesses the accuracy and reliability of the prediction. The other prediction tools including CAMEO, Robetta, and QMEAN are among the first-line by time-based statistical confidence and reliable performances. The InterEvScore used in the study has determined the heteromeric protein interfaces (polar contacts) and the integration of the evolutionary information obtained from the multiple sequence alignments of each protein in the clusters with a residual-based multi-body statistical potential. In this prediction server, docking searching is systematically implemented utilizing the FRODOCK2 and the outcomes are re-calculated by InterEvScore [22] and SOAP_PP atom-based statistical potential to enhance the confidence level of the

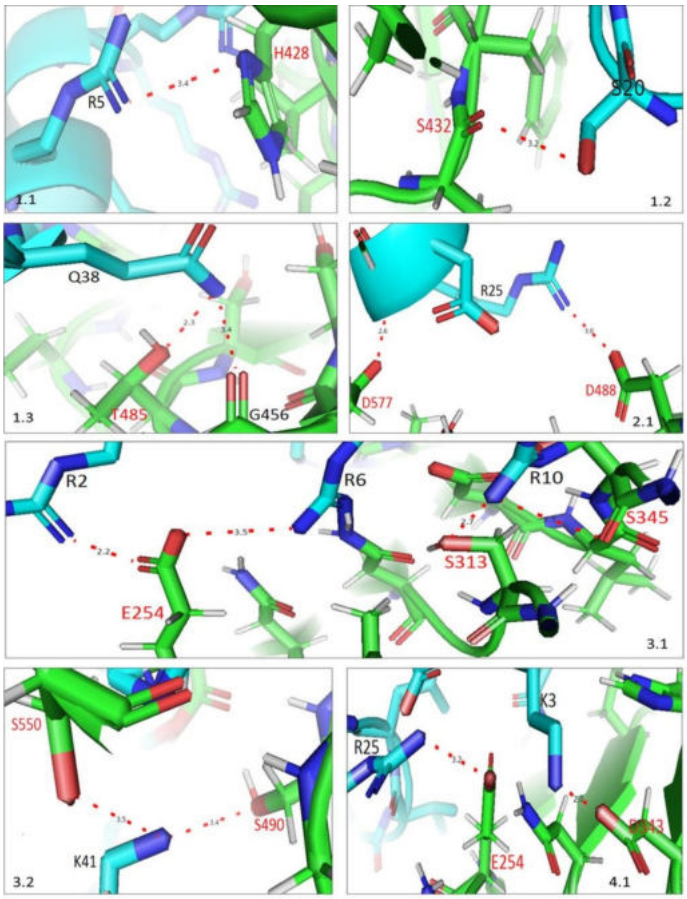

Fig. 4. The InterEvDock2, SOAP_PP, and FRODOCK2 model results of CARMIL1 protein and LRR peptide. The docking complexes are represented in a protein interface representation by PyMOL, colored by (CARMIL1 in green, its interaction residues font in LRR regions in red, and LRR peptide in cyan, and possible polar contacts (dash lines) in red). The sections in the figures are enumerated in the order of the list in Table 1 (Such 1.1-4.1). Only figures from docking pose related to LRR regions are concerned.

predictions.

The structural details present in the predicted protein model are significant for defining the biological function of the modeled protein and its use in future experiments, model verification has a critical step in protein structure prediction. To assess the quality level of secondary structure of model CARMIL1 protein and LRR peptide, we drew the Ramachandran plots through the webserver at https://swissmodel.expasy.org. The Ramachandran plots are represented as an x–y plot of the Phi (φ) and Psi (ψ) of the dihedral angles between N-Ca and Ca-C planar peptide bonds in a protein's backbone. The Phi (φ) and Psi (ψ) are regarded as conformational angles that determine the conformation of the whole chain of a protein. All theoretically probable secondary structures are shaded in orange and dark blue as the most favored regions in the plots (Figs. 8 and 9). To interpret these plots, we used the outputs of MolProbity [25] tool, which is a structure-validation web service that provides evaluation of model quality. Ramachandran Favoured score is 96.66% for the CARMIL1, while this score is 100.00 % for LRR peptide. The Ideal score for the Ramachandran Favoured in the MolProbity tool has been reported > 98% as an ideal case.

Protein structures can contain multiple intense foldable parts, namely domains. These domains comprise typical hydrophobic cores, and can be folded free of each other, and are nearly always connected to establish diverse roles [26]. In this manner, our first pre-

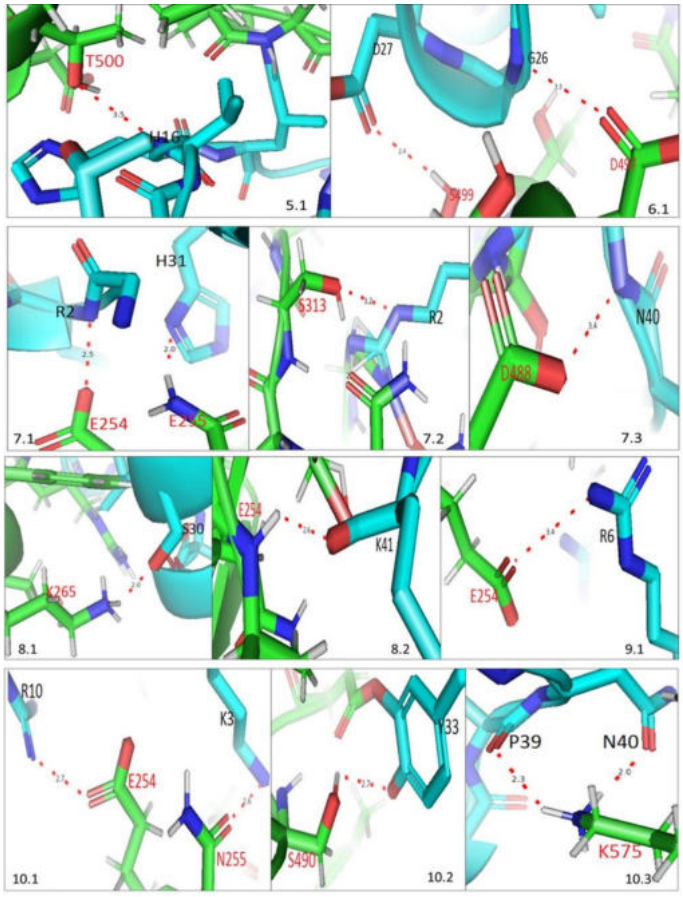

Fig. 5. The docking complexes are represented in a protein interface representation by PyMOL, colored by (CARMIL1 in green, its interaction residues font in LRR regions in red, and LRR peptide in cyan, and possible polar contacts (dash lines) in red). The sections in the figures are enumerated in the order of the list in Table 1 (Such 5.1-10.3). Only figures from docking pose related to LRR regions are concerned.

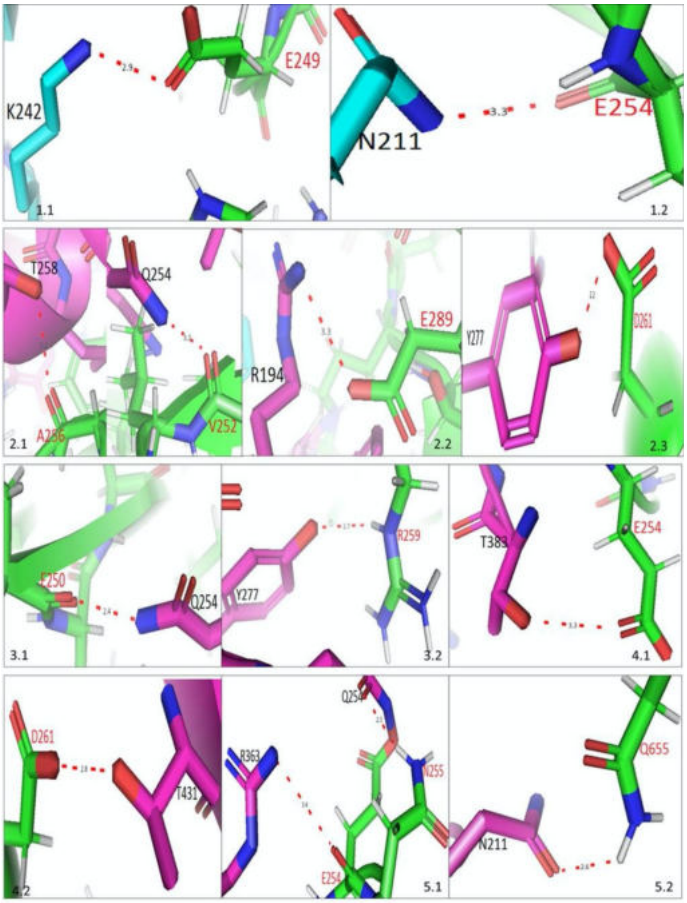

Fig. 6. The InterEvDock2, SOAP_PP, and FRODOCK2 model results of the CARMIL1 and IRAK1 protein. Binding modes of the CARMIL1 and IRAK1 in the protein interface representations are shown, colored by (CARMIL1 in green, its interaction residues font in LRR regions in red, and IRAK1 protein in cyan and magenta). The sections in the figures are enumerated in the order of the list in Table 2. (Such 1.1-5.2) Only figures from docking pose related to LRR regions are included.

liminary approach in this study was to analyze the LRR regions in CARMIL1 through Uniprot and PDBe-knowledge base (see Fig. 3, section A) since Wang *et al*. [6] demonstrated that the LRRs of CARMIL1 associate with IL-1 signaling proteins.

LRRs are nearly 21-28 residues (Figs. 6 and 7).s sequence motifs existing in various proteins with distinct functions. The main role of these motifs seems to implement a multidirectional structural framework for the composition of protein-protein interactions. Recent structural information in the LRR proteins has heightened the need for our opinion concerning the structural factors, our experience to model such proteins with uncharacterized structures, and has illuminated how these proteins attend in protein-protein interactions [27]. For this purpose, the molecular docking process may be a satisfactory and robust computational approach to recognize the function of LRRs in protein-protein interaction at the atomic level.

The cell-permeable, CARMIL1 binding LRR peptide, has been reported to inhibit IL-1-induced collagen degradation by MMPs [6]. The novel LRR peptide consists of 41 amino acids, most of which are basic and hydrophilic (see part 2.1.). The results, as shown in Figs. 4 and 5, indicate that lysine(K) and arginine(R) in the LRR peptide are the basic amino acids, whereas glutamate (E) and aspartate(D) in the CARMIL1 are acidic amino acids in the most parts of whose interaction poses in the complexes. Besides, according to the calculation results, the LRR peptide mostly interacts with the residues in the LRR 1 and LRR 7 regions (see Table 1).

Binding modes of CARMIL1 with the IRAK1 protein by molecular docking simulation are displayed in Figs. 3 and 4. In general, IRAK1 interacted with the protein kinase domain between the residue number of 212-521 (see Table 2). As a computational output, residues Q254 and Y277 in the protein kinase domain are the most common polar interactions with CARMIL1, while CARMIL1 binds to IRAK1 through

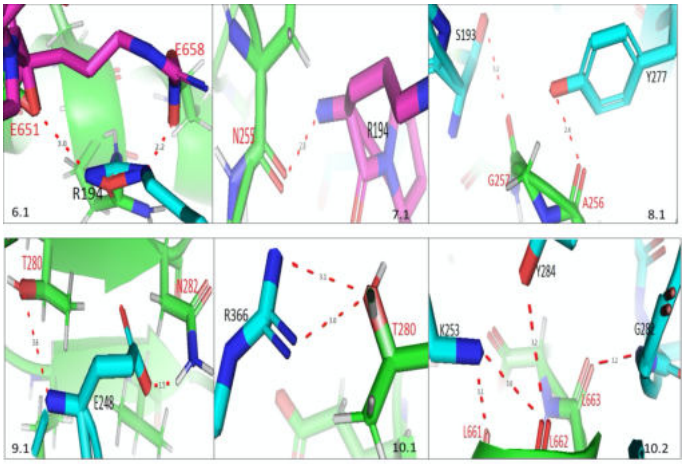

Fig. 7. The InterEvDock2, SOAP_PP, and FRODOCK2 model results of the CARMIL1 and IRAK1 protein. Binding modes of the CARMIL1 and IRAK1 in the protein interface representations are shown, colored by (CARMIL1 in green, its interaction residues font in LRR regions in red, and IRAK1 protein in cyan and magenta). The sections in the figures are enumerated in the order of the list in Table 2. (Such 6.1-10.2) Only figures from docking pose related to LRR regions are included.

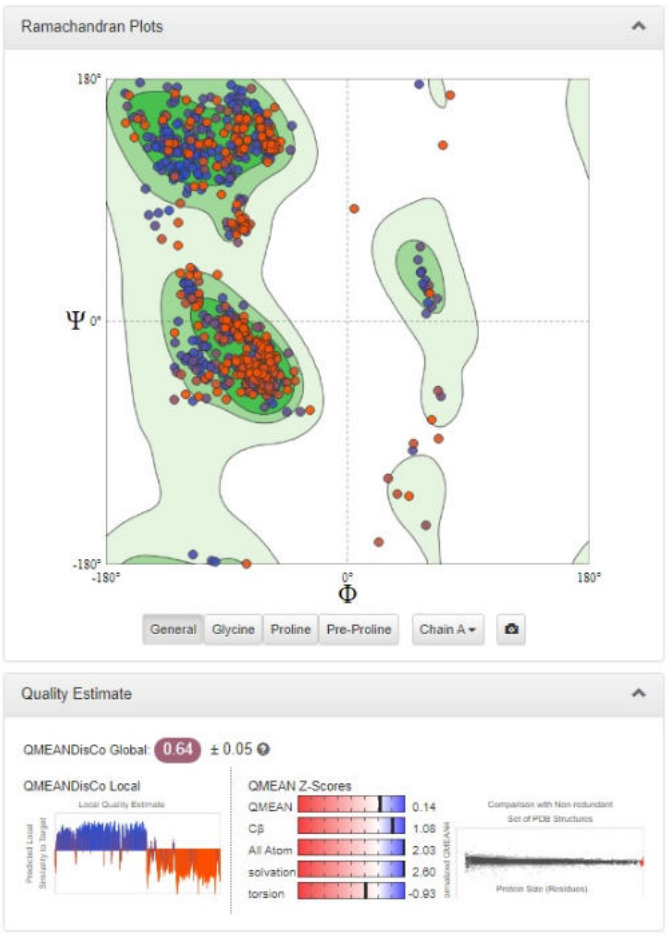

Fig. 8. Ramachandrant and structure quality estimate plot of CARMIL1 from the webserver at https://swissmodel.expasy.org.

Fig. 9. Ramachandrant and structure quality estimate plot of LRR peptide from the webserver at https://swissmodel.expasy.org.

V252, E254 residues in LLR1 (see Figs. 6 and 7). According to the results of both the LRR peptide and IRAK1 protein docking with the model of human CARMIL1 protein, E254, N254, and R630 commonly interacted residues. All docked complexes with the LRR peptide and IRAK1 are related to LRRs regions in CARMIL1. These calculations would seem to suggest that the primary function of the LRRs performs a sophisticated structural frame for the formation of protein-protein interactions.

Limitations

As limitations of the study, to be sure and explain the stability of the molecular docking complexes, classical molecular dynamic (MD) process is advised to conduct. InterEvDock2 docking server is presently not able to dock nucleic acids or small molecules. When nucleic acids or ligands are present in a protein chain, they will be kept only as steric objects. In this study, as only one ligand is conducted, comparison assessment is recommended by targeting a similar ligand to the LRR region of the CARMIL1 protein just as the LRR peptide is targeted.

CONCLUSION

Inflammation is normally a response of the body to infection and ailment. However, it may be seldomly misled, hereby the immune system preferably attacks healthy tissues. In the current study, an in-silico analysis was performed to predict the polar contacts to evaluate the binding mode and affinities of the CARMIL1 with the IRAK1 protein and the novel LRR peptide. The docking score and protein-protein interaction with significant amino acid residues were identified as hits

through the docking server. Both the LRR peptide and IRAK1 protein contacted with the residues of E254 and N255 in the LRR1 region of human CARMIL1. The outcomes indicate that the LRR1 region is more significant in evaluating the LRR peptide for its effectiveness in inhibiting the interaction of IRAK1 and CARMIL1 protein. Taken together, as three scoring programs in the docking process have confirmed each other, we conclude that the models with the highest binding energy are the complexes that interact with residues in the LRRs regions of the CARMIL1. This study may contribute to future studies, as CARMIL1 being a promising target for anti-inflammatory drug development.

Authors' Contribution

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

Ethical approval

There are no studies with the human participant or animal performed by any of the authors in this paper.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):821-827 Original Article

DOI: 10.18621/eurj.1027730 *Medical Biology and Genetics*

Investigation of the effect of vascular endothelial growth factor gene 936 C/T polymorphism in familial Mediterranean fever patients

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ABSTRACT

Objectives: This study aims to investigate the effect of vascular endothelial growth factor (VEGF) gene 936C/T polymorphism (rs3025039) on the appearance of phenotypic characteristics of familial Mediterranean fever (FMF) patients that differ with respect to MEditerranean FeVer (*MEFV*) gene mutations. Here, we investigated a single functional polymorphism in the VEGF gene.

Methods: The study group consisted of 223 FMF patients with definite diagnosis according to Tel-Hashomer criteria who carried *MEFV* gene mutations, while 208 FMF patients with definite diagnosis of FMF but without any mutations, making up the control group, were included in the study. The VEGF gene 936C/T polymorphism was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

Results: Genotype and allele frequencies of the VEGF rs3025039 polymorphism between the two groups were significantly different ($p = 0.03$ and $p = 0.011$, respectively). The TT genotype was found to be more frequent in the study group than in controls (4.9% vs. 3.3%, respectively).

Conclusions: Our results seem to indicate that the VEGF 936C/T polymorphism affects the appearance of the phenotypic characteristics of FMF. It is possible that other variants of this gene may also have similar effects.

Keywords: Genotyping, hereditary, inflammation, *MEFV* gene, VEGF gene

Autoinflammatory diseases are diseases in which systemic symptoms such as recurrent fever, high acute phase reactants are observed and also accompanied by pathologies such as rash, serositis and arthritis affecting various organs. [1]. Familial Mediterranean fever (FMF) is the most widespread autosomal recessive, inheritable autoinflammatory disease caused by MEditerranean FeVer (*MEFV*) gene mutations [2].

FMF (OMIM 249100) is characterized by recurrent self-limiting attacks of fever, peritonitis, pleuritis, arthritis and erysipelas-like skin lesions [3, 4]. It is estimated that around 150,000 people in the world have this disease, although its prevalence is uneven among ethnic groups [5]. FMF is most frequently observed in individuals of Turkish, Arab, non-Ashkenazi Jewish and Armenian origin [6-9].

The pathogenesis of FMF is not completely understood although the gene responsible for FMF has been identified [10]. *MEFV* gene located on the short arm of chromosome 16, comprising 10 exons and respon-

Received: ***November 25, 2021;*** *Accepted:* ***April 28, 2022;*** *Published Online:* ***August 8, 2022***

How to cite this article: *Yüce M, Bağcı H. Investigation of the effect of vascular endothelial growth factor gene 936 C/T polymorphism in familial Mediterranean fever patients. Eur Res J 2022;8(6):821-827. DOI: 10.18621/eurj.1027730*

e-ISSN: 2149-3189

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sible for FMF was cloned by two independent groups [11, 12]. The *MEFV* gene encodes a protein called pyrin or marenostrin with 781 amino acids. It is expressed in polymorphonuclear leucocytes and cytokine-activated monocytes and acts as an anti-inflammatory protein. More than 70 FMF-related *MEFV* gene mutations have been reported [13, 14].

Vascular endothelial growth factor (VEGF) polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) technique or VEGF, produced by different cell types, has an important role in both physiological and pathological angiogenesis. [15]. The regulation of angiogenic processes is complex and requires a fine balance between pro-angiogenic and antiangiogenic mediators. One of the most important pro-angiogenic factors is the VEGF [16]. VEGF plays a central role in human rheumatoid arthritis (RA) and animal models of arthritis [17, 18]. VEGF is one of the main regulators of angiogenesis, stimulating the formation of new vessels and increasing the permeability of existing blood vessels. This, in turn, causes the increased and continuity of inflammatory conditions. Due to this, the contribution of VEGF in the pathogenesis of various immunological and inflammatory diseases has been demonstrated. [19].

Single nucleotide polymorphisms (SNPs) of the *VEGF* gene are associated with the production of *VEGF* protein and are reported to be involved in susceptibility to several disorders in which angiogenesis may be critical with regard to pathogenesis [20-23]. *VEGF* gene polymorphisms have been shown to be associated with susceptibility to diseases and angiogenesis is an important process in the pathogenesis of chronic inflammatory disorders such as rheumatoid arthritis [20, 22, 24].

VEGF gene is located on chromosome 6p21.3 and contains 8 exons. The coding region spans approximately 14 kb [25, 26]. Functional polymorphisms of the *VEGF* gene have been associated with low or high *VEGF* protein synthesis. Regarding the V*VEGF* gene 3'- untranslated region C/T polymorphism at 936. position, low plasma levels of VEGF have been reported in carriers of T allele when compared with non-carriers [27].

Neutrophils are the main cell types that play important role in the acute inflammation of FMF. It is also known that VEGF plays a significant role in these cells. Therefore, in this study, our aim is to investigate the possibility of functional *VEGF* gene 936C/T polymorphism being is effective in the emergence of phenotypic characteristics of the FMF disease.

METHODS

Study Populations

Patients who were referred from various clinics to Ondokuz Mayıs University Faculty of Medicine, Department of Medical Biology, Molecular Genetics Laboratory for *MEFV* gene mutation analysis were included in the study. Ethical Board approval was taken for the study from Faculty of Medicine, Ondokuz Mayıs University. In addition, informed consents were taken from patients and each FMF patient filled in an FMF information survey before giving a blood sample. After signing the form, the patients were examined according to Tel-Hashomer criteria and the patients and the controls with definitive diagnosis were evaluated for *VEGF* gene 936C/T polymorphism (rs3025039).

The study group included 223 unrelated FMF patients (SG) with a definitive diagnosis according to the Tel-Hashomer criteria. These patients have two of the twelve most commonly occurring *MEFV* gene mutations (E148Q, P369S, F479L, M680IG/C, M680IG/A, I692del, M694V, M694I, K695R, V726A, A744S, and

Table 1. The demographical characteristics of the FMF patient and control groups

Demographical characteristics	Study group (n = 223)	Control group (n = 208)
Age (years), median (minimum-maximum)	25 (2 - 69)	15 (2 - 88)
Gender (male/female), n (%),	120/103 (53.8/46.2)	91/117 (43.8/56.2)

The study group (FMF patients) = 223 FMF patients with definitive diagnosis according to Tel-Hashomer criteria and carrying two of the 12 mutations commonly seen in MEFV gene, Control group = 208 symptomatic FMF patients who had definitive diagnosis according to the same criteria but did not carry any of the 12 mutations tested.

R761H). The control group (CG) consisted of 208 symptomatic FMF patients who had a definitive diagnosis according to the same criteria but who did not carry any of the twelve most commonly occurring *MEFV* gene mutations indicated above.

Genotyping

Genomic DNA was extracted from peripheral blood cells according to kit directive using Vivantis GF-1 Nucleic Acid Extraction Kit (Qiagen, Istanbul, Turkey). *VEGF* gene 936C/T polymorphism was genotyped using the previously described polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) technique with minor modifications [28]. The PCR was performed in a total volume of 25 µL using 100 ng of genomic DNA, 5 µL of 10× reaction buffer, 2 mM magnesium chloride (Fermentas), 10 pmol of each primers, 0.5 mM of each dNTP and 2 U of Taq DNA polymerase (Fermentas). The PCR conditions were: 1 cycle at 95°C for 12 minutes; 35 cycles, each consisting of denaturation at 95°C for 30 seconds, annealing at 57°C for 40 seconds, and synthesis at 72°C for 30 seconds; and a final cycle at 72°C for 10 minutes. The PCR products were digested with restriction endonuclease NIaIII (Fermentas), in a 37°C incubator 24 hours. With this enzyme, the C allele remained uncut (198bp), while the T allele was cut into 2 fragments of 114bp and 84bp. The fragments were loaded onto 3% agarose gel, electrophoresed and observed over an UV-transilluminator.

Statistical Analysis

Analyses of data were performed using the Statistical Package Program (SPSS, version 23.0, Chicago, IL, USA). Continuous data were given as mean ± standard deviation and median (min-max). Allele and genotype frequencies of patients and controls were compared with chi square (χ^2) test. All p values were 2-tailed and *p* values less than 0.05 were considered significant. The Hardy-Weinberg equilibrium (HWE) was evaluated by χ^2 test

RESULTS

DNA samples of 223 FMF patients (SG) and 208 con-

Table 2. Clinical characteristics of the FMF patient groups and control group

Clinical characteristics	Study group (n = 223)	Control group (n = 208)
Fever, n (%)		
pozitif	213 (95.5)[a]	202 (97.1)[a]
negatif	10 (4.5)	6 (2.9)
Abdominal pain, n (%)		
pozitif	214 (96.0)[a]	198 (95.2)[a]
negatif	9 (4.0)	10 (4.8)
Amyloidosis, n (%)		
pozitif	20 (9)[a]	23 (11.1)[a]
negatif	203 (91)	185 (88.9)
Arthritis/arthralgia, n (%)		
pozitif	169 (75.8)[a]	149 (71.6)[a]
negatif	54 (24.2)	59 (28.4)
Erysipelas-like erythema, n (%)		
pozitif	59 (26.5)[a]	63 (30.3)[a]
negatif	164 (73.5)	145 (69.7)

[a,b,c]There is no difference between groups with the same letter.

The study group (FMF patients) = 223 FMF patients with definitive diagnosis according to Tel-Hashomer criteria and carrying two of the 12 mutations commonly seen in *MEFV* gene, Control group = 208 symptomatic FMF patients who had definitive diagnosis according to the same criteria but did not carry any of the 12 mutations tested.

trols (CG) were genotyped with regard to VEGF 936C/T polymorphism. In the study group, there were 120 (53.8%) males and 103 (46.2%) females. There were 91 (43.8%) males and 117 (56.2%) females in the control group. Demographic data of the patients are shown in Table 1. The clinical features of FMF patients who had a definitive diagnosis according to Tel-Hashomer criteria were shown in Table 2. In terms of clinical findings (fever, abdominal pain, amyloidosis, arthralgia and erysipelas-like erythema), there was no difference between study groups. The genotype distribution shown in patients who carry mutation in *MEFV* gene (homozygote, heterozygote, and compound heterozygote) in the Table 3.

When the study and the control groups were assessed in terms of genotype and allele frequencies, the difference was found to be statistically significant (Table 4). Based on the findings of the study: of the study group, 152 (68.2%) had CC genotype, 60 (26.9%) had CT genotype and 11 (4.9%) had TT genotype; while, of the control group, 165 (79.3%) had CC genotype, 36 (17.3%) had CT genotype and 7 (3.4%) had TT genotype. When the genotype frequencies of *VEGF* gene 936C/T polymorphism in the study group were compared with those of the control group, the difference between the results of the two groups was found to be statistically significant (χ^2=7.06; p = 0.03).

The study group had a lower frequency of CC genotype when compared with the control group (p = 0.022). CT heterozygous genotype frequency of VEGF gene was observed at a rate of 26.9% in the study group and 17.3% in the control group and was found to be statistically significantly higher (p = 0.015). TT genotype frequency was found to be higher in the control group (3.4%) when compared with the study group (4.9%); however, this difference was not statistically significant (p = 0.41) (Table 4).

When the study group was compared with controls, we observed significant difference between the C and T allele frequencies, with the allele C present in 81.61% of the study group and 88% of the controls and allele T present in 18.38% of the study group and 12% of the controls. T allele was found to be statistically higher in the study group as compared with the control group (χ^2= 6.39; p = 0.011).

Table 3. The distribution of MEFV mutations

	Frequency	%
Mutation non detected	208	48.3
Mutation detected	223	51.7
M694V, M694V	77	34.5
M680I(G/C), M694V	48	21.5
M680I(G/C), M680I(G/C)	20	9.0
M694V, E148Q	16	7.2
M680I(G/C), V726A	16	7.2
M694V, V726A	16	7.2
E148Q, P369S	4	1.8
M694V, R761H	4	1.8
E148Q, M680I(G/C)	3	1.3
E148Q, V726A	2	0.9
F479L, V726A	2	0.9
E148Q, M694I	2	0.9
M680I(G/C), R761H	2	0.9
M680I(G/C), M680I(G/A)	2	0.9
E148Q, E148Q	2	0.9
M694V, A744S	1	0.4
E148Q, R761H	1	0.4
M694V, E148Q, P369S	1	0.4
M694I, M694I	1	0.4
F479L, F479L	1	0.4
M694V, P369S	1	0.4
M680I(G/A), M694V	1	0.4

DISCUSSION

The VEGF plays a significant role in affecting the endothelial cells. The proliferation of endothelial non-proliferative cells on culture deprived of oxygen and food shows that it causes network formation and stimulates ramification. Neovascularization that occurs as a result of the angiogenesis following the abundant production of VEGF is significant in the development of chronic inflammation [24]. Studies have shown that some SNPs in the *VEGF* gene are predisposing factors in rheumatic diseases such as ankylosing spondylitis and rheumatoid arthritis [20, 22]. There are studies which report that *VEGF* gene polymorphisms are associated with VEGF production [24]. However, there is only one study that examines the relationship between *VEGF* gene polymorphism and FMF. This study

Table 4. The comparison of genotype and allele frequencies of VEGF 936C/T polymorphism between FMF patients and the control group

VEGF gene 936 C/T	Study group (n = 223)	Control group (n = 208)	χ^2	*p* value
Genotype, n (%)			7.06	**0.03**
CC	152 (68.2)	165 (79.3)		**0.022**
CT	60 (26.9)	36 (17.3)		**0.015**
TT	11 (4.9)	7 (3.4)		0.41
Allele, n (%)			6.39	**0.011**
C	364 (81.61)	366 (88)		
T	82 (18.38)	50 (12)		

The study group (FMF patients) = 223 FMF patients with definitive diagnosis according to Tel-Hashomer criteria and carrying two of the 12 mutations commonly seen in MEFV gene, Control group = 208 symptomatic FMF patients who had definitive diagnosis according to the same criteria but did not carry any of the 12 mutations tested.

examines whether there is a difference between the study and the control groups of FMF patients regarding VEGF 936C/T polymorphism.

One study showed that the C→T transition at position 936 which is one of the 3 polymorphisms located at 3'UTR is significantly associated with VEGF plasma level. The same study reported that 936C/T polymorphism was associated with significantly lower VEGF plasma levels in healthy men and thus this polymorphism could be a significant genetic indicator for diseases associated with angiogenesis [27].

The purpose of this study was to examine whether *VEGF* gene 936C/T functional polymorphism was associated with the basic phenotypic characteristics of FMF disease. A statistically significant difference was found between the study and control groups in terms of genotype and allele frequencies ($p = 0.03$ and $p = 0.011$, respectively). Our study showed that the study group had lower rates of CC genotype when compared with the control group, while they had higher rates of CT genotype. Although *VEGF* gene 936TT genotype was found to be higher in the study group when compared with the control group, the difference was not statistically significant. In terms of allele frequencies, C allele was found to be lower in the study group when compared with the controls while T allele was found to be higher ($p = 0.011$).

In their study with FMF patients and healthy control groups in 2007, Gunesacar *et al*. [19] did not find a significant difference between the genotype and allele frequencies of *VEGF* gene 936C/T polymorphism. In addition, they reported that 936TT genotype of this gene was found to be higher in FMF patients when compared with the healthy controls; however, this difference was not statistically significant [19]. This result is in parallel with the results of our study.

In a study on patients with rheumatoid arthritis (RA) by Han *et al*. [20] in 2004, the 936T allele was found to be associated with lower production of VEGF in controls; while, compared with controls, the same allele was found to be in significantly higher frequency in patients with RA (22.7 and 13.4%, $p = 0.002$). The results of this study asserted that *VEGF* gene can play a role in RA development. The researchers also examined the two SNPs (at - 2578 and -1154 positions) located in the promoter region and one SNP (at -634 position) in 5'-untranslated region and suggested that these polymorphisms were not associated with RA [20].

In another study conducted with Chinese RA patients in 2013, 3 SNPs (-2578C/A, - 634G/C and 936C/T) were examined and these polymorphisms were not found to be associated with the risk of RA [29].

In a meta-analytic study of the relationship between disease activity and VEGF levels in rheumatoid arthritis in 2018, circulating VEGF levels were found to be significantly higher in RA patients. However, there is no association between VEGF-2578A/C, -634C/G, +936T/C, and -1154A/G polymorphisms and RA development [30].

In another study conducted in 2016, the relation-

ship between serum VEGF protein levels and *VEGF* gene polymorphisms was examined in patients with RA. Studies have shown that VEGF -1154A/G and -2578A/C genetic variants may be genetic susceptibility factors for RA, and increased serum levels of VEGF in RA patients with high disease activity [31]. Similar results in a similar study of the relationship between ankylosing spondylitis and *VEGF* gene polymorphism in the Chinese population and serum VEGF levels in the same year showed that VEGF levels are significantly associated with AS in the inflammatory process [32].

In a study of female RA patients in 2017, an increase in the frequency of VEGF 936CT and a decrease in the 936CC genotype were observed in seronegative patients as compared to healthy controls. Investigators have stated that the presence of certain *VEGF* gene variants in the regulatory region may reflect the nature of immunopathological mechanisms in RA [33].

Many studies have investigated the frequency of *MEFV* gene mutations in Turkey and other countries. A study reported that M694V, M694I, M680I, V726A and E148Q gene mutations were the most frequently observed mutations [8]. In the studies of Federici *et al*. [34], it was shown that five mutations constitute 85% of all *MEFV* gene mutations. It has been reported that M694V, V726A, M680I, E148Q, R761H and P369S are the most frequently observed mutations in Turkey [35]. It is understood that these mutations are much more common in FMF patients than other mutations. The results reported above support that the 12 *MEFV* gene mutations we screened in our study constitute the majority (at least 95%) of *MEFV* gene mutations that can be seen in a population. Therefore, it brings to mind the possibility that patients who do not have any of the 12 *MEFV* gene mutations screened but show FMF phenotype according to Tel-Hashomer criteria may show a phenotype similar to the FMF phenotype due to different factors. We aimed to test the influence of *VEGF* gene 936C/T polymorphism on the occurrence of the FMF symptoms definitely diagnosed but genotypically different (whether or not carrying the 12 most commonly occurring *MEFV* gene mutations) FMF patient groups.

Limitations

Our study assessed the study and control groups based on Tel-Hashomer criteria and the patients who had a definitive diagnosis were included in the study. Thus, the clinical features of the both groups were similar. Hence, no intergroup comparison was done.

CONCLUSION

VEGF gene 936C/T polymorphism might affect the occurrence of phenotypic features of FMF. There is a possibility that the VEGF stimulation of the leucocyte chemotaxis might be the cause of this effect. This may, in turn, cause an increase in the occurrence of the FMF symptoms in the control group that does not carry any of the 12 most commonly occurring *MEFV* gene mutations.

Recommendations

Here, we investigated a single functional polymorphism in the VEGF gene. It is possible that other variants of this gene may also have similar effects. For this reason, other variants can work in FMF patients.

Authors' Contribution

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

Conflict of interest

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

Financing

This work was supported by Ondokuz Mayıs University Scientific Research Funding (PYO.TIP.1904.12.002).

Acknowledgements

We would like to thank the patients, researchers and laboratory technicians.

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The European Research Journal 2022;8(6):828-836 Original Article

DOI: 10.18621/eurj.876332 *Public Health*

Assessment of knowledge level and behavior about vaccines of mothers applying to the children's hospital

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ABSTRACT

Objectives: The aim of this study is to evaluate the knowledge levels of mothers who applied to a university hospital about childhood vaccinations and the sociodemographic characteristics affecting them.

Methods: The population of the descriptive cross-sectional study consists of mothers who applied to the outpatient clinics of Ondokuz Mayıs University Children's Hospital between February 1 and March 31, 2019. A 32-item questionnaire developed by the researchers was applied to 338 mothers who agreed to participate in the study. Ethics committee and institution permissions were obtained. Data were presented with descriptive statistics.

Results: Ninety-five point three percent of the mothers stated that the vaccine was necessary. While 52.7% of the individuals stated that they were aware of the existence of self-paid vaccines, it was determined that 84.0% of the participants did not have self-paid vaccines for their children. Thirty-seven point six of the participants knew that the first vaccine of the newborn was given at birth. Healthcare staffs were among the sources of information for 94.7% of the mothers who stated that they received information about vaccination.

Conclusions: It was found out that the mothers had information about the vaccine. However, the rate of vaccination other than routine vaccines was low. In order to combat vaccine refusals and prevent misinformation, it is considered to be important to provide information at every opportunity, and especially about paid vaccines, parents should be given more information.

Keywords: Vaccine, knowledge level, mother

The response caused by the administration of antigen to the body to protect against a microorganism is called "immunization", and the procedure to obtain this response is called "vaccination". Vaccination is one of the most cost-effective health interventions available, saving millions of people from illness, disability and death each year [1]. Despite the current availability of effective and safe vaccines that protect against a range of serious diseases and the development of many promising new vaccines, these diseases still pose significant threats to developed and developing countries [2]. Routine vaccination services are provided against 13 diseases (diphtheria, pertussis, tetanus, polio, hepatitis B, hepatitis A, H. influenzae type b, tuberculosis, measles, mumps, rubella, chickenpox and pneumococcus) in infants and children within the scope of the "Expanded Immunization Program", which was initiated by the Ministry of Health

Received: ***February 8, 2021;*** *Accepted:* ***April 14, 2021;*** *Published Online:* ***January 16, 2022***

How to cite this article: *Topaktaş B, Özdemir Ş, Hasdemir S, Arslan HN, Terzi Ö, Dündar C. Assessment of knowledge level and behavior about vaccines of mothers applying to the children's hospital. Eur Res J 2022;8(6):828-836. DOI: 10.18621/eurj.876332*

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e-ISSN: 2149-3189

in 2008 in our country. Although it varies by region, it is reported that the childhood vaccination rate in 2019 is between 96-99% on average [3]. However, the number of fully vaccinated children is below the desired targets. For example, according to Turkey Demographic and Health Survey 2020, it is stated that in 15% of registered infants who received the first dose of DTaP-IPV-Hib vaccine (Diphtheria, tetanus, acellular pertussis, inactive polio virus, Hemophilus influenza type b), the third dose was left missing, therefore, full protective resistance cannot be achieved against these five diseases [4].

Among the reasons why children are not fully vaccinated at the appropriate time, it is stated that there are various factors such as the socioeconomic structure of the family, the education level of the parents, the number of children, problems in accessing the health institution, crowded family, parents being young, being a single parent, insufficient or misinformation [5]. In addition, it has been stated that the increasing number of vaccination refusals around the world are due to the lack of sufficient and accurate information about the vaccines by the parents and the speculative news on vaccines in the media [2].

Especially the knowledge, attitude and behavior of mothers about vaccination are important because mothers play an important role in improving the health of children. The practices of mothers to cope with side effects, their negative perception of mild illnesses, negative attitudes towards vaccination, and misconceptions of mothers on vaccination are some of the most important obstacles to childhood vaccination [6]. It is important for mothers to obtain precise and accurate information about vaccines and to develop positive perceptions about vaccines, thus it will contribute to the prevention of many infectious diseases that cause mortality and morbidity in childhood.

In this study, it was aimed to evaluate the knowledge level and influencing sociodemographic characteristics of mothers who applied to a tertiary health institution, which is an important reference hospital in the Black Sea region, about childhood vaccines.

METHODS

Mothers who applied to Ondokuz Mayıs University Children's Hospital outpatient clinics between February 1 and March 31, 2019 constituted the population of the study, which is a descriptive cross-sectional type. Considering the average monthly number of patients examined in pediatric outpatient clinics and using the data of a study conducted in our country [7], it was calculated that at least 338 mothers should be reached.

The questionnaire form, which was used as data collection tool in the study, was composed of 32 questions developed by the researchers by reviewing the literature. The survey included 11 questions about the socio-demographic characteristics of the mothers and 21 questions about childhood vaccinations. The answer of the participants who replied the question "What is the first vaccination time of children" in the questionnaire as "At birth" was accepted as correct. After obtaining the permission of the ethics committee (verdict number: 2019/98), the questionnaire application was initiated by the researchers using the face to face interview method. Beforehand, explanatory information was given to the mothers about the study and verbal consent was obtained from all mothers who agreed to participate.

Statistical Analysis

After the data were encoded, they were transferred to the SPSS (Version 22 for Windows, SPSS Inc, Chicago, IL, USA) program and analyzed. Continuous variables were expressed as mean ± standard deviation and discrete variables as numbers and percentages (%). Analysis of data was made using Pearson chi-square and Fisher's exact test. The statistical significance level for all tests was accepted as $p < 0.05$.

RESULTS

The mean age of 338 mothers was 33.6 ± 6.8 years, and 33.4% were primary school graduates. It was determined that 79.6% of the participants did not have a job, 46.2% had two children, and the household income of 43.2% was between 2,001 and 3,000 TL (Turkish Lira) (Table 1).

Ninety-five point three percent of the mothers stated that the vaccine was necessary, while 4.7% stated that it was not. When Table 2, in which the ques-

Table 1. Sociodemographic characteristics of the mothers (n = 338)

Characteristics	n	%
Age group (years)		
18-34	199	58.9
35-64	139	41.1
Education status		
Primary education	113	33.4
Secondary education	65	19.2
High school	87	25.7
University	73	21.6
Working status		
Not working	269	79.6
Officer	42	12.4
Worker	10	3.0
Other	17	5.0
Total household income (TL)		
0-2,000	83	24.6
2,001-3,000	146	43.2
3,001-4,500	41	12.1
Above 4,500	68	20.1
Number of children		
1	80	23.7
2	156	46.2
3	75	22.2
4 and above	27	7.9

tions and answers are presented measuring the knowledge level of the participants, is examined, 90.1% of the people who considered the vaccine necessary answered the question "Why do you think vaccination is necessary" as "The vaccine protects from infectious diseases". The answer of 73.3% of the mothers to the question "What would be the harm if the vaccine is not given" was "Infectious diseases increase". While the answer to the question about whether vaccines have any side effects was "yes" with a rate of 85.5%, the most commonly said side effect was "fever" (90.6%). Thirty-seven point six percent of the participants stated that the first vaccine of the newborn was given at birth, 95.6% of the participants stated that the vaccines were administered in family health centers and 74.5% of the participants said that the vaccines were administered by nurses. Fifty-two point seven percent of the individuals stated that they were aware of the existence of self-paid vaccines and 70.3% of them specified that vaccines could be delayed in the presence of disease.

According to the statements of the participants, the number of children who developed side effects after any vaccination was found to be 150 (44.4%), and the number of children who had the related disease despite vaccination was 27 (8.0%). Healthcare staff were among the sources of information for 305 (94.7%) of 322 (95.3%) mothers who stated that they had received information about vaccination. Other sources of information were listed as internet (5.0%), neighbors and friends (1.9%) and television, radio, and newspaper (1.6%). It was determined that 284 of the participants (84.0%) did not have self-paid vaccines for their children. The most common reasons for this were the lack of knowledge about self-paid vaccines (63.4%) and not thinking that it was necessary (38.4%).

In Tables 3 and 4, the distribution of knowledge levels, attitudes and behaviors of the mothers on vaccination according to their sociodemographic characteristics are presented. Accordingly, those between the ages of 35-64 were more aware of the existence of self-paid vaccines and the frequency of self-paid vaccination was higher than those in the 18-34 age group ($p = 0.009$ and $p < 0.001$, respectively). On the other hand, mothers between the ages of 18-34 knew the time of first vaccination more accurately ($p = 0.005$). Participants with high school education and above gave more accurate answers about the availability of self-paid vaccines ($p < 0.001$), time of first vaccination ($p < 0.001$) and delay of vaccines ($p = 0.017$). The frequency of getting self-paid vaccination ($p < 0.001$) and getting information about vaccination ($p = 0.004$) was also higher in this group. It was determined that working mothers had more information about the side effects of vaccines ($p = 0.036$), existence of self-paid vaccines ($p < 0.001$) and time of first vaccination ($p = 0.011$) and got more self-paid vaccinations for their children ($p < 0.001$). Individuals with a total household income above 3,000 TL were more successful in self-paid vaccination ($p < 0.001$) and vaccination postponement questions ($p = 0.012$); it was found out that they got more self-paid vaccines ($p = 0.003$), got more information about vaccination ($p = 0.023$), and bene-

Table 2. Distribution of mothers' responses to questions about vaccines

Questions	n	%†	Questions	n	%†
Are there self-paid vaccines? (n =336)			**Are there any side effects of vaccines? (n = 338)**		
Yes	177	52.7	Yes	299	88.5
No	159	47.3	No	39	11.5
What will be the harm if the vaccine is not given?* (n = 322)			**What are the side effects of vaccines?* (n = 299)**		
Infectious diseases increase	236	73.3	Fever	271	90.6
The disease is severely cured	50	15.5	Rash	103	34.4
Disabilities - fatal risks occur	34	10.6	Allergy	91	30.4
I don't know	19	5.9	Gets sick	6	2.0
Other	11	3.4	Other	33	11.0
Who applies the vaccine?* (n = 333)			**Where are vaccines administered?* (n = 338)**		
Nurse	248	74.5	Family Health Center	323	95.6
Midwife	151	45.3	Hospital	72	21.3
Physician	33	9.9	Maternity and Child Health Center	4	1.2
Why are vaccinations necessary?* (n = 322)			**When is the first vaccination of newborn? (n = 338)**		
Protect against infectious diseases	290	90.1	At birth	127	37.6
The disease is mildly cured	10	3.1	One month old	82	24.3
Other	24	7.5	One week old	38	11.2
I don't know	5	1.6	Two weeks old	3	0.9
Can vaccinations be delayed? (n = 333)			**I don't know**	**88**	**26.0**
When gets sick	234	70.3			
Cannot be delayed	18	5.4			
I don't know	81	24.3			

*Multiple choices, †Calculated on valid respondents

fited more from healthcare staff as a source of information ($p = 0.016$).

DISCUSSION

The majority of mothers in the study stated that vaccination was necessary. In a study conducted in Zonguldak, all mothers and 98% in Hatay stated that they considered vaccination necessary [8, 9]. In a study in Italy, 86.2% of the parents found it necessary to apply vaccines even if a healthy lifestyle was followed and natural treatments were used [10]. Considering that the anti-vaccine tendency has increased in the world and in our country especially in recent years [11], it is a positive finding that vaccines are considered necessary by the majority of the mothers included in our study, regardless of sociodemographic characteristics such as education level, age group, employment status or household income.

Local side effects such as pain, swelling and erythema at the injection site, mostly mild and temporary, and systemic side effects such as fever and rash may occur after vaccination applications. Much less often than these side effects, there is a risk of developing anaphylaxis to the vaccine or its components, with about one in a million doses [12]. In order not to take a negative attitude about vaccination, we think it is im-

Table 3. Distribution of mothers who gave correct answers according to their sociodemographic characteristics

Characteristics	Correct Answers to Questions (%)					
	Are there any side effects of vaccines?	Are there any self-paid vaccines?	When is the first vaccine given?	Can vaccination be postponed?	n	%
Age group (years)						
18-34	85.9	46.7	43.7	69.1	199	58.9
35-64	92.1	61.2	28.8	71.9	139	41.1
p value	0.081	0.009	0.005	0.572		
Education status						
Secondary school and below	87.6	36.4	25.3	64.6	178	52.7
High school and above	89.4	70.6	51.3	76.6	160	47.3
p value	0.618	< 0.001	< 0.001	0.017		
Working status						
Not working	86.6	46.3	34.2	68.8	269	79.6
Working	95.7	77.9	50.7	76.1	69	20.4
p value	0.036	< 0.001	0.011	0.241		
Total household income (TL)						
Below 3,000	87.8	41.2	34.5	65.9	229	67.8
3,000 and above	89.9	76.9	44.0	79.4	109	32.2
p value	0.566	< 0.001	0.091	0.012		
Number of children						
1	95.0	55.0	40.0	66.7	80	23.7
2	86.5	57.8	41.0	71.4	156	46.2
3 and above	86.3	43.1	30.4	71.3	102	30.2
p value	0.111	0.064	0.198	0.728		

portant for parents to know these symptoms and to be aware that these side effects are expected and temporary. Eighty-eight point five percent of the mothers in the study stated that vaccines had side effects, and this rate was found to be 73.7% in another study [9]. In studies involving parents and adults, the rate of those who stated that vaccines had side effects was 53.6% and 67.7%, respectively [7, 13]. In our study, it was determined that working mothers knew the side effects of vaccines with a higher frequency. Similarly, correct answers to other questions of knowledge were received with high frequency in this group. This may be due to working mothers' communication with mothers who have similar experiences in the working environment, or different factors such as education and economic levels. As a limitation of the study, working mothers constituted approximately one-fifth of the participants in this study. However, the participation of working mothers in similar studies was also low [7, 9]. Different study designs in which the two groups show a similar distribution are needed to determine the factors affecting knowledge levels of working and non-working mothers.

The answers given to the question of which side effects can be seen in vaccines, although the order varies, were found to be generally similar to those in

Table 4. Distribution of mothers' attitudes and behaviors towards vaccination according to sociodemographic characteristics

Characteristics	Questions					
	Is vaccination necessary?	Have you got a self-paid vaccine?	Do you obtain information about vaccination?	Who is your source of information on vaccination?	n	%
	Yes (%)	Yes (%)	Yes (%)	Healthcare staff (%)		
Age group (years)						
18-34	94.5	8.7	95.0	90.4	199	58.9
35-64	96.4	23.9	95.7	91.4	139	41.1
p value	0.411	< 0.001	0.763	0.752		
Education status						
Secondary school and below	95.5	8.6	92.1	89.2	178	52.7
High school and above	95.0	22.0	98.8	92.5	160	47.3
p value	0.827	0.001	0.004	0.297		
Working status						
Not working	95.2	11.2	95.2	90.7	269	79.6
Working	95.7	29.9	95.7	91.2	69	20.4
p value	1.000*	< 0.001	1.000*	0.898		
Total household income (TL)						
Below 3,000	96.5	11.0	93.4	88.2	229	67.8
3,000 and above	92.7	23.4	99.1	96.3	109	32.2
p value	0.120	0.003	0.023	0.016		
Number of children						
1	95.0	16.3	96.3	92.5	80	23.7
2	96.2	17.6	94.9	89.0	156	46.2
3 and above	94.1	9.9	95.1	92.2	102	30.2
p value	0.747	0.223	0.891	0.571		

*Fisher's exact test

other studies [7-9, 13]. It was determined that 44.4% of the children of the participants developed side effects after vaccination. This was higher than the two domestic studies, in which the related rates were 13.3% and 15.0% [8, 14]. In a study carried out in Egypt, this rate was found to be much higher with 94.4%, and most of these side effects were fever [15]. The reason for these differences may be related to the difference in the options presented in the closed-ended questions, the memory factor or the application of the vaccines at the country level and the difference in the vaccines administered.

In studies involving mothers and parents across the country, the rate of awareness of self-paid vaccines outside the Expanded Immunization Program varies between 27.8% and 63.3% [7-9, 16]. In our study, this

rate was found to be 52.7% and the rate of the participants who had their children vaccinated at least once for a fee was 16.0%. Similarly, the frequency of self-paid vaccination was found as low as 9.7% and 15.6% in other studies [7, 9]. In one of those studies, similar to the this study, the most common reason was not having knowledge about self-paid vaccines [9]. The higher frequency of self-paid vaccination among mothers in the 35-64 age group, with higher education and income levels, and those working in a job is generally compatible with the literature [7, 9, 16]. These findings show that awareness about paid vaccines, income and education level play an important role in the administration of these vaccines.

Hepatitis B vaccine, the first vaccine given to babies, should be administered within the first 72 hours after birth, preferably within the first 24 hours [17]. While the rate of knowing that the first vaccine was given at birth was found to be 65.4% and 92.2% in studies conducted in our country [8, 9], this rate ranged between 68.5% and 97.1% in studies abroad [18-20]. The rate of knowing the time of first vaccination was found to be 37.6% in this study, and it was quite low compared to the literature. Although individuals in the 35-64 age group, who are not working and have a secondary school or less education level, give correct answers to this question at a lower rate, it is an issue that should be emphasized that almost half of the mothers with high school and above education level could not give the correct answer.

In conditions such as severe illness or high fever, it is preferable to postpone vaccinations until recovery [21]. Seventy point three percent of the mothers knew that vaccines could be delayed when the disease develops. In this respect, a similar result was obtained in a study conducted on parents in Istanbul, and 71.6% of the participants stated that vaccines could be delayed when fever or disease occurred [22]. The point that should not be overlooked in this regard is that diseases such as mild upper respiratory tract infections do not prevent vaccination, and the decision to postpone in cases of severe illness and high fever should be made by the physician [21]. In the questions posed in our study, not making a distinction between disease and severe disease can be considered as a limitation.

Ninety-five point three of the participants stated that they received information about vaccination. In studies involving mothers or parents in our country, the rate of those who declared that they received information varies between 58.3% and 88.6%, and similar to our study, it is seen that healthcare staff are the most benefited as a source of information [7-9, 14]. There are different findings about the source of information abroad. For example, in studies involving pregnant women, it was determined that word-of-mouth information was obtained most frequently in Italy, and more frequently from family or friends in China [23, 24]. In Ethiopia, it was observed that mothers or caregivers received the most information about vaccination from healthcare workers [18]. It is of utmost importance to obtain accurate information in order to reduce the increasing number of vaccine rejection and to eliminate false hesitations about vaccination. For this purpose, we think that the primary source of information should be health professionals. As a matter of fact, even if information is obtained from different sources, it has been shown that physicians are trusted the most in this regard [24] and that giving positive opinions by physicians increases the rate of vaccination [25].

Among the mothers, those with high school or higher education, those working in a job, and those with higher income were more knowledgeable in most of the information questions. This situation is similar in many studies carried out in our country and abroad, and it is seen that people with high education or income levels are more conscious about vaccination [19, 20, 23, 26-28]. Assuming that people with a high level of education are more involved in business life and have higher income, we think that the main determinant variable in this issue is the level of education.

We found that the number of children did not affect the level of knowledge or attitudes and behaviors about vaccination. There are different findings on this subject in similar studies. For example, as the number of children increased in the parents who applied to the pediatric health and diseases outpatient clinic in İzmir, there was a decrease in the rate of parents who know about self-paid vaccines and have these vaccinations for their children [7]. In a study conducted in Lebanon, as the number of children increased, it was seen that the knowledge, attitude and behavior scores of the parents about vaccination decreased [26]. It was determined that the number of children did not affect the knowledge level of vaccination in a study carried out in Kars, similar to our study [27].

Limitations

This study has several limitations. Since the sample of the study consists of mothers who applied to the hospital, the results can be generalized to the population they belong to. Post-vaccination side effects and disease development is a finding based on mothers' statements. In the question about vaccination postponement, no distinction was made between illness and severe illness.

CONCLUSION

As a result, it was found out that the majority of the mothers in this study considered the vaccine necessary, had information about the side effects of vaccines, but did not have enough information about the time of the first vaccination, and although almost half of the participants were aware of the existence of paid vaccine, a small portion of them had paid vaccines for their children. It turned out that those with higher education and income levels and those who work were generally more knowledgeable about vaccination, almost all mothers were informed about vaccination in some way, and healthcare staff ranked first as the source of information. In order to prevent vaccination refusals and misinformation, it is thought that the continuity of first-hand information provided by healthcare professionals is of great importance and the information needs of parents should be met, especially about paid vaccines.

Authors' Contribution

Study Conception: BT, ŞÖ, SH, HNA, ÖT, CD; Study Design: BT, ŞÖ, SH, HNA, ÖT, CD; Supervision: BT, ŞÖ, SH, HNA, ÖT, CD; Funding: CD; Materials: HNA; Data Collection and/or Processing: ŞÖ; Statistical Analysis and/or Data Interpretation: SH; Literature Review: BT; Manuscript Preparation: ÖT and Critical Review: BT, ŞÖ, SH, HNA, ÖT, CD.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):837-844 Original Article

DOI: 10.18621/eurj.893220 *Audiology*

Audiological differences in healthy individuals with generalized joint hypermobility: a case-control study

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ABSTRACT

Objectives: Despite the prevalence of generalized joint hypermobility (GJH), the audiological functions of individuals with GJH have not been documented. This study aimed to investigate audiological findings in individuals with GJH.

Methods: This observational, cross-sectional, controlled study was conducted between May 2017 and August 2017. The mean age of all participants was 20.25 ± 0.75 years (range: 19-22 years). The generalized joint hypermobility consisted of individuals with a Beighton score of ≥ 5, while the controls with a Beighton score of ≤ 4. Pure-tone audiometry, immittance audiometry, and Transient Evoked Otoacoustic Emsission (TEOAE) testing were performed on subjects with generalized joint hypermobility (n = 25, mean age: 20.24 ± 0.72 years) and sex- and age-matched healthy controls ((n = 31, mean age: 20.26 ± 0.77 years).

Results: There were no significant differences in the mean hearing thresholds between the groups, although six (5.4%) ears in the GJH group had thresholds > 15 dB at one (five ears) or more frequencies. Significant differences were detected between the groups in the left ear for TEOAEs at 4 kHz and acoustic reflex thresholds.

Conclusions: Individuals with GJH have some audiological differences that may be a predictor of changes related to future hearing loss. Further studies that involve larger samples and include participants of different ages are needed in order to determine whether individuals with GJH are more prone to hearing loss.

Keywords: Audiometry, joint laxity, generalized joint hypermobility, hearing loss, otoacoustic emissions

Generalized joint hypermobility (GJH) is characterized by an exaggerated ability to move the joints beyond the normal range of motion as a result of increased connective tissue flexibility and/or capsular or ligamentous looseness [1, 2]. GJH is often hereditary and thought to be caused by genetic alterations related to collagen [3]. It may occur without complications as an asymptomatic condition or may be accompanied by musculoskeletal symptoms, such as muscle or joint pain. GJH is commonly encountered in many other disorders [4]. It may exist as a part of genetic disorders that affect connective tissue (e.g., Ehlers-Danlos syndrome (EDS), Marfan syndrome, osteogenesis imperfecta (OI) or other syndromes (e.g., Down syndrome, bony dysplasias, velocardiofacial syndrome) [5].

GJH is defined by a Beighton score ≥ 5/9 [4, 6]. In a healthy university population, the prevalence of GJH was 33.0% for females and 12.3% for males [7]. Individuals with GJH are prone not only to musculoskeletal complaints, but also to manifestations such as cardiovascular dysautonomia, gastrointestinal

Received: ***March 10, 2021;*** *Accepted:* ***June 18, 2021;*** *Published Online:* ***February 1, 2022***

How to cite this article: *Taş M, Tuna F, Yılmaz Ş. Audiological differences in healthy individuals with generalized joint hypermobility: a case-control study. Eur Res J 2022;8(6):837-844. DOI: 10.18621/eurj.893220*

e-ISSN: 2149-3189

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motility issues, psychological distress, and fatigue [8]. However, the susceptibility to damage and the effect on hearing function have not been investigated. Although a few studies have shown sensorineural hearing impairment affecting predominantly high frequencies as an association between collagen tissue disorders featuring hypermobility (e.g. EDS, Stickler syndrome, Down syndrome, and OI) and audiological dysfunction [9-14], the association has not been examined in nonsyndromic, asymptomatic healthy individuals with GJH.

GJH is the best-known clinical manifestation of inherited defects of the connective tissue [15]. It has recently been shown that gene expressions of the asymptomatic healthy individuals with GJH (higher TNXB and SLC39A13 and lower COL1A1, COL1A2, COL5A1, FKBP14, and DSE) differ from those of the healthy controls, suggesting that genetic difference should not be underestimated [16]. As in all parts of the body, connective tissue is found in the ear. Collagen is the main component of connective tissue. Various collagen types have been found in multiple structures of the ear, such as the tympanic membrane, interossicular joints in the middle ear, and the cochlea [17]. Because of the genetic and acquired features that define joint hypermobility, which is related to connective tissue disorders, questions about the relationship between ear involvement and joint hypermobility are not unexpected. The aim of current study was to describe the relationship between GJH and audiological functions compared with age- and sex-matched controls.

METHODS

Participant Selection

Participants aged between 19 and 22 years of age were selected from a total of 131 students in the Audiometry Department from Health Services Vocational College of University. Students were evaluated according to the Beighton score, and those with a score ≥ 5/9 were considered to have GJH [4, 6]. A total of 25 participants with GJH were enrolled in the study. From the same source, 31 age- and sex-matched healthy volunteers with a Beighton score ≤ 4/9 and no risk for hearing deficit were selected. The exclusion criteria for both groups were a history of ototoxic drug use or ear

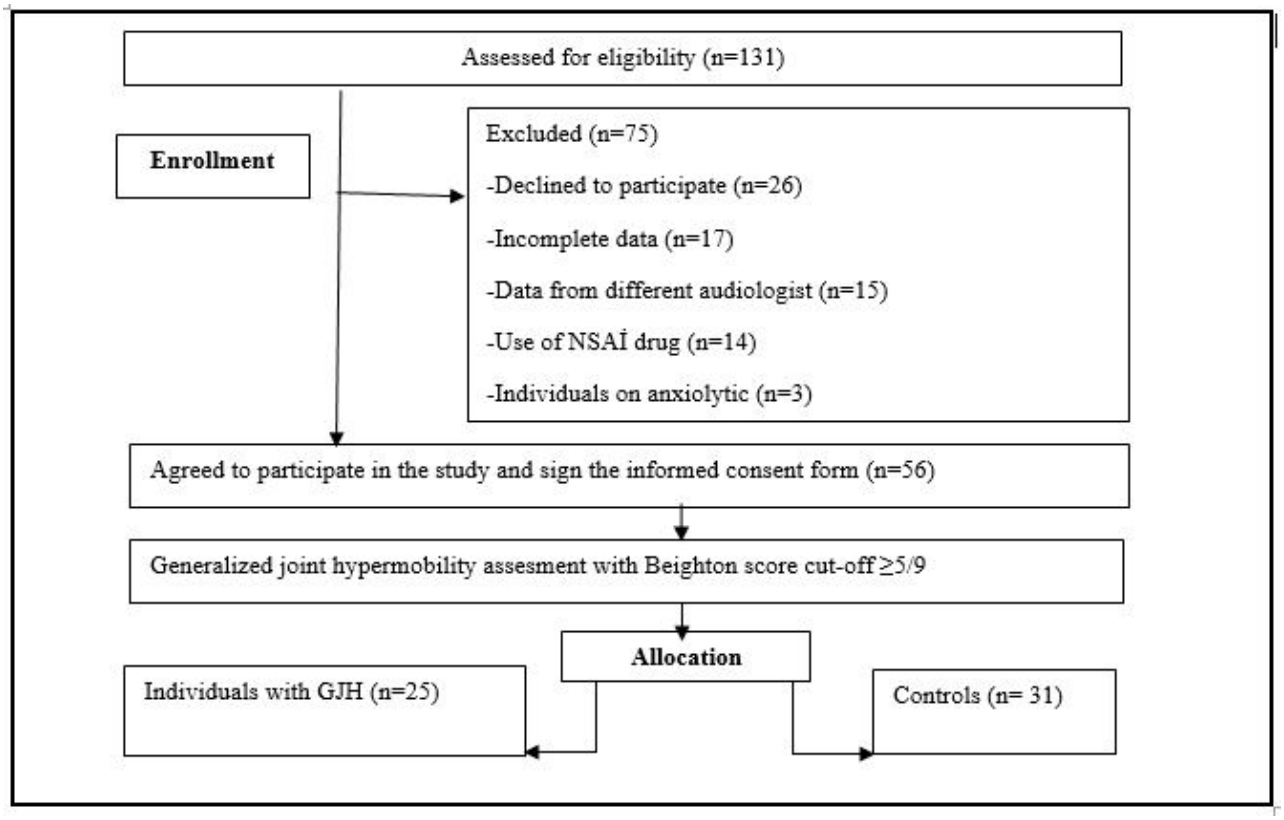

Fig. 1. The flowchart of the study.

surgery, congenital or acquired ear disease, and the presence of a known disease (Fig. 1). This observational and case control study was approved by the Ethics Committee of the University Medical Faculty (TUTF-BAEK-2017/146), and written informed consent was obtained from each student.

Clinical Evaluation

A total of 131 students from Health Services Vocational College of University underwent an initial clinical interview and musculoskeletal evaluation. All participants were evaluated and underwent examination by the second author. The Beighton score was determined by evaluating nine joints and the following items: I- Placement of hands flat on the floor without bending the knees, II- Hyperextension of the elbow to ≥ 10°, III- Hyperextension of the knee to ≥10°, IV- Opposition of the thumb to the volar aspect of the ipsilateral forearm, V- Passive dorsiflexion of the fifth metacarpophalangeal joint to ≥ 90° [2, 6]. During the physical examination, in order to exclude symptomatic GJH or Hypermobile Ehlers-Danlos syndrome, we investigated the presence of features used in the diagnosis of Hypermobile Ehlers-Danlos syndrome according to the 2017 International Classification of Ehlers-Danlos Syndrome [18]. Therefore, only participants with asymptomatic, nonsyndromic/isolated GJH were included in the study.

Evaluation of Hearing

Audiological measurements were performed in sound-isolated test rooms by a certified audiologist. The test procedure included otoscopy, impedance audiometry (tympanometry and acoustic reflexes), pure-tone audiometry, and transient evoked otoacoustic emission (TEOAE) tests.

Behavioral Audiometry

Air-conduction hearing thresholds were evaluated in both ears of the participants at 0.5-, 1-, 2-, 4-, and 8-kHz frequencies using Inter-acoustic Clinical Audiometer (AC-40, Denmark) and TDH-39 (Telephonics, USA) earphones. Bone conduction thresholds were measured if the air conduction thresholds were greater than 15 dB, using Radioear B-71 (Radioear, USA) bone vibrator.

Immittance Audiometry

Middle-ear function and acoustic reflexes were assessed using an AT235H impedance audiometer device (Interacoustics, Denmark). The tympanometry was performed with a 226 Hz probe tone. The measurement pressure range was set at + 200 daPa to -400 daPa. Type A tympanograms (peak pressure: between +100 daPa and -50 daPa) were accepted as normal. Contralateral acoustic reflex thresholds were measured at 1 kHz.

Otoacoustic Emission (OAE) Measurements

Transient Evoked Otoacoustic Emission (TEOAE) measurements were binaurally performed using the ILO 292 Echoport USB II and ILO V6 Clinical OAE software (Otodynamics, London), with non-linear click stimuli at 80 dB. TEOAEs were considered present if overall reproducibility was ≥ 70% and signal-to-noise ratio (SNR) was > 3 dB in at least three of the measured frequencies.

Statistical Analysis

Statistical evaluations were performed using IBM SPSS version 20.0 (IBM Corporation, Armonk, NY, USA). The normality of the distribution of the data was examined using the Kolmogorov-Smirnov (K-S) test. The TEOAE measurement results, which satisfy the conditions of normal distribution and variance equality, were compared using an independent samples t-test. Because data sets related to subjective audiometry and tympanometry measurements were not normally distributed and because no equality of variance was provided in the measurement of the acoustic reflex threshold (Levene test, $p < 0.05$), the Mann–Whitney U test was used in the comparisons of these measurements (K–S test, $p < 0.05$). In all comparisons between the groups, the data obtained from the left and right ears were compared separately. A p - value of < 0.05 was considered statistically significant.

RESULTS

The present study enrolled 56 participants: 37 (66.1%) females and 19 (33.9%) males. The GJH group consisted of 25 participants (17 females and 8 males), and the healthy control group consisted of 31 participants (20 females and 11 males). None of the participants met the criteria for Hypermobile Ehlers-Danlos syn-

drome. The mean age of all the participants was 20.25 ± 0.75 (range: 19-22) years; 20.24 ± 0.72 years; the mean age of the GJH group and control group were 20.24 ± 0.72 years and 20.26 ± 0.77 years, respectively. There was no difference between groups with respect to sex or age ($=\chi^2 0.36$, $p = 0.85$ and t-test, $p = 0.93$, respectively). The results of Beighton score evaluation are shown in Table 1. The median Beighton score of participants with GJH was 6 (min: 5, max: 8), and that of the controls was 2 (min: 0, max: 3). The mean body mass indexes of the GJH and control groups were 21.12 ± 2.28 and 21.84 ± 2.17 kg/m^2, respectively (t = 1.20, $p = 0.24$).

As table 2 shows, there were no significant differences in the mean hearing thresholds between the groups ($p > 0.05$). Six (5.4%) ears in the GJH group had thresholds above 15 dB at one or more frequencies (one at 0.5 kHz, four at 8 kHz, and one at all frequencies), whereas all subjects in the control group had hearing thresholds below 15 dB at all frequencies tested. One subject in the GJH group had a 55 dB threshold in the left ear at 8 kHz, despite normal values (≤ 15 dB HL) at the remaining frequencies. According to the pure tone average (PTA) across 0.5, 1, 2, and 4 kHz, another subject in the GJH group had unilateral minimal hearing loss (PTA for the left ear = 23 dB). None of the ears with elevated thresholds had air–bone gaps greater than 10 dB HL.

The mean reproducibility scores of the TEOAE measurements was as follows: right ear = 97.20 ± 2.93, left ear = 96.48 ± 5.35 in the GJH group, and right ear = 97.94 ± 1.31, left ear = 97.35 in the control

Table 1. Results of Beighton score evaluation

Beighton score items	GJH n (%)	Control n (%)	Total n (%)	OR (95% CI)	LR	LR *p* value
Placement of hands flat on the floor without bending the knees	7 (28.0)	2 (6.5)	9 (16.1)	0.177 (0.033-0.950)	4.896	**0.027**
Hyperextension of the right elbow to ≥10°	16 (64.0)	13 (41.9)	29 (51.8)	0.406 (0.137-1.202)	2.725	0.099
Hyperextension of the left elbow to ≥10°	14 (56.0)	12 (38.7)	26 (46.4)	0.496 (0.170-1.447)	1.669	0.196
Hyperextension of the right knee to ≥ 10°	11 (44.0)	6 (19.4)	17 (30.4)	0.305 (0.093-1.005)	3.994	**0.046**
Hyperextension of the left knee to ≥ 10°	9 (36.0)	6 (19.4)	15 (26.8)	0.427 (0.127-1.429)	1.952	0.162
Opposition of the right thumb to the volar aspect of the ipsilateral forearm	23 (92.0)	3 (9.7)	26 (46.4)	0.009 (0.001-0.061)	43.696	**< 0.001**
Opposition of the left thumb to the volar aspect of the ipsilateral forearm	21 (84.0)	2 (6.5)	23 (41.1)	0.013 (0.002-0.079)	39.022	**< 0.001**
Passive dorsiflexion of the right fifth metacarpophalangeal joint to ≥ 90°	17 (68.0)	5 (16.1)	22 (39.3)	0.090 (0.025-0.323)	16.306	**< 0.001**
Passive dorsiflexion of the left fifth metacarpophalangeal joint to ≥ 90°	21 (84.0)	9 (29.0)	30 (53.6)	0.078 (0.021-0.292)	18.012	**< 0.001**
Total	25 (100.0)	31 (100.0)	26 (100.0)			

GJH = generalized joint hypermobility

Table 2. **Results of pure-tone audiometry**

	Frequency (kHz)	Mean ± SD		Mean Ranks		*p* value
		GJH	Control	GJH	Control	
Left ear	0.5	7.00 ± 4.56	6.00 ± 2,38	29.00	28.10	0.79
	1	5.80 ± 3.12	5.19 ± 1.58	28.78	28.27	0.83
	2	6.00 ± 4.08	5.35 ± 1.80	28.30	28.66	0.89
	4	6.20 ± 4.15	5.19 ± 1.58	29.40	27.77	0.52
	8	7.20 ± 12.51	2.58 ± 4.44	31.96	25.71	0.11
Right ear	0.5	6.20 ± 3.61	5.48 ± 2.99	29.36	27.81	0.66
	1	5.40 ± 1.38	5.48 ± 2.37	28.16	28.77	0.83
	2	5.60 ± 2,20	5.16 ± 2.73	29.64	27.58	0.44
	4	5.00± 1.44	5.00 ± 1.83	28.50	28.50	1.00
	8	4.20 ± 5.89	3.06 ± 4.02	29.64	27.58	0.60

GJH = generalized joint hypermobility, SD = standard deviation

group ($p > 0.05$). The mean SNR values of TEOAEs by frequency are shown in Table 3 (after excluding one subject with a type B tympanogram). TEOAE amplitudes of the left ear at 4 kHz were significantly lower in the GJH group compared to the control group (t = 2.56, $p = 0.01$). In the immitancemetric measurements, no statistically significant difference was found between the groups with respect to the mean values of static compliance, which indicates the highest peak of the curve of the tympanogram. The mean values of static compliance in the GJH and control group, respectively, were as follows: for the left ear, M = 0.89 ± 0.61 and M = 0.71 ± 0.43; for the right ear, M = 0.89 ± 0.75 and M = 0.79 ± 0.43 ($p > 0.05$). All participants had bilateral type A tympanograms, with the exception of one subject in the GJH group, who had a type B curve (flat curve without peak) in the left ear. Acoustic reflex could not be obtained from the left ear of one

Table 3. **Mean signal to noise (SNR) values of TEOAEs by frequency and by ears in the groups**

Frequency (Hz)	Ear	GJH L ear (n =24) R ear (n = 25 (Mean ± SD)	Control (n = 31) (Mean ± SD)	T	*p value*
1000	Right	21.12 ± 6.63	21.25 ± 5.32	0.76	0.94
	Left	17.36 ± 8.61	17.96 ± 6.15	0.13	0.78
1400	Right	22.42 ± 6.25	23.49 ± 5.46	0.68	0.50
	Left	18.61 ± 8.37	21.05 ± 4.97	1.09	0.18
2000	Right	19.87 ± 4.88	20.76 ± 5.99	0.60	0.55
	Left	19.00 ± 5,95	18.19 ± 4.81	0.61	0.58
2800	Right	18.34 ± 5.41	20.19 ± 5.40	1.27	0.21
	Left	17.16 ± 5,41	19.79 ± 4.79	1.96	0.06
4000	Right	14.36 ± 5.17	17.02 ± 6.72	1.63	0.11
	Left	13.61 ± 6.60	17. 97 ± 6.01	2.56	**0.01***

GJH = generalized joint hypermobility, SD = standard deviation, L = left, R = right

Table 4. Acoustic reflex thresholds (dB) in the left and right ear for both groups

	Mean ± SD		Mean Ranks		*p value*
	GJH L ear (n =24) R ear (n = 25	Control (n = 31)	GJH	Control	
Left ear	96.46 ± 6.51	99.35 ± 3.59	24.08	32.03	0.018*
Right ear	95.40 ± 8.40	98.87 ± 5.43	25.78	30.69	0.12

GJH = generalized joint hypermobility, SD = standard deviation, L = left, R = right

subject with GJH. Therefore, when comparing the acoustic reflex thresholds (ARTs) for the left ear, the GJH group was taken as N = 24. As Table 4 shows, the ARTs for the left ears in the GJH group were significantly lower than in the control group ($p = 0.018$).

DISCUSSION

In this study, the mean hearing thresholds were statistically similar in the GJH and control groups. All subjects had normal hearing, with the exception of two subjects in the GJH group (one with slight unilateral hearing loss and the other with an elevated threshold of 55 dB at 8 kHz). However, significant differences were detected between the groups in the left ear for TEOAEs at 4 kHz and acoustic reflex thresholds.

Despite the lack of data about audiological features of individuals with nonsyndromic GJH, hearing loss is seen in patients who have genetic syndromes with joint hypermobility, including OI, EDS, and Stickler syndrome [13, 14, 19, 20]. Hearing loss in these disorders is thought to be related to structural changes in the middle and inner ear. However, the tympanometry results revealed that the middle ear system in the great majority of our study participants was normal. Only one subject in the GJH group had a type B curve, indicating little or no movement in the tympanic membrane. Although deep type A tympanograms were obtained from five subjects in the GJH group and from four subjects in the control group, there was no difference in the average compliance between groups. Static compliance represents the mobility of the middle ear, and a peak exceeding the upper compliance limit (> 1.6 cc) indicates an excessively flaccid middle ear system. This is a common finding in patients with Stickler syndrome [9, 19, 21]. Acke *et al*. [9] argued that the hypermobile middle ear system of Stickler patients may result from previous otitis media but that the collagen defect may also contribute. In the absence of hearing loss, as in this study's subjects, a deep type A tympanogram can be considered a result of minor tympanic membrane abnormalities, such as scar tissue and thin or single layer eardrum. The findings also showed that acoustic reflex thresholds (ARTs) were lower in the GJH group. Although acoustic reflex occurs at a lower sensation level in patients with cochlear pathology, it is broadly accepted that it varies from 70–80 to 100 dB HL in patients with normal hearing [22].

When comparing groups in terms of TEOAE measurement, SNR amplitudes in the left ear at 4 kHz were found to be lower in the GJH group than in the control group. In addition, pure-tone thresholds above 15 dB were observed only in the GJH group. These findings raise the question of whether individuals with GJH have an increased susceptibility to hearing loss. It is known that otoacoustic emissions are highly sensitive to cochlear pathology, and minimal amounts of cochlear damage that cannot be detected by behavioral pure-tone audiometry may cause measurable changes in OAE responses [23]. OAE testing permits early detection of cochlear dysfunction of genetic origin, as carriers of gene mutations who have normal behavioral hearing sensitivity may display subtle auditory abnormalities that can be observed in OAEs [24, 25]. Sensorineural hearing loss affecting mainly high frequencies has been detected in some patients with OI, EDS, and Stickler syndrome [13, 14, 18]. In a study of 141 children with EDS, Weir *et al*. [14] found that

the majority of patients had slight and mild hearing loss and that the type of hearing loss was equally divided between pure conductive and pure sensorineural deficits. Acke *et al*. [19] detected mild and predominantly high frequency (3-8 kHz) sensorineural hearing loss in Stickler patients. The same study, taking into account the normal pure-tone thresholds for several frequencies in most patients, found that OAEs were absent or had very low amplitudes, even in frequency bands with normal hearing. The pathogenesis of sensorineural hearing impairment associated with the above-mentioned disorders is not fully understood, but possible causes include hair cell loss, abnormalities of the tectorial membrane, and disruption of the stria vascularis [9, 13, 14, 21]. The functional integrity of outer hair cells is essential for the generation of OAEs. In addition, stria vascularis and spiral ligament, which are critical structures for the function of the cochlea, are thought to play an important role in the mechanisms of OAEs [26]. In this study, reduced TEOAE amplitudes at 4 kHz may be related to possible changes in these structures.

Limitations

This study has several limitations. First, the research conducted with a small sample characterized by a narrow age range. Second, there are no comparable studies, because previous research has focused only on syndromes associated with hypermobility. In addition, considering that acoustic reflex thresholds were found to be lower in the GJH group, it would have been useful to compare acoustic reflexes of the groups for all frequencies. Third, we didn't use the Auditory Brainstem Response (ABR) test. However, as we mentioned, some studies have detected a hearing loss in some patients with genetic syndromes with joint hypermobility. It is thought to be related to structural changes in the middle and inner ear. In the light of these studies indicating middle and inner ear pathologies, we aimed to investigate whether GJH patients have hearing loss and any audiological differences that could be specifically related to the middle and inner ear. For this reason, we thought that pure-tone audiometry, immittance audiometry, and OAE test could be enough to examine middle and inner ear function. Since OAE measures are sensitive indicators of cochlear function and thought to be an additional cross-check measure, we included the OAE test. In addition, the ABR test can provide additional information about the status of the auditory pathway, including the neural pathway. To our knowledge, this is the first report of audiological features of individuals with non-syndromic GJH. We believe that future studies could explore this issue further by investigating ABR differences in this population.

CONCLUSION

In conclusion, the findings of the current study showed that individuals with GJH have some audiological differences that may be a predictor of changes related to future hearing loss. Further studies that involve larger samples and include participants of different ages are needed in order to confirm the result and determine whether individuals with GJH are more prone to hearing loss.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):845-850 Original Article

DOI: 10.18621/eurj.870909 *Chest Diseases*

Contribution of bronchoscopic lavage to the diagnosis of smear negative pulmonary tuberculosis

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ABSTRACT

Objectives: Sputum acid-fast bacilli (AFB) and Xpert examinations are the most commonly used methods in early diagnosis for pulmonary tuberculosis. It becomes difficult for the clinician to initiate treatment in patients with negative sputum smear and Xpert examinations at the stage of diagnosis. Waiting for culture results for diagnosis causes delay in treatment and increases the risk of disease transmission. We aimed to investigate the contribution of bronchoscopic lavage (BL) obtained with flexible fiberoptic bronchoscopy (FOB) to the diagnosis of tuberculosis.

Methods: The BL results of 36 patients hospitalized in the tuberculosis clinic who were clinically suspected of tuberculosis, but whose sputum AFB and Xpert results were negative, and whose bronchoscopic lavages were taken by FOB, were retrospectively evaluated.

Results: BL Xpert examination was the method that made the most contribution to the diagnosis with a rate of 27.7%. BL smear AFB results did not contribute to the diagnosis statistically. Sputum and BL tuberculosis culture results were found to be close to each other. In total, the diagnosis of 50% of the patients was confirmed.

Conclusions: Performing Xpert examinations on bronchoscopic materials of patients with negative sputum examinations significantly increase the rate of early diagnosis.

Keywords: Sputum, smear negative, pulmonary tuberculosis, acid-fast bacilli, bronchoalveolar lavage, MGIT Culture, Xpert

Tuberculosis (TB) is one of the top 10 causes of death worldwide, and nearly a quarter of the world's population is infected with *Mycobacterium tuberculosis* [1]. Although mycobacterium culture is the gold standard in the diagnosis of pulmonary tuberculosis, the growth of *M. tuberculosis* in cultures occurs after 6-8 weeks. Therefore, sputum acid-fast bacilli (AFB) and Xpert examinations are the most commonly used methods in early diagnosis. Sputum smear is important for early diagnosis, but in order to observe the bacillus in the smear, there should be 5000 bacilli per ml [2]. Approximately 50-60% of patients with pulmonary tuberculosis can be diagnosed with sputum smear examinations [3]. Xpert test is more sensitive than microscopy [4]. However, even if there is a bacillus in the sputum, a small number of bacilli may not be detected. It becomes difficult for the clinician to initiate treatment in patients with negative sputum smear and Xpert examinations at the stage of diagnosis. Waiting for culture results for diagnosis both causes delay in treatment and increases the risk of disease transmission. Flexible fiberoptic bronchoscope (FOB) can be used to perform mycobacterial examinations in these patients [5, 6]. In addition, broncho-

Received: *January 30, 2021;* ***Accepted:*** *January 24, 2022;* ***Published Online:*** *February 9, 2022*

How to cite this article: *Şahin ME, Bulut S. Contribution of bronchoscopic lavage to the diagnosis of smear negative pulmonary tuberculosis. Eur Res J 2022;8(6):845-850. DOI: 10.18621/eurj.870909*

e-ISSN: 2149-3189

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scopic lavage (BL), broncho-alveolar lavage (BAL), and bronchial brushing methods can be applied for early diagnosis. Considering the efficacy/complication rate, trans-bronchial needle aspiration (TBNA) or trans-thoracic needle aspiration biopsy (TNAB) has been used for a long time because of its contribution to both early diagnosis and differential diagnosis [7-10]. These methods are important both in detecting tuberculosis and in differential diagnosis. Averagely 30-40% untreated smear negative patient will develop active TB within first two year period [11].

METHODS

Patients

In the institution where we work, a committee called the Medical Specialty Education Board is convened and it checks the appropriateness of the planned studies. So we applied to this board and got the approval of our study with the decision (date-number Jan 25, 2020-659). Thirty-six patients with a clinical and radiological suspicion of pulmonary tuberculosis, who received inpatient treatment at our Ankara Ataturk Chest Diseases and Thoracic Surgery Training and Research Hospital 2-TB clinic between 2018-2019 years, and whose smear AFBs and sputum Xperts found negative in 3 sputum samples was included. Sputum induction was applied to all patients who could not produce sputum or had negative sputum smears. The additional contribution of BL application to sputum examinations was investigated. Therefore, patients who could not produce sputum in any way were excluded from the study. Less involvement in the upper right zone was evaluated as limited involvement on chest radiography. Those with more involvement were defined as diffuse involvement. Those with and without cavities in the radiography were recorded. Smear AFB and Xpert positivity of BL were accepted as early diagnosis.

We included 36 patients who had negative sputum AFB and Xpert's but had a pre-diagnosis of pulmonary tuberculosis to our study and we aimed to evaluate the contribution of smear, culture and Xpert results of their FOB lavages' to the diagnosis.

Bronchoscopic Lavage Technique

Written informed consent was obtained from all patients before the bronchoscopic procedure. Premedication was performed with diazepam before FOB. Also local anesthesia was applied to the upper respiratory tract with 10% lidocaine. After examining the tracheobronchial tree with Olympus 1T260 type FOB, BL was performed with 15-20 cc saline solution from the lobe bronchus, where lesions were detected by radiological methods before the procedure. During the procedure, 2% lidocaine was applied as needed for local anesthesia. Lidocaine was not applied during lavage.

Microbiological Testing

The lavage smears were stained with Ziehl-Neelsen (Z-N) to search for acid-fast bacilli. BL samples were seeded on solid (Lowenstein-Jensen) and liquid (MGIT 960) media for tuberculosis culture.

Xpert MTB/RIF Test

This test is a nucleic acid amplification (NAA) test. Disposable cartridge is used. It is based on heminested real-time PCR that amplifies the rpoB gene target. Sample is taken from the patient with suspected TB. The material is mixed with the reagent provided by the test and a cartridge containing this mixture is placed in the Xpert machine. From this point on, all transactions are fully automated. The Xpert MTB/RIF test detects Mycobacterium Tuberculosis Complex (MTBC) and rifampine (RIF) resistance in less than 2 hours. It has an excellent accuracy when made in sputum and has been approved by the World Health Organization (WHO) [12, 13]. The general sensitivity and specificity of the Xpert Ultra test we used were reported as 87.5% and 98.7% respectively. This sensitivity was found to be 78.9% in smear negative samples. The limit of detection (LOD) is reported as 15.6 CFU/ml for sputum [14].

Cepheid Xpert MTB / RIF Ultra, GXMTB / RIF-ULTRA-10 devices and cartridges were used for Xpert evaluation. After the material was treated with NaOH, it was mixed with the reagent provided with the test and loaded into the system with a disposable cartridge and the samples were evaluated by automatic reading by the system.

Statistical Analysis

Categorical independent data were analyzed using the chi-square test. Categorically dependent data were

analyzed using the McNemar test. Normal distribution of numeric variables was evaluated with Shapiro-Wilk test, Skewness and Kurtosis. Normally-distributed numeric variables were expressed as mean and standard variation while non-normally distributed variables were expressed as median. The correlation between two numeric variables with normal distribution and a linear correlation was analyzed using Pearson's test. The correlation between variables that did not show normal distribution was evaluated with Spearman's test. All analyses were performed using SPSS 22.0 statistical software (SPSS Inc, Chicago, IL, USA). A $p < 0.05$ was considered statistically significant.

RESULTS

Of the patients included in the study, 17 (47.2%) were female and 19 (52.7%) were male. The mean age of the female patients was 42.2 and the mean age of the male patients was 47.9. The youngest patient was 18 and the oldest patient was 83 years old. The most common symptom was cough (88.9%), followed by weight loss (69.4%) and fever (55.6%) (Table 1).

Comorbid diseases are chronic obstructive pulmonary disease (1 patient), asthma bronchiale (1 patient), cervix Ca. (1 patient) and non-hodgkin lymphoma (1 patient). Two patients were diagnosed with diabetes mellitus and were using oral anti-diabetic agents. None of the patients were HIV positive but close contact was detected in the histories of six patients. There was a cavity in the chest radiograph of 21 patients. There was no correlation between the presence of cavity and BL AFB smear/Xpert or culture positivity ($p = 0.768$ and $p = 0.571$, respectively). Radiologically, seven patients had less involvement than right upper zone. Wider involvement was detected in 29 patients (Table 2). The relationship between the bacteriological positivity rates of the patients and the CRP, sedimentation rate, PPD values, the presence of cavity in the chest radiographs and the amount of involved zones were evaluated. There was no statistically significant relationship between these parameters ($p = 0.484$, $p = 0.733$, $p = 0.059$ and $p = /0.079$ respectively).

Table 1. Demography and symptoms

	Data
Gender, n (%)	
Male	19 (52.7)
Female	17 (47.2)
Age (years) (mean±SD)	
Male	47.94 ± 22.27
Female	42.11 ± 19.64
Symptoms, n (%)	
Cough	32 (88.9)
Weight loss	25 (69.4)
Fever	20 (55.6)
Weakness	17 (47.2)
Hemoptysis	7 (19.4)
Dyspnea	4 (11.1)
Chest pain	2 (5.6)

Sputum tuberculosis culture result was positive in 12 (33.3%) patients. On the other hand, lavage tuberculosis culture was positive in 9 (25%) patients. There was no statistically significant difference between lavage and smear culture positivity ($p > 0.05$). In one patient, MBT and non-tuberculous mycobacteria (NTM) were found together in mycobacterial cultures. In 3 (5.5%) patients, lavage smear was positive for AFB. This rate was not statistically significant ($p = 0.25$). Two of these 3 patients had positive lavage Xpert results. In the third patient, the sputum culture was positive, so lavage AFB contributed to early diagnosis in only 1 patient. In 10 (27.7%) patients, lavage Xpert was positive. This rate was statistically significant ($p = 0.002$).

The examinations of 13 (36.1%) patients were positive and 11 (30.5%) patients were diagnosed early with the applied BL. Most of the patients who can be diagnosed early are diagnosed with Xpert positivity. AFB positivity in bronchoscopic lavage was detected in only 3 (8.3%) cases. With lavage Xpert and AFB positivity, this rate was 30.5% with 11 patients (Table 3). In total, 18 (50%) patients were diagnosed with sputum culture and lavage examinations. Clinical improvement and radiological regression were detected in all patients during their 6-month follow-up.

DISCUSSION

In this study, we aimed to evaluate the contribution of

Table 2. **Bacteriological positivity according to chest radiography findings**

(n = 36)	Cavitary	Non-cavitary	Correlation of the cavity (*p* value)	Involvement less than the upper right zone	Involvement greater than the upper right zone	Correlation of the involvement (*p* value)
Chest radiography, n (%)	21 (58.3)	15 (41.6)		7 (19.4)	29 (80.6)	
BL AFB smear or Xpert +	6	4	0.768	1	9	0.311
BL Culture +	5	4	0.571	0	9	0.094

AFB = Acid fast bacilli, BL = Bronchoscopic lavage

bronchial lavage results to the diagnosis of pulmonary tuberculosis in patients with negative AFB smear in sputum.

In our study, lavage smear AFB was found to be positive in 3 (8.3%) patients. Two of these three patients had positive lavage Xpert results and one had positive sputum culture results. Previous studies show that BL smear AFB exams can range from 4% to 60% [6, 15, 16]. In the study of Yuksekol *et al*. [17], one of the studies conducted in our country, AFB direct examination of bronchial lavage was found to be positive in 23.2% of cases. While this rate was 19% in Alp *et al*.'s [8] study, Balbay *et al*. [7] Found that BL smear positivity rate was 29% which was higher than ours. However, in the study of Intepe *et al*. [18], bronchoalveolar lavages of 83 patients were examined and no smear positivity was found in any of the patients. Studies show that the rate of AFB smear positivity in BL or BAL can vary in a wide range.

Although it has been reported that bronchoscopic examinations may contribute more to the diagnosis than sputum examinations, the positivity of induced sputum smear is considerably higher. In the comparative study published in 2017 by Luhadia *et al*. [19], a microbiological comparison of induced sputum and bronchial aspirate was performed on 100 patients (60/40, respectively) with negative sputum smear AFB results, and the diagnostic value of bronchial aspirate was found to be significantly higher. It has been suggested that FOB examinations can be preferred according to the cost-complication/efficiency ratio. In the study of Gopathi *et al*. [20], the positivity of AFB smear in BL is slightly higher than in induced sputum. The results of sputum induction and bronchoscopy were similar in the meta-analysis performed by Luo *et al*. [21].

We think that our AFB smear results can be compared with the results of Luhadia, Gopathi and Lua's studies, as sputum induction is applied to patients who cannot produce sputum or who are negative for sputum AFB smears.

In our study, smear AFB results were positive only in 3 patients, but when this result is evaluated together with BL AFB, smear and Xpert results, the positivity rate increases from 8.33% to 30.5%. This ratio is also the rate of patients who were diagnosed early. Early diagnosis was obtained mostly (27.7%) by Xpert positivity.

The Xpert MTB / RIF tests, which started to become widespread in the 2000s and are now being used in many centers in our country, can detect a higher rate of positivity in materials taken with FOB than in AFB smear examinations [22-25]. There are many publications that support this. In the study of Lee *et al*. [26]

Table 3. **Bacteriological results**

	Smear AFB + n (%)	Xpert + n (%)	Solid and liquid culture + n (%)
Sputum (n = 36)	0 (0)	0 (0)	12 (33.3)
BL (n = 36)	3 (8.3)	10 (27.7)	9 (25)

AFB = Acid fast bacilli, BL = Bronchoscopic lavage

published in 2013, 50 of 132 patients were diagnosed with tuberculosis with BL + BAL, and the sensitivity of AFB smear and Xpert tests in this study was found to be 13.2-81.6%, respectively. In the study of Khalil and Butt [24] these rates were found to be 36% and 87%, respectively. In the study published by Le Palud *et al*. [23] in 2014 and evaluated 162 patients retrospectively, BL or BAL were used as bronchoscopic material. In this study in which 30 tuberculosis patients were identified, 16% of the patients were AFB smear positive, while 80% were found to be Xpert positive [23]. However, the sensitivity of the Xpert test in smear-negative samples is reduced [14].

The patients included in almost all of the other studies were those who sesputum smears were negative or who were unable to produce sputum. However, patients who could not produce sputumwere not included in our study. We think this is an important reason why our BL smear results were less positive than previous studies. Although the sputum examinations of all patients were examined together with the AFB smear and the Xpert, the negative sputum examinations suggest that the number of bacilli in the respiratory secretions of the patients included in the study may be low. This is also observed in our FOB-BL results.

The inhibitory effect of lidocaine, which is used as a local anesthetic in bronchoscopy, to the growth of MTB in cultures, has been known for a long time [27-29]. The possibility that the patients may have a low number of bacilli in respiratory secretions or that the patients have not been able to produce high-quality sputum do not completely eliminate the possibility of lidocaine inhibition. In the study of Schmidt *et al*. [30], it is stated that lidocaine at concentrations of 1% and above inhibits MBT growth. In our study, we tried to minimize the local anesthetic exposure of the bacilli during the bronchoscopic procedure. In addition, positive results of sputum and BL cultures decrease this possibility. The results of our study exclude the negative effect of local anesthesia, with a lower rate of smear-AFB positivity in lavage, and a higher rate of culture positivity. We consider the inhibition of MTB growth due to lidocaine as a poor possibility.

In our study, BL AFB smear examinations did not significantly contribute to early diagnosis. However, the Xpert results made a statistically and clinically significant positive contribution. Although the disadvantage of Xpert examinations is that they can not detect non-tuberculosis mycobacteria, it's advantage is the detection of rifampicin resistance and rapid results less than two hours.

CONCLUSION

Bronchoscopic lavage Xpert examination contributes significantly more to the diagnosis than sputum Xpert examination. In centers where Xpert MTB/RIF can be examined, bronchial lavage statistically increases the rate of early diagnosis. It is also important to learn about RIF resistance at an early stage, in terms of the continuation and direction of treatment.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):851-858 Original Article

DOI: 10.18621/eurj.1132682 *Public Health*

Post-COVID-19 vaccine SARS-CoV-2 antibody investigation in healthcare professionals

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ABSTRACT

Objectives: Main purpose of this study was evaluating inactive severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccine subsequent anti-S1 IgG feedback and the aspects involved in such reactions for professionals in healthcare (HCP) as the dominant risk group.

Methods: Thirty-six HCPs with previous COVID-19 infection and 164 with no priors, 200 in total, who was working in the Ankara Public Health Molecular Diagnosis Laboratory were included. Main tool of identifying humoral immune response quantifably in serum samples which were obtained 28 days after administering each of two doses of vaccine was Roche Elecsys SARS-CoV-2 kit.

Results: Average antibody levels of 164 negative HCPs were 15.82 ± 8.59 IU/mL and 26.042 ± 10.73 IU/mL while 36 positive HCPs demonstrated antibody responses as 66.083 ± 33.927 IU/mL and 90 ± 27.012 IU/mL 28 days after each of two doses of vaccine for both individual groups respectively. A statistically meaningful difference was found in antibody levels after two vaccine doses in both groups ($p < 0.0001$). The authors observed statistically higher average antibody levels after initial vaccine dosage in HCPs with infection than the antibody levels of naive individuals after second dose ($p < 0.0001$). Age, gender and vaccination feedback did not have a statistically meaningful disparity ($p > 0.05$).

Conclusions: It was concluded that the average antibody level achieved after inital dose n HCPs with COVID-19 infection was surpassing the average antibody level obtained after the second dose in naive HCPs. The authors recommend further clinical researches on antibody levels and the extent of protection to prohibit COVID-19.

Keywords: SARS-COV-2, anti-S1 IgG response, health care professionals, COVID-19

New severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is an enveloped and single-stranded RNA virus that belongs to Coronaviridae family that causes the infectious respiratory disease Coronavirus disease 2019 (COVID-19) [1]. More than 430 million confirmed cases of COVID-19 have been reported worldwide with more than five and a half million deaths by the end of February 2022 according to World Health Organization (WHO) data [2] for coronavirus disease which was declared a pandemic by the WHO on March 11, 2020 [3]. Pandemic lead to an increased danger of vocational liability

Received: ***June 18, 2022;*** *Accepted:* ***August 17, 2022;*** *Published Online:* ***October 19, 2022***

How to cite this article: *Gürer Giray B, Güven Açık G, Baş SM, Bulut YE, Kotanoğlu MS. Post-COVID-19 vaccine SARS-CoV-2 antibody investigation in healthcare professionals. Eur Res J 2022;8(6):851-858. DOI: 10.18621/eurj.1132682*

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e-ISSN: 2149-3189

against a novel and rapidly advancing infection for health and care workers (HCPs) worldwide and thus establishing a set of requirements to accommodate new obligations and aspects for a spacious scope of duties and professional services [4]. Research carried out earlier demonstrate the escalation of infection rates in symptomatic and asymptomatic HCPs up to 14% and 7.1% respectively which surpass the percentages reported by the prevailing demographic studies to date and proposes a professional hazard [5, 6]. Preventing COVID-19 infections in hospitals for HCPs and their families is significant but surging numbers of HCP demise nationally and internationally indicate this premise is being disregarded [7].

Steep movement of proinflammatory immune cells induces acute respiratory distress syndrome, septic shock, bleeding and coagulation dysfunction in intense conditions [8]. 16 nonstructural proteins four structural proteins as of spike (S), envelope (E), membrane (M), and nucleocapsid (N) are encoded by Sars-CoV-2 genome [9]. Viral spike (S) protein engagement with host angiotensin-converting enzyme 2 (ACE2) receptor commences host cell infections by SARS-CoV-2 [10]. The S glycoprotein weighs 180 kDa and consists of two subunits as S1 and S2 [11]. The S1 subunit is one of the best immunological targets for antibodies that neutralize SARS-CoV-2 due to its neutralizing antibody induction capacity and species-specific antigenic specificity. while containing the receptor-binding domain (RBD) responsible for virus entry into the host cell via the ACE2 receptor [12, 13].

The humoral immune response can hinder contagion by subduing antibodies that restricts the virus in a way that inhibits host cell infection [2]. This condition can be fulfilled by obstructing spike-ACE2 receptor interaction or by disturbing fusion system the virus fancies to infiltrate host cell cytoplasm for SARS-CoV-2 [14]. Antibodies targeting the spike (S) glycoprotein and the nucleocapsid (N) protein play a role in the humoral immune response against SARS-CoV-2. Such antibodies neutralize viral infection of human cells and tissues that express angiotensin converting enzyme 2 (ACE2) [12, 15]. Efforts to develop vaccines to control the pandemic started early and today vaccines based on different principles such as mRNA vaccines, adenoviral vector-based vaccines and inactivated virus vaccines are being utilized after phase studies [16, 17].

SARS-CoV-2 vaccines depend on a strategy that induces humoral and cellular immune response and neutralizes antibodies against the virus' S protein which plays an important role in infecting cells or RBD based on its strategy [18, 19]. CoronaVac is an inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences Co., Ltd. (Beijing, China). CoronaVac vaccine phase 1/2 studies were initiated in China and the vaccine was demonstrated inducing neutralizing antibodies after it was shown to be safe and immunogenic in different animal models such as rodents and non-human primates [20, 21].

SARS-CoV-2 vaccine administration have been initiated after phase 3 with HCPs priority and then to high-risk groups on January 11, 2021 in Turkey. This program was composed of two intramuscular doses of CoronaVac 600 U/0.5 mL (Sinovac Life Science Co, Ltd, Beijing, China) vaccine 28 days in between each dose. The BNT162b2 vaccine (Pfizer-BioNTech) was included in the immunization program with two doses administed with four-week intervals. 52,798,119 people in our country were vaccinated with two doses of SARS-CoV-2 vaccine as of February 28, September 2022 [22]. In this study, it was aimed to determine the antibody responses that occur after two doses of inactivated SARS-CoV-2 vaccine (CoronaVac) administration in HCPs in the COVID-19 risk group and to assess factors leading to this response.

METHODS

Study Design and Ethics

This study was performed with the approval of the TR Ministry of Health COVID-19 Scientific Research Evaluation Commission (Decision No: 2021-12-07T01_19_42) and the approval of the TR Ministry of Health Sciences University Ankara Training and Research Hospital Clinical Research Ethics Committee (Decision No: E-21-859). Samples were obtained after written informed consent had been obtained, and all procedures were performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

Study Population and Samples

200 HCPs working in Ankara Provincial Health Directorate Public Health Molecular Diagnosis

COVID-19 Laboratory were included in the study after obtaining informed consent. Blood samples were collected 28 days after both initial and follow-up vaccine doses administered to HCPs. Age, gender, smoking and COVID-19 infection status were recorded to determine the factors that may affect the SARS-CoV-2 antibody response.

Antibody Measurements

Four to five ml blood samples of health worked included in the study were taken into ethylenediamine tetra acetic acid (EDTA) tubes, centrifuged at 4000 rpm for 10 minutes and serum samples were separated. Serum samples were stored at -80°C until they were included in the study. SARS-CoV-2 total antibodies (IgM and IgG) were quantitatively identified by the electrochemiluminescence immunological (ECLIA) process and the Elecsys® Anti-SARS-CoV-2 kit (Roche Diagnostics GmbH, Germany) involving recombinant protein representing the receptor-binding site (RBD) of S1 antigen. The assessment scope of the kit is between 0.40-250 U/mL and values above 0.80 U/mL are acknowledged as positive in result details as recommended by the manufacturer Roche. Results are automatically calculated in the form of a cut-off index (COI) [23].

Statistical Analysis

Percentage and frequencies were used for categorical variables in statistical analysis. Normality assumption was carried out with Shapiro-Wilk and Kolmogorov-Smirnov tests. Independent t test was used to compare independent variables and paired sample t-test was used to compare dependent variables. One-way variance analysis and bonferroni post-hoc analyses were applied in cases where there were more than two groups. The statistical significance level (*p* value) was determined as 0.05 in all analysis, and they were performed with the R software version 3.6.0.

RESULTS

One hundred twenty-two (62%) of the 200 HCPs included in the study were female and 76 (38%) were male with a mean age of 43.69 ± 1.17 years (range: 24 to 65 years) (Table 1). The occurrence of having pre-

Table 1. Demographic characteristics of healthcare professionals

	Total (n = 200)	COVID-19 negative (n = 164)	COVID-19 positive (n = 36)	*p value*
Age (years), n (%)				0.71
Mean ± SD (range)	43.69 ± 1.17 (24-65)	43.78 ± 1.34 (24-64)	43.17 ± 2.27 (28-57)	
24-35	40 (20)	34 (20.7)	6 (16.7)	
36-45	74 (37)	60 (36.6)	14 (38.9)	
≥ 46	86 (43)	70 (42.7)	16 (44.4)	
Gender, n (%)				**0.004**
Female	124 (62)	104 (63.4)	20 (55.6)	
Male	76 (38)	60 (36.6)	16 (44.4)	
Smoking status, n (%)				0.97
No	146 (73)	130 (79.3)	16 (44.4)	
Yes	54 (27)	34 (20.7)	20 (55.6)	
Adverse effect, n (%)				0.36
First post-dose	54 (27)	32 (19.5)	22 (61.1)	
Second post-dose	68 (34)	44 (26.8)	24 (66.7)	

SD = standard deviation

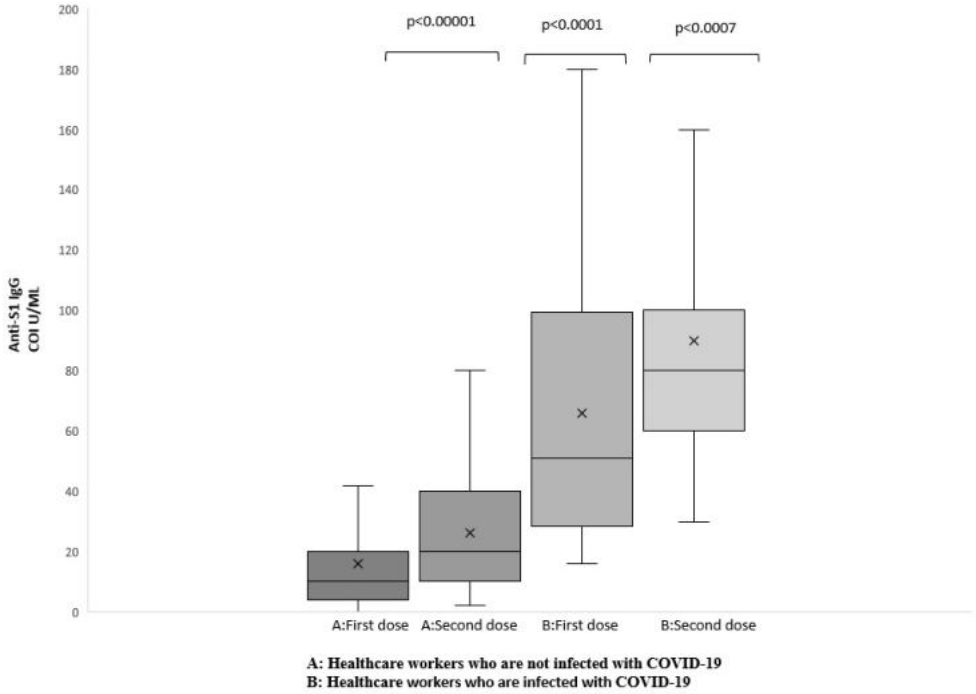

Fig. 1. Antibody response after first and second doses of vaccine.

vious COVID-19 infection confirmed with real-time reverse transcriptase polymerase chain reaction (rT-PCR) was determined as 18% (n = 36). There was no significant difference between the groups with and without COVID-19 infection in terms of age ($p = 0.71$) and smoking ($p = 0.97$) except for gender ($p = 0.004$) according to the data collected to identify the factors affecting the antibody response to the inactivated SARS-CoV-2 vaccine (Table 1). It was found out that 36 HCPs who had COVID-19 infection 65 days (day = 15-150) ago on average had 100% antibody response after the first and second doses of vaccine and the average antibody levels were 66.083 ± 33.927 and 90 ± 27.012. The antibody responses of 164 (82%)

Table 2. Factors affecting inactivated SARS-CoV-2 vaccine response

	Individuals without COVID-19 infection (n = 164)					Individuals with COVID-19 infection (n = 36)				
	n	First dose PVAE, COI (mean + SD)	*p value*	Second dose PVAE, COI (mean + SD)	*p value*	n	First Dose PVAE, COI (mean + SD)	*p value*	Second Dose PVAE, COI (mean + SD)	*p value*
Age (years)										
24-35	34	18.35 ± 8.011	0.49	28.38 ± 9.03	0.74	6	54.5 ± 20.32	0.76	73.33 ± 9.65	0.22
36-45	60	16.13 ± 8.59		25.83 ± 11.39		14	66.07 ± 32.19		85 ± 24.74	
≥ 46	70	14.32 ± 8.78		25.08 ± 11.09		16	70.43 ± 38.72		100.62 ± 31.05	
Gender										
Female	104	14.62 ± 7.55	0.24	24.74 ± 10.23	0.27	20	72.25 ± 29.95	0.35	92 ± 25.66	0.7
Male	60	17.97± 10.50		28.3 ± 11.54		16	58.375± 35.49		87.5 ± 25.04	
Smoking status										
Yes	34	21.32 ± 12.13	0.08	34.55 ± 11.32	**0.03**	20	67 ± 38.57	0.89	90 ± 29.73	1
No	130	14.38 ± 7.36		18.84 ± 10.23		16	64.937 ± 27.72		90 ± 23.31	

COI = cut-off index, PVAE = post-vaccination adverse effect

HCPs who did not have COVID-19 infection were 97.56% (n = 160) and 100% (n = 164) 28 days after each of the two vaccine doses while average antibody levels were determined as 15.82 ± 8.59 and 26.042 ± 10.73 respectively (Table 2). Moreover, it was determined that the average antibody level of HCPs with COVID-19 infection after the first dose of vaccination was higher than the average antibody levels of HCPs who did not have COVID-19 infection after the second vaccine dose and there was a statistically meaningful variance (Fig. 1). ($p < 0.001$).

A statistically important discrepancy was detected between the antibody levels of initial and following vaccine dose in both groups (Fig. 1). Average antibody levels of HCPs aged 24-35, 36-45 years and ≥ 46 years old who were not infected with COVID-19 were determined as 28.38 ± 9.03 IU/mL 25.83 ± 11.39 IU/mL, 25.08 ± 11.09 IU/mL respectively after second vaccine dose. There was no statistically compelling difference between average antibody level and age (Table 2) ($p > 0.05$). 20 (55.6%) of the HCPs with COVID-19 infection were female and 16 (44.4%) were male. The average antibody levels after the first dose of vaccination were 72.25 ± 29.95 and 58.375 ± 35.48 IU/mL while the antibody levels were determined as 92 ± 25.66 IU/mL and 87.5 ± 25.04 IU/mL respectively after the second dose of vaccination. It was found out determined that there was no statistically significant difference in the antibody response after the first and second doses of vaccine (Table 2) ($p > 0.05$).

Post-vaccination adverse effect (PVAE) was observed in 27% (n = 54) and 34% (n = 68) of the patients after two doses (Table 2). It was observed that there was no statistically significant difference in terms of PVAE after the first and second doses of inactivated SARS-CoV-2 vaccine in all HCPs with respect to PVAE ($p > 0.05$). The distribution of PVAE observed after the first and second vaccine doses is given in Fig. 2. The average post-dose antibody levels of smoking HCPs with and without COVID-19 infection were 34.55 ± 11.32 IU/mL and 90 ± 29.73 IU/mL while non-smokers were determined as 18.84 ± 10.23 IU/mL and 90 ± 23.312 IU/mL respectively. A statistically convincing divergence was determined between smoking and antibody feedback in HCPs who did not have COVID-19 infection (Table 2) ($p = 0.03$).

DISCUSSION

Investigation of definitive antibodies ensuing active immunization in managing COVID-19 pandemic has an important place both in the vaccine development and approval process and in the follow-up of vaccinated individuals [24]. In this study, it was determined that 95.12% (n = 156) and 100% (n = 164) antibody response was achieved 28 days after the first and second dose of inactivated SARS-CoV-2 vaccine administration in HCPs who did not have COVID-19 infection respectively while 100% antibody response was obtained after initial vaccine dose in HCPs with previous COVID-19 infection. 100% anti-RBD IgG seroconversion was reported after the second vaccine in the 18-59 age cluster in the phase 1 and phase 2

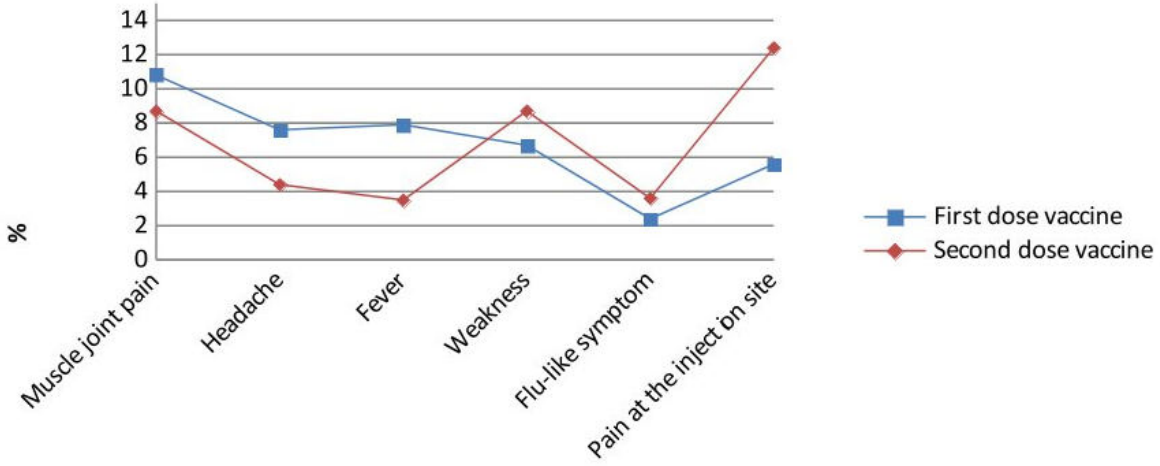

Fig. 2. The distribution of post-vaccination adverse effect (PVAE) (%) after the first and iconic dose of vaccine administration.

clinical trials of the inactivated SARS-CoV-2 vaccine [25].

Antibody responses were found as %92 for BNT162b2/Pfizer-BioNTech and %100 for 1273/Moderna as SARS-CoV-2 mRNA vaccines; 92.9% AstraZeneca and %100 for Sputnik V (rAd26-S and rAd5-S) as viral vector vaccines while 96% and %100 for BBIBP-CorV/Sinopharm as inactivated virus vaccines used worldwide [26-29].

The fact that SARS-CoV-2 vaccines provide different levels of seroconversion shows that the technology used in vaccine production and target antigens affect the antibody response. Antibody level variation after the initial vaccine dose can be explained by the rapid and strong antibody response of individuals with COVID-19 infection due to the secondary immune response. HCPs without previous SARS-CoV-2 infection demonstrated substantially less anti-S antibodies levels at the second (mean 73.2 vs 55.2 U/mL; $p < 0.05$) and third (mean 73.2 vs 55.9 U/mL; $P < 0.01$) measurements when compared with a first-time measurement in another study performed with a similar method to our study. HCPs with prior SARS-CoV-2 infection yielded higher anti-S antibody concentration levels at different measurements. Similar to the results of our study, the outcome of this study proposes that vaccines may promote the memory immune cells that flourish post-infection those are achieving more antibody response which is possibly more vigilant and durable against SARS-CoV-2 infection [30].

In this study, the authors resolved that the average antibody level obtained after first vaccine dose in HCPs with COVID-19 infection was statistically significantly greater than the average antibody level achieved after the second vaccine dose in naïve HCPs. Secondary immune reaction in individuals with COVID-19 infection due to memory B cells activated after vaccination leads to higher antibody response compared to naive individuals. Similar to our study, only one dose of inactivated SARS CoV-2 vaccine was determined to provide rapid and high humoral response because of secondary immune activity in individuals with COVID-19 infection and antibody levels after a single dose vaccine was higher than the second vaccine of naïve individuals [31].

Most of the vaccine studies adopts neutralizing antibody activation approach. Plaque reduction neutralization and microneutralization tests based on the use of live virus particles are virological fundamental methods of showing distinct SARS-CoV-2 neutralizing antibodies. 5-7 days long incubation duration and biosafety level III laboratory obligation of these tests highly limits their routine use [32, 33]. Therefore, it may be an alternative to use serological SARS-CoV-2 tests which do not require an equipped infrastructure, have high efficiency and are cheaper than neutralization tests as a marker to show neutralizing antibody presence [32]. IgM, IgG, and IgA response can be demonstrated by using varying antigenic targets such as C and N proteins, S1 subunit of protein S and RBD in serological tests [34].

In a meta-analysis [35], it was reported that antibodies against N protein do not have a neutralizing effect on SARS-CoV-2 since this antigen is in the envelope structure. Therefore, serological tests seeking surface structures such as SARS-CoV-2 S1 antigen and RBD are recommended if it is not possible to carry out neutralization tests. It was found that the humoral immune response was statistically significantly higher in women and individuals under 37 years of age in a study using mRNA vaccine [36].

In another study using mRNA-based vaccine [26] it was reported that there was a statistically significant relationship between antibody response and age, and the highest antibody levels were found in the cluster below 30 years of age like our study. Twenty (37.03%) smokers were SARS-CoV-2 positive in our study and it has been determined that smokers who have not had COVID-19 infection previously have a higher antibody response to the inactive SARS-CoV-2 vaccine. In other studies smoking is correlated with lower ab titres after COVID-19 vaccination contrary to our findings [37, 38].

CONCLUSION

The effect of various factors such as race, ethnicity, age, gender and smoking status on the antibody response to vaccines based on different principles needs to be investigated in SARS-CoV-2 vaccine studies. The most important limitation of this study is that the SARS-CoV-2 antibody levels of the vaccinated individuals were not identified before the vaccine administration. However, high anti-S IgG levels detected 28 days after the first dose of vaccine in individuals di-

agnosed with COVID-19 infection with RT-PCR positivity can be explained by secondary immune response which distinguishes the naive individuals. Administration of a single dose of subdued SARS-CoV-2 vaccine in COVID-19 positive individuals accomplished higher antibody levels achieved with two doses in naive individuals. However, antibody level that provides protection in COVID-19 infection or the duration of protection has not yet been fully explained. Consequently, there is a need for prospective studies on how long the immunity provided by SARS-CoV-2 vaccines will continue.

Authors' Contribution

Study Conception: BGG, GGA; Study Design: BGG, MSK; Supervision: GGA, YEB; Funding: N/A; Materials: GGA, SMB; Data Collection and/or Processing: BGG; Statistical Analysis and/or Data Interpretation: BGG, YEB; Literature Review: GGA, SMB, YEB; Manuscript Preparation: BGG and Critical Review: MSK.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgment

We would like to thank to our colleagues who have not spared their days and nights, who have been away from their children, homes and loved ones since the pandemic started, who did not leave the laboratory for months and all the healthcare workers in our country who struggled under the same conditions.

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The European Research Journal 2022;8(6):859-868 Original Article

DOI: 10.18621/eurj.874472 *Cell Biology*

Regulatory effects of laminin derived peptide on microtissue formation for tissue engineered scaffold-free constructs

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ABSTRACT

Objectives: Vascularization is an important stage for tissues and organs. The vascular network is succeeded by the attachment, spreading, proliferation of endothelial cells, and the completion of endothelialization. Endothelization can be mediated by laminin-derived peptides on microtissues. It is known that laminin-derived Tyr-Ile-Gly-Ser-Arg (YIGSR) peptide contributes to endothelial microtissue formation by promoting increased adhesion and proliferation of endothelial cells. This study aims to determine the efficacy of the laminin-derived YIGSR peptide in Human Umbilicial Vein Endothelial Cell (HUVEC) scaffold free microtissues (SFMs).

Methods: After solid phase synthesis of YIGSR, microtissues were formed as SFMs. SFMs were cultured with 0 mM (control group), 1.5 mM and 3 mM YIGSR peptide. Diameters and viability analysis of HUVEC SFMs were performed on the 1st, 4th and 7th days.

Results: The diameters of control SFMs group decreased day by day. Diameters of 3 mM YIGSR SFMs increased on the 1st and 4th days but significantly decreased on the 7th day. On the other hand, 1.5 mM YIGSR had a tendency on tissue formation because of increased diameter. As a result of the viability, YIGSR peptide increased cell viability.

Conclusions: It has been determined that 1.5 mM YIGSR is the optimum amount for enlargement and viability of HUVEC SFMs. The concentration has contributed to proliferation and viability of endothelial SFMs. Thus, 1.5 mM YIGSR has been found as the most promising peptide concentration for increasing vascularization.

Keywords: Scaffold free microtissues, HUVEC microtissue formation, YIGSR peptide synthesis

Tissue engineering studies have become popular in the scientific world with the increasing need for organ transplantation. One of the difficulties of organ transplantation is that the tissue cannot be vascularized [1, 2]. However, vascularization is essential for tissue viability. Nutrient and oxygen deficiency causes loss of functions of organs or tissues and necrosis. However, the formation of a stable microvascular network is required for long-term viability and cellular function [3, 4]. To secure tissue functions, the vascularization period should be understood clearly.

Vascularization is a process that plays a key role for viability of tissues. It is known that the signal network that endothelial cells establish with the extracellular matrix (ECM) is the beginning of new vessel formation. Endothelial cells are flat epithelial cells

Received: ***February 6, 2021;*** ***Accepted:*** ***February 17, 2022;*** ***Published Online:*** ***February 18, 2022***

How to cite this article: *Yaralı Çevik ZB, Ördek A, Karaman O. Regulatory effects of laminin derived peptide on microtissue formation for tissue engineered scaffold-free constructs. Eur Res J 2022;8(6):859-868. DOI: 10.18621/eurj.874472*

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e-ISSN: 2149-3189

covering the inner surface of the vessel, located between blood and vein walls. Human Umbilical Vein Endothelial Cells (HUVECs) are the most commonly used cell types in vascular research. HUVECs have high proliferation capacity during vascularization [3]. The formation of vasculogenesis mainly begins with the proliferation of endothelial cells and continues with the migration of cells to the vascular formation region. Eventually forms a tubular structure in response to vascular growth factors [5, 6]. Therefore, the proliferation and viability of HUVECs are critically important to understand the vascular mechanisms.

Although preliminary experiments of tissue engineered studies are mostly conducted in two dimensional (2D) cell culture [7, 8], fundamental behaviours and functional properties of cells are restricted by 2D culture. Traditional 2D culture is also limited for extracellular matrix (ECM) production [9]. The lack of ECM affects proliferation, cell survival mechanism, differentiation of cells and mechano responses. Moreover, the limitations of the 2D monolayer system engenders non-transferable nutrients, oxygen, and waste. The limitation causes to break down cell metabolism [10]. However, Baker and Chen [11] showed that 3D culture improved the physiological structures and functional properties of the cells . One of the types of 3D is scaffold free microtissues (SFMs). SFMs can be regarded as an ideal model because they have similarities to the in vivo cells in terms of expression of genes and production of proteins [12, 13]. Since ECM is one of the main factors that play a role in the vascularization process, limited production of ECM may cause the process to be poorly understood. Therefore, considering architecture and cell to matrix interaction of vessels, SFMs may be more realistic approaches than traditional 2D methods. SFMs can mimic natural tissue responses and productions of ECM proteins. It allows intercellular interactions and synthesizes their own ECM [13].

Proteins, one of the basic components of living things, have various mechanisms of action in endothelial cells. Proteins consist of different bioactive sequences. Specific chains allow for easier observation of the effects of the protein. Proteins or protein fragments which are located in the basal membrane are known to play an important role in cellular behavior. Laminin, consisting of three polypeptide chains, is one of the main glycoproteins of the basal lamina. It has the effects of increasing the lifespan and quality of endothelial cells [3, 14]. The glycoprotein affects

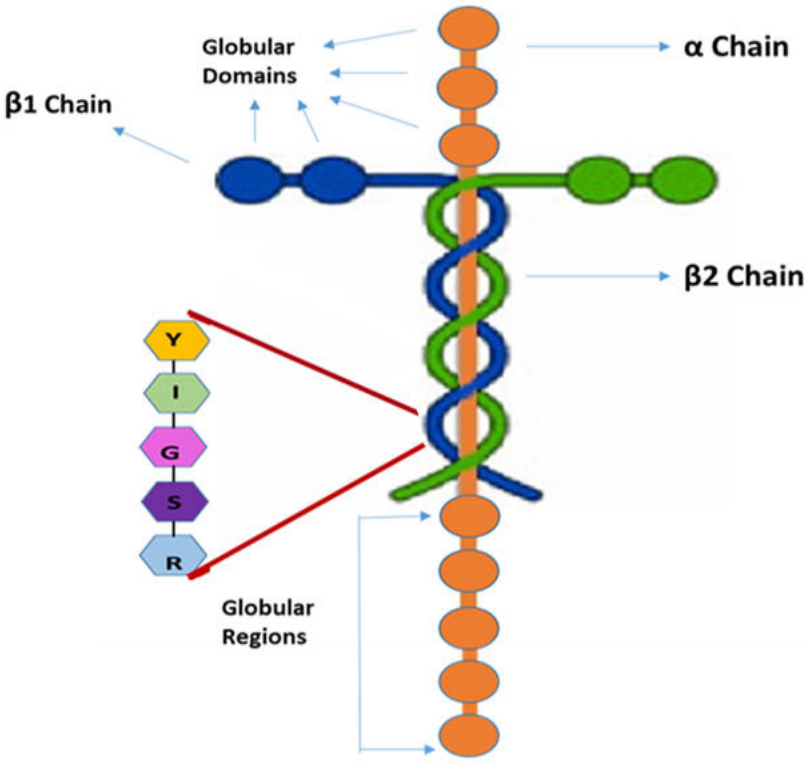

Fig. 1. YIGSR sequence configuration on laminin structure.

growth, differentiation, adhesion, proliferation and spreading of cells [15, 16]. Tyr-Ile-Gly-Ser-Arg (YIGSR) sequence is one of the active parts of the laminin protein. The part is found in the β1 chain of the laminin (Fig. 1) [17]. Previous studies have been shown that YIGSR has effects on various cellular events such as differentiation, migration and proliferation [18-21]. The peptide has also the ability of spreading and growth-promoting effects of endothelial cells [3, 22, 23]. Identification of concentration of YIGSR sequence is important in the activity of endothelial cells. Previous studies have shown that the growth, adhesion and migration of endothelial cells depend on YIGSR concentrations [3, 22, 24, 25]. YIGSR leads to proliferation and endothelialization of HUVECs in the monolayer system. In light of this information, laminin-derived YIGSR peptide can be used as a regulator for the micro-vascularization in tissue-engineered constructs. However, the effects of YIGSR on the fabrication of HUVECs SFMs has not been determined in the literature yet.

The study aims to determine the optimum laminin-derived YIGSR peptide amount on scaffold-free HUVEC microtissue. The viability and proliferation level of endothelial cells are important for prevascularization. Martin, F. *et al* showed that the differentiation state and development of the generated SFMs can be identified by diameter calculation and SFMs maturation [26]. Moreover, Karaman and Yarali [27] demonstrated that SFMs diameter measurement can relate to SFMs growth and viability analysis. Therefore, diameter analysis can be used to identify healthy growing SFMs [27, 28]. In this context, the diameter and viability analyzes have been performed on HUVEC SFMs with different YIGSR peptide concentrations.

METHODS

Synthesis of YIGSR Peptide

All chemicals used for peptide synthesis were purchased from AAPPTEC (Louisville, KY, USA). Tyr-Ile-Gly-Ser-Arg (YIGSR) sequences were added on 9 fluorenyl methoxycarbonyl (Fmoc) protected 4-Methylbenzhydrylamine (MBHA) resin (0.67 mmol/g loading capacity) as previously described procedure [28]. Briefly, the resin was swollen by dimethylformamide (DMF) for 30 minutes. Then, deprotection process was performed to separate the Fmoc from resins. Under the nitrogen gas, 2 eq. (based on loading capacity of resin) Fmoc-protected amino acid, 2 eq. 1 Hydroxy benzotriazole (HOBt), 2 eq. O-Benzotriazol-1-yl-N,N,N,N' tetrametiluronyum-hexafluorophosphate (HBTU) and 4 eq. N,N Diisopropylethylamine (DIEA) was added in DMF. This mixture was incubated for 4 hours to bind amino acids. After determining whether the amino acid was bound with the ninhydrin test, Fmoc protecting group ends were removed by using cyclohexylamine. To complete the process, the amino acids of YIGSR were added sequentially. After peptide synthesis was carried out, the obtained sequence was cleaved from resin by using 2.5% triisopropylsilane (TIPS), 2.5% distilled water (DIW), and 95% trifluoroacetic acid (TFA). This mixture was shaken gently 1 hour per 20 min. Then, ice cold (-20°C) diethyl ether was added. After the suspension was centrifuged at 4500 rpm and 25°C for 10 minutes. Finally, the obtained pellet was freeze-dried for 5 hours.

Culture of Human Umbilical Vein Endothelial Cells (HUVECs) SFMs

All cell culture equipment and reagent were taken from Sigma (St. Louis, Missouri, ABD) and HUVECs were taken from Ege University Bioengineering Department. HUVECs were cultured at 36°C, 5% carbon dioxide, 70-80% humidity. Dulbecco's Modified Eagle Medium (DMEM) which contains 10% Fetal Bovine Serum (FBS), 1% L-Glutamine, 1% Penicillin/Streptomycin was used to culture HUVECs. The medium was refreshed per 2 days. After reach to 80-90% confluency, cells were washed with Phosphate Buffer Saline (PBS). After trypsinization with Trypsin/EDTA, 60.000 /75 µl cells added into 3D agarose mold.

Agarose mold which is prepared as 2% agarose was constructed by sterilized 3D Petri Dish. Agarose mold was incubated in Endothelial Basal Medium (EBM-2, CC3156, Lonza) media without serum. After removing media from agarose, the molds were incubated for 30 minutes to allow the cells to settle into the wells. Subsequently, EBM-2 was added on agarose mold.

1.5 mM YIGSR and 3 mM YIGSR peptide concentrations were prepared with EBM-2 as serum free (Fig. 2). Then the mixtures were filtered by 0.22 µm

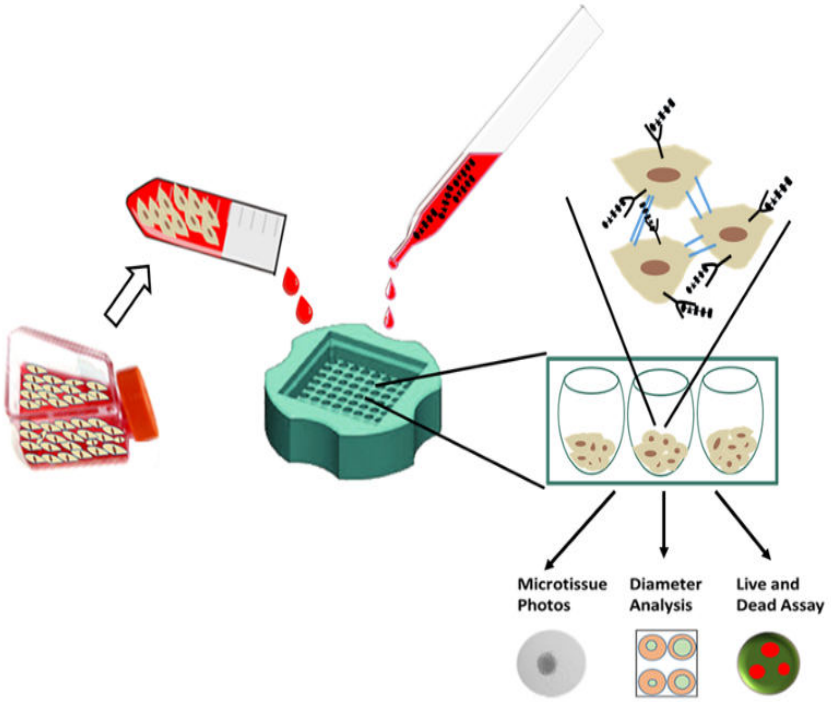

Fig. 2. Diagram of microtissue fabrication and application of YIGSR peptide on HUVEC SFMs: After monolayer culture of HUVEC, SFMs were fabricated in 3D Petri Dish. Control, 1.5 mM and 3 mM YIGSR concentrations were analyzed by images, diameter and viability analysis of SFMs.

microfilter. Only EBM-2 were used without YIGSR concentration as a control group. Each experimental group was performed as triplet treatments (n = 3).

Diameter Analysis of HUVEC SFMs

To calculate and analyze enlargements of microtissue, diameter analysis was performed. Microscopic images of the microtissues were taken on the 1st, 4th and 7th days by using Cell Sense Entry software (Olympus, Germany). Diameter lengths of different YIGSR containing microtissues in each group were measured by Image-J (National Institutes of Health, Bethesda, MD, USA) software. Average lengths and standard errors were calculated as presented in Fig. 3.

Viability effects of YIGSR peptides on HUVECs SFMs

To understand the effects of different YIGSR concentrations to the viability of HUVEC SFMs, viability assay was performed. The protocol was applied as described in previous studies [27, 28]. The rate of live or dead cells in microtissue was indicated by Double Staining Kit (Dojindo, Molecular Technologies, Inc, Japan). After media was removed, the agarose molds were washed 5 times with PBS. Images of live and dead cells were taken by Olympus fluorescent microscope. Images were taken 3 times for each SFMs (n = 9). Each live and dead cell images were merged. Additionally, fluorescence pixel intensities for each microtissue group were measured by Image-J software.

Statistical Analysis

All experimental data was subjected to a one-way analysis of variance (ANOVA) with Tukey's post hoc test at a significance level of $p^* < 0.05$, $p^{**} < 0.01$ and $p^{***} < 0.001$.

RESULTS

HUVEC SFMs with concentrations of 0 mM, 1.5 mM and 3 mM YIGSR were cultured as shown in Fig. 2. 0 mM YIGSR peptide concentration was used as negative control. Images of SFMs were taken on the 1st, 4th and 7th days as shown in Fig. 3. Three images of three microtissues from each plate were taken (n = 9) by using an inverted microscope. As it is seen, all microtissue formations were greatly accomplished on the

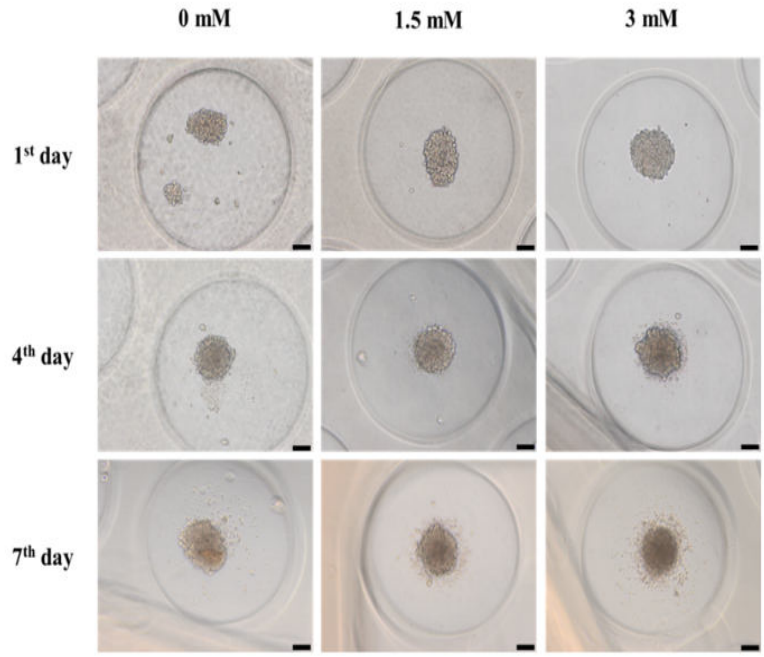

Fig. 3. Micrographs of SFMs at different concentrations on 1st, 4th and 7th days. Scale bar represents 100 µm size.

first day. In the following days, microtissue formation continued successfully.

The diameters of the microtissues were measured with Image-J Software (NIH) for each experimental group. The average diameters of microtissues in 0 mM, 1.5 mM and 3 mM YIGSR peptide concentrations on the first day are 219.89 ± 4.07 µm, 252.2 ± 10.2 µm, 291.5 ± 8.9 µm, respectively. On the fourth day, average diameters are 217.35 ± 4.15 µm, 264.8 ± 3.6 µm, 284.5 ± 2.65 µm, respectively. At the end of the experiments, diameter measurements are 206.38 ± 5.8 µm, 288.66 ± 6.9 µm, 280.14 ± 3.14 µm, respectively.

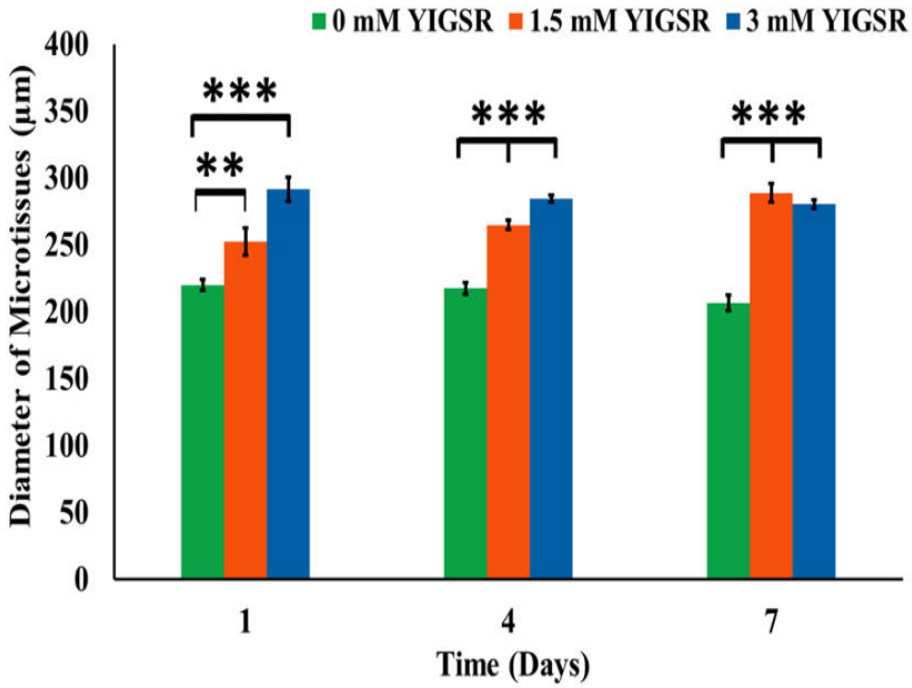

Fig. 4. Effects of 1.5 mM and 3 mM YIGSR concentration on HUVEC microtissue diameters.

On the 1st, 4th and 7th days, the highest significant differences were observed at 0 mM and 3 mM YIGSR concentrations (***$p < 0.001$). It is also seen that there is a significant difference at 1.5 mM (***$p < 0.001$), on the 4th and 7th days. On the 1st day, there was also a significant difference in microtissue diameters including 1.5 mM YIGSR (**$p < 0.05$).

As a result of the diameter analysis, increases in SFM sizes were observed at 0 mM and 1.5 mM peptide concentrations (Fig. 4). However, SFMs diameters were decreased in the 3 mM YIGSR group. The most effective amount of used peptide concentrations was found as 1.5 mM. Because the 1.5 mM YIGSR was statistically significant to increase in microtissue sizes. The viability of SFMs was performed on microtissues which contained 0 mM, 1.5 mM and 3 mM peptide concentrations. The fluorescent microscopy images showing the live and dead cells for experimental groups of YIGSR concentrations at day 7 were shown in Fig. 5. A, B, and C, respectively.

Fig. 5 also showed that the merge of red and green images of SFMs. Red and green images were taken singly for each SFMs and then these images merged using Image J. The visual comparisons of Fig. 5. A, B, and C show that varying concentrations of YIGSR have noticeable effects on endothelial cell viability. Pixel density analysis of each fluorescent image was performed to quantify these visual comparisons. Mean fluorescence pixel intensity values of 0 mM, 1.5 mM and 3 mM YIGSR microtissues were determined as shown Fig. 6.

The green and red color intensity of the 0 mM YIGSR (control group) was accepted as 100 % and this ratio was used for other peptide group comparisons. Among the used concentrations, 1.5 mM has a higher green color density. According to pixel intensity analysis, the amount of 1.5 mM YIGSR green color intensity was 23.12 % higher than the green color intensity of the control group. The excess of green color has indicated that 1.5 mM YIGSR group enhanced cell

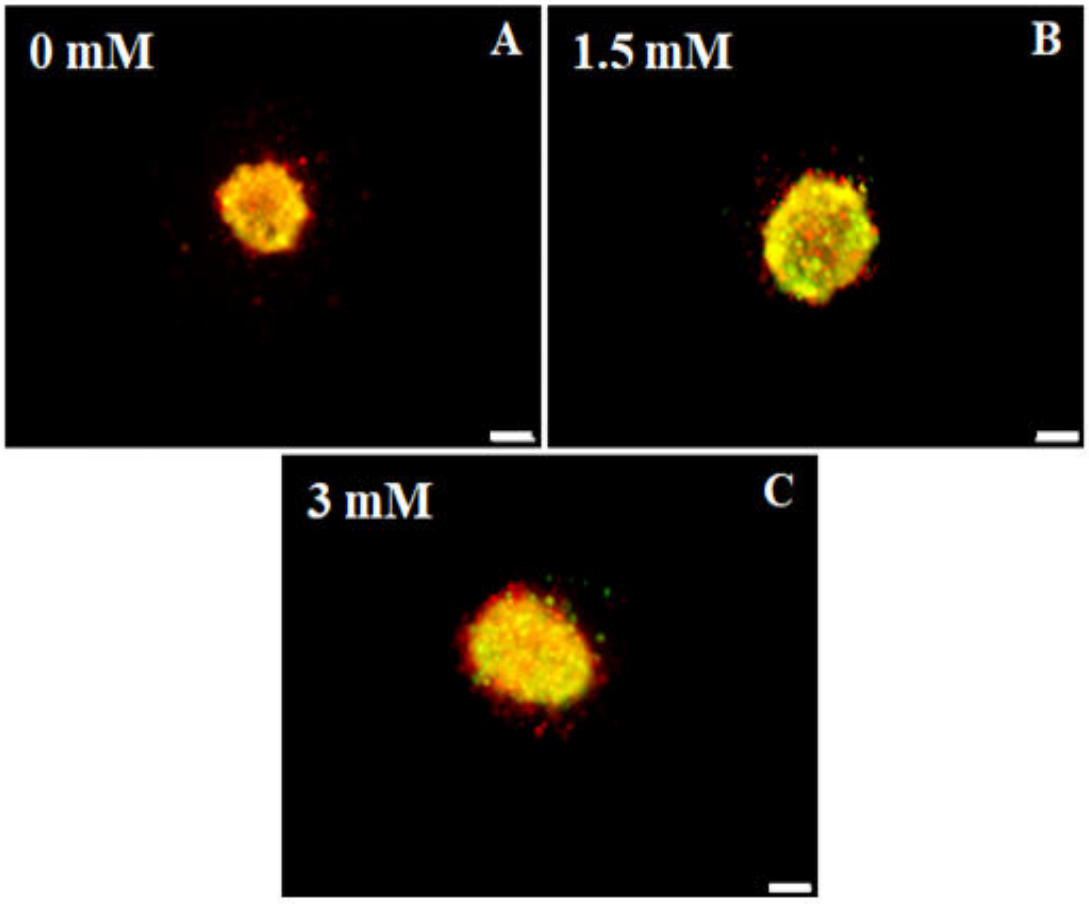

Fig. 5. Viability Assay (A) at 0 mM YIGSR concentration, (B) at 1.5 mM YIGSR concentration, and (C) at 3 mM YIGSR concentration. The scale bar represents 100 µm.

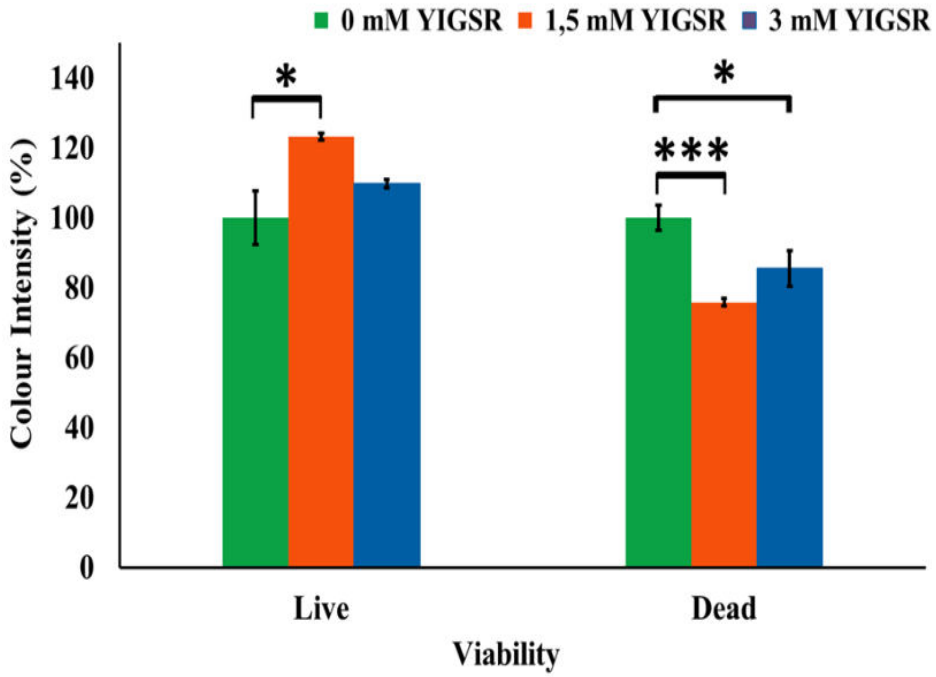

Fig. 6. Effects of 1.5 mM YIGSR and 3 mM YIGSR concentrations on SFMs viability.

viability. In addition, it is seen that the 1.5 mM YIGSR group has a significantly low red color intensity compared to untreated microtissues. 3 mM YIGSR increased the viability rate by 9.77 % compared to the control group. It was observed that the microtissues with 3 mM YIGSR had approximately 14.5 % less red color intensity than the 0 mM group. Both the decrease in red color and the increase in green color indicated that the use of YIGSR enhances the cellular vitality of HUVEC SFMs. Although 3 mM contributed to viability, it caused a decrease in microtissue diameters. When all the results of used concentrations have been evaluated, it has been determined that the most effective YIGSR concentration was 1.5 mM. This peptide concentration contributed to viability significantly and caused an increase in SFM diameter.

DISCUSSION

Biomaterials regulate cellular response and induce migration, differentiation, and proliferation through increased cell-matrix and cell-surface interactions with specific molecules. Surface coating with functional parts of major extracellular matrix proteins such as laminin, collagen and fibronectin can increase cell proliferation, migration, and intercellular communication [29]. Therefore, the use of bioactive peptides for enhanced tissue regeneration has recently taken an essential trend in tissue engineering studies [3, 24, 30, 31]. For cell proliferation, attachment and spreading, ECM mimicking bioactive peptides are important for tissue engineered constructs. One of the ECM proteins is laminin which affects cell attachment and spreading [3, 30]. The reason is that cells with high surface adhesion and spreading rate are more prone to forming advanced tissues [32-35].

YIGSR is a pentapeptide, a laminin-derived peptide, is found in the β1 chain of laminin protein. It has a key role in cell metabolism. Ali *et al*. [3] and Jun and West [22] indicated that YIGSR has important effects in endothelial cell proliferation, cell-to-cell interactions, adhesion, production of the extracellular matrix, and migration. Although YIGSR is a known peptide, the mechanisms of YIGSR have not been clearly enlightened, yet. Actually, YIGSR may interact with ECM proteins such as integrin and laminin which is mostly expressed in endothelial cells. Those molecules binds cells onto ECM so they can enhance cell viability and proliferation through cell receptor and ligand interaction [36, 37]. The interactions of YIGSR and integrin promote ECM connection which is critically important for HUVEC microenvironments [38]. Moreover, the immobilization of YIGSR on ECM can

strikingly enhance HUVEC proliferations and cell movements [39]. Expression level of this receptor is associated with cell proliferation and migration. Correspondingly, Jun and West [22] showed that laminin-derived YIGSR peptide increased the endothelial cell proliferation and ECM production.

The study examined that 1.5 mM and 3 mM YIGSR peptide concentrations enhanced the proliferation potency of HUVECs SFMs. SFMs with 1.5 mM, and 3 mM YIGSR were used to understand the effects of the YIGSR peptide on the diameter differences of SFMs. Each experimental group was checked on the 1st, 4th, and 7th days. All SFMs were created from the first day. It was clearly seen that the diameters of 1.5 mM and 3 mM YIGSR increased at day 1 significantly, but then there was decrease in diameter of SFMs in 3 mM YIGSR group. The diameter of the control group also decreased. At the end of 7th day, less efficacy was seen in 3 mM YIGSR peptide concentration compared with 1.5 mM peptide concentration. Studies of Martin *et al*. [26], Karaman and Yarali [27], and Yarali *et al*. [28] demonstrated that formation of SFMs develops depending on the diameter enlargement of SFMs. In light of that information, we concluded that both of YIGSR peptide concentrations provide enlargement of HUVEC SFMs and contributed to developing SFMs formation until the end of the 4th day. However, 3 mM YIGSR peptide did not show spread of SFMs. We predicted that 3 mM YIGSR peptide may have accumulated among SFMs because Iwamoto *et al*. [40] demonstrated that YIGSR may block binding proteins on cell membranes.

Andukuri *et al*. [41] indicated that YIGSR increased attachment, proliferation and also viability of HUVECs. YIGSR is a peptide that supports endothelial tissue formation. A similar study was reported that YIGSR promotes the activity of cellular metabolism and contributes to HUVECs viability [42]. Ovadia *et al*. [43] proved that 2 mM CGKGYIGSR peptide combination in 3D hydrogel promotes induced pluripotent stem cell (iPSC) viability. The increase in SFM viability with the use of YIGSR peptide may also be associated with rising cellular metabolic activity. The vascularization process, which is a dynamic event, is very important for the sustainability of cellular vitality for vascular tissue formation [5]. Our study also showed that both 1.5 mM YIGSR and 3 mM YIGSR groups increased the viability of cells. End of the 7th day, 1.5 mM YIGSR and 3 mM YIGSR had more viability than the control group. Particularly, 1.5 mM YIGSR peptide showed a significant augmentation in SFMs viability [44]. According to the results, we predicted that the YIGSR peptide enhances the survival of SFMs.

In this study, we examined the effects of the laminin-derived YIGSR peptide on HUVECs SFMs. The effects of 1.5 mM YIGSR and 3 mM YIGSR were determined in terms of diameter and viability analysis on HUVEC SFMs. As a conclusion, 1.5 mM YIGSR peptide was the most effective concentration compared with 3 mM YIGSR group and control group without YIGSR.

CONCLUSION

Briefly, this study described the effect of laminin derived YIGSR peptide concentration in vitro development of HUVEC SFMs. YIGSR concentration contributed to cellular metabolism and proliferation of SFMs. Additionally, results confirmed that formation and viability of SFMs could be supported by YIGSR. Moreover, this study aimed to clarify the optimum YIGSR concentration on developing SFMs. Consequently, successful development of SFMs was observed in the 1.5 mM YIGSR group. The results observed that SFM formation and viability relates with YIGSR concentration. We estimated that 1.5 mM YIGSR concentration may promise to increase vascularization since it contributed to proliferation and viability of SFMs, which is the first step of vascularization. In the prevascularization of the HUVECs SFMs, 1.5 mM YIGSR can be used to mimic the native ECM protein structures for fabrication of tissue-engineered constructs.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):869-881 Original Article

DOI: 10.18621/eurj.910253 *Otorhinolaryngology*

The effectiveness of platelet rich plasma therapy in chronic sinusitis patients with odor disorder undergoing endoscopic sinus surgery

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ABSTRACT

Objectives: Objectives: In this study, the objective was to compare the effectiveness of fluticasone dipropionate and platelet-rich plasma treatments added to the treatment in patients undergoing functional endoscopic sinus surgery in patients with chronic sinusitis involving odor dysfunction, different stages and types of sinusitis.

Methods: The study included a total of 60 patients between 18 and 60 years who underwent endoscopic sinus surgery due to chronic paranasal sinus infection followed by olfactory dysfunction. Group 1: paranasal sinus surgery + steroid therapy (first 30 patients) and Group 2: paranasal sinus surgery + steroid therapy + PRP therapy (second 30 patients) were grouped together without the patients' knowledge of the operating physician and of the treatment protocol. For the paranasal sinus CT evaluation, Lund-Mackey staging was used and the degree of the disease was determined using the Kennedy staging system. Modified Sniffin Stick test was applied to all patients in the preoperative 1st week and in the 3rd month postoperatively. The Modified Sniffin Stick test was conducted on all patients during the preoperative 1st week and the postoperative 3rd month. The modified Sniffin Stick test and endoscopic evaluation score were evaluated and whether or not the patients benefited from PRP treatment were compared.

Results: According to the postoperative endoscopy score, there was a difference between the experimental group and the control group in terms of postoperative endoscopy score, and it was found to be statistically significant. Besides, the postoperative endoscopy score of the experimental group was found to be lower than the control group. The average of Modified Sniffin' Stick Test scores in the postoperative period of the patients in the study was 28.27 ± 7.88 for the experimental group, while it was determined as 20.08 ± 5.75 for the control group, and this difference was statistically significant. The average anosmia times of the experimental and control group patients in the study were compared, and the mean duration of anosmia was 48.53 ± 20.40 (6-96) for the experimental group and 44.27 ± 19.45 (6.96) for the control group. The difference in the mean duration of anosmia between the experimental and control groups was not found statistically significant.

Conclusions: In this research, PRP, which is applied to functional endoscopic sinus surgery and fluticasone dipropionate treatment, has been shown to be a readily applicable, safe and highly efficient method of treatment in patients with chronic sinusitis accompanied by smell dysfunction.

Keywords: chronic sinusitis, platelet-rich plasma, functional endoscopic sinus surgery, odor

***Received:** April 6, 2021; **Accepted:** May 21, 2021; **Published Online:** May 27, 2021*

***How to cite this article:** Gökçe Kütük S, Topuz MF, Güvey A, Açıkgöz Ç. The effectiveness of platelet rich plasma therapy in chronic sinusitis patients with odor disorder undergoing endoscopic sinus surgery. Eur Res J 2022;8(6):869-881. DOI: 10.18621/eurj.910253*

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e-ISSN: 2149-3189

The natural sense of smell is defined as Normosmia. Smell dysfunction, anosmia (complete loss of smell), hyposmia (reduced smell ability), hyperosmia (improved smell perception), pantosmia (improved smell perception without stimuli), parosmia (different smell stimulus perception), phantosmia (negative smell perception with no stimuli) [1, 2]. Anosmia and hyposmia are the most common feelings of odor disorders. 20 percent of the general adult population is known to be affected [3]. With the growth in the prevalence of olfactory disorder in recent years, its meaning has also been understood. In many neurodegenerative disorders such as Alzheimer's and Parkinson's disease, it is a biological marker and its physiological significance has been proved that it helps to diagnose early [4]. Also, a person's impaired eating habits have a direct effect on quality of life and vital mortality by social behavior deficits and environmental hazard exposure [5].

Many underlying diseases can trigger smell dysfunction. It can be considered similar to patients presenting with hearing loss when researching the etiology of these patients. Two major types of olfactory dysfunction may be categorized as conductive-environmental (anatomical barriers preventing odors from reaching the olfactory epithelium and receptors) or sensorineural (central; olfactory receptors, olfactory neurons, or disruption of the pathways to the olfactory centers of the central nervous system) [1, 6].

Odor loss has been associated with chronic rhinosinusitis (CRS), nasal polyposis, allergic rhinitis, inflammatory and neoplastic paranasal sinus diseases, which are obstructive nasal diseases [1, 7, 8]. The most common cause of odor dysfunction among sinonasal diseases is chronic rhinosinusitis (CRS) with or without nasal polyposis. Olfactory dysfunction is actually thought to affect 61 to 83 percent of CRS patients and 95 percent of nasal polyposis, irrespective of their subtype. It can be due to histological changes in the neuroepithelium and to cytokine-mediated dysfunction of the olfactory receptor associated with mechanical obstruction and inflammation in pathophysiology caused by polyps or edematous mucosa [9, 10].

Asthma, nasal polyposis, and smoking are described as characteristics associated with smell dysfunction in patients with CRS at the age of 65. The level of mucosal eosinophilia in these patients has been shown to predict olfactory impairment [6]. Although mechanical obstruction associated with edema and infection may cause conductive smell loss, there is significant evidence that hyposmia or anosmia is due to the effects of inflammation on olfactory neurons in some forms of chronic rhinosinusitis [11].

Diagnosis of olfactory dysfunction requires a detailed history, and a comprehensive systematic physical examination including nasal endoscopy to evaluate the olfactory bulb and sinonasal region [12]. Olfactory evaluation includes olfactory tests, which are consists of self-assessment tools (Sino-Nasal Outcome Test, Rhinosinusitis Disability Index test, Questionnaire of Olfactory Disorders, etc.), functional olfactory tests (testing true olfactory function) and physiological tests (mucociliary function, nasal airflow, or brain activity during sniffing) [9]. Psychophysical tests are performed in four groups as odor perception tests, discrimination tests, odor identification and odor identification tests. Computed Tomography is the gold standard for patients with sinus disease and is also the first study proposed to identify anatomical occlusion [2, 5].

In order to prevent exposure to smoke and natural gas, to avoid consuming spoiled foods, and to maintain nutritional health and quality of life, patients should be given a first step in the treatment of odor disorders as to the potential causes, degree and prognosis of odor loss, and patients should receive safety advice [13].

The treatment should be based on etiology and, first of all, it should be eliminated if there is an underlying pathology. For medical treatment, intranasal calcium buffers such as corticosteroids, zinc, theophylline, minocycline, vitamins, lipoic acid, phosphodiesterase inhibitors, pentoxifylline and sodium citrate are known to be effective in the treatment of olfactory dysfunction, but further studies are required [3, 11].

While odor disorders of the conductive type have a relatively good prognosis, odor disorders of the neural type have a worse prognosis and often do not improve with treatment. The patients who can benefit the most from the treatment are patients with sinonasal diseases such as septum deviation, concha bullosa, nasal stenosis, allergic rhinitis, nasal polyposis, and chronic sinusitis. In the medical treatment of the disease, systemic and intranasal steroids, antiallergic

drugs and antibiotherapy for rhinosinusitis infections are given [2].

The combined use of oral steroid sprays and topical nasal steroid sprays in the treatment of CRS patients has been shown to be more effective than nasal sprays alone. There is no consensus, however, on the duration and dosage of steroid therapy, and guidelines for treatment vary greatly. Furthermore, long-term use is discouraged in patients with CRS due to the potential side effects of oral steroids [12].

In the treatment of odor disorders that are thought to develop due to mechanical causes, surgical interventions may also be performed. By improving ventilation and probably reducing inflammation in the olfactory bulb area, functional endoscopic sinus surgery positively improves the olfactory dysfunction associated with CRS. There are studies showing that nasal polyp patients are more likely to recover after ESS from olfaction [11].

Treatment of platelet rich plasma (PRP); in recent years, it has become a favorite material for clinicians and researchers since it accelerates tissue healing, decreases bleeding, edema and pain, and has been used in many specialties and operations with increasing frequency. In orthopedic, maxillofacial, periodontic, plastic, thoracic, vascular and neurosurgery, ophthalmology and dermatology, PRP is currently being used [15-17].

Due to its advantages in wound healing, angiogenesis, use as glue material, post-operative pain and bleeding, PRP is also one of the interesting materials in otorhinolaryngology and head and neck surgery. A significant improvement in odor function was observed following platelet-rich plasma injection treatment applied in patients with anosmia [18].

PRP was first used in 2016 by Mavrogeni *et al*. [18], based on the view that platelets can accelerate the regeneration of the olfactory nerves in anosmia patients by secreting various growth factors and active metabolites, especially transforming growth factor. Following the application of PRP in the olfactory area of 5 patients with idiopathic anosmia in 4 sessions, the sense of smell returned in 4 patients and one patient stated that he could smell a little but could not smell all. In a similar study conducted in 2020, although there was no significant improvement in Sniffin Sticks score in 2 patients with anosmia, it was observed that all 5 patients with hyposmia reached normosmia after 3 months of follow-up [19]. In a study conducted by Yasak *et al*. [20] on mice, the effects of PRP on the olfactory nerve were histological. When examined, it was found that epithelial damage was significantly less in the PRP group and epithelial thickness was higher. As a result of the studies, it was concluded that the use of PRP in the treatment of odor dysfunction has curative effects.

The aim of this study was to investigate the effects of treatment with fluticasone dipropionate and platelet-rich plasma in combination with functional endoscopic sinus surgery in the treatment of cases of chronic sinusitis accompanied by odor disorder at different stages and types of sinusitis.

METHODS

This prospective study was conducted with the permission of the Local Ethics Committee with the number 3049 and 25/10/2019 date ethics document, between 16.09.2019 and 30.01.2021. The study included a total of 60 patients between the ages of 18 and 60 who underwent endoscopic sinus surgery due to chronic paranasal sinus infection followed by olfactory dysfunction. For each patient, demographic data such as age and gender, detailed history and physical examination, head and neck examination, diagnostic nasal endoscopy and computed tomography (CT) axial-coronal non-contrast scan were performed. The patients were evaluated for preoperative mean duration of anosmia, history of nasal and paranasal sinus surgery for any cause, smoking, allergic rhinitis, history of asthma, septal deviation, turbinate hypertrophy, and presence of a nasal polyp. Concomitant medical conditions, chronic systemic diseases, other potential for anosmia (tumors, Alzheimer's disease, intracranial aneurysms, brain tumors, clinical exposure to suppositories and solvents, diabetes, hormonal disorders and some drug therapies [nifedipine, terbinafine, others, Multiple sclerosis etc.]), these patients were excluded from the study if they could not be excluded. Information on the presence and severity of paranasal sinus symptoms, use of medication for the treatment of symptoms, allergy and allergy treatments were recorded. All patients were medically treated with oral antibiotics, antihistamines, nasal steroid spray and, in some cases, oral steroid combination for at least 6

months prior to the decision on operation, but the decision on surgery was taken when this medication did not improve the general clinical picture. Intranasal endoscopic exam and paranasal sinus computed tomography methods were used in the diagnosis of chronic paranasal sinus infection. In the preoperative and postoperative 3rd month, Lund-Mackey staging was used for the evaluation of paranasal sinus CT and the degree of the disease was determined by the Kennedy staging system. Modified Sniffin Stick test was applied to all patients for the odor test in the first week before the operation and in the third month after the operation to investigate their sense of smell.

All patients were informed and their consent forms were obtained about the surgery. Endoscopic sinus surgery was conducted as a surgical treatment using the Messerklinger technique. Uncinectomy, anterior ethmoidectomy, posterior ethmoidectomy, middle meatus antrostomy, frontal recess opening, sphenoidectomy, partial concha resection, polypectomy, septoplasty and concaplasty operations have been added in all cases included in our study and in cases needed according to the prevalence of infection in the paranasal sinuses.

All patients received broad-spectrum antibiotic therapy and nasal irrigation with ocean water at least twice a day for 10 days at the end of the operation. Throughout the postoperative period, all patients were administered fluticasone dipropionate, a topical steroid in the form of two sprays into each nostril once a day for three months, starting from the first postoperative week, and all patients were started on oral prednisolone at 1 mg/day/kg during the postoperative period, and the doses were gradually reduced and stopped during the 10-day treatment period.

Patients are unaware of the physician performing the operation and the treatment protocol applied to the patients; Group 1 was separated into paranasal sinus surgery + steroid therapy (first 30 patients) and Group 2: paranasal sinus surgery + steroid therapy + PRP treatment group (second 30 patients).

Thirty patients in the PRP group received a total of 3 PRP injections at the end of the operation and at the postoperative 1st month and 2 months in controls in addition to the surgical and post-medical treatments (antibiotherapy + ocean water washing + topical steroid + oral prednisone). PRP injection was applied by direct injection method to the "Regio olfactory" area. Imaging was conducted with the endovision system we used in sinus surgery operations during the application of PRP to this area in the nasal cavity, and during this process, 4 mm and 2.7 mm 0°, 30°, 45° and 70 Storz endoscopes were used as required. 3 ml of PRP obtained by soft and hard spin method with the endovision system to the olfactory mucosa covering an area of 5 cm^2 where the anatomical region called Regio olfactory is located, with a dental tip (black tipped) injector, by passing the olfactory epithelium in a thick columnar structure pseudoatrophy. It was applied to the 'Regio olfactory' area by the direct injection method by injecting approximately 0.5 ml of PRP per cm^2 to each injection by going deep under the epithelium. The Modified Sniffin Stick test was applied to all patients in the postoperative 3rd month and evaluated with an endos copic evaluation score, examining whether the patients benefited from PRP medication.

Statistical Analysis

Statistical analysis of the data used in the study was made with the SPSS 25.0 program. The distribution of information about the sociodemographic and health status of the patients was analyzed with descriptive statistics. The Shapiro- Wilk test was used to examine whether the obtained data fit the normal distribution. The mean comparisons between the two independent groups, the Independent Samples t Test if the continuous data conformed to the normal distribution, and the Mann- Whitney U test if it did not. Chi-Square Test was used for comparison of categorical data.

RESULTS

Half of the patients in the study constitute the experimental group, and the other 50% (30 patients) constitute the control group. The distribution of demographic characteristics of the patients (gender, age), Kennedy staging and Lund-Mackey staging are given in Table 1. According to the results obtained; 41.7% (25 patients) of the patients in the study were women and 58.3% (35 patients) were men. In addition, the average age of women in the study was 43.34 ± 7.24 (28-59) years, and the average age of men was 43.34 ± 6.71 (24-60) years.

Fourty percentage of the experimental group of

Table 1. Distribution of socio-demographic characteristics of experimental and control groups

Group	Gender	N	% (Group)	% (Total)	Age			
					X	SD	Min	Max
PRP Group	Female	12	40	20	43.67	7.95	28	55
	Male	18	60	30	44.06	7.21	24	60
Control Group	Female	13	43.4	21.7	42.69	6.82	34	59
	Male	17	56.6	28.3	42.59	6.28	25	51
Total	Female	25	83.4	41.7	43.16	7.24	28	59
	Male	35	116.6	58.3	43.34	6.712	24	60

PRP = platelet rich plasma, X = mean, SD = Standard deviation, Min = minimum, Max = maximum

the study (12 patients) were women and 60% (18 patients) were men. The average age of women was 43.67 ± 7.95 (28-55) years, the average age of men was 44.06 ± 7 years. Of the control group, 43.4% (13 patients) were women and 56.6% (17 patients) were men. The mean age of female patients in the control group was 42.69 ± 6.82 (34-59) years, and the mean age of male patients was 42.59 ± 6.82 (25-51) years.

The distribution of the patients included in the study regarding the operation history, smoking status, allergic rhinitis, asthma, septum deviation, concha hypertrophy, environmental allergic history, nasal discharge, nasal obstruction, congestion, headache and infection findings are given in Table 2. According to the results, 10% (3 patients) of the patients in the experimental group have a history of operation and 30% (9 patients) smoke. On the other hand, 13.4% of the control group (4 patients) had an operation history and 30% (9 patients) were smoking. In addition, 86.6% of the patients in the experimental group (26 patients) had an environmental allergic history, 36.6% (11 patients) had allergic rhinitis, 23.4% (7 patients) had asthma and 76.6% (23 patients) have a runny nose. In the control group, 83.4% (25 patients) had an environmental allergic history, 33.4% (10 patients) allergic rhinitis, 23.4% (7 patients) asthma and 33.4% (10 patients) patient) has a runny nose.

Septum deviation in 36.6% (11 patients) of the experimental group patients included in the study, concha hypertrophy in 43.4% (13 patients), nasal obstruction in 80% (24 patients) and 53.4% (16 patients) have congestion. Septum deviation in 36.6% (11 patients) of the control group patients, concha hypertrophy in 43.4% (13 patients), nasal obstruction in 80% (24 patients) and congestion in 50% (15 patients) has. In addition, 40% of the experimental group patients (12 patients) have headache and 33.4% (10 patients) have signs of infection. In the control group, 43.4% (13 patients) had headache and 30% (9 patients) had symptoms of infection.

Kennedy and Lund- Mackey staging results of the patients in the study are given in table 3. According to the Kennedy staging results of the patients; 16.7% of the patients in the experimental group (5 patients) were "stage 1", 20% (6 patients) "stage 2", 33.4% (10 patients) "stage 3" and 30% (9 patients) are in "stage 4". Of the patients in the control group, 16.7% (5 patients) were stage 1, 23.4% (7 patients) stage 2, 30% (9 patients) stage 3 and 30% (9 patients) It is found in stage 4 ($X^2 = 0.130$; $p = 0.998$).

According to Lund-Mackey staging results of the patients; While the Lund-Mackey score of 36.6% (11 patients) of the patients in the experimental group was below 11, 63.4% (19 patients) were 11 and above. The Lund-Mackey score of 40% (12 patients) of the patients in the control group was below 11, while 60% (18 patients) were 11 and above ($X^2 = 0.071$; $p = 0.791$).

The postoperative endoscopy scores of the experimental and control group patients included in the study were compared and the results obtained are given in Table 4. According to these results; The mean postoperative endoscopy score was 3.03 ± 1.71 (0-7) in the experimental group and 4.93 ± 2.38 (0-7) in the control group. This difference between the experimental group and the control group in terms of postopera-

Table 2. Distribution of the information on the health status of the experimental and control groups

Health Information		PRP Group			Control Group			Total	
		N	% (Group)	% (Total)	N	% (Group)	% (Total)	N	%
Operation Story	Yes	3	10	5	4	13.4	6.7	7	11.7
	No	27	90	45	26	86.6	43.3	53	88.3
Smoking Use	Yes	9	30	15	9	30	15	18	30
	No	21	70	35	21	70	35	42	70
Allergic Rhinits	Yes	11	36.6	18.3	10	33.4	16.7	21	35
	No	19	63.4	31.7	20	66.6	33.3	39	65
Asthma	Yes	7	23.4	11.7	7	23.4	11.7	14	23.3
	No	23	76.6	38.3	23	76.6	38.3	46	76.7
Septum Deviation	Yes	11	36.6	18.3	11	36.6	18.3	22	36.7
	No	19	63.4	31.7	19	63.4	31.7	38	63.3
Concha Hypertrophy	Yes	13	43.4	21.7	13	43.4	21.7	26	43.3
	No	17	56.6	28.3	17	56.6	28.3	34	56.7
Environmental Allergy Story	Yes	26	86.6	43.3	25	83.4	41.7	51	85
	No	4	13.4	6.7	5	16.6	8.3	9	15
Runny Nose	Yes	23	76.6	38.3	10	33.4	16.7	33	45
	No	7	23.4	11.7	20	66.6	33.3	27	55
Nasal Obstruction	Yes	24	80	40	24	80	40	48	80
	No	6	20	10	6	20	10	12	20
Congestion	Yes	16	53.4	26.7	15	50	25	31	51.7
	No	14	46.6	23.3	15	50	25	29	48.3
Headache	Yes	12	40	20	13	43.4	21.7	25	41.7
	No	18	60	30	17	56.6	28.3	35	58.3
Signs of Infection	Yes	10	33.4	16.7	9	30	15	19	31.7
	No	20	66.6	33.3	21	70	35	41	68.3

PRP = platelet rich plasma

tive endoscopy score was found to be statistically significant (t = 3.553; $p = 0.001$). The postoperative endoscopy score of the experimental group was found to be lower than the control group.

Modified Sniffin Stick Test results in the preoperative and postoperative periods of the patients in the study are given in Table 5. According to the results obtained; In the preoperative period, the mean odor threshold score was 7.69 ± 1.94 in the experimental group and 7.66 ± 1.98d in the control group, and this difference between them was not statistically significant (t = -0.059; $p = 0.953$). However, the mean odor threshold score in the postoperative period was 12.58 ± 3.03 in the experimental group and 9.53 ± 2.28 in the control group. The difference in postoperative odor threshold scores between the experimental group and the control group was found to be statistically significant (t = -4.327; $p < 0.001$). The mean odor discrimination score in the preoperative period was 6.53 ± 2.30 in the experimental group and 6.55 ± 2.32 in the

Table 3. Chi-square test of the experiment and control group Kennedy and Lund-Mackey staging

Kennedy and Lund-Mackey Staging	Stage	PRP Group		Control Group		Total		X^2	*p value*
		N	%	N	%	N	%		
Kennedy Staging	Stage 1	5	8.3	5	8.3	10	16.7	0.130	0.998
	Stage 2	6	10	7	11.7	13	21.7		
	Stage 3	10	16.7	9	15	19	31.7		
	Stage 4	9	15	9	15	18	30		
	Total	30	50	30	50	60	100		
Lund – Mackey Staging	Under 11	11	18.3	12	20	23	38.3	0.071	0.791
	11 and Above	19	31.7	18	30	37	61.7		
	Total	30	50	30	50	60	100		

PRP = platelet rich plasma

control group. In the postoperative period, themean odor discrimination score was calculated as 9.71 ± 3.14 in the experimental group and 8.02 ± 2.67 in the control group, and this difference was statistically significant (t = -2.244; p = 0.029). . The average scent recognition score for the experimental group was 2.02 ± 1.10 in the preoperative period and 5.98 ± 3.25 in the postoperative period; For the control group, it was 2.03 ± 0.89 in the preoperative period and 2.48 ± 1.28 in the postoperative period. The difference in odor recognition scores between the postoperative experimental group and the control group was found to be statistically significant (t = -5.496; p < 0.001).

The average of Modified Sniffin Stick Test scores in the postoperative period of the patients in the study was 28.27 ± 7.88 for the experimental group; It was determined as 20.08 ± 5.75 for the control group, and this difference was statistically significant (t = -4.959; p < 0.001).

The average anosmia times of the experimental and control group patients in the study were compared and the results obtained are given in Table 6. According to these results; The mean duration of anosmia was 48.53 ± 20.40 (6-96) for the experimental group and 44.27 ± 19.45 (6.96) for the control group. The difference in mean duration of anosmia between the experimental and control groups was not found statistically significant (t = -0.829; p = 0.829).

DISCUSSION

Olfaction is one of the most important basic life functions in a very large part of living things in nature, while it is relatively less important for humans. Compared to living things in nature, the human olfactory system is less developed, and it is estimated that it can distinguish about 10,000 odors 16. As a result of the stimulation of olfactory molecules by the olfactory field, cells have evolved to receive the sense of smell.

Table 4. Independent t-test used for comparison of postoperative endoscopy scores of experimental and control groups

Group	Postoperative Endoscopy Score				y	*p value*
	X	SD	Min	Max		
PRP Group	3.03	1.71	0	7	3.553	**0.001**
Control Group	4.93	2.38	1	9		
Total	3.98	2.27	0	9		

PRP = platelet rich plasma, X = mean, SD = Standard deviation, Min = minimum, Max = maximum

Table 5. Independent sample t-test performed for comparison of preoperative-postoperative modified sniffin stick test results of experimental and control group patients

Content	Period	Group	N	X	SD	t	*p value*
Odor threshold	Preoperative	PRP Group	30	7.69	1.94	-0.059	0.953
		Control Group	30	7.66	1.98		
	Postoperative	PRP Group	30	12.58	3.03	-4.327	**< 0.001**
		Control Group	30	9.58	2.28		
Discriminating smell	Preoperative	PRP Group	30	6.53	2.30	0.048	0.962
		Control Group	30	6.55	2.32		
	Postoperative	PRP Group	30	9.71	3.14	-2.244	0.029
		Control Group	30	8.02	2.67		
Smell recognition	Preoperative	PRP Group	30	2.02	1.10	0.069	0.946
		Control Group	30	2.03	0.89		
	Postoperative	PRP Group	30	5.98	3.25	-5.496	**< 0.001**
		Control Group	30	2.48	1.28		
Modified Sniffin Stick test total score	Preoperative	PRP Group	30	16.23	4.82	0.013	0.990
		Control Group	30	16.24	4.83		
	Postoperative	PRP Group	30	28.27	7.88	-4.595	**< 0.001**
		Control Group	30	20.08	5.75		

PRP = platelet rich plasma, X = mean, SD = Standard deviation, Min = minimum, Max = maximum

This specialized sense is located in the respiratory system, providing integrity of function. However, a decrease in the sense of olfaction can affect people's vital functions and quality of life. Hyposmia, defined as a decrease in smell, is observed in 16% of the general population, while the prevalence of anosmia, defined as a loss of smell, is about 5% although it varies in studies [21, 22]. Anosmia can be temporary or permanent, depending on the degree of degeneration. Although there are studies showing that systemic steroids work in the treatment of these conditions, there is no clear medical treatment option defined yet [23].

Since the 1970s, when endoscopic sinus surgery was defined, it has been the first choice within the limits of indication in the surgical procedure of diseases located in the paranasal sinus and nasal cavity. Today, its indications have exceeded the nasal and paranasal structures and have become to cover many areas, primarily the skull base, pterygopalatine and infratemporal fossa, and orbita. The main reason for this is that endoscopic surgeries are less invasive compared to open surgical procedures, and the developing endo-

Table 6. Independent t-test used for comparing the average anosmia times of the experimental and control groups

Grup	Average Anosmia Duration				t	*p value*
	X	SD	Min	Max		
PRP Group	48.53	20.40	6	96	-0.829	0.411
Control Group	44.27	19.45	6	96		
Total	46.40	19.88	6	96		

PRP = platelet rich plasma, X = mean, SD = Standard deviation, Min = minimum, Max = maximum

scope and imaging technologies are increasing this technique day by day [24, 25].

Functional Endoscopic Sinus Surgery (FESS) is applied for treatment in many diseases such as chronic rhinosinusitis, nasal polyposis, paranasal tumors, pituitary tumors, cerebrospinal fluid (CSF) rhinorrhea, and encephalocele. Epistaxis, choanal atresia, angiofibroma, Thornwaldt's cyst treatment, septoplasty, dacryocystorhinostomy, and turbinoplasty applications are among the other usage areas of FESS [26]. After FESS, properly applied postoperative care shortens the recovery time of patients and decreases the frequency of revision surgery [27]. Nasal irrigation with saline, crust debridement, systemic and local steroids, and antibiotics can be used for early postoperative care [27, 28].

There are experimental and clinical studies investigating the effects of various materials on nasal mucosal healing in the current literature. In the study conducted by Yılmaz *et al*. [29] on rats, it was shown that systemically administered N-acetylcysteine reduced goblet cell loss and inflammatory cell migration and also had positive effects on wound healing in the nasal mucosa by decreasing the subepithelial thickness index. Cassano *et al*. [30] suggested that postop hyaluronic acid nasal washing in patients who underwent endoscopic turbinoplasty (turbinate reduction) shortened mucociliary transport time and accelerated mucosal cell regeneration compared to saline irrigation, and thus had positive effects on mucosal healing. Rezaeian [31] found out that spray cryotherapy is an affordable and effective method that increases mucosal healing rates in patients undergoing FESS due to nasal polyposis.

Grzeskowiak *et al*. [32] reported that the application of soluble tampons impregnated with antibiotics and steroids in patients who underwent FESS provided a better improvement compared to saline-soaked tampons, based on the postop endoscopic examination scores, and also gave more positive results by patient satisfaction. Testa *et al*. [33] compared topical gomenol oil with topical vitamin E application in patients who underwent FESS for chronic rhinosinusitis and showed that the application of vitamin E accelerated the restoration process of the sinonasal mucosa and was more effective on healing than other treatments.

Choi *et al*. [34] reported in an experimental study performed in rabbits that applying a silastic sheath to septal perforation provided significant early closure by leading to the acceleration of healing. Chan *et al*. [35] observed in an experimental study in rabbits that the application of Spongostan impregnated with hepatocyte growth factor to the damaged area in the maxillary sinus, compared to the saline-impregnated tampon, reduced fibrosis and accelerated wound healing and ciliogenesis, especially for the first 3 days.

There are studies reporting that systemic steroids reduce symptoms due to obstruction by limiting the postoperative mucosal edema, thanks to their anti-inflammatory effects [36]. However, systemic use of systemic steroids is controversial, considering their potential for side effects [37]. Topical steroids, on the other hand, are the most common agents accepted for use in the postoperative period due to their anti-inflammatory effects and lack of systemic side effects [36].

The use of platelet-rich blood products in medicine started with their derivatives produced for hemostasis. Its effects on wound healing also began with the increase in awareness of the growth factor and cytokines of platelets. PRP is a cellular plasma component that is obtained by centrifugation of whole blood and contains a higher platelet concentration than whole blood. Normally, the cellular component of plasma consists of 93% erythrocytes, 6% platelets, and 1% leukocytes. PRP has a 3-5 times higher rate of platelets compared to normal whole blood [38]. PRP contains growth factors in hyperphysiological ratio due to its high platelet concentration. With this feature, its antibacterial, anti-inflammatory, and accelerating effect on many tissues has been proved in various animal and human studies [38].

Lund-Kennedy endoscopic scoring system is widely used in the literature for objective evaluation of functional sinus surgery. In a study conducted by Poetker *et al*. [39], who investigated the recovery after functional endoscopic sinus surgery in chronic rhinosinusitis patients with polyps, it was reported that patients significantly improved in endoscopic findings after the operation [40].

The Lund-Mackey staging was used in the evaluation of paranasal sinus CT in the preoperative and postoperative 3rd month, and Kennedy staging system was used to determine the degree of the disease in the evaluation of the patients constituting the sample of

the study. According to the Kennedy staging results of the patients, 16.7% of the patients in the experimental group (5 patients) were in "stage 1", 20% (6 patients) in "stage 2", 33.4% (10 patients) in "stage 3", and 30% (9 patients) in "stage 4". Of the patients in the control group, it was found out that 16.7% (5 patients) were in stage 1, 23.4% (7 patients) in stage 2, 30% (9 patients) in stage 3, and 30% (9 patients) in stage 4. With regards to Lund-Mackey staging results of the patients, while the Lund-Mackey score of 36.6% (11 patients) of the patients in the experimental group was below 11, 63.4% (19 patients) were 11 and above. The Lund-Mackey score of 40% (12 patients) of the patients in the control group was below 11, while 60% (18 patients) were 11 and above.

The patients who constitute the sample of the study were investigated for their preoperative sense of olfaction and at the end of the third postoperative month. According to the postoperative endoscopy score, there was a difference between the experimental group and the control group in terms of postoperative endoscopy score, and it was found to be statistically significant. Besides, the postoperative endoscopy score of the experimental group was found to be lower than the control group. Additionally, the Modified Sniffin' Stick test was applied to all patients for the smell test in the first week before surgery and in the third postoperative month. The average of Modified Sniffin' Stick Test scores in the postoperative period of the patients in the study was 28.27 ± 7.88 for the experimental group, while it was determined as 20.08 ± 5.75 for the control group, and this difference was statistically significant.

The average anosmia times of the experimental and control group patients in the study were compared, and the mean duration of anosmia was 48.53 ± 20.40 (6-96) for the experimental group and 44.27 ± 19.45 (6.96) for the control group. The difference in the mean duration of anosmia between the experimental and control groups was not found statistically significant.

A significant improvement in the loss of smell after the operation in the patient groups can be attributed to the endoscopic sinus surgery. Although there are controversial results in the literature, there are many clinical studies reporting improvement in olfaction after endoscopic sinus surgery. In a study conducted by Litvack *et al*. [41], it was reported that, especially in anosmic patients, the loss of smell significantly improved after the operation. It was also deduced that improvement in anosmia is more pronounced, especially in nasal polyposis errors. Similar results were reported in a review by Rudmik *et al*. [42] In patients with anosmic and nasal polyps, a significant improvement in anosmia was reported after endoscopic sinus surgery.

There are many studies investigating the effect of functional endoscopic sinus surgery on postoperative symptoms. In 58 studies conducted with 3 different quality of life scales, Soler *et al*. [43] reported that the most significant improvement in symptoms after sinus surgery occurred in the first 6 months, and no difference developed in symptoms after this period. In their cohort study, Smith *et al*. [44] reported that in all patients with all phenotypes of chronic rhinosinusitis, functional endoscopic sinus surgery revealed a significant increase in the postoperative quality of life in patients with both general and disease-specific quality of life. Kennedy *et al*. [45] suggested that in their study on 104 chronic rhinosinusitis patients, they found 51% improvement after functional endoscopic sinus surgery according to the results of the SNOT22 questionnaire.

Most of the studies in the literature indicated that PRP could contribute positively to wound healing. There are few studies in the literature regarding the use of PRP in different disciplines of Otorhinolaryngology. As an example of these studies that aim to benefit from the regenerative properties of PRP, Yan *et al*. [19] injected a single dose of PRP into the olfactory mucosa of seven patients who had complained of anosmia and hyposmia for more than 6 months, had no sinonasal inflammatory disease, and did not respond to topical steroid therapy in the pilot study. Although all patients reported subjective improvement in their sense of olfaction after injection, the findings stabilized later. At the 3rd month follow-up, the patient's symptoms did not change, and 5 patients with hyposmia showed 60% improvement in olfaction tests and reached the level of normosmia. The limitations of the study include being conducted in one field and the limited number of patients, and the absence of a control group. Since the primary purpose is seen as demonstrating the reliability and tolerability of PRP use, it lays the groundwork for more detailed studies.

In the study of Kütük *et al*. [46], consisting of 80

patients, the effect of topical PRP application to the tonsillar lodge after pediatric tonsillectomy on short-term postoperative pain, appetite, need for analgesia, and bleeding rates was examined. The patients were divided into two as only tonsillectomy and tonsillectomy in addition to PRP treatment groups, each consisting of 40 patients. Postoperative pain and appetite scores, analgesic requirement, and bleeding were recorded. When compared with the tonsillectomy group, the pain scores of the tonsillectomy in addition to the PRP treatment group were significantly better on days 1-10, appetite on days 1-6, and analgesic need on days 1-10. One bleeding case in the experimental group was recorded compared to the four bleeding cases in the control group. When the parameters were statistically compared, they were found to be significant [46].

In the study of Ricci *et al*. [47], which included 59 patients who underwent superficial parotidectomy due to benign parotid tumor, the effect of PRP application to the parotidectomy lodge on hospitalization, drain duration, and postoperative facial paresis rates was investigated. Superficial parotidectomy was applied to 38 patients in the control group, superficial parotidectomy was applied to the experimental group consisting of 21 patients, and PRP was applied to the surgery site. The mean drain time was three days in the control group and two days in the experimental group ($p < 0.05$). The average hospitalization time was four days in the control group and five days in the experimental group ($p < 0.05$). Postop facial paresis was observed in four (10.5%) patients in the control group and in two (9.5%) patients in the experimental group. At the end of 24 hours, facial paresis developed in 2 more patients in the control group, and the total number of patients increased to six (15.7%). Paresis was not permanent in any patient and resolved within two months. Sialocele was observed in seven (18.4%) patients in the control group and in two (9.5%) patients in the experimental group ($p < 0.05$). Frey's syndrome and keloid formation were not observed in any of the patients. Consequently, it is thought that patients benefit clinically from the application of PRP to the surgery site [47].

In 2018 the two groups allocated to the topical subjects on oil myringoplasty results PRP effect in a study conducted in Turkey on 20 rats were studied [48]. Myringotomy was performed in the left ears of the subjects randomly divided into two groups of 10 subjects each. In the control group, the fat graft taken from the inguinal region after myringotomy was shaped like an hourglass and placed in the myringotomy area. Although the same procedure was applied to the experimental group, it was kept in PRP obtained from the subject for five minutes before applying the fat graft. After 21 days of follow-up, the eardrums of the subjects who were sacrificed were extracted and examined, and the groups were compared. Adipocyte area and the number of mature vessels were higher in the experimental group compared to the control group [48].

In a study examining the effect of PRP on the healing of acute tympanic membrane perforation in 2017, 12 New Zealand rabbits were used [49]. Perforation was created on the pars tensa part of the bilateral eardrums of the subjects examined in 2 groups with the help of a needle. Then, PRP obtained from the subject itself was applied to its right ear, and the left ear was left as the control group. Ear examination was performed on the subjects on the 1st, 4th, 7th, 10th, 13th, 16th, 21st, and 35th days and the time to close the perforation was noted. The subjects were sacrificed at 2 months after the procedure, and eardrums were extracted and subjected to histopathological examination. Perforation closure time was 12 days in the experimental group and 17.7 days in the control group, and this difference was statistically significant ($p = 0.0145$) [49].

Based on the view that it may accelerate the regeneration of olfactory nerves in anosmia patients due to its inclusion of growth factors, PRP was first used in 2016 by Mavrogeni *et al*. [18] Following the application of PRP in the olfactory area of 5 patients with idiopathic anosmia in 4 sessions, the sense of smell returned in 4 patients, and one patient stated that he could smell a little but could not smell all. In a similar study conducted in 2020, although there was no significant improvement in Sniffin Sticks score in 2 patients with anosmia, it was observed that all 5 patients with hyposmia reached normosmia after 3 months of follow-up.46 In a study conducted by Yasak *et al*. [20] on mice, the effects of PRP on the olfactory nerve were examined histologically, and it was found that epithelial damage was significantly less and epithelial

thickness was higher in the PRP group. It was concluded that PRP use in anosmia patients has curative effects.

CONCLUSION

The use of blood products rich in thrombocytes, which accelerates postoperative recovery by inducing healthy epithelial healing, is gradually increasing after otorhinolaryngology surgery. Studies in this area have been increasing in recent years, and the use of these autologous products is introduced to the clinical practice of surgeons. Our research has shown that PRP has an effect on relieving the symptoms of patients with chronic sinusitis and olfactory dysfunction. We think that PRP is a material that can be used in endoscopic endonasal surgical interventions since it does not have undesirable side effects such as allergic reaction or foreign body reaction due to its preparation in autogenous blood tissue, and it is easy to prepare and cheap. Our study has shown the effectiveness of PRP and it is thought that it will shed light on future studies to become a practically usable method.

Authors' Contribution

Study Conception: SGK; Study Design: SGK; Supervision: SGK; Funding: SGK, MFT, AG, ÇA; Materials: SGK, MFT, AG, ÇA; Data Collection and/or Processing: SGK, MFT, AG, ÇA; Statistical Analysis and/or Data Interpretation: SGK, MFT, AG, ÇA; Literature Review: SGK; Manuscript Preparation: SGK and Critical Review: SGK

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):882-891 Original Article

DOI: 10.18621/eurj.933709 *Radiology*

Comparison of apparent diffusion coefficient and relative apparent diffusion coefficient values for differential diagnosis of breast lesions

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ABSTRACT

Objectives: The purpose the study was to evaluate the role of diffusion weighted magnetic resonance imaging (DW-MRI) in diagnosis of benign and malignant breast lesions, to calculate a cut-off apparent diffusion coefficient (ADC) value and to explore use of relative ADC (r ADC) for improving sensitivity and specificity of MRI in diagnosis of breast cancer.

Methods: This retrospective study based on a cohort of patients who underwent dynamic contrast enhanced (DCE)-MRI having suspicious breast mass by ultrasonography and mammography to whom DWI sequence was added to the routine diagnostic MRI. ADC and r ADC (lesion/normal breast tissue) values of breast masses were calculated. The threshold ADC values used to differentiate benign and malignant lesions were determined using receiver operating characteristic analysis, sensitivity, specificity, positive predictive value and negative predictive value were calculated.

Results: Malignant masses had significantly lower ADC (mean: $1.03 \pm 0.36 \times 10^{-3}$ mm^2/s) and r ADC (mean: $0.66 \pm 0.22 \times 10^{-3}$ mm^2/s) values than those of benign masses with ADC (mean: $1.50 \pm 0.56 \times 10{-}3$ mm^2/s) and r ADC (mean: $0.97 \pm 0.31 \times 10^{-3}$ mm^2/s) values, respectively ($p = 0.001$ for both). The best cut-off value for the lesion ADC was 1.09×10^{-3} mm^2/s with a sensitivity of 72.73%, and specificity of 79.17%. The best cut-off value for r ADC was 0.83 with sensitivity of 78.79% and specificity of 70.83%.

Conclusions: DWI has high diagnostic value with high sensitivity and specificity differentiating benign and malignant breast lesions. ADC and r ADC values can improve the diagnostic accuracy of differentiating benign and malignant breast lesions.

Keywords: Diffusion weighted imaging, MRI, apparent diffusion coefficient, relative ADC, breast mass

Breast cancer is still a common malignancy and cause of cancer death. Despite improvements in detection of breast cancer with the widespread application of mammography and ultrasonography (US), other screening methods may contribute to early diagnosis for women at increased risk of breast cancer. Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) plays a significant role in breast lesion characterization in those with high risk factors including family history or genetic predisposition and young women with dense breast tissue and it has higher sensitivity over both mammography and US [1]. DCE-MRI can also support breast cancer staging, solving the question of the actual size of the lesion, multicen-

Received: *May 22, 2021;* ***Accepted:*** *November 26, 2021;* ***Published Online:*** *March 28, 2022*

How to cite this article: *Parlak AE, Yağcı B. Comparison of apparent diffusion coefficient (ADC) and relative ADC values for differential diagnosis of breast lesions. Eur Res J 2022;8(6):882-891. DOI: 10.18621/eurj.933709*

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e-ISSN: 2149-3189

tricity, residual tumor distinction better than mammography and US [2-4]. Nonetheless, several studies have shown that conventional breast MRI, including T2-weighted imaging and contrast-enhanced T1-weighted imaging, is constrained in terms of breast tumor specificity [5-7]. Consequently, significant interest has been expressed in the development of adjunct MRI methods to improve the specificity of DCE-MRI and diffusion weighted breast imaging has been investigating for its potential to boost breast disease diagnosis at the expense of a slight increase in examination time. Diffusion weighted magnetic resonance imaging (DW-MRI) is based on the random Brownian motion of water molecules which has potential to alter the signal intensity [8, 9]. This motion of water molecules is more restricted in highly cellular tissues (e.g., high grade tumors) or in case with intact cellular membrane whereas water molecules can easily diffuse in low cellular environment or where there is cell membrane destruction. A low cellular environment enables a greater extracellular space for water molecules to disperse, which can also freely move through destructed cellular membranes from the extracellular to the intracellular compartment. Hence the degree of tissue water diffusion is inversely associated with the cellularity of the tissue and the cell membrane integrity. Based on this phenomenon, DW-MRI can be used to evaluate many pathologic conditions in the body and can help differentiate cellularity of the histologic structure [8-12].

Diffusion is quantified by calculating what is known as the apparent diffusion coefficient (ADC) value in square millimeter per second, which describes the average area covered by a molecule per unit time. The ADC value can be calculated by assessing the signal attenuation that occurs at diffusion-weighted imaging performed at different b values [10-12].

Recent studies have shown that ADC values were significantly lower in malignant breast lesions compared to benign breast lesions because of the higher cellular density (due to the intensity of the tumor tissue) in malignant lesions [13-15].

Unfortunately, menstrual cycle and hormone-replacement therapy influence the ADC values obtained from diffusion weighted imaging (DWI) [16, 17]. Relative ADC (r ADC) value is defined to optimize ADC value, which is calculated by dividing ADC value of the breast lesion by adjacent breast parenchyma therefore minimizing the individual differences as well as the potential therapy effects. Furthermore, the r ADC value has been supposed to be unaffected by the menstrual cycle [18, 19].

The purpose of this study was to evaluate the role of DW-MRI in the diagnosis of benign and malignant breast lesions, to calculate a cut off ADC value and to explore use of r ADC for improving sensitivity and specificity of MRI in diagnosis of breast cancer.

METHODS

Study Population

This retrospective study was based on a cohort of patients who underwent DCE-MRI having suspicious breast mass by ultrasonography and mammography to whom a specific DWI sequence was added to the routine diagnostic focused MRI. The lesions categorized as Breast Imaging Reporting and Data System (BI-RADS) 3, 4 or 5 were included in the study. Part of the BIRADS 3 lesions which were followed for 2 years and decided as stable thus re-categorized as BI-RADS 2 were excluded from the study.

A 110 breast masses of 107 adult female patients with histopathological proven diagnosis were retrieved from the database over a 3-year-old period and retrospectively reviewed for the breast masses on the DWI images. Over the 110 breast masses, 22 were excluded due to the inability to reach the raw data of the images, 18 excluded due to poor image quality, 8 excluded because of uncertainty in identifying the match lesion with the pathology and 5 excluded as the lesions were smaller than 10 mm in size and diffusion weighted images were not identifiable. Eventually, 24 benign breast masses of 24 patients and 33 malignant breast masses of 32 patients were included in our study.

Magnetic Resonance Imaging

All individuals underwent a diagnostic focused MRI performed with a 1.5-Tesla (T) superconducting 8 channel MRI system (Phillips, Achieva) equipped with high-speed gradients. The MR images of breast in the sagittal and axial planes were obtained in the supine positions with a high-resolution breast-array coil. Turbo spin-echo T1-weighted (TR/TE, 514/10), turbo spin-echo T2-weighted (TR/TE, 4044/70), T1-weighted SPIR (600/minimum) with and without

gadolinium Gd-based contrast agents (0.1 mmol/kg of body weight) were acquired. DWI using single-shot spin-echo echo-planar imaging (EPI) was performed in axial plane with diffusion gradient b values of 0 and 800 mm²/s. The following DWI parameters were used: field of view (FOV) 175 (R-L) × 278 (AP) mm; number of excitations (NEX) 2; matrix size, 116 × 185; slice thickness, 3 mm intersection gap, none.

Image Interpretation

Before evaluating MR images, identifying information was removed from images. A radiologist (seven years of experience in breast imaging) evaluated the images for the quality, and to locate and mark the matched histopathological masses from the pathology records as well as evaluation of masses. After all images were reviewed, the diffusion-weighted images were transferred to a separate workstation (Phillips, Extended MR workspace, 2.6.3.4, Netherlands). Apparent diffusion coefficient (ADC) maps were generated. After four-week period, the same radiologist without looking at any patient data, measured the ADC values on the previously marked images. A circular region of interest (ROI) with a value of 50-70 mm² was placed on the center of the mass. We placed a single ROI smaller than lesion in the solid tumor and care was taken to avoid calcified, hemorrhagic or necrotic areas of the masses or the breast parenchyma while placing the ROI. Another ROI was placed on the mass free breast parenchyma. Measurements were repeated three times for both masses and the breast parenchyma. The average values were calculated. ADC values were expressed as square millimeters per second. Relative ADC (r ADC) values were calculated by dividing the mean ADC values of each patient's mass by the mean ADC values of each patient's parenchyma.

Ethics Statement

This study was approved by the Institutional Ethics Committee (2014- 41/ 8) written informed consent was obtained from all subjects prior to MRI examination. Patient records and information were anonymized and de-identified prior to analysis.

Statistical Analysis

Data were analyzed using the Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA). Descriptive statistics included frequency, percentage, mean, median, standard deviation (SD) minimum, maximum. Kolmogorov-Smirnov and Shapiro Wilks test was used for the normality of the dependent and independent measures. Independent samples t-test and Mann–Whitney U test were used to compare the measurements of benign and the malignant group. Pearson Chi-Square test was used to compare categorical variables. The threshold ADC values used to differentiate between benign and malignant lesions were determined using receiver operating characteristic (ROC) analysis, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. All differences associated with a chance probability of 0.05 or less were considered statistically significant.

RESULTS

Fifty-seven breast lesions of 56 patients with a mean age of 45.54 ± 11.03 years were included in this study. Four-two percent of 24/57 breast masses of 24 female patients were benign (mean age: 39.96 ± 9.81 years) and 33/57 (58%) breast masses of 32 female patients were malignant (mean age: 49.72 ± 10.12 years) were included.

The benign lesions included 11 fibroadenomas, 2 sclerosing adenosis, 1 adenoid ductal hyperplasia, 5 granulomatous mastitis, 2 mastitis, 2 papilloma, 1 radial sclerosing lesion. The malignant lesions included 23 invasive ductal carcinomas (IDC), 5 ductal carcinoma in-situ (DCIS), 4 invasive lobular carcinomas and 1 invasive mucinous carcinoma (Table 1) (Fig. 1).

The ADC of breast masses ranged between 0.5 × 10^{-3} mm²/s and 2.6 × 10^{-3} mm²/s (mean: 1.23 ± 0.51 × 10^{-3} mm²/s). The r ADC values of breast masses ranged between 0.3 × 10^{-3} mm²/s and 1.6 × 10^{-3} mm²/s (mean: 0.79 ± 0.31 × 10^{-3} mm²/s).

As for the ADC measurements of the lesions, the lowest ADC lesion value was 0.49 × 10^{-3} mm²/s with IDC, and the highest ADC lesion value was 2.62 × 10^{-3} mm²/s with granulomatous mastitis. The lowest (lesion/normal breast tissue) r ADC rate was (0.30) in an IDC case, whereas the highest was (1.61) in a case of granulomatous mastitis.

The mean ADC value was found to be 1.50 ± 0.56 × 10^{-3} mm²/s for all the benign lesions. Among the be-

Table 1. Histopathologic distribution, age, apparent diffusion coefficient (ADC) and relative ADC values of benign and malignant lesions

	Malignant lesions (n = 33)	Benign lesions (n = 24)	*p value*
Breast Lesions (n = 57)	Invasive ductal carcinoma (n = 23), Invasive lobular carcinoma (n = 4), Ductal carcinoma in-situ (n = 5), Invasive mucinous carcinoma (n = 1)	Fibroadenoma (n = 11), Granulomatous mastitis (n = 5), Mastitis (n = 2), Papilloma (n = 2), Adenoid ductal hyperplasia (n = 1) Radial sclerosing lesion (n = 1) Sclerosing adenosis (n = 1), Adenosis (n = 1)	
Age (year) (mean ± SD)	39.96 ± 9.81	49.72 ± 10.12	[a]**0.001****
Lesion ADC (mean ± SD) × 10^{-3} mm^2/sec	1.03 ± 0.36	1.50 ± 0.56	[b] **0.001****
Parenchyma ADC (mean ± SD) × 10^{-3} mm^2/sec	1.57 ± 0.22	1.54 ± 0.28	[a] 0.551
Relative ADC (mean ± SD)	0.66 ± 0.22	0.97 ± 0.31	[b] **0.001****

[a]Student t Test, [b]Mann Whitney U Test, ** $p < 0.01$

n = Number of cases, ADC = Apparent diffusion coefficient, SD = Standard deviation,

$p < 0.05$ was considered as statistically significant.

nign lesions, the lowest ADC value was belonged to a mastitis with 0.57 × 10^{-3} mm^2/s and the highest ADC value was 2.62 × 10^{-3} mm^2/s in a granulomatous mastitis. The mean (lesion/normal breast tissue) r ADC rate was 0.97 ± 0.31 in these lesions. The lowest rate among all the benign lesions was 0.41 in an intraductal papilloma and the highest rate was 1.61 in a granulomatous mastitis.

The mean ADC value was 1.03 ± 0.36 × 10^{-3} mm^2/s for all the malignant lesions. Among the malignant lesions, the lowest ADC value was 0.49 × 10^{-3} mm^2/s in an IDC, and the highest ADC value was 2.13 × 10^{-3} mm^2/s in an invasive mucinous carcinoma. The mean (lesion/normal breast tissue) r ADC rate was 0.66 ± 0.22 in these lesions. The lowest rate was 0.30 in an IDC and the highest rate was 1.34 in an invasive mucinous carcinoma among all the malignant lesions (Figs. 2 and 3).

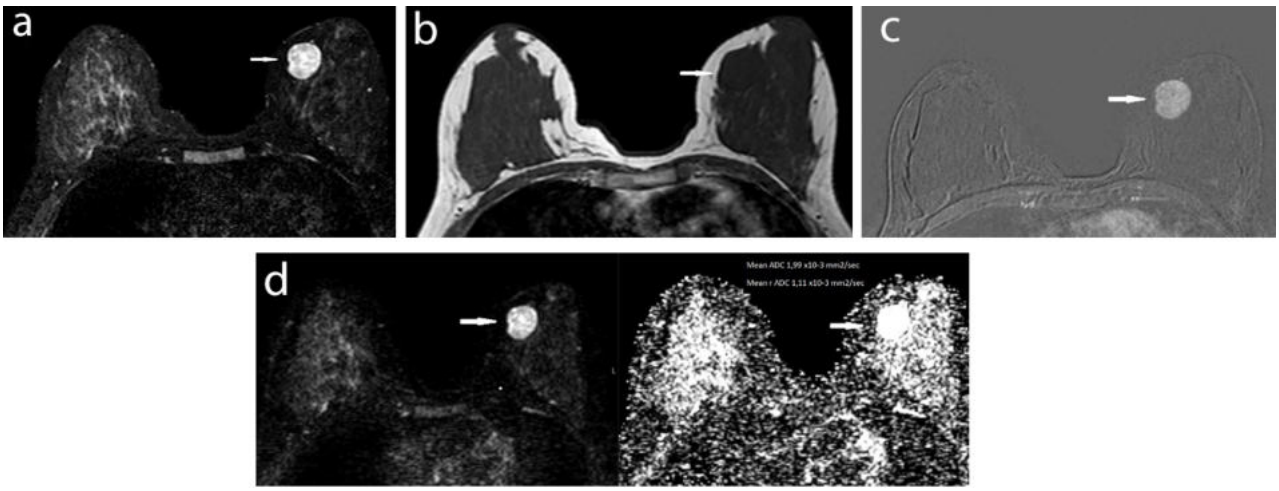

Fig. 1. (a) A well circumscribed 20 × 15 mm mass in the left upper-inner-quadrant of left breast, hyperintense in T2 weighted images; (b) hypointense in T1 weighted images; (c) homogeneously diffuse enhancing (arrow) in T1 weighted post contrast images; and (d) Non-restricted in DWI with ADC 1.99 × 10^{-3} mm^2/s and r ADC 1.11 × 10^{-3} mm^2/s . The pathology report showed fibroadenoma.

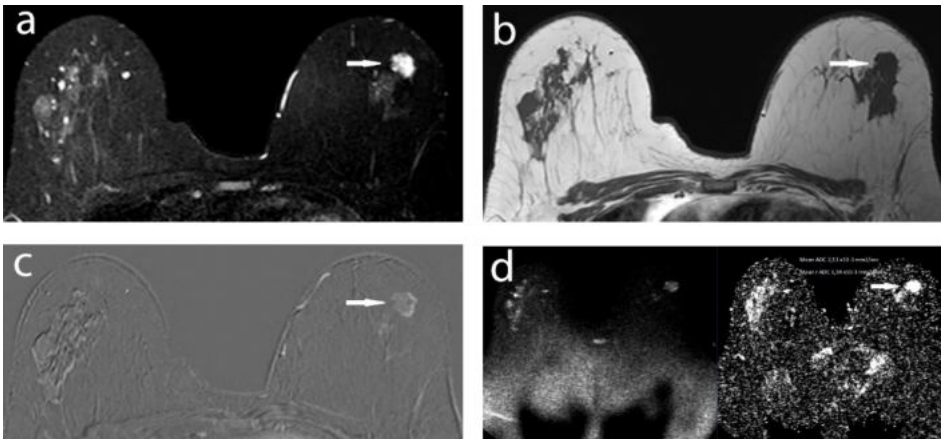

Fig. 2. (a) A 18 × 15 mm mass in the left upper-outer quadrant of the left breast, very high signal in T2 weighted images; (b) Low signal in T1 weighted images and has lobulated not well circumscribed contour; (c) rim-like enhancing pattern in post contrasted T1 weighted images; and (d) In DWI shows non-restricted diffusion (arrow). The ADC was 2.13 × 10^{-3} mm^2/s and r-ADC was 1.34 × 10^{-3} mm2/s. It was an invasive mucinous carcinoma, according to histopathology.

The difference between the mean ADC values of the malignant lesions and those of benign lesions was statistically significant (p = 0.001). Also, the difference between the (lesion/normal breast tissue) r ADC values of the malignant lesions and those of benign lesions was statistically significant (p = 0.001) (Table 1).

Malignant masses had a significantly lower ADC (mean: 1.03 ± 0.36 × 10^{-3} mm^2/s) and r ADC (mean: 0.66 ± 0.22 × 10^{-3} mm^2/s) values than those of benign masses (ADC, mean: 1.50 ± 0.56 × 10^{-3} mm^2/s and r ADC, mean: 0.97 ± 0.31 × 10^{-3} mm^2/s; respectively, p = 0.001) (Table 1).

Benign lesions showed a similar or slightly lower signal intensity than parenchyma in ADC maps, while the vast majority of malignant lesions showed a significantly lower signal intensity than those of parenchyma. No significant differences were observed between the normal breast tissue of the malignant and the benign group (Table 1).

Of the benign lesions, 5 (20.8%) had lower ADC values than the determined threshold value of 1.09 × 10^{-3} mm^2/sec. Of these 5, 2 were intraductal papilloma, 2 were mastitis, and 1 was granulomatous mastitis. Of the malignant lesions, 9 (27.3%) had higher ADC values than the determined threshold value of 1.09 × 10^{-3} mm^2/s. Of these 9, 3 were DCIS, 3 were IDC, 2 were invasive lobular carcinoma and 1 was invasive mucinous carcinoma.

Of the benign lesions, 7 (29.2%) had lower r ADC

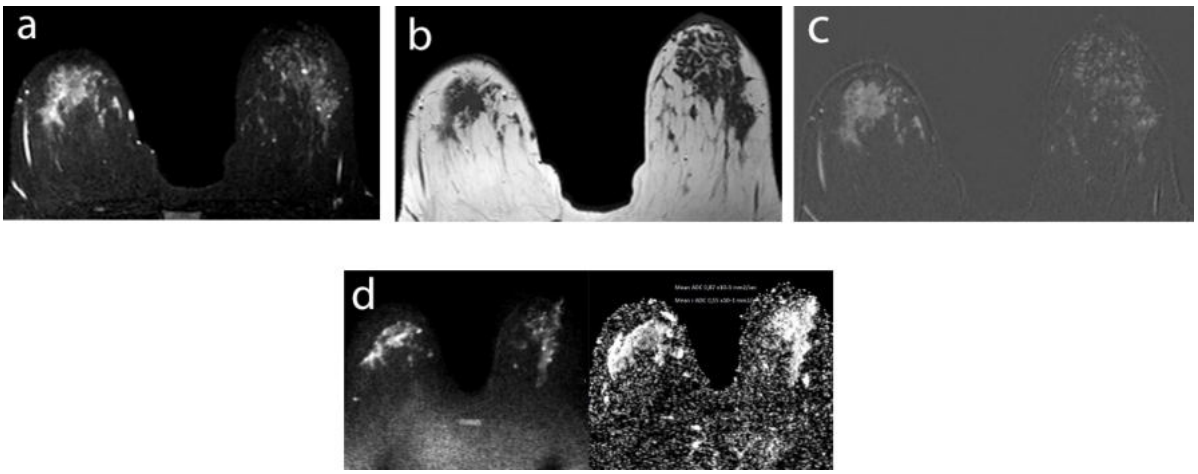

Fig. 3. (a, b) A non-mass like lesion in the upper-outer-quadrant of the right breast in T1 and T2 weighted images; (c) Clumped pattern enhancement in post-contrast images; and (d) it is mildly restricted in DWI with ADC 0.87 × 10^{-3} mm^2/s and r-ADC 0.55×10^{-3} mm^2/s. . The histopathology result was invasive ductal carcinoma.

Table 2. Receiver operating characteristic analysis for apparent diffusion coefficient (ADC) and relative ADC values for benign and malignant lesions by pathology

	Diagnostic Scan					ROC Curve		*p value*
	Cut-off	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Area	95% Confidence Interval	
Lesion ADC	≤ 1.09	72.73	79.17	82.76	67.86	0.758	0.622-0.894	**0.001****
Relative ADC	≤ 0.83	78.79	70.83	78.79	70.83	0.804	0.685-0.924	**0.001****

***p* < 0.05 was considered as statistically significant.

values than the determined threshold value 0.83 × 10^{-3} mm^2/s. Of these 7, 2 were intraductal papilloma, 2 were mastitis, 2 were granulomatous mastitis and 1 was fibroadenoma. Of the malignant lesions, 7 (21.2%) had higher ADC values than the determined threshold value 0.83 × 10^{-3} mm^2/s. Of these 7, 3 were DCIS, 2 were IDC, 1 were invasive lobular carcinoma and 1 was invasive mucinous carcinoma (Figs. 2 and 3).

Using ROC analysis, we compared the ability of DWI to differentiate malignant and benign lesions by pathology, and found that the best cut-off value for the lesion ADC measurement was 1.09 × 10^{-3} mm^2/s his resulted in sensitivity of 72.73 %, specificity of 79.17%, PPV of 82.76% and NPV of 67.86 % (Table 2). Area under curve (AUC) was 0.758 (95% CI 0.622-0.894, $p = 0.001$) which was statistically significant and indicated that using ADC value, DWI could discriminate benign and malignant lesions with high probability (Fig. 4). The odds of a lesion with an ADC value of ≤ 1.09 × 10^{-3} mm^2/s receiving a malignant diagnosis were 10.133 times (95% CI: 2.909-35.296)

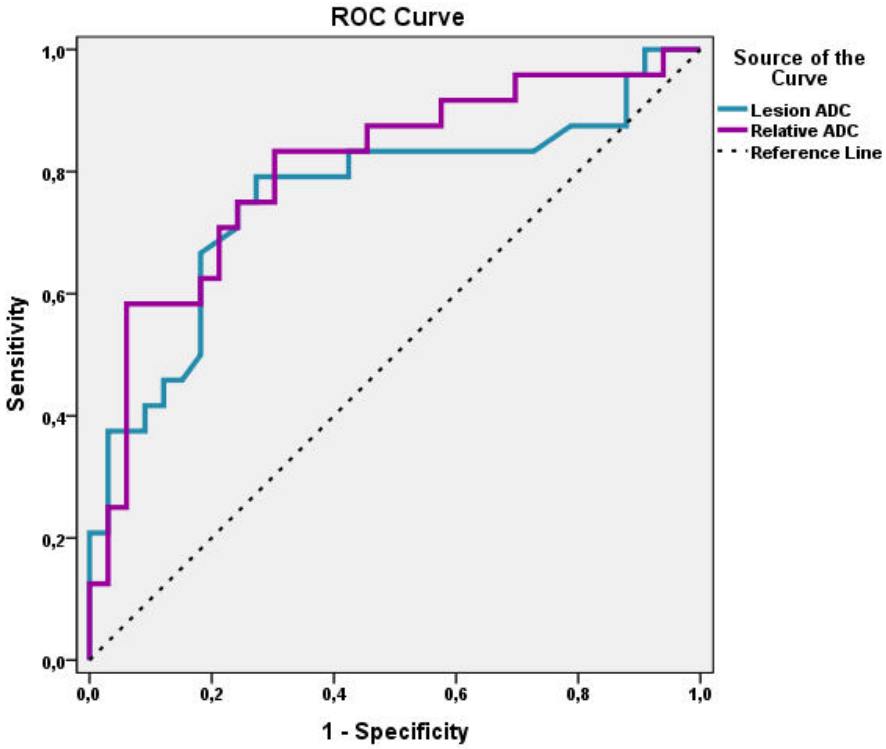

Fig. 4. ROC curve of DWI for discriminating benign and malignant breast lesions. Diagonal lines denote apparent diffusion coefficient (ADC) and r-ADC values.

Table 3. The best cut-off values for lesion apparent diffusion coefficient (ADC) and relative ADC values for benign and malignant lesions by pathology

		Pathological Results				*p value*
		Benign		Malignant		
	Cut-off	n	%	n	%	
Lesion ADC	**> 1.09**	19	79.2	9	27.3	**0.001****
	≤ 1.09	5	20.8	24	72.7	
Relative ADC	**> 0.83**	17	70.8	7	21.2	**0.001****
	≤ 0.83	7	29.2	26	78.8	

Pearson's Chi-square Test **$p < 0.01$

that of a benign lesion (Table 3).

We also determined best cut-off value for r ADC using ROC analysis and found that 0.83 was the best cut-off value for discriminating malignant and the benign lesions with sensitivity of 78.79 %, specificity of 70.83%, PPV of 78.79% and NPV of 70.83% (Table 2). AUC was 0.804 (95% CI, 685-0.924, $p = 0.001$) which was statistically significant and indicated that use of r ADC could discriminate benign and malignant lesions with high probability (Fig. 4). The odds of a lesion with a r ADC value of ≤ 0.83 receiving a malignant diagnosis were 9.020 times (95% CI: 2.682-30.340) that of a benign lesion (Table 3).

DISCUSSION

Diffusion weighted imaging (DWI) based on the random and thermal (Brownian) motion of water which can be quantified by calculating apparent diffusion coefficient (ADC) values in square millimeter per second depending on the degree of water molecule diffusion at in vivo MRI [8-12]. ADC values obtained from DWI can be affected by menstrual cycle and hormone replacement therapy [16, 17]. Relative ADC (r ADC) value has been defined to optimize ADC value, and supposed to be unaffected by the menstrual cycle [18-20].

Recent studies have shown that ADC values in malignant breast lesions were substantially lower than in benign breast lesions due to the higher cellular density in malignant lesions [21-23]. Our study results revealed that ADC values and r ADC values were significantly lower in malignant breast lesions compared to benign breast lesions. Our findings were consistent with those of past studies in literature [24-26].

Akin *et al*. [26] published a research, the threshold value for the mean ADC value of the lesions was considered 1.08×10^{-3} mm^2/s, in the ROC analysis the AUC was 0.95 and the sensitivity and specificity of detecting malignant lesions were 92.1% and 92.4%, respectively. The difference between the mean ADC values of the malignant lesions and benign lesions was statistically significant ($p = 0.001$). Also, the difference between the r ADC values of the malignant lesions and benign lesions was statistically significant ($p = 0.001$).

According to the ROC curve in our study, the best threshold value was 1.09×10^{-3} mm^2/s for lesion ADC. Akin *et al*. [26] revealed the similar results with high sensitivity and specificity provided that threshold ADC 1.08×10^{-3} mm^2/s. There was a statistically significant difference between the ADC values of the malignant breast lesions and those of the benign breast lesions ($p < 0.01$), and the diagnostic value of the ROC analysis for ADC values yielded AUC value of 0.758 with a sensitivity of 72.73% and specificity of 79.17% in our study. These results are within the range of previously reported values in literature and is closer to lower values [27, 28]. Our results are much more similar with Yilmaz *et al*. [28].

Şahin and Arıbal [29] revealed significant differences in ADC and r ADC ratios of benign and malignant lesions in their study. They found threshold ADC value of mass/ normal fibro glandular tissue was 0.8 with 91.4% sensitivity and 100% specificity for differentiating between benign and malign lesions. Although our results were consistent with their study in

terms of statistically significant differences of ADC and r ADC values for malignant and benign lesions, sensitivity and specificity values of the current study are not as high as Cennet S *et al*. study. However, the results of this study were also higher than those of many literature studies results probably as they used the minimum ADC and r ADC values while the others and we used mean ADC and r ADC values [25, 26]. Nadrljanski and Milosevic [30] published a research, the matched female premenopausal patients with confirmed histological diagnosis of either BIRADS 3 or BIRADS 5 lesions. They reported the selected parameters: r ADC and ADC for N1 and N2 and the differences between B3 and B5 lesions were considered highly statistically significant, with p-values ($p <$ 0.00001 for both). The data was regarding the selected parameters for the group of patients with B3 lesions (N1 = 52, ADC = 1.45 ± 0.13 × 10^{-3} mm2/s; r ADC = 0.81 ± 0.08 × 10^{-3} mm^2/s) and for the group of patients with B5 lesions (N2 = 52, ADC = 1.00 ± 0.11 × 10^{-3} mm^2/s; r ADC = 0.58 ± 0.07 × 10^{-3} mm^2/s). In our study the mean r ADC values for the benign lesions were 0.97 and 0.66 for the malignant lesion which were consistent with their study.

DW-MRI provides information on microstructure such as tissue cellularity, which has been shown to be an important index of tumor grade and local tissue architecture, which is a sensitive early indicator of abnormality [15, 21-24]. The lowest cellular zone has the maximum ADC value, while the highest cellular zone has the minimum ADC value. In addition, the ADC value may be affected by the components of fibrosis and necrosis in tumors [25]. Yoshikawa *et al*. [31] study's results support these findings. ADC values of IDC were significantly lower than those of non-IDC. Also, ADC values of both types were significantly lower than those of normal breast parenchyma.

Yoshikawa *et al*. [31] reported that the mean ADC values of the histological types were calculated as follows: The mean ADC values for IDC, NIDC, and normal breasts were 1.07 ± 0.19 × 10^{-3} mm^2/s, 1.42 ± 0.17 × 10^{-3} mm^2/s, and 1.96 ± 0.21 × 10^{-3} mm^2/s, respectively. The ADC values for IDC and NIDC were significantly different from those of normal breasts ($p <$ 0.001 each). Mean ADC values were also significantly different between IDC and NIDC ($p < 0.001$). In our study mean ADC values for NIDC and IDC were 1.22 ± 0.47 × 10^{-3} mm^2/s and 0.99 ± 0.33 × 10^{-3} mm^2/s, respectively and mean r ADC values for NIDC and IDC were 0.73 ± 0.27 and 0.64 ± 0.22, respectively. In our study the results were lower for non-IDC similar to Yoshikawa *et al*. [31] results however we could not find a statistically significance. Infect the number of cases in their study was also low similar to our study. However, in the current study ADC and r ADC values for one of the non-IDC lesions were much smaller than those of all the IDC since the non-IDC lesions especially frequently in DCIS it is hard to evaluate the lesion with DWI.

In a study of Tao *et al*. [32] made with only DCIS lesions, they found that most middle and high-grade DCIS lesions showed non mass like enhancement so it is hard to be recognized by DWI. They used DCE-MRI and intravoxel incoherent motion DWI (IVIM-DWI) and reduce the misdiagnosis of DCIS. We thought maybe we could hardly find DCIS lesions and ADC and r ADC values were not truly evaluated. Maybe a study using IVIM, DWI must be done and evaluate ADC and r ADC value with this technique [32].

There was histological diversity in malignant breast masses. The number of cases for IDC, invasive lobular carcinoma, DCIS and invasive mucinous carcinoma was 23, 4, 5, and 1, respectively which leads to influence on statistical analysis. For instance, ADC and r ADC values for invasive mucinous carcinoma were 2.13 × 10^{-3} mm^2/s and 1.34 respectively which was significantly higher than other types of malignant diagnosis. If the number of invasive mucinous carcinoma was higher, cut-off value would be significantly increased. The same condition applies for benign lesions as well. Two lesions with histological diagnosis of papilloma had ADC values of 0.99 × 10^{-3} mm^2/s and 0.83 × 10^{-3} mm^2/s and r ADC values of 0.69 and 0.53 which were quite low value compared to remaining benign cases.

Hatakenaka *et al*. [33] reported a study about tumor cellularity and tumor ADC for the differential diagnosis of breast tumors and they found that ADC values correlate inversely with tumor cellularity. Mucinous carcinoma demonstrates lower cellular density and higher extracellular water content. They also have very high signal intensity on T2- weighted images. The increase in extracellular water in stroma may have contributed to higher ADC values [33]. Similar to this past study, ADC values of mucinous carcinoma and

granulomatous mastitis resulted in increase of mean ADC values in our study. Further studies with more homogenously distributed group of histological diagnosis with larger sample size would be beneficial.
Another limitation was that the diagnostic accuracy of DWI may decrease due to diameter of lesions, Kinoshita *et al* [34] have reported that the lesions < 10 mm in diameter cannot be demonstrated by DWI .
Another concern was that, non-IDCs, including lobular carcinoma in situ, atypical ductal hyperplasia, as well as mastitis and granulomatous mastitis may enhance similar to the parenchyma due to the lower cellular density, and these lesions may show less restricted diffusion. In our study we have the similar results because of the patients with mastitis and granulomatous mastitis. In these lesions we had less restricted diffusion but in Yilmaz *et al*. [28] study, the granulomatous mastitis showed restricted diffusion and r ADC values were calculated as 0.927. We had 2 mastitis and 2 granulomatous mastitis with r ADC values ranged 0.63 to 1.61. The number of cases in the current study was not enough for further analysis and a study with only mastitis patients would better clarify the condition .

Limitations

Our study presents a number of limitations. Firstly, it was a retrospective study which to some extent, facilitates the evaluation, since the histological findings were readily available. However, this resulted in exclusion of number of patients due to the lack of patients' data. Another limitation was that IDC was dominant in the histopathologic subgroup distribution of malignant lesions, and the number of other lesions was small compared to IDC which leads to inevitable election bias.

CONCLUSION

There is rapidly growing evidence of the potential value of DWI to improve breast cancer detection and characterization. The technique is relatively easy for incorporation into clinical breast MRI protocols and provides complementary information to conventional breast MRI examinations. Furthermore, diffusion characteristics of malignant and benign lesions can be quantified by ADC measurements and using both ADC and r ADC techniques together can increase the diagnostic performance of breast MRI in the diagnosis of breast lesions.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgement

We thank Dr. Iclal Erdem Toslak for her contributions.

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The European Research Journal 2022;8(6):892-897 Original Article

DOI: 10.18621/eurj.1170080 *Family Medicine*

Perinatal deaths in Bursa Province, Turkey: an analysis by applying the International Classification of Diseases-perinatal mortality (ICD-PM) system

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ABSTRACT

Objectives: The International Classification of Diseases for Perinatal Mortality (ICD-PM) system is a globally used classification based on the International Statistical Classification of Diseases and Related Health Problems (ICD-10) codes. Moreover, it focuses on the time of death and maternal conditions. Here, we analyzed perinatal deaths by using the ICD-PM system.

Methods: This is a retrospective study, performed between January 1, 2020, and March 30, 2022, in Bursa. Perinatal characteristics and the causes of perinatal deaths were recorded. The perinatal deaths were classified according to the ICD-PM system and descriptives were given.

Results: The majority of perinatal death cases (119 cases) occurred in the antepartum period. The leading cause of antepartum deaths was unspecified causes (62.2%) followed by fetal growth disorders (9.3%). A total of 63 (53.7%) mothers were healthy (M5) while 27 (22.7%) mothers had medical or surgical conditions (M4). Acute intrapartum events (33.4%) were the commonest cause of intrapartum deaths followed by unspecified causes (26.6%). When neonatal deaths were analyzed, low birth weight/prematurity constitute 59.6% of neonatal deaths. The largest proportion of mothers was healthy in the intrapartum (40%) while maternal complications of pregnancy (M2) was the most commonest classification for neonatal deaths.

Conclusions: ICD-PM is a globally used system for classifying perinatal deaths. The time of perinatal death used in this system provides focus on interventions in perinatal care and it encourages comparison between perinatal care centers. We suggest that we might use resources truly to prevent perinatal deaths in our country by using this system.

Keywords: ICD-10, ICD-PM, perinatal death, perinatal mortality

The perinatal period covers the late pregnancy period after the 24th gestational week, the birth time, and the first week of the postnatal interval. Thus, perinatal mortality is composed of stillbirths, intrapartum and early neonatal mortality. The perinatal mortality rate is known to be one of the indicators of the development of a country. As a consequence of this, the analysis of perinatal deaths have a crucial role for both physicians and governments [1-3]. Defining the reason by autopsy is too important for detecting malpractice, inheritance law, and preventive medicine in perinatal deaths [4, 5]. Nevertheless, perinatal deaths have been inadequately recorded all around the world.

Received: ***September 2, 2022;*** ***Accepted:*** ***October 6, 2022;*** ***Published Online:*** ***October 11, 2022***

How to cite this article: *Metin S. Perinatal deaths in Bursa Province, Turkey: an analysis by applying the International Classification of Diseases-perinatal mortality (ICD-PM) system. Eur Res J 2022;8(6):892-897. DOI: 10.18621/eurj.1170080*

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e-ISSN: 2149-3189

Classification systems have been tried to be used to clarify the cause of perinatal deaths. Some high-income countries have suggested local classification systems such as the Codac system for the United Kingdom, the Stockholm system for Sweden, and the Tulip system for the Netherlands [6]. In 2016, the World Health Organization offered a globally used classification system named the International Classification of Diseases for Perinatal Mortality (ICD-PM) [7]. This system was based on the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). Moreover, the cause of death was classified by the time of death such as antepartum, intrapartum, or neonatal period, and maternal conditions such as complications of placenta, cord, and membranes; complications of pregnancy, the other complications of labor and delivery; maternal medical and surgical conditions.

To the best of our knowledge, there is only one study in the literature analyzing the cause of perinatal deaths with the ICD-PM classification system in Turkey. Considering the importance of perinatal deaths, the importance of identifying their causes is extremely important. Here, we presented the perinatal characteristics of perinatal death cases of Bursa and classified these with the universal ICD-PM system.

METHODS

This study was designed as a retrospective study reflecting the data of a city in Turkey. The ICD-PM system was applied to the perinatal deaths between January 1, 2020, and March 30, 2022, in Bursa. The study was approved by the local ethics committee with an approval number of 2019-KAEK-140. Since the patients did not participate actively in the study, verbal or written informed consent was not obtained. Stillbirths, intrapartum deaths, and neonatal deaths were included in the study while pregnancy terminations were not taken into account. Deaths without sufficient clinical data were excluded from the study. Stillbirth was defined as intrauterine deaths that occurred after the 24th gestational week. Intrapartum death was diagnosed when the death takes place during labor or cesarean section while neonatal death was defined the babies born alive and dying within 28 days of life.
The maternal age, any previous medical disease, smoking habit, parity, the time interval between pregnancies, any need for artificial reproductive techniques, education status of mother and father, the number of antenatal visits, the vaccination status, presence of consanguineous marriage, Rh isoimmunization, gestational age at the time of death, delivery mode,gender and birth weight of the baby were recorded from the medical records. Moreover, the presence of congenital anomalies, infection or growth restriction, any birth trauma, and neonatal disorders such as convulsions, and respiratory or cardiovascular conditions were noted.

The deaths were classified according to the ICD-PM system. The system was initially composed of three groups named as A (antepartum), I (intrapartum), and N (neonatal). Then, these groups were subdivided in terms of causes (6 groups for A, seven groups for I and 11 groups for N). All causes were divided according to the maternal conditions (M1 to M5). The number of cases and percentages were written in each section.

Statistical Analysis

Statistical analyses were performed by using SPSS version 25.0. Shapiro Wilk test was used to assess the distribution of variables. Normally distributed, continuous variables were presented with mean ± standard deviation while non-normally distributed ones were shown with median (min-max) values. Categorical variables were expressed as frequency or percentages.

RESULTS

A total of 233 perinatal death cases were analyzed in the present study. The mean maternal age was 33.8 ± 6.1 years. The median gravida was 2 (1-7), and the median number of antenatal visits was 6 (0-12). Smokers were 26 women which constitute 11.2% of the patients. A total of 23 (9.9%) of mothers underwent artificial reproductive techniques. For multiparous pregnant women, the time interval between pregnancies was lower than 1 year in 3 (4.3%) of patients, 1-2 years in 7 (10%) of patients, and more than 2 years in 60 (85.7%) patients. Consanguineous marriage was present in 38 (16.3%) couples. The median gestational week was 31 (24-41) and the median birth weight was 1427.5 (500-3995) grams. According to the education

status; 3% of mothers were illiterate, %30.9 were from primary school, 23.2% from secondary school, 23.6% from high school, and 19.3% from university while 9% were illiterate, %28.8 were from primary school, 21.5% from secondary school, 25.8% from high school, and 23.2% from university. Rh isoimmunization was detected in 25 (10.7%) cases and tetanus toxoid was fully administered in 170 (73%) cases. The delivery mode was composed of 96 (41.2%) vaginal delivery and 137 (58.8%) cesarean sections. A total of 42.9% of the babies were female while the remaining is male.

Table 1. The classification of perinatal deaths according to the ICD-PM system

Cause of perinatal death	Maternal conditions					
	M1	M2	M3	M4	M5	Total n (%)
Antenatal death (A)						
A1: congenital malformations, deformations, chromosomal abnormalities	0	0	0	0	8	8 (6.7)
A2: infectio	1	0	0	00		1 (0.8)
A3: antepartum hypoxia	0	0	0	0	0	0 (0)
A4: other specified antepartum disorder	9	1	0	4	11	25 (21)
A5: disorders related to fetal growth	0	0	0	10	1	11 (9.3)
A6: fetal death of unspecified cause	9	8	0	13	44	74 (62.2)
Total, n (%)	19 (16)	9 (7.6)	0 (0)	27(22.7)	64(53.7)	119 (100)
Intrapartum death (I)						
I1: congenital malformations, deformations, chromosomal abnormalities	0	0	0	1	1	2 (13.3)
I2: birth trauma	0	0	0	0	0	0 (0)
I3: acute intrapartum event	3	0	2	0	0	5 (33.4)
I4: infection	1	0	0	0	0	1 (6.7)
I5: other specified intrapartum disorder	1	0	0	1	1	3 (20)
I6: disorders related to fetal growth	0	0	0	0	0	0 (0)
I7: intrapartum death of unspecified cause	0	0	0	0	4	4 (26.6)
Total, n (%)	5 (33.4)	0 (0)	2 (13.3)	2 (13.3)	6 (40)	15 (100)
Neonatal death (N)						
N1: congenital malformations, deformations, chromosomal abnormalities	0	2	0	0	11	13(13.1)
N2: disorders related to fetal growth	0	0	1	0	0	1 (1)
N3: birth trauma	0	0	0	0	0	0 (0)
N4: complications of intrapartum events	0	0	0	0	0	0 (0)
N5: convulsions and disorders of cerebral status	0	0	0	0	0	1 (1)
N6: infection	3	0	0	0	2	5 (5.1)
N7: respiratory and cardiovascular disorders	2	2	0	4	7	15 (15.2)
N8: other neonatal conditions	0	0	0	0	2	2 (2)
N9: low birth weight and prematurity	10	23	20	0	3	59 (59.6)
N10: miscellaneous	1	0	0	0	2	3 (3)
N11: neonatal death of unspecified cause	0	0	0	0	0	0 (0)
Total, n (%)	16 (16.1)	30 (30.3)	21 (21.2)	5 (5.1)	27 (27.3)	99 (100)

M1 = complications of placenta, cord, and membrane, M2 = maternal complications of pregnancy, M3 = other complications of labor and delivery, M4 = maternal medical and surgical conditions, M5 = no maternal conditions

The classification of perinatal deaths according to the ICD-PM system was demonstrated in Table 1. According to this table, the majority of perinatal death cases (119 cases) occurred in the antepartum period. The leading cause of antepartum deaths was unspecified causes (62.2%) followed by fetal growth disorders (9.3%). A total of 63 (53.7%) mothers were healthy (M5) while 27 (22.7%) mothers had medical or surgical conditions. Acute intrapartum events were the commonest cause of intrapartum deaths with an incidence of 33.4% followed byunspecified causes (26.6%). When neonatal deaths were analyzed, low birth weight/prematurity constitute 59.6% of neonatal deaths. Similar to antepartum deaths, the largest proportion of mothers was healthy in the intrapartum (40%) while maternal complications of pregnancy (M2) was the most commonest classification for neonatal deaths.

DISCUSSION

Perinatal death is an indicator of the quality of maternal and fetal well-being. Proper detection and classification of perinatal deaths have become a priority for public health. Although most perinatal deaths occur in low and middle-income countries, nearly 5 million deaths persist worldwide each year [8, 9]. Unfortunately, the number of deaths could be much more than expected due to the poor recording system.

Perinatal deaths can arise from maternal, fetal, or placental reasons. The underlying reason is generally complex and multifactorial. Many classification systems have been introduced for the classification of perinatal deaths. In 2016, World Health Organization suggested a new, global classification system which was named ICD-PM. This system had many advantages as compared to others. The perinatal deaths were classified in terms of the time of death, this system took maternal conditions into account, allowed postmortem and histological investigations, and provided international comparisons for perinatal deaths [10].

In 2016, a pilot database study using ICD-PM system was performed for perinatal deaths in South Africa and the United Kingdom. Both in South Africa and the United Kingdom, most of the deaths occurred in the antepartum period. The most common causes of death in the antepartum period were unspecified causes and chromosomal abnormalities. In the intrapartum period, the leading cause of death was acute intrapartum events. In the same study, the most common reason for neonatal death was low birthweight/prematurity followed by chromosomal abnormalities. Commonly, there was no maternal condition for antepartum, intrapartum, and neonatal deaths. Maternal medical conditions and complications of the placenta, cord, and membranes were common accompanying conditions [11].

A study from South Africa reported similar results to these studies. According to this study, antepartum deaths with unspecified causes and M5 maternal condition were the most common form of antepartum perinatal death. In this study, acute intrapartum events was the leading reason for intrapartum deaths while it was low birth weight or prematurity for neonatal deaths [12].

Luk *et al*. [13] performed a validation study and showed that the proportion of unknown causes of perinatal deaths decreased by nearly 3 folds with ICD-PM system as compared to local systems. Likewise the previous study, the majority of perinatal deaths were found to occur in the antepartum period with an unspecified causes in this study. Moreover, they reported that the most common cause of neonatal death was low birth weight/prematurity while it was congenital malformations in the intrapartum period. According to the maternal conditions, M1 was commonest in the antepartum and neonatal period and M3 in the intrapartum period [13].

Contrary to these studies, Aminu *et al* demonstrated that most of the cases were intrapartum deaths. Infection was the most common reason while M1 was the most common maternal condition for antepartum stillbirths. Acute intrapartum events compose the largest proportion for intrapartum deaths and M3 was the most common maternal condition [14]. Different from this study, the study evaluating the classification of stillbirths in Northeast Nigeria retrospectively found that M4 was the commonest reason for antepartum stillbirths while M3 was the most common cause of intrapartum stillbirths. Both antepartum and intrapartum deaths occurred owing to disorders of fetal growth [15].

In the study of Priyani *et al*. [16], antepartum hypoxia was found to be the commonest cause of stillbirths. Fetal death of unspecified cause was the third

common cause of stillbirths. Congenital malformations followed by prematurity were the common reasons for neonatal deaths, while congenital malformation was the most common reason for intrapartum deaths [16].

A study from developing countries demonstrated that preterm birth (60.5%), birth asphyxia (22.5%) and congenital malformations (12.7%) were the leading causes of death in newborns [17]. There is only one study in the literature applying ICD-PM system in Turkey. This study reported that most perinatal deaths were in the antepartum period and the most common cause was fetal development disorders. Intrapartum deaths were shown to occur owing to extremely low birth weight [18].

In our study, we found that the majority of perinatal death cases occurred in the antepartum period. The leading cause of antepartum deaths was unspecified causes followed by fetal growth disorders.

Commonly, there was no maternal condition for antepartum and intrapartum deaths. Acute intrapartum events were the commonest cause of intrapartum deaths followed by unspecified causes. When neonatal deaths were analyzed, low birth weight/prematurity constitute 59.6% of neonatal deaths. Maternal complications of pregnancy (M2) was the most commonest classification for neonatal deaths.

Comparison of the results in various studies is difficult. The study populations were not the same and the management of pregnancy or labor is different among countries. However, prematurity, asphyxia, infections, birth trauma, and congenital anomalies were reported to be common causes of perinatal death in many studies.

Limitations

The present study has some limitations. First of all, it has a retrospective design. Second, it consists of a limited number of cases. Last, it reflects the data of only one region of Turkey.

CONCLUSION

ICD-PM is a globally used, comparative system for classifying perinatal deaths. The time of perinatal death used in this system provides to focus on interventions in perinatal care. Although it is not a perfect classification system, it encourages comparison between perinatal care centers. Thus, we suggest that we might use resources truly and drive interventions timely to prevent perinatal deaths in our country by using this system.

Authors' Contribution

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

Conflict of interest

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

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):898-901 Case Report

DOI: 10.18621/eurj.1013221 *Child Psychiatry*

A case of anorexia nervosa whose body image deteriorated after being weighed with classmates at school

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ABSTRACT

Anorexia nervosa is an eating disorder characterized by a heightened desire to have a lean body structure that leads to an extreme fear of becoming fat. The patients with this disorder have a severely impaired perception of their bodies. Negative behaviors and attitudes in peer communication may hugely impact the affected individual at every stage of this disorder. The level of peer communication quality could be shaped by the conditions prevalent in the school, particularly the approaches undertaken by the educators and the staff at school. However, it would be incorrect to state that wrong communication styles adopted by the friends of an individual or unsuitable conditions in the school environment alone would be sufficient for the development of anorexia nervosa in adolescents. A 16-year-old girl with anorexia nervosa was followed up for 1.5 years after the diagnosis of the disorder, a period during which she had begun exhibiting improvement in her condition. However, her symptoms reappeared after she underwent height and weight measurements at school during the physical education and sports class to determine her physical health. This case is presented (1) to remind that anthropometric measurements are personal data and (2) to emphasize that it would be appropriate to provide privacy in this respect at schools.

Keywords: Anorexia nervosa, eating disorders, adolescents, weigh at school

Adolescence is a period of human life that is characterized by rapid changes in the physical structure. These changes lead to the adolescents being highly focused on their bodies, with their weight, height, and general body ratio becoming important factors impacting their personal sense of competence. The changes occurring in their body structure alter theirself-perceived body image and raise concerns regardingtheir appearance [1]. It is, therefore, necessary for adolescents to adapt to these rapid and intense physical body changes and regulations. However, such adaptation is highly sensitive, with any external factor capable of disruptingthe harmony achieved and thereby causing eating disorders [2]. Moreover, the development of body perception is influenced by several factors other than physical development. Problems with one or more of these factors during the development of body image in the minds of adolescents leads to a sense of dissatisfaction with the body, whichmay culminate into eating disorders [3].

The difference between one's perceived image of the body and the body shape one wishes to have, or a

Received: ***October 22, 2021;*** *Accepted:* ***December 8, 2021;*** *Published Online:* ***August 4, 2022***

How to cite this article: *Kaymaz N, Uzun ME. A case of anorexia nervosa whose body image deteriorated after being weighed with classmates at school. Eur Res J 2022;8(6):898-901. DOI: 10.18621/eurj.1013221*

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e-ISSN: 2149-3189

negative presentation of this difference by different environmental factors may lead to eating disorders in the adolescent age group, which is already sensitive to the physical changes occurring in their bodies as body image problems are common in this age group [4-6].

In the present report, a case of a 16-year-old girl with anorexia nervosa is discussed. The girl was followed up for 1.5 years after the diagnosis of the disorder, during which her condition had begun improving. However, her symptoms reappeared after she underwent height and weight measurements along with other students in the physical education and sports class at school. Through this case, we would like to emphasize two issues. The first is that we should keep in mind that anthropometric measurements are personal data. Therefore, it is appropriate not to share it with anyone else without the person's permission. It is important to follow the health indexes in schools in the early diagnosis of some diseases as growth retardation, short stature, eating disorders etc as is known. However, care should be taken to make the measurements individually, not in groups, and to share them only to the individual and the caregivers. And the second thing that we want to discuss via this paper is that if to provide privacy of anthropometric measurements of students is not possible, such health status follow-up can be provided carried out by family physicians more frequently.

CASE PRESENTATION

A 16-year-old girl who was followed upfor 1.5 years after the diagnosis of anorexia nervosa demonstrated restricted food intake during the last two weeks of the follow-up period. Her refusal to eat food had led to a bodyweight loss of 2 kg as revealed in her last evaluation in this period. At that time, her daily calorie consumption, as stated by her,had been halved (400 kcal/d) in the last 5 days. She explained that this behavior developed after she underwent a weight measurement in her physical education and sports class along with her friends at school. She further stated that after the measurements, her friends compared their body weights, and a few of them decided to restrict their diet. Although at that time, it had been long since she had checked her weight, she stated that when she learned her weight during the measurements conducted at school and heard the conversation of her friends, her fear of gaining weight increased. As a consequence, while she stated that it had been long since she had calculated her daily calorie intake, after the measurements at school, she began the calculations again and decided to reduce her calorie consumption thereafter. It is noteworthy that while there was evident refusal to eat food, no binge eating/vomiting and/or laxative/purgative/diuretic usage was reported in her case. Moreover, at that point, she had not been using any medication for her illness for a long time.

According to the height and weight measurements recorded at school, she (who had a history of losing up to 28 kg at the beginning of the disorder) was underweight (weight 42 kg; height 155 cm; body mass index (BMI) = 17.5 kg/cm^2). Her vital signs and systemic examination were normal. She appeared fatigued, and her movements were slow. Her face had a calm and unhappy expression. Her thoughts included those of being overweight and that there would be no end to her weight gain. Her mother is a housewife, and her father is a long-distance driver. Although she stated that her relationship with her mother was normal, she also stated that her mother was controlling in her behavior. Moreover, she stated that she did not often meet her father due to his preoccupation with his job, although she again stated that there was no problem in her relationship with her father, in general, and that it was a normal father-child relationship. She also has a 23-year-old brother, with whom, as she stated, she did not share a normal brother-sister relationship as they arguedseveral times. She expressed that she felt no strong bonding among her family members. She also felt that none of her family members understood her. There was no history of sexual or physical abuse. Her success in school was satisfactory, and she aimed to become an architect. She was a hostel resident who, as stated by her, did not get along quite well with her peers in general, had no best friend or boyfriend, and no hobby that she enjoyed. She expressed having thoughts of harming herself sometimes, particularlyat times when she would be angry. The laboratory investigations conducted on admission revealed a normal complete blood count, electrolytes, sedimentation rate, liver and kidney function tests, and levels of amylase, cholesterol, and thyroid-stimulating hormone. The

case was subsequently referred to the child and adolescent psychiatry department. However, she refused drug treatment. Therefore, weekly individual supportive therapy and concurrent family counseling were recommended to her.

DISCUSSION

Adolescence is a period in human life in which body image problems are common. Adolescents are particularly sensitive to the physical changes occurring in their bodies [6]. This leads to the adolescents being highly sensitive to the opinions of others while they attempt to find their place in the world. Meanwhile, peer groups begin to gain huge importance during adolescence. Adolescents are affected greatly by the thoughts, behaviors, and attitudes of the individuals constituting their surrounding environment. Since studies have reported a significant association between self-perception of appearance and self-confidence [7], it would not be wrong to state that perception of physical appearance is a variable that could dampen the self-confidence of adolescents [8].

Anorexia nervosa is a disorder that commonly commences during adolescence and, therefore,requires particular attention when diagnosed in this age group [9]. The disorder is characterized by low body weight, excessive fear of gaining weight, persistent behaviors that prevent weight gain, and/or a distorted body image. It is a critical mental disorder that turns into a chronic condition and, as a consequence, significantly impacts the affected patients and their families [10].Among the premorbid characteristics prevalent in the affected individuals, perfectionism and obsessiveness are the most remarkable ones. The feeling of worthlessness appears to be the dominant one [11]. Although various treatment options are variable for this disorder, the mortality rate remains high to date [12].

The origin of the disorder might be in certain triggering factors,including criticism from other people, such as friends and family members, or hearing jokes related to weight or weight gain [13]. Effective preventive policies would positively support the school environment and enable countering the communication problems that might occur among peers. A positive school environment could be created by spreading awareness regarding the disorder among the staff members of all departments at different schools.

The idealized and standardized body perception prevalent in the current world has taken almost everyone under its influence and given rise to a certain level of mental pressure regarding maintaining such a body image. Weight loss, diet, sports, and aesthetic applications are marketed with almost no impact from any economic crisis. Internalizing the ideal body forms and attempting to achieve such aims causes individuals to develop unhealthy behaviors, dissatisfaction with their bodies, and reduced self-confidence [14].

CONCLUSION

It is common for schools in certain countries to conduct height and body weight measurements periodically for students to evaluate their physical health status and to subsequently provide timely interventions,including appropriate diet recommendations and exercise/sports suggestions wherever necessary. However, it is important to remember that anthropometric measurements belong to the category of personal data and, therefore, protecting the confidentiality of such data should be mandatory. The case discussed in the present study suggests that it might be more appropriate to conduct such measurements in community health centers rather than at school. Nevertheless, if such measurements are planned for schools, it must be ensured that the adolescents undergoing the measurements are not present in the same room with their adolescent friends during the measurements. It is recommended not to conduct such measurements with in the same classroom on the same day and rather schedule appointments, which would cause no inconvenience as these are individual assessments independent of group dynamics. These recommendations would prevent the possible events of peer bullying. These simple preventive approaches are important for preventing the distortion of body image and subsequent life-altering eating disorders in adolescents.

Authors' Contribution

Study Conception: NK; Study Design: NK; Supervision: MEU; Funding: N/A; Materials: N/A; Data Collection and/or Processing: NK; Statistical Analysis

and/or Data Interpretation: NK; Literature Review: MEU; Manuscript Preparation: NK and Critical Review: MEU.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2022;8(6):902-905 Case Report

DOI: 10.18621/eurj.1038522 General Surgery

A rare long-term complication in a patient with gastric bypass: remnant gastric perforation

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ABSTRACT

Roux-en-Y gastric bypass (RYGB), one of the most frequently performed operations within the scope of bariatric surgery, creates a remnant stomach containing the fundus, corpus and antrum where gastric acid and pepsinogen are synthesized in significant amounts. Although rare complications such as bleeding, perforation and ischemia occur regarding the remnant stomach. A 47-year-old male patient who was operated on for open RYGB 10 years ago was admitted to the emergency department with complaints of abdominal pain and deterioration in his general condition. The patient who had widespread tenderness in the abdomen was unstable on physical examination. It was observed that the remnant stomach was perforated 2.2 cm from the anterior surface of the corpus at emergency laparotomy after defining the upper gastrointestinal anatomy with the aid of peroperative endoscopy. The patient whose remnant stomach was resected was discharged uneventfully on the 5th postoperative day. The approach to remnant gastric perforations due to benign causes is the same as for gastric perforations. Laparotomy is indicated in unstable patients.

Keywords: Gastric bypass, perforation, complication, remnant stomach

Roux-en-Y gastric bypass (RYGB), one of the most frequently performed operations within the scope of bariatric surgery, creates a remnant stomach containing the fundus, corpus and antrum where gastric acid and pepsinogen are synthesized in significant amounts. Although rare, complications such as bleeding, perforation and ischemia occur regarding the remnant stomach [1]. In a study, it was stated that the incidence of non-functioning remnant gastric perforation was 0.25% [2].

Although the underlying mechanisms are not clearly revealed, *H. pylori*, NSAID use, smoking and alcohol are considered risk factors in remmant gastric perforations [3]. Diagnosis of remnant gastric perforations is difficult because there are no specific physical examination and radiological findings [1]. The surgical treatment of remnant gastric perforations varies in the literature. There are cases with primary closure, omentopexy or resection [1, 2].

In this article, we evaluated the management of remnant gastric perforation in a patient who underwent open gastric bypass surgery 10 years ago.

CASE PRESENTATION

A 45-year-old male patient with no history of smoking or NSAID use, diagnosed with iron deficiency anemia, underwent open RYGB surgery 10 years ago, and was negative for H.pylori in his gastroscopy performed in

Received: ***December 19, 2021;*** *Accepted:* ***June 8, 2022;*** *Published Online:* ***September 26, 2022***

How to cite this article: *Ay OF, Erdoğdu UE, Tezer H, Şen S. A rare long-term complication in a patient with gastric bypass: remnant gastric perforation. Eur Res J 2022;8(6):902-905. DOI: 10.18621/eurj.1038522*

e-ISSN: 2149-3189

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2017, applied to the emergency department with abdominal pain and general condition disorder for about two days. The patient had a history of intermittent abdominal pain for about three years before applying to the emergency department.

On physical examination, arterial tension was 100/70 mmHg, pulse: 110 beat/min, body mass index (BMI): 29 kg/m^2. The patient was tachypneic and agitated. There was widespread tenderness and distension on abdominal examination. Bowel sounds were decreased.

It was seen that white blood cells: 12000 [4-10, 103], hemoglobin: 9.4 [12-16] gr/dL, middle corpuscular column: 64 fL [80-120], C- reactive protein: 9 (0-3) mg/L in his laboratory tests. Millimetric air densities and free fluid were observed in the remnant stomach region on CT. (Fig. 1).

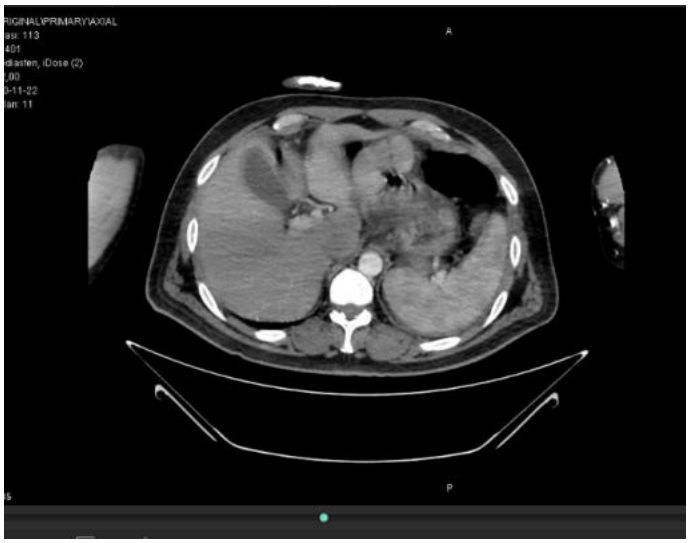

Fig. 1. CT image in emergency room application perigastric minimal air densities.

Emergency laparotomy was decided based on the patient's septic clinic and findings. After the anatomy was defined with the help of gastroscopy in the operation, and it was observed that the blood supply of the remnant stomach was impaired and there was a 2.2 cm perforation in the corpus region (Figs. 2 and 3).

The remnant stomach was resected divided from duodenum with stapler because of considering that primary repair would not be appropriate due to intra-abdominal contamination. Oral intake was started on the second postoperative day, and the patient was discharged on the 5th postoperative day uneventfully. No malignancy was detected in the pathological examination.

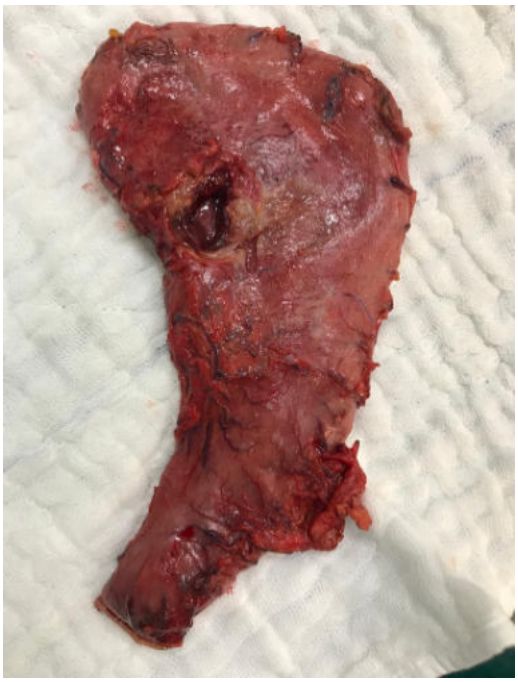

Fig. 2. Image after resection perforated 2.2 cm area on the anterior surface of the corpus.

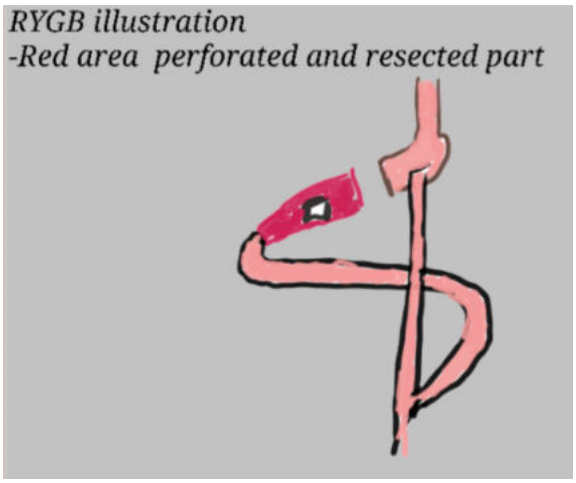

Fig. 3. IOperation illustration.

DISCUSSION

Although the underlying mechanisms are not clearly revealed *H. pylori*, NSAID use, smoking and alcohol are considered risk factors in remmant gastric perforations. In a study comparing the presence of *H. pylori* in the remnant and functional stomach; it was reported

that all cases with *H. pylori* positive in the remnant stomach were also positive in the functional stomach. Recent studies on remnant mucosal histology have reported that chronic gastritis, pangastritis, atrophy, and intestinal metaplasia are seen [3]. Biopsy taken from the functional stomach part of the patient in 2017 and pathological examination was negative for H. pylori and pathological examination revealed inflammatory granulation tissue.

In a study, it was stated that the incidence of nonfunctioning remnant gastric perforation was 0.25% [2]. Iranmanesh *et al*. [1] state that acute remnant gastric perforations occur approximately two years after RYGB [1]. Remnant gastric perforation developed in our patient approximately one years after the RYGB operation.

Diagnosis of acute remnant gastric complications is difficult because there are no specific clinical and radiological findings [1]. Since the remnant stomach does not contain air, air densities may not be seen in the tomography of remnant gastric perforations [4].

Primary repair and omentopexy is the first recommended treatment in the surgical treatment of remnant gastric perforation since the remnant stomach is a low-pressure region. However, remnant gastrectomy can be performed in cases of bleeding, necrosis, gastrogastric fistula. Some authors even recommend remnant gastrectomy in the presence of unexplained abdominal pain in RYGB operated patients after certain diagnoses such as cholelithiasis and internal herniation have been excluded [1]. In addition, performing total gastrectomy instead of partial in perforated remnant stomachs provides definitive treatment as it will prevent peptic ulcer disease that may develop due to the residual antral mucosa [2]. In our case, remnant gastrectomy was performed because signs of necrosis were observed during the operation. However, the patient also had a history of intermittent abdominal pain for about three years.

Delayed gastric necrosis and perforation cases have poor outcomes and the mortality rate is 50-80% [4]. Since RYGB is the most frequently performed operation for bariatric surgeons worldwide, general surgeons should be alert to complications [5]. Surgeons managing bariatric surgery patients should have remnant gastric pathologies in their differential diagnosis [1].

CONCLUSION

RYGB is one of the most common operations performed by bariatric surgeons. General surgeons should be alert for possible complications associated with these procedures. The approach to remnant gastric perforations due to benign causes is the same as for gastric perforations.

Authors' Contribution

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

Informed consent

Written informed consent was obtained from the patient for publication of this case and any accompanying imagest.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgment

We would like to thank Deniz Y Fırat, MD for the visuals and mentorship of the operation.

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The European Research Journal 2022;8(6):906-908 Case Report

DOI: 10.18621/eurj.1049854 *Pediatric Neurology*

Generalized tetanus in an eight-year-old girl: a case report

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ABSTRACT

Tetanus, a vaccine-preventable disease threatens life. Tetanus has four clinical presentations: neonatal, localized, cephalic, and generalized. Generalized tetanus was the most common presenting feature on admission to the hospital. We report a case with generalized tetanus with difficulty swallowing and sore throat by admission. She was eight years old. She had symptoms on the fifth day of nail soak. She rapidly developed an opisthotonic posture on the first day of admission. She was discharged on foot on the seventieth day. Generalized tetanus should be kept in mind that a patient may be admitted to a hospital with difficulty swallowing and a sore throat.

Keywords: Generalized tetanus, childhood, tetanus toxoid, muscle spasm

Tetanus is a vaccine-preventable disease which is caused by *Clostridium tetani*. It often causes disease through infected wounds [1]. The exotoxin tetanospasmin blocks the presynaptic release of neurotransmitters, causing uncontrolled muscle contraction and clinical spasms [2]. Tetanus is not transmitted from person to person. Prevention of tetanus in public is by vaccination after injury [3].

Tetanus has four clinical presentations: neonatal, localized, cephalic, and generalized. Generalized tetanus constitutes 94-95% of cases admitted to the hospital after the neonatal period [4].

We present a case of generalized tetanus who presented with sore throat and difficulty in swallowing and was discharged after recovery.

CASE PRESENTATION

An eight-year-old Syrian girl with a painful throat, difficulty swallowing, and tongue motions, as well as neck pain, fever, and shivering, was admitted to the hospital. She had the symptoms for three days.

Eight days ago, a nail became lodged in her right foot. She had never been immunized against tetanus before. She couldn't open her mouth during the physical checkup. She had grimacing on her face. She couldn't show her teeth, couldn't sit without assistance, and was bending backwards. She was suffering from diaphoresis. She had a Dakar score of 3. The findings of the initial laboratory tests were normal; *C. tetani* (Tetanus) IgG was 0.05 IU/mL (0.01-0.15 IU/mL). No organism was found in blood or sputum cultures.

The wound was cleaned. The treatment was promptly supplemented with aqueous penicillin (400.000 units/kg/day, 4 divided doses) and metronidazole (30 mg/kg/day, 3 divided doses). For muscle stiffness and tachycardia, midazolam infusion (0.1 mg/kg/h-0.4 mg/kg/h) and tizanidine (2 mg/day, 3 split doses) were added. A dose of 500 IU of human tetanus

Received: ***December 28, 2021;*** *Accepted:* ***February 15, 2022;*** *Published Online:* ***September 28, 2022***

e-ISSN: 2149-3189

How to cite this article: *Yimenicioğlu S, Turhan S, Sağlam C, Bildirici Y, Akdemir B. Generalized tetanus in an eight-year-old girl: a case report. Eur Res J 2022;8(6):906-908. DOI: 10.18621/eurj.1049854*

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immunoglobulin was given. She developed opisthotonus after a few hours (Fig. 1). She was intubated in the twelfth hour of her admission. Rocuronium bromide was used to provide skeletal muscular relaxation (starting at 7 micrograms/kg/min and increasing to 12 micrograms/kg/min). Phentanyl was utilized for pain. An extra dose of 1000 IU antitoxin was given because of spasms in the neck and abdominal muscles. The infusion of esmolol (200 micrograms/kg/min) had begun. When necessary, amiodarone was used to treat tachycardia. With loud stimuli or external movement, she had myoclonus in her arms, which moved towards her legs. Myoclonus was treated with a single dosage of rocuronium administered intravenously. Because the tachycardia persisted, adenosine and lidocaine were administered. A single dose of captopril was given because hypertension had developed. Penicillin and metronidazole were withdrawn on the 14th day. The patient could not be removed from the ventilator. A tracheostomy was put in place. She had pulmonary edema and cardiac arrest on the sixteenth day of her admission. For the persisting myoclonus in the arms, legs, and around the mouth, gabapentin and levetiracetam were added. With a decrease in rocuronium infusion, she was able to open her eyes when she heard her name called on the twentieth day of admission. Trismus had returned, and she was still experiencing autonomic symptoms (diaphoresis, tachycardia, and hypertension). The tracheostomy was removed on the 45th day of admission. Her legs hurt as she moved them. She exhibited gripping incompetence in her right hand, and her muscle strength in the right leg was 3/5, while it was 4-5/5 in the left leg.

On the 50th day of hospitalization, a brain MRI indicated hyperintensities in the periventricular deep white matter, the centrum semiovale, and the subcortical white matter. The diffusion MRI and MRI venography of the brain were both normal. She was able to sit without assistance on the 70th day, when she was discharged, although she still needed help. She had right hemiparesis.

DISCUSSION

Tetanus is a vaccine-preventable infectious disease. In 2015, 79% of deaths due to tetanus were seen in developing countries like South Asia and sub-Saharan Africa [4].

Because some developing countries' reporting systems are inefficient, the incidence may be higher than estimated [4]. The classic form of tetanus is generalized tetanus, which accounts for more than 80% of cases. It takes 3 to 21 days for symptoms to appear after infection, with symptoms often intensifying over a week [1]. Our patient had symptoms on the fifth day of nail wound.

Toxin accumulation in the central nervous system causes autonomic dysfunction [5]. Our patient had shivering, diaphoresis, and tachycardia. Diaphoresis, increase in temperature, arrhythmias, and changing blood pressure are all autonomic nervous system signs of generalized tetanus [1]. Reflex spasms, risus sardonicus (continuous facial contraction), opisthotonos (backward arching of the head, neck, and spine due to strong muscular spasms), and generalized seizure-like activity have all been reported [1, 5]. All of these symptoms were present in our case. The Dakar scoring system is a prognostic scoring system that takes into account the incubation period and the period of onset as well as neurological and cardiac manifestations [6]. Our patient had a Dakar score of 3. Farrar et al. [7] reported a mortality rate of 59% with a Dakar score of ≥ 3 and a mortality rate of 14% with a Dakar score of < 3. Tetanus is a life-threatening illness. Wound care, management of muscle spasm and consequences, res-

Fig. 1. Wound under right foot and opisthotonic posture of the patient.

piratory support, metabolic state balancing, and avoidance of the toxin's ongoing spread are all part of the treatment [4]. Tetanus cannot be diagnosed with a specific test [5]. In the general population, protective antibody levels range from 0.01 to 0.15 IU/mL. The majority of vaccinated people should have protective antibody levels of > 0.15 IU/mL [5]. If serum antibody titers are greater than 0.1 IU/mL, a diagnosis of tetanus should be considered doubtful [4, 5]. Our patient's Tetanus IgG was 0.05 IU/mL. She was not vaccinated before. The patient must stay in a calm place. The number of manipulations and procedures performed on patients must be kept to a bare minimum. Patients may have myoclonus. Myoclonus is a condition that begins in the upper extremities and extends lower. Symptoms may appear sooner or later depending on the distance between the damage site and the central nervous system, with an incubation period of 3-21 days [5].

Tetanic seizures are a sign of a worsening prognosis. The patient is in excruciating pain and does not lose consciousness throughout this seizure [5]. Our patient suffered tetanic seizures as well, but the prognosis was not as bad as it may have been. On house visits after discharge, she was vaccinated.

CONCLUSION

Tetanus can be prevented with vaccination. It should be kept in mind that patients with tetanus may present with difficulty swallowing and a sore throat.

Informed consent

Written informed consent was obtained from the patient's family for publication of this case and any accompanying imagest.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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