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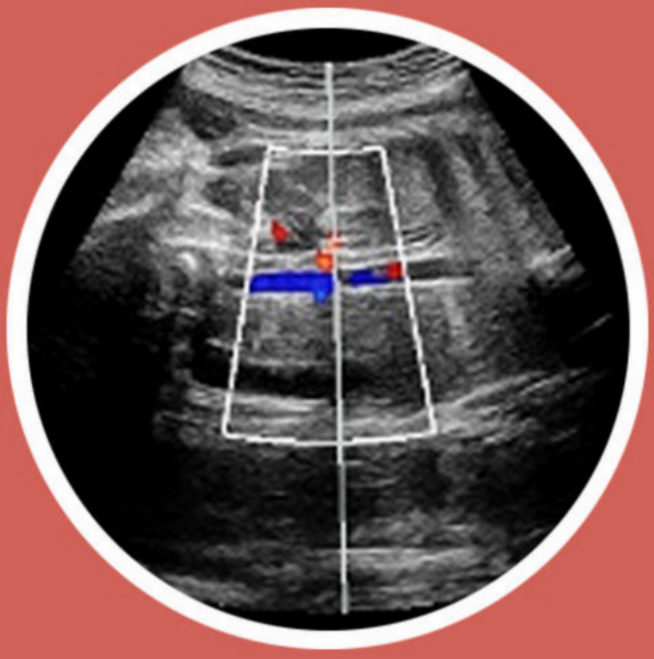

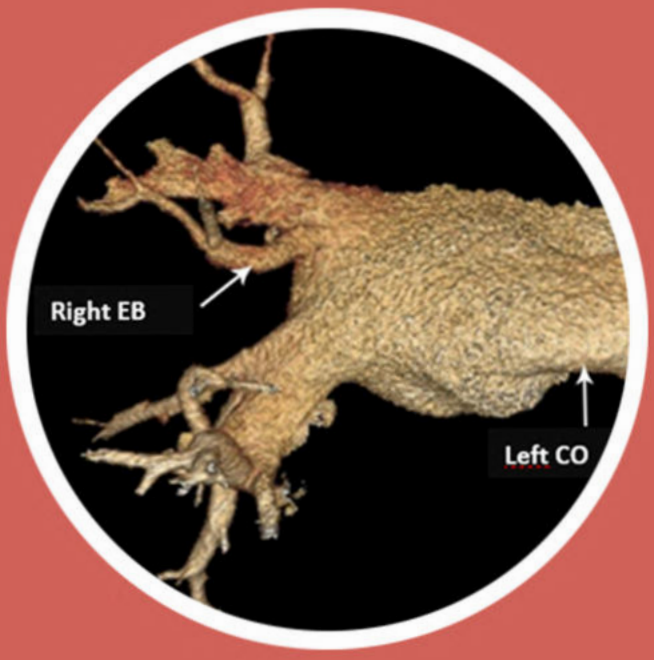

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The European Research Journal 2022;8(5):554-559 Original Article

DOI: 10.18621/eurj.866522 *Obstetrics and Gynaecology*

Investigating the quality-of-life scale in patients with placental adhesion disorder undergoing a cesarean hysterectomy and partial uterine resection

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ABSTRACT

Objectives: This study aimed to investigate the postoperative quality-of-life of patients with placental adhesion disorder undergoing a cesarean hysterectomy and partial uterine resection.

Methods: This was a prospective study performed on 40 patients aged from 25 to 35 years. The subjects were divided into two groups based on whether the uterus was preserved or not following a cesarean hysterectomy and on partial uterine resection. The Turkish version of SF-36 Quality-of-life Scale consisting of 36 items and 8 subscales was applied to the patients.

Results: The participants had a mean age of 31.3 ± 3.2 years. The mean parity, number of cesarean sections and body mass index was 3.45, 3.22 and 29.1 ± 2.9, respectively. The mean physical function score was 83.4 ± 11.61 in the cesarean hysterectomy group and 93.67 ± 8.55 in the partial uterine resection group. A significant difference was found between the cesarean hysterectomy and partial uterine resection groups in terms of their health-related quality-of-life ($p = 0.005$).

Conclusions: Patients who underwent partial uterine resection had improved health-related quality-of-life compared to patients who had a cesarean hysterectomy. Choosing uterus-preserving surgery in suitable patients by evaluating the degree of placental adhesion and adhesion size may have a positive effect on the quality-of-life after surgery.

Keywords: cesarean hysterectomy, partial uterine resection, placenta accreta, placental adhesion disorder, quality of life

Placental adhesive disorder (PAD) is a serious complication of pregnancy occurring when the chorionic villi invades the myometrium [1]. Patients who are exposed to PAD have a scarred uterus. The two major risk factors of PAD are placenta praevia and a prior cesarean section, as well as curettage and previous uterine rupture, uterine artery embolization and conservative myomectomy [2]. The changing risk factors, such as the growing rate of cesarean delivery, have increased the rate of placenta accreta in the past forty years [3]. Depending on the extent of uterine invasion by trophoblastic tissue, PAD is divided into placenta increta, accreta and percreta lesions [4]. Placenta accreta can occur in cases of implantation of the chori-

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onic villi on the myometrium without involving the decidua. The placental villous tissues cause increta to invade the myometrium while the uterus has a percreta serosal layer in the adjacent organs caused by the chorionic villi [5]. The incidence of PAD has increased tenfold during the past fifty years despite its being regarded as a rare condition [6]. PAD is related to a high risk of emergency hysterectomy, massive post-partum hemorrhage (PPH), multiple blood transfusions, and maternal mortality and morbidity [7]. In the UK, this condition is the second most common cause of hemorrhage that results in peripartum hysterectomy [8].

PAD has traditionally been managed with different techniques such as a hysterectomy and a cesarean section causing hemorrhage and morbidity. Other therapeutic options, such as cesarean section avoiding the removal of the placenta combined with compression sutures, methotrexate, balloon tamponade and B-Lynch suture where the placenta remains in situ, have all been proposed in the past few decades [9, 10]. One of the other conservative treatment strategies is reconstruction of the uterine wall and resection of the retained adherent placenta [11-13]. Moreover, the emphasis is more on improving the quality-of-life and the tendency to preserve the uterus. Therefore, in this study, the appropriateness of more conservative management of PAD surgery is discussed [14].

This study aimed to investigate the quality-of-life of PAD patients who had partial uterine resection and those who underwent cesarean hysterectomy to see if the partial uterine resection method positively affected the postoperative quality-of-life compared to cesarean hysterectomy.

METHODS

This study was a prospective one conducted in a tertiary hospital between January 2018 and December 2019 (Approval date:13.09.2017/Decision No:113). Postoperative Quality-of-life Scales were conducted and compared between patients who underwent cesarean hysterectomy or partial uterine excision due to PAD in our tertiary hospital between January 2013 and December 2018. The Quality-of-life Scale questionnaire was given to patients referred to the hospital for treatment at least 6 months after surgery and accepted to be included in the study. Those with psychiatric disorders, such as depression, anxiety disorder, chronic disease and multiple pregnancies were excluded from the study. Forty-six patients were contacted through electronic records. Six patients could not participate in the study for different reasons (living in a different city, diagnosis of depression, etc.). Therefore, forty patients aged from 25 to 35 years were included in the study. Written consents of the patients was obtained. The patients were divided into two groups depending on the operative methods: cesarean hysterectomy or partial uterine resection+bilateral tubal ligation. The SF-36 Quality-of-life Scale was developed for the first time by Stewart *et al*. [15]. Its Turkish validity was studied by Koçyiğit *et al*. [16]. The Turkish version of the SF-36 Quality-of-life Scale, consisting of 36 items, 8 subscales including physical role performance, general health, social function, energy, emotional role performance, physical function, pain and mental health, was applied to the patients. Scores related to the subscales vary from 0 to 100, in which higher scores indicate a better level while lower scores indicate deteriorating health.

Statistical Analysis

The SPSS 18.0 statistical software package was used for data analysis. Continuous variables with normal data distribution were determined using mean ± standard deviation, while variables with non-normal distribution were determined using median and lowest–highest values and categorical variables were determined with numbers and percentages. In univariate comparisons between the groups, t-test and variance analysis were applied to the groups independently of parametric tests based on the distribution of continuous variables, and the non-parametric Kruskal-Wallis test and the Mann-Whitney U-test were used. Categorical variables were compared using the Chi-square test. $P < 0.05$ was considered to be significant.

RESULTS

Table 1 shows the demographic variables of the groups. No statistically significant difference was found between the groups in terms of gravidity, age, BMI, parity and number of cesarean sections (see Table 1). Based on the results obtained, no statistically significant difference was found between the groups

Table 1. Descriptive statistics of demographic variables between groups

	Cesarean hysterectomy (n = 25)			Partial uterine resection (n = 15)			*p value*
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	
Age (years)	26	38	31.76 ± 3.20	25	36	30.73 ± 3.12	0.326
BMI (kg/m^2)	25	39.2	29.11 ± 3.30	25	33.6	29.08 ± 2.36	0.975
Gravidity	3	7	5.2 ± 1.04	3	7	4.7 ± 1.16	0.166
Parity	2	5	3.48 ± 0.96	2	5	3.40 ± 0.73	0.782
Number of living children	2	5	3.44 ± 1.00	2	5	3.40 ± 0.73	0.893
Abortion	0	3	0.72 ± 0.79	0	2	0.4 ± 0.63	0.190
Number of cesarean section	2	5	3.2 ± 0.76	2	5	3.26 ± 0.79	0.813

Min = minimum, Max = maximum, SD = standard deviation, BMI = Body Mass Index, $p < 0.05$ statistically significant.

in terms of systemic disease, incision type and pathology results (see Table 2).

The research variables were studied according to physical function, physical problems due to restriction, physical pain scale, general health perception, Energy/Fatigue Scale, social function, emotional problems due to restriction and emotional well-being between the groups. The t-test of two independent populations was used to investigate the difference in these variables between the groups. The results are shown in Table 3.

The results obtained showed that the mean physical function was statistically higher in the partial uterine resection group compared to the cesarean hysterectomy group ($p = 0.005$). The groups showed no statistically significant difference in other subscales ($p > 0.05$) (Table 3).

DISCUSSION

This study showed a significant difference between the two cesarean hysterectomy and partial uterine resection groups. The mean physical function in

Table 2. The number and percent of the respondents stratified by systemic disease and incision type

	Cesarean hysterectomy (n = 25)		Partial uterine resection (n = 15)		*p value*
	Frequency	%	Frequency	%	
Systemic disease					0.707
0	24	96.0	14	93.3	
1	1	4.0	1	6.66	
Total	25	100	15	100	
Incision type					0.819
Midline	21	84.0	13	86.66	
Phannenstiel	4	16.0	2	13.3	
Total	25	100	15		
Pathology results					0.078
Accreta	16	64.0	14	93.3	
Increta	7	28.0	1	6.66	
Percreta	2	8.0	0	0	

Table 3. Descriptive statistics of the research variables and - test for Equality of Means in two test groups

	Group	n	Mean	SD	Std. Error Mean	*p* value
Physical function	Cesarean hysterectomy	25	83.40	11.61	2.32	0.005*
	Partial uterine resection	15	93.67	8.55	2.21	
Physical problems due to restriction	Cesarean hysterectomy	25	72.00	37.72	7.54	0.694
	Partial uterine resection	15	76.67	33.36	8.61	
Physical pain scale	Cesarean hysterectomy	25	69.30	20.74	4.15	0.282
	Partial uterine resection	15	76.33	17.90	4.62	
General health perception	Cesarean hysterectomy	25	66.20	20.52	4.10	0.906
	Partial uterine resection	15	65.33	24.89	6.42	
Energy/Fatigue scale	Cesarean hysterectomy	25	53.40	19.45	3.89	0.302
	Partial uterine resection	15	60.33	21.67	5.59	
Social function	Cesarean hysterectomy	25	79.00	20.64	4.12	0.383
	Partial uterine resection	15	84.16	12.01	3.10	
Emotional problems due to restriction	Cesarean hysterectomy	25	76.00	32.66	6.53	0.702
	Partial uterine resection	15	80.00	30.34	7.83	
Emotional well-being	Cesarean hysterectomy	25	62.08	17.29	3.46	0.281
	Partial uterine resection	15	68.13	16.36	4.22	

SD = standard deviation, *$p < 0.05$ statistically significant.

the partial uterine resection group was statistically higher than that in the cesarean hysterectomy group.

PAD is a life-threatening condition that occurs when the placental villi is abnormally adherent and results in a myometrium defect or an absence of myometrial tissue [17]. In our previous case series where the surgical technique and results of partial uterine resection were evaluated retrospectively, it was concluded that the local resection technique was an effective and safe method for treating anterior PAD [13]. Moreover, Acar *et al*. [18] showed that partial uterine resection was an alternative, acceptable and conservative management technique in the case of PAD. Also, Karaman *et al*. [12] found that an effective, fertility-preserving and safe approach was the local resection of the percreta site.

In our current study comparing the postoperative quality-of-life of patients with partial uterine resection and cesarean hysterectomy, it can be said that partial uterine resection positively affected the quality-of-life. This may be answered simply by sparing the patient uterine surgery, especially in a population where the uterus is one of the important organs for life and continuity of reproduction. On the other hand, the partial resection method may cause abnormal uterine bleeding or infection due to longer operating times and more sutures on the uterus.

While we expected a positive effect in patients spared uterine surgery, we wanted to consider and evaluate the real quality-of-life based upon fit and tested scales. In the quality-of-life scoring, the difference in physical function scores was significant between the two groups. The statistically significantly higher physical function score in the group spared uterine surgery suggested that organ-preserving surgery may have had a positive effect on the physical function scores of the patients where the problem was unknown. Shabana *et al*. [19], who reported a modified surgical approach, i.e. stepwise cesarean section, emphasized that conservative surgery may be a modern medical approach option. Our findings are in line with the opinion by Matsubara *et al*. [20] stating that retention of the uterus may improve women's quality-of-life and preserve their sexual identity, irrespective

of fertility.

Su *et al*. [21] suggested the use of primary cesarean hysterectomy as the treatment of abnormal invasive placenta, but conservative treatment may be used in women with a strong desire for fertility. This is in line with results of the study by Cui *et al*. [22] who concluded that the implanted placenta remaining in situ should preferably be chosen for PAD patients with a desire for fertility and those undergoing termination of pregnancy in the second trimester. Tong et al. [23] concluded that it was feasible to have placental retention and uterine conservation in carefully selected cases, which can avoid the complications of a cesarean hysterectomy.

There is a consensus among experts on a planned preterm cesarean hysterectomy as the approach recommended before the 35th gestational week [24, 25]. On the other hand, several studies have discussed the conservative method in different case series and case reports, claiming that conservative management that leaves the placenta in situ causes harmful effects. A review of all the studies reported between January 1985 and May 2006 by Timmermans *et al*. [26] showed that 80% of the 60 cases could preserve the uteri. Kuppermann *et al*. [27] compared health-related quality-of-life scores for patients after hysterectomy and uterus-preserving surgery (for benign gynecologic surgery). They found more dramatic improvements in hysterectomy and concluded that women undergoing hysterectomy had a 6-month delay in improvement compared to the women undergoing uterus-preserving surgery who tended to show immediate improvement. However, the patients in this study consisted of benign conditions, not PAD or obstetrical reasons. Matsuzaki *et al*. [28] found that conservative management had serious complications leading to unplanned hysterectomies, were there was a reported success rate of 61.8% for those undergoing the conservative technique, which is not in line with our study that resulted in increased physical function with the conservative technique.

Limitations

Our study had some limitations. Firstly, the posterior placenta was not evaluated due to the technical limitations of the partial resection surgery method. Secondly, we could not evaluate the preoperative quality-of-life scales of the patients. As far as we know, this study is the first one to evaluate the quality-of-life functions after cesarean hysterectomy and partial resection surgery due to placental adhesion disorders.

CONCLUSION

It was concluded that the partial uterine resection group had a higher health-related quality-of-life than the cesarean hysterectomy group. Studies are needed with large patient groups in which patients with placental adhesive disorder would be appropriate for uterus-preserving surgery, the postoperative quality-of-life scales and even evaluating the sexual functions of patients selected for uterus-preserving surgery.

Authors' Contribution

Study Conception: NTO, GU, TG; Study Design: NTO, HN, RN; Supervision: NTO, GU, TG; Funding: N/A; Materials: NTO, GU, HN, RN; Data Collection and/or Processing: NTO, GU, TG, HN, RN; Statistical Analysis and/or Data Interpretation: NTO, GU, HN, RN; Literature Review: NTO, GU, TG; Manuscript Preparation: NTO, TG, HN and Critical Review: NTO, GU, RN.

Conflict of interest

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

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The European Research Journal 2022;8(5):560-566 Original Article

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Is there a difference between tranexamic acid application routes in hip hemiarthroplasty?

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ABSTRACT

Objectives: This study aimed to define the optimal efficacy route of tranexamic acid treatment given during hemiarthroplasty after femoral neck fracture.

Methods: This study examined the files of patients with hip fractures over 65 years of age and treated surgically in our clinic between 2017 and 2019. Patients included in these files were grouped as non-tranexamic acid and topical and systemic tranexamic acid. Then, the demographic information, height and weight of the patient files, haemoglobin and hematocrit levels before and after the surgery, bleeding profiles, tranexamic acid dose and the route of administration, complications in postoperative follow-up, the amount of fluid coming from the drain and duration of drainage, postoperative intensive care follow-up duration of hospitalisation was investigated.

Results: A total of 100 patients, 50 of whom were in the control group, 25 of whom were treated with topical tranexamic acid, and 25 of whom were treated with intravenous tranexamic acid, were included in this study. Postoperative blood transfusion was applied to 60% (n = 30) of the control group, 20% (n = 5) of the topical group, and 24% (n = 6) of the intravenous group. When compared statistically, it was found that topical and intravenous groups were lower than the control group ($p = 0.001$ and $p = 0.002$, respectively), but there was no significant difference between them ($p = 0.759$). When the blood loss calculations made by the Gross method were examined, the average of the control group was 1011.5 ml (179-1837 ml), the topical group was 695.7 ml (11-2503 ml), and the intravenous group was 710.9 ml (173-11315 ml) calculated as. When analysed statistically in terms of blood loss, it was found that the control group was significantly higher than the topical and intravenous groups, but there was no significant difference between the topical and intravenous groups ($p = 0.002$).

Conclusions: Tranexamic acid applied to reduce blood loss during arthroplasty surgery can be used effectively either by topical or systemic methods.

Keywords: Femoral neck fracture, hip hemiarthroplasty, tranexamic acid

The number of hip fractures due to osteoporosis increases in the ageing world population [1]. A femoral neck fracture is a common type of hip fracture in ageing populations and is most effectively managed with hemiarthroplasty, which is reported to have relatively good outcomes [2, 3].

One of the complications of hip hemiarthroplasty in the perioperative period is bleeding [4]. Especially

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bleeding complications may cause additional problems as the patient affects early mobilisation. Due to blood loss, the postoperative blood transfusion rate is estimated to be about 18% for total hip arthroplasty (THA). Intra-operative blood loss from THA can range from 700 to 900 ml [5, 6]. In recent years, many methods have been tried to prevent this loss of blood in arthroplasty surgeries and reduce the number of allogeneic blood transfusions [7-9]. Tranexamic acid (TA), an antifibrinolytic agent, is one of these methods. TA is a small molecule that inhibits plasminogen activation and plasmin activity. In recent years, there have been publications on the use of TA during the surgical treatment of hip fractures. However, these are studies with different treatment methods or in which only TA is applied in one way [10-15]. A study comparing hemiarthroplasty with local and systemic applications with the diagnosis of femoral neck fracture could not be seen in the literature. We hypothesise that TA reduces blood loss in hip hemiarthroplasty regardless of the route of administration. This study aimed to compare TA's topical and systemic application with each other and with the control group in terms of blood loss.

METHODS

The study was approved by the local institutional review board (date – number: 18/12/2018 - 1069) and performed under the ethical standards laid down in the Declaration of Helsinki. All patients provided written informed consent before their inclusion in the study. In the study, the files of patients treated with a diagnosis of femoral neck fracture between 2017 and 2019 were retrospectively analysed. Patients over 65 years of age who underwent cemented hemiarthroplasty with the diagnosis of displaced femoral neck fracture were included in the study. From the patient files examined; patients with a pathological femoral neck fracture, history of anticoagulant and antiaggregant treatment, history of a bleeding disorder, history of ischemic heart disease, history of cerebrovascular disease, stage IV and V Chronic renal failure, non-surgical treatment, cases undergoing osteosynthesis, under 65 years of age were excluded. In the analysis based on inclusion and exclusion criteria, the data of 25 patients who were administered topical and systemic TA were obtained. In order to compare the treatment groups, the data of 50 patients with the same characteristics, who were not administered TA by any means, were examined.

All patients underwent hip hemiarthroplasty with a posterolateral approach under spinal anaesthesia in the lateral decubitus position. Antibiotic prophylaxis (2 g cefazolin) was administered to all patients an hour before the incision. A cemented femoral stem (TST®) was performed by three senior surgeons for all patients included in the study. If tranexamic acid was administered intravenously to the patient, it was administered in 100 ml saline at a 10 mg/kg dose 10 minutes before the start of surgery. Topical TA was applied to the group after capsule repair, followed by fascia repair and 2 g/50 ml TA under the fascia. All patients underwent wound drain located under the fascia for postoperative follow-up. The drain was activated 1 hour after surgery. Complete blood count and biochemical values were checked daily at postoperative 2nd hour, 6th hour and postoperative hospital stay. Allogeneic blood transfusion was performed in patients with control haemoglobin values < 8 g/dl or who developed signs of anaemia (chest pain, tachycardia and orthostatic hypotension not responding to fluid resuscitation). It was removed if the drain was below 50 cc on the first postoperative day. On the second postoperative day, the patient was mobilised under the supervision of a physiotherapist.

The Gross method was used to measure the amount of blood loss [16]. Nadler Formula was used to calculate the blood volume required for the method [17]. This formula;

Blood volume (l) = height (m) 3 x k1 + body weight (kg) x k2 + k3

The fixed values in the formula are for female gender; k1: 0.356, k2: 0.033, k3: 0.183 for the male gender as k1: 0.367, k2: 0.032, k3: 0.604 were taken as. After calculating the blood volumes of all patients, preoperative and postoperative blood losses were calculated.

The formula of the Gross method is

$V_{\text{total blood loss}}$ (ml) = Blood volume x ($Hct_{\text{preop}} - Hct_{\text{postop}}$) / Hct_{average}

Patients included in the study; demographic characteristics (age, sex, height, weight, laterality), hospitalisation and intensive care unit stay, usage and doses of tranexamic acid during surgical treatment, haemo-

globin, hematocrit, INR, PT, aPTT values, amount of fluid from surgical drains, allogeneic blood transfusion rates and amounts in postoperative follow-up, and postoperative deep vein thrombosis and periprosthetic infection complications were evaluated.

Statistical Analysis

SPSS (Statistical Package for the Social Sciences) 25.0 package program and Microsoft Excel 2016 programs were used for statistical analysis. Assuming an α error of 0.05 and 1 minus β of 0.80 when calculating power, we assumed that the mean change in haemoglobin level in patients undergoing hip fracture surgery would be 4 g/dl (standard deviation [SD] 1 g/dl). Assuming a 20% reduction in blood loss would be clinically significant (i.e. 8 g/dl), 25 patients in each group would be required to detect a change. Shapiro-Wilk test was used to determine whether or not it was in a normal distribution. These findings were compared between the independent groups using student t-test or ANOVA. The Chi-square test was used for categorical variables between independent groups. The significance limit for all statistical tests was $p = 0.05$. Results will be analysed based on a 95% confidence interval.

RESULTS

A total of 100 patients were included in this study, including 25 patients receiving topical tranexamic acid, 25 patients receiving intravenous administration, and

Table 1. Demographic data of patients

	Control	Topical	Intravenous	*p* value
Gender, n				0.079*
Female	27	20	17	
Male	23	5	8	
Age (year)	80.1 ± 8.4	79.4 ± 8.5	78.7 ± 9.6	0.807**
Laterality				0.503*
Right	19	8	13	
Left	31	17	12	
Hospitalization time (day)	11.5 ± 5.1	10 ± 3.4	9.6 ± 3.5	0.167**
Height (m)	1.64 ± 0.08	1.63 ± 0.07	1.62 ± 0.06	0.540**
Weight (kg)	68.5 ± 11.5	64.9 ± 9.9	64.9 ± 12.8	0.309**
Surgery time (min)	85.2±15.1	87.4±13.2	86.7±14.3	0.845**
Preoperative Hgb (g/dL)	11.5 ± 1.2	11.3 ± 1.2	11.8 ± 0.9	0.267**
Preoperative Hct (%)	35.8 ± 3.3	34.2 ± 3.2	35.2 ± 2.7	0.130**
Preoperative PT (sec)	12.2 ± 1.1	11.8 ± 0.8	11.7 ± 1.4	0.152**
Preoperative aPTT (sec)	27.2 ± 3.2	26.6 ± 3.9	26.7 ± 2.7	0.731**
Preoperative INR	1.05 ± 0.08	1.06 ± 0.09	1.06 ± 0.1	0.813**
Postoperative (2nd day) Hgb (g/dL)	9.6 ± 1.3	9.8 ± 1.3	9.9 ± 1.2	0.570**
Postoperative (2nd day) Hct	29.2 ± 3.9	29.7 ± 3.6	29.8 ± 3.7	0.771**
Postoperative (2nd day) PT (sec)	13.7 ± 1.6	13.4 ± 1.3	13.1 ± 1.6	0.310**
Postoperative (2nd day) aPTT (sec)	29.8 ± 4.5	28.5 ± 3.9	28.7 ± 2.0	0.331**
Postoperative (2nd day) INR	1.19 ± 0.15	1.16 ± 0.13	1.16 ± 0.13	0.680**

Hgb = hemoglobin, Hct = hematocrit, aPTT = activated partial thromboplastin time, PT = prothrombin time, INR = international normalized ratio
* Kruskal Wallis test
** Oneway ANOVA test

50 patients who had never received topical tranexamic acid. 64 (64%) of the patients were female, 36 (36%) were male, and the mean age was 79.6. Trauma causing fracture was found to be a simple fall in all patients. Forty (40%) of the fractures are right, and 60 (60%) are left femoral neck fractures. The distribution of some sociodemographic and fracture characteristics of the patients is shown in Table 1. As indicated in the table, no statistically significant difference was found between the patients divided into three groups in terms of gender, age, laterality, hospitalisation time, height and body weight. Blood samples taken before and after surgical treatment were examined in all patients. No significant difference was observed among the groups (Table 1).

Wound drainage was applied to all patients after routine surgical treatment. The drain was recorded as a mean of 246.5 ml (50-650 ml) in the control group, 124 ml (5-250 ml) in the topical group and 101.4 ml (20-250 ml) in the intravenous group. When compared statistically, it was found that topical and intravenous groups were lower than the control group ($p < 0.001$), but there was no significant difference between them ($p = 0.487$). Allogeneic blood transfusion was applied to the patients who developed anaemia during clinical follow-up. It was observed that transfusion was applied to 60% of the patients in the control group (n = 30, 1 unit of erythrocyte suspension in 6 patients, two units of erythrocyte suspension in 21 patients, three units of erythrocyte suspension in 2 patients, and four units of erythrocyte suspension in 1 patient were replaced.), 20% of the patients in the topical group (n = 5, 1 unit of erythrocyte suspension in 2 patients, and two units of erythrocyte suspension in 3 patients were replaced.), and 24% of the patients in the intravenous group (n = 6, 1 unit of erythrocyte suspension in 2 patients, and two units of erythrocyte suspension in 4 patients were replaced.) (Table 2).

Blood loss and haemoglobin differences of all patients included in the study were calculated. While calculating the haemoglobin difference, the lowest postoperative blood and preoperative blood results were used. The mean difference in haemoglobin values before and after surgery in the control group was calculated as 2.3 g/dL (0.1- 4.5 g/dL). The haemoglobin difference of patients in the topical group is 1.6 g/dl (0.1- 4.7 g/dl); patients in the intravenous group were calculated as 1.8 g/dL (0.1-3.9 g/dL). When the calculated values were analysed statistically, it was found that haemoglobin decrease significantly decreased (Table 2).

When the blood loss calculations made by the Gross method were examined, the average of the control group was 1011.5 ml (179-1837 ml), the topical group was 695.7 ml (11-2503 ml), and the intravenous group was 710.9 ml (173-11315 ml) calculated as. When analysed statistically in terms of blood loss, it was found that the control group was significantly higher than the topical and intravenous groups, but there was no significant difference between the topical and intravenous groups ($p = 0.002$) (Table 2).

In the postoperative follow-up of the patients, deep vein thrombosis (DVT) was observed in 4% (n = 2) of the control group. Similarly, when the topical and intravenous groups were examined, this complication was observed in one patient in both groups. When analysed statistically, it was calculated that there was no significant difference ($p > 0.99$) (Table 3).

Patients included in the study were examined in terms of periprosthetic infection. As a result of this examination, no complication was encountered in any patient in groups treated with topical and intravenous

Table 2. Fluid amount from patients' drains, blood transfusion rates, haemoglobin difference and blood loss

	Control	Topical	Intravenous	*p* value
Drain (ml)	246.5 ± 1.2	124 ± 83	101.4 ± 77	**< 0.001***
ABT* **	60% (n = 30)	20% (n = 5)	24% (n = 6)	**0.001****
Hgb difference (g/dL)	2.3 ± 1.12	1.6 ± 1.01	1.8 ± 1.2	**0.031***
Blood loss (ml)	1011.5 ± 419	695.7 ± 478	710.9 ± 374	**0.002***

ABT = Allogeneic blood transfusion, Hgb = hemoglobin
*Oneway ANOVA test
**Chi-square test

Table 3. Complication and mortality rates of patients

	Control	Topical	Intravenous	*P* value
DVT	4% (n = 2)	%4% (n = 1)	4% (n = 1)	> 0.99*
Periprosthetic joint infection	10% (n = 5)	0% (n = 0)	0 (n =0)	0.074* **0.028****
30-day mortality	12% (n = 6)	24% (n =6)	16% (n = 4)	0.409*
90-day mortality	20% (n = 10)	28% (n =7)	16 (n = 4)	0.564*
1-year mortality	44% (n = 22)	28 (n = 7)	24 (n = 6)	0.161*

DVT = deep vein thrombosis
*Chi-square test
**Fisher'sExact test

tranexamic acid. Periprosthetic infection developed in 10% of the patients in the control group, and a second surgical intervention was required. Allogeneic blood transfusion was applied to all of these cases during the perioperative period. When analysed statistically, no significant difference was found when the three groups were compared (p = 0.074). However, it was calculated that the infection rate was higher in the control group than patients who used tranexamic acid regardless of the route of administration (p = 0.022) (Table 3).

When the 30-day, 90-day and 1-year mortality rates of the patients included in the study were examined, it was calculated as 12%, 20% and 44% in the control group, respectively. In the topical group, these rates are 24%, 28% and 28%; in the intravenous group, it was observed as 16%, 16% and 24%. When analysed statistically, no significant difference was observed between the groups (Table 3).

DISCUSSION

Arthroplasty is used in the current treatment of hip fractures which are increasingly observed in the elderly. Blood loss during and after arthroplasty can reach up to 1800 ml [10, 18-20]. At the same time, allogenic blood transfusion rates in these patients have been reported in the literature between 20% and 60% [9, 18]. In this case, both haemorrhage amount and high blood transfusion rates may cause multiple complications [21]. Hemodynamic instability can cause severe complications in these patients whose general condition is fond [22]. This study found more blood loss and blood transfusion rates in patients in the group that we did not apply TA. Regardless of the method of administration, its application significantly reduced the need for blood transfusion.

It is available in the literature that TA used during hemiarthroplasty reduces the amount of haemoglobin decrease [10, 11]. It has also been shown that different TA administration route does not provide any advantage [23]. It has been shown that there is no difference between the method of TA application and the complication rates [23]. In this case, it may be an appropriate strategy to reduce the amount of bleeding while performing hemiarthroplasty in femoral neck fractures. The route of administration can be left to the surgeon's preference.

Emara *et al*. [23] reported in their study that the drain follow-up of control groups and tranexamic acid-treated groups decreased by approximately 50%. In our study, when we compared the control group with both the topical group and the intravenous group, half of the reduction in fluid from the drain was found. There was a difference of approximately 20 ml between the groups in which TA was applied in this study, and no statistically significant difference was found.

Our study showed a more significant decrease in blood transfusion rates in TA groups than in the literature [24, 25]. In fact, in these two studies, 15 mg/kg tranexamic acid was used as two intravenous doses, and in our study, the intravenous dose was applied as 10 mg/kg. In their studies, Kang *et al*. [12] reported that they topically applied 3 g TA and decreased the blood transfusion rate by 25%. In our study, we applied 2 gr TA and detected a 66% reduction in blood transfusion rate. Achieving the same effect with a lower dose can help reduce complications after sur-

gery.

Vascular embolism and thrombosis are the most severe complications reported after tranexamic acid administration [26]. In the literature, although it has been reported that there is no increase in complications and mortality rates of TA application in orthopaedic procedures, studies are showing that topical application may be safer [5, 10, 11, 20, 25]. There may be no difference between the application route and the possibility of developing complications in hemiarthroplasty patients. Even so, the possibility of complications associated with the use of tranexamic acid can be reduced by appropriate patient selection and appropriate administration.

Considering the data on the duration of hospitalisation, it has been reported that the administration of tranexamic acid did not significantly reduce these times. In their study, Liu *et al*. [10], the meantime of hospitalisation was 5.5 days, Lee *et al*. [11], while it was found to be approximately 20 days in their study, this average was 10.6 days in our study. This difference may have resulted from countries' health systems and sociocultural differences.

Limitations

The main limitations of our study are that the research was conducted retrospectively and a single surgeon could not perform the surgical treatments of the patients included in the study. The small number of samples included in the study, different surgical techniques and approaches are not included, and the effect of reducing bleeding on functional results is unknown. This situation can only be demonstrated by randomised controlled prospective studies with many cases.

CONCLUSION

As a result of our study to evaluate the effectiveness of tranexamic acid administration during partial arthroplasty application as a surgical method in femoral neck fractures. It has been concluded that both topical and systemically used tranexamic acid reduces blood loss and reduces blood transfusion rates.

Authors' Contribution

Study Conception: MY, Yİ, HÇ, SSD, AY, TOB, NE, HG; Study Design: MY, Yİ, HÇ, SSD, AY, TOB, NE, HG; Supervision: MY, Yİ, HÇ, SSD, AY, TOB, NE, HG; Funding: MY, Yİ, HÇ, SSD, AY, TOB, NE, HG; Materials: MY, Yİ, HÇ, SSD, AY, TOB, NE, HG; Data Collection and/or Processing: MY, Yİ, HÇ, AY, TOB, NE; Statistical Analysis and/or Data Interpretation: MY, Yİ, HÇ, AY, TOB, NE; Literature Review: MY, Yİ, HÇ, AY, NE; Manuscript Preparation: MY, Yİ, HÇ, AY, NE and Critical Review: MY, Yİ, HÇ, AY, TOB, NE.

Conflict of interest

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

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The European Research Journal 2022;8(5):567-572 Original Article

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Regional distribution of glucose-6-phosphate dehydrogenase deficiency in Turkey and evaluation of clinical findings: a multicenter study

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ABSTRACT

Objectives: The single most inherited enzyme deficiency is that of glucose-6-phosphate dehydrogenase (G6PD) with a presence in almost 400 million of the world'spopulation. The number of reported G6PD mutations is 186. Furthermore, geographical location is a determining factor for the prevalence of G6PD. Therefore, much of the existing epidemiological literature concerning this issue in Turkey has reported data specific to cities and regions. The purpose of this study was to examine G6PD deficiency in a sample of subjects. Outcome measures reported in this study include the clinical factors associated with the deficiency, as well as in geographical dispersion across regional locations in Turkey.

Methods: This is a retrospective, cross-sectional study. The sample comprised 308 subjects with a G6PD diagnosis. Data collection commenced in January 2011, and was completed by May 2020.

Results: In Turkey, the Mediterranean region has the greatest prevalence of G6PD enzyme deficiency. Subjects presenting with this deficiency were also diagnosed with haemolytic anaemia that was attributed to favism. Subsequently, drug and neonatal hyperbilirubinemia-induced haemolysis ensued. Over 90% of subjects diagnosed with a critical G6PD deficiency and recurrent haemolysis were allocated to the Class II variant.

Conclusions: The Mediterranean, along with Agean and Marmara regions are where the highest prevalence of G6PD enzyme deficiency are observed. Favism-induced haemolytic anaemia is the most often identified clinical precursor to diagnosis of G6PD deficiency in Turkey. The most common clinical feature after this condition is drug related haemolysis and the onset neonatal hyperbilirubinemia.

Keywords: Favism, glucose phosphate dehydrogenase deficiency, hemolytic anaemia

The first enzyme encountered in the pentose phosphate pathway is Glucose-6-phosphate dehydrogenase (G6PD). It is also the most rate-limiting enzyme in the pathway. For RNA, DNA and nucleotide synthesis to take place in this same pathway, ribose-5- phosphate must also be produced. Another product of this pathway is nicotinamide adenine dinucleotide phosphate (NADPH), which increases reduced glutathione (GSH) and thus prevents oxidative stress [1]. Due to the fact that there are no mitochondria within the erythrocytes, the pentose phosphate pathway is the sole route of production of NADPH.

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Inhibition of G6PD restricts NADPH production and thus, when exposed to drugs and infections, erythrocytes are subject to oxidative stress.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an inherited disorder. Men are mostly subject to G6PD deficiency due to its X-linked transmission. To date the number of reported G6PD mutations is 230, and it is present in 0.5 billion of the world's population-making it the most prevalent inherited enzyme deficiency [2,3]. Geographical dispersion is an influential factor in G6PD deficiency incidence, while ethnicity also impacts on the prevalence of the deficiency too. Findings concerning the geographical dispersion of plasmodium falciparum and G6PD deficiency are comparable. In patients with G6PD deficiency, there is an argument to support the effectiveness of plasmodium falciparum resistance [4, 5]. In 1989, the World Health Organizations (WHO) reported that one or more G6PD deficiencies were present in at least 5% of the world's population [2]. The most affected populations were those of sub-Saharan Africa with a prevalence of 15%, and Southeast Asia at 26% [2]. This contrasted with Turkey with a percentage ranging from 0.5% to 2.9%, while similar findings were reported in other countries in proximity to the Mediterranean Sea, as well as the United States of America [2].

The majority of existing epidemiological data collected in Turkey concerning G6PD deficiency has focused on geographical distribution across regions, with the greatest frequency being reported in Cukurova (5.8-8.5%), while other regional frequencies include the Aegean at 2.3%, and the Black Sea at 5% [6, 7]. Additionally, in those screened for neonatal hyperbilirubinemia, G6PD deficiency was present in 1.12% [8]. Due to the high prevalence of G6PD in other countries in the Mediterranean region, nearby countries to Turkey, such as Italy and Greece have incorporated G6PD deficiency screening into their standard screening programme for newborn children.

There is a general consensus that G6PD deficiency is unlikely to negatively impact on a patient's quality of life, or their life expectancy [9]. However, recently, a study has reported that the risk of cardiovascular disease is observed more frequently in men with G6PD deficiency than in the control group (odds ratio, 1.39; confidence interval, 1.04-1.87) [10]. Diagnosis of the deficiency is often not made until the patient is in the neonatal or paediatric phase due to its asymptomatic nature. For example, G6PD deficiency is often undiagnosed until after patients are presenting with neonatal jaundice, favism, non-spherocytic chronic hemolytic anaemia or acute hemolytic episodes, the onset of which can be triggered by infections, or the ingestion of chemicals or medication [3].

Lack of enzymes is the marker used to specifically diagnose G6PD deficiency, which can be assessed by qualitative fluorescent spot test, methemoglobin reduction test or quantitative spectrophotometric measurements. Although it is not a preferred diagnostic option, it is also possible to use the family screening method in prenatal diagnosis. This method utilises the polymerase chain reaction to identify specific gene mutations [11].

The purpose of this study was to examine G6PD deficiency in a sample of G6PD deficient subjects. Outcome measures included in the analysis were clinical factors associated with G6PD deficiency, and geographical dispersion across the country of Turkey.

METHODS

All subjects included in this study had been applicants between the dates of January 2011 and March 2020. Data were retrieved restrospectively from hospital patient files stored on the internal information system. Demographic data analysed included patient age, home city and medical history. Clinical histories prior to diagnosis of G6PD deficiency were questioned in the anamnesis of the patients and noted. The clinical measures from each patient included in the analysis were; complete blood count, serum AST, LDH, total and direct bilirubin levels. Quantitative spectrophotometric methods were utilised to measure G6PD levels - a reading of over 4.6 IU/gHb was recommended to be a normal value for G6PD levels by the testing kit manufacturer.

There are five variants of G6PD enzyme deficiency as described by WHO – all of which are characterised by enzyme activity levels and measures of clinical factors [2]:

Class I: Severe deficiency - presents as less than 10% activity alongside chronic (nonspherocytic) hemolytic anaemia (Chicagovariant).

Class II: Severe deficiency - presents as more than

10% activity along side intermittent haemolysis, and subsequent to infection, or administration of drugs or chemicals (Mediterranean, Mahidol variant).

Class III: Mild deficiency – presents as between 10 and 60% activity, and alongside haemolysis in the presence of stressors.

Class IV: Non-deficient variant with no associated pathologies.

Class V: Elevated enzyme activity with no associated pathologies (Hektoen variant).

The study protocol was approved by the Institutional Review Board of Etlik Zübeyde Hanim Training and Research Hospital (IRB no. 2020/79).

Statistical Analysis

Quantitative data are reported in values of mean ± standard deviation, numerical units and percentage distribution n (%). Descriptive statistical methods were used at statistical evaluation of the patients.

RESULTS

Data from 316 subjects was selected for analysis. Of these, 308 male subjects, each aged above 18 years old were included in the final data set, as 8 of the original sample did not present clinically significant measures to enable diagnosis of G6PD deficiency. Regional distribution of the subjects is presented in Table 1. The distribution of the cities where the cases were living, was given on the map in Fig. 1.

Table 1. Regional distribution of subject diagnosed with G6PD deficiency

Region	Number of Subjects n (%)
Mediterranean	113 (36.7)
Aegean	85 (27.6)
Marmara	50 (16.2)
Central Anatolia	33 (10.7)
Southeastern Anatolia	18 (5.9)
Black Sea	8 (2.6)
East Anatolia	1 (0.3)
Total	**308 (100)**

Table 2 presents sample data for age, hemoglobin, hematocrit, serum AST, LDH, total, indirect bilirubin and G6PD levels.

In 67 (21.8%) of the subjects, diagnosis of G6PD had followed one or more episodes of acute haemolysis, of which 41 had been attributed to drugs, 23 to infection and 3 to chemicals. Diagnosis of G6PD deficiency following favism-related haemolysis accounted for 184 (59.7%) of the subjects' diagnoses, while 39 (12.7%) followed haemolysis after prolonged neonatal jaundice. Family history of G6PD deficiency was the preceding factor to diagnosis in 18 (5.8%) of the subjects – all of whom had not previously experienced haemolysis. Table 3 presents the percentage dis-

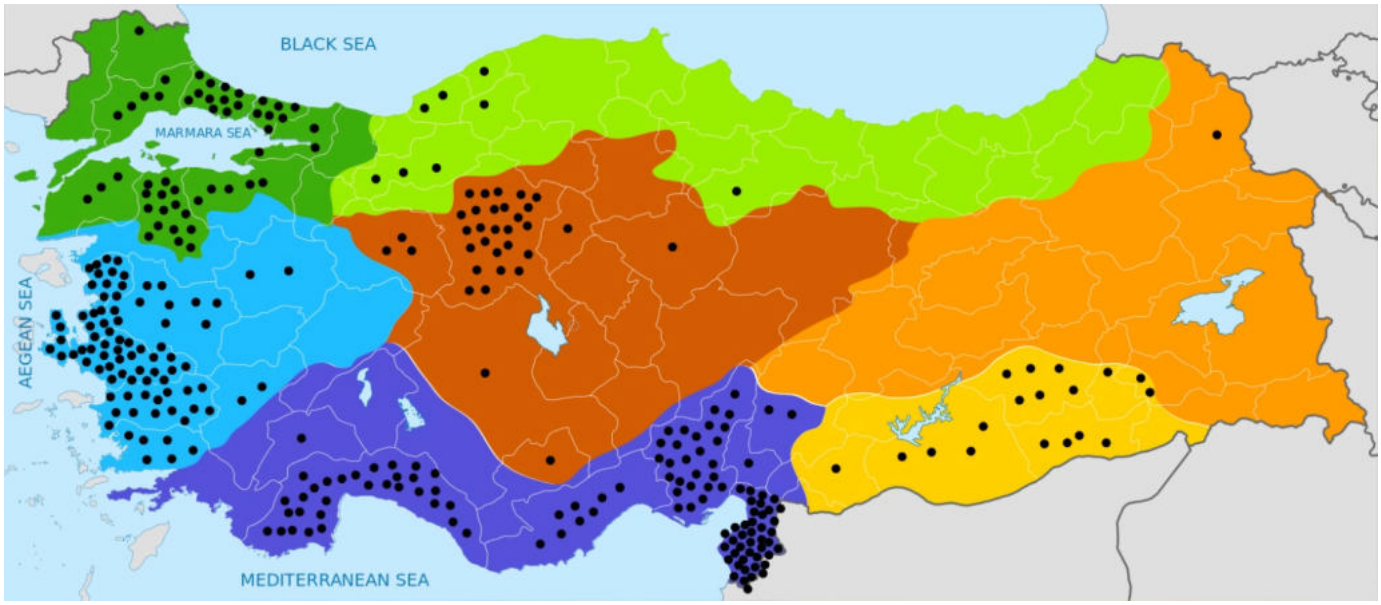

Fig. 1. Distribution of patients by geographical region/city (Every dots belongs to one patient).

Table 2. Age and laboratory data

Parameter	Unit	Mean ± SD
Average age	Year	25.23 ± 4.54
G6PD (4.60-13.50)	IU/gram hb	0.94 ± 1.00
Hemoglobin (11.6-17.0)	gr/dL	14.73 ± 1.44
Hemotocrit (34.9-50.1)	%	43.8 ± 3.76
AST (14-35)	U/L	24 ± 8.1
LDH (90-240)	U/L	251 ± 127
Total bilirubin (0.2-1.2)	mg/dL	1.44 ± 1.41
Indirect bilirubin (0.2-0.8)	mg/dL	0.27 ± 0.18

Table 3. Distribution of subject clinical history prior to diagnosis of G6PD deficiency

Clinical History	Number of cases n (%)
Acute hemolytic episodes	67 (21.8)
Drugs	41 (13.3)
Infection	23(7.5)
Chemical	3 (1.0)
Favism	184 (59.7)
Neonatal jaundice	39 (12.7)
Family history	18 (5.8)
Total	308 (100)

tribution of clinical precursors prior to diagnosis.

Analysis of the subjects' clinical presentations showed that 4 (1.3%) of G6PD deficiency in chronically haemolytic subject belonged to the Class I variant. In 271 subjects, G6PD deficiency was categorised as belonging to the Class II variant and 15 subjects belongs to Class III variant. There were 18 subjects whose G6PD deficiency could not be categorised due to an absence of haemolytic episodes, although each had a history of severe G6PD deficiency that was linked to a family history of G6PD deficiency.

DISCUSSION

There have been multiple studies conducted to examine the frequency of G6PD deficiency in numerous countries around the world, including Turkey. One or more gene G6PD deficiencies are present in almost 5% of the world's population, which means that around 500 million people are influenced by this deficiency [3, 12]. Geographical location and ethnicity of the population are both influential factors in the prevalence of G6PD deficiency. Jews represent the population with the greatest prevalence, with over 60% of this group having a G6PD deficiency, which is in contrast to the Pacific countries of Australia and New Zealand, where prevalence falls to as little as 0.1% [13, 14].

The findings of Aksu *et al*. [15] identified that in the city of Antalya around 7.4% of the male population was G6PD deficient. The data reported here by the authors of this study demonstrated that prevalence was greatest in the Mediterranean region, with the Agean and Marmara regions placed second and third in terms of prevalence. As we expected, the rate of G6PD was found to be higher in the regions adjacent to the Mediterranean, and was observed at a lower rate in the neighboring regions. Interestingly, the prevalence of G6PD deficiency in both these regions was above the Turkish average of 2.9%. However, prevalence has been reported to be higher still at 6.9% in Balikesir and Canakkale of the Marmara region in a healthy sample of 1421, while populations in the Black Sea region have demonstrated prevalence of G6DP deficiency at around 5% [7, 16]. The research evidence reported here has shown lower prevalence of G6DP deficiency than values reported elsewhere. It also appeared that the subjects of this study had attempted to conceal their diagnosed G6DP deficiencies during their health screening processes.

Those diagnosed with G6PD deficiency do not always present with precursors of acute hemolytic anaemia, neonatal jaundice or chronic non-spherocytic anaemia. Despite the relevance to the Turkish population this is not an issue that has been reported in any other published research findings. As predicted, the most frequent clinical feature reported prior to diagnosis of G6PD deficiency was favism at 59.7% of all cases. Favism is the onset of haemolytic anaemia in the 24 to 48 hour period following ingestion of fava beans – most commonly occurring in children aged between 2 and 5 years old, it is not often a fatal condition [17]. Favism in G6PD deficient people is determined by their genetic make up; not every individual who has a G6PD deficiency has favism, but every individual with favism will be G6PD deficient [18, 19].

The data reported here shows that favism is most common in those with a Class II variant of G6PD deficiency.

The frequency of neonatal jaundice in cases of pre-diagnosis of G6PD deficiency has been reported as between 1.12% and 3.8% [8, 20, 21] which is in contrast to the prevalence of 12.7% reported in the findings here. Thirteen percent of patients who present with neonatal jaundice are later diagnosed with G6PD deficiency, and therefore particular attention must be paid to those newborn patients presenting with hyperbilirubinemia, as this may lead to more successful rates of diagnosis for G6PD deficiency. It has been shown that a G6PD-deficient newborn who develops jaundice has higher bilirubin at admission, requires more exchange transfusion and prolonged phototherapy, and associated with a longer hospital stay [22]. In order to address the high rates of G6PD deficiency in Turkey it is important that G6PD deficiency tests are incorporated into the standardized health screening processes deployed at birth, as they are already in countries such as Italy and Greece. Most critically, this is to prevent life-threatening episodes of haemolysis – it is especially pertinent given the high prevalence of G6PD deficiency in the Mediterranean region. The data reported here identifies that the third most likely cause of heamolytic anaemia prior to diagnosis of G6PD deficiency is the administration of drugs (13.3%), of which antibiotics and anesthetic products were the most frequently recalled, although not officially documented. In those where haemolysis was caused by chemicals, the subjects reported that the chemical ingested was naphthalene. Eighteen did not experience haemolytic episodes, and therefore were not classified to this variant of G6PD deficiency, although they were screened and consequently diagnosed with the deficiency due to a family history of deficiency. Every subject in this sample presented levels of G6PD enzyme no greater than 10%, while all subjects reported favism to be a feature in their family history.

Based on the relevant levels of G6PD enzymes and their clinical features, there was only four (1.3%) Class I G6PD deficiency identified from the sample of 308 subjects – which were diagnosed due to his presentation of neonatal jaundice, and presented reports of haemolysis. There were 271 subjects who presented with a ClassII G6PD deficiency. Most existing studies of G6PD deficiency conducted in Turkey focused on distribution in regions and cities. The subjects in this study had been provided with different diagnoses in different regions of Turkey in order to explain their episodes of haemolysis. This was prior to successful diagnosis of G6PD deficiency through this research study, which was made based on their low enzyme levels and clinical symptoms. As such, it is possible that, the prevalence of ClassI G6PD deficiency identified here could represent general prevalence of ClassI G6PD deficiency across the regions and cities of Turkey.

There are limited reports on the prevalence of G6PD deficiency in women, due to the necessity of genetic diagnosis in studies and the rarer clinical manifestations of G6PD deficiency in women. However, data from a study of 355 female participants from previously malaria endemic areas in Northeast Thailand showed that the prevalence of G6PD Deficiency was much higher than expected (18% by fluorescent spot test, 29.6% by quantitation of G6PD activity) [23].

Limitations

A key limitation of these findings is that the data were analysed retrospectively. Therefore it was not possible to gain other measures at the point of data collection that may have proven to be valuable, such as genetic data. Because homozygous female genotypes are extremely rare, this study was limited to male patients only, and it is a limiting factor that it could not be included due to the lack of a diagnosed female patient.

CONCLUSION

In conclusion, these findings have demonstrated a higher prevalence of G6PD deficiency in the Mediterranean, Aegean, Marmara and Central Anatolia regions respectively. Furthermore, the most common clinical symptom to present prior to diagnosis of G6PD deficiency in the Turkish population is favism-induced haemolytic anaemia. Other common clinical symptoms prior to diagnosis are haemolytic anaemia attributable to drugs, neonatal hyperbilirubinemia and infections.

Authors' Contribution

Study Conception: CB; Study Design: MY; Super-

vision: SS; Funding: MKK, MA; Materials: MY; Data Collection and/or Processing: SS, EK; Statistical Analysis and/or Data Interpretation: MY, GÖ; Literature Review: MY, GÖ; Manuscript Preparation: SS and Critical Review: MKK, CB, MA.

Conflict of interest

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

Financing

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Short-term effectiveness of auricular vagus nerve stimulation in patients with myofascial pain syndrome

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ABSTRACT

Objectives: To evaluate the effect of auricular vagus nerve stimulation (VNS) applied in addition to ischemic compression and stretching exercises on pain, trigger point (TP) sensitivity, grip strength, quality of life and autonomic functions in patients with myofascial pain syndrome (MPS).

Methods: Sixty patients, who had neck pain, met the diagnostic MPS criteria of Travell and Simons were included in the study. The subjects were randomly divided into VNS group (n = 30) or control group (n = 30). Each group performed 10 sessions of TP ischemic compression and stretching exercises (5 days/week). Ten sessions of 30-minute long auricular VNS were added to the treatment in VNS group. Pain severity [Visual Analogue Scale (VAS)], TP sensitivity (algometer), grip strength (Jamar dynamometer), quality of life [Short Form-36 (SF-36)] and autonomic function [Composite Autonomic Symptom Scale-31 (Compass-31)] were evaluated before and after 10 sessions of treatment.

Results: The VAS, algometer and Jamar measurements showed significant improvement in both groups. A statistically significant improvement was found in orthostatic intolerance, secretomotor and pupillomotor subscales of Compass-31 scale in the VNS group following the treatment ($p < 0.05$) while no significant difference was observed in the control group ($p > 0.05$). The control group showed significant improvement in all parameters of SF-36 scale, while the VNS group showed significant improvement in physical function, social functionality and pain parameters ($p < 0.05$). The changes in the VAS, algometer, Jamar scores and secretomotor subscale of the Compass-31 scale were statistically higher in the VNS group than in the control group ($p < 0.001$, $p < 0.001$, $p = 0.001$ and $p = 0.011$, respectively).

Conclusions: It can be argued that auricular VNS increases the effectiveness of ischemic compression and stretching exercises in patients with MPS. Further and detailed studies are needed in which the effect of VNS alone or in combination with other treatments in patients with MPS is examined and the physiological mechanisms are investigated.

Keywords: Myofascial pain syndrome, auricular vagus nerve stimulation, ischemic compression, autonomic nervous system, quality of life

Myofascial pain syndrome (MPS) is a painful syndrome caused by taut bands and trigger points (TP) in the muscles, tendons, ligaments, and fascia [1]. Having a prevalence of 12% in the community, MPS has been reported to be mostly seen in individuals between the ages of 30-50 and to be 2 times

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more common in women than in men. All or a combination of muscle spasm, muscle tenderness, limitation of movement, stiffness, fatigue, and autonomic dysfunction may accompany this painful syndrome [2].

The aim of MPS treatment is to inactivate the trigger points that cause pain, to reduce the tension in the muscles and to restore tissue elasticity, to use these muscles with the correct posture and to enable the person to perform daily life activities without any problems. Various medical and conservative treatments are used in the treatment of MPS. Hot and cold pack application, spray and stretching, ischemic compression, ultrasound, therapeutic massage, transcutaneous electrical nerve stimulation, trigger point injection with local anesthetic, dry needling, botulinum toxin injection, stretching exercises, biofeedback are the most commonly used ones. However, in addition to these methods, reducing stress and investigating psychological factors are of significance in the regression of these symptoms [3].

Stimulation of the tenth cranial nerve, the vagus, may affect the autonomic nervous system, reducing the perception of pain and increasing recovery in patients with MPS. It has been shown in the studies conducted on animals that beyond the inhibitory effect the vagus nerve creates through its central connections, it may also have a peripheral effect on nociceptors [4]. In light of these data, it can be thought that auricular vagus nerve stimulation (VNS) will contribute to the MPS treatment. VNS can modulate dystonic symptoms as well as afferent inputs that affect brain regions involved in the formation of sympathovagal balance [5]. The mechanisms (central or peripheral) that provide the analgesic effect with the stimulation of the vagus nerve are still unclear. Very few studies have been conducted on this issue [4]. Offering the most appropriate and effective treatment method to patients suffering from this syndrome, which causes a decrease in the quality of life and loss of workforce, will both reduce the treatment costs and save time for the patient. This study was conducted to evaluate the additional benefits of auricular VNS in patients with MPS.

METHODS

The study was planned in accordance with the Principles of the Declaration of Helsinki. Approval was obtained from the Clinical Research Ethics Committee of Bahçeşehir University on 04.04.2018 with the decision numbered 2018-07/01. The study was carried out between June 2018 and April 2019. All volunteers presented their informed consent before their enrollment in the study.

Sixty patients aged 20-60 who checked into to the Physical Therapy and Rehabilitation Unit of Istanbul Memorial Hospital with the complaint of neck pain, who met the diagnosis and MPS criteria of Travell and Simons, and who had at least one active TP palpable on the trapezius muscle and a taut band were included in this study [6]. Exclusion criteria were as follows: having cervical disc herniation, radiculopathy or myelopathy, having acute inflammatory disease, use of antispasmothic and analgesic medications, and pregnancy.

Randomization of the study was done using the closed-envelope method through odd and even numbers. The subjects were randomly divided into two groups as VNS and control group. Thirty individuals were assigned to each group. Trigger point ischemic compression and stretching exercises were performed for 10 sessions 5 days a week in the control group. In addition to the treatment used in the control group, a total of 10 sessions of auricular VNS were performed for 30 minutes in the VNS group.

All participants were evaluated before the first session, and the same parameters were re-evaluated after 10 sessions of treatment. The participants were evaluated for pain, pressure pain threshold, grip strength, quality of life, and autonomic functions.

Application of Ischemic Compression

The most sensitive TP of the cases with active TPs in the upper fibers of the M. trapezius was determined through palpation. Progressive ischemic compression was applied to this determined TP was 10 times for at least 20 seconds in each session. During the application, a constant pressure was applied with the thumb on the palpable trigger point on the M. trapezius for a certain period of time. As the pain began to subside, the pressure was gradually increased to maintain the same level of pain [7].

Stretching Exercises

The subjects participating in the study were first shown how to do stretches, then they performed them

as self-stretches. In order to provide relaxation by reducing the pain on the trigger points and to increase the range of motion of both active and passive joints by bringing the muscles to their normal length [8], M. Trapezius muscle stretching exercises were performed with 10 repetitions in each session, and the hold time at the last point was determined as 20 seconds.

Vagus Nerve Stimulation

In our study, auricular VNS was applied to both ears simultaneously from the tragus and concha parts by using a specially manufactured earpiece, the size of which can be adjusted according to the ear size, and the Vagustim® device, which is connected to the earpiece. In order for the earpiece connected to the device to conduct the electric current well, conductive gel was applied to the metal part of the earpiece that would contact the patient and placed in a way that coincided with the inner outer surface of the tragus and the concha. The frequency of the Vagustim device connected to the earpiece was set to 10 Hz and its pulse duration to less than 500 microseconds, in modulated TENS mode and biphasic asymmetric waveform. The intensity of the current was adjusted by questioning the sensory threshold of the participants.

Pain Assessment

Visual analog scale (VAS) was used in evaluating the severity of pain. Participants were asked to mark their mean pain during last 48 hours on the line on a 10 cm Likert scale, with "no pain" at the left end (0 cm) and "worst pain" (10 cm) at the right end. Measurements from the starting point of the scale (left end) to the patient's marks were measured in centimeters and recorded as pain intensity [9].

Evaluation of Pressure Pain Threshold

Pain threshold measurements of the participants were performed using the digital algometer branded Algometer Commander Jtech Medical 801-478 USA. The taut bands in the upper fibers of the M. trapezius were found by palpation, and painful trigger points here were marked. The algometer was placed at these points with an angle of 90° and measurements were made. Pain threshold measurements were repeated 3 times and the arithmetic average was taken. The subjects were asked to report the first time they felt pain, and the pain threshold was recorded by reading the value displayed on the device. In all three measurements, a 30-second rest period was given in between [10].

Grip Strength Evaluation

The grip strength measurement of the participants was made using the Jamar hydraulic dynamometer. The measurement was performed with the patient in a sitting position, with the elbow with strong grip at 90° flexion, the forearm neutral and the arm adjacent to the trunk [11]. The patient was asked to grasp the dynamometer and squeeze it with all his/her strength, the measurements were repeated 3 times and the arithmetic averages were taken. These force measurement results were recorded in kilograms.

Questioning of Autonomic Functions

The autonomic functions of the participants were evaluated with COMPASS-31. The Autonomic Symptom Profile (ASP) is a questionnaire designed to comprehensively assess the severity, distribution, and autonomic functional capacity of symptoms in patients with autonomic disorders. The Composite Autonomic Symptom Score (COMPASS) is derived from the ASP and questions autonomic functions with 84 selected questions. Complexity in the scoring algorithm of COMPASS resulted in ambiguous and inconsistent scores. Because the scoring algorithm of this test is complex and requires computer analysis, COMPASS-31 was developed. Selected from ASP and COMPASS to evaluate autonomic function, 31 questions were grouped under 6 areas. A maximum raw score was determined for all areas individually, and a weighting factor was assigned to each area based on the existing perception of the importance of the areas to reflect autonomic failure. The maximum weighted score ranges from 0 to 100. A high score indicates autonomic failure [12].

Questioning of Quality of Life

The Short Form-36 (SF-36) questionnaire was used to question the quality of life. The SF-36 is one of the most widely used and validated forms of questioning quality of life. It was developed and brought into use as a questionnaire by Rand Corporation in 1992 [13].

Statistical Analysis

IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) and MS-Excel 2007 programs were used for statistical analysis and calculations. Statistical significance level was set at $p < 0.05$. The Shapiro-Wilk test was used to evaluate whether the variables in the study were in accordance with the normal distribution. The median (Interquartile Range - IQR) values were used to display the descriptive statistics of the variables that were found not to show normal distribution, and the mean ± SD (Standard Deviation) values were used for the variables with normal distribution. In the comparison of the measurement values before and after the application, the t-test results were used for the normally distributed variables, and the Wilcoxon-Signed Rank test results for those not normally distributed. In the comparison of VNS and control groups before and after the treatment, the t-test results were used for normally distributed variables, and Mann-Whitney U-test results for non-normally distributed variables.

RESULTS

In the VNS group, 27 patients (mean age: 38.14 ± 9.94 years) and 26 patients in the control group (mean age: 35.42 ± 10.74 years) completed the study. The CONSORT flow diagram of the study was given in Fig. 1. There was no significant difference in demographic data between the VNS and control groups ($p > 0.05$) (Table 1).

When the baseline values of the groups were compared, a statistically significant difference was observed only in the gastrointestinal subscale of the Compass-31 scale ($p = 0.027$) and the energy/vitality and mental health subscales of the SF-36 scale ($p = 0.025$ and $p = 0.004$, respectively) (Table 1).

In the VNS group, the pre- and post-treatment VAS scores, algometer and grip strength values showed significant differences ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) (Table 2).

When the Compass-31 scale was analyzed for the VNS group, a statistically significant difference was

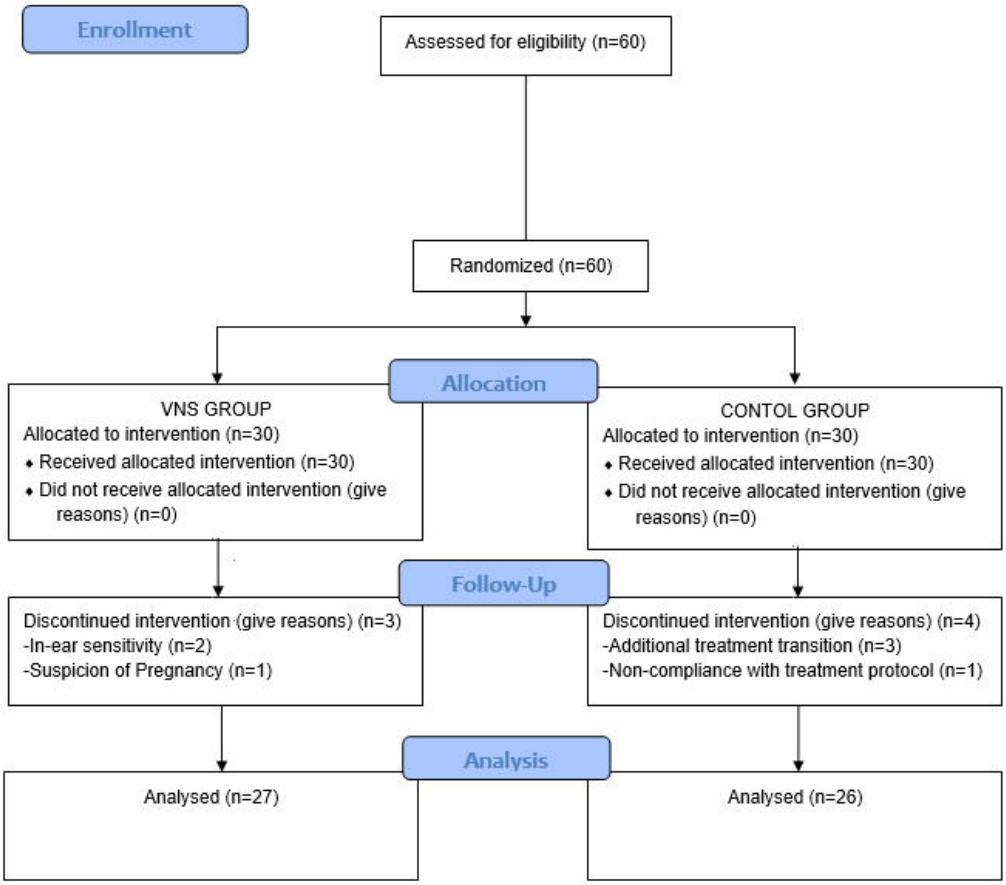

Fig. 1. Consort flow diagram.

Table 1. Comparison of demographic parameters and baseline characteristics of the groups

		VNS Group (n = 27) Mean ± SD / Median (Min-Max)	Control Group (n = 26) Mean ± SD / Median (Min-Max)	Z / t	*p* value
Age (year)		38.14 ± 9.94	35.42 ± 10.74	0.959	0.342[i]
BMI (kg/m$^{2)}$		24.82 ± 4.37)	24.19 ± 4.68	0.511	0.584[i]
Symptom Duration (month)		6.00 (0.25-36)	5.50 (0.50-60)	-0.411	0.681[m]
VAS		6.00 (4-9)	6.00 (5-8)	0.779	0.436[m]
Algometer (kg/cm²)		6.81 ± 2.57	7.48 ± 2.63	-0.926	0.359[i]
Grip strength (kg)		23.32 ± 5.56	24.00 ± 9.67	0.274	0.785[i]
Compass-31	**Orthostatic intolerance (0-10)**	4.00 (2-7)	4.00 (2-8)	1.634	0.102[m]
	Vasomotor (0-6)	2.50 (2-4)	3.00 (2-4)	1.010	0.313[m]
	Secretomotor(0-7)	2.00 (0-5)	0.00 (0-3)	0.684	0.494[m]
	Gastrointestinal(0-28)	9.00 (6-16)	10.00 (9-11)	2.207	**0.027[m]**
	Bladder (0-9)	1.00 (0-4)	0.00 (0-5)	1.116	0.264[m]
	Pupilomotor(0-15)	5.59 ± 2.69	5.04 ± 2.57	0.766	0.447[i]
SF-36	**Physical function**	80.00 (45-100)	90.00 (40-100)	1.875	0.061[m]
	Role physical	25.00 (0-100)	25.00 (0-100)	0.604	0.546[m]
	Emotional function	100 (0-100)	66.66 (0-100)	0.650	0.515[m]
	Energy/vitality	42.22 ± 17.12	53.65 ± 18.84	2.313	0.025[i]
	Mental health	56.29 ± 13.76	68.46 ± 15.15	3.062	0.004[i]
	Social function	75.00 (25-100)	62.50 (25-100)	0.207	0.836
	General health	49.81 ± 17.62	51.15 ± 23.38	0.236	0.814[i]
	Pain	47.50 (12.50-70)	47.50 (22.50-80)	0.207	0.836[m]

BMI = Body Mass Index, VAS = Visual Analog Scale, Min = Minimum, Max = Maximum, SD = Standard deviation.
[i]Independent sample t test / [m]Mann Whitney U test

observed only between the pre- and post-treatment orthostatic intolerance, secretomotor and pupillomotor subscales ($p = 0.004$, $p = 0.002$ and $p = 0.007$, respectively) (Table 2).

When the SF-36 scale was analyzed for the VNS group, a statistically significant difference was observed only between the pre- and post-treatment physical function, social functionality and pain subscales ($p = 0.009$, $p = 0.011$ and $p = 0.002$, respectively) (Table 2).

In control group, there were statistically significant differences between the pre- and post-treatment VAS scores, algometer and grip strength values ($p < 0.001$, $p < 0.001$ and $p = 0.003$, respectively) (Table 3).

When the SF-36 scale was analyzed for the control group, a statistically significant difference was observed between the pre- and post-treatment values of all subscales ($p < 0.05$ for all) (see Table 2).

When the changes observed in the VNS and control groups following the treatment were compared, the changes in the VAS, algometer and Jamar scores in the VNS group were found to be statistically significantly different compared to the control group ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively). Similarly, a significant change was found in favor of the VNS group in the secretomotor subscale of the Compass-31 scale ($p = 0.011$). When the changes observed

Table 2. Comparison of pre-treatment and post-treatment values of VAS, Algometer, Grip strength, Compass-31 and SF-36 scores within VNS group

		Pre-treatment Mean ± SD/ Median (Min-Max)	Post-treatment Mean ± SD/ Median (Min-Max)	Z/t	*p* value
VAS		6.00 (4-9)	4.00 (2-6)	-4.615	**< 0.001[w]**
Algometer (kg/cm²)		6.82 ± 2.57	10.04 ± 2.95	9.278	**< 0.001[p]**
Grip strength (kg)		23.32 ± 8.56	25.39 ± 8.27	5.540	**< 0.001[p]**
Compass-31	**Orthostatic intolerance (0-10)**	4.00 (2-7)	3.00 (2-6)	-2.897	**0.004w**
	Vasomotor (0-6)	2.50 (2-4)	3.00 (2-3)	0.0000	1.000[w]
	Secretomotor (0-7)	2.00 (0-5)	1.50 (0-3)	-3.035	**0.002[w]**
	Gastrointestinal (0-28)	9.00 (6-16)	8.50 (9-11)	-1.633	0.102[w]
	Bladder (0-9)	1.00 (0-4)	0.00 (0-5)	-1.642	0.101[w]
	Pupillomotor (0-15)	8.00 (6-10)	6.50 (5-9)	-2.699	**0.007[w]**
SF-36	**Physical function**	80.00 (45-100)	85.00 (60-100)	-2.600	**0.009[w]**
	Role physical	25.00 (0-100)	50.00 (0-100)	-1.930	0.054[w]
	Emotional function	100 (0-100)	100 (0-100)	-1.813	0.070[w]
	Energy/vitality	42.22 ± 17.12	48.33 ± 19.75	-1.849	0.076[p]
	Mental health	56.29 ± 13.76	59.70 ± 15.73	-1.524	0.140[p]
	Social function	75.00 (25-100)	87.50 (50-100)	-2.530[W]	**0.011**
	General health	49.81 ± 17.62	50.93 ± 16.23	-0.560[p]	0.580
	Pain	47.50 (12.50-70)	67.50 (22.50-90)	-3.109[W]	**0.002**

VAS = Visual Analog Scale, Min = Minimum, Max = Maximum, SD = standard deviation.
[w]Wilcoxon-Signed Rank test / [p]Paired t test

following the treatment in the other subscales of the Compass-31 scale and the SF-36 scale were compared, no significant difference was found between the two groups ($p > 0.05$) (Table 4).

DISCUSSION

To the best of our knowledge, this study is the first to examine the effect of VNS on MPS. VNS was applied from both ears noninvasively so the possible asymmetric effects (central and peripheral) of unilateral stimulation were avoided. In addition, tragus and concha regions were stimulated concomitantly to increase the affected auricular vagus nerve fibers. Besides these strengths of the study; the limited number of participants in the groups, the absence of a group in which VNS was applied alone, the non-similarity of the effect mechanisms of the treatments applied in the VNS group, and the absence of sham treatments can be considered among the limitations.

MPS is a complex syndrome characterized by TPs and taut bands. Individuals with clinical MPS have a high recurrence rate. Patients may have muscle stiffness, muscle pain, headache, dizziness, nausea, vomiting and sleep problems that affect their daily activities [14]. Variable sympathetic hyperactivity, along with sweating, vasoconstriction, vasodilation, and piloerection, has also been reported in the myofascial TP regions [15].

Studies in rabbits and humans have shown that the increase in sympathetic activation contributes to the

Table 3. Comparison of pre-treatment and post-treatment values of VAS, Algometer, Grip strength, Compass-31 and SF-36 scores within Control group

		Pre-treatment Mean ± SD/ Median (Min-Max)	Post-treatment Mean ± SD/ Median (Min-Max)	Z/t	*p* value
VAS		6.00 (5-8)	4.00 (3-7)	-4.572	**< 0.001**[w]
Algometer (kg/cm²)		7.48 ± 2.63	9.09 ± 2.65	7.888	**< 0.001**[p]
Grip strength (kg)		24.00 ± 9.67	24.53 ± 9.47	3.341	**0.003**[p]
Compass-31	**Orthostatic intolerance (0-10)**	4.00 (2-8)	3.00 (2-6)	-1.518	0.085[w]
	Vasomotor (0-6)	3.00 (2-4)	3.00 (2-4)	-1.000	0.317[w]
	Secretomotor (0-7)	0.00 (0-3)	0.50 (0-3)	-0.758	0.448[w]
	Gastrointestinal (0-28)	10.00 (9-11)	9.00 (7-11)	-1.000	0.317[w]
	Bladder (0-9)	0.00 (0-5)	0.00 (0-4)	0.000	1.000[w]
	Pupillomotor (0-15)	7.00 (5-9)	6.00 (4-10)	-1.207	0.227[w]
SF-36	**Physical function**	90.00 (40-100)	92.50 (50-100)	-2.124	**0.034**[w]
	Role physical	25.00 (0-100)	62.50 (0-100)	-2.701	**0.007**[w]
	Emotional function	66.66 (0-100	100 (0-100)	-2.373	**0.018**[w]
	Energy/vitality	53.65 ± 18.84	58.26 ± 17.20	-2.150	**0.041**[p]
	Mental health	68.46 ± 15.15	74.15 ± 13.58	-3.335	**0.003**[p]
	Social function	62.50 (25-100)	81.25 (37.50)	-3.508[W]	**< 0.001**
	General health	51.15 ± 23.37	54.81 ± 23.68	-2.774[p]	**0.010**
	Pain	47.50 (22.50-80)	68.75 (35-90)	-4.536[w]	**< 0.001**

VAS = Visual Analog Scale, Min = Minimum, Max = Maximum, SD = standard deviation.
[w]Wilcoxon-Signed Rank test / [p]Paired t test

modulation of motor activity in TPs. However, there is little evidence of sympathetic-sensory interaction in myofascial TP to explain local and referred pain. Autonomic nervous system dysfunctions are thought to be effective in maintaining chronic musculoskeletal pain. It is believed that increased sympathetic activity exacerbates the spontaneous pain seen in TN in patients with chronic neck and shoulder pain [16]. Thus, autonomic nervous system abnormalities are thought to play a role in myofascial TP-related chronic pain [17].

The vagus nerve has a wide distribution throughout the body and, with its central connections, the vagus plays an important role in the control of homeostasis. Functional disorders in the autonomic nervous system are associated with the disruption of this balance. Stimulation of the vagus nerve via the cervical or auricular pathway can contribute to homeostasis and modulate pain and inflammation [5, 18]. Thanks to the neurophysiological data obtained in recent years, the effects of VNS on pain have become more comprehensible [19].

In our study, we aimed to examine the effect of auricular VNS applied in addition to ischemic compression and stretching exercises on pain, TP sensitivity, and grip strength in patients diagnosed with MPS. In addition, the SF-36 and COMPASS-31 scales were used to evaluate quality of life and autonomic functions. When we reviewed the literature, we observed that although there were many studies on the treatment

Table 4. Comparison of the post-treatment and pre-treatment differences of the groups

		VNS Group mean ± SD	Control Group mean ± SD	*p* value
VAS		-2.77 ± 0.80	-1.96 ± 0.66	**< 0.0010[m]**
Algometer (kg/cm²)		3.22±1.80	1.61 ± 1.04)	**< 0.0010[i]**
Grip strength (kg)		2.07±1.94	0.52 ± 0.79	**0.001[i]**
Compass-31	**Orthostatic intolerance (0-10)**	-1.095 ± 1.48	-0.625 ± 1.06	0.496[m]
	Vasomotor (0-6)	0.000 ± 0.632	-0.285 ± 0.755	0.429[m]
	Secretomotor (0-7)	-0.875 ± 1.295	-0.115 ± 0.816	**0.011[m]**
	Gastrointestinal (0-28)	-2.333 ± 0.577	-1.000 ± 1.414	0.197[m]
	Bladder (0-9)	-0.407 ± 1.185	0.000 ± 0.282	0.211[m]
	Pupillomotor (0-15)	-1.900 ± 1.370	-0.625 ± 1.597	0.102[m]
SF-36	**Physical function**	3.70 ± 7.01	1.92 ± 4.70	0.456[m]
	Role physical	13.88 ± 40.03	15.38 ± 24.57	0.924[m]
	Emotional function	16.04 ± 42.73	14.10 ± 26.95	0.992[m]
	Energy/vitality	6.11 ± 17.17	4.61 ± 10.94	0.708[i]
	Mental health	3.40 ± 11.61	5.69 ± 8.70	0.423[i]
	Social function	12.03 ± 21.78	11.05 ± 12.41	0.926[m]
	General health	1.11 ± 10.31	3.65 ± 6.71	0.295[i]
	Pain	16.85 ± 20.50	21.34 ± 10.95	0.522[m]

VAS = Visual Analog Scale, Min = Minimum, Max = Maximum, SD = standard deviation.
[i]Independent sample t test / [m]Mann Whitney U test

of MPS, there was no study on the use of VNS. There have been opinions arguing that sympathetic activity in intramuscular TPs in MPS increases and there is an increase in pain that develops because of this increase [16, 20]. Therefore, we investigated the change in pain in patients with MPS through auricular VNS application.

Hanten *et al*. [21] reported that a home program consisting of ischemic compression and stretching exercise was effective in reducing TP tenderness and pain intensity in individuals with neck and upper back pain. In another randomized controlled study, it was found that the application of ischemic compression in 41 patients with chronic myofascial shoulder pain can reduce symptoms in the treatment of myofascial pain [22]. In our study, the ischemic compression and stretching application applied in the control group yielded results similar to the literature. There was a decrease in the patients' pain after the treatment. In addition to the reduced pain, an increase in grip strength was also observed.

In a randomized controlled study examining cervicogenic headache caused by myofascial TPs, it was reported that the application of ischemic compression was effective [23]. Hodgson and Fryer [24] used an algometer to measure the effect of ischemic compression on pressure sensitivity on latent TPs in the upper trapezius muscle in 37 subjects with myofascial TP. As a result, they found that application of ischemic compression can be an effective treatment for TPs [24]. Similarly, there was a decrease in the TP sensitivity and an increase in the pain pressure threshold in the control group in our study. When the patients were compared before and after the treatment, statistically significant changes were found in VAS, algometer and Jamar grip strength measurements. It is believed that the decrease in pain and sensitivity in TPs causes an increase in grip strength.

When the responses given by the control group on the Compass-31 scale were examined, no significant change was observed. Although there was a decrease in pain, there was no change in the scores of the Compass-31 scale, which reflects the autonomic nervous system activity, suggesting that the application of trigger point ischemic compression and stretching exercises have a local effect. When the SF-36 scores in the

control group were examined, statistically significant improvements were observed in all subscales. This could have occurred as a result of a reduction in pain.

There is a case report in the literature in which percutaneous VNS was applied to a patient with treatment-resistant cervical dystonia. The patient had a subjective improvement in mobility, sleep and mood, in addition to dystonia. A decrease in muscle tonus was observed [59. In a study by Busch *et al*. [19], a decrease in mechanical and pressure pain sensitivity and an increase in mechanical pain threshold were found in healthy individuals through noninvasive VNS. Clancy *et al*. [25] revealed that transcutaneous VNS reduces sympathetic activity in healthy individuals. Similarly, a decrease in pain, an increase in pain pressure threshold and grip strength, and an improvement in autonomic nervous system functions were observed in the VNS group in our study. In addition, when the changes observed as a result of the treatment between the two groups were compared, clinically more improvement was achieved in the VAS, algometer and grip strength measurements in the VSS group. It can be stated that VNS increases the effectiveness of application of trigger point ischemic compression and stretching exercises in the treatment of MAS. Although the control group showed more improvement in the SF-36 scale, there was no difference between the two groups when compared in terms of the amount of change. We think that this may be due to the differences that existed between the groups at the beginning. When the changes in the Compass-31 scale were compared, there was no statistically significant difference between the groups, except for the secretomotor subscale. Myofascial TP treatment may cause physiological changes in the autonomic nervous system [26]. Although ischemic compression therapy applied to the control group is a peripheral and local treatment method, it had a central effect on the body and may have affected Compass-31 scores accordingly.

It was observed in our study that the application of VNS in patients with MPS increased the effectiveness of ischemic compression and stretching therapy in terms of pain, algometry and grip strength. This may have occurred as a result of combining a central treatment (VNS) and a local treatment. VNS plays an important role in the modulation of pain and nociception thanks to its central effects [27, 28]. However, there are also studies in the literature indicating that VNS application reduces ischemia [29, 30]. Further studies are needed to more clearly reveal the physiological mechanisms by which VNS increases the effectiveness of ischemic compression and stretching exercises.

Limitations

The limited number of participants in the groups, the absence of a group in which VNS was applied alone, the non-similarity of the effect mechanism that the treatments applied in the VNS group created on the body, and the absence of sham treatments can be considered among the limitations of the study. No side effects related to the treatments were detected in the participants.

CONCLUSION

The application of ischemic compression and stretching stands out as an effective method in the treatment of MPS. This result is consistent with previous studies in the literature. However, no studies examining the effectiveness of VNS in MPS have been found in the literature. Our study is the first of its kind in this regard. It can be stated that VNS increases the effectiveness of the application of trigger point ischemic compression and stretching exercises in the treatment of MPS. More detailed studies are needed to examine the effect of VNS alone or in combination with other treatments in patients with MPS, and to investigate the physiological processes.

Authors' Contribution

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

Conflict of interest

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

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Evaluation of the relationship between Doppler predictors with human papillomavirus types, smear and cervical biopsy results

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ABSTRACT

Objectives: The study aims to investigate whether there is any difference between human papillomavirus (HPV) types, smear results, and colposcopic biopsy results in terms of iliac, uterine, and cervical artery pulsatility and resistive index values in high-risk HPV positive patients.

Methods: Iliac, uterine, and cervical artery pulsatility and resistive index values were determined by pelvic Doppler ultrasonography in patients who applied for high-risk HPV positivity and underwent colposcopy-guided cervical biopsy.

Results: There was no difference between HPV types and Pap-smear results and the pulsatility and resistive indices of the iliac artery, uterine artery, and cervical artery. It was observed that the mean cervical artery pulsatility index of the patients whose colposcopic cervical biopsy result was cervical intraepithelial neoplasia (CIN) 1 was 1.61 ± 0.43 and the cervical artery pulsatility index of the patients with CIN 2-3 was 1.15 ± 0.28, and a statistically significant difference was found between them ($p = 0.038$). There was no difference between other Doppler indices and colposcopic cervical biopsy results.

Conclusions: Doppler indices such as cervical artery pulsatility index may be helpful in the evaluation of cervical cancer precursor lesions.

Keywords: Doppler ultrasonography, human papilloma virus, cervical intraepithelial neoplasia, Pap smear

Cervical cancer is the 4th most common cancer among women worldwide [1]. Almost all cervical cancers are associated with human papillomavirus (HPV). HPV 16 50%, HPV 18 20% and other high-risk types such as HPV 31, 33, 45, 52 and 58 are also responsible for 19% [2, 3]. The HPV test, cervical cytology (PAP-test), or a combination of the two tests can be used in cervical cancer screening. The colposcopic examination is the most valuable method in managing HPV positivity and/or abnormal pap-smear test results.

Angiogenesis is the production of new vessels in a specific area and is required for tumor growth and progression [4]. It has been shown that the progression of the lesion from cervical intraepithelial neoplasia (CIN) to cervical cancer is accompanied by angiogenesis [5, 6]. It has been reported that angiogenesis in cervical cancer is an independent prognostic factor and can predict recurrence [7-9].

Color Doppler ultrasonography (US) is a sonogra-

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phy technique used to evaluate blood flow in the area of interest semi-quantitatively. Pulsatility Index (PI) and Resistive Index (RI) are flow parameters used in doppler ultrasound, mainly used to assess resistive in the vascular system. It is an inexpensive, easily available, and non-invasive examination. Doppler US is an effective method to evaluate cervical carcinoma vascularization, and is associated with specific tumor characteristics and is effective in predicting therapeutic response to treatment [10].

In our study, we aimed to determine whether there is any difference between HPV types, smear results, and colposcopic biopsy results in terms of iliac, uterine, and cervical artery PI and RI values detected by Doppler US in high-risk HPV positive patients.

METHODS

Patients aged between 30-65 years, who applied to the gynecological oncology outpatient clinic due to high-risk HPV positivity and underwent colposcopy-guided cervical biopsy between June 2020 and August 2020 at Zonguldak Maternity and Child Health Hospital, were included in this prospective study. The smear and HPV information of the patients were obtained from the results of the national cervical cancer screening program that the patients had at the time of application. According to the American Society for Colposcopy and Cervical Pathology (ASCCP) guideline, a colposcopy and accompanied cervical biopsy were performed by a gynecologist oncologist (A.T.Ç.). Patients who underwent hysterectomy for any reason, patients with a history of using birth control pills or vaginal drugs, patients with a history of cervical precancerous lesions or cervical cancer, patients who underwent uterine artery embolization, or who received chemoradiotherapy were excluded from the study.

Before colposcopy, all of the patients underwent sonography. Pelvic ultrasound was routinely performed using an Acuson S3000 Ultrasound System (Siemens Healthineers, Erlangen, Germany) equipped with an 8C3 HD convex transducer probe (Siemens Healthineers, Erlangen, Germany), which has color and pulsed Doppler capabilities in the supine position. The procedure was performed after ideal bladder filling. The same radiologist (6 years of experience in genitourinary sonography) evaluated all scans and quantitative measurements and was blinded to the patient's clinical information. Additionally, Doppler parameters are standardized. Morphological evaluation of both ovaries and uterus was performed in systematic gray-scale 3D US examination; to rule out fibroids, endometriosis, adenomyosis, or tumors that may affect vascularization. Color flow Doppler was activated in all cases after morphological evaluation. First, the iliac, uterine, and cervical arteries were identified by color Doppler US, then spectral Doppler parameters were calculated automatically as resistive index (RI) and pulsatility index (PI), and the lowest parameters were used for analysis.

The study protocol was approved by the Ethics Committee of Zonguldak Bülent Ecevit University (Number: 2020/12, Date: 10/06/2020). Written informed consents were obtained from all patients. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows version 20 software (IBM Corp., Armonk, NY, USA). Descriptive statistics for continuous variables were expressed as mean ± standard deviation or median (minimum-maximum), whereas nominal variables were expressed as number and percentage (%). The variables were investigated using visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's tests) to determine whether or not they are normally distributed. ANOVA test was used for variables with normal distribution, and Kruskal-Wallis test was used for those that did not show normal distribution. Tukey's test performed pairwise post-hoc tests when an overall significance was observed. A p value of < 0.05 was considered statistically significant.

RESULTS

A total of 45 patients aged 30-65 years who applied for high-risk HPV positivity and underwent colposcopy and cervical biopsy were included in the study. The mean age of the patients was 42.58 ± 7.31. Twenty-three (51%) patients were HPV 16+, 3 (7%) patients were HPV 18+, 19 (42%) patients were high-risk (other than HPV 16/18) HPV+. Smear results of

Table 1. Doppler indices according to HPV types

	HPV-16	HPV-18	Other HR* HPV	*p* value
IA PI	2.34 (1.94-5.16)	2.78 (2.22-2.98)	2.51 (1.62-3.42)	0.704
IA RI	0.90 ± 0.04	0.86 ± 0.09	0.89 ± 0.04	0.274
UA PI	1.97 ± 0.40	2 ± 0.09	2.10 ± 0.50	0.634
UA RI	0.81 ± 0.07	0.81 ± 0.02	0.82 ± 0.08	0.967
CA PI	1.35 ± 0.34	1.32 ± 0.25	1.43 ± 0.43	0.745
CA RI	0.72 ± 0.10	0.73 ± 0.09	0.76 ± 0.10	0.571

HPV = human papillomavirus, IA = iliac artery, UA = uterine artery, CA = cervical artery, PI = pulsatility index, RI = resistive index

Values are presented as median(minimum-maximum) or mean±standard deviation

*high risk

29 (64%) of the patients were normal, 8 (18%) were inflammation, four (9%) were low-grade squamous intraepithelial lesions (LGSIL), three (7%) were atypical squamous cells of undetermined significance (ASCUS), and one (2%) was insufficient. According to the results of cervical biopsy performed with colposcopy, it was chronic cervicitis in 29 (64%) cases, CIN-1 in 7 (16%) cases, and CIN 2-3 in 9 (20%) cases.

There was no difference between HPV types in terms of the iliac artery, uterine artery and cervical artery pulsatility and resistive indices (Table 1). No difference was found between Pap-smear results in terms of the iliac artery, uterine artery, and cervical artery pulsatility and resistive indices (Table 2).

When colposcopic cervical biopsy results and iliac artery, uterine artery, and cervical artery pulsatility and resistive indices were examined; the mean cervical artery pulsatility index of the patients with CIN-1 was 1.61 ± 0.43, and patients with CIN 2-3 was 1.15 ± 0.28, and a significant difference was found between them (p = 0.038). There was no difference between other Doppler indices and colposcopic cervical biopsy results (Table 3).

DISCUSSION

Cervical cancer continues to be a significant cause of cancer morbidity and mortality in countries without screening programs. HPV is the primary etiological agent of cervical intraepithelial lesion and cervical cancer, and it can be detected in 99.7 percent of cervical cancers [1-3]. Cytology and HPV or both (co-test) are used in cervical cancer screening. A direct biopsy performed under colposcopy is accepted as the gold standard method in the diagnosis of cervical intraep-

Table 2. Doppler indices according to Pap-smear results

	Normal	Chronic Cervicitis	LGSIL	ASCUS	Insufficient	*p* value
IA PI	2.61 (1.66-5.16)	2.50 (2.24-3.38)	2.22 (2.13-2.94)	2.05 (1.62-2.37)	2.78	0.246
IA RI	0.90 ± 0.04	0.92 ± 0.04	0.84 ± 0.05	0.89 ± 0.01	0.88	0.055
UA PI	2.07 ± 0.46	1.83 ± 0.30	2.31 ± 0.46	1.82 ± 0.26	1.85	0.335
UA RI	0.80 ± 0.6	0.80 ± 0.9	0.84 ± 0.5	0.92 ± 0.5	0.83	0.065
CA PI	1.40 ± 0.40	1.38 ± 0.32	1.53 ± 0.34	1.06 ± 0.36	1.45	0.588
CA RI	0.74 ± 0.11	0.75 ± 0.07	0.75 ± 0.05	0.70 ± 0.17	0.73	0.981

LGSIL = low-grade squamous intraepithelial lesions, ASCUS = atypical squamous cells of undetermined significance, IA = iliac artery, UA = uterine artery, CA = cervical artery, PI = pulsatility index, RI = resistive index

Values are presented as median(minimum-maximum) or mean ± standard deviation

Table 3. Doppler indices according to colposcopic cervical biopsy results

	Chronic Cervicitis	CIN-1	CIN-2,3	*p* value
IA PI	2.42 (1.66-5.16)	2.51 (1.94-2.98)	2.22 (1.62-3.45)	0.901
IA RI	0.89 ± 0.04	0.90 ± 0.04	0.89 ± 0.05	0.896
UA PI	2 ± 0.44	2.03 ± 0.23	2.11 ± 0.55	0.804
UA RI	0.8 ± 0.08	0.80 ± 0.03	0.83 ± 0.09	0.785
CA PI	1.40 ± 0.35	1.61 ± 0.43*	1.15 ± 0.28*	**0.038**
CA RI	0.73 ± 0.10	0.81 ± 0.04	0.71 ± 0.12	0.105

CIN = cervical intraepithelial neoplasia, IA = iliac artery, UA = uterine artery, CA = cervical artery, PI = pulsatility index, RI = resistive index
Values are presented as median(minimum-maximum) or mean±standard deviation
*$p = 0.038$ (Tukey Test)

ithelial lesions. However, misdiagnosis and over-treatment may also be mentioned.

Angiogenesis is a rate-limiting step for various pathological conditions, including cancer growth. As the tumor grows and the cells in the center of the tumor become hypoxic, the tumor begins to supply its blood requirement by shifting the angiogenesis stimulator-inhibitor balance in favor of stimulation [11]. Neovascularization is a priority and necessary for tumor progression and metastasis [12]. Angiogenesis is a complex process controlled both negatively and positively by growth factors. The dominant growth factor that regulates angiogenesis is the vascular endothelial growth factor (VEGF) [13].

Transvaginal Doppler ultrasound evaluates the vascularization of the tumor non-invasively. Studies have shown that women with cervical cancer have a lower average PI in the uterine and cervical arteries than healthy women [14, 15]. Hsieh *et al*. [16] reported that intratumoral blood flow evaluated by transvaginal color Doppler ultrasonography correlated with higher proliferation index, higher HPV infection incidence, and pelvic lymph node metastasis in cervical cancer.

Wu *et al*. [17] compared the intratumoral blood flow in the cervical cancer group with the cervical blood flow in the control group. They found that PI and RI values were significantly lower in the cervical cancer group. The authors stated that the evaluation of the intratumoral blood flow of the cervix could be helpful in the early diagnosis and treatment of cervical cancer [17].

Liang *et al*. [18] investigated the role of transvaginal 3D Doppler ultrasonography in the diagnosis of cervical intraepithelial neoplasia. Vascularization index (VI), flow index (FI) and vascularization flow index (VFI) were found to be significantly higher in the early cervical cancer (stage 1a-2a) group than in the control group and high grade CIN group. (CIN 2-3) ($p < 0.01$). Again, when compared with the control group, the VI, FI, and VFI parameters of the high-grade CIN group were found to be significantly higher ($p < 0.01$) [18].

Ping *et al*. [19] investigated the relationship between VI and VEGF expression in three-dimensional color angiography in chronic cervicitis, CIN, and cervical carcinoma. It was observed that VEGF expression gradually increased in chronic cervicitis, CIN, and cervical carcinoma, also VI was correlated with VEGF expression level [19].

In the study conducted by Doğan *et al*. [20], HPV positive patients had higher uterine and cervical artery RI values than the control group. Additionally, they investigated the diagnostic effectiveness of uterine and cervical artery vascularity alone or combined with HPV DNA test and smear. As a result, they observed that combining cervical artery RI with high-risk HPV or smear reduced sensitivity but increased specificity. They also found that combining uterine artery PI with high-risk HPV slightly increased the positive predictivity compared to the high-risk HPV test alone [20].

Although colposcopy-guided cervical biopsy is important for early diagnosis of high-grade CIN and cervical cancer, it scares the patients and is not always accepted because it is an invasive procedure. There is a search for non-invasive examinations to reduce the

colposcopy process. In our study, we examined the difference between HPV types, smear results, and colposcopic biopsy results in terms of the iliac, uterine, and cervical artery PI and RI values detected by pelvic Doppler US, which is a non-invasive and easily tolerated examination by patients. Consistent with the literature, we found a significant difference in cervical artery pulsatility index between patients with cervical biopsy results CIN-1 and CIN 2-3.

Limitations

Our study had some limitations. The main limitation of this study was the smaller sample size (45 cases). Only one radiologist evaluated all scans; interobserver or intraobserver reproducibility could not be evaluated. Sonography was not reproducible since it was an operator-dependent and real-time examination. The data were collected from a single center. In our study, we could not distinguish between premenopausal and postmenopausal, which can affect blood circulation. Our data needs to be supported by prospective, multicenter studies with a large patient population.

CONCLUSION

If our results support multicenter studies, pelvic doppler US can be considered an important step in selecting patients to be referred for colposcopy after cervical cancer screening.

Authors' Contribution

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

Conflict of interest

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

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The investigation of thiol-disulfide homeostasis in patients with diabetic peripheral neuropathy

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ABSTRACT

Objectives: Oxidative stress plays a significant role in the pathogenesis of chronic diabetic complications. Hyperglycemia induced oxidative stress is prominent for the development of diabetic polyneuropathy (PNP). Thiol disulfide homeostasis plays a vital role in antioxidant defense. In this study, we aimed to investigate thiol-disulfide homeostasis, total antioxidant capacity (TAC), and advanced oxidant protein products (AOPP) in patients with PNP.

Methods: Eighty patients with T2DM and 19 healthy controls were included in the study. PNP was assessed by using the Michigan Neuropathy Screening Instrument and Electroneuromyography. TAC, AOPP, and total thiols, native thiols and disulfide levels of thiol-disulfide homeostasis parameters were studied with serum samples. The results were compared in patients with/without PNP and control group.

Results: Serum HbA1c (9.5 ± 2.0% vs 8.0 ± 1.8%; $p = 0.019$) and triglyceride levels (204.4 ± 77.0 vs 151.7 ± 58.5 mg/dL, $p = 0.014$) were significantly higher and serum total thiol levels (540.4 ± 9.9 vs 566.7 ± 2.6 µmol/L, $p = 0.038$) were significantly lower in patients with PNP. Serum TAC, AOPP, native thiol, and disulfide levels were comparable among patients with/ without PNP. Serum CRP, AOPP, total thiol, and native thiol levels were found to be higher in patients with type 2 DM ($p = 0.001$, $p = 0.002$, $p = 0.02$ and $p = 0.03$; respectively) compared to the control group. No correlation was observed between serum thiol-disulfide homeostasis parameters and serum glucose and HbA1c levels.

Conclusions: Our study reveals that oxidative stress markers such as serum TAC, AOPP, and disulfide levels are closely related to the existence of diabetes. No significant difference was noted among patients with and without diabetic PNP.

Keywords: Diabetic peripheral neuropathy, oxidative stress, thiol-disulfide homeostasis, total antioxidant capacity, advanced oxidation protein products

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from insufficient insulin secretion and/or resistance in the body. Previous studies report that oxidative stress,

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imbalance of oxidants, and antioxidants in favor of oxidants in the body, plays a major role in the development, pathogenesis, and progression of chronic complications of DM [1-4].

Diabetic peripheral neuropathy is one of the most common chronic microvascular complications of type 2 DM (T2DM). The pathogenesis of diabetic neuropathy is still unclear. However, currently accepted hypotheses focus on hyperglycemia-induced oxidative stress and their interactions for the development of nerve damage [5].

Proteins, glutathione, homocysteine, and some other molecules contain thiols groups, which are organic compounds that contain functional sulfhydryl groups. Thiol groups may form disulfide bonds under oxidative conditions. These formed disulfide bonds can be reduced back to thiol groups to maintain thiol-disulfide homeostasis. Thiol disulfide homeostasis plays an important role in many physiological processes such as antioxidant defense, protein structures stabilization, and enzymatic activity management [6].

Imbalance in thiol-disulfide homeostasis is thought to play a role in the pathogenesis of DM and obesity [7, 8]. Ates *et al*. [9] assessed the role of thiol-disulfide homeostasis in prediabetic patients in a recent study. Their results suggest that impaired thiol-disulfide homeostasis is important in the development of diabetes and diabetes-related diseases.

Advanced oxidation protein products (AOPP) and total antioxidant capacity (TAC) are also important markers in assessing oxidative stress in diabetic patients [3, 10, 11]. Hyperglycemic state in DM leads to non-enzymatic glycolysis, oxidative and carbonyl stress [3, 10]. The amino acids in the structure of proteins are altered due to oxidative stress and form advanced glycation end products (AGE) and AOPP. The structural alterations in the molecules damage biologically important compounds [10, 11]. AOPP is a cross-linked di-tyrosine protein product and is considered to be a reliable marker for the detection of protein damage [12, 13]. TAC shows the total activity of all enzymatic or non-enzymatic antioxidants [14].

The relationship between DM and thiol-disulfide homeostasis studied in recent studies [15, 16]. To the best of our knowledge, there are no studies that investigated the relationship between diabetic polyneuropathy (PNP) and thiol-disulfide homeostasis.

In the current study, we investigate the relationship between diabetic PNP and thiol-disulfide homeostasis, AOPP, and TAC.

METHODS

Eighty T2DM patients between the ages of 40 and 55 years from the Endocrinology outpatient clinic and 19 healthy volunteers were included in this cross-sectional study. Local Ethical Committee approval was received (02.11.2016 and 2011-KAEK-25 2016/19-03), and informed consent was taken from all patients and healthy participants.

Diagnosis of acute or chronic infectious disease, malignancy, chronic liver disease (AST and ALT ≥ 3×ULN), severe renal insufficiency [glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2], decompensated heart failure, severe cardiac arrhythmia, having a history of acute coronary events within the last six weeks, surgery, burn or severe trauma within the last one month, pregnancy, lactation, smoking and endocrine diseases other than T2DM, hypertension and dyslipidemia were the exclusion criteria for the patients.

The Control group is selected from non-smoker healthy volunteers aged between 40 to 55 years. Any known acute and chronic illnesses, pregnancy, lactation, and usage of antioxidant medication were the exclusion criteria for healthy volunteers.

A detailed physical examination of all participants in the study was performed. Blood pressure was measured using a sphygmomanometer at an upright sitting position after at least 5 minutes of rest. Repeated blood pressure measurements within 2 minutes were obtained by the same physician, and the average of readings was recorded. A platform scale was used for weight measurements. Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared and recorded. The body fat ratio was measured by the bioimpedance method using the Tanita® instrument.

Feldman *et al*.'s [17] two-steps quantitative clinical and electrophysiological assessment was used for the diagnosis and staging of diabetic PNP. The Michigan Neuropathic Screening Instrument (MNSI) and electroneuromyography (EMG) was performed to all

participants with T2DM. Diabetic PNP diagnosis was confirmed in patients with both positive MNSI assessment and EMG results. MNSI, which is used for the assessment of distal symmetrical peripheral neuropathy, includes two separate assessments consist of a 15-item questionnaire and lower extremity physical examination. It is used to assess distal symmetrical peripheral neuropathy in diabetes. EMG study was performed using the Nihon Kohden MEB9102K device. All simulations were performed supra-maximally with bipolar stimulus electrodes. The nerve conduction study was performed in the unilateral upper and lower extremities. Neuro-conduction study protocol includes unilateral studies of sural sensory, ulnar sensory, and median sensory nerves, and peroneal, tibial, median, and ulnar motor nerves with F waves. The minimum case definition criterion for electrodiagnostic confirmation of distal symmetric polyneuropathy is an abnormality ($\geq 99^{th}$ or $\leq 1^{st}$ percentile) of any attribute of nerve conduction in 2 separate nerves, one of which must be the sural nerve 18. T2DM patients were grouped as PNP and without PNP (woPNP) according to MNSI and EMG results.

Laboratory Analysis

Venous blood samples were taken for the measurement of the serum thiol-disulfide homeostasis parameters, TAC, and AOPP levels after 8-12 hours of fasting. Serum samples were centrifuged for 10 minutes at 3000 rpm and stored at -80 ºC. The serum levels of triglyceride (TG), total cholesterol (TChol), HDL-cholesterol (HDL-C), ALT, creatinine and fasting plasma glucose (FPG) were determined using commercially available assay kits with an Olympus AU 2700 auto-analyzer (Olympus Diagnostics, GmbH, Hamburg, Germany). The LDL-cholesterol (LDL-C) was calculated using the Friedewald formula 19. HbA1c level was determined by Adams HA-8160 (Arkray KDK, Shiga, Japan), which uses a cation exchange HPLC method.

Total antioxidant capacity was measured with the ferritic reducing ability of plasma method applied to micro ELISA on a Read well Touch Elisa plate analyzer (Robonik PVT Ltd. Mumbai, India) [20]. The AOPP levels of the samples were determined by the spectrophotometric method developed by Witko-Sarsat *et al*. [21] and defined as µmol/L in chloramine-T equivalents. The thiol/disulfide homeostasis assay was studied according to the method described by Erel and Neşelioğlu [22]. Serum CRP levels were measured by the BN II system nephelometric analyzer (Dade Behring, Germany).

Statistical Analysis

The distribution of continuous data was assessed with the Shapiro-Wilk test of normality. The Mann–Whitney U test or Independent sample t-test was used, when appropriate, to compare differences between the two groups. One-way ANOVA was used to compare more than two independent groups in a normal distribution, and the Bonferroni test was used when significance was found. Kruskal-Wallis test was used for non-normal distribution data, and the Mann-Whitney U test was used in binary comparisons when significant differences were found. Variables are given as mean ± standard deviation. Pearson Chi-square test, Fisher's exact chi-square test, and Fisher-Freeman-Halton test were used for comparison of categorical variables, and data were given with frequency and percentage values. Relations between variables were examined by Spearman's correlation coefficient. $\alpha = 0.05$ was considered as statistically significant. Statistical analyzes were performed in the IBM SPSS Statistics 22 program.

RESULTS

Eighty patients with T2DM (female/male = 46/34) and 19 healthy volunteers (female/male = 15/4) were included in the study. Patients with discordant EMG and MNSI results were excluded, and statistical analysis was performed with 31 patients with PNP, 24 patients woPNP, and 19 healthy volunteers. The demographic characteristics and laboratory findings of the participants are summarized in Table 1.

The mean age of the PNP, woPNP, and control groups were comparable (49.1 ± 4.6 years, 49.3 ± 4.0 years, and 46.5 ± 4.1 years; respectively). There was no significant difference in age, gender, and blood pressure control among the three groups. The mean BMI was significantly higher in PNP group (32.5 ± 7.8 kg/m^2) compared to woPNP (30.8 ± 2.7 kg/m^2) and control group (27.3 ± 4.8 kg/m^2, $p = 0.005$).
While FPG levels were similar (218.1 ± 85.5 mg/dL vs 177.4 ± 67.3 mg/dL, $p = 0.067$), HbA1c levels were

Table 1. Demographics and laboratory results of patients with type 2 diabetes mellitus and controls

	Patients without PNP (n = 24)	Patients with PNP (n = 31)	Controls (n = 19)	*p* value
Age (years)	49.3 ± 4.0	49.1 ± 4.6	46.5 ± 4.1	0.092
Diabetes duration (years)	4.6 ± 3.1	7.2 ± 6.1	-	
Female, n (%)	11(45.8)	19 (61.3)	15 (78.9)	0.087
Male, n (%)	13 (54.2)	12 (38.7)	4 (21.1)	0.080
BMI (kg/m²)	30.8 ± 2.7	32.5 ± 7.8	27.3 ± 4.8	**0.005**
SBP (mmHg)	115.4 ± 8.8	117.7 ± 9.5	107.9 ± 25.7	0.131
DBP (mmHg)	75.0 ± 11.4	75.8 ± 8.8	77.8 ± 10.8	0.655
FPG (mg/dL)	177.4 ± 67.3	218.1 ± 85.5	94.8 ± 9.2	**< 0.001**
HbA1c (%)	8.0 ± 1.8	9.5 ± 2.0	-	
TChol (mg/dL)	218.6 ± 40.5	228.5 ± 45.1	222.3 ± 25.01	0.753
LDL-C (mg/dL)	137.4 ± 30.1	165.3 ± 116.0	138.5 ± 26.3	0.362
TG (mg/dL)	151.7 ± 58.5	204.4 ± 77.0	105.4 ± 52.2	**< 0.001**
HDL-C (mg/dL)	52.0 ± 14.4	45.3 ± 9.3	59.1 ± 16.7	**0.04**
ALT (U/L)	37.2 ± 19.9	37.0 ± 29.4	20.6 ± 9.9	**0.004**
Cr (mg/dL)	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.398
GFR (mL/dk/1.73 m²)	96.3 ± 16.8	96.5 ± 22.7	89.3 ± 14.4	0.248
Total protein (g/dL)	7.2±0.5	7.9±3.5	7.2 ± 0.4	0.921
Albumin (g/dL)	4.5 ± 0.3	4.7 ± 0.2	4.3 ± 0.2	0.212
CRP (mg/dL)	4.29 ± 2.17	5.5 ± 2.97	3.15 ± 0.5	**0.001**
TAC (µmol/L)	946.4 ± 163.6	929.6 ± 231.0	939.5 ± 131.2	0.883
AOPP (µmol/L)	120.3 ± 45.5	122.8 ± 41.5	79.9 ± 35,0	**0.001**
Total Thiol (µmol/L)	566.7 ± 52.6	540.4 ± 39.9	508.4 ± 70.7	**0.001**
Native Thiol (µmol/L)	532.2 ± 43.7	510.4 ± 44.2	470.5 ± 70.9	**0.001**
Disulfide (µmol/L)	17.1 ± 10.20	14.9 ± 8.2	18.9 ± 8.1	0.218

Data are expressed as mean±standard deviation. PNP = Diabetic Peripheric Polyneuropathy, GFR = Glomerular filtration rate, BMI = Body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, FPG = fasting plasma glucose, LDL-C = Low density Lipoprotein, TG = Triglyceride, HDL-C = High density Lipoprotein, TChol = Total cholesterol, ALT = alanine aminotransferase, Cr = creatinine, HbA1c = hemoglobin A1c, TAC = Total antioxidant capacity, AOPP = Advanced oxidation protein products

significantly higher in PNP group (9.5 ± 2.0 % vs 8.0 ± 1.8 %, $p = 0.019$) (Table 1). Although serum TChol and LDL-C levels were comparable, differences in serum TG levels reach statistical significance between the PNP, woPNP, and control groups (204.4 ± 77.0 mg/dL, 151.7 ± 58.5 mg/dL, and 105.4 ± 52.2 mg/dL; respectively, $p < 0.001$) (Table 1). Intergroup comparison reveals that TG levels were significantly higher in the PNP group compared to woPNP ($p = 0.014$). It was observed that HDL-C levels were lower in the PNP group compared to woPNP, but the difference could not reach statistical significance (45.3 ± 9.3 mg/dL vs. 52.0 ± 14.4 mg/dL, $p = 0.132$). HDL-C levels were found to be significantly lower in the PNP group compared to the control group (45.3 ± 9.3 mg/dL vs. 59.1 ± 16.7 mg/dL, $p < 0.001$) (Table 1). The evaluation of serum CRP level shows a significant difference among the groups ($p = 0.001$) (Table 1).

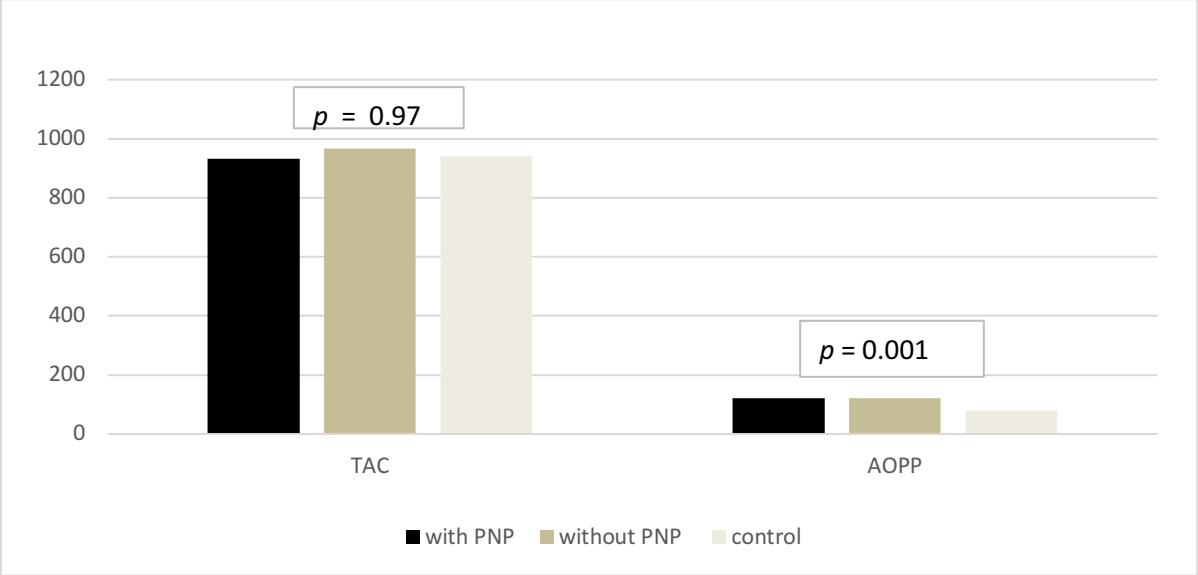

Fig. 1. Serum total antioxidant capacity and advanced oxidation protein products levels of patients with type 2 diabetes mellitus and controls. PNP = Diabetic peripheric polyneuropathy, TAC = Total antioxidant capacity, AOPP = Advanced oxidation protein products.

CRP levels were significantly higher in the PNP and woPNP group than the control group ($p = 0.001$ and $p = 0.02$, respectively).

No significant difference was noted in terms of serum TAC levels among the groups. Serum AOPP level was 122.8 ± 41.5 µmol/L in PNP, 120.3 ± 45.5 µmol/L in woPNP and 79.9 ± 35.0 µmol/L in the control group. While AOPP levels were similar between PNP and woPNP groups, it was higher in PNP and woPNP groups compared to the control group ($p = 0.001$ and $p = 0.002$, respectively) (Fig. 1).

Serum total thiol and native thiol levels were significantly different among the groups ($p = 0.001$) (Table 1). Total thiol levels in woPNP were significantly higher than PNP group (566.7 ± 52.6 µmol/L vs 540.4 ± 39.9 µmol/L, $p = 0.038$) and control group (566.7 ± 52.6 µmol/L vs 508.4 ± 70.7 µmol/L, $p = 0.001$). The native thiol levels were significantly higher in PNP and woPNP groups compared to the control group ($p = 0.004$ and $p = 0.001$; respectively), while no difference was observed between PNP and woPNP group ($p = 0.093$) (Fig. 2).

No significant difference between the serum disulfide levels, disulfide/native thiol ratio, disulfide/total thiol ratio, and native thiol/total thiol ratio when the three groups were compared. No correlation was observed between thiol-disulfide homeostasis parameters and FBG and HbA1c levels.

In terms of medical treatment history, oral antidiabetic drug (OAD) usage was higher in woPNP compared to the PNP group (87.5% vs. 41.9%, $p = 0.003$). Insulin usage with and without OAD treatment (41.9% and 16.1%, respectively) was higher PNP group (4.2% and 13.9%, respectively). The comparison of antihypertensive treatment shows no significant difference between PNP and woPNP group ($p = 0.179$).

DISCUSSION

The development of diabetic neuropathy is closely related to diabetes duration and glycemic control, similar to the other microvascular complications of DM [23]. Studies showed that the duration and severity of hyperglycemia is the most important factor in the development of neuropathy in patients with T2DM [23, 24]. Furthermore, neuropathy development is also shown to be associated with HbA1c, TG levels, BMI, smoking history, and the presence of hypertension [23-25]. In another prospective study, after one year follows up, serum triglyceride levels were found correlated with neuropathy progression independent of glycemic control, diabetes type, and insulin administration [26]. These results consider that classic vascular risk factors are also increasing the chance of diabetic neuropathy development. Consistent with the

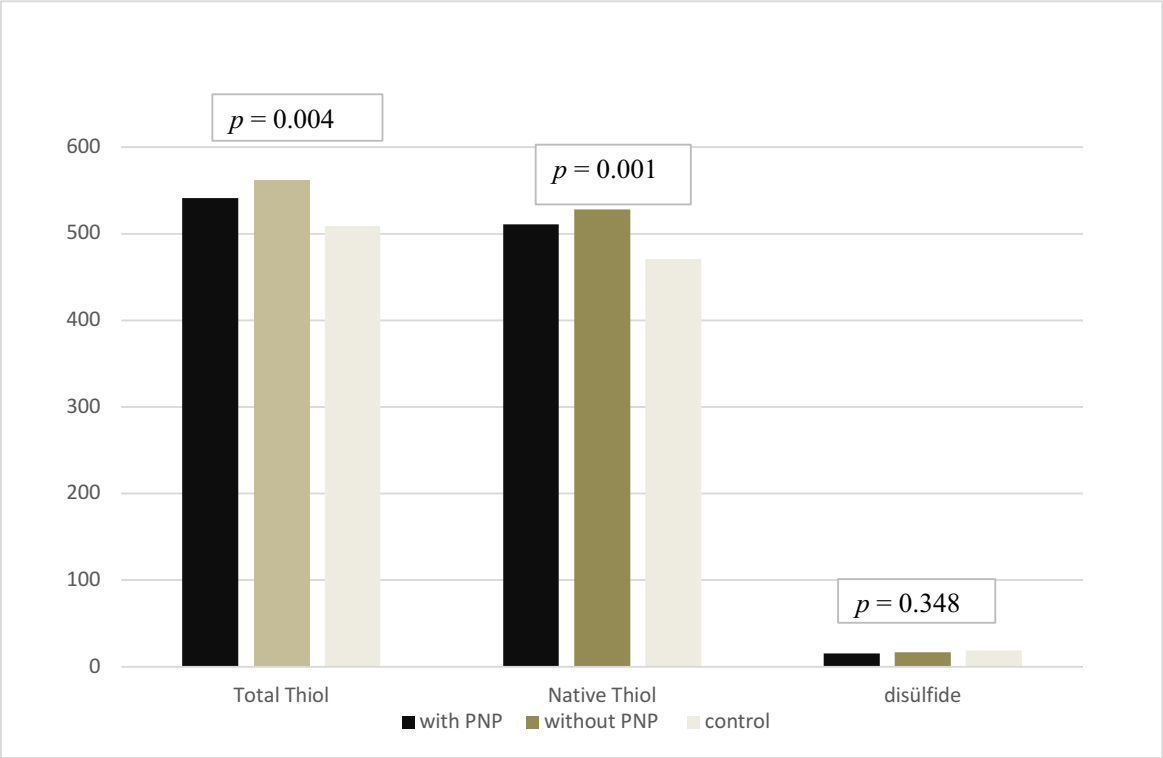

Fig. 2. Thiol-disulfide homeostasis parameters of patients with type 2 diabetes mellitus and controls. PNP = Diabetic peripheric polyneuropathy.

previously published studies, our study also proves that the patients with PNP had worse glycemic control, higher serum TG and lower HDL-C levels. Our results show no relationship between PNP and duration of DM, BMI, and blood pressure measurements.

Dynamic thiol-disulfide homeostasis has shown to contribute to antioxidant protection, detoxification, and apoptosis processes [6-8, 22]. Some molecules, such as proteins, glutathione, and homocysteine, contain thiol groups. Thiols are functional sulfhydryl group, which is oxidized under oxidative conditions and converted to disulfide bonds. The resulting disulfide bonds can be reduced again to the thiol groups, thus attempting to preserve the thiol-disulfide balance. The sum of the existing thiol groups and the reduced thiol groups, also called native thiols, gives the total thiol level. Oxidizing agents in the medium can convert the native thiols to reduced thiol groups, while with the existence of antioxidants, these reduced thiol groups can be converted back to native thiols. Any imbalance in thiol-disulfide homeostasis has also shown to be associated with T2DM and the pathogenesis of obesity [7, 8]. However, there are not enough studies in the literature investigating the relationship between thiol-disulfide homeostasis and diabetic microvascular complications.

Ergin *et al*. [16] showed that patients with T2DM had significantly lower serum total and native thiol levels, higher disulfide, disulfide/native thiol and disulfide/total thiol ratios compared to the control group. Disulfide levels were significantly lower in the newly diagnosed group than the other patients with T2DM. Ateş *et al*. [15] also demonstrated that serum native thiol levels were statistically lower in patients with prediabetes compared to controls. In another study conducted with 30 obese, 27 gestational DM and 68 healthy pregnant women, serum disulfide, disulfide/native thiol and disulfide/total thiol levels increased, and native/total thiols decreased in cord blood of pregnant women with obesity or gestational DM. In addition, the increased levels of disulfide in the cord blood and the reduction of native/total thiol ratio were associated with the poor perinatal outcomes [27]. It has also been reported that serum total and native thiol levels decreased, and disulfide levels increased in patients with type 1 DM [15]. In contrast to these published studies, we observe no significant difference in serum disulfide levels between both patients with and

woPNP and the patients with T2DM and the control group. While native thiol levels were found to be higher in T2DM participants, no significant difference was observed between PNP and woPNP group in our study. The properties of the studied population may have affected our study results. The mean age of the patients was higher, but glycemic control was better in our patients compared to the study population of the Ates *et al*. [15]. Discordant results in our study might be due to the fact that the relationship between PNP and oxidative stress is weaker in advanced age. Moreover, Chakraborty *et al*. [28] showed that metformin treatment restores the altered antioxidant status and inflammatory parameters in patients with T2DM and has antioxidant activity. All of our patients were using metformin treatment, which might explain increased levels of native thiols and decreased disulfide levels in diabetic patients compared with the controls.

Mean serum CRP levels of patients with PNP were found to be significantly increased compared to controls, but no statistical difference was found between the patients with and woPNP in our study. These results may suggest that DM is an inflammatory condition, and the increase of CRP is due to the presence of diabetes rather than diabetic complications. AOPP is an early marker for oxidative stress and is used as a measure of protein damage (predominantly albumin and its aggregates) [13, 21]. Since protein function is strictly dependent on conformation and folding pattern; structural changes in proteins are thought to be among the molecular mechanisms that lead to the complications of diabetes [29]. In addition, the biological effects of AOPPs are similar to those of AGEs and are considered to have a role in inflammatory processes [30]. In our study, mean serum AOPP levels were significantly higher in patients with T2DM. However, there was no difference between those with and woPNP. In our study, HbA1c levels were higher in PNP compared to woPNP, but glycemic control in both groups was not optimal. These results suggest that the AOPP increase is related to poor glycemic control rather than the presence of PNP.

Dordevic *et al*. [31] reported that serum TAC levels, which indicate the total activity of all enzymatic or non-enzymatic antioxidant substances, decreased in patients with T2DM and PNP but serum TAC level did not correlate with blood glucose, diabetes duration, and grade of nerve damage [31]. Although serum TAC level was lower in patients with PNP, no significant difference was noted among the three groups.

Serum CRP, AOPP, total thiol, and native thiol levels were found to be higher in patients with T2DM compared to healthy volunteers. However, no significant difference was observed in terms of serum CRP, AOPP, TAC, native thiols, and disulfide levels between the patients with and woPNP. Although HbA1c levels were significantly higher in PNP group, some studies show that it's possible to occur PNP in prediabetic patients [32].

Limitations

Since our study has a cross-sectional design, there is a need for prospective studies with long-term follow-up and a large number of cases with multiple blood samples taken at different times in order to determine the true role of thiol-disulfide homeostasis in the development of diabetic microvascular complications.

CONCLUSION

These results suggest that oxidative stress parameters assessed in the present study are more closely related to the presence of DM rather than diabetic complications. As a deficiency the example size of the control group is smaller compared to the patient group due to strict exclusion criteria. But, to the best of our knowledge, this is the first study investigates the relationship between dynamic thiol-disulfide homeostasis and PNP.

Highlights

•Serum CRP, AOPP, total thiol, and native thiol levels were found to be higher in patients with T2DM. But the difference between PNP and woPNP group was not significant.

•Oxidative stress parameters assessed in the present study are more closely related to the presence of DM rather than the diabetic complications.

Authors' Contribution

Study Conception: DÜE; Study Design: DÜE; Supervision: SK; Funding: YÜ; Materials: ÖE, NBP; Data Collection and/or Processing: DÜE; Statistical Analysis and/or Data Interpretation: DS; Literature

Review: GE; Manuscript Preparation: DÜE and Critical Review: NK.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Local Ethical Committee (02.11.2016 and 2011-KAEK-25 2016/19-03). Informed consent was taken from all patients and healthy volunteers.

Conflict of interest

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

Financing

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The European Research Journal 2022;8(5):598-605 Original Article

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Understanding the paradigma of opportunistic screening

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ABSTRACT

Objectives: To provide demographical and pathological characteristics of breast cancer patients diagnosed in a tertiary clinic with opportunistic screening and diagnostic workup and compare the results with the available national and global breast cancer statistics.

Methods: Clinical and pathological data of breast cancer patients diagnosed in our tertiary breast clinic between March 14, 2017 and February 28, 2020 have been entered into a database and analyzed retrospectively. Results were analyzed and compared with the national and global statistics.

Results: The total number of patients included in this study were 137 and the number of tumors was 145. Sixty-four (46.7%) patients were detected in screening. All of the patients were female. The mean age was 51.8 years. Eighteen (13.1%) patients were young females (< 40 years), 55 (40.1%) were in 40-49 years, 26 (18.9%) in 50-59 years, 24 (17.5%) in > 60-69 years, 14 (10.2%) in > 70 years. Of the invasive cancers, 100 (79.4%) were invasive ductal, 15 (11.9%) invasive lobular, 6 (4.8%) pleomorphic lobular, 4 (3.2%) papillary, and 1 (0.8%) tubular cancer. Distribution of stages were: 13.1% stage 0, 38.6% stage I, 29.6% stage II, 10.3% stage III, and 8.2% stage IV. The mean tumor diameter was 26.6 mm. Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) were positive in 82.5%, 61.9% and 15.8% of the tumors respectively.

Conclusions: Results of this study are in accordance with the latest results of the National Breast cancer database, a project governed by the Turkish Federation of Breast Disease Societies (TMHDF), considering the tumor size, age distribution, histologic subtype analysis, receptor status. However, the percentage of early-stage tumors was higher in this study.

Keywords: Breast cancer, histology, stages, molecular subtypes, opportunistic screening

Breast cancer is the most frequently diagnosed cancer in women in almost all regions of the world and the most frequent cause of death from cancer [1, 2]. Genetic, geographic, racial, and ethnic differences influence breast cancer characteristics and prognosis. Therefore breast cancer control plans may vary in different regions of the World. To obtain clinical and pathological profiles of breast cancer in different populations is a critical step in determining better screening and disease management protocols.

The Cancer Control Department (CCD) was founded in 1983 to keep reliable cancer records for cancer control. After that National Breast cancer database, a project governed by the Turkish Federation of

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Breast Disease Societies (TMHDF) was embarked. Breast cancer records from 36 centers throughout the country have been collected in this database since 2005 [3, 4]. The final up to date results of the breast cancer registry program were published in 2019 [4]. The reported incidence of breast cancer in Turkey increased more than 2-fold from 24/100.000 in 1993 to 50/100.000 in 2017. Almost 20.000 patients were diagnosed with breast cancer in Turkey between May 1, 2005, and April 17, 2017, according to data recorded by NBCRP. The majority of patients (68%) were at stage 2 or higher and almost half of the patients (48.6%) had axillary lymph node involvement accordingly.

The Bahcesehir Breast Cancer Screening Project (BBCSP) is the first organized population-based breast cancer mammographic screening project in the country and provided data on mammography screening in Turkey. It is a 10-year-long program (2009-2019) implemented in Bahçeşehir, a large region of Istanbul, Turkey. Healthy women aging between 40-69 were invited and screened in every two years of the 10 years. Their first results were published in 2014 [5]. BBCSP resulted in a change in the stage distribution of breast cancers with a significant increase in early-stage cancers. Based on these results Ozmen *et al*. [6] showed the efficacy and cost-effectiveness of breast cancer screening between ages 40 and 69 in their study and concluded that an organized population-based screening program may be cost-effective in Turkey and other developing countries.

This study aims to compare the types and stages of clinically detected and opportunistic screening-detected breast cancers diagnosed in a tertiary reference breast clinic to the national data of TMHDF and the population-based screening of BBCSP.

METHODS

In this study, we analyzed the data of 137 breast cancer patients who were registered in the period from March 14, 2017 to February 28, 2020. The patients who applied to our clinic for screening or diagnostic purposes or who were referred for biopsy after a diagnostic workup in another center were included in this study. A total of 10,015 patients referred to the clinic during this period and 5,984 patients had an opportunistic mammography screening. Histopathologic confirmation was made after US-guided core needle, US-guided or stereotactic vacuum-assisted biopsies. Patients' age, gender, tumor size, stage, histologic type and grade, receptor status, molecular subtype were recorded in a database.

Histologic types and staging were done according to WHO classification and American joint committee on Cancer and histologic grade according to Scarf Bloom-Richardson classification [7].

Estrogen receptor (ER) and progesterone receptor (PR) expression values higher than 1% was accepted as positive. Human epidermal growth factor receptor-2 (HER-2) expression with a (+++) results in immunohistochemistry method or suspected cases a (++) result in immunohistochemistry method) a positive SISH or FISH evaluation were considered as positive Her 2 receptor status. Molecular subtypes were classified as; Luminal A (ER or PR positive + HER-2 negative, ki-67 < 14%), luminal B (ER or PR positive and ki-67 ≥ 14%), luminal B HER-2 enriched (ER or PR positive, HER-2 positive), triple-negative (ER, PR and HER-2 negative) and HER-2-positive (ER and PR negative, HER-2 positive).

This study was approved by the Institutional review board (Acıbadem Mehmet Ali Aydınlar Univer-

Table 1. Age distribution of breast cancer patients

Age groups	Number of cancers	%	Number of women	%
20-29	2	1.4	2	1.5
30-39	18	12.4	16	11.7
40-49	56	38.6	55	40.1
50-59	28	19.3	26	18.9
60-69	26	17.9	24	17.5
> 70	15	10.3	14	10.2
Total	145	100	137	100

sity, decision number: 2020-05/30). The study was conducted according to the Declaration of Helsinki.

Statistical Analysis

For statistical analysis, Pearson chi-square analysis was used in data analysis, and asymptotic or exact *p* values were given. Definitive statistics were shown as frequency and percentile. $p < 0.05$ was accepted as statistically significant.

RESULTS

The number of primary breast malignancies diagnosed after the biopsies carried out in our tertiary breast clinic was 145 in the time interval between March 14, 2017 to February 28, 2020. Eight patients had bilateral involvement wherein six bilateral invasive breast tumor was found, in one patient one side was invasive cancer while the contralateral side was DCIS and one

Table 2. Comparison of data of breast cancer patients in current study with the national statistics

	National Data	Overall Cancers
Number of patients	19503	137
Mean age	51.8	51.8
< 40 (years) (%)	16.6	13.1
≥ 40 (years) (%)	83.4	86.8
≥ 70 (years) (%)	10	10.2
Mean tumor size (cm)	2.5	2.6
DCIS (%)	4.7	13.1
PN0 (%)	51.4	64.3
Stage (%)		
0	4.7	13.1
I	28.5	38.6
II	48.3	29.6
III	14.5	10.3
IV	4	8.2
Histologic subtype (%)		
IDCa	76.9	79.4
ILCa	6.5	11.9
IMCa (IDCa+ILCa)	4.2	4.8
Others	12.4	4.0
Expression status (%)		
ER+	72.5	82.5
PR+	62.3	61.9
HER-2 positive	21.8	15.8
Ki-67 < 14 (%)	35	32.5
Molecular subtype (%)		
Luminal A/B	78.3	80.1
HER-2	9.6	9.5
Triple negative	12.1	10.3

IDCa = invasive ductal carcinoma, ILCa = invasive lobular carcinoma, IMCa = invasive mixed carcinoma, ER = Estrogen Receptor, PR = Progesterone Receptor, HER-2 = human epidermal growth factor receptor-2

Luminal A = ER+, HER-2 (-), Ki67 <14%, Luminal B = ER+, HER-2 (+/-), Ki67 ≥ 14%

patient had bilateral DCIS. The total number of patients diagnosed with breast cancer or DCIS was 137. Of these cancer patients, 64 (46.7%) were diagnosed in screening and the rest 73 (53.3%) were diagnosed after a clinical finding. Clinical findings of the diagnostic group patients were palpable mass and/or skin changes such as retraction or discoloration. A total of 279 biopsy procedures were performed for screening-detected lesions. Of these; 217 were done by US-guided core needle, 11 US-guided vacuum, and 41 stereotactic-assisted vacuum biopsies were performed.

Two interval cancers showed due to high breast density (BI-RADS type B breast density in one and C in the other). Neither of the lesions were visible in the screening mammograms. These two patients presented with palpable lesions in the breast 9 months and 11 months after the screening.

The ages of the patients ranged between 27 and 93. The median and mean ages were 48 and 51.8, respectively. The mean age of patients was 53.2 for the screening group and 50.6 for the diagnostic group. The percentage of patients younger than 40 was 13.1%. The age distribution of the patients is given in Table 1.

Both breasts were affected similarly (51.7% right, 48.3% left). Tumors were found to be located most commonly in the upper outer quadrants of both breasts (50.3%). Thirty-two (25.3%) invasive cancers were multifocal or multicentric.

The pathologic diagnosis of 19 (13.1%) of the tumors were DCIS. The rest 126 (86.9%) lesions were invasive breast cancer. Of the invasive cancers, 100 (79.4%) were invasive ductal, 15 (11.9%) invasive lobular, 6 (4.8%) pleomorphic lobular, 4 (3.2%) papillary, and 1 (0.8%) tubular cancer. Histologic subtype distribution of the invasive tumors are given in Table 2 and Table 3. Multifocal/multicentric invasive carcinomas had the same histology and a single phenotype

Table 3. Stages, lymph node involvement and distribution of molecular subtypes according to the detection method

	Screening -detected cases	Diagnostic cases
Stage *		
0	11 (16.1%)	8 (10.3%)
I	38 (55.8%)	18 (23.3%)
II	13 (19.1%)	30 (38.9%)
III	3 (4.4%)	12 (15.5%)
IV	3 (4.4%)	9 (11.6%)
Total	68	77
Regional Lymph Nodes of Invasive cancers**		
N0	45 (78.9%)	36 (52.2%)
N1	9 (15.8%)	24 (34.8%)
N2	1 (1.8%)	1 (1.5%)
N3	2 (3.5%)	8 (11.6%)
Total	57	69
Molecular Subtypes of Invasive cancers***		
Luminal A	25 (43.9%)	23 (33.3%)
Luminal B (Her-2 negative)	21 (36.8%)	24 (34.8%)
Luminal B (Her-2-positive)	2 (3.5%)	6 (8.7%)
HER-2	5 (8.8%)	7 (10.1%)
Triple negative	4 (7%)	9 (13%)
Total	57	69

*Stages are given for each breast in bilateral cases (145 cases). ($p < 0.001$) ** Lymph node involvement is given for the axillary finding of each breast (126 invasive cancers – 6 bilateral). ($p = 0.009$) ***Molecular subtypes ($p = 0.489$)

in terms of hormone receptors, human epidermal growth factor receptor 2, and molecular subtypes except for cases with associating DCIS; thus, immunohistochemical analyses of the index tumor was sufficient for invasive cancers.

Table 2 shows the comparison of data of breast cancer patients in the current study with the national statistics. The distribution of the stages of these patients at diagnosis was as follows: Stage 0: 13.1%, Stage I: 38.6%, Stage II: 29.6%, Stage III: 10.3%, and Stage IV: 8.2%. The stages according to the detection method (screening or diagnostic) are given in Table 3. Eight patients had bilateral tumors and each breast was staged separately. Lymph node involvement rates of invasive cancers were given in Tables 2 and 3.

Stage 0+1 cancer (TisN0M0 and T1N0/N1miM0) rates for screening and diagnostic group were 72.0% and 33.6% ($p < 0.001$) respectively while pN0 tumor rates were 78.9% and 52.2% respectively ($p = 0.009$). The mean tumor diameter was 26.6 mm and the median tumor size was 20 mm (6-110 mm). Mean and median tumor sizes in screening group were 21.4 mm and 15 mm (ranging between 6 mm and 60 mm) whereas 30.8 mm and 22 mm (ranging between 7 mm and 100 mm) in diagnostic patients respectively.

The rate of Ki-67 value equal to and higher than 14% was in 67.5% of the tumors, while those with a Ki-67 value of > 20% was 42.8 %. Estrogen (ER), progesterone (PR), and HER-2 receptor expression were positive in 82.5%, 61.9 %, and 15.8% of the tumors respectively. The molecular subtype distribution of the overall cancers are given in Table 2. The distribution of molecular subtypes was not significantly different in screening and diagnostic groups ($p = 0.489$) (Table 3).

Summary profile of the screening group and diagnostic group patients for comparison are given in Table 4: 72% and 33.6 % were early-stage, 78.9% and 52.2% were pN0, 84.2% and 76.8% were luminal type, the mean age was 53.2 and 50.6 years, the mean tumor diameter was 21.4 mm and 30.8 mm, median tumor diameter was 15 mm and 22 mm respectively.

DISCUSSION

Breast cancer incidence and mortality rates are rising in developing countries such as Turkey in contradiction to decreasing breast cancer-related mortality rates in developed countries [8]. Westernized lifestyle (weight gain and increasing age at first birth and decreasing number of children born to women), aging population, and opportunistic screening may explain the increase in breast cancer incidence [9]. Downward mortality trends in developed countries reflect the success of screening and improvements in breast cancer management.

The median age of breast cancer in the USA is 62 which means that 50% of patients are over 62. However, in Turkey, the national data showed the median age as 51 and the most populated age group was 45-49 [4]. This can be attributed to the young population age in Turkey. The national breast cancer screening period was changed from 50-69 to 40-69 years of age based on the national data which revealed that the breast cancer cases under 50 years of age constituted 48% of all cases in Turkey [3]. Furthermore, BBCSP showed that more than half of the cancers (55.6%) were detected between 40-49 years in screening [5]. In this study, the results are in accordance with previous findings such as 40-49 being the most populated age group with a 51 year mean age for cancer detection. On the other hand, 13.1% of the patients in our study were younger than 40 while 53.2 % of patients

Table 4. Summary profile of the cancers in screening and diagnostic group for comparison

	Screening-detected	Diagnostic
Stages 0 + 1 cancers	72.0%	33.6%
pN0	78.9%	52.2%
Luminal type cancers	84.2%	76.8%
Mean Age (years)	53.2	50.6
Mean tumor diameter (mm)*	21.4	30.8
Median tumor diameter (mm)*	15	22

*Invasive cancers only

were below 50. The results of this study also support the earlier onset of screening age before 50. The rate of female patients younger than 40 is 13.1% and in line with the 16.6% derivated from the national data [4]. An average of 20% of breast cancer cases in Europe occur in women younger than 50 while 36% is seen between ages 50-64 and the remaining over 64 [10]. In the US the percentage of young female patients under 40 is 4% [2]. This dramatic difference between national and US and European statistics may be attributed to a relative over-population in the younger women in Turkey. On the other hand, similar findings are reported in the Asian women stating the peak of incidence of breast cancer in between 40 and 50 years with an increase in incidence and rising mortality [11]. The authors remark the merit of further studies evaluating a possible contribution of environmental, genetic, or biologic factors.

In the United States, DCIS accounts for almost 20% of all newly diagnosed breast cancers [2]. However, DCIS patients constituted only 4.7% of all patients diagnosed with breast cancer in Turkey [4]. In our study 13.1% of tumors were DCIS. A relatively low percentage of DCIS patients in Turkish national statistics compared to US statistics can be explained by the lack of population-based screening programs and low breast cancer awareness. BBCSP showed high detection of DCIS and early-stage cancers in screening where 22% had DCIS, and 61% had stage I invasive breast cancer while only 16.6% of invasive cancers were axillary node-positive [5]. In the screening group of the current study, 16.1% of patients had DCIS, 55.8% had stage I cancer and 21.1% were node-positive cancers. Compared to BBCSP results we had a relatively lower percentage of stage 0 and 1 cancer but higher than the national data. A recent study showed similar findings with a lower DCIS detection rate in the opportunistic screening group compared to population-based organization [12]. They have explained this difference with a possible more effective evaluation of the mammograms in organized screening programs by specifically trained screening radiologists. Opportunistic screening was found less sensitive in detecting occult cancers compared to organized screening due to the lack of experience of the radiologists in the clinical setting because of fewer readings. [13]. However, this is not the case in breast specific radiology units. This may be true for centers where mammograms are read by general radiologists with fewer mammography reading experience. On the other hand, another study showed higher rates of DCIS in favor of the opportunistic screening compared to the organized program [14]. We agree on the differences in evaluating screening mammograms in a clinical setting and an organized screening program. However, it is unlikely to produce such an outcome as the evaluation in a breast clinic is long and detailed compared to screening settings and done by skilled radiologists on breast imaging. We can explain the difference in our clinic with the contamination of opportunistic screening by the attendance of women with hidden or unclaimed symptoms or findings. It is a high probability that women with findings may apply for annual screening mammograms without claiming their complaints and the rate of such application is not low in opportunistic settings. ACR benchmarks for mammography screening are as follows: median size of invasive cancers (in mm) 14.0, percentage node-negative invasive cancers 77.3%, percentage stage 0+1 cancer 74.8% [15]. Our results of screening-detected cancers are 15mm, 78.9%, and 72% accordingly. The percentage of pN0 and stage 0+1 cancers obtained by BBCSP are 83.4% and 83% [5]. Although our rates for both DCIS and stage 1 cancers were relatively lower than organized screening results of the BBCSP they were within the acceptable limits of international benchmarks [15].

The rate of stage 0+1 and pN0 cancers were higher in this study compared to the national data: pN0 cancers (64.3% and 51.4%, respectively) and stage 0+1 cancers (51.7% and 33.2%, respectively) [4]. In US and Europe, pN0 tumors were consisted of 53% and 46% while locally advanced tumors were twice as frequent in Europe (8%), and metastatic tumors were of similar frequency (5-6%) [16]. According to the SEER data from 1975 to 2012, the rate of T0 and T1 tumors increased from 36% to 68% and the rate of T3 or larger tumors decreased from 64% to 32% [17]. This significant change in favor of smaller tumors are attributed to the initiation of screening. Our findings are in line with the SEER data showing that 33.6% of the tumors detected in the diagnostic group are smaller than 2 cm while 72.0% in the screening group. Accordingly, findings of decreasing tumor size and stage are reported from various countries in Europe [18-23]. A similar finding is reported from Asia where stage III

cancers decreased from 40% to 20% from 1970 to 1990 in China and stage I cancers increased from 19.3% to 36% from 1970 to 1990 1996 to 2004 in South Korea [24].

In histologic subtype analysis of the current study invasive ductal carcinoma (79.4%) was the most frequent type similar to national (76.9%) statistics. Invasive lobular cancer (11.9%) was almost two-fold higher compared to the national data (6.5%). The percentage of mixed/pleomorphic type (IDca +ILca) was similar in both studies (4.8 and 4.2). According to US statistics, more than 75% of invasive breast cancers are invasive ductal carcinomas and invasive lobular carcinoma represents about 15% of invasive breast cancers [2].

When molecular subtypes obtained in this study are compared to national data the rate of Luminal type (80% vs 78%), HR(hormone receptor)negative/HER-2 positive (9.5 % vs 9.6%), and triple-negative (10.3% vs. 12.1%) tumors were similar [4]. According to SEER breast cancer subtype HR+/HER2- was the most common subtype, representing 73% of all cases, triple-negative breast cancer 12%, HR+/HER2+ breast cancer 11% HR-/HER2+ breast cancer 4% [2]. HR-/HER2+ cancer rate of this study and national statistics was higher than US results.

This study showed a significant difference between the opportunistic screening and diagnostic patients in terms of the axillary involvement ($p = 0.009$), stage of the cancer ($p < 0.001$), DCIS detection rate and mean tumor size which are the main benchmarks of a better outcome. A recent study evaluating the opportunistic screening showed its efficacy with a reduced mortality which was comparable to population-based screening outcomes [12]. Although our study does not provide its effect on mortality our screening results are comparable with the population-based screening of BBCSP and showed significant differences compared to diagnostic patients. On the other hand, studies show that opportunistic screenings are less cost-effective and end up with higher costs compared to an organized program. However, participation higher than 55% of the targeted population is recommended for the efficacy of an organized screening over an opportunistic approach [25]. We believe that raising the awareness and improvement of the facilities for opportunistic screening will be effective in the detection of earlier cancers in countries where participation of at least 55% of the targeted population is far from reach.

Limitations

The main limitation of this study is a possibility of contamination of the screening group by women with unclaimed symptoms or findings. As this is a tertiary diagnostic clinic this possibility has a higher potential. However, the similarity of our findings with the BBCSP lowers the likelihood of its unfavorable effects. Besides this, we have meticulously evaluated the patient files for the differentiation of screening and diagnostic applications. The second limitation of the study is its retrospective and one center design.

CONCLUSION

Results of this study showed that the cancers detected in this study are similar to the national data considering the tumor size, age distribution, histologic and molecular subtypes. To our knowledge, this is the first study in Turkey comparing opportunistic screening to clinical breast cancer detection and organized screening. Opportunistic screening in a tertiary clinic shows comparable results with the organized screening program. We believe that it is an effective screening method for countries with limited resources where participation of the critical mass (55% of the targeted population) is far from reach.

Main Points

*In this study there is a significant difference between the opportunistic screening and diagnostic patients in terms of the axillary involvement, stage of the cancer, and tumor size.

*Characteristics of cancers detected in this study are similar to the national data considering the tumor size, age distribution, histologic and molecular subtypes.

*Opportunistic screening in a tertiary clinic may show comparable results with the organized screening program.

Authors' Contribution

Study Conception: NG, İD, AA; Study Design: NG, EY; Supervision: EY; Funding: AS; Materials: İD; Data Collection and/or Processing: AA; Statistical

Analysis and/or Data Interpretation: NG; Literature Review: NG; Manuscript Preparation: NG and Critical Review: NG, AA.

Conflict of interest

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

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Effects of intrauterine devices on female sexual function: a cross-sectional study

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ABSTRACT

Objectives: To examine the differences in sexual functions between women using copper intrauterine device (Cu-IUD) and levonorgestrel intrauterine system (LND-IUS).

Methods: A total of 125 women between the ages of 20 and 40 were included in the study. The demographic data of all participants were recorded. Female Sexual Function Index (FSFI) was performed to all participants. Study participants were divided into 3 groups; 42 Cu-IUD users, 42 LND-IUS users, and 41 control (not use contraceptive) group, then compared. Women with a total score lower than ≤ 26.5 were considered as having sexual dysfunction.

Results: The prevalence of female sexual dysfunction (FSD) was 56.8% among the participants. The total FSFI scores of Cu-IUD and LNG-IUS groups were similar. Moreover, the FSFI score of both IUD users was lower than the control group, and the difference was statistically significant ($p < 0.001$). The presence of Cu-IUD affected arousal more than pain, satisfaction, lubrication and orgasm scores ($p = 0.016$). Pain score was similar among IUD groups and significantly lower than controls ($p < 0.001$). Moreover, orgasm and satisfaction scores were found to be significantly higher in the control group than in the both IUD groups ($p < 0.001$). All of three groups were also similar about desire and lubrication domains of FSFI.

Conclusions: In conclusion, this study found that Cu-IUD and LNG-IUS users did not differ in terms of sexual function according to scores calculated by FSFI.

Keywords: Female sexual dysfunction, female sexual function index, copper intrauterine device, levonorgestrel intrauterine device, sexuality

Intrauterine device (IUD) is the second most widely used modern contraceptive method worldwide [1]. IUD is a safe and effective method of contraception that primarily acts by inhibition of fertilization mechanisms [2, 3]. Approximately 23% of women in the world prefer the IUD as a contraceptive method, this ratio is around 14% in our country, Turkey [4].

Intrauterine contraceptives (IUCs) include the copper intrauterine device (Cu-IUD) and the levonorgestrel-releasing intrauterine system (LNG-IUS). The LNG-IUS could induce amenorrhea or irregular and mild intermenstrual bleeding in most of its users. The Cu-IUD users may complain about intermenstrual or prolonged menstrual bleeding and pelvic discomfort [5]. The features such as being long-acting, safe, cost-effective, independent from sexual intercourse, not inhibiting breastfeeding, rapid return of fertility after the method is stopped, make this method preferred by millions of women. Despite having several advantages, IUD might have some adverse effects on

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women's overall health, especially on their sexual health [6].

Sexual health is defined by the World Health Organization (WHO) as a state of physical, emotional, mental and social well-being with sexuality [7]. Sexual life is one of the important factors affecting the women's quality of life. Female sexual dysfunction (FSD) is a group of psycho-sexual disorders that include some or all of the problems such as sexual desire, orgasm, arousal disorders and sexual pain [8]. FSD is a common problem worldwide, and its prevalence varies by ethnicity, race, religion and region of residence [9]. Various scoring systems have been developed for the diagnosis of FSD. The most widely used of these is "Female Sexual Function Index" (FSFI). FSFI is an index that investigates the physiological and psychological aspects of patients' sexual functions together and was first defined by Rosen *et al* in 2000 [10].

Contraception method preferences of women may be affected by the physical and psychological side effects that the current method might cause. In this context, the fact that the method may cause sexual dysfunction emerges as an important factor that may affect the choice decision. Although there are various studies on the adverse effects of the IUD on women's health, there seems to be insufficient data on its effects on sexual function. Therefore, we aimed to investigate whether the use of Cu-IUD or LNG-IUS in women has any effects on sexual function. For this aim, we calculated and compared the FSFI scores of women using the IUDs and not using any contraceptive methods.

METHODS

This cross-sectional study was conducted in Bursa Yüksek Ihtisas Training and Education Hospital gynecology clinic after obtaining local ethics approval (2018/12-35). Women applying for routine gynecology examination using any contraceptive methods for at least six months were asked to fill the FSFI. 38 women did not want to fill the questionnare, 125 women aged between 20-40 years were included in the study. Each participant's age, body mass index (BMI), parity, educational status, income, partner's age, duration of sexual intercourse, contraceptive method, duration of contraception were recorded. Income was divided into three subgroups as low- < 2000 Turkish liras (TL) per month, medium- 2000-5000 TL per month, high- > 5000 TL. Education status was defined as primary school- five or eight years, high school and college.

Women diagnosed with systemic diseases, endometriosis, pelvic inflammatory disease, previous pelvic surgery, premature menopause, pelvic organ prolapse, incontinence and infertility, smokers, women taking any medicine, obese patients were excluded from the study. Individuals were grouped according to their contraceptive methods. Cu-IUD (Nova TCu380A®) group consisted of 42 women. 42 women were in LNG-IUS (52 mg, Mirena®; Bayer) group. 41women using no contraception (either using traditional methods or desiring pregnancy) were defined as control group.

Female sexual function was evaluated using a validated FSFI questionnaire [10]. FSFI is a questionnaire that consists of nineteen multipl choice questions, includes desire, arousal, lubrication, orgasm, satisfaction and pain subtitles. A total score less than ≤ 26.5 was considered as FSD. Higher scores indicated beter sexual function [11]. The scale shows the sexual function of women in the last 4 weeks by calculating 6 subgroup scores and FSFI score. After the first evaluation, women who were eligible for the study were asked to fill in FSFI in a private, quiet hospital room under the supervision of the researchers. After the questionnaires were filled, they were taken by the researchers and archived to form a database.

Statistical Analysis

Data were analyzed by SPSS 22.0 for Windows (SPSS Inc, Chicago, IL, USA) statistics programme. The normality of distribution was assessed by Shapiro-Wilk test. Analysis of variance (ANOVA) and Kruskall-Wallis tests were used for analysis of continuous variables. Chi- square test was used for categorical data. Spearman rho coefficients were calculated for correlation analysis. $p < 0.05$ was considered statistically significant.

RESULTS

Both IUD users and control individuals were similar among age, parity, BMI, partner age, duration of using

Table 1. Demographic, clinical characteristics and FSFI scores of patients among groups

Characteristics	Copper IUD (n = 42)	LNG-IUS (n = 42)	Control (n = 41)	*p value*
Age (years)	33.50 ± 4.64	34.69 ± 4.80	32.70 ± 4.13	0.139
Parity	2 (1-5)	2 (1-4)	2 (1-5)	0.149*
BMI (kg/m^2)	25.88 ± 4.55	25.17 ± 3.16	26.39 ± 4.37	0.125
Partner's age (years)	37.09 ± 4.92	38.00 ± 6.33	35.34 ± 6.42	0.121
Duration of relationship (years)	12.97 ± 5.47	13.40 ± 7.01	10.31 ± 6.01	0.059
Duration of use (months)	29.35 ± 10.56	31.50 ± 10.19	32.07 ± 10.93	0.467
Income				0.061**
Low	13 (31.0)	5 (11.9)	5 (12.2)	
Medium	26 (61.9)	28 (66.7)	27 (65.9)	
High	3 (7.1)	9 (21.4)	9 (22.0)	
Education				0.216**
Primary school (5 years)	18 (42.9)	16 (38.1)	9 (22.0)	
Primary school (8 years)	11 (26.2)	8 (19.0)	11 (26.8)	
High school	9 (21.4)	11 (26.2)	10 (24.4)	
College	4 (9.5)	7 (16.7)	11 (26.8)	
FSFI subgroups				
Desire	6.83 ± 1.32	6.40 ± 1.90	6.65 ± 1.23	0.432
Arousal	13.85 ± 2.48	12.35 ± 1.97	12.90 ± 2.61	**0.016**
Lubrication	14.40 ± 2.06	13.38 ± 2.32	14,31±2,69	0.095
Orgasm	9.35 ± 2.26	10.30 ± 1.99	11.31 ± 1.75	**< 0.001**
Satisfaction	8.47 ± 2.72	10.42 ± 2.50	11.85 ± 1.74	**< 0.001**
Pain	9.83 ± 2.36	9.95 ± 2.42	12.43 ± 2.11	**< 0.001**
Total FSFI Score	23.64 ± 3.48	23.84 ± 3.58	26.40 ± 2.95	**< 0.001**

Values are given as mean±standart deviation or number (%). IUD = intrauterine device, LNG = levonorgestrel, FSFI = female sexual function index.

*Kruskal-Wallis test was used, values are given as median (range).

**Chi square test was performed.

the contraceptive method, income and educational status (Table 1).

FSFI scores of patients are also given in Table 1. Groups were similar among desire and lubrication domains of FSFI. Arousal score was 13.85 ± 2.48 in Cu-IUD group and significantly higher than the other groups (p = 0.016). Orgasm and satisfaction scores were 11.31 ± 1.75 and 11.85 ± 1.74 in the control group. Post-Hoc analysis revealed that orgasm score and satisfaction score were significantly high in control groups ($p < 0.001$). Pain score was similar among IUD groups and significantly lower than controls ($p < 0.001$). According to the post-hoc analysis total FSFI score was highest in the control group, significantly different from IUD groups. The total FSFI scores of Cu-IUD and LNG-IUS groups were similar (Table 1). Seventy-one of 125 women had sexual dysfunction. Percentage of women with FSD were higher than women without FSD in both IUD groups (p = 0.049). On the other hand in the control group 41.5 percent of women had FSD (Table 2).

The relationship between FSFI domains were calculated. There was positive significant correlation between pain and satisfaction scores, likewise in orgasm and satisfaction scores (r = 0.490, $p < 0.001$; r = 0.664, $p < 0.001$). No correlation was found between desire

Table2. Distribution of female sexual dysfunction among groups

Groups*	Women with FSD n (%)	Women without FSD n (%)
Copper IUD	28 (66.7)	14 (33.3)
LNG-IUS	26 (61.9)	16 (38.1)
Control	17 (41.5)	24 (58.5)
Total	71 (56.8)	54 (43.2)

Data are given as number (%). FSD = female sexual dysfunction, IUD = intrauterine device, LNG = levonorgestrel

*Chi square test was performed ($p = 0.049$).

and satisfaction. Also arousal and lubrication scores were correlated (r = 0.410, $p < 0.001$).

DISCUSSION

In this study, we aimed to examine the sexual function differences among women using the Cu-IUD and the LND-IUS. Approximately 40% of women in the world experience one or more sexual problems [9]. While the FSD rate is reported to be approximately 43-57% in Turkey [12-14], these rates are given as 34-40% for women in the USA and Europe [15]. Consistent with the literature, the prevalence of FSD among all participants in our study was determined to be 56.8%. The sexual dysfunction is affected by various individual factors such as psychological, biological, social, economic, political, ethnic characteristics, and religious beliefs. Therefore, the regional differences in incidence remain acceptable.

In many previous publications, it has been reported that LNG-IUSs worsen sexual function and have a higher quite rate than Cu-IUDs [16, 17]. These results are mainly due to unplanned bleeding effect and possibly other progestogenic side effects of LNG-IUSs [17, 18]. In a cross-sectional study, in 153 women with Cu-IUDs and LNG-IUSs, it was reported that the women using LNG-IUS as a contraceptive method were five times more likely to report a subjectively negative effect on sexual function compared to those using Cu-IUD [19]. However, inclusion criteria and characteristics of the patient population were not clearly specified in this study, and a valid questionnaire was not used. On the other hand, there are several studies reporting that LNG-IUSs have no effect on sexual function. In an observational study, the authors analyzed whether 31 users of the LNG-IUS showed any differences in quality of life and sexual function 12 months after the IUD implantation, and whether IUD had any effect on these variables. They found no significant difference in the results [20]. Furthermore, Sanders *et al*. [21] followed the LNG-IUS and the Cu-IUD users for one-year, they could not detect any differences between the compliance rates of the users. Bastianelli *et al*. [22] applied the FSFI questionnaire to 158 women before and one year after the LND-IUS implantation and reported that there was no significant difference between the two questionnaire scores. Similarly, in our study, there was no significant difference between the total FSFI scores of the Cu-IUD and the LNG-IUS groups. In this study, risk factors that may cause FSD were examined and there was no significant difference between individual characteristics of all groups.

There are many studies in the literature evaluating the effects of the Cu-IUD, the LNG-IUS or other contraceptive methods on female sexuality. However, there are few data comparing the effects of two types of IUD on sexual dysfunction with control (not use contraceptıve) group [6]. In this study although the IUDs groups had similar FSFI scores, the total FSFI score was the highest in the control group, significantly different from the IUDs groups ($p < 0.001$). Menstrual abnormalities (hypermenorrhea or menorrhagia, etc.) caused by the Cu-IUD might be the reason for the lower FSFI scores of these women. So considering that the Cu-IUD may increase menstrual flow or LNG-IUS may cause spotting and irregular bleeding, the lower scores of sexual function in women using IUDs could be attributed to this situation. According to the epidemiological studies, we could mentioned that the possible side effects of the IUDs on female sexuality have conflicting results and are still controversial.

In this study, the scores of the FSFI domains were also evaluated separately. The presence of Cu-IUD seems to effect arousal score. All groups were also similar among desire and lubrication domains of FSFI. But in another research, it was reported that the desire

and arousal scores of women using LNG-IUS were higher than Cu-IUD and control groups [6]. In our study, pain score was similar among IUD groups and significantly lower than controls ($p < 0.001$). Likewise, several previous studies have found that IUDs can reduce pain scores in sexual function [6, 23, 24]. On the other hand, Sakinci *et al*. [25] reported that Cu-IUDs increased sexual pain compared to women with no contraception, and this finding may negatively affect female sexuality. The patient's age and Cu-IUD status were found to be correlated with FSFI domain of pain. Elnashar *et al*. [26] found that 31.5% of the healthy women experienced pain problems during sexual intercourse, while Valadares *et al*. [27] was determined this rate as 39.5%. Valadaras *et al*. [27] stated in their study that the risk of experiencing dyspareunia decreased in those whose frequency of sexual intercourse was more than 3 times a week. In our study and most previous studies, there is no data about the frequency of sexual intercourse for all participants [23-25]. More studies are needed to explore the physical and psychological aspects of partners' sex lives.

In the present study, we showed that there might be a decrease in sexual function scores and an increase in the rate of FSD in the presence of IUDs in women. The data of previous reports are challenging due to the heterogenity of individuals' sociodemographic characteristics. Moreover, our groups were similar among some characteristics such as age, parity, BMI, partner's age, duration of contraceptive use, income, and education level. Thus, the present research could contribute to a better understanding of the effects of both IUDs on sexual function scores, unlike many literature publications.

The strength of this study is the use of valid standardized questionnaire to investigate the level of sexual dysfunction in infertile women. Moreover, the addition of a control group contributed to the power of the study.

Limitations

There are also some limitations of this study such as being single center and having a relatively small sample size. If an additional quality of life assessment questionnaire had been administered to the participants, more information about the participants could have been obtained and a more reliable interpretation of the results could have been made. In addition, the educational status, socio-cultural and socio-economic levels of the participants might have affected the answers given and indirectly the results of the study.

CONCLUSION

This study found that the FSFI scores of women using the Cu-IUD and the LNG-IUS were similar and these results were significantly lower than women who did not use any contraceptive method. The potential effects of a contraceptive method on a woman's quality of life and sexual function might influence the choice of the method. In this respect, current literature data is still insufficient to understand and manage the relationship between IUD types and sexual dysfunction, and further studies with larger populations are needed.

Authors' Contribution

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

Conflict of interest

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

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Investigation of the relationship between contrast nephropathy development and body mass index in patients receiving contrast media in the emergency department

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ABSTRACT

Objectives: This study aims to investigate the risk factors in patients presenting to the emergency department, undergoing contrast-enhanced computed tomography (CT), and developing contrast-induced nephropathy (CIN) and whether there is a relationship between CIN and body mass index (BMI).

Methods: A total of 336 patients presenting to the emergency department between 15.3.2019 -31.12.2019 and underwent CT by administering intravenous (IV) contrast agent (CA) were prospectively analyzed. Patients' age, gender, chronic diseases, height, weight, BMI, and hospitalization or discharge information were recorded. Control creatinine was measured at 72nd and 120th hours. Patients who developed CIN were recorded.

Results: The mean age of the patients was 57 years (min: 18-max: 96) and 56.5% were male. CIN developed in 6.5% of the patients. Congestive heart failure (CHF) was associated with the increased risk of CIN (p = 0.045). There was a significant increase in CIN risk in patients aged 40-70 years (p = 0.008). The risk of CIN development was increased with advanced age (p = 0.002). Dialysis was required in 13% of patients who developed CIN. There was no significant relationship between BMI and CIN development (p = 0.740).

Conclusions: We did not find a significant relationship between BMI and CIN. However, the risk of CIN development was higher in patients over 40 and especially in patients with CHF.

Keywords: Contrast-induced nephropathy, body mass index, emergency medicine, contrast-enhanced computed tomography.

Globally, contrast agent (CA) is used to monitor approximately 60 million patients per year [1]. All water-soluble, nephrotropic iodinated CAs have a direct toxic effect on renal epithelial cells and may cause contrast-induced renal medullary ischemia [2]. With the spread of diagnostic and therapeutic imaging methods and the effectiveness of contrast agents, the use of imaging methods is increasing, which causes an increase in side effects related to CA. Early and late side effects or even death can be seen due to CA use. The most important side effect is contrast-induced nephropathy (CIN) [3]. CIN is a 25% increase in basal serum creatinine value or at least 0.5 mg/dl increase in absolute serum creatinine value within the first 48 hours after radiographic contrast agent use [4]. The incidence of CIN has been calculated as 2% in the gen-

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eral population, but its incidence reaches 20-30% in high-risk groups such as elderly patients and patients with diabetes, congestive heart failure, and chronic kidney disease [5].

The use of contrast media for diagnostic purposes is increasing day by day in emergency departments. However, the number of studies on the frequency of CIN in the emergency department is very limited in the current literature. CIN is a potentially preventable clinical condition. The first step to prevent CIN is to identify risk factors. Mortality and morbidity associated with CIN will be reduced by identifying high-risk patients, taking necessary precautions, and applying appropriate treatments. Therefore, we aimed to evaluate the frequency of CIN occurring after contrast-enhanced tomography, to contribute to predict CIN risk, and to investigate whether there is a relationship between body mass index (BMI) and CIN. Our study is the first study conducted in an emergency service in terms of determining whether there is a relationship between CIN and BMI.

METHODS

Six thousand two hundred patients patients who presented to the Emergency Service of the University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital between 15.03.2019-31.12.2019 and underwent contrast-enhanced computed tomography were prospectively included in the study. Five thousand eight hundred and sixty-four patients were excluded because they did not meet the criteria. A total of 336 patients were included in the study. Ethics committee approval was obtained with the date and number of 2011-KAEK-25 2019/03-16 prior to the study.

Patients with a history of chronic renal failure (CRF), who were pregnant, who were under the age of 18, who refused to give consent, who received contrast media in the last two weeks, whose creatinine value could not be measured before or after contrast agent application at 72nd and 120th hours, who were discharged from the emergency department and were not followed up, who were in shock, and who had a diagnosis of sepsis were excluded.

The Patient Information and Consent Forms prepared according to the Declaration of Helsinki were read to the patients and their signed consents were obtained. PHILIPS 128 Multislays computed tomography device was used in the emergency radiology unit. Nonionic contrast agent (iopromide, Ultravist™; 370 mg I/ml -100 mL vial Bayer Schering Pharma and Iohexol, Omnipaque GE Healthcare 350mg I/ml-100ml) was used as intravenous contrast agent. Saline was intravenously infused to the patients before and after tomography scanning. Age, gender, chronic disease history, height, weight, BMI, creatinine value before tomography, creatinine values at 72nd and 120th hours after contrast-enhanced tomography were recorded.

Statistical Analysis

Data were analyzed with SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.), and type I error level was accepted as $\alpha = 0.05$ in statistical analysis. The conformity of height, weight, and body mass index measurements to normal distribution was examined with the Shapiro Wilk test. Descriptive statistics were expressed as median (minimum: maximum) and mean ± standard deviation, while the Mann Whitney U test was used for comparisons between groups with and without contrast nephropathy. Categorical variables were expressed as n (%), and Pearson chi-square, Fisher's exact chi-square and Fisher Freeman-Halton tests were used for comparisons between groups. As other causes that could cause acut renal failure (ARF) were used as exclusion criteria, in contrast-enhanced CT group, those with a 25% increase in serum creatinine values measured before the contrast agent administration (0 hour) and at 72nd and 120th hours after administration were accepted as having contrast agent nephropathy. In the study, the body mass index was calculated as kg/m^2 in all patients.

RESULTS

A total of 336 patients were included in the study. 56.52% (n = 190) of the patients were male while 43.5% (n = 146) were female. There was no statistically significant difference in the development of CIN in patients in terms of gender ($p = 0.255$).

The mean age was 57 years (min: 18-max: 96). Patients were divided into three as young (18-40 years), middle aged (41-70 years) and old (71 years and over) and risk analyses were performed. There

Table 1. Age groups and comorbidities

Age group	n	%
18-40	68	20.2
40-70	183	54.5
71 and over	85	25.3
Total	336	100
Comorbidities	**n**	**%**
DM	87	25.8
HT	144	42
CHF	12	3.5
CVD	65	19.3
CAD	27	8
Other	57	16.9

DM = diabetes mellitus, HT = hypertension, CHF = congestive heart failure, CVD = cerebrovascular disease, CAD = coronary artery disease

was no statistically significant difference in the development of CIN in young patients ($p = 0.255$) while a statistically significant increase in terms of the CIN development risk was found in middle-aged patients ($p = 0.008$). In addition, the risk of developing CIN in elderly patients increased even more ($p = 0.002$). 110 (32.7%) of 336 patients did not have any comorbidities. The distribution of comorbidities and age groups of the cases are shown in Table 1.

It was seen that 100 (30%) of 336 patients were discharged from the emergency department, while 236 (70%) were hospitalized. 22 (6.5%) of the patients developed CIN. The mean age of the patients with CIN was 64. 68.2% (n = 15) of the patients were males. 14 (4.1%) of the hospitalized patients developed CIN.

When the relationship between CIN and comorbidities (diabetes mellitus [DM], hypertension [HT], congestive heart failure [CHF], cerebrovascular disease [CVD], coronary artery disease [CAD]) in 22 patients who developed CIN was examined, a significant statistical relationship was found between CHF and CIN development ($p = 0.037$). The mean height of the group with CIN was found to be 167.09 cm. The mean height was 161 cm for females and 172 cm for males. No statistically significant difference was found between the CIN development and height ($p = 0.991$). The mean weight in the group with CIN was 77 kg. The mean weight was 76 kg for females and 78 kg for males. No statistically significant relationship was found between the development of CIN and weight ($p = 0.835$).

The mean BMI of the patients with CIN was found to be 26.05 kg/m^2. The mean BMI was found to be 29.3 kg/m2 for females and 26.4 kg/m^2 for males. The relationship between CIN and BMI was examined and no statistically significant relationship was found ($p = 0.740$). Table 2 shows the relationship between CIN with comorbidities in addition to the height, weight, and BMI of patients. Using BMI, the patients were divided into four as underweight, normal weight, overweight and obese and their CIN development risk was evaluated. No statistically significant relationship was found ($p = 0.886$). Table 3 shows the results of the statistical analysis between BMI and CIN. The basal creatinine and glomerular filtration rate (GFR) values of the patients and the control creatinine and GFR values are shown in Table 4.

Table 2. Data regarding CIN, comorbidities, height, weight, and BMI

Comorbidities	With CIN	Without CIN	*P* value
DM	9	78	0.096
HT	13	131	0.111
CHF	3	9	0.037
CVD	2	63	0.272
CAD	3	24	0.404
BMI	26.05	26.85	0.74
Height	167.50	167	0.991
Weight	77	75	0.835

DM = diabetes mellitus, HT = hypertension, CHF = congestive heart failure, CVD = cerebrovascular disease, CAD = coronary artery disease, BMI = body mass index, CIN = contrast-induced nephropathy

Table 3. Data regarding the relationship between CIN and weight

Weight	With CIN	Without CIN	*p* value
≤ 18 (underweight)	0	8	0.886
19-25 (normal weight)	7	97	
25-30 (overweight)	9	118	
31 ≤ (obese)	6	91	

CIN = contrast-induced nephropathy

Tablo 4. Creatinine and GFR values

	Basal	Control
Creatinine (mean ± SD)	0.94 ± 0.39	0.90 ± 0.51
GFR (mean ± SD)	86.01 ± 26.14	89.91 ± 27.18

GFR = Glomerular filtration rate

DISCUSSION

In this study, we aimed to investigate the risk factors and the relationship between CIN and BMI in patients who presented to the emergency department and developed CIN after contrast-enhanced tomography.

CIN is defined as an increase in serum creatinine greater than 25% or ≥ 0.5 mg/dL occurring within 3 days of intravenous contrast agent administration without an alternative cause and is defined as the third cause of hospital-acquired ARF [6]. It is associated with hospitalization, increased length of hospital stay, and high mortality rates [7].

The mechanism of formation of CIN is not known precisely and in detail. However, hemodynamic changes in the kidney (contrast-induced biphasic response in renal blood flow, shunt of medullary blood flow to the cortex, tubulo-glomerular feedback), free radicals and reperfusion damage (the formation of free oxygen radicals as a result of hypoxia, resulting in oxidative stress and apoptosis), tubule direct toxicity and immunological damage to cells, hematological factors (increase in blood viscosity as a result of decrease in erythrocyte flexibility and development of medullary hypoxia as a result) are blamed in the physiopathogenesis of CIN [8].

Although renal functions usually return to their former state with appropriate treatment, CIN development is clinically associated with long and short-term survival, the need for hemodialysis, prolonged hospitalization, increased cost, mortality, and morbidity [9].

Volume expansion and the use of low osmolar contrast media are the most effective methods to prevent CIN development [10]. However, in our study, despite intravenous saline infusion and the use of low osmolar contrast media before the procedure, CIN developed in some patients, which makes the data even more valuable. Nash *et al*. [11] determined that the third most common cause of ARF in hospitalized patients was CIN. The number of studies investigating the relationship between the development of CIN and the use of contrast media in the emergency department is very limited. Most studies have been conducted on patients undergoing percutaneous coronary intervention. Mitchell *et al*. [12] reported the incidence of CIN due to contrast-enhanced CT scan as 11% in outpatients in the emergency department. In their study in which the frequency of CIN related to the use of contrast agent for abdominal CT in the emergency department was investigated, Kim *et al*. [13] reported the frequency of CIN as 4.5%. In our study, we found the frequency of CIN to be 6.5%, which is consistent with the literature.

The incidence of contrast media nephropathy increases with age. Although there is no certain age limit, it has been shown in the literature that the risk increases in patients aged 60-75 years [14]. Being 75 and over is accepted as a risk factor in many scoring system. In our study, the incidence of CIN was seen to increase with age ($p = 0.004$). Studies have reported that the rate of CIN development is higher in women [15]. Kiski *et al*. [16] found a higher rate of CIN development in women in their study, and stated that increase might be due to the fact that female patients included in the study were older, had lower eGFR, had a higher DM diagnosis, and most of the female patients were using loop diuretics. In the study of Isler *et al*. [17], on the other hand, the risk of CIN was found to be higher in men. In our study, no significant

relationship was found between the development of CIN and gender ($p = 0.225$).

In the study of Marenzi *et al*. [18], which included 208 patients who underwent percutaneous coronary intervention, DM was not defined as a risk factor. In Koruk's thesis study [19], which included 342 patients in the emergency room, DM was not found to be a risk factor. Similarly, in our study, when diabetic patients were compared with non-diabetic patients, no risk was found in terms of CIN development in patients with diabetes ($p = 0.096$).

Rihal *et al*. [9] reported that HT is a risk factor for the development of CIN [9]. Additionally, Mehran *et al*. [20] expressed that HT is a risk factor for the development of CIN. In the study of Marenzi *et al*. [21], on the other hand, hypertension was not defined as a risk factor for the development of CIN. In our study, no CIN development risk was found in patients with HT ($p = 0.111$).

In literature, heart failure has been reported as an increased risk factor for the development of CIN [14]. There are some studies reporting a left ventricular ejection fraction (LVEF) < 40% as a risk factor for CIN [22]. Those studies were performed on patients undergoing percutaneous coronary intervention. In our study, an increased risk was found for the development of CIN in patients with CHF ($p = 0.037$).

In a retrospective study on 7586 patients who underwent percutaneous coronary intervention Rihal *et al*. [9] reported that patients with a history of CVDs or transient ischemic attack were at increased risk for the development of CIN. In their study including 8357 patients who underwent percutaneous coronary intervention, Mehran *et al*. [23] did not define previous CVDs as a risk factor for the development of CIN. In our study, there was no statistical significance in terms of CIN development risk in patients with a history of CVDs ($p = 0.272$).

We did not find any studies investigating the relationship between CIN and CAD in the literature, and no relationship was found between CIN and CAD in our study ($p = 0.404$).

BMI is a good indicator of intravascular volume. Considering the data showing that the risk of developing CIN increase in patients with intravascular volume deficiency, it may be a guide in identifying risky patients [24].

When the publications comparing kidney size and body parameters were examined, it was found that there was a linear relationship between kidney size and BMI [25]. Accordingly, it is reported that in the evaluation of kidney dimensions, the values measured by ultrasonography should be examined by considering BMI. In the study of Weisenbach *et al*. [26], in which they evaluated 330 normal children, kidney size was found to be associated with body weight and height. Schmidt *et al*. [27], examined 717 infants aged between 0 and 18 months, and kidney sizes were found to be associated with age, gender and BMI in this study. In the study of Cohen *et al*. [25], in which kidney volume was measured by magnetic resonans, a statistically significant correlation was found between kidney volume and height, weight and BMI. While a strong relationship was found between kidney volume and body weight, a moderate relationship was found with height and a weak relationship with BMI [25]. In their study, Şengül *et al*. [28] showed a significant relationship between BMI with serum uric acid level, glucose, and HbA1c in patients with chronic renal failure. In another study, Güngören *et al*. [29], demonstrated that BMI was an independent predictor of CIN development in patients undergoing cardiac catheterization. In our study, however, BMI was not found to be as an independent predictor of CIN.

In the study of Kandemir *et al*. [30], on the prevalence of contrast nephropathy in patients who underwent percutaneous coronary intervention in acute coronary syndrome, a significant correlation was found between CIN and BMI ($p = 0.044$). In our study, however, no significant relationship was found between the development of CIN and BMI ($p = 0.740$). We think that the application of hydration in all patients in our clinic may have played a role.

In the study of Nikolsky *et al*. [31], 3.1% of patients who developed CIN required dialysis. In the study of Marenzi *et al*. [32], hemofiltration was started four to six hours before the procedure and continued for 18-24 hours in the patients in the intensive care unit for angiography. Hemofiltration was stopped during angiography. As a result, a 45% reduction in the development of CIN, a 22% decrease in the need for renal replacement therapy, and a 20% lower mortality rate were reported [32]. In the study of Gruberg *et al*. [33], it was reported that the need for dialysis was up to 35%. In our study, dialysis need developed in 3 of the patients who developed CIN. One of these patients

died on the 45th day of intensive care follow-up, and one required permanent dialysis (operated with aortic dissection).

It has been reported that ARF developing after surgical intervention is most commonly seen in patients undergoing cardiovascular surgery. Major surgery has been identified as a risk factor for the development of postoperative renal failure and the most common cause is hemodynamic instability observed during the operation [34]. During our study, one of the patients who underwent cardiac surgery needed permanent hemodialysis. We think that the majority of the previous studies were on patients who underwent percutaneous coronary intervention and the frequent use of cardiac surgery after the procedure in these patients may have been the cause.

Limitations

The fact that our study was single-centered and some patients who were discharged from the emergency department did not come for follow-up and thus were excluded may have affected the results. In addition, since our hospital is a regional trauma and stroke center, the inability to give preventive treatment to patients because of an urgent need for imaging before CT angiography may have had an impact on the results.

CONCLUSION

CIN is a condition that can lead to mortality and morbidity. However, it is preventable on condition that causes are known and precautions are taken. Therefore, identifying the causes is of the utmost importance. In our study, we found that the risk starts in patients over 40 years old and especially in those with CHF. In addition, patients over 70 have more risks compared to the other age groups. However, we did not find a significant relationship between BMI and CIN. Considering the results, mortality and morbidity associated with CIN can be reduced by measures to be taken before and after contrast-enhanced CT scan in elderly patients with a history of CHF. This should also be considered, since there is no significant relationship between BMI and CIN.

Authors' Contribution

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

Conflict of interest

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

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Traditional and complementary medicine use for knee osteoarthritis

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ABSTRACT

Objectives: This study aims to determine the traditional and complementary medicine (TCM) use in patients with knee osteoarthritis, by whom these methods are recommended, which methods provide the patients with the best outcome, and to contribute to the awareness of physicians about TCM methods.

Methods: One hundred four patients over the age of 40 who were diagnosed with knee osteoarthritis and had complaints for at least 6 months were included in the study. The TCM applications in the previous treatments of the patients, and whether they used additional medications or not, and finally, the TCM method they benefited from the most, and who recommended and applied these methods were questioned and recorded.

Results: In our study, we found that the most commonly used treatments were phytotherapy products and supportive drugs, in line with the literature. We did not find the use of hypnosis, hirudotherapy, reflexology, homeopathy, osteopathy, chiropractic, maggot applications, apitherapy, or music therapy methods. TCM methods of patients, we determined that they learned from their families and close circles rather than the doctors they applied to.

Conclusions: Patients diagnosed with knee osteoarthritis commonly use TCM methods, phytotherapy being in the first place.

Keywords: Alternative treatment, phytotherapy, pain, musculoskeletal diseases, degenerative diseases, arthralgia

Knee osteoarthritis is one of the most common forms of arthritis, with pain and limited function [1]. Knee osteoarthritis incidence and prevalence have been increasing due to aging, an increase in life expectancy, and the epidemic of obesity [2]. It is reported that approximately 250 million people suffer from knee osteoarthritis throughout the world and the associated treatment costs sum up to $185,5 billion per year only in the United States of America (USA) [3, 4], whereas World Health Organization (WHO) estimates that 9.6% of the males and 18% of the females, over 60 years of age, get diagnosed with osteoarthritis [5]. The main focus of treatment is to relieve pain, restore function, and slow down disease progression [6, 7]. There are six key recommendations comprising diagnosis, self-management, physiotherapy, pharmacotherapy, orthobiology, and complementary and integrative health care available in the latest guide published in the USA in 2020 for the non-surgical treatment of knee osteoarthritis (Table 1) [8]. Most commonly, pharmacological treatments have been adopted. However, the potential side effects of pharmacological treatment (digestive problems, heart failure, and renal impairment) limited their use. In

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addition, the dissatisfaction with traditional treatments due to medical, psychological, social, and economic costs leads patients and physicians to seek alternative methods [8, 9]. Traditional medicine is defined as the sum of knowledge, skills, and practices based on the theories, beliefs, and experiences of different cultures in the prevention, diagnosis, and treatment of physical and mental diseases an in the protection and improvement of health [9]. Complementary medicine comprises the applications and products that are not available in the country's tradition or currently used modern medicine but available in various fields of medicine and the health care system [9]. These practices were adopted also by WHO, whereas a field of study called Traditional, Complementary and Integrative Medicine has been constituted [9]. Complementary/ alternative/ traditional/ functional/ integrative medicine/ folk medicine/ non-traditional medicine terms are being used interchangeably in various countries [10]. WHO reported that 88% of its 170 member states use complementary treatment [11]. Evidence-based assessment is usually difficult due to the poor methodology of studies in this area; however, these treatments are being considered natural and adopted by patients and specialists. Besides, patients may believe that these treatments are natural and safe, even though they may be unwilling to communicate with their health care professionals regarding medication use. The characteristics of these methods such as easier access, cost efficiency, and a longstanding increase in their usage rates. Traditional and complementary medicine (TCM) applications, known as GETAT in Turkey, have been gradually increasing throughout the world since the 1990s. The Regulation comprises acupuncture, ozone, mesotherapy, prolotherapy, hypnosis, hirudotherapy, reflexology, homeopathy, phytotherapy, osteopathy, chiropractic, maggot applications, apitherapy, cupping, and music therapy

Table 1. Non-surgical treatment suggestions for knee osteoarthritis

Diagnosis
-Self- management: Exercise, weight loss and bracing
-Physical therapy
-Pharmacotherapy
a. Topical pharmacotherapy
b. Oral pharmacotherapy (acetaminophen and/or oral non-steroidal anti-inflammatory drugs), duloxetine, opioids (including tramadol)
c. Intra-articular injections
1. intra-articular corticosteroid injection
2. intra-articular viscosupplementation injection
-Orthobiologics:
a. platelet-rich plasma injections
b. stem cell injections (eg, mesenchymal adipose-derived, and bone marrow-derived)
-Complementary and Integrative Health:
a. Dietary Supplements, and Nutraceuticals: Avocado and soybean extract, Boswellia serrata, Cannabidiol, Chondroitin, Curcumin, Collagen, Glucosamine, Glucosamine plus chondroitin, Methylsulfonylmethane, Omega-3 fatty acid, Pycnogenol, Rosehip, Traditional Chinese medicine, Vitamin D, Vitamin E, Willow bark extract.
b. Acupuncture
c. Massage
d. Light touch
e. Meditation
f. Tai chi
g. Yoga

methods. Even though the TCM methods seem to be accepted among physicians, many health specialists are devoid of the necessary information required to help patients make decisions. Despite the best application recommendations, the integration of complementary treatments into knee osteoarthritis management may be controversial and there is no international consensus on whether the complementary treatments must be included in knee osteoarthritis management and when or how this must be done [10, 11].

This study aims to determine the treatment management and TCM use in patients with knee osteoarthritis, by whom these methods are recommended, which methods provide the patients with the best outcome and to contribute to the awareness of physicians about TCM methods.

METHODS

This prospective, cross-sectional study has been conducted upon the ethics committee's approval between 01/06/2021 and 01/11/2021. All procedures have been carried out in compliance with the Principals of the Helsinki Declaration developed by the World Medical Association. 104 patients over the age of 40, who applied to the Physical Medicine and Rehabilitation (PMR) outpatient clinic with knee pain, were diagnosed with knee osteoarthritis, had complaints for at least 6 months, and had been followed up for 3 months, were included in the study. In all the patients, knee osteoarthritis was diagnosed and classified by clinical evaluation and anteroposterior radiograph of the osteoarthritic knee. The radiographic imaging of the stiffer knee was evaluated based on the Kellgren-Lawrence grading system (K-L) [12]. According to K-L, knee osteoarthritis can be doubtful (grade I); mild (grade II); moderate (grade III); or severe (grade IV). Patients with severe systemic disease, malignant disease, acute infection, being pregnant or in breastfeeding period, with psychiatric and neurological diseases causing communication challenges, patients who had knee joint replacement, suffering from acute meniscus and ligament injuries were excluded from the study. It was questioned whether the patients had been treated before with one of the TCM methods recommended by the Ministry of Health. The study was introduced to the patients who had been treated with at least one TCM method in the last five years and met the required criteria, whereas the patients who agreed to participate were included in the study and were asked to sign an informed consent form. Patients were questioned in terms of age, gender, height, weight, body mass index BMI), educational background, employment status, previous job if not employed (office or physical labor), presence of additional disease, smoking, major injury in the knee (fracture and/or soft tissue trauma in the knee that requires a cast or splint application) or recurrent minor injuries (fall, sprain history that does not require a cast or splint application), number of pregnancy and number of children, age of menopause, history of stress/anxiety/depression (the patients undergoing treatment due to the presence of any of these complaints), hobby activities (activities performed by bending the knees or kneeling), the manner of praying (regular or seated), toilet type (European or squat type), use of high heels, drugs used, whereas the obtained information was recorded in a form. Besides, all participants were questioned in terms of previous physical therapy, spa treatment, whether they exercise regularly, intraarticular (IA) injections (corticosteroid, viscosupplementation), orthobiological treatments (platelet-rich plasma injections (PRP), stem cell injections (mesenchymal adipose-derived, and bone marrow-derived) (MSC), dietary supplements (glucosamine sulfate, chondroitin sulfate, and collagen of animal origin). Knee pain severity was evaluated according to the Visual Analogue Scale (VAS). VAS; A common, simple method with a well-established validity and reliability. The physician described each one of the numbers from 0 to 10 marked on a 10 cm line and asked the patients to mark the severity of pain on the move, at rest, and at night separately (0 = no pain, 5 = moderate pain, 10 = very severe pain). The presence of varus/valgus deformities was checked and recorded during the examinations performed while the K-L score and presence of chondrocalcinosis were checked and recorded in the x-rays taken. The TCM applications (acupuncture, ozone, mesotherapy, prolotherapy, hypnosis, hirudotherapy, reflexology, homeopathy, phytotherapy, osteopathy, chiropractic, maggot applications, apitherapy, cupping, and music therapy) in the previous treatments of the patients, and whether they used additional medications or not, and finally, the TCM method they bene-

Table 2. Distribution of demographic characteristics

		n (%)
Age	54 and younger	22 (21.2)
	55-64	45 (43,3)
	65 and older	37 (35.6)
Gender	Female	89 (85.6)
	Male	15 (14.4)
Educational background	Primary School	83 (79.8)
	High School	17 (16.3)
	Undergraduate	4 (3.8)
Employment	Employed	11 (10.6)
	Unemployed	93 (89.4)
Previous employment	Office	7 (6.7)
	Housewife	67 (64.4)
	Physical labor	30 (28.8)
BMI	Normal	11 (10.6)
	Overweight	33 (31.7)
	1st degree obese	33 (31.7)
	2nd degree obese	27 (26)
Smoking	Smoker	14 (13.5)
	Non-smoker	90 (86.5)
Diabetes	Diabetic	33 (31.7)
	Non-diabetic	71 (68.3)
Hypertension	Available	52 (50)
	Not available	52 (50)
Major trauma in the knee	Available	5 (4.8)
	Not available	99 (95.2)
Repeating knee trauma	Available	29 (27.9)
	Not available	75 (72.1)
Stress/anxiety/depression	Available	80 (76.9)
	Not available	24 (23.1)
Number of pregnancy	0	18 (17.3)
	1-2	32 (30.8)
	3-4	36 (34.6)
	5 and more	18 (17.3)
Number of children	0	19 (18.3)
	1-2	42 (40.4)
	3-4	34 (32.7)
	5 and more	9 (8.7)
Age of menopause	40 and younger	15 (14.6)
	41-50	68 (65.2)
	51 and older	21 (20.2)

BMI = Body Mass Index

fited from the most, and who recommended and applied these methods were questioned and recorded.

Statistical Analysis

Data analysis was performed with SPSS 26.0 software and the study was carried out with a 95% confidence level. Frequency (n) and percentage (%) are provided for categorical (qualitative) variables while average (X), standard deviation (ss), minimum and maximum statistics are provided for numeric variables. Chi-square test techniques and independent samples t-test were used in the study. In the study, independent samples t-test was used to compare the benefits from the applications according to VAS and K-L measurements, and the chi-square test was used for the relationship between the benefit from the applications and demographic characteristics

RESULTS

The distribution of demographic and some clinical characteristics of the 104 patients included in the study is shown in Tables 2 and 3. The mean value of the knee VAS was 5.93 ± 1.21 (range: 3-9), and the mean value of the KL score was 2.71 ± 0.63 (range: 2-4). It is determined that 50.96% of the patients had at least one PMR before, and the treatments they received and benefited from are listed in Table 4. Although the most received treatment method is phytotherapy (59.6%), the most beneficial treatment is IA VS treatment (25.9%). When TCM treatments were evaluated (Table 5), it was determined that the first preferred treatment was phytotherapy (59.6%), and the most beneficial methods were phytotherapy (20%) and mesotherapy (20%). In Table 6, however, the comparison of VAS and KL measurements according to the benefits of the treatment methods applied is shown. There was a significant difference in VAS score between patients who used and did not use phytotherapy. It was determined that patients with mild pain preferred this treatment. On the contrary, it was determined that those with severe pain preferred this treatment in patients who had leeches (Table 6). In addition, patients with high K-L scores preferred viscosupplementation, while patients with lower K-L scores preferred cupping therapy (Table 6).

Table 3. Distribution of other clinical features

		n (%)
History of swelling in the knee	Available	56 (53.8)
	Not available	48 (46.2)
Hobby activities	Available	21 (20.2)
	Not available	83 (79.8)
Manner of prayer	Regular	33 (31.7)
	Seated	50 (48.1)
	Not available	21 (20.2)
High heel usage	Available	13 (12.5)
	Not available	91 (87.5)
Toilet type	European style	86 (82.7)
	Squat toilet	18 (17.3)
Medication	Paracetamol	18 (17.3)
	NSAID	68 (65,4)
	Not available	18 (17.3)
Exercise therapy	Available	23 (22.1)
	Not available	81 (77.9)
Number of TCM treatment	1	27 (26)
	2	52 (50)
	3-4	25 (24)
Varus/valgus	Varus	55 (52.9)
	Valgus	7 (6.7)
	Not available	42 (40.4)
Chondrocalcinosis	Available	22 (21.2)
	Not available	82 (78.8)
Medical advice	Available	25 (24)
	Not available	79(76)
TV, internet	Available	42 (40.4)
	Not available	62 (59.6)
Neighbor, relative advice	Available	60 (57.7)
	Not available	44 (42.3)

TCM = Traditional Complementary Therapy

DISCUSSION

Due to the potential side effects of pharmacological and surgical treatments, ACR reported that it supports the use of non-pharmacological treatments, including physiotherapy and exercising (13). The prevalence of physiotherapy intended for knee osteoarthritis has been reported in the literature at rates varying between 24.8% and 52% [14-16]. Consistent with the literature, this rate is found to be 50.96% in our study. Numerous studies are showing that spa treatment is beneficial in knee osteoarthritis, and it is widely being used throughout the world [17, 18]. In our study, 47.1% of our patients had received spa treatment at least once before. Numerous studies are evaluating the clinical effects of VS, steroids, PRP, or MSC injections in the treatment of knee osteoarthritis. In a meta-analysis performed by the use of steroids and VS is recommended for suitable patients with knee osteoarthritis [19]. For pain and inflammation, it is of the opinion that steroids are probably the best treatment whereas it is followed by VS. It could not be shown that single PRP, multiple PRP, and adipose MSC interventions cause a significant reduction in joint pain or improvement of joint function compared to placebo. Nevertheless, the differences in treatment effects are small, clinically insignificant, and there are other factors such as cost and patient preferences. These facts may change the treatment preferences of patients with knee osteoarthritis. In our study, 56.7% of the patients were treated with VS, 54.8% with steroids, and 18.3% with PRP. When the treatment that the patients benefited from the most was questioned, 25.9% of the patients preferred VS, 14.4% preferred steroid, and 6.7% preferred PRP in the first place. The prevalence of TCM use is particularly high among osteoarthritis patients throughout the world [20-23]. A recent study has emphasized that more than one-third of adults with osteoarthritis seek complementary care to manage their health and that dialogues that encourage open and non-judgmental communication and sharing between health care providers and patients to provide the best possible patient care are necessary [22]. Also in these studies, considering the fact that patients do not provide accurate information about the use of TCM, and they hide that they benefit from TCM, it is thought that the rate of resorting to these methods may be even higher. WHO states that the integration of these applications into health systems will promote the safe and effective use of TCM [11, 24, 25]. In countries such as China, Korea, USA, Germany, Switzerland, Cuba, Japan, and Chile, 40-86% of the population resort to TCM at least once a year [26]. The methods used vary

Table 4. Distribution of the patients who resort to intra-articular injections, orthobiologics, diet supports, Spa and TCM methods and the most effective therapies

		n (%)	n (%)*
Phytotherapy	Available	62 (59.6)	3 (2.9)
	Not available	42 (40.4)	
Viscosupplementation	Available	59 (56.7)	27 (25.9)
	Not available	45 (43.3)	
Steroid	Available	57 (54.8)	15 (14.4)
	Not available	47 (45.2)	
Spa	Available	49 (47.1)	10 (9.6)
	Not available	57 (52.9)	
Supportive medication	Available	49 (47.1)	2 (1.9)
	Not available	57 (52.9)	
Cupping/bloodletting	Available	43 (41.3)	10 (9.6)
	Not available	61 (58.7)	
Leech	Available	32 (30.8)	2 (1.9)
	Not available	72 (69.2)	
Prolotherapy	Available	25 (24)	7 (6.7)
	Not available	79 (76)	
Acupuncture	Available	20 (19.2)	7 (6.7)
	Not available	84 (80.8)	
PRP	Available	19 (18.3)	7 (6.7)
	Not available	85 (81.7)	
Mesotherapy	Available	18 (17.3)	10 (9.6)
	Not available	86 (82.7)	
Ozone	Available	9 (8.7)	4 (3.8)
	Not available	95 (91.3)	
Massage	Available	6 (5.8)	0
	Not available	98 (94.2)	
Stem Cell injections	Available	1 (0.96)	0
	Not available	103 (99)	

*1st most effective therapy . PRP = Platelet-Rich Plasma, TCM = Traditional Complementary Therapy

according to the country's geographical localization, ethnic origin, educational and socioeconomic factors, and religious beliefs, lifestyles, and cultures. Paltiel *et al*. [27] determined that the types of TCM used the most in Israel were homeopathy, relaxation therapy, and reflexology, while spiritual treatments, vitamins and herbs, and mind/body approaches are being used in the USA. Frass *et al*. [28] state that an increase in using TCM was observed in all countries between 1990 and 2006, whereas the methods being used the most are herbal therapy, chiropractic, massage, and homeopathy. While the TCM methods used the most in Western countries are multivitamins, meditation, hypnotherapy, homeopathy, acupuncture, relaxation exercises, and aromatherapy, while herbal therapies seem to be prioritized in the East. In a study conducted, the rate of TCM usage in Turkey was found to be 60.5% [29]. In another study, it was found that 48%

Table 5. Distribution of the most preferred and most effective TCM applications

	n (%)*	n (%)**
Phytotherapy	62 (59.6)	20 (19.2)
Supportive medication	49 (47.1)	10 (9.6)
Cupping/bloodletting	43 (41.3)	19 (18
Leech	32 (30.7)	5 (4.8)
Prolotherapy	25 (24)	13 (12.5)
Acupuncture	20 (19.2)	12 (11.5)
Mesotherapy	18 (17.3)	20 (19.2)
Ozone	9 (8.6)	6 (5.7)
Massage	6 (5.8)	2 (1.9)

TCM = Traditional Complementary Therapy
*Distribution of most preferred applications, **Distribution of most effective applications.

of cases with osteoarthritis resorted to the TCM method [30]. In patients diagnosed with osteoarthritis, knee osteoarthritis is the disease in which the most complementary medicine method is used, whereas TCM was used in approximately 1/3 of the patients [31-33]. In recent years, significant results have been obtained in the treatment of knee osteoarthritis due to the continuous development of TCM studies [34, 35]. In the studies conducted in our country, it has been determined that the most commonly used TCM methods are herbal treatments and nutritional supplements, while other methods comprise bodybased applications, prayer, religious practices, massage, vitamins, and special diets [29-32]. In our study, unlike other studies, we questioned only the TCM applications approved by the Ministry of Health, and consistent with the literature, we found that the most frequently used treatments were phytotherapy products and supportive drugs. Herbal treatments have been one of the most frequently used traditional methods from past to present, since they are considered cheaper, easily accessible, and have fewer side effects compared to other treatment methods [36, 37]. Individuals have been trying to relieve the symptoms of their diseases by benefiting from the analgesic and anti-inflammatory properties of these treatments [29, 35]. The absence of the methods such as hypnosis, hirudotherapy, reflexology, homeopathy, osteopathy, chiropractic, maggot applications, apitherapy, and music therapy in our study may be related to the fact that these methods are not yet widely known by patients and physicians in our country, and that the number of professionals who will apply these methods is insufficient. The ways to access the TCM method used show a great variety. In the study of Ulusoy *et al*. [31], most of the individuals who resorted to TCM were encouraged by their relatives or the mass media, while 13.6% reported that they resorted to it in line with the recommendations of the physicians. Similarly, in the study of Karadağ *et al*. [30], the recommendation by the family and social sphere took the first place with a rate of 52.5%]. 2.8% of the patients reported that they resorted to TCM upon the recommendation of a physician. In the study conducted by Dikici *et al*. [32], nearly half of the patients had resorted to TCM methods with the influence of family and friends, while 21.8% resorted to these methods upon the recommendation of a physician. Similarly, in our study, the most frequent motivation to resort to TCM was upon the recommendation of neighbors/relatives (57.7%), while the rate of the patients being treated upon the recommendation of a physician was 24%. Tekçi [38], in his study, examined the knowledge and attitudes of research assistant physicians about TCM applications and found that 66% of physicians knew about leech application, 63% about acupuncture, 62.5% about hypnosis, 9.5% about ozone therapy, and 47% about music therapy. The least known methods were found to be chiropractic, apitherapy, prolotherapy, osteopathy, and homeopathy. Despite the best application recommendations, the integration of complementary treatments into osteoarthritis management may be controversial and there is no international consensus on whether the complementary treatments must be included in osteoarthritis managements and when or how this must be done. Despite several well-written and wellconsidered guides, there is no direct recommendation for TCM applications. This lack of appropriate clinical advice and information is a challenge for clinicians on how to advise patients the best since some of these treatments have a particularly high profile in the common press. Therefore, understanding the patterns of TCM users provides health care professionals with the opportunity to make more comprehensive treatment decisions and to improve relationships with patients. As a result, patients diagnosed with knee osteoarthritis

Table 6. Comparison of VAS, K-L measurements according to the effectiveness of the therapy methods applied

		Knee VAS		K-L Score	
		Mean ± SD	*p value*	Mean ± SD	*p value*
Spa	Available	5.88 ± 1.33	0.804	2.76 ± 0.66	0.663
	Unavailable	5.95 ± 1.18		2.7 ± 0.63	
Mesotherapy	Available	5.68 ± 1.09	0.195	2.68 ± 0.61	0.749
	Unavailable	6.03 ± 1.24		2.72 ± 0.64	
Phytotherapy	Available	5.19 ± 1.3	**< 0.001**	2.56 ± 0.64	0.138
	Unavailable	6.19 ± 1.06		2.77 ± 0.63	
Viscosupplementation	Available	5.95 ± 1.19	0.910	2.87 ± 0.61	**0.005**
	Unavailable	5.92 ± 1.24		2.53 ± 0.62	
Steroid	Available	6.02 ± 1.08	0.535	2.76 ± 0.54	0.545
	Unavailable	5.87 ± 1.29		2.68 ± 0.69	
Prolotherapy	Available	6.11 ± 1.05	0.494	2.89 ± 0.66	0.164
	Unavailable	5.89 ± 1.24		2.67 ± 0.62	
PRP	Available	6.5 ± 1.24	0.084	2.58 ± 0.79	0.459
	Unavailable	5.86 ± 1.19		2.73 ± 0.61	
Supportive Medication	Available	6.12 ± 1.11	0.493	2.76 ± 0.75	0.707
	Unavailable	5.9 ± 1.23		2.7 ± 0.61	
Bloodletting	Available	6.03 ± 1.24	0.596	2.52 ± 0.57	**0.043**
	Unavailable	5.89 ± 1.2		2.79 ± 0.64	
Leech	Available	6.53 ± 1.13	**0.037**	2.87 ± 0.74	0.308
	Unavailable	5.83 ± 1.2		2.69 ± 0.61	
Ozone	Available	6.33 ± 1.5	0.300	2.56 ± 0.53	0.442
	Unavailable	5.89 ± 1.18		2.73 ± 0.64	
Acupuncture	Available	6.16 ± 1.07	0.372	2.63 ± 0.5	0.545
	Unavailable	5.88 ± 1.24		2.73 ± 0.66	

PRP = Platelet-Rich Plasma, VAS = Visual Analog Scale, K-L = Kellgren-Lawrence grading system, SD = standard deviation

commonly use TCM methods, phytotherapy being in the first place. However, they often learn about these methods from their families and close circles rather than the physicians they consulted with. Physicians must have sufficient knowledge about and equipment for these methods, which are increasingly being used in our age, and must be able to inform and guide their patients on this subject. Thus, the level of awareness of the patients about the use of TCM will increase and possible unexpected situations will be able to be prevented by consulting with physicians. Evidence-based recommendations cannot be made well enough due to the conducted scientific studies not having the quality high enough and to the insufficiency of randomized controlled studies.

Limitations

The shortcomings of the study are; being cross-sectional, the benefit rate of patients not depending on objective evidence, herbal products in phytotherapy treatment being provided from herbalists and not being under the supervision of doctors and the number of patients being low.

CONCLUSION

The treatment to be applied for knee osteoarthritis must be planned according to the individual, whereas pharmacological and non-pharmacological treatments must be applied to the patients together, by considering the current treatment options. Study reports presenting new treatment recommendations for knee osteoarthritis patients continue to be published in the literature, whereas the guidelines containing treatment options for these patients are updated day by day. It is crucial to follow current guidelines and literature to achieve success in the treatment of knee osteoarthritis patients. The use of TCM methods is increasing day by day in many countries. Among the reasons why people resort to TCM services, it can be listed that it is compatible with their culture, less costly, easier to access and it involves no or less interventional procedures, furthermore, it is being seen as a hope for the treatment of chronic diseases. It is aimed to evaluate the patient's condition more efficiently and thus to determine the optimal treatments, to improve the quality of life and health outcomes, and to prevent complications while minimizing morbidity.

Authors' Contribution

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

Conflict of interest

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

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The European Research Journal 2022;8(5):629-635 Original Article

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How to differentiate the B.1.1.7 variant from COVID-19 in hospitalized patients?

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ABSTRACT

Objectives: Little is known about COVID-19 and less about the B.1.1.7. There is a need for clinical information and tests to help doctors deal with the pandemic. This study aimed to investigate clinical and laboratory differences between hospitalized non-variant COVID-19 and the B.1.1.7 variant.

Methods: Data of 173 hospitalized non-variant COVID-19 and 176 B.1.1.7 variants were retrospectively investigated. D-dimer monocyte ratio (DMR) and ferritin monocyte ratio (FMR) values were calculated by dividing D-dimer and ferritin levels to monocyte count, respectively. Monocyte eosinophil ratio (MER) was obtained by dividing monocyte count by eosinophil levels.

Results: Clinical stay, intensive care unit (ICU) stay, and severe disease rates were found to be higher in the non-variant COVID-19. Eosinophil and basophil levels remained lower, whereas ferritin, FMR, and MER were more elevated in the same group. On ROC analysis, areas under the curve (AUC) of ferritin and FMR were found as 0.7 ($p = 0.001$) and 0.75 ($p = 0.001$), respectively.

Conclusions: The present study revealed that the B.1.1.7 variant had milder clinical manifestations, shorter clinic and ICU stay, and less severe disease rates than the non-variant COVID-19. Higher levels of ferritin, FMR, and MER may indicate the B.1.1.7 variant.

Keywords: The B.1.1.7, variant, COVID-19, monocyte eosinophil ratio, eosinophils

Coronavirus disease-2019 (COVID-19), caused by the severe acute respiratory syndrome−coronavirus 2 (SARS-CoV-2), is a reason for the illness and death of millions since it was identified. Later in the pandemic, genetic variants of SARS-CoV-2 have emerged and circulated the World. About a year later, on December 14, 2020, the United Kingdom (UK) Government was notified of the emergence of a SARS-CoV-2 variant under investigation, which was later defined as lineage B.1.1.7. [1]. In the following period, new concerns have emerged regarding the infectivity, pathogenicity, and mortality of the B.1.1.7 variant [2]. Undoubtedly, recognizing the disease has importance in both preventive medicine and treatment. Polymerized chain reaction (PCR) and genetic studies diagnose the illness and variant analysis. Yet genetic analysis is a time-consuming, expensive procedure and not available in every healthcare institution. There is a need for more clinical information and tests to help doctors during the diagnosis or give an idea about which patients should undergo a genetic study. Biomarkers of inflammation derived from the peripheral blood hemogram parameters have been investigated as independent predictors for the prognosis of systematic inflammatory diseases [3, 4]. In a previous study, high levels of PDW have been associated with

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COVID-19 mortality [5]. All parameters mentioned above are studied by routine complete blood count tests that clinicians might overlook. This study aimed to investigate clinical and laboratory differences between non-variant COVID-19 and the B.1.1.7 variant else-more to determine whether C-reactive protein (CRP) monocyte ratio (CMR), D-dimer CRP ratio (DCR), D-dimer monocyte ratio (DMR), ferritin monocyte ratio (FMR) and monocyte eosinophil ratio (MER) can help to differentiate non-variant COVID-19 from the B.1.1.7 variant. Also, to explore the most useful diagnostic biomarkers, optimal cut-off values, and correlations between these biomarkers.

METHODS

Data of randomly chosen unvaccinated hospitalized 173 Non-variant COVID-19 and 176 B.1.1.7 variants between January-April 2021 were retrospectively investigated. Patients who were found to be positive for SARS-CoV-2 polymerized chain reaction test (PCR) and who were found to have non-variant COVID-19 or B.1.1.7 as a result of genetic analysis between the specified dates were included in the study. Even if the clinical and tomographic findings were compatible, patients with negative SARS-CoV-2 PCR results and pregnant women were excluded from the study. In Turkey, vaccination against COVID-19 was started on14.01.2021 with healthcare providers and continued with citizens over the age 65 on 11.02.2021. Intensive vaccination was applied in march-april 2021.Vaccination rates were low during the data collection period and were not statistically sufficient for analysis, so individuals who were vaccinated at a very low rate were not included in the study. The demographic and clinical data of all patients are shown in table 1. The decision to hospitalize or home follow-up was given according to the general condition of the patients, blood analysis including a complete blood count, CRP, D-dimer, ferritin levels, the percent saturation of oxygen in the blood values (SpO2), and the severity of tomographic involvement. Patients with one of the following criteria; oxygen saturation below 93% in room air, C-reactive protein (CRP) value above 50 mg/L, D-dimer above one ug FEU/ml, ferritin above 500 ng/mL, lymphocyte values belove 500 103/µL, severe involvement on computed tomography were hospitalized. Severe disease is defined as suspected respiratory infection symptoms, plus any of the following; shortness of breath, respiratory rate above 30 breaths/min; at rest, oxygen saturation belove 93%; Pa02/Fi02 belove 300 mmHg (1 mmHg Z 0.133 kPa). The groups were compared by examining the hemogram parameters lymphocyte, monocyte, eosinophil, and SpO2 obtained on the same day of the diagnosis. The values of CMR, DMR, and FMR were calculated by dividing CRP, D-dimer, and ferritin levels by monocyte count. MER was obtained by dividing monocyte count by eosinophil, and DCR was obtained by dividing D-dimer by CRP. The ethical committee approval was obtained from Bursa City Hospital Clinical Research Ethical Committee (Date: 20.05.2021, No: 2021-9/2).

Statistical Analysis

All the statistical analyses were carried out using SPSS 25.0 software. A Kolmogorov-Smirnov test was performed for the normality of the sample data; continuous variables were defined by the mean ± standard deviation, median (interquartile range %25–%75), while the categorical variables were expressed as frequency and percent. A Student t-test for parametric assumptions and Mann Whitney U test for non-parametric hypotheses compared the independent groups. The Roc analysis was performed for optimal cut-off values to predict the B.1.1.7 variant.

RESULTS

The median age turned out to be 49 (range; 18-88) years in the non-variant COVID−19 group and 42 (range; 18-87) years in the B.1.1.7 variant group (Table 1). The mean age was significantly higher in the non-variant COVID-19 group ($p < 0.02$). Although the gender distribution in the non-variant COVID−19 group was 49.7% (n = 86) women and 50.3% (n = 87) men, it was 51.7% (n = 91) women and 48.3% (n = 85) men in the B.1.1.7 variant group. Hypertension (HT) was higher in the non-variant COVID-19 group ($p = 0.001$). No statistically significant difference was found between chronic obstructive pulmonary disease (COPD), asthma, diabetes mellitus (DM), coronary artery disease (CAD), and malignancy rates. Although no statistical differences were found, mortality rates

were higher in the non-variant COVID-19 group. Clinic stay and intensive care unit (ICU) stay were higher in the non-variant COVID-19 ($p = 0.001$ and $p = 0.046$, respectively). SpO_2 was lower in the non-variant COVID-19 ($p < 0.002$). Severe disease rates were higher in the non-variant COVID-19 group ($p = 0.04$). Lymphocyte, eosinophil, basophil levels and DCR remained lower in the non-variant COVID-19 ($p = 0.05$, $p = 0.001$, $p = 0.001$ and $p = 0.004$; respectively). D-dimer, CRP, ferritin, CMR, FMR, and MER were higher in the non-variant COVID-19. Optimal cut-off values calculated by the ROC analysis and the ROC curves are presented in Fig. 1. When non-variant COVID-19 patients were compared to the B.1.1.7 variants, the areas under the curve (AUC) of ferritin, CMR, FMR, and MER were found as 0.7 ($p = 0.001$),

Table 1. Demographic, clinical data, and laboratory findings of nonvariant COVID-19 and the B.1.1.7 variants

Variable	Non-variant COVID-19 (n = 173)	The B.1.1.7 variant (n = 176)	*p* value
Age (years)	49 (18-88)	42 (18-87)	**0.02***
Female	86 (49.7%)	91 (51.7%)	0.7
Male	87 (50.3 %)	85 (48.3%)	
Lymphocyte (10^3/µL)	1.58 ± 0.71	1.7 ± 1	**0.05***
Monocyte (10^3/µL)	0.51 (0.14-1.44)	0.63 (0.13-4.85)	0.07
Eosinophil (10^3/µL)	0.02 (0-1.45)	0.06 (0-3.2)	**0.001***
Basophil (10^3/µL)	0.01 (0-0.07)	0.03 (0-0.9)	**0.001***
D-dimer (ug FEU/ml)	0.45 (0.15-8.36)	0.42 (0.01-4.76)	0.3
CRP (mg/L)	18 (0.3-434)	12 (0.6-234)	**0.02***
Ferritin (ng/mL)	246 (8.2-1941)	101 (6-1438)	**0.001***
CMR	25 (0.43-1287)	17.4 (0.72-1021)	**0.001***
DCR	0.021 (0-2.6)	0.042 (0-4.2)	**0.004***
DMR	0.6 (0.14-519	0.56 (0.013-2.5)	0.06
FMR	530 (7.9-8695)	153 (12-6252)	**0.001***
MER	13 (0.14-82)	7.3 (0.18-98)	**0.003***
Death rate	24 (13.9%)	16 (9.1%)	0.16
Severe disease	32 (18.5%)	19 (10.8%)	**0.04***
Clinic stay	7.7 (0-40)	2.2 (0-35)	**0.001***
ICU stay	2.1 (0-61	1.3(0-13)	**0.046***
SpO2	94 (45-100)	98 (75-100)	**0.002***
COPD	1 (0.6%)	0 (0%)	0.3
Asthma	8 (4.6%)	14 (8%)	0.2
DM	15 (8.7%)	22 (12.5%)	0.24
HT	40 (23%)	15 (8.5%)	**0.001***
CAD	9 (5.2%)	9 (5.1%)	0.97
Malignancy	0 (0%)	2 (0.6%)	0.16

Data are shown as mean ± standard deviation or mean (ninimum-maximum or n (%). CRP = C-Reactive Protein, CMR = CRP monocyte ratio, DCR = D-dimer CRP ratio, DMR = D-dimer monocyte ratio, FMR = Ferritin monocyte ratio, MER = monocyte eosinophil ratio, ICU = Intensive Care Unit, SpO2 = median oxygen saturation, COPD = Chronic Obstructive Pulmonary Disease, DM = Diabetes Mellitus, CAD = Coronary Artery Disease.

*$p < 0.05$ statistically significant

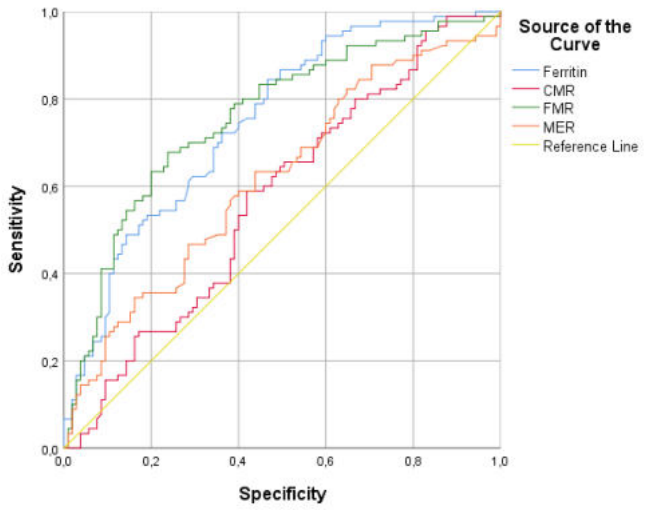

Fig. 1. ROC curves comparing the prediction of B.1.1.7 Variant, variables for ferritin, CMR = C-reactive protein monocyte ratio, FMR = ferritin monocyte ratio, MER = monocyte eosinophil ratio. Diagonal segments are produced by ties.

0.67 (p = 0.001), 0.75 (0.001), and 0.61 (p = 0.004), respectively (Table 2). The correlation analysis is shown in Table 3. Negative correlation detected between lymphocyte and CMR (r = 0.29, p = 0.001), DCR and CMR (r = 0.84, p = 0.001). Positive correlation detected between CRP and CMR (r = 0.59, p = 0.001), D-dimer and DMR (r = 0.51, p = 0.001), CMR and FMR (r = 0.36, p = 0.001). DMR and FMR (r = 0.28, p = 0.001).

DISCUSSION

When COVID-19 first appeared, little was known about the disease, making management difficult. Accumulation of knowledge has increased over time, but the emergence of new variants such as the B.1.1.7 variant has raised further questions about the disease.

The SARS-CoV-2 B.1.1.7 variant cases have increased rapidly worldwide and are reported to be more contagious than the non-variant COVID-19. To the best of our knowledge, no studies investigating CMR, DCR, DMR, FMR, and MER of COVID-19 cases infected with the B.1.1.7 variant have been published. This study examined clinical and blood parameter differences between the non-variant COVID-19 and the B.1.1.7 variants. According to a report from seven European countries, increased hospitalizations and ICU admission risk was associated with the SARS-CoV-2 variants, including the B.1.1.7. [6]. In the present study, the clinic stay and ICU hospitalization time were longer in non-variant COVID-19. More non-variant COVID-19 patients had more severe disease rates and lower median SpO2 levels. Lymphocytes are cells of immunity that play an essential role in the fight against pathogens. Following viral infections, different virus types may cause changes in total lymphocyte count. In a study, Wang *et al*. [7] reported decreased lymphocyte levels in patients with COVID-19. In the present study, lymphocyte levels were higher in the B.1.1.7 variant group. Current data suggest an essential role for monocyte activation in developing immunopathology of patients with COVID-19 [8]. According to the present study results, no difference was detected between groups regarding monocyte levels. Eosinophils are circulating cells with many functions. Some of them are; antiviral activity and immunoregulation. But there is limited information about their role in COVID-19. In a study, Xie *et al*. [9] reported a decrease in circulating eosinophil counts in COVID-19 patients more frequently than other types of pneumonia patients. Previously eosinopenia was reported in patients with acute respiratory deterioration during infection with SARS-CoV-2 [10]. In the present study, non-variant, COVID-19 patients had lower

Table 2. ROC analysis of non-variant COVID-19 patients versus B.1.1.7 variant

Variables	AUC (95% CI)	Cut-off	Sensitivity%	Specificity %	*p* value
Ferritin	0.7 (0.62-0.77)	172	63	63	**0.001***
CMR	0.67(0.6-0.75)	23.6	64	63	**0.001***
FMR	0.75 (0.68-0.81)	277	68	32	**0.001***
MER	0.61 (0.53-0.69)	9.7	59	58	**0.004***

AUC = Area Under ROC Curve, CMR = C-reactive protein monocyte ratio, FMR = ferritin monocyte ratio, MER = monocyte eosinophil ratio.

*p < 0.05 statistically significant

Table 3. Spearman correlations between laboratory findings of non-variant COVID-19 and the B.1.1.7 variants

		LYMPH	CRP	D-dimer	Ferritin	CMR	DCR	DMR	FMR	MER
LYMPH	r	1	-0.24	-0.09	-0.09	-0.29	0.18	-0.21	-0.12	-0.14
	p		**0.001***	0.16	0.09	**0.001***	**0.002***	**0.001***	**0.04***	**0.03***
CRP	r	-0.24	1	0.08	0.20	0.59	-0.55	0.04	0.12	0.16
	p	**0.001***		0.245	**0.001***	**0.001***	**0.001***	0.46	**0.04***	**0.02***
D-dimer	r	-0.09	0.08	1	0.10	0.08	0.20	0.51	0.12	0.04
	p	0.16	0.24		0.12	0.22	0.006*	0.001*	0.07	0.59
Ferritin	r	-0.09	0.2	0.1	1	0.08	-0.05	0.002	0.6	0.12
	p	0.09	**0.001***	0.12		0.17	0.39	0.97	**0.001***	0.07
CMR	r	-0.29	0.59	0.08	0.08	1	-0.84	0.24	0.36	0.12
	p	**0.001***	**0.001***	0.22	0.17		**0.001***	**0.001***	**0.001***	**0.05***
DCR	r	0.18	-0.55	0.20	-0.05	-0.84	1	0.26	-0.19	-0.20
	p	**0.002***	**0.001***	**0.006***	0.39	0.001*		**0.001***	**0.001***	**0.002***
DMR	r	-0.21	0.04	0.51	0.002	0.24	0.26	1	0.28	-0.15
	p	**0.001***	0.46	**0.001***	0.97	**0.001***	**0.001***		**0.001***	**0.02***
FMR	r	-0.12	0.12	0.12	0.60	0.36	-0.19	0.28	1	-0.04
	p	**0.04***	**0.04***	0.07	**0.001***	**0.001***	**0.001***	**0.001***		0.95
MER	r	-0.14	0.16	0.04	0.12	0.12	-0.20	-0.15	-0.04	1
	p	**0.03***	**0.02***	0.59	0.07	**0.05***	**0.002***	**0.02***	0.9	

LYMP = lymphocyte, CRP = C-reactive protein, CMR = CRP monocyte ratio, DCR = D-dimer CRP ratio, DMR = D-dimer monocyte ratio, FMR = ferritin monocyte ratio, MER = monocyte eosinophil ratio.

*$p < 0.05$ statistically significant

eosinophil levels than the B.1.1.7 variants. In a study Song *et al*. [11] reported higher levels of CRP and D-dimer in the B.1.1.7 variants compared to those non-variants. Unlike the study conducted by Song *et al*. [11] in the present research, non-variant, COVID-19 patients had higher levels of CRP, but no difference was detected between D-dimer levels. In some patient groups, especially in severe disease, COVID-19 is associated with inflammatory cytokine storm in which the inflammatory response may change the iron homeostasis. Zhou *et al*. [7] reported patients diagnosed with severe COVID-19 had higher serum ferritin levels than in other groups. In the present study, ferritin levels were lower in the B.1.1.7 variants. Different results might be due to heterogeneity of COVID-19 or the difference in the behavior of SARS-CoV-2 in geographical regions.

Increased risk of hospitalization, intensive care admission, and mortality rates were previously reported for B.1.1.7 [12, 13]. In the present study, severe disease rates were lower in the non-variant COVID-19 than in the B.1.1.7 variant. Even though no statistical difference was detected, death rates were also found to be higher in the non-variant COVID-19. In a study, Nyberg *et al*. [14] reported an increased risk of hospital admission for people infected with the B.1.1.7 variant. Different results between studies might be due to heterogenicity and other behavior of SARS-CoV-2.

Although similar case numbers are reported in different countries, mortality rates may differ.

Recently, ratios that are more accessible to researchers have been used in diagnosis and prognosis assessment. A high neutrophil-lymphocyte ratio (NLR) was reported in patients who tested positive for SARS-CoV-2 than controls [15]. A study from Wuhan/China by Yang *et al*. [16] reported elevated NLR was significantly associated with illness severity. Yang *et al*. [16] investigated 93 COVID-19 patients and found out that the lymphocyte to monocyte ratio (LMR) of severe patients was significantly higher than non-severe patients. This study thought that the proportions of essential parameters in terms of disease severity and prognosis in COVID-19 might help differentiate non-variant COVID-19 from the B.1.1.7 variant. CMR, DMR, FMR, and MER were higher in the non-variant COVID-19, whereas DCR was lower in the same group. CMR was moderately positively correlated with CRP, and ferritin was with FMR. CMR was strongly negatively correlated with DCR. To the best of our knowledge, this is the first study analyzing above mentioned ratios in patients with COVID-19.

Limitations

This study has limitations; besides being a retrospective study, even though all parameters belong to the first admission before undergoing an in-hospital treatment, they still might have been affected by age difference, comorbidities or medications used for these comorbidities. SpO2 measured at the first admission are instant measurements and can be affected by conditions such as the temperature and nail polish of the fingers and are not as objective as blood gas analysis. Since the study included unvaccinated patients, it does not provide information about vaccinated patients.

CONCLUSION

In conclusion, the present study revealed that the B.1.1.7 variant had less severe disease rates, and even though no statistical difference was found, fewer death rates were compared to non-variant COVID-19. Higher levels of ferritin, CMR, FMR, and MER might be indicating the B.1.1.7 variant.

Authors' Contribution

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

Conflict of interest

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

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Renal artery Doppler findings in fetuses of mothers with preeclampsia

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ABSTRACT

Objectives: Preeclampsia (PE), which affecting multi-organ systems, is one of the most common causes of feto-maternal morbidity and mortality. The fetal kidney is one of the vulnerable organs in PE caused by sustained vasospasm of the renal arteries. In this study, it was aimed to reveal the changes in the renal vascular bed with renal artery Doppler examinations in fetuses of pregnant women with PE.

Methods: Fifty-five pregnant women with PE and 60 healthy pregnant women were included in this prospective study. Multiple pregnancies, those who did not want to participate in the study, and those with other co-morbidities were excluded from the study. Fetal renal artery Doppler studies included renal artery systolic/diastolic (S/D) ratio, pulsatility index (PI) and resistance index (RI) of the control and PE groups, and findings such as week of birth and birth weight were recorded and analyzed statistically.

Results: Fetal renal artery PI values were found to be higher in pregnant women with PE compared to the control group (2.93 in the patient group, 2.28 in the control group, $p < 0.001$). There was no significant difference between RI values and S/D ratios between the two groups. In the preeclampsia group, gestational week and baby weight at birth were significantly lower.

Conclusions: Due to preeclampsia, hypoxia occurs in peripheral tissues and organs at the maternal level. Fetal organs are also affected by these hypoxic conditions. Doppler is an extremely useful examination tool in the evaluation of the status of peripheral organs such as the kidney. This study suggests that PE increases the resistance of renal arteries in fetuses of mothers with PE compared to fetuses of mothers without PE, which may contribute critically to kidney disease later in life.

Keywords: Preeclampsia, Doppler, fetal renal artery

Preeclampsia (PE), which increases morbidity and mortality in mothers and fetuses due to a multi-systemic disease involving more than one organ, is a picture that occurs with proteinuria, thrombocytopenia, kidney failure, liver failure, pulmonary edema, and brain/visual symptoms [1, 2]. The treatment of preeclampsia, the etiology of which has not been fully elucidated, has not yet been found. The only known definitive solution is delivery and separation of the placenta, which is thought to play a role in the etiology [3].

PE causes a higher risk of chronic hypertension, cardiovascular disease [4], chronic kidney disease [5] and end-stage renal disease [6] on maternal health not

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only during pregnancy but also in subsequent years. Fetuses of mothers with PE have a higher lifetime risk for pulmonary, cardiovascular, and metabolic diseases, systemic vascular dysfunction, and obesity [7-9]. Additionally, hypoxia caused by placental pathology in PE reduces nephrons in the fetal kidneys, which may affect the long-term health of fetuses at lifetime risk [10]. Some animal experiments have shown that this damage causes a decrease in size of fetal kidneys and glomerular number [7, 11].

Doppler ultrasonography is one of the most appropriate examination methods to show vascular resistance anomalies in fetal kidneys due to preeclampsia, since it does not contain ionizing radiation and is a noninvasive method. Resistance index (RI) and pulsatile index (PI) values, which mean high values measured by Doppler examination, show increased resistance in the vascular bed, are the best indicators of resistance in the vascular bed [12].

In this study, it was aimed to reveal the changes caused by PE in the fetal renal vascular bed by Doppler examination measurements made from fetal renal arteries in pregnancies complicated by PE.

METHODS

A prospective study was conducted between March-2021 and December-2021 in a tertiary hospital with PE and control group to evaluate the changes caused by PE in fetal renal vascular bed with Doppler examination measurements made from fetal renal arteries. Ethical approval was obtained from the local institutional ethics committee (number E-2021/87).

Written informed consent was obtained from all participants before the examination. Fifty-five patients with preeclampsia and 60 normal pregnant women were included in the study. The diagnosis of PE was made according to the guidelines of the International Federation of Gynecology and Obstetrics for the Study of Hypertension in Pregnancy. As a result of the data scans, the routine laboratory, ultrasonographic measurements and birth follow-up data of the pregnant women who applied to the hospital were recorded. Gestational age was confirmed by first trimester sonographic measurements of pregnancy.

Inclusion criteria were women with a singleton pregnancy complicated with PE, and the control group consisted of mothers of matched gestational age. Fetal anomaly, accompanying maternal comorbidity (such as diabetes, ischemic heart disease, kidney disease and autoimmune diseases), multiple pregnancies, pregnant women with smoking and alcohol use, and pregnant women who did not want to participate in the study were excluded.

All patients underwent an ultrasonographic examination using a Mindray Resona 7 ultrasound (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China), diagnostic apparatus with a 1,2-6 MHz convex abdominal probe. Fetal renal artery Doppler assessments were performed by a fetal medicine specialist (HAS) using a method similar to that previously described by Azpurua *et al*. [4]. The ultrasound parameters were performed gestational weeks in between 27-40. All pregnant women were placed in the supine position and respiratory levels were kept constant to avoid noise and artifacts.

The color Doppler range (1.5 to 2.0 mm) was in-

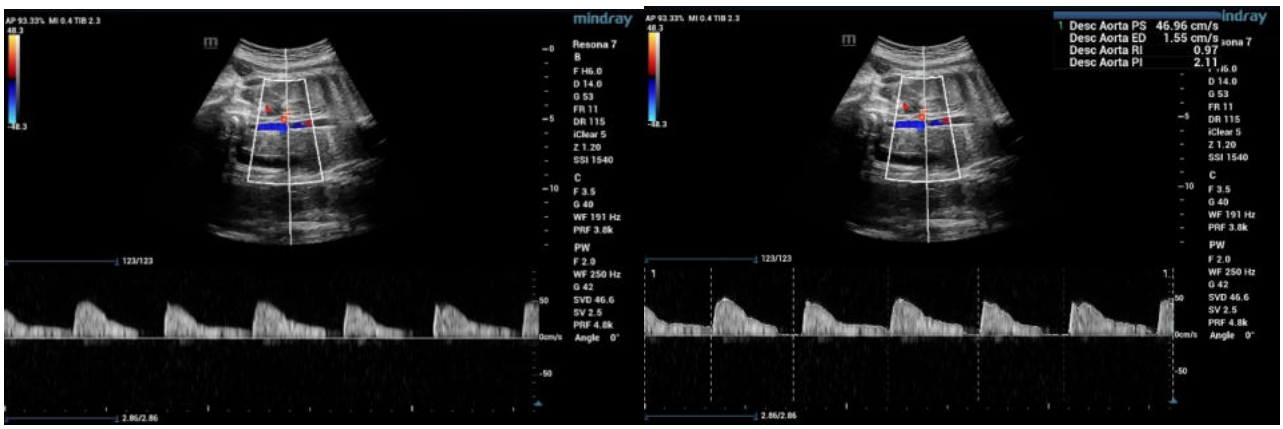

Fig. 1. Fetal renal artery doppler imaging.

serted into the lumen of the renal artery, just after the aortic outlet, in the midsection of the renal artery before intrarenal branching (Fig. 1)

The scan plane was set in the range of 30°-60°. Low filter (50-75Hz) settings were used to preserve the end-diastolic values of the renal artery waveform. Renal artery PI, RI, and systolic diastolic (S/D) velocity ratios were measured.

Statistical Analysis

The IBM SPSS Statistics program (Version 11.0, SPSS; Chicago, IL, USA) was used for the statistical evaluation of the data obtained in this study. The Shapiro-Wilk test was used to test whether the variables of the patient and control groups fit the normal distribution, and the variables that fit the normal distribution were given as mean ± standard deviation values, and the variables that did not fit the normal distribution were given as their median (minimum-maximum) values. "Mann Whitney U" and "Independent Sample t" tests were used to analyze the differences between the two groups.

Table 1. Demographic data averages of the study and control groups

Variables	Preeclamptic Patient (n = 55)	Control Group (n = 60)
Age (years)	30.16	30.05
Weight (kg)	81.83	79.93
Height (cm)	162.63	159.21
Gravity	2.61	2.73
Parity	1.1	1.26
Living child	1.03	1.16

RESULTS

Table 1 shows the demographic characteristics. Maternal age and demographic characteristics were similar between the study and control groups (Table 1). It was found that the renal artery Doppler PI values of the fetuses of mothers with PE were higher than the control group (2.93 in the patient group, 2.28 in the control group, $p < 0.001$). Week of birth and fetal weight at birth were significantly lower in the PE group ($p < 0.001$). The RI and S/D values of the participants did not differ between the patient and control groups ($p > 0.05$) (Table 2).

DISCUSSION

In the normal physiology of the fetus, the renin-angiotensin system activity is high in early pregnancy, so urine production is low due to high resistance in the renal arteries during early pregnancy. In the later weeks of pregnancy, renal artery resistance decreases due to low renin-angiotensin system activity and this causes an increase in urine production [6, 13].

In PE high systemic vascular resistance and low cardiac output are seen, which causes hypoxia in peripheral tissues and organs. Animal experiments show an increased risk of impaired fetal kidney development due to increased renal vascular tone due to hypoxia, therefore an increase in Doppler values of the fetal renal artery was expected. These changes in the kidney may be the cause of hypertension in both childhood

Table 2. The values belonging to both groups and the analyses

Variables	Preeclamptic Patient (n = 55)	Control Group (n = 60)	*p* value
RI	0.87 (0.67-0.98)	0.85 (0.61-0.99	0.569
PI	2.93 (1.47-3.72)	2.28 (1.43-3.71)	**< 0.001**
Renal arter S/D	9.9 (6.21-21.3)	11.8 (6.3-22.4)	0.083
Birth Weight (g)	1949.47 ± 803.02	2704.9 ± 735.57	**< 0.001**
Week of Birth	34.71 (27-40.28)	37.14 (26.85-41.28)	**< 0.001**

The RI, PI and S/D values belong to fetal renal arteries. RI = Resistance index, PI = pulsatility index, S/D = systolic/diastolic ratio

$p < 0.05$, *Mann Whitney *U* test, ** Independent Sample *t* test

and adulthood of fetuses with PE [9, 11].

Our study is similar to the fact that fetal renal blood flow Doppler indexes are higher in pregnant women with PE compared to normal pregnancy. When PI, RI values and S/D ratios obtained in fetal renal artery Doppler examinations were compared with the control group, an increase was observed in fetal PI values in patients with PE, but no significant difference was found in RI and S/D values.

Our results are also in line with the results of another study, Boubred *et al*. [14] showed that PI indices in the renal arteries increased in the PE animal group compared to the control group. This increase in PI indices is explained as the deterioration of the balance between vasoconstrictive factors and vasorelaxing factors, including the renin-angiotensin system (RAS) and renal sympathetic nervous system [14].

Several other studies have shown similar results to our study and these studies also explained this increase in renal artery tension in PE, the imbalance of vasoconstrictors, and the increased vascular sensitivity to angiotensin II induced by placental hypoxia [15, 16].

Although the pathophysiological mechanisms responsible for PE are not yet known, it has been described in some studies that prenatal hypoxia has a significant effect on PE-mediated blood pressure and that some ion channel activities cause an increase in renal vascular tension [9, 11].

Some studies examining maternal renal artery Doppler indices, such as Sohn and Fendel [17], showed maternal renal artery blood flow velocity in normal and hypertensive pregnancies, and also found an increase in resistance indices compared to normotensive ones as in fetuses.

RI, PI, and S/D values indicate impedance in the vascular bed, the best indicators of resistance in the vascular bed. In our study the finding that only PI was the meaningful indices. However, at the same time, RI and S/D indices are also important in terms of their correlation with arterial pressure. Since PI indicates changes in the total velocity waveform, some studies have described PI as a more relevant index for renal Doppler velocity measurement, but RI and S/D are measured based on two points on the maximum velocity curve [18].

Interstingly, contrary to our results, some studies show reduced renal artery resistance in fetuses of mothers with PE. Like Kaya *et al*. [19], they showed that the renal artery PIs, RIs and S/D ratios were significantly lower in fetuses of mothers with PE ($p < 0.001$ and $p = 0.013$, respectively). In another similar study, Afsari *et al*. [20] reported that renal artery S/D ratio, RI and PI were significantly decreased in the PE group compared to the control group ($p < 0.001$). Ma'ayeh *et al*. [21] showed that renal artery RIs and S/D ratios were significantly lower in the PE group ($p = 0.003$), but they did not show a statistically significant difference in PI. The explanation may highlight these discrepancies, that pregnant women diagnosed with PE had abnormal AF index and fetal growth excluded from these studies, and all participants with PE had normal AF index and fetal weight.

However, many previous studies have shown that patients with PE have a higher incidence of oligohydramnios, and in one study, this was associated with reprogrammed AQP1 (aquaporin 1) expression through a DNA methylation-mediated epigenetic mechanism [22].

In a study conducted with pregnant women with intrauterine growth restriction and oligohydramnios, renal artery Doppler indexes were found to be higher [23], and in another similar study examining fetal renal blood flow in hypoxemia, high renal artery PI due to renal hypoperfusion was shown [24].

Part of the discussion about decreased renal artery resistance may be explained by Platt *et al*. [25], who stated that Doppler indices are affected by the region of the disorder within the kidney, not the degree of renal dysfunction.

It seems that more research is needed to say the end point in the changes caused by PE in the fetal renal vascular bed with Doppler examination measurements.

The strengths of this study are that the Doppler studies were performed by a fetal medicine specialist and the gestational age of the control group was similar.

Limitations

Our study had some limitations. Pregnant women with PE were not differentiated according to mild or severe PE. Peak systolic velocities and renal volume were not studied in this study. In addition, the number of patients was small and the participant's maternal renal artery blood flow rate were not examined.

CONCLUSION

This study suggests that PE increases the resistance of renal arteries in fetuses of mothers with PE, which may contribute critically to kidney disease later in life. A future study investigating renal artery Doppler values for fetuses after delivery may provide important implications for understanding renal vascular blood flow changes in pregnancy complicated by PE.

Authors' Contribution

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

Conflict of interest

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Safety of allergen immunotherapy in patients with SARS-CoV-2 infection

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ABSTRACT

Objectives: The aims of presenting study were trying to expose the course of SARS-CoV-2 (severe acute respiratory syndrome–related coronavirus) in patients with allergic rhinitis (AR), to compare the prevalence of SARS-CoV-2 infection, hospitalization and pneumonia rates in patients with AR receiving allergen immunotherapy (AIT) and patients did not receiving AIT (non-receivers) and to define possible risk factors for SARS-CoV-2 positivity in patients with AR.

Methods: A total of 419 patients with AR who were being followed-up in a tertiary allergy clinic between June 1, 2020 and December 31, 2020, were selected for the study.

Results: Seventy-nine (18.9%) patients became infected with the SARS-CoV-2 [32 (19.6%) patients in AR patients with AIT and 47 (18.4%) patients in non-receivers] and the rate of pneumonia was 2.4% [12.7% of SARS-CoV-2 (+) patients]. There was no significant difference was determined between the AR patients with AIT and the non-receivers in regard of the rate of SARS-CoV-2 infection, pneumonia and hospitalization (p = 0.864, p = 0.055 and p = 0.075; respectively). There was a significant difference between the groups in terms of gender, duration of disease, sensitivity to allergens (atopy) and serum IgE levels (p = 0.009, p = 0.001, p = 0.001 and p = 0.001; respectively). The accompanying comorbidities, eosinophil cout, AIT and duration of AIT were not found to be associated with an increased risk SARS-CoV-2 PCR positivity. However, female gender was shown to be associated with an decreased risk for SARS-CoV-2 PCR positivity (OR, 0.571; 95% confidence interval, 0.330-0.987; p = 0.045)

Conclusions: The course of SARS-CoV-2 is similar in patients with AR who underwent AIT and patients with AR who did not undergo AIT, and AIT does not seem to increase the risk for SARS-CoV-2 infection.

Keywords: Allergic rhinitis, allergy immunotherapy, subcutaneous immunotherapy, therapeutics

Although unprecedented efforts have been made all over the world to prevent the spread of and contain COVID-19, the number of cases continues to rise, and since it was first identified COVID-19 has become the most pressing health issue globally [1, 2]. There is still no effective treatment for the disease, and due to different virus variants and numerous socioeconomic inequalities, vaccination efforts against the virüs currently do not have the expected speed and effects. Therefore, in terms of both reducing mortality and morbidity, as well as for the efficient utilization of resources, it is very important to identify special patient groups, especially those with chronic diseases and who may be affected to a greater extent by COVID-19, and to investigate the effect of the treatment received by these patiet groups during the course

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of COVID-19.

Allergic rhinitis (AR) is a common allergic disease that affects approximately 10-30% of the pediatric and adult population [3, 4]. Among patients with AR, Allergen Immunotherapy (AIT) can be applied to those who do not possess sufficient clinical benefit despite minimal allergen exposure and optimal pharmacological treatment, or who have side effects related to these treatments. Patients with AR tend to produce lower levels of type 1 interferon (IFN) during upper respiratory viral infections than those without AR [5, 6]. This may put allergic patients at higher risk for COVID-19-related morbidity and mortality [7, 8]. In contrast, low expression of angiotensin converting enzyme (ACE) 2 was detected in airway cells of AR patients, and this is thought to be protective against COVID-19.

In patients with seasonal/perennial allergic rhinitis, AIT is the only therapy that demonstrates both disease-modifying and therapeutic potential, as well as inducing long-term tolerance of the immune system to allergens. AIT exerts these effects with an increase in B regulatory (Breg) and T regulatory (Treg) cell numbers and functions, and IL-10 levels in the foreground [9, 10]. AIT inhibits the activation of allergen-specific Th2 cells through B and T reg cells, as well as suppressing the T cell response directed by Th1 and Th17 cells. Besides causing a cytokine storm of dysregulated Th1 immune response and ARDS (Acute Respiratory Distress Syndrome), ARDS remains an prominent cause of SARS-CoV-2 related mortality [11]. It can therefore be argued that AIT may have a positive effect on the course of SARS-CoV-2. Moreover, the adenomatous and secretory structure of the nasal mucosa in AR patients due to allergen exposure, local anti-inflammatory effects of intranasal steroids used for treatment, type 2 inflammation dominance in AR and the effects of AIT on Breg and Treg suggest that the course of SARS-CoV-2 may differ in patients with AR.

Therefore, the aim of presenting this study was to attempt to expose the course of SARS-CoV-2 in patients with allergic rhinitis, to compare the prevalence of SARS-CoV-2/COVID-19 infection, hospitalization and mortality rates in patients with allergic rhinitis receiving AIT and in patients not receiving AIT (non-receivers), and finally to define possible risk factors for SARS-CoV-2 positivity in patients with allergic rhinitis.

METHODS

Selected for the study were 419 adult patients with allergic rhinitis who were being followed-up in a tertiary allergy clinic in Konya, located in the central Anatolia, Turkey, between June 1, 2020 and December 31, 2020, in a retrospective manner. Only patients who were receiving active-continuous treatment for allergic rhinitis during the study period were included in the study.

Patients with nasal discharge, sneezing attacks, burning, nasal congestion and stinging in the eyes, accompanying itching in the ears, eyes and palate were evaluated. Patients with skin prick tests or allergen-specific IgE measurement, which were found to be compatible with the patient's clinical condition, were included in the study. Patients with nasal discharge, sneezing attacks, burning, nasal congestion but not allergen sensitivity were not included in the study.

Demographic (age, gender, duration of allergic rhinitis and AIT, accompanying comorbidities, atopy) and clinical data (serum IgE and blood eosinophil counts in patients prior to initiation of treatment for allergic rhinitis and/or AIT) were retrieved from medical files. A skin prick test was performed using standardized inhalant allergens (ALK, Madrid, Spain), House dust mite (Dermatophagoides (D) farinae, D. pteronyssinus), cat (Felis domesticus), dog (Canis familaris), cockroach (Blatella germanica), fungi (Alternaria, Cladosporium, Aspergillus) and pollen mixtures (tree, weed, grass). It was performed subcutaneously with the conventional protocol in all patients undergoing AIT. Specific IgE measurement was carried out in patients where systemic atopy could not be demonstrated via skin prick tests. We did not have any patients who underwent sublingual immunotherapy, rush or ultra-rush immunotherapy. The diagnosis of SARS-CoV-2 was made by a positive Polymerase Chain Reaction (PCR) test in patients with consistent clinical presentation for COVID-19.

Venous blood samples for biochemical analyses were drawn after at least 10 h of fasting before taking any medication. Abbott Cell Dyn 3700 series (Sheath reagent) and Siemens BN II/ BN ProSpec system (using particle-enhanced immunonephelometry) were used for whole blood count and quantitative determination of serum immunoglobulin (IgE).

The study was approved by Karatay University Ethics Committee (Decision number 2021/36, date

19.11.21).

Statistical Analysis

Statistical analysis was performed with the IBM SPSS Statistics Version 22 software package. Normally distributed parameters were presented as mean ± standard deviation and data that were not normally distributed were expressed as median (interquartile range: minimum–maximum). Descriptive data were presented as frequencies and percentages and compared using a Chi-square test. Comparisons between baseline characteristics were performed by independent Student t, Mann-Whitney rank-sum, Fisher exact or Chi-square tests where appropriate. As a result of these statistical analysis, parameters with $p < 0.2$ between SARS-CoV-2 (+) patients and SARS-CoV-2 (-) patients were subjected to regression analysis. Binomial logisticegression analysis was performed to determine independent predictors for SARS-CoV-2 positivity.

RESULTS

A total of 419 patients with allergic rhinitis were included in the study [Female: 266 (63.5%), Male: 153 (36.5%)]. The mean age was 30 years (18 to 76 years). The mean duration of disease was 5 years (0.6-35). One hundred sixty-three (38.9%) patients were receiving AIT. In patients undergoing AIT, the duration of immunotherapy was 15 months (3-58 months). The most common allergen sensitivity in patients was found to be pollen mixture sensitivity (58.7%) and house dust mite sensitivity (23.2%).

Seventy-nine (18.9%) patients became infected with the SARS-CoV-2 virus during the study period [32 (19.6%) patients in allergic rhinitis patients with AIT and 47 (18.4%) patients in non-receivers]. There was no significant difference was determined between the allergic rhinitis patients with AIT and the non-receivers in regard to the rate of SARS-CoV-2 ($p = 0.864$). Clinical characteristics of the patients are summarized in Table 1.

Table 1. Demographic, clinical and laboratory parameters of allergic rhitis patients

Parameters	Total (n = 419)	Allergic rhinitis patients with AIT (n = 163)	Allergic rhinitis patients without AIT (n = 256)	*p* value
Age (years), mean (range)	30 (18-76)	31 (18-69)	28 (18-76)	0.070
Gender, Female, n (%)	266 (63.5)	91 (55.8)	175 (68.4)	**0.009**
Duration of disease (years), mean (range)	5 (0.6-35)	6 (1-35)	4 (0.6-24)	**0.001**
Allergen sensivity, n (%)				**0.001**
Pollen mixtures	246 (58.7)	115 (70.6)	131 (51.2)	**0.001**
House dust mite	97 (23.2)	41 (25.2)	56 (21.9)	0.438
Mold	6 (1.4)	0	6 (2.3)	0.086
Animal dander	7 (1.7)	1 (0.6)	6 (2.3)	0.178
Venom	20 (4.8)	3 (1.8)	17 (6.6)	**0.032**
Multiple	43 (10.3)	3 (1.8)	40 (15.6)	**0.001**
IgE at diagnosis (IU/ml), mean (range)	90 (10-2020)	118 (17-2020)	78 (10-1143)	**0.001**
Eosinophil count (cell/ml), mean (range)	170 (3.40-1110)	160 (3.40-1110)	170 (10-980)	0.971
SARS-CoV-2, n (%)	79 (18.9)	32 (19.6)	47 (18.4)	0.745
Pneumonia, n (%)	10 (2.4)	7 (4.3)	3 (1.17)	0.055
Hospitalization (days), mean (range)	7 (1.7)	5 (3.1)	2 (0.77)	0.075

AIT = Allergen immunotherapy, Ig = immunoglobulin, SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2

Table 2. Demographic and clinical characteristics of allergic rhinitis patients according to SARS-CoV-2

Parameters	Total (n = 419)	SARS-CoV-2 (+) (n = 79)	SARS-CoV-2 (-) (n = 340)	*p* value
Age (years), mean (range)	30 (15-76)	32 (17-69)	29 (15-76)	0.244
Female gender, n (%)	266 (63.5)	58 (73.4)	208 (61.2)	**0.042**
Duration of disease (years), mean (range)	5 (0.6-35)	6 (2-32)	5 (0.6-35)	0.093
Comorbidities, n (%)	33 (7.9)	8 (10.1)	25 (7.4)	0.410
Hypertension	13 (3.1)	1 (1.3)	12 (3.5)	0.296
Type 2 diabetes mellitus	5 (1.2)	2 (2.5)	3 (0.9)	0.239
CAD	12 (2.9)	4 (5.1)	8 (2.4)	0.193
Hypothyroidism	3 (0.7)	1 (1.3)	2 (0.6)	0.112
Atopy, n (%)				0.752
Pollen mixtures	246 (58.7)	45 (57.0)	201 (59.1)	0.726
House dust mite	97 (23.2)	22 (27.8)	75 (22.1)	0.272
Mold	6 (1.4)	0	6 (1.8)	0.599
Animal dander	7 (1.7)	1 (1.3)	6 (1.8)	0.755
Venom	20 (4.8)	3 (3.8)	17 (5)	0.652
Multiple	43 (10.3)	8 (10.1)	35 (10.3)	0.965
IgE at diagnosis (IU/ml), mean (range)	90 (10-2020)	83 (15-2020)	96.5 (10-1380)	0.713
Eosinophil count (cell/ml), mean (range)	170 (3.40-1110)	180 (20-980)	165 (3.40-1110)	0.130
Immunotherapy, n (%)	163 (38.9)	32 (40.5)	131 (38.5)	0.745
Duration of AIT (months), mean (range)	15 (3-58)	14 (5-40)	17 (3-58)	0.714

SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2, Ig = immunoglobulin, CAD = Coronary artery disease, AIT = Allergen immunotherapy

In the 419 patients included in the study, the rate of pneumonia was 2.4% (10 patients) [12.7% of SARS-CoV-2 (+) patients]. During the study period, seven patients [21.9% of SARS-CoV-2 (+) patients] in AIT group and three patients [6.4% of SARS-CoV-2 (+) patients] in non-AIT group had pneumonia due to SARS-CoV-2. Five patients [15.6% of SARS-CoV-2 (+) patients] in AIT group and two patients [4.3% of SARS-CoV-2 (+) patients] in non-AIT group were hospitalized. No significant difference was determined between allergic rhinitis patients with AIT and the non-receivers in regard to the rate of pneumonia and hospitalization ($p = 0.055$ and $p = 0.075$). During the study period, there were no patients admitted to the intensive care unit or who had died.

We divided the study participants into two groups, as the allergic rhinitis patients on AIT and allergic rhinitis patients not receiving AIT; no significant difference was determined between the groups in terms of age, baseline eosinophil count and frequency of infection with SARS-CoV-2 virus, SARS-CoV-2 related pneumonia and SARS-CoV-2 related hospitalization. However, there was a significant difference between the three groups in terms of gender, duration of disease, sensitivity to allergens (atopy) and serum IgE levels ($p = 0.009$, $p = 0.001$, $p = 0.001$ and $p = 0.001$, respectively) (Table 1).

When SARS-CoV-2 positive and SARS-CoV-2 negative allergic rhinitis patients were compared, there were no significant differences between both groups in terms of age, duration of disease, accompanying comorbidities, sensitivity to allergens (atopy), serum IgE levels, eosinophil counts, rate of patients receiving AIT and duration of AIT. A significant difference was determined in terms of gender ($p = 0.042$) (Table 2).

It was found that according to univariant and mul-

Table 3. Logistic regression analysis of possible risk factors associated with SARS-CoV-2 in allergic rhinitis patients

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Gender	0.571 (0.331-0.984)	**0.043**	0.571 (0.330-0.987)	**0.045**
Duration of disease	1.041 (1.000-1.083)	0.048	1.040 (0.999-1.082)	0.056
CAD	2.213 (0.649-7.543)	0.204		
Eosinophil count	1.001 (1.000-1.002)	0.073		
Immunotherapy	1.086 (0.659-1.790)	0.745		
Duration of immunothrapy	0.987 (0.953-1.022)	0.470		

CAD = Coronary artery disease

tivariant regression analysis, the accompanying comorbidities (coronary artey disease), eosinophil count, AIT and duration of AIT were not found to be associated with an increased risk SARS-CoV-2 PCR positivity. On the other hand, female gender was shown to be associated with an decreased risk for SARS-CoV-2 PCR positivity (OR, 0.571; 95% confidence interval, 0.330-0.987; $p = 0.045$) (Table 3).

DISCUSSION

To the best our knowledge, our study is the only study evaluating the course of COVID-19 in patients undergoing AIT, and this study highlights three important findings: (1) Allergic disease duration, sensitized allergen types, and IgE levels at the time of diagnosis are higher in allergic rhinitis patients undergoing AIT. (2) There is no significant difference between the patients who underwent AIT and the non-AIT patient group in terms of SARS-CoV-2 prevalence, SARS-CoV-2-associated pneumonia, and SARS-CoV-2-associated hospitalization. (3) Although female gender is protective for SARS-CoV-2 positivity in allergic rhinitis patients, AIT or duration of AIT is not a risk factor for SARS-CoV-2 (+) in this patient group.

AIT is recommended for patients who use optimal pharmacological treatment for allergic respiratory diseases and minimize allergen exposure but do not get enough benefit from these treatments or have side effects related to these treatments. Thus, AIT can be considered as the next step of pharmacological treatment in patients with AR. It is therefore expected that the duration of illness in patients with AR who underwent AIT would be longer than in patients without AIT. In patients with a long disease duration, higher serum IgE levels may be achieved due to increased allergen exposure.

In our study, the prevalence of SARS-CoV-2, SARS-CoV-2-associated pneumonia, and hospitalizations between patients with and without AIT were found to be normal between groups. In a retrospective study conducted in Wuhan, no difference was found between patients with AR and non-AR patients in terms of severe cases, need for mechanical ventilators, and complications [12]. Ren *et al*. [13] reported that AR has a protective effect for COVID-19 infection in all age groups and that drugs used in the treatment of AR (antihistamines and intranasal steroids) do not affect COVID-19 severity and mortality. A study conducted in Turkey showed that there was no significant difference between patients with and without allergic rhinitis in terms of SARS-CoV-2-related hospitalizations and COVID-19 severity [14]. It was reported by Vezir *et al*. [15] that COVID-19 is more asymptomatic/mild in pediatric patients with aeroallergen sensitivity. A number of hypotheses have been proposed in these studies to explain the relatively positive effect of AR on the course of COVID-19. The first of these is on nasal steroids. Intranasal steroids are the most commonly used drugs in allergic rhinitis patients. In an ARIA-EAACI statement, it has been suggested that patients infected with COVID-19 can use intranasal steroids at recommended doses, since there is no evidence that the immune system is suppressed by these agents, patients with allergic rhinitis should not discontinue the use of intranasal steroids [16]. Moreover, some nasal steroids such as mometasone have been

shown to inhibit SARS-CoV-2 replication [17]. It has been suggested by Straus *et al*. that the use of nasal steroids reduces COVID-19-related hospitalizations, intensive care admissions, and mortality [18]. Another hypothesis is about eosinophils. With experimental studies, eosinophils have been shown to have a potential role in viral clearance and antiviral host defense [19]. Clinical prevalence in patients with AR correlates with blood and nasal eosinophil counts [20]. Therefore, it can be speculated that increased eosinophil counts in the respiratory tract may be protective against COVID-19 [21]. Another hypothesis proposes that ACE2 expression is decreased due to Type 2 inflammation in airway cells of patients with AR, and that allergen-specific T cells show a rapid and effective memory response to heterogeneous SARS-CoV-2 epitopes [13]. It has been shown by Kimura *et al*. [22] that IL-13 exposure reduces ACE2 expression in patients with asthma and AR. Contrary to these data, Yang *et al*. [23] suggested that allergic rhinitis has an increased risk in terms of SARS-CoV-2 positivity and more severe disease, and that hospitalizations are longer in patients with allergic rhinitis.

As another result of our study, it was found that female gender is protective in terms of SARS-CoV-2 (+) in patients with AR. Many reasons have been suggested in the differences of pathophysiology between genders in COVID-19 [24-26]. Many studies have shown that since the beginning of the pandemic male gender is a risk factor for COVID-19-related morbidity and mortality. The reason for these differences between genders may be immunological, hormonal or genetic differences or a combination of these. The effects of sex hormones on pattern recognition receptor and type I IFN responses are different. Sex hormones may affect immune cells in different ways. Estrogen is immunosuppressive at high doses and activates the immune system at low levels. On the other hand, testosterone suppresses natural immunity at all levels. Also, estrogen has been shown to inhibit ACE2, a functional receptor of SARS-COV-2, but androgens upregulate ACE2 activity [27].

As a result of the study, we found that AIT or AIT duration in patients with AR is neither a risk nor protective factor for SARS-CoV-2 (+). There is no study in the literature on the course of COVID-19 in patients with allergic rhinitis who underwent AIT. There is increasing evidence that AIT induces IgG4-positive regulatory B cells (Bregs), and regulatory B cells suppress antigen-specific T cell proliferation by producing IL-10. Also, AIT induces Treg cell formation [10, 28]. Treg cells are an indispensable subset of T cells that weaken the excessive immune response to pathogens, develop immune tolerance against environmental proteins, cancer cells and transplanted organs, and prevent and control the occurrence of autoimmune and allergic diseases [29]. Treg cells can inhibit ongoing inflammation in various steps by secreting suppressive mediators such as IL-10, TGF-β, and IL-35, by suppressing and/or cytolyzing dendritic cells through membrane molecules such as CTLA-4, PD-1, and enzymes such as granzymes A and B [9, 30, 31]. T reg cells use these mechanisms to suppress all effector cell types (directly or indirectly), eosinophils, B cells, DCs, T cells as well as inflamed resident tissue cell [29]. Therefore, despite the fact that it is thought that AIT can contribute positively to the course of COVID-19 by preventing the exaggerated cytokine response via Treg, we could not obtain such a result in our study, and we found the COVID-19 course of patients with AR who underwent AIT and patients with AR who did not receive AIT to be similar. We believe that more comprehensive studies should be conducted on this subject.

Limitations

Our study has some limitations. The first of these is the cross-sectional design. Secondly, the age range of the study population is much younger compared to older patients who are vulnerable to COVID-19, and they have fewer accompanying comorbid non-allergic comorbidities. Another thing that may have negatively affected the prevalence of SARS-CoV-2 is that rapid diagnostic tests like PCR tests were not widely used at the beginning of the pandemic.

CONCLUSION

In conclusion, we would like to highlight that in patients with AR who underwent AIT and in patients with AR who did not undergo AIT, the course of COVID-19 is similar, and AIT does not seem to increase the risk for COVID-19 infection. As such, it could be safely used in patients with AR, compatible with the data in the literature.

Authors' Contribution

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

Conflict of interest

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

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Comparison of pulmonary veins in patients with and without atrial fibrillation using multidedector computed tomographic angiography

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ABSTRACT

Objectives: Atrial fibrilation (AF) develops from an arrhythmogenic ectopic focus, which triggers the vicious circle that creates arrhythmias. Arrhythmogenic foci are often located in the transition areas between the pulmonary veins and the left atrial endothelium. This study aims to compare the pulmonary vein anatomy of patients with and without AF using multidetector computed tomographic (MDCT) angiography and to evaluate the relationship between the presence of pulmonary vein variations and the development of AF.

Methods: Seventy cases (38 males, 32 females) aged between 23 and 75 (mean age: 49.9 ± 13.3) were included in this study. This study consisted of 20 patients undergoing endovascular radiofrequency catheter ablation with AF and 50 participants (control) without AF. MDCT angiography examination was performed for the evaluation of pulmonary vein anatomy and variations.

Results: Normal pulmonary vein anatomy was observed in 30% (n = 6) of the study group, 60% (n = 30) of the control group, and 51.4% (n = 36) of the total of both groups. Variation in pulmonary vein anatomy (accessory pulmonary vein or common ostium) was detected in 48.6% (n = 34/70) of the cases. The most common variation was the presence of accessory pulmonary vein (35.7%). Common ostium was found to be the second most common variation (12.8%). All common ostia were localized on the left side. Early branching of pulmonary veins was detected in 41 (58.5%) of 70 cases.

Conclusions: Accesory pulmonary vein, common ostium and early branching are more frequently present in patients with AF.

Keywords: Atrial fibrillation, pulmonary veins, multidetector computed tomographic angiography

Atrial fibrillation (AF) is the most common rhythm disorder in the community. The prevalence of AF varies between 0.4% and 1% in general, and its incidence rises to 8% after the age of 80. AF, which is responsible for 15% of all strokes, is a clinical entity with a high morbidity and mortality rate, doubling the mortality rates due to cardiovascular causes [1-3]. The main mechanism underlying the pathophysiology of AF is the arrhythmogenic ectopic focus, which triggers the vicious circle that creates arrhythmias. Arrhythmogenic foci are often located in the transition areas between the pulmonary veins and the left atrial endothelium. These transition zones, which correspond to the ostia where the pulmonary veins open to

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the left atrium, are tissues that tend to produce arrhythmias, which are covered like a shirt by the myocardial tissue embryologically [4-7].

Although there are various antiarrhythmics that can be used to control the ventricular response and maintain normal sinus rhythm in the treatment of AF, the success rate of medical treatment is low and there are many side effects. This situation has brought the search for different treatments to the agenda. Jais et al.'s [6] demonstration of the presence of ectopic pulses originating from the pulmonary veins in patients with AF not only gave a new direction to the treatment of AF, but also opened a new window in understanding the mechanism of AF. Today, endovascular radiofrequency catheter ablation (RFCA) of the arrhythmia focus located in the transition region of the pulmonary vein ostia is the most effective method used in the treatment of medically resistant AF. The success rate of RFCA depends on knowing the anatomy of the pulmonary veins and left atrium before the procedure. Today, multidetector computed tomographic (MDCT) angiography is the most preferred method for revealing the pulmonary vein anatomy before RFCA in detail and creating two- and three-dimensional images that will serve as a guide during the procedure. In recent years, various studies have been conducted to question the relationship between pulmonary vein anatomy and the development of AF, based on the basic mechanism in the pathophysiology of AF. Some authors indicated a possible role of anomalies in the number and insertion of pulmonary veins in initiating AF. It has been shown also well that changes in anatomy of pulmonary veins such as enlargement, may have an effect on arrhythmogenesis [8-14].

In this study; it was aimed to compare the pulmonary vein anatomy of patients with and without AF using 64-slice MDCT and to evaluate the relationship between the presence of pulmonary vein variations and the development of AF.

METHODS

Patient Population

Seventy cases (38 males, 32 females) aged between 23 and 75 (mean age: 49.9 ± 13.3) were included in this study. The study group consisted of 20 consecutive patients (mean age: 44.5 ± 12.5 years [range, 26-68 years]; 11 males, 9 females) who were diagnosed with AF and planned for RFCA treatment [AF(+) group], MDCT angiography examination was performed for the evaluation of pulmonary vein anatomy and variations. MDCT images of the control group [AF(-) group] consisting of 50 cases (mean age: 46.4 ± 11.7 years [range, 22-71 years]; 27 males, 23 females) who had no history of AF and underwent cardiac MDCT angiography examination with another preliminary diagnosis were retrospectively analyzed. There were no statistically significant differences between the study group and the control group in regard to age, sex, presence of hypertension or left ventricular systolic dysfunction. Patients with clinically important valvular disease, coronary artery bypass grafts and severe left ventricular dysfunction were not included to the study.

Informed consent was obtained from all patients and the study was approved by the local Ethics Committee of our hospital. (2010/247).

MDCT Scanning Protocol

A 64-detector CT scanner (Aquilion; Toshiba Medical Systems; Tokyo, Japan) and the same protocol were used for the examination of all patients. While patients were laid down in the supine position electrocardiography (ECG) electrodes were attached and they were monitorized. Firstly, antero-posterior and lateral scanogram images were obtained in order to determine the position of the heart and the borders of the examination area. Images were obtained during a single breath hold from the top of the diaphragm to the top of the aortic arch. An 80-100 ml iodinated contrast agent (Iomeron, Iomeprol 400 mgI/ml, Bracco, Italy veya Iopromid, Ultravist 370 mgI/ml, Schering AG, Germany) was given at a flow rate of 4-5 ml/sec through an 18-20 G cannula that was placed inside the right antecubital vein, and then 40 ml saline was given at the same rate. Optimal scan time was determined by using the automatic bolus tracking method (Sure Start, Toshiba Medical System). The region of interest (ROI) was placed over the left atrium in study group and over the descending aorta in control group, and an adjustment was carried out so that scanning would start automatically when maximum contrasting reached 180 HU. Scanning parameters were as follows: collimation 64×0.5 mm, tube voltage: 120 kV,

tube current: 300-500 mA, tube rotation time: 400 ms, slice thickness: 0.5 mm, increment 0.3 mm.

MDCT Image Analysis

A retrospective ECG-gated technique was used for the reconstruction of images. The raw data obtained from the coronary CTA examination were reconstructed at the 75% phase (mid-diastolic phase) of R-R interval by using 0.5 mm slice thickness and 0.3 mm increment. Two- and three-dimensional images were rendered by using multiplanar reformat (MPR), maximum intensity projection (MIP), and volume rendering (VR) methods by transferring the obtained axial images to a separate workstation (Vitrea 2, Vital Images, Minnesota, USA). Scans were analyzed by consensus of two observers unaware of the clinical data.

Assessment of the pulmonary veins was first conducted by examining the anatomy of the pulmonary veins and their insertion into the left atrium on three-dimensional VR images. After, two-dimensional MPR images in three different orthogonal planes (transverse, coronal and, sagittal) were evaluated to determine the number of pulmonary veins, the number of ostia and branching pattern of the pulmonary veins. Normal pulmonary venous anatomy was defined as the presence of single right and left superior and inferior pulmonary veins that drain into the left atrium without a common ostium or any accessory pulmonary veins. The ostial insertion of the pulmonary veins was defined as either separate insertion or common ostium. Pulmonary veins that either entered this virtual border of the left atrium separately or bifurcated within a distance of less than 5 mm from the border were defined as having separate ostia. If the distance between the virtual border of the left atrium and and the bifurcation of both pulmonary veins was 5 mm or larger on transverse and coronal planes, the ostium was defined as a common ostium. Accessory pulmonary vein was defined as a additional pulmonary vein entered the left atrium with a separate ostium from the superior and inferior pulmonary veins. Early Branching was defined as bifurcation of the pulmonary vein within 10 mm of origin from the virtual border of the left atrium. Measurements of pulmonary vein diameters were made at the level of the ostium. The diameter of the ostium of each pulmonary vein was measured in antero-posterior (AP) and supero-inferior (SI) directions. MPR images were used to obtain images in planes that were perpendicular to the course of the veins to allow measurements in two orthogonal directions. To determine the shape of the pulmonary vein ostia the venous ostium index (VOI) was calculated for all pulmonary veins by dividing AP measurements by SI measurements. By comparing the data obtained, it was investigated whether there was a statistically significant difference between the two groups in terms of the presence of pulmonary vein variations, pulmonary vein diameters and VOI.

Statistical Analysis

Statistical analyses were performed using the SPSS 15.0 software pack (SPSS Inc., Chicago, Ill, USA). Continuous variables are expressed as means ± standard deviation (SD) and dichotomous data are expressed as numbers and percentages. Paired Student t-test was used to compare continuous variables between AF(+) and AF(-) groups. Comparisons of categorical variables between the two groups were performed by Chi-square test. Values of $p < 0.05$ were considered significant.

RESULTS

Normal pulmonary vein anatomy was observed in 30% (n = 6) of the study group, 60% (n = 30) of the control group, and 51.4% (n = 36) of the total of both groups. In the evaluation made considering the sum of both groups (n = 70); variation in pulmonary vein anatomy (accessory pulmonary vein or common os-

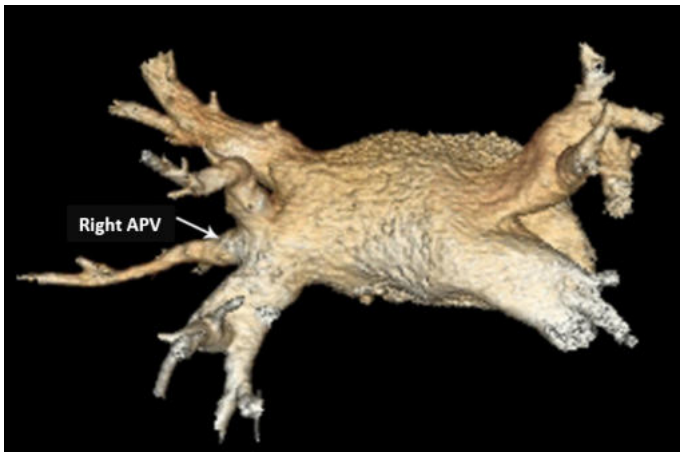

Fig. 1. Three-dimensional MDCT angiography image of a 50-year-old female patient with atrial fibrillation shows an accessory pulmonary vein (APV) on the right.

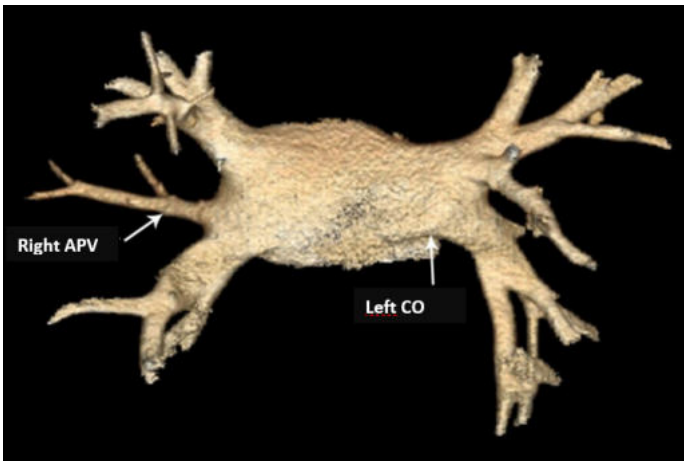

Fig. 2. Three-dimensional MDCT angiography image of a 30-year-old male patient with atrial fibrillation shows the accessory pulmonary vein (APV) on the right and the common ostium (CO) on the left.

tium) was detected in 48.6% (n = 34/70) of the cases (Figs. 1 and 2).

The rate of variation was 70% in the AF(+) group and 40% in the AF(-) group. The most common variation was the presence of accessory pulmonary vein (35.7%). Accessory pulmonary vein was present in 8 (40%) of AF(+) cases and 17 (34%) of AF(-) cases. Although the rate of accessory pulmonary vein was higher in the AF(+) group, the difference between the two groups was not significant in the statistical evaluation (p = 0.844). Common ostium was found to be the second most common variation (12.8%) (Figs. 2 and 3). Common ostium was present in 6 (30%) of AF(+) cases and 3 (6%) of AF(-) cases. All common ostia were localized on the left side. When the AF(+) group and the AF(-) group were compared in terms of having common ostium, the difference between the two groups was found to be statistically significant (p = 0.021). Early branching of pulmonary veins was detected in 41 (58.5%) of 70 cases (Fig. 4).

The right pulmonary veins in 28 (40%) cases, the left in 8 (11.4%) cases, and bilateral pulmonary veins in 5 (7.1%) cases gave early branches. Early branching was mainly (68.2%) localized on the right side. Right early branching was present in 10 (50%) AF(+) cases and 18 (36%) AF(-) cases. Although the rate of right early branching was higher in the AF(+) group, the difference between the two groups was not significant in the statistical evaluation (p = 0.265). It was determined that 85% of the right-sided localized early branches originate from the inferior pulmonary vein and 15% from the superior pulmonary vein, while all of the left-sided early branches originate from the inferior pulmonary vein. Comparison of two groups in terms of the presence of accessory pulmonary vein, common ostium and early branching is seen in (Table 1).

When the sum of both groups (n = 70) was evaluated, the mean AP diameters were 15.7 ± 2.4 mm for the right superior pulmonary vein (RSPV); 15.3 ± 2.4 mm for right inferior pulmonary vein (RIPV); 14.4 ± 2.5 mm for left superior pulmonary vein (LSPV); for the left inferior pulmonary vein (LIPV), it was found to be 13.0 ± 2.2 mm. SI diameter mean was 17.4 ± 2.5 mm for RSPV; 16.7 ± 2.7 mm for RIPV; 17.5 ± 2.6 mm for LSPV; it was found to be 16.4 ± 2.5 mm for LIPV. According to these findings, in general, the

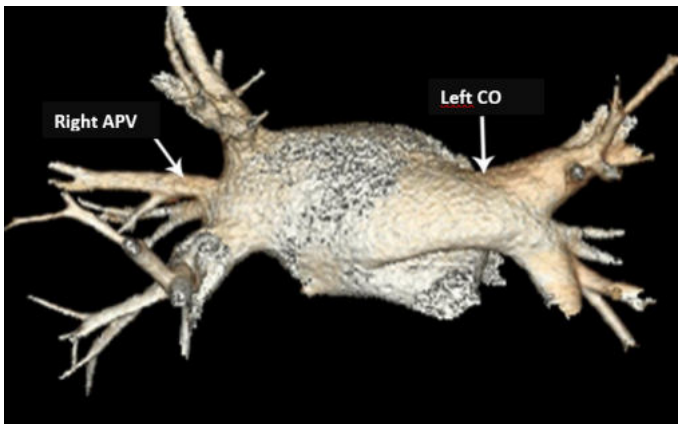

Fig. 3. Three-dimensional MDCT angiography images (a, b) of a 59-year-old male patient with atrial fibrillation showing the accessory pulmonary vein (APV) on the right and the common ostium (CO) on the left.

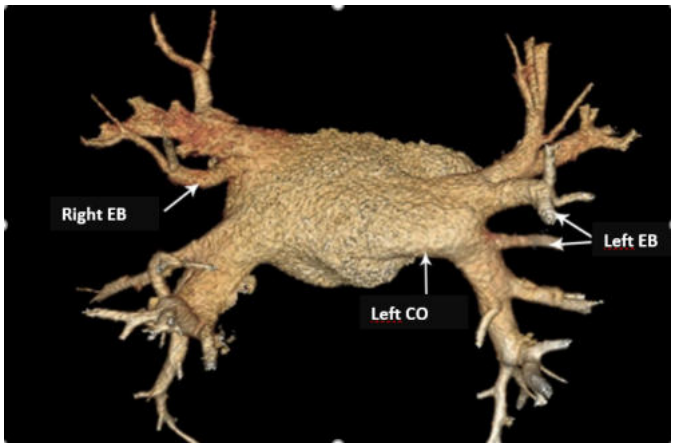

Fig. 4. TEarly branching (EB) on both sides and common ostium (CO) on the left in three-dimensional MDCT angiography image of a 50-year-old male patient with atrial fibrillation. (APV) on the right and the common ostium (CO) on the left.

Table 1. Comparison of AF (+) group and AF (-) group in terms of APV, CO and EB variant

	AF(+)	AF (-)	*p* value
Right APV, n (%)	8 (40)	17 (34)	0.844
Left CO, n (%)	**6 (30)**	**3 (6)**	**0.021**
Right EB, n (%)	10 (50)	18 (36)	0.265
Right + left EB, n (%)	**2 (50)**	**2 (50)**	

APV = accessory pulmonary vein, CO = common ostium, EB = early branching

mean diameter of the superior pulmonary veins was larger than the mean diameter of the inferior pulmonary veins, and the mean diameters of the right pulmonary veins were larger than the mean diameters of the left pulmonary veins. When AF(+) and AF(-) group pulmonary veins were compared in terms of AP diameters; there was no significant difference between the two groups in terms of AP diameters of RSPV, RIPV, LSPV and LIPV (p = 0.672, p = 0.136, p = 0.820 and p = 0.782, respectively). In the comparison made in terms of SI diameters of pulmonary veins, no significant difference was found between the two groups in terms of SI diameters of RSPV, LSPV and LIPV (p = 0.220, p = 0.953 and p = 0.627, respectively), while SI diameters of RIPV were found in the AF(+) group was found to be significantly higher than the AF(-) group (p = 0.016) (Table 2).

Table 2. Comparison of antero-posterior (AP) and supero-inferior (SI) diameters and venous ostium index (VOI) of pulmonary veins (PV) in AF(+) and AF(-) patients

x	Number	Mean	SD
Age (years)	70	50.0	13.3
SI-RSPV (mm)	70	17.4	2.5
SI-RIPV (mm)	70	16.7	2.7
SI-LSPV (mm)	61	17.5	2.6
SI-LIPV (mm)	61	16.4	2.5
AP-RSPV (mm)	70	15.7	2.4
AP-RIPV (mm)	70	15.3	2.4
AP-LSPV (mm)	61	14.4	2.5
AP-LIPV (mm)	61	13.0	2.3
VOI-RSPV	70	0.9	0.1
VOI-RIPV	70	0.9	0.2
VOI-LSPV	61	0.8	0.2
VOI-LIPV	61	0.8	0.1

PV = pulmonary vein, RSPV = right superior pulmonary vein, RIPV = right inferior pulmonary vein, LSPV = left superior pulmonary vein, LIPV = left inferior pulmonary vein, VOI = venous ostium index, SD = standard deviation

When the AF(+) and AF(-) groups were compared in terms of the VOI value obtained by dividing the AP diameter measurements by the SI diameter measurements; the VOI value of RIPV was lower in the AF(+) group than in the AF(-) group, and the difference was statistically significant ($p < 0.001$). However, the difference between the two groups in terms of VOI values of RSPV, LSPV and LIPV was not statistically significant (p = 0.078, p = 0.931 and p =0.739, respectively) (Table 2).

DISCUSSION

Today, it is known that the main localizations of ectopic foci, which are the triggers of most paroxysmal AF, are the ostia of the pulmonary veins. The answer to why ectopic foci that give rise to AF are located in the ostia, which is the opening of the pulmonary veins into the left atrium, is hidden in the embryological development stages. At the beginning of embryological development, the pulmonary vein confluences are covered by the atrial wall [13].

In the later stages of embryological development, the atrial wall is covered by myocardial tissue. In this phase, which is called musculization or atrialization, the pulmonary vein ostia are surrounded by myocardial tissue like a shirt. Therefore, the localizations where ectopic foci arise are localizations where myocardial tissue remnants from past stages of embryological development are present. Since this myocardial tissue around the ostia of the superior pulmonary veins covers a larger area compared to the myocardial tissue around the ostia of the inferior pulmonary veins, especially the superior pulmonary veins have an important place in the pathogenesis of AF [14-18].

Normally, there are two superior pulmonary veins, one on the right and one on the left, and two inferior pulmonary veins, one on the right and one on the left. The right superior pulmonary vein drains the superior

and middle lobes of the right lung, while the left superior pulmonary vein drains the superior lobe of the left lung, including the lingula. Inferior pulmonary veins on both sides drain the lower lobe on their side [19, 20]. In our study, 51.4% of the total of AF(+) and AF(-) groups; when the groups were considered separately, we observed one superior pulmonary vein with separate ostiums on the right and left and one inferior pulmonary vein with separate ostiums at a rate of 30% in the AF(+) group and 60% in the AF(-) group.

When compared to pulmonary artery anatomy, variations in pulmonary vein anatomy and developmental anomalies are observed more frequently. One of the main variations that we may encounter regarding pulmonary veins is the presence of an accessory pulmonary vein. When the veins draining the right lung middle lobe, left lung lingula or smaller segments open into the left atrium with a separate ostium instead of opening into the superior or inferior pulmonary vein, this vein is called the accessory pulmonary vein. In the literature, the most common variation was reported to be accessory pulmonary vein in some studies, while the presence of common ostium was reported more frequently in some studies.has been reported [21-23].

In patients with AF, the incidence of right accessory pulmonary vein was reported as 16% by Scharf *et al*. [24], 18.6% by Tsao *et al*. [25], 23% by Mclellan *et al*. [26], and 26% by Marom *et al*. [21]. In a study of 51 patients with AF, the incidence of right accessory pulmonary vein was found to be 7.8%, while the incidence of left accessory pulmonary vein was found to be 3.9% by Skowerska *et al*. [8]. The researchers did not find the presence of accessory pulmonary veins in any of the control group cases without AF. In our study, we found the presence of accessory pulmonary vein as the most common (35%) variation. This ratio was determined by Altinkaynak and Koktener [27]. It was found to be 20.4% in their study. While we detected the presence of right accessory pulmonary vein in 40% of the AF(+) group and 34% of the AF(-) group, we did not find the presence of left accessory pulmonary vein in any of the cases. In a study by Koçyiğit *et al*. [23], accessory veins were observed only on the right. The rate we found for the incidence of right accessory pulmonary vein is well above the rates reported in the literature (7.8-26%). When we compared the AF(+) and AF(-) groups in terms of the frequency of accessory pulmonary veins, we found a higher incidence of accessory pulmonary veins in the AF(+) group, but we did not find a significant difference between the two groups as a result of the statistical evaluation ($p = 0.844$).

A variation encountered in pulmonary vein anatomy is the common ostium. The common ostium, which is defined as the fusion of the superior and inferior pulmonary veins on one side and opening into the left atrium with a single ostium, is usually seen on the left side. The presence of right common ostium is a very rare condition and we did not find a right-sided common ostium in our study. In our study, we found the left common ostium to be the second most common (12.8%) variation. Left common ostium was present in 30% of AF(+) cases and 6% of AF(-) cases and we found that common ostium was significantly more common in the AF(+) group, consistent with previous studies ($p = 0.021$).). Similarly, the presence of the left common ostium was found 27.4% in AF(+) cases and 12.9% in AF(-) cases by Skowerski *et al*. [8]. In the study of Jongbloded *et al*. [28], involving 23 AF(+), 11 AF(-) cases, left common ostium incidence was found 83% in AF(+) cases, 55% in AF(-) cases. In Thai society, Wannasopha *et al*. [22] revealed that the rate of single ostium on the left (59%) is higher than the rate of single ostium. The most common variation was the left common vein, while the rate was 32.2% in the study by Altinkaynak and Koktener [27], this rate was found to be 35.6% in the study by Koçyiğit *et al*. [23].

Another variation that can be seen in pulmonary veins is early branching. Early branching is defined as bifurcation of the pulmonary vein within 10 mm of origin from the left atrium. In our study, we found early branching pattern most frequently (68.2%) in the right inferior pulmonary vein. We also detected early branching in the right superior pulmonary veins and left inferior pulmonary veins, at lower rates compared to the right inferior pulmonary veins. In some studies, it has been suggested that early branching of the left superior pulmonary vein is similar to the left inferior pulmonary vein in terms of its incidence. However, we did not find any early branch in the left superior pulmonary vein in our study.

While discussing the relationship between pulmonary vein variations and AF, one of the issues that was especially emphasized was the ostial diameter

measurements of the pulmonary veins. In our study, we found the diameters of the right pulmonary veins to be larger than the diameters of the left pulmonary veins and the diameters of the superior pulmonary veins to the diameters of the inferior pulmonary veins, according to the AP and SI diameter measurements measured from MPR images. Koçyiğit *et al*. [23] also found similar findings in their study. This situation is associated with myocardial sheath covering a wider and denser area especially in the superior vein ostia during the gestational development stages. As a result, the place and importance of the superior pulmonary veins in the development of AF is particularly emphasized. Skorewski *et al*. [8] found the diameters of the left atrium and pulmonary vein to be larger (larger) in the AF(+) group than in the AF(-) group.

The structural remodeling of the left atrium and pulmonary veins in patients with AF may be an explanation of these changes. While it is well known that the diameter of the left atrium increases with long-standing AF, less information is available if is there a correlation between such morphological changes and the degree of left atrium and pulmonary vein enlargement. In our study, although we found the SI diameters of the pulmonary veins to be higher in the AF(+) group, we found that the difference between the two groups was not statistically significant for veins other than RIPV. There are other studies that support larger SI diameters. [23, 29]

Another concept discussed in the literature in the context of pulmonary vein AF relationship is VOI. The VOI used to evaluate the shape of the pulmonary vein ostium is the ratio of the AP diameter of the pulmonary vein to the SI diameter (VOI=AP\SI). When we evaluate the sum of both groups together; We found that the VOI values of the left pulmonary vein were smaller than those of the right pulmonary veins. Based on this result, we can say that left pulmonary vein ostia tend to be more oval shaped than right pulmonary vein ostia. Similarly, Jongbloed *et al*. [28] and Skorewski *et al*. [8]. It was also reported in the studies performed by the left pulmonary vein that the ostia of the left pulmonary vein are more oval shaped than the ostiums of the right pulmonary veins. It has been determined that the ostia on the right side are more rounded in a study by by Koçyiğit *et al*. [23]. When we compare the AF(+) and AF(-) groups in terms of VOI value; while we found the VOI value of RIPV to be significantly lower in the AF(+) group compared to the AF(-) group, we found that there was no significant difference between the two groups in terms of the VOI values of RSPV, LSPV and LIPV.

Although AF is such a frequently encountered arrhythmia, the desired level of success in the medical treatment of AF has not yet been achieved and various complications caused by drug therapy have led to a series of discussions about AF treatment. Today, endovascular RFCA of the arrhythmia focus located in the transition region of the pulmonary vein ostia is accepted as one of the effective methods in the treatment of treatment-resistant AF cases. The success rate of RFCA is closely related to knowing the anatomy of the left atrium and pulmonary veins in detail before the procedure.

Conventional catheter angiography, echocardiography, MDCT, and magnetic resonance imaging (MRI) are among the main methods that can evaluate this complicated pulmonary vein anatomy. Although conventional pulmonary vein angiography is considered a standard technique for evaluating pulmonary veins, it has some disadvantages such as being an invasive method and difficult measurement due to projection errors. Echocardiography, on the other hand, is not an appropriate method to evaluate the atriovenous junction. In addition, the evaluation of the proximal parts of the pulmonary veins by echocardiography is also insufficient.

MRI and MDCT are the most appropriate methods for evaluating the relationship between the left atrium and pulmonary veins, as well as for the morphological and dimensional evaluation of pulmonary veins, with reconstructed three-dimensional images. These two methods stand out as more preferable methods compared to other methods in that they allow the acquisition of images that can be considered as a "road map" before ablation We analyzed the pulmonary vein anatomy of the patients who will undergo RFKA with MDCT before the procedure, from both axial images and MPR and three-dimensional volume rendering images.

As a result of our study; We found that a very high rate (48.6%) of variation (accessory pulmonary vein or common ostium) was seen in pulmonary vein anatomy. The rate of variation was higher in the AF(+)

group than in the AF(-) group (70%, 40%, respectively). The most common variations are; presence of common ostium on the left, accessory pulmonary vein on the right. The incidence of premature branching in the pulmonary veins was higher in the AF(+) group than in the AF(-) group (75%, 52%, respectively). When we compare the AF(+) and AF(-) groups in terms of the ostial diameters of the pulmonary veins; While we found the SI diameter of RIPV to be higher in the AF(+) group, we found that there was no significant difference between the two groups in terms of other diameters.

Limitations

Since the study was conducted with a small number of cases, more studies are needed on this subject.

CONCLUSION

In conclusion, there is a greater variability in the pulmonary vein anatomy and detailed knowledge of the pulmonary vein anatomy and variations is required to maximise the safety and efficacy of RFCA procedure. MDCT angiography enables a valuable road map for pulmonary vein anatomy prior to RFCA in patients with AF. The number, location, and size of pulmonary veins and pulmonary vein branching anomalies are easily and accurately depicted with MDCT angiography. Accesory pulmonary vein, common ostium and early branching are more frequently present in patients with AF.

Authors' Contribution

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

Conflict of interest

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

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The relationship between platelet indices and residual SYNTAX score in patients with ST-segment elevation myocardial infarction

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ABSTRACT

Objectives: We aimed to investigate the relationship between thrombocyte indices, which have previously been proven to be associated with many cardiovascular diseases and adverse events, and residual SYNTAX (SYNergy between percutaneous coronary intervention with TAXus and cardiac surgery) score (rSS) in patients with ST-segment elevation myocardial infarction (STEMI).

Methods: Our study included 534 patients who underwent primary percutaneous coronary intervention (PCI) for STEMI between January 2018 and June 2021. In our study, only patients who underwent infarct-related coronary artery revascularization in the index procedure were evaluated. First of all, patients were compared into two groups low rSS (rSS ≤ 8) and high rSS (rSS > 8). Our definition of platelet indices includes mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (Pct), MPV to platelet ratio (MPVPR), platelet to lymphocyte ratio (PLR), and MPV to lymphocyte ratio (MPVLR).

Results: The mean age of the study patients was 56.4 ± 10.3 years and 78.8% were male. The high rSS group had higher C-reactive protein, lower lymphocyte count, and significantly higher platelet indices other than PLR and MPV. Among the platelet indices, MPVLR was found to have the best correlation with rSS (r: 0.398, $p < 0.001$). MPVLR (AUC: 0.820, 95% CI: 0.701-0.899) was determined as the best diagnostic power index with 5.08 cut-off value in predicting high rSS with 88% sensitivity and 76% specificity (Youden index: 0.64). Age, right coronary artery involvement as culprit lesion, ejection fraction, diabetes mellitus, and MPVLR (OR: 5.966 [2.489-8.413], $p < 0.001$) and PDW were identified as independent risk factors for predicting high rSS.

Conclusions: In conclusion, increased MPVLR is associated with high rSS in STEMI patients. There is a significant positive correlation between MPVLR and rSS. MPVLR is an independent predictor of high rSS.

Keywords: Coronary artery disease severity, incomplete revascularization, mean platelet volume to lymphocyte ratio, platelet distribution width, residual SYNTAX score

Worldwide, ischemic heart disease is the most common cause of death and its incidence is increasing. Ischemic heart disease is the cause of about 20% of all deaths in Europe, although there is great variation between countries. ST-segment elevation myocardial infarction (STEMI) is a subgroup of ischemic heart disease with a high mortality and morbidity rate, and a clinical and hemodynamic response can be achieved dramatically with primary percutaneous coronary intervention (PCI)-based revascularization [1, 2]. Multi-vessel coronary artery disease occurs in 40–65% of STEMI patients undergoing pri-

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mary PCI and is associated with adverse prognosis [3]. Multi-vessel disease, complex coronary anatomies, and the approach of artery revascularization responsible for isolated infarcts can cause incomplete revascularization.

The extent of coronary artery disease after infarct-related coronary artery PCI, or in other words, incomplete revascularization, can be evaluated with the residual SYNTAX (SYNergy between percutaneous coronary intervention with TAXus and cardiac surgery) score. The ACUITY (Acute Catheterization and Urgent Intervention Triage strategY) study identified that a high residual SYNTAX score (rSS > 8) is associated with major adverse cardiovascular events (MACE) and poor prognosis in patients with moderate-to-high risk acute coronary syndrome (ACS) [4]. It has also been proven that rSS is an independent predictor of MACE and mortality in STEMI patients [5].

Inflammation and thrombosis are two major mechanisms that play an important role in different stages of atherosclerotic plaque formation, progression, rupture, and thrombosis [6]. Platelet activation plays an important role in the formation and progression of atherosclerosis and thrombus formation on ruptured atherosclerotic plaques [7]. Therefore, it has been suggested that platelet dysfunction is an important factor in the pathogenesis of STEMI [8]. Platelet volume indices; Mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (Pct), and mean platelet volume to platelet ratio (MPVPR) are simple indices that express platelet activity and can be obtained in routine complete blood count tests [9]. The increase in mean platelet volume (MPV) is also an indicator of an increase in platelet activity and aggregation tendency [10]. A relationship between MPV and coronary artery disease has been demonstrated by previous studies [11]. PDW refers to platelet activity as a measure of circulating platelet size variation [12]. Increased PDW values indicate anisocytosis and are associated with increased thrombosis propensity and severity of coronary artery disease in ACS patients [13]. Pct is a measure of total platelet mass. Increased Pct values are associated with major adverse cardiac and cerebrovascular events [14]. Lymphocytes are the earliest cell type involved in the formation of atherosclerotic plaque. A decrease in their number is an indicator of the inflammatory response and contributes to plaque unstabilization. In the inflammatory phase of thrombosis, platelets are accompanied by lymphocytes [6, 15, 16].

Unlike platelet volume indices, platelet to lymphocyte ratio (PLR) and MPV to lymphocyte ratio (MPVLR), which are indices that can evaluate thrombosis and inflammation together, have been shown to have prognostic importance in ACS patients [17, 18]. There is insufficient evidence to show the relationship between rSS, which is a measure of incomplete revascularization, and these platelet indices, which can affect the extent of coronary artery disease. In this study, we aimed to investigate the relationship between rSS and platelet indices and to determine the index with the highest diagnostic power for high rSS prediction.

METHODS

Study Population

In our study, patients who underwent primary PCI for STEMI between January 2018 and June 2021 were retrospectively screened. In the index procedure, only culprit lesion revascularization was our prerequisite, so those who underwent multivessel revascularization in the same procedure, those who were given medical follow-up after their diagnostic angiography, or those who decided to undergo coronary bypass graft surgery (CABG) were excluded from the study. Patients with a previous history of PCI or CABG, those with hematologic/autoimmune/infectious or inflammatory diseases, advanced renal or hepatic disease, a history of malignancy, or those receiving immunosuppressive therapy were excluded from the study. As a result, 534 patients were included in our study. Our study was carried out in accordance with the principles of the Declaration of Helsinki, with the decision of the local ethics committee numbered 2022/126.

The diagnosis of STEMI was determined in accordance with the 2017 European Society of Cardiology (ESC) recommendations, in the presence of characteristic symptoms and signs of myocardial ischemia, by detecting ST segment elevation in at least 2 consecutive electrocardiographic leads consistent with coronary anatomical localization [1]. In patients with multivessel disease who had only culprit lesion revascularization, non-infarct coronary revascularization was planned to be performed before discharge or under elective conditions, depending on the patients'

anginal complaints and hemodynamic status.

Patient Characteristics

Demographic and clinical parameters at admission were recorded from the hospital database. All blood samples were collected before patients underwent coronary angiography. In biochemical analyzes: total cholesterol, low-density lipoprotein cholesterol (LDL-C) (calculated with the Friedewald equation), high-density lipoprotein cholesterol (HDL-C), triglyceride, creatinine, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP) and albumin results were evaluated. Hematological data included hemoglobin, leukocyte, lymphocyte, thrombocyte, PDW, and MPV measurements. Platelet indices were calculated from these hemogram data. Definition of platelet indices: MPV, PDW, plateletcrit (Pct: platelet count × MPV / 10,000), mean platelet volume to platelet ratio (MPVPR: MPV / platelet count x 100), platelet to lymphocyte ratio (PLR: Platelet count / lymphocyte count) and mean platelet volume to lymphocyte ratio (MPVLR: MPV / lymphocyte count). Echocardiography measurements were performed immediately after performing PCI during hospitalization at the coronary intensive care unit.

Coronary Angiographic Evaluation

Coronary angiography was performed via femoral or radial access for each patient within 90 minutes of admission. Two independent, interventional cardiologists blinded to patient clinical data individually evaluated coronary angiographic images to calculate coronary artery disease severity [19]. Firstly, the SYNTAX score I was calculated using the online SYNTAX score calculator (http://www.syntaxscore.com, version 2.1). for each patient. The rSS was calculated based on the remaining coronary artery lesions after performing PCI for the infarct-related coronary artery. Coronary arteries were evaluated as 16 distinct segments, and segments with luminal stenosis of 50% or more and > 1.5 mm in diameter were evaluated. An rSS >8 was considered a high rSS [4].

Study patients were primarily evaluated as two groups according to the calculated rSS levels: Low rSS (rSS ≤ 8) and high rSS (rSS > 8). According to the MPVLR index cut-off value, which was determined to be the best predictor of incomplete revascularization expressed with high rSS, the study patients were again formed into two groups and compared in terms of their angiographic data: Low MPVLR (MPVLR ≤ 5.08) and high MPVLR (MPVLR > 5.08).

Statistical Analysis

Statistical analyses were performed using SPSS 21 for Windows (SPSS Inc., Chicago, IL). The conformity of the data to the normal distribution was determined by the Kolmogorov-Smirnov test. Continuous data are presented as mean ± standard deviation (SD) for variables with normal distribution and as median (25th-75th percentiles) for variables without normal distribution. The independent sample t-test was used for comparing quantitative variables with normal distribution, while the Mann-Whitney U test was used for comparing the means between groups without normal distribution. Categorical data are presented as "number (%)" and were compared using Pearson's χ^2 test or Fisher's exact test. Spearman analysis was used to evaluate the correlation between rSS and platelet indices. Univariate and multivariate logistic regression analyses were used to evaluate independent risk factors for a high rSS. Receiver operating characteristic (ROC) curve analysis was conducted to determine the optimal platelet indices values to indicate high rSS in terms of both sensitivity and specificity. Intra-observer and inter-observer agreements for 2 cardiologists were calculated using Cohen's kappa coefficient. *P* value < 0.05 was considered statistically significant.

RESULTS

Our study included 534 patients who underwent primary PCI for ST-segment elevation myocardial infarction. The mean age of the patients was 56.4 ± 10.3 years, and 78.8% (n = 421) were men. Our study patients were evaluated into two groups 'low rSS' with rSS ≤ 8 and 'high rSS' with rSS > 8. The mean age of the patients in the high rSS group was significantly higher than that of the low rSS group (58.3 ± 10.3 years vs 55.8 ± 10.2 years, $p = 0.032$). Gender distribution was dominant in terms of the male gender in both groups, but there was no significant difference between the groups. There was no significant difference in terms of BMI, and systolic and diastolic blood

Table 1. Baseline clinical, demographic and laboratory characteristics of the study population

	All patients (n = 534)	Low rSS (n = 397)	High rSS (n = 137)	*p* value
Age (years)	56.4 ± 10.3	55.8 ± 10.2	58.3 ± 10.3	**0.032**
Gender (male), n (%)	421 (78.8)	312 (78.5)	109 (79.5)	0.101
BMI (kg/m^2)	25.6 ± 3.6	25.9 ± 3.8	25.2 ± 4.4	0.233
Heart rate (BPM)	79.2 ± 15.8	78.4 ± 15.1	79.8 ± 14.7	0.101
SBP (mmHg)	121.2 ± 21.4	123.2 ± 21.2	121.3 ± 20.6	0.216
DBP (mmHg)	79.0 ± 12.3	78.7 ± 11.5	78.4 ± 11.4	0.289
Hypertension, n (%)	219 (41)	160 (40.3)	59 (43.1)	0.390
DM, n (%)	91 (17)	67 (16.8)	24 (17.5)	0.603
PAD, n (%)	21 (3.9)	15 (3.7)	6 (4.3)	0.087
Hyperlipidemia, n (%)	379 (70.9)	280 (70.5)	99 (72.2)	0.581
Smoking, n (%)	278 (52)	206 (51.8)	72 (52.5)	0.263
Total cholesterol (mg/dL)	178.7 ± 39.7	178.1 ± 40.8	180.2 ± 40.4	0.408
LDL-C (mg/dL)	105.9 ± 36.1	104.2 ± 36.7	110.2 ± 35.1	**0.009**
HDL-C (mg/dL)	43.2 ± 11.8	42.6 ± 11.6	42.9 ± 11.9	0.355
Triglycerides (mg/dL)	145.1 (95.7 - 245.3)	146.8 (93.1 - 241.2)	142.6 (97.3 - 249.1)	**0.021**
Creatinine (mg/dL)	0.92 ± 0.22	0.91 ± 0.19	0.92 ± 0.21	0.134
Glucose (mg/dL)	117.5 ± 26.1	119.4 ± 25.9	116.5 ± 25.8	0.188
ALT (U/L)	22.1 (17.1 - 31.5)	21.5 (15.5 - 27.2)	22.1 (16.3 - 28.2)	0.097
AST (U/L)	26.4 (20.2 - 32.6)	27.2 (21.8- 32.7)	26.3 (20.1- 33.5)	0.128
CRP (mg/L)	4.4 (1.83 - 9.24)	3.8 (2.45 - 11.8)	6.9 (2.75 - 12.7)	**0.006**
Albumin (g/dL)	3.76 (1.71- 6.76)	3.61 (1.56 - 5.77)	3.87 (1.84 - 6.96)	0.111
Hemoglobin (g/dL)	14.5 (13.5 -15.8)	14.4 (13.3 - 15.4)	14.7 (13.7 - 15.8)	0.094
Leukocytes x10^3/mm^3	11.4 (9.3 - 13.6)	9.8 (8.8 - 11.9)	12.9 (10.1 - 16.6)	**< 0.001**
Lymphocyte x10^9/L	2.56 ± 0.51	3.72 (2.67 - 5.18)	1.72 (1.54 - 2.61)	**0.006**
Platelets x10^3/mm^3	219.5 (174.3 – 261)	218.2 (170 - 254.6)	222.7 (185.6 - 270.2)	0.075
PLR	163.23 ± 35.48	161.44 ± 32.24	165.12 ± 35.48	0.121
MPV (fL)	10.37 ± 1.63	10.23 ± 1.69	10.59 ± 1.59	0.091
MPVLR	3.58 ± 1.52	3.27 ± 1.32	5,66 ± 2.15	**< 0.001**
MPVPR	4.44 ± 1.61	3.21 ± 1.43	5,52 ± 2.06	**< 0.001**
Pct (%)	0.246 ± 0.72	0.217 ± 0.082	0.264 ± 0.064	**0.003**
PDW (%)	14.3 ± 2.9	12.36 ±2.72	17.38 ±2.94	**0.005**
β-blocker	64 (11.9)	47 (11.8)	17 (12.4)	0.096
Calcium channel blocker	85 (15.9)	62 (15.6)	23 (16.7)	0.421
ACEI	59 (11)	45 (11.3)	14 (10.2)	0.313
ARB	74 (13.8)	55 (13.8)	19 (13.8)	0.524
Statin	144 (26.9)	105 (26.4)	39 (28.4)	0.362

rSS = Residual SYNTAX score, BMI = Body mass index, BPM = Beats per minute, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, DM = Diabetes mellitus, PAD = Peripheral arterial disease, LDL-C = Low-density lipoprotein cholesterol, HDL-C = High-density lipoprotein cholesterol, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, CRP = C-reactive protein, PLR = Platelet to lymphocyte ratio, MPV = Mean platelet volume, MPVLR = Mean platelet volume to lymphocyte ratio, MPVPR = Mean platelet volume to platelet ratio, Pct = Plateletcrit, PDW = Platelet distribution width, ACEI = Angiotensin-converting enzyme inhibitor; ARB = Angiotensin receptor blocker

pressures. Risk factors such as hypertension, diabetes mellitus (DM), peripheral arterial disease (PAD), hyperlipidemia, and smoking did not differ significantly between the groups. While no difference was found in total cholesterol and HDL cholesterol, it was found that LDL cholesterol was significantly higher in the high rSS group (110.2 ± 35.1 mg/dL vs 104.2 ± 36.7 mg/dL, $p = 0.009$) and triglyceride level was significantly higher in the low rSS group [146.8 (93.1 - 241.2) mg/dL vs 142.6 (97.3 - 249.1) mg/dL, $p = 0.021$]. While there was no significant difference between the groups in other biochemical parameters, it was observed that the CRP level was higher in the high rSS group [6.9 (2.75 - 12.7) mg/L vs 3.8 (2.45 - 111.8) mg/L, $p = 0.006$]. From the hemogram data, hemoglobin and platelet counts did not differ significantly between the groups, and the leukocyte counts in the high rSS group [12.9 (10.1-16.6) ×103/mm3 vs 9.8 (8.8 - 11.9) ×103/mm3, $p < 0.001$] and the lymphocyte count in the low rSS group [3.72 (2.67 - 5.18) ×109/L vs 1.72 (1.54 - 2.61) ×109/L, $p = 0.006$] were found to be significantly higher. There was no significant difference in the medical treatments of the study patients before the index procedure. Baseline clinical, demographic, and laboratory data of our study patients and rSS groups are presented in Table 1.

The distribution of hemogram data and rates, which we define as platelet indices, between the groups is as follows: (i) Although numerical superiority in PLR and MPV is observed in the High rSS group, the difference between the groups is not statistically significant. (ii) MPVLR ($p < 0.001$), MPVPR ($p < 0.001$), Pct ($p = 0.003$) and PDW ($p = 0.005$) were observed to be significantly higher in the high rSS group. Platelet indices data distribution is presented numerically in Table 1 and graphically in Fig. 1.

MPVLR, MPVPR, Pct, and PDW seem to be ahead of other platelet indices in our search for the most appropriate platelet index for the prediction of the high level of rSS (> 8), due to their different distributions among the rSS groups and the correlation relationships detected with rSS. The diagnostic power of thrombocyte indices in predicting high rSS was tested by ROC analysis. According to the analysis results, it was found that the platelet indices MPVLR [Area under the curve (AUC): 0.820], Pct (AUC: 0.712), and PDW (AUC: 0.754) had a significant diagnostic value for the prediction of high rSS (for each $p < 0.001$). MPVLR was determined as the index with the best diagnostic power in predicting high rSS with a cut-off value of 5.08, sensitivity of 88% and specificity of 76% (Youden index: 0.64). Platelet indices

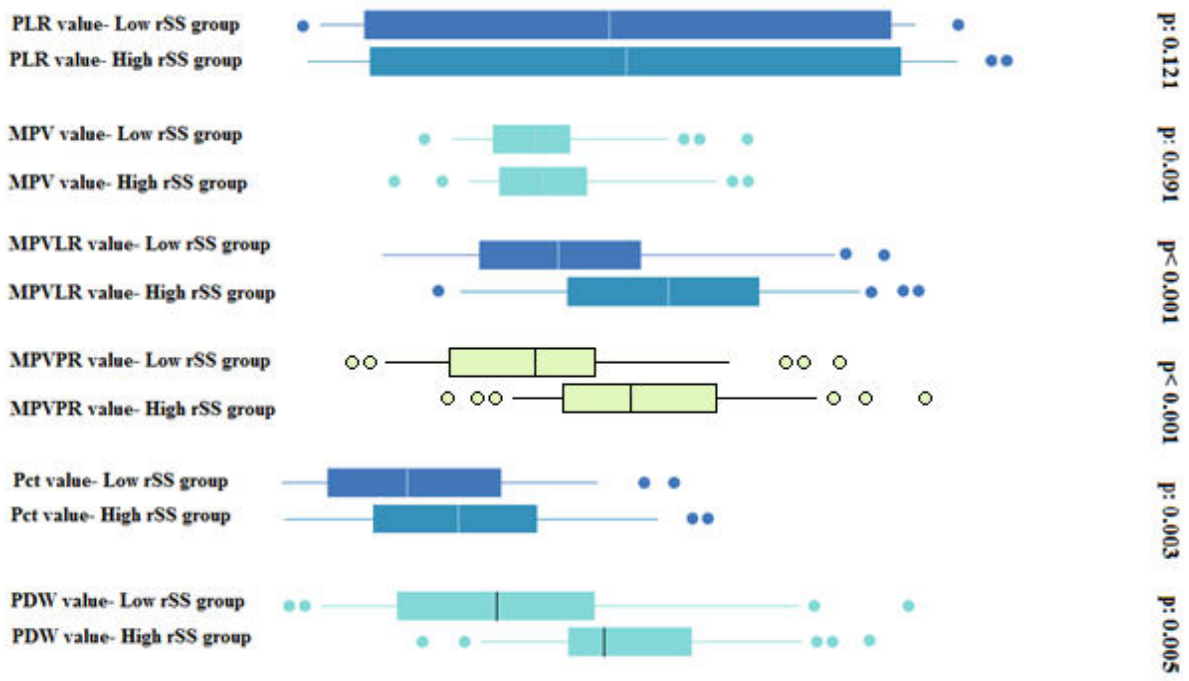
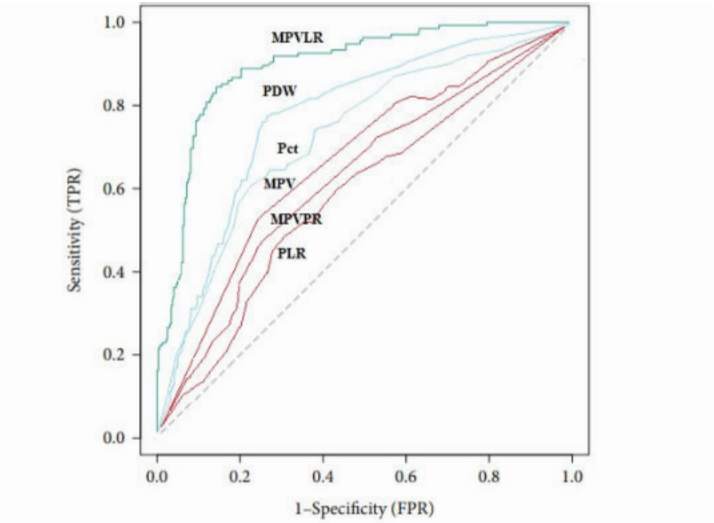


Fig. 1. Platelet indices values of patients with low and high rSS. PLR = Platelet to lymphocyte ratio, MPV = Mean platelet volume, MPVLR = Mean platelet volume to lymphocyte ratio, MPVPR = Mean platelet volume to platelet ratio, Pct = Plateletcrit, PDW = Platelet distribution width.

Table 2. Baseline echocardiographic and angiographic characteristics of the study population

	All patients (n = 534)	Low MPVLR (n = 321)	High MPVLR (n = 213)	*p* value
LVEF (%)	53.4 ± 6.9	54.8 ± 6.1	53.6 ± 6.5	0.129
Culprit vessel, n (%)				
LAD	254 (47.6)	170 (52.9)	84 (39.4)	**< 0.001**
CXA	164 (30.7)	94 (29.3)	70 (32.9)	
RCA	116 (21.7)	57 (17.8)	59 (27.7)	
Coronary artery lesions, n (%)				
SVD	231 (43.2)	154 (48)	77 (36.2)	**< 0.001**
DVD	175 (32.8)	103 (32.1)	72 (33.8)	
TVD	128 (24)	64 (19.9)	64 (30)	
SYNTAX score	16.1 (10.6 - 21.6)	15.5 (10.1- 22.2)	17.1 (11.1 - 24)	**0.003**
SYNTAX score group, n (%)				
Low (≤ 22)	360 (67.4)	250 (77.9)	110 (51.6)	**< 0.001**
Moderate-High (> 22)	174 (32.6)	71 (22.1)	103 (48.4)	
rSS	3.4 (0 - 7.9)	4.4 (2.6 - 8.1)	6.15 (3.6 - 9.6)	**< 0.001**

MPVLR = Mean platelet volume to lymphocyte ratio, LVEF = Left ventricular ejection fraction, LAD = Left anterior descending, CXA = Circumflex artery, RCA = Right coronary artery, SVD = Single-vessel disease, DVD = Double-vessel disease, TVD = Triple-vessel disease, SYNTAX = SYNergy between percutaneous coronary intervention with TAXus and cardiac surgery, rSS = Residual SYNTAX score



Variables	Cut-off value	AUC	95% CI	Youden Index	*p* value
PLR	162.12	0.602	0.524 - 0.706	0.52	0.164
MPV	10.41	0.644	0.542 - 0.714	0.54	0.244
MPVLR	5.08	0.820	0.701 - 0.899	0.64	**< 0.001**
MPVPR	4.64	0.628	0.520 - 0.726	0.52	0.256
Pct	0.236	0.712	0.623 - 0.807	0.59	**< 0.001**
PDW	15.6	0.754	0.601 - 0.812	0.58	**< 0.001**

Fig. 2. Receiver operating characteristic curve showing the optimal values of platelet indices for a high rSS prediction. AUC = Area under the curve, CI = Confidence interval, PLR = Platelet to lymphocyte ratio, MPV = Mean platelet volume, MPVLR = Mean platelet volume to lymphocyte ratio, MPVPR = Mean platelet volume to platelet ratio, Pct = Plateletcrit, PDW = Platelet distribution width.

Table 3. Correlation between rSS and clinical variables

	Correlation coefficient	*p* value
Age	0.204	**0.012**
LDL-C	0.096	0.356
Triglycerides	-0.124	0.402
CRP	0.234	**0.009**
Leukocytes	0.145	0.224
Lymphocyte	-0.201	0.102
Platelets	0.087	0.508
PLR	0.230	0.078
MPV	0.198	0.184
MPVLR	0.398	**< 0.001**
MPVPR	0.240	**0.024**
Pct	0.286	**0.008**
PDW	0.292	**< 0.001**

LDL-C = Low-density lipoprotein cholesterol, CRP = C-reactive protein, PLR = Platelet to lymphocyte ratio, MPV = Mean platelet volume, MPVLR = Mean platelet volume to lymphocyte ratio, MPVPR = Mean platelet volume to platelet ratio, Pct = Plateletcrit, PDW = Platelet distribution width

ROC analysis details are presented in Fig. 2.

Study patients were divided into two subgroups according to the determined MPVLR cut-off value: 'low MPVLR' with MPVLR ≤ 5.08 and 'high MPVLR' with MPVLR > 5.08. Baseline echocardiographic and angiographic measurements of study patients were evaluated comparatively between MPVLR subgroups (Table 2). The mean ejection fraction (EF) of the study patients was 53.4 ± 6.9 %, and there was no significant difference between the 'low' and 'high MPVLR' groups. It was observed that LAD was involved at a rate of 47.6% as the culprit coronary artery. LAD involvement was found to be higher in the low MPVLR subgroup. It was found that RCA involvement was 21.7%, and the high MPVLR subgroup had a significantly higher rate of involvement. While single-vessel involvement was observed more frequently in the low MPVLR subgroup, three-vessel involvement was observed more frequently in the high MPVLR subgroup ($p < 0.001$). The SYNTAX score was found to be 17.1 (11.1 - 24) in the high MPVLR group and 15.5 (10.1 - 22.2) in the low MPVLR group ($p = 0.003$). When SYNTAX score ≤ 22 was grouped as low SYNTAX and SYNTAX score >22 as moderate-high SYNTAX: It was found that 67.4% of the study patients were in the low SYNTAX group and patients in the low MPVLR group were significantly higher in the low SYNTAX group ($p < 0.001$). It was determined that the patients in the high MPVLR group had a higher rate in the moderate-high SYNTAX group ($p < 0.001$). rSS was found to be significantly higher in the high MPVLR subgroup compared to the low MPVLR group [6.15 (3.6 - 9.6) vs 4.4 (2.6 - 8.1), $p < 0.001$]. The residual SYNTAX score and correlation analysis of clinical variables are presented in Table 3. It was observed that age and CRP had a significant positive correlation with rSS. Although they were significantly different between the residual SS groups, LDL, triglyceride, leukocyte, and lymphocyte levels were not found to be significantly correlated with rSS. The correlation between hemogram data and rates, which we define as platelet indices, and rSS is as follows: (i) PLR and MPV did not make a significant difference between the rSS groups, nor did it show a significant correlation with rSS. (ii) MPVLR (r: 0.398, $p < 0.001$), MPVPR (r: 0.240, $p = 0.008$), Pct (r: 0.286, $p = 0.008$) and PDW (r: 0.292, $p < 0.001$) had a significant positive correlation with rSS.

Logistic regression analysis was performed to identify independent risk factors in the prediction of high rSS and to compare these effects of platelet indices (Table 4). From clinical and demographic factors: Age [OR: 1.298, 95% CI: 1.013 - 1.796, $p = 0.002$], Culprit vessel: RCA involvement [OR: 2.210, 95% CI: 1.594 - 4.223, $p < 0.001$], EF [OR: 1.066, 95% CI: 0.903 -1.190, $p = 0.015$] and DM [OR: 1.640, 95% CI: 1.138 - 3.136, $p = 0.009$] were defined as independent risk factors for high rSS. Among the platelet indices: MPVLR [OR: 5.966, 95% CI: 2.489 - 8.413, $p < 0.001$] and PDW [OR: 2.540, 95% CI: 1.318 - 3.236, $p = 0.009$] were detected as independent risk factors for high rSS.

Intra-observer and inter-observer agreements for SYNTAX score (SS) and rSS measurements were calculated [Intra-observer agreement: kappa coefficient ($\varkappa$) for SS measurement: 0.85 ($p = 0.013$) (for ZYG) and 0.87 ($p = 0.024$) (for AK) and $\varkappa$ for rSS measurement: 0.82 ($p = 0.005$) (for ZYG) and 0.85 ($p = 0.011$)

Table 4. Univariate and multivariate logistic regression analyses for independent predictors of high residual SYNTAX score

	Univariate analysis			Multivariate analysis		
	Odds ratio	**95% CI**	***p* value**	**Odds ratio**	**95% CI**	***p* value**
Gender (male)	1.513	1.027 - 2.785	0.042	1.107	0.692 - 2.011	0.484
Age	1.068	1.012 - 1.126	0.008	1.298	1.013 - 1.796	**0.002**
Culprit vessel: RCA	2.161	1.344 - 3.162	< 0.001	2.210	1.594 - 4.223	**< 0.001**
EF	0.997	0.857 - 1.498	0.022	1.066	0.903 - 1.190	**0.015**
Smoking	1.566	1.112 - 2.282	0.004	1.056	0.756 - 1.583	0.447
Hypertension	1.898	1.388 - 2.841	0.021	1.377	0.873 - 2.422	0.076
DM	2.124	1.418 - 3.586	< 0.001	1.640	1.138 - 3.136	**0.009**
Albumin	0.984	0.802 - 1.309	0.365			
Hemoglobin	0.962	0.753 - 0.998	0.031	1.098	0.862 - 1.438	0.863
Leukocytes	0.998	0.844 - 1.303	0.208			
Platelets	1.043	0.894 - 1.172	0.182			
Platelet indices						
MPVLR	4.102	2.428 - 6.552	< 0.001	5.966	2.489 - 8.413	**< 0.001**
PDW	3.214	1.148 - 3.856	< 0.001	2.540	1.318 - 3.236	**0.009**
Pct	2.142	1.481 - 3.568	< 0.001	1.340	1.183 - 3.163	0.079
MPV	0.989	0.831 - 1.330	0.282			
MPVPR	0.976	0.735 - 0.989	0.013	1.089	0.826 - 1.484	0.836
PLR	0.968	0.824 - 1.283	0.249			

CI = Confidence interval, RCA = Right coronary artery, EF = Ejection fraction, DM: Diabetes mellitus, MPVLR = Mean platelet volume to lymphocyte ratio, PDW = Platelet distribution width, Pct = Plateletcrit, MPV = Mean platelet volume, MPVPR = Mean platelet volume to platelet ratio, PLR = Platelet to lymphocyte ratio

(for AK); Inter-observer agreement (between ZYG and AK): ϰ for SS measurement: 0.84 ($p = 0.021$) and ϰ for rSS measurement: 0.79 ($p = 0.017$)].

DISCUSSION

In this study, in which we examined the relationship between platelet indices and rSS, MPVLR and PDW were defined as independent predictors of high rSS. We found that MPVLR was the index with the best correlation with rSS and the best diagnostic power for rSS prediction.

Platelet activation plays an important role in the formation and progression of atherosclerotic plaques and thrombus formation when these plaques rupture [7, 20]. An increase in MPV is one of the indicators of increased activation and aggregation of platelets [10]. In a well-designed study conducted at the cellular level, larger platelets were shown to be more metabolically and enzymatically active [21]. Platelets with increased size and metabolic activity can accelerate the thrombosis and inflammatory response. The formation of ACS is facilitated and its course is adversely affected due to more active platelets. Previous studies have shown that MPV is associated with mortality and MACE in patients with myocardial infarction with and without ST-segment elevation [8, 22]. Inflammation and thrombosis mechanisms are involved in the formation and destabilization of atherosclerotic plaque, its rupture, and finally thrombosis [6, 23]. Platelets are accompanied by lymphocytes in thrombosis formation [15]. Lymphocytes are one of the most important cell types that play a role in the formation of atherosclerotic lesions, and their number decreases as a result of the increased inflammatory response [16]. Inflamma-

tory responses cause lymphopenia due to increased lymphocyte apoptosis as a result of stress-induced cortisol secretion associated with ACS [24, 25]. This reduction is one of the indicators of growth, destabilization, and rupture of atherosclerotic plaques. The lower the lymphocyte count, the more unstable the plaque [26]. In other words, the increase in MPV and the decrease in the number of lymphocytes indicate that a suitable environment is ready for the formation of atherosclerotic lesions. MPV and lymphocyte count are included in routine hemogram data. MPVLR, which shows the MPV / lymphocyte count, is an index that can be easily calculated from routine hemogram data and can show the thrombosis and inflammation responses alone [27]. In a study by Hudzik *et al*. [28]; MPVLR was found to be an independent predictor of mortality, no-reflow, and MACE in ST-segment elevation myocardial infarction patients.

The prevalence of multivessel coronary artery disease in STEMI patients undergoing PCI is approximately 40-65%. As the number of diseased vessels increases, the risk of cardiac death and MACE also increases [3, 29]. The optimal management of residual stenoses after infarct-related artery reperfusion in STEMI patients remains a matter of debate [30]. rSS indicates residual coronary atherosclerotic load after PCI. The prognostic value of rSS was investigated for the first time in the ACUITY study, and high rSS (> 8) was found to be a strong predictor of 1-year mortality, cardiac mortality, unplanned revascularization, and MACE [4]. Loutfi *et al*. [31] showed significant reductions in 1-year MACE in STEMI patients with lower rSS. Burgess *et al*. [5] reported the rates of cardiac death and myocardial infarction (MI) at follow-up as 5%, 15%, and 26% for MVD and STEMI patients with rSS of 0, 1-8, and > 8, respectively. Significantly higher rates of cardiac mortality and MI were found in the group with rSS > 8. Braga *et al*. [32] found that rSS was positively correlated with all-cause mortality and MACE in patients who underwent primary PCI for STEMI and were found to have MVD. rSS has also been identified as an independent predictor of these outcomes during follow-up.

PDW refers to platelet activity as a measure of circulating platelet size variation [12]. Elevated PDW values indicate anisocytosis, indicating increased platelet activity, which is associated with an increased propensity for thrombosis and severity of coronary artery disease in ACS patients [13]. In our study, PDW was higher in the high rSS group and was found to be an independent predictor of high rSS.

DM is closely associated with cardiovascular diseases, and it has been shown that the risk of coronary artery disease increases 2 to 4 times in patients with DM [33]. The addition of DM to existing risk factors leads to increased severity of coronary artery disease and the number of diseased vessels is high in these patients [34]. Our results support this situation and rSS was found to be higher in patients with DM.

The first variable that comes to mind when atherosclerosis is mentioned is advanced age. As age increases, the presence and prevalence of atherosclerotic disease increases. In addition, the frequency of diabetes, hypertension, and hyperlipidemia increases with age. The most common cause of death in the elderly is cardiovascular diseases caused by atherosclerotic changes. As expected, the mean age was higher in the high rSS group, as seen in our study [4, 35].

Ischemic heart disease is one of the most important causes of EF decrease, and as the number of diseased vessels increases, EF is expected to decrease [36]. As previously shown [37], low EF is one of the independent predictors of high rSS in our study as well.

To the best of our knowledge, this is the first study to examine the relationship between MPVLR and rSS. In our study, we demonstrated a positive correlation between MPVLR and the degree and complexity of residual coronary stenosis in patients with STEMI after PCI. We found that higher MPVLR levels were more common in STEMI patients with high rSS and that increased MPVLR levels independently predicted high rSS.

Limitations

In this retrospective study, the relatively small sample size and absence of a control group are our main limitations. In addition, it is difficult to make a causal inference, since we examined the relationship between platelet indices and rSS cross-sectionally. The effect of increased MPVLR on short- and long-term cardiovascular events has not been evaluated; therefore, we cannot comment on the prognostic effects of our results. This was a retrospective single-center analysis with no potential to avoid selection bias. Even

after the adjusted analysis, some confounding factors may have affected the results.

CONCLUSION

In conclusion, increased MPVLR is associated with higher rSS in STEMI patients. There is a significant positive correlation between MPVLR and rSS. MPVLR is an independent predictor of high rSS. With our results, we can suggest that MPVLR may be a valuable and easily applicable biomarker that can provide additional information in the risk stratification of patients with STEMI after PCI.

Authors' Contribution

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

Conflict of interest

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

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The relationship of platelet to lymphocyte ratio with the presence and extent of coronary atherosclerosis detected by coronary computed tomography angiography

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ABSTRACT

Objectives: Platelet-lymphocyte ratio (PLR) combines the predictive risk of platelet and lymphocyte counts into a single risk index. PLR has been studied as a predictive marker in a variety of cardiovascular diseases. However, our understanding of the link between PLR and coronary artery disease (CAD) remains limited. The present study aimed to evaluate the relationship between PLR and intensity of coronary atherosclerosis in patients with suspected CAD.

Methods: In this retrospective study, we included 221 patients undergoing dual-source 64-slice coronary computed tomography angiography (CCTA). Total and different types of leukocyte counts were measured with an automatic blood counter. Based on a modified version of the American Heart Association's categorisation, the coronary artery tree was divided into 16 segments. To assess the extent of coronary atherosclerosis, the number of affected coronary segments was counted. Coronary artery plaques were classified into three categories: (1) calcified plaque, (2) non-calcified plaque, and (3) mixed plaque.

Results: After multivariable backward stepwise regression analysis, PLR remained as an independent predictor for both the presence and extent of coronary atherosclerosis (OR = 2.38, 95% CI: 1.27-4.47 and OR = 1.66, 95% CI: 1.10-2.51, respectively). There was no significant relationship between PLR and plaque morphology. Conclusions: Higher PLR was associated with the intensity of coronary atherosclerosis detected by CCTA. Further research is necessary to determine the optimal approach to using PLR in medical practice.

Keywords: Platelet-lymphocyte ratio, coronary artery disease, atherosclerosis, computed tomography angiography

Coronary artery disease (CAD), which is pathologically characterized by atherosclerosis, is the leading cause of death worldwide. Chronic low-grade inflammatory state plays a central role in the onset and progression of atherosclerosis [1]. Inflammation's role in CAD has been thoroughly investigated, and a con-

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sistent relationship between several pro-inflammatory markers and CAD has been established [2, 3]. Platelet (pro-inflammatory) and lymphocyte (regulatory and protective) counts have been identified as biological markers in a variety of cardiovascular diseases and inflammatory conditions [4-6]. Platelet-lymphocyte ratio (PLR), which is calculated by dividing the absolute platelet count by the absolute lymphocyte count, is an integrated manifestation of two considerable inflammatory pathways. Therefore, a raised PLR may play a more decisive role in forecasting CAD than each parameter alone.

Recently, the PLR has been investigated as a predictive marker for a variety of numerous cardiovascular conditions, such as acute coronary syndromes, peripheral vascular diseases, atrial fibrillation, and heart failure [7]. Moreover, PLR has been shown to be associated with the severity of atherosclerotic disease in stable angina pectoris [8]. In the majority of these studies, conventional angiography was preferred over coronary computerized tomography angiography (CCTA) to evaluate coronary artery lesions. CCTA can provide more accurate details about atherosclerotic plaque burdenas it allows direct assessment of the vascular wall, and thus positive remodeling.

This study aimed to investigate the association between PLR and the intensity of coronary atherosclerotic lesions in patients with suspected CAD undergoing CCTA. We hypothesized that higher PLR levels would be a reliable and independent risk factor for coronary atherosclerotic plaque burden. Meanwhile, we also examined the link between atherosclerotic plaque morphology and PLR levels.

METHODS

Study Population

In this retrospective study, we enrolled 295 consecutive patients undergoing CCTA for CAD from February 2018 to May 2020. All the patients had been referred to our outpatient clinic with the complaint of chest pain. A detailed physical examination was performed, and a detailed medical history was recorded for each patient. Patients with any history of CAD, acute/chronic kidney insufficiency, congestive heart failure, severe valvular heart disease, active infection or systemic inflammatory conditions, hematologic disorders, and active malignancy were excluded from the study. Following the application of the inclusion criteria, a total of 221 cases were admitted to the study. Diabetes mellitus was defined as a fasting plasma glucose level of more than 126 mg/dL on two separate tests, or the active use of any antidiabetic drug. Hypertension was considered to be a systolic blood pressure of ≥ 140 mm Hg and/or a diastolic blood pressure of ≥ 90 mm Hg, or the active use of any antihypertensive medication. Dyslipidemia was defined as a total cholesterol level of ≥ 200 mg/dL or the active use of any active lipid lowering medication. Body mass index (BMI) was calculated by the formula of weight (kg)/ height² (m²).

Ethics Committee Approval

All participants gave informed consent to participate in the research. The research protocol was approved by theInstitutional Research Ethical Committee (code: 2021/10/525).

Laboratory Analysis

Blood samples were drawn after an overnight fasting. Total and different types of leukocyte counts were measured with an automatic blood counter. PLR was calculated by dividing the platelet count by the lymphocyte count. All laboratory analyses, including biochemical parameters and lipid profiles, were performed using an automatic biochemistry analyzer (Cobas 8000; Roche Diagnostics, Basel, Switzerland).

CCTA and Assessment of Coronary Atherosclerosis

CCTA was performed with a dual-source 64-slice multidetector CT scanner (Aquilion; Toshiba Medical Systems, Japan). In case of heart rate higher than 65 beats per minute, an intravenous beta-blocker (metoprolol, 5-25 mg) was administered. Sublingual nitroglycerine (0.4 mg) was given just before scanning unless there were any contraindications. During scanning, 80-110 mL (weight-based dosing) of nonionic contrast agent (350 mgI/mL iomeprol; Bracco Imaging, Milan, Italy) was injected via venous route at a flow rate of 5.5 mL/s followed by 50 mL of isotonic bolus. A radiologist who specializes in cardiac imaging evaluated all angiographic images immediately after data collection. The relationship between PLR and CAD was studied separately based on the pres-

ence and extent of atherosclerotic lesions. Any apparently distinguishable structure attributed to the vessel wall in at least two different imaging planes was defined as coronary arterial plaque. Based on a modified version of the American Heart Association's categorisation, the coronary artery tree was divided into 16 segments [9]. To determine the extent of coronary atherosclerosis, the number of affected coronary segments was counted. Coronary artery plaques were classified into three categories: (1) calcified plaque (which has a higher Hounsfield unit (HU) than the contrast-enhanced coronary artery lumen); (2) non-calcified plaque (which has a higher HU than the adjacent connective tissue but a lower HU than the contrast-enhanced coronary artery lumen); and (3) mixed plaque (which has both calcified and noncalcified plaque components).

Statistical Analysis

The analysis was performed out using R software (version 4.0.1). Normality was analyzed using the Shapiro-Wilk's test. Continuous variables were expressed as mean ± standard deviation (SD) and categorical data as numbers and percentages (%). The

Table 1. Baseline clinical and laboratory characteristics of the study population

Variable	Coronary plaque (+) (n = 144)	Coronary plaque (-) (n = 77)	*p*value
Clinical characteristics			
Age (years)	57.2 ± 9.7	50.0 ± 9.8	**< 0.001**
Gender (male)	86 (59.7)	36 (46.8)	0.065
BMI (kg/m^2)	27.5 ± 3.4	27.1 ± 3.7	0.474
Cigarette smoking[a]	62 (43.1)	24 (31.2)	0.084
Hypertension	101 (70.1)	40 (51.9)	**0.007**
Diabetes mellitus	34 (23.6)	8 (10.4)	**0.017**
Dyslipidemia	94 (65.3)	36 (46.8)	**0.008**
Medication			
Acetylsalicylic acid	93 (64.6)	49 (63.6)	0.889
ACE inhibitors/ARBs	76 (52.8)	36 (46.8)	0.393
Beta blockers	64 (44.4)	32 (41.6)	0.680
Calcium antagonists	22 (15.3)	13 (16.9)	0.755
Statin	67 (46.5)	31 (40.3)	0.371
Laboratory characteristics			
Hemoglobin, g/dL	14.1 ± 1.6	13.7 ± 1.4	0.111
WBC count, ×10^3/mL	7.75 ± 1.75	7.39 ± 2.10	0.174
Platelet count, ×10^3/mL	257.9 ± 61.6	242.9 ± 56.2	0.079
Lymphocyte count, ×10^3/mL	2.15 ± 0.59	2.57 ± 0.84	**< 0.001**
Platelet–lymphocyte ratio	129.9 ± 50.6	100.8 ± 28.7	**< 0.001**
Creatinine, mg/dL	0.86 ± 0.17	0.84 ± 0.19	0.451
Total cholesterol, mg/dL	200.5 ± 47.6	183.1 ± 43.7	**0.008**
LDL cholesterol, mg/dL	133.1 ± 36.1	116.2 ± 35.5	**0.001**
HDL cholesterol, mg/dL	43.2 ± 12.8	46.6 ± 11.4	0.053
Triglyceride, mg/dL	161.5 ± 81.2	144.6 ± 76.5	0.134

[a]Active smokers. Data are expressed as mean ± SD and n (%). ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker, BMI = body mass index, CAD = coronary artery disease, HDL = high-density lipoprotein, LDL = low-density lipoprotein, WBC = white blood cell.

students' t-test and the Mann-Whitney U-test were used to analyze continuous variables with normal and non-normal distributions, respectively. The chi-square test was used to analyze categorical variables and proportions. The Kruskal-Wallis test was used to compare the difference in PLR between coronary plaque morphology subgroups.Univariable and multivariable backward stepwise proportional odds regression analysis was performed to identify the predictive variables of coronary atherosclerosis. For backward elimination, a 0.20 alfa level was chosen. The results of regression analyses were reported as odds ratios (OD) with their respective 95% confidence intervals (CI). The partial effects plot was used to demonstrate the relative importance of some of the variables analyzed in the regression model. For all analyses, two-tailed statistically significant threshold was set at p - value < 0.05.

RESULTS

The baseline clinical and laboratory characteristics of the study population are shown in Table 1. The participants were divided into two groups based on the presence of coronary atherosclerotic plaque. Gender, BMI, and the frequency of cardiovascular medication were not significantly different between the two groups. The coronary atherosclerotic group had a higher prevalence of cardiovascular risk factors such as hypertension, diabetes, and dyslipidemia. Among hematological parameters, patients with coronary atherosclerosis had higher platelet counts ($p < 0.001$) and lower lymphocyte counts ($p < 0.001$). PLR levels were also significantly different between the groups (129.9 ± 50.6 versus 100.8 ± 28.7, $p < 0.001$). The results of univariable and multivariable regression analyses to predict the independent variables associated with the presence and extent of coronary atherosclerosis are presented in Table 2 and Table 3, respectively. After multivariable backward stepwise regression analysis, PLR remained as an independent predictor for both the presence and extent of coronary atherosclerosis (OR = 2.38, 95% CI: 1.27-4.47 and OR = 1.55, 95% CI: 1.10-2.51, respectively). Apart from the PLR level, other variables including age, smoking, diabetes mellitus, hypertension, and low-density lipoprotein (LDL) level were found to be statistically significant (Figs. 1 and 2). Also, no significant relationship was found between the PLR and coronary plaque morphology (Fig. 3).

DISCUSSION

In this study, PLR was found to be an independent predictor of the presence and extent of CAD, independent of traditional cardiovascular risk factors. Moreover, there was no meaningful association between coronary plaque morphology and PLR levels. We evaluated the lesions with CCTA, which is a unique aspect of this study. In this regard, our results contribute to the cur-

Table 2. Univariable and backward stepwise multivariable analyses demonstrating the association between cardiovascular risk factors, including PLR and the presence of coronary plaque

Variable	Univariable		Stepwise multivariable	
	OR (95% CI)	*p* value	OR (95% CI)	*p*value
Age (from 47 to 62)	3.09 (1.93-4.93)	< 0.001	3.51 (1.99-6.20)	**< 0.001**
Gender (male)	1.68 (0.96-2.95)	0.064		
Smoking	1.66 (0.93-2.99)	0.071	2.57 (1.25-5.26)	**0.010**
Hypertension	2.12 (1.22-3.84)	**0.001**	2.59 (1.29-5.20)	**0.007**
Diabetes mellitus	2.66 (1.16-6.09)	**0.001**	2.56 (0.95-6.90)	0.063
Creatinine	1.18 (0.76-1.81)			
LDL (from 104 to 151)	1.86 (1.27-2.74)	**0.001**	2.42 (1.50-3.92)	**< 0.001**
PLR (from 87.4 to 146.9)	3.09 (1.72-5.55)	**< 0.001**	2.38 (1.27-4.47)	**0.006**

CI = confidence interval, LDL = low-density lipoprotein, OR = odds ratio, PLR =platelet-lymphocyte ratio

Table 3. Univariable and backward stepwise multivariable analyses demonstrating the association between cardiovascular risk factors, including PLR and the extent of coronary atherosclerosis

Variable	Univariable		Stepwise multivariable	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age (from 47 to 62)	2.39 (1.66-3.43)	**< 0.001**	2.19 (1.49-3.21)	**< 0.001**
Gender (male)	1.52 (0.94-2.45)	0.083		
Smoking	1.62 (1.004-2.64)	**0.042**	2.22 (1.33-3.71)	**0.002**
Hypertension	2.00 (1.21-3.30)	**0.006**	1.75 (1.04-2.95)	**0.036**
Diabetes mellitus	2.81 (1.54-5.12)	**0.007**	2.63 (1.40-4.96)	**0.003**
Creatinine	1.26 (0.86-1.84)	0.244		
LDL (from 104 to 151)	1.66 (1.21-2.28)	**0.001**	1.72 (1.25-2.37)	**0.001**
PLR (from 87.4 to 146.9)	2.13 (1.42-3.18)	**<0.001**	1.66 (1.10-2.51)	**0.025**

CI = confidence interval, LDL = low-density lipoprotein, OR = odds ratio, PLR = platelet-lymphocyte ratio

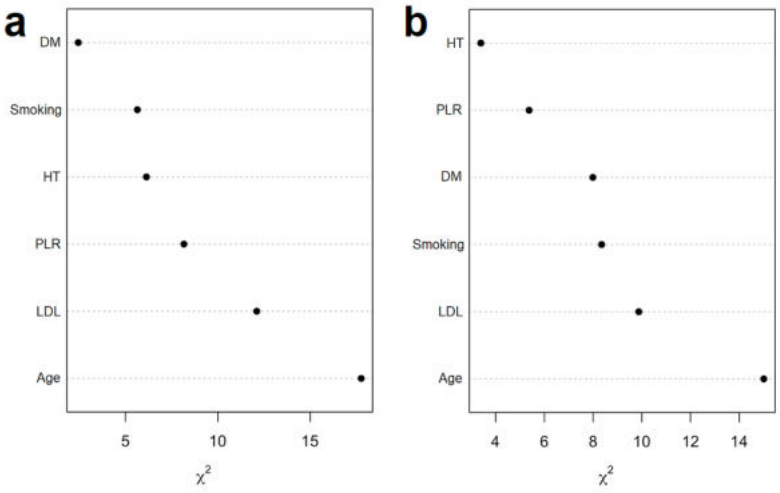

Fig. 1. The relative importance of each factor associated with the presence (a) and extent of coronary artery disease (b). DM = diabetes mellitus, HT = hypertension, LDL = low-density lipoprotein, PLR = platelet-lymphocyte ratio.

rent literature.

Platelet activation is a pivotal stage in the onset and progression of cardiovascular diseases [4]. During inflammation, platelets are stimulated by endothelial substances and secrete inflammatory mediators and cytokines [10]. Increased platelet count accelerates the formation, progression and instability of atherosclerotic lesions and has also been shown to be associated with adverse outcomes of CAD [11]. On the other hand, lymphocytes have been shown to play an active role in modulating inflammatory responses at each stage of the atherosclerotic process. Lymphopenia has been identified as a typical response to systemic stress, and has been linked to a poor prognosis, particularly in processes where inflammation is a major pathophysiological factor [12]. As an independent predictor, the PLR combines the predictive risk of platelet and lymphocyte counts into a single risk index. In this regard, some previous studies reported a positive correlation between PLR and commonly used inflammatory markers [13, 14].

Patients with high PLR levels have been shown to have an increased atherosclerotic burden on coronary angiography [15]. Akboğa *et al*. [16] investigated the association between high PLR and the presence/severity of coronary atherosclerosis; concluding that preprocedure PLR levels were independently associated with the Gensini score. Likewise, Yüksek *et al*. [8] reported the relationship between PLR and the severity of CAD in patients with stable angina pectoris. How-

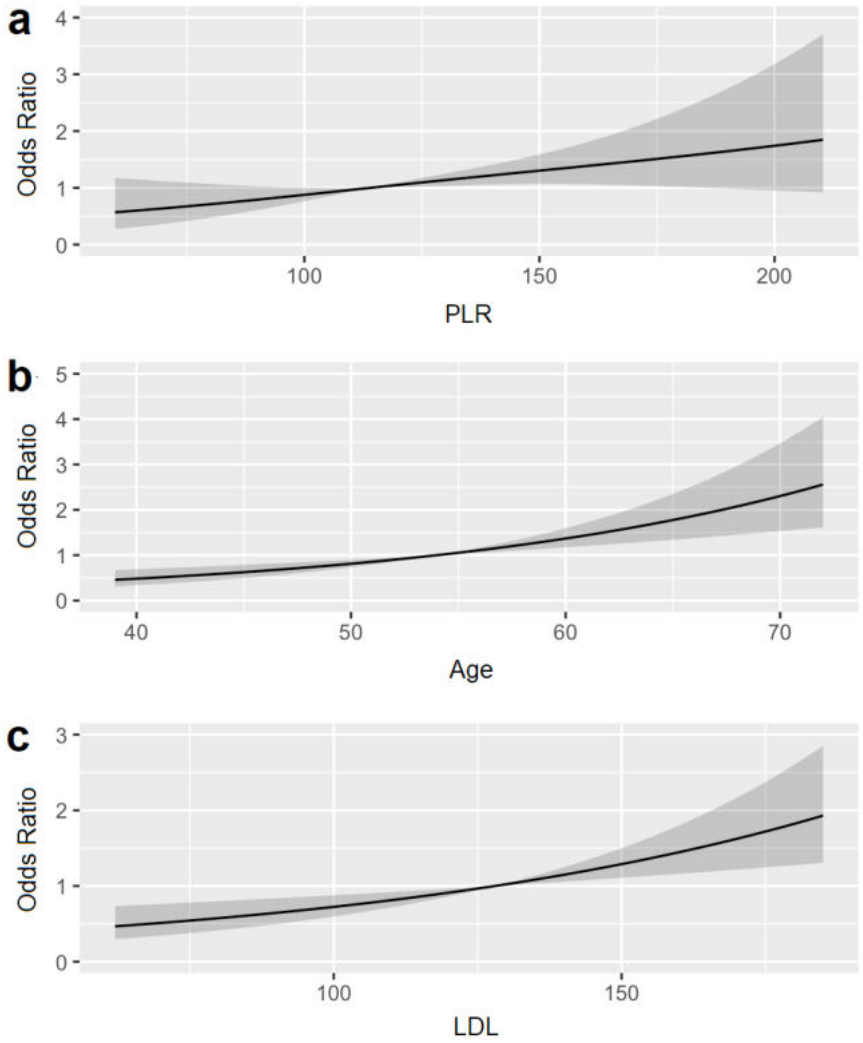

Fig. 2. Partial effect plots of PLR (a), age (b) and LDL (c) showing association with the extend of coronary artery disease in a multivariable model. LDL = low-density lipoprotein, PLR = platelet-lymphocyte ratio.

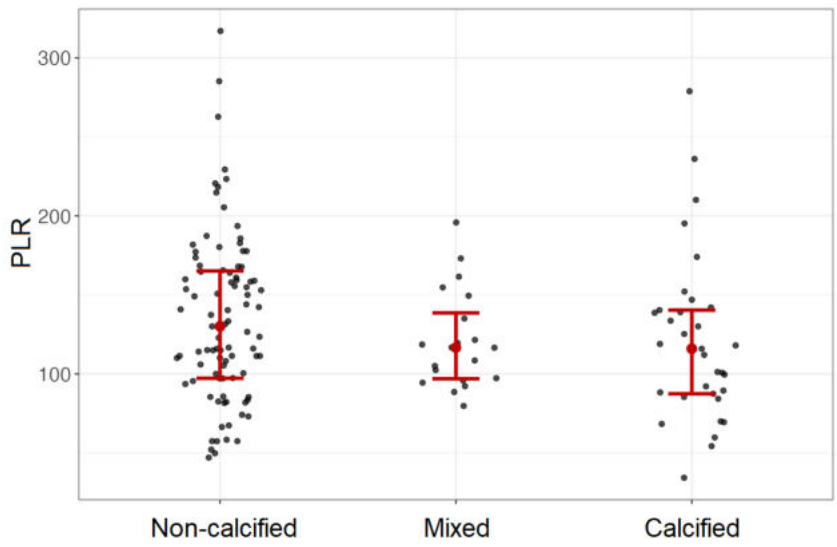

Fig. 3. Scatter plot of the relationship between PLR and plaque morphology (p> 0.05). PLR = platelet-lymphocyte ratio.

ever, the association between the PLR and CAD is not fully elucidated; the above-mentioned mechanisms related to platelets and lymphocytes in inflammation and atherosclerosis can shed light on these interactions. Our findings confirm and strengthen several previous studies consistently demonstrating PLR as a predictor of CAD. The identification of coronary artery lesions by CCTA helped us to make a more sensitive assessment compared to earlier studies, as CCTA provides more reliable qualitative and quantitative data about plaque morphology and the whole coronary system as compared to invasive approaches [17].

Coronary plaque content is a critical determinant of clinical progression and prognosis in CAD. However, the role of calcium deposits on plaque vulnerability is debatable, as several studies have shown inconsistent results regarding the effect of calcified plaques on major adverse cardiac event rates [18-20]. It has been previously shown that inflammatory markers are only weakly correlated with plaque composition and coronary calcification, and mostly determined by the existence of coronary risk factors [21]. The relationship between the coronary plaque morphology and PLR was also analyzed separately, but no association was detected. Further investigation of reliable and cost-effective inflammatory biomarkers predicting the presence of a vulnerable plaque with greater sensitivity and specificity is warranted.

Limitations

The present study had some limitations that have to be addressed. First, this research was conducted at a single center using a cross-sectional design with a small study sample size. Further randomized prospective studies are required to confirm our outcomes. Second, the presence or absence of calcification was the sole feature used to classify plaque morphology. As a result, we did not consider other plaque characteristics (such as a large lipid core and a thin fibrous cap) that could be assessed using imaging techniques like optical coherence tomography or intravascular ultrasonography. Third, our results are based on calculating PLR from a single blood sample before CCTA. Evaluating the change in PLR over time may provide useful information. Fourth, our study did not include all pro-inflammatory mediators associated with atherosclerosis, such as interleukin-1, C-reactive protein, and tumor necrosis factor. As a result, PLR could not be compared to these well-known inflammatory markers. Finally, additional longitudinal cohort studies investigating the associations of PLR with both cardiovascular events and mortality are required to support these findings.

CONCLUSION

According to the findings of the present study, increased PLR was found to be associated with the intensity of coronary atherosclerosis detected by CCTA in patients with no prior history of CAD. Thus, PLR can be useful in predicting the coronary atherosclerosis in addition to traditional cardiovascular risk factors. Further research is necessary to determine the optimal approach to using PLR in medical practice.

Authors' Contribution

Study Conception: HÇ, CT; Study Design: HÇ, NH; Supervision: HÇ, CT; Funding: N/A; Materials: SU, NH; Data Collection and/or Processing: SU, MK; Statistical Analysis and/or Data Interpretation: AK; Literature Review: HÇ; Manuscript Preparation: HÇ and Critical Review: CK, MD.

Conflict of interest

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

Financing

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The European Research Journal 2022;8(5):678-684 Original Article

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Evaluation of the effect values of risk factors by clustering method in patients who died due to COVID-19 disease

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ABSTRACT

Objectives: The aim of this study is to determine the factors that may be associated with mortality in patients who died due to COVID-19 and to determine the effect sizes of the factors that make a statistically significant difference.

Methods: The patients who died due to COVID-19 between 01.03.2020 and 01.03.2021 in Bursa province were evaluated retrospectively. In addition to demographic information such as age, gender, nationality, existing chronic diseases of the patients, COVID- PCR test results, length of hospital stay, intensive care unit follow up times, intubation application times were recorded. The effect size of the variables on mortality were evaluated.

Results: Total of 3,510 deaths due to COVID-19 were evaluated. Of these, 2107 (60%) were male and 1403 (40%) were female. Three thousand three hundred and seventy-four (96.12%) patients are 50 years or older. In both sexes, the highest number of deaths were in the age range of 70-79. The most common comorbidities were hypertension (HT) (n = 1,182; 34.16%) and diabetes mellitus (DM) (n = 776; 22.43%). HT and DM had a strong effect value between the groups ($p < 0.001$ and $p < 0.001$, phi effect values: 0.661 and 0.681, respectively). Although there was a statistically significant difference for the age variable, it had an insignificant effect value ($p = 0.008$, $\delta = 0.074$).

Conclusions: Risk factors frequently reported for COVID-19 deaths but there are no studies showing the true effect values. In this study, HT and DM had a strong effect separately, gender and coronary artery disease (CAD) variables were moderate, chronic obstructive pulmonary disease (COPD), lung cancer and other chronic disease variables had weak effect values, age and non-lung cancers had insignificant effect.

Keywords: Chronical disease, COVID-19, COVID-19 related deaths, effect value, risk factors

Coronavirus disease 2019 (COVID-19), a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, was first reported in December 2019 in Wuhan, Hubei Province, China, and has spread rapidly since then [1]. A global pandemic was declared after its uncontrolled spread around the world. After the rapid spread of COVID-19 infection globally, more than 505 million confirmed cumulative

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cases of coranavirus disease (COVID-19) were diagnosed and more than 6 million people died worldwide [2]. Turkey was one of the countries most affected by COVID-19; Starting with the first case in Turkey on March 11, 2020, and at the end of the last 25 months, as of April 2022, over 14.7 million cumulative confirmed cases were detected and 97,666 deaths occurred [3].

Hospitalization and death rates for COVID-19 patients differ between populations and studies. Mortality rates vary between countries between 1% and 10% [4]. A systematic study conducted in China found the mortality rate to be 3-4% [5]. According to reports from Italy and the United Kingdom, death rates range from 10% to 20% [6, 7]. The death rate in Turkey has been estimated to be around 1% [3].

Fever, cough, respiratory difficulty, muscle soreness, and exhaustion are the most common symptoms of COVID-19 infection [8]. Although the majority of COVID-19 patients recover in a short time, it can lead to fatal results in some patients [9].

The severity and mortality of the COVID-19 disease have been attributed to a variety of demographic variables and co-morbidities. Advanced age is the factor most closely associated with COVID-19 mortality [10, 11]. Other factors linked to mortality include the presence of comorbidities such as diabetes mellitus (DM), cardiovascular and chronic respiratory system diseases, hypertension (HT), obesity, kidney and liver disease, cancer, chronic obstructive pulmonary disease (COPD), asthma [12-15]. In a retrospective study of 828 confirmed cases of COVID-19 from open-access individual-level data worldwide, male, older age, hypertension, diabetes, and USA patients identified as independent risk factors for death among COVID-19 patients [16]. It is stated that neurological and chronic respiratory system diseases are also associated with COVID-19 death [17]. In several studies patients with comorbidities; has been shown that there is a relationship between the severity of the disease and the occurrence of death in those with obesity and also smoking history [18-22].

The aim of this study is to determine the factors that may be associated with mortality in patients who died due to COVID-19 and to determine the effect sizes of the factors that make a statistically significant difference.

METHODS

In this study, the patients who died due to COVID-19, which occurred between 01.03.2020 and 01.03.2021 in Bursa province, were evaluated retrospectively. Study data includes data from a total of 14 hospitals, including 2 training and research hospitals, two central state hospitals and 12 district state hospitals across Bursa. A total of 3,510 deaths were examined and 139 (3.96%) of these patients were foreign nationals. During the planning phase of the study, the Ministry of Health COVID-19 scientific studies permission and the approval of the ethics committee in our hospital were obtained (2019- KAEK-140). The medical records of patients were evaluated retrospectively from the patient files registered in the public health management system and hospital information management system. Patients with at least one positive COVID-PCR sample between March 2020 and March 2021 were considered as COVID positive.

Demographic information of patients like gender, age, nationality, existing chronic diseases of the patients, COVID-PCR test results of the patients, length of hospital stay, intensive care follow-up times, intubation application times and hospitals where they were followed were recorded. Presence of chronic disease of the patients were classified as HT, DM, COPD, malignancy (lung malignant neoplasm, extrapulmonary malignancy) and other chronic diseases. The patients' existing chronic conditions were obtained from hospital information management systems and reported using International Classification (ICD 10) codes.

Statistical Analysis

Cluster analysis was used to show the relationships in a multivariate way, as the data set includes

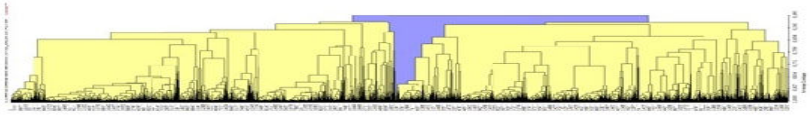

Fig. 1. Hierarchical cluster analysis dendrogram.

both continuous and nominal type variables. Hierarchical clustering methods were designed as part of multivariate statistical analysis to generate homogeneous groups of cases or entities called clusters. The Gower similarity coefficient was used to calculate the similarities between the deaths caused by COVID-19. Clusters were combined with the full linkage method and a dendrogram was created showing the clustering of cases according to their similarity levels (Fig. 1). Clustan Graphics V.8.00 software was used for cluster analysis. Clusters (Cluster-1 and Cluster-2) were statistically tested to identify variables that were influential in clustering.

The conformity of the variables to the normal distribution was examined with the Shapiro Wilk test and the Kolmogorov-Smirnov test. Because the data did not show normal distribution, Mann Whitney U test was used for comparisons between the two groups. Descriptive statistics are given as median (minimum-maximum) because the data are not normally distributed and nonparametric tests are used. Pearson Chi Square test was used to compare categorical variables between groups. Categorical variables were expressed as n (%). Because the sample size was too large and the p-value was statistically significant, the effect size was calculated. In the case of non-parametric Cliff's Delta effect size and Phi effect size for categorical data are given. Statistical analyzes were made with SPSS v22.0 program and R v3.6.3 program "effsize" package. In statistical analyzes, $\alpha = 0.05$ was taken as the level of significance. In the interpretation of Cliff's Delta effect size values; $\delta < 0.147$ unimportant, $\delta < 0.330$ small, $\delta < 0.474$ medium, $\delta \geq 0.474$ intervals are taken into account.The intervals specified in Table 1 were taken into account in the interpretation of the Cliff's Delta effect size and Phi effect size values [23].

Table 1. Interpretation of Cliff's delta effect size and Phi effect size

Effect Size	Interpretation
Cliff's delta effect size	
$\delta < 0.147$	Negligible
$\delta < 0.330$	Small
$\delta < 0.474$	Medium
$\delta \geq 0.474$	Large
Phi effect size	
0.0-0.1	Negligible association
0.1-0.2	Weak association
0.2-0.4	Moderate association
0.4-0.6	Relatively strong association
0.6-0.8	Strong association
0.8-1.0	Very strong association

Interpretation of Cliff's delta effect size and Phi effect size

RESULTS

Total of 3,510 deaths due to COVID-19 were evaluated in this study. When the historical and monthly distributions of deaths were examined, it was seen that the highest values were reached in November with 32.99% (n = 1,158) cases, followed by December with 29.66% (n = 1,041) cases. The lowest mortality rates were recorded in March 2020, at 0.14% (n = 5), and in June of 1.08% (n = 38).

When the gender distribution of our study population is examined, 2,107 (60%) were male and 1,403 (40%) were female (male/female ratio 1.5). Three thousand three hundred and seventy-four (96.12%) patients are 50 years or older (Fig. 2). In both sexes, the highest number of deaths were in the age range of 70-79. In this age group, 34.08% of men (n = 718) and 33.14% of female (n = 465) died due to COVID-19. In the age range of 60-69 years for males (n = 613; 29.09%) and 80-89 years for female (n =349; 24.88%) was the second highest number of deaths were recorded. Fig. 3 shows death distributions by age and gender.

The most common comorbidities were HT (n = 1,182; 34.16%) and DM (n =776; 22.43%). Cancer was present in 130 patients, with 48 (1.39%) having lung cancer and 82 (2.43%) having extra-pulmonary cancer. Five hundred and sixteen (14.91%) patients did not have any concomitant chronic disease. The distributions of chronic diseases accompanying the patients are given in Fig. 4.

When Cluster-1 and Cluster-2 groups are compared, a statistically significant difference was found in terms of age and time to death after COVID-19 diagnosis ($p < 0.008$ and $p < 0.001$, respectively). The effect size was found to be insignificant between the two groups according to Cliff's Delta. There was no statistically significant difference between the groups

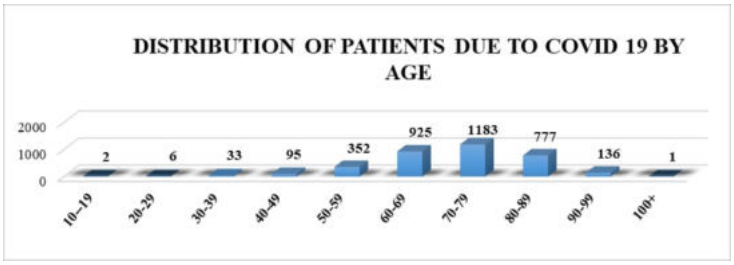

Fig. 2. Age distribution of study population.

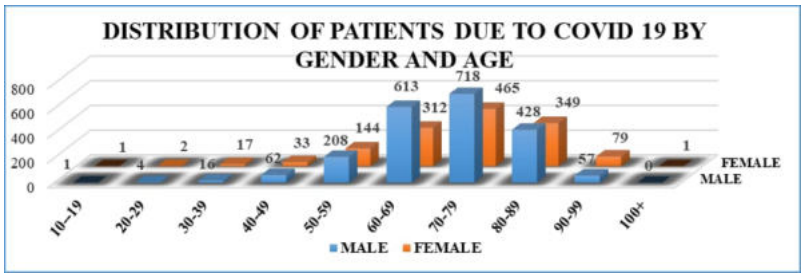

Fig. 3. Age distribution of study population by gender.

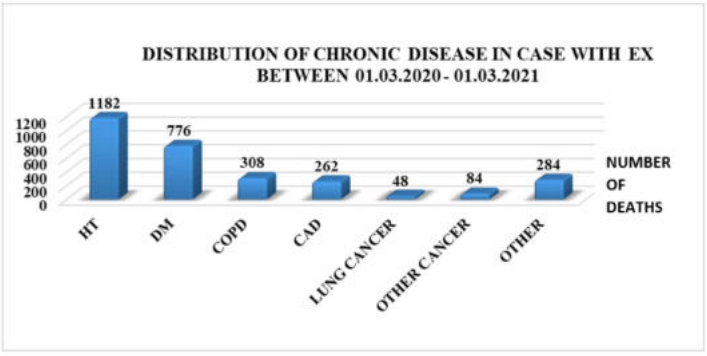

Fig. 4. Distribution of existing chronic diseases in the study population. HT = Hypertension, DM = Diabetes Mellitus, CAD = Coronary artery disease, COPD = Chronic obstructive pulmonary disease.

Table 2. Comparisons of Cluster-1 and Cluster-2 groups

	Cluster-1 (n = 854)	Cluster-2 (n = 842)	*p* value	Cliff's Delta	
				δ	%95 CI.
Age (years)	71 (14-97)	72 (35-102)	**0.008**	0.074	(0.019-0.129)
Intubation time (time from hospitalization to intubation) (hours)	0 (0-49)	0 (0-46)	0.144	-	-
Death time (time from hospitalization to death) (days)	7 (0-90)	8 (0-132)	**< 0.001**	0.097	(0.042-0.151)

Data are given as mean (minimum-maximum)

in terms of intubation time ($p = 0.144$) (Table 2). When Cluster-1 and Cluster-2 groups were compared, there was a statistically significant difference in terms of gender, HT, DM, CAD, COPD, lung cancer, extra-pulmonary cancer and other chronic disease variables ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.006$ and $p < 0.001$, respectively). When the effect sizes were evaluated, insignificant correlation between the two groups in terms of extra-pulmonary cancer. Weak correlation between the two groups in terms of COPD, lung cancer and other chronic disease; moderate correlation between the two

groups in terms of gender and CAD; A strong correlation was observed between the two groups in terms of HT and DM (Table 3). The rate of Gender-1 observation was higher in the Cluster-1 group than in the Cluster-2 group ($p < 0.001$) (Table 3). The incidence of HT, DM, CAD, respiratory and other chronic diseases was found to be lower in the Cluster-1 group than in the Cluster-2 group ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively). Lung cancer and extrapulmonary cancer observed rates were higher in the Cluster-1 group than in the Cluster-2 group ($p < 0.001$ and $p = 0.006$, respectively) (Table 3).

DISCUSSION

During the pandemic process, many studies have been conducted investigating the mortality and morbidity relationships of patients due to COVID-19 [9-11]. Many studies have found that severe COVID-19 disease and variables linked to death, such as HT, DM, Obesity, COPD, CAD, advanced age, and other chronic diseases, are statistically significant. However, the literature on the actual effect values could not be reached. In this study, we examined the effect values in COVID-19 patients who died, in addition to the statistical significance of these variables. HT and DM had a significant effect value between groups, whereas gender and CAD variables had a medium effect value, COPD, lung cancer, and other chronic illness variables had weak effect values, and age and non-lung cancer variables had no effect value.

In the meta-analysis examining on the intensity of underlying diseases in hospitalized 76993 patients for COVID-19, HT, cardiovascular diseases, DM, smoking, COPD, malignancy and chronic kidney disease were detected respectively [24]. The most common comorbidities in COVID-19 patients, according to various studies, are HT, DM, and CAD, with HT being the most common. However, no research has been carried to establish that HT and DM have an independent effect on mortality [25, 26]. In this study, it was determined that HT and DM have a strong effect on deaths related to COVID-19 and pose a statistically significant risk in deaths caused by COVID-19.

In their meta-analysis, Nakanishi *et al*. [27] they

Table 3. Comparisons of Cluster-1 and Cluster-2 groups

		Cluster-1 (n = 854)	Cluster-2 (n = 842)	*p* value	Phi (*p*)
Gender, n (%)	**Male**	607 (71.1)	403 (47.9)	**< 0.001**	0.237 (**< 0.001**)
	Female	247 (28.9)	439 (52.1)		
HT (available), n (%)		93 (10.9)	643 (76.4)	**< 0.001**	0.661 (**< 0.001**)
DM (available), n (%)		12 (1.4)	552 (65.6)	**< 0.001**	0.681 (**< 0.001**)
CAD (available), n (%)		111 (13)	306 (36.3)	**< 0.001**	0.271 (**< 0.001**)
COPD (available), n (%)		69 (8.1)	166 (19.7)	**< 0.001**	0.168 (**< 0.001**)
Lung cancer (available), n (%)		30 (3.5)	4 (0.5)	**< 0.001**	0.108 (**< 0.001**)
Extrapulmonary cancer (available), n (%)		67 (7.8)	39 (4.6)	**0.006**	0.066 (**0.006**)
Other chronical diseases (available), n (%)		114 (13.3)	236 (28)	**< 0.001**	0.181 (**< 0.001**)

HT = Hypertension, DM = Diabetes Mellitus, CAD = Coronary artery disease, COPD = Chronic obstructive pulmonary disease

reported the effect of age-related severity and mortality of the main genetic risk factor for COVID-19. They stated that the risk of mortality increased in those carrying the RS10490770 allele gene on the chromosome 3 locus. They also stated that the effect size is more common than other similar clinical risk factors and is associated with an increased risk of morbidity and mortality, which is more pronounced among individuals aged 60 years and younger [27]. In this study, we found that although the age variable was statistically significant in deaths due to COVID-19, the effect value was insignificant. Therefore, we think that the age variable can be reported as a mortality risk due to strong risk factors such as risky allele, HT, and DM.

Limitations

The main limitation of this study was that when evaluating chronic diseases in our patient population, data on drug use, treatment compliance and disease duration could not be recorded. The other limitation is that treatment differences or additional treatments were not taken into account, since the treatments received by our patients were applied in accordance with standard treatment algorithms.

CONCLUSION

It's critical to understand the disease's epidemiology in order to predict what to expect in the event of a worldwide pandemic. Knowing who is at risk of morbidity and mortality can help with patient management. Risk factors frequently reported for COVID-19 deaths in the literature are HT, DM, CAD, COPD, advanced age, and cancer. Although these variables are statistically significant in the studies, there are no studies showing the true effect values on mortality from COVID-19. In this study, in which 3,510 COVID-19-related deaths were examined, HT and DM had a strong effect separately, gender and CAD variables were moderate, COPD, lung cancer and other chronic disease variables had weak effect values, age and non-lung cancers had insignificant effect.

Understanding the epidemiology of the disease is essential to assist the effort to manage the 2019 coronavirus pandemic and to determine what to look out for in a global new outbreak. We need to know which individuals are at high risk of SARS-CoV-2 infection, as well as the morbidity and mortality risks if infected. The number of articles describing these aspects is increasing, almost similar to the epidemic, revealing the uncertainty about this disease.

Author' Contribution

Study Conception: SM; Study Design: SM, HA; Supervision: SE; Funding: CD; Materials: HA, İE; Data Collection and/or Processing: HA, CD; Statistical Analysis and/or Data Interpretation: SM, İE; Literature Review: SE, CD; Manuscript Preparation: SM and Critical Review: SE, İE.

Ethical disclosure

Ethical committee approval was obtained from Bursa City Hospital Ethical committee during the study planning phase. An informed consent form has been signed by the parents of the cases involved.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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(5):685-694 Original Article

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Investigation of the relationship of frontal QRS-T angle and digoxin use and blood digoxin level

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ABSTRACT

Objectives: Digoxin is an antiarrhythmic drug with a narrow therapeutic range and used in clinical conditions such as heart failure and atrial fibrillation. The planar frontal QRS-T angle reflects the deviations between the depolarization and repolarization of the ventricles, and it has been reported that an increase in this angle is associated with an increase in mortality. In our study, the relationship between frontal QRS-T angle and digoxin use and blood digoxin level was investigated.

Methods: The study included 105 digoxin users who used digoxin, whose levels were measured, who had an electrocardiogram (ECG) on the system, and 15 patients with similar characteristics, who had an ECG and did not use digoxin. Patients using digoxin and whose levels were measured were also divided into three groups as < 0.8 ng/mL, 0.8-1.2 ng/mL, and > 1.2 ng/mL. The absolute value of the value obtained by subtracting the axis of the T wave from the axis of the QRS angle indicated on the paper, calculated automatically on the 12-lead ECG, was accepted as the frontal QRS-T angle value.

Results: Planar frontal QRS-T angle measured by 12-lead ECG in digoxin users was 120º (55.5º-155.5º), while it was 106º (32º-163º) in non-users, and there was no statistical difference between the two groups ($p = 0.833$). In the evaluation made according to different blood drug levels as < 0.8 ng/mL, 0.8-1.2 ng/mL, > 1.2 ng/mL in digoxin users, no significant difference was observed between the frontal QRS-T angle between the groups (109.5° [60.25°-154.25°] for < 0.8 ng/mL, 136.5° [48.5°-158.5°] for 0.8-1.2 ng/mL, 117° [34°-154°] for 1.2 ng/mL) ($p = 0.773$)

Conclusions: There was no significant difference in frontal QRS-T angle between digoxin users and non-users. There was no significant relationship between different blood digoxin levels and frontal QRS-T angle.

Keywords: Frontal QRS-T angle, digoxin, digoxin level, intoxication, electrocardiogram

Digoxin is an antiarrhythmic drug with positive inotropic, negative dromotropic, and chronotropic effects, used in the treatment of heart failure (HF), atrial fibrillation (AF), supraventricular tachycardia. The therapeutic range is narrow and in some cases measurement of its level may be required. Moreover, the serum drug level may reach high levels and cause intoxication. In clinical practice, measurement of blood level is not always possible due to reasons such as laboratory conditions in the center and patient-related factors.

Planar frontal QRS-T angle can be defined as the absolute value of the number obtained by subtracting the QRS complex axis and the T wave axis, which can be calculated by superficial 12-lead electrocardiogram (ECG) [1]. The planar frontal QRS-T angle reflects

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the deviations between depolarization and repolarization of the ventricles, and an increase in this angle has been reported to be associated with increased mortality [2].

Although the effects of digoxin on the ECG have been clearly defined, its effects on the frontal QRS-T angle and the relationship between this value and the blood level have not been adequately studied. In our study, we aimed to investigate the relationship between blood digoxin level and frontal QRS-T angle.

METHODS

Study Population

This study was designed as a single-center retrospective study. A total of 400 patients who were referred to Bursa City Hospital between July 2019 and February 2022, who received oral or intravenous digoxin due to heart failure and AF, and whose blood digoxin levels were studied have been evaluated. Patients with blood digoxin levels in the system but no ECG and those with bundle branch block and pre-excitation pattern on the basal ECG and individuals under the age of 18 years were excluded from the study. Finally, 105 patients were included in the study.15 patients with similar patient characteristics but not using digoxin were also comprised in the control group. The necessary eligibility decision was taken from the Clinical Research Ethics Committee of Bursa City Hospital (decision no: 2022-4/2). The study was conducted by the Helsinki Declaration principles.

Laboratory Evaluation

Serum digoxin levels, which were evaluated by the homogeneous immunoassay method, were recorded. Complete blood count, liver and kidney function tests, electrolytes, lipid profile, and thyroid function tests determined from the samples taken during the same period were also noted.

Electrocardiography and Echocardiography

On the day the digoxin level was studied, 12-lead surface ECG (GE Healthcare, MAC 2000 ECG System, 2063587-001) was taken in supine position at 25 mm/s paper speed and 10 mm/s voltage was evaluated. ECGs, which were transferred to personal computers afterward and magnified by 300%, were examined under the Adobe Acrobat DC program. The QT interval was measured as the time from the beginning of the QRS complex to the end of the T wave. The corrected QT interval was calculated with Bazett's formula in patients with sinus rhythm. In AF patients, the corrected QT interval was determined by averaging 10 beats using the Bazett formula. The planar frontal QRS-T axis was found by taking the absolute value of the difference between the automatically calculated QRS complex axis and the T wave axis on the superficial 12-lead ECG. If this value was above 180, the number found by subtracting 360 was accepted as the frontal QRS-T axis.

Echocardiograms of the patients recorded in the system during the period in which digoxin levels were measured were examined. Left ventricular ejection fraction calculated using the modified Simpson method was recorded.

Other Parameters

The age and gender data of the patients were noted. Concomitant diseases such as hypertension (HT), diabetes mellitus (DM), hyperlipidemia (HL), coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI), and heart failure (HF), which required continuous drug use were detected. If there is heart failure, its etiology (ischemic/nonischemic) and NYHA class were determined. If AF was present on ECG, whether it was valvular or non-valvular and EHRA class was determined In addition, if there are drugs used concomitantly with digoxin, it was recorded.

Statistical Analysis

All statistical tests were conducted using the Statistical Package for the Social Sciences 25.0 for Windows (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to analyze the normality of the data. Normally distributed variables were expressed as mean ± standard deviation (SD), while non-normally distributed variables were expressed as median with interquartile range (IQR). The categorical variables are presented as percentages. The Chi-square test was used to assess differences in categorical variables between groups. Student’s t-test or Mann Whitney U test was used to compare unpaired samples as needed. The relationships among parameters were assessed using Pearson’s or Spearman's cor-

Table 1. Clinical demographic characteristics of patients in digoxin users and non-users

	Using digoxin (n = 105)	Not using digoxin (n = 15)	*p value*
Clinical characteristics			
Age (years)	72 ± 14.1	48.4 ± 14.0	0.292
Male, n (%)	41 (39)	8 (53.3)	0.292
HT, n (%)	58 (55.2)	12 (80)	0.069
DM, n (%)	41 (39)	4 (26.7)	0.408
HL, n (%)	1 (3.8)	0 (0)	0.582
CABG, n (%)	12 (11.5)	2 (13.3)	0.690
PCI, n (%)	17 (16.3)	6 (40)	**0.03**
CKD, n (%)	29 (27.6)	2 (13.3)	0.349
AF (non valvular), n (%)	69 (71.9)	13 (86.7)	0.225
HF, n (%)			
Ischemic	23 (26.4)	7 (46.7)	0.112
Nonischemic	43 (49.4)	8 (53.3)	0.780
Digoxin intoxication, n (%)	15 (14.3)	0 (0)	0.211
NYHA class	2.37 ± 0.94	2.4 ± 0.6	0.912
EHRA class	2.33 ± 0.9	2.2 ± 0.68	0.602
LVEF, (%)	43.8 ± 13.2	41 ± 12	0.443
Laboratory findings			
Hgb, g/dL	11.9 ± 2	12.4 ± 2.5	0.43
WBC, $\times 10^3$	9.6 ± 3.9	7 ± 2.2	**0.022**
PLT, $\times 10^3$	263 ± 125.7	220 ± 99	0.222
Urea, mg/dL	45 (31-70)	35 (29-52)	0.258
Creatinin, mg/dL	1 (0.8-1.4)	1 (0.9-1.4)	0.653
GFR (mL/min/1.73 m^2)	59.7 ± 26.7	57.4 ± 26.3	0.771
Na, mEq/L	137.3 ± 6.4	138.1 ± 3.2	0.64
K, mEq/L	4.3 ± 0.6	4.6 ± 0.5	0.176
Ca, mg/dL	9 ± 0.7	9 ± 0.4	0.794
TC, mg/dL	164.8 ± 5	133.7 ± 34.4	0.6
LDL, mg/dL	97 ± 39	79 ± 27.7	0.159
HDL, mg/dL	40.6 ± 12.2	40.5 ± 17.2	0.972
TG, mg/dL	126.5 (97.2-194.5)	73 (61.2-131)	0.017
AST, IU/L	20 (16-30)	23 (15-37)	0.762
ALT, IU/L	16 (12-24)	13 (11-22)	0.489
INR	1.3 (1.1-1.9)	1.1 (1.1-1.3)	0.192
TSH, mIU/L	1.4 (0.8-2.6)	1.1 (1.6-2.1)	0.723
Blood digoxin level, ng/mL	1.11 (0.7-1.7)	-	**< 0.001**
Concomitant medications			
ASA	23 (22.8)	4 (26.7)	0.739
Clopidogrel	12 (18)	3 (20)	0.407
Ticagrelor	1 (1)	0 (0)	1.000
Warfarin	27 (26.5)	0 (0)	0.021
NOAC	49 (48)	11 (73.3)	0.067
ACEi	45 (44.1)	8 (53.3)	0.584
ARB	12 (11.8)	0 (0)	0.359
BB	71 (69.6)	9 (60)	0.455
CCB	32 (31.4)	2 (13.3)	0.225
MRA	46 (45.1)	7 (46.7)	0.909
ARNI	4 (3.9)	0 (0)	0.503
Ivabradine	4 (3.9)	0 (0)	0.435
Amiodaron	12 (11.8)	2 (13.3)	0.861
SGLT-2i	6 (5.9)	0 (0)	0.335
Furosemide	77 (71.6)	9 (64.3)	0.548
Thiazide	27 (26.5)	3 (20)	0.757
Statin	14 (13.7)	2 (13.3)	0.967

Data are shown as mean ± standard deviation or n (%) or mean (minimum-maximum). HT = hypertension, DM = diabetes mellitus, HL = hyperlipidemia, CABG = coronary artery by-pass grefting, PCI = percutaneous coronary intervention, CKD = chronic kidney disease, AF = atrial fibrilation, HF = heart failure, LVEF = left ventricular ejection fraction, NYHA = New York Heart Association, EHRA = European Heart Rhythm Association, Hgb = haemoglobin, WBC = white blood cell, PLT = platelet, GFR = glomerular filtration rate, TC = total cholesterol, LDL = low density lipoprotein, HDL = high density lipoprotein, TG = triglyceride, AST = aspartate aminotransferase, ALT = alanine aminotransferease, INR = international normalized ratio, TSH = thyroid stimulating hormone, ASA = acetylsalicylic acid, NOAC = novel oral anticoagulants, ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, MRA = mineralocorticoid receptor antagonist, ARNI = angiontensin receptor neprilysin inhibitor, BB = beta-blocker, CCB = calcium channel blocker, SGLT2i = sodium/glucose cotransporter-2 inhibitors

relation analysis according to the normality of the data. The significance of the difference between digoxin levels groups was evaluated using one-way ANOVA followed by Bonferroni corrected post-hoc test for normally distributed numerical parameters. Parameters that did not show normal distribution were evaluated with the Kruskal–Wallis test, and the significance between the groups was determined using the Mann–Whitney U test pairwise comparison. The significance of differences between groups for ordinal parameters was assessed by using the chi-square test. Significance was assumed at a 2-sided $p < 0.05$.

RESULTS

The clinical and demographic characteristics of the patients who used and did not use digoxin in the study are shown in Table 1. While the mean age of individuals using digoxin was 72 ± 14.1 years, it was 48.4 ± 14.0 years in those who did not use digoxin. The male sex ratio was 39% in the digoxin group. This rate was 53.3% in the group that did not use digoxin. The rate of percutaneous coronary intervention history was higher in the group that did not use digoxin. In addition, there was no statistical difference between the group's HT, DM, HL, CABG, CKD, non-valvular AF, HF. Also EHRA, NYHA class, and LVEF were similar. When the laboratory parameters were examined, no difference was found between the two groups, except that the triglyceride and WBC value was statistically higher in the group using digoxin. There was no statistical difference between the two groups in terms of drugs used other than digoxin.

The electrocardiographic findings of the patients are given in Table 2. The corrected QRS duration was detected to be longer in the group that did not use digoxin than in those who used it. Other measurements were similar.

The clinical, demographic and electrocardiographic findings of individuals using digoxin according to blood digoxin levels are given in Table 3. The mean age was higher in the group with blood digoxin level >1.2 ng/mL than in the group with < 0.8 ng/mL (76.2 ± 11.2 years vs. 69.4 ± 13 years, $p = 0.029$). The frequency of CKD was statistically higher in the > 1.2 ng/mL group than in the other groups (16.7% for < 0.8 ng/mL, 17.9% for 0.8-1.2 ng/mL, and 40.4% for > 1.2 ng/mL, $p = 0.03$). EHRA class was statistically higher in the > 1.2 ng/mL group compared to the < 0.8 ng/mL group (2.6 ± 0.9 vs. 2 ± 0.6, $p = 0.024$). In electrocardiographic findings the mean heart rate was found to be significantly higher in the < 0.8 ng/mL group than in the >1.2 ng/mL group (94.8 ± 27 beats/min vs. 71 ± 22.6 beats/min, $p = < 0.001$). While the QRS duration was shorter in the <0.8 ng/mL group compared to the other groups ($p = 0.033$), the QT interval was longer in the >1.2 ng/mL group than in the < 0.8 ng/mL group ($p = 0.031$). The QTc interval was longer in the 0.8-1.2 ng/mL group than in the >1.2 ng/mL group ($p = 0.046$). Urea, creatinine and GFR values, which indicate kidney functions in laboratory parameters, were statistically higher in the >1.2 ng/mL group (61.4 mg/dL, $p = < 0.009$; 1.17 mg/dL, $p = < 0.003$;48.4 ± 23.1 mL/min/1.73 m2, $p = < 0.001$, respectively). There is no significant difference in other laboratory parameters. No significant difference was observed in the drugs used, except that amiodarone was used more in the 0.8-1.2 group than in the other groups (3.4%, 25.9% and 8.7%, respectively) (p =

Table 2. Comparison of ECG findings of groups using and not using digoxin

	Using digoxin (n = 105)	Not using digoxin (n = 15)	*p value*
Mean HR, beat/min	82.40 ± 25.8	88.73 ± 23.5	0.372
QRS duration, ms	101.06 ± 25.5	99.4 ± 29.3	0.818
QT, ms	378.42 ± 67.8	384.93 ± 84.7	0.737
QTc, ms	426.3 ± 46.7	461.8 ± 58.6	**0.009**
Frontal QRS-T Axis	120 (55.5-155.5)	106 (32-163)	0.833

Data are shown as mean±standard deviation or mean (minimum-maximum). HR = heart rate, ECG = electrocardiogram, min = minute, ms = miliseconds

Table 3. Demographic and ECG characteristics of patients using digoxin according to their blood levels

	< 0.8 ng/mL (n = 30)	0.8-1.2 ng/mL (n = 28)	> 1.2 ng/ml (n = 47)	*p value*
Clinical characteristics				
Age (years)	69.4 ± 13 [a]	70.8 ± 11	76.2± 11.2 [a]	0.029
Male, n (%)	12 (40)	8 (28.6)	21 (44.7)	0.381
HT, n (%)	17 (56.7)	19 (67.9)	22 (46.8)	0.204
DM, n (%)	14 (46.7)	14 (50)	13 (27.7)	0.095
HL, n (%)	0 (0)	3 (10.7)	1 (2.1)	0.075
CABG, n (%)	2 (6.7)	6 (21.4)	4 (8.7)	0.154
PCI, n (%)	6 (20)	4 (14.3)	7 (15.2)	0.809
CKD, n (%)	5 (16.7)	5 (17.9)	19 (40.4)	0.03
AF (non valvular), n (%)	23 (85.2)	16 (64)	30 (68.2)	0.180
HF, n (%)				
Ischemic	9 (37.5)	7 (28)	7 (18.4)	0.247
Nonischemic	11 (45.8)	13 (52)	19 (50)	0.907
NYHA class	2.1 ± 0.9	2.4 ± 1	2.5 ± 0.8	0.197
EHRA class	2 ± 0.6 [a]	2.2 ± 0.9	2.6 ± 0.9 [a]	0.024
LVEF, (%)	48.2 ± 12.2	40.5 ± 14	42.6 ± 13	0.09
ECG findings				
Mean HR, beat/min	94.8 ± 27[a]	87.8 ± 22.1	71 ± 22.6[a]	**< 0.001**
QRS duration, ms	90.9 ± 19.9	106.3 ± 27.1	104.5 ± 26.4	**0.033**
QT, ms	354 ± 69.3 [a]	376 ± 47.5	395 ± 73 [a]	**0.031**
QTc, ms	426.4 ± 42.4	443.9 ± 40.4[*]	415.9 ± 51[*]	**0.046**
Frontal QRS-T Axis	109.5 (60.25-154.25)	136.5 (48.5-158.5)	117 (34-154)	0.773
Laboratory findings				
Hgb, g/dL	12.3 ± 2.4	12 ± 1.8	11.8 ± 2.0	0.526
WBC, $\times 10^3$	8.9 ± 3.9	9.9±4	9.9 ± 3.9	0.569
PLT, $\times 10^3$	257 ± 104.5	286 ± 126	254 ± 138.1	0.53
Urea, mg/dL	38 (30-55.25)	34.4 (28.5-55.1)	61.4 (39.75-82)	**0.009**
Creatinin, mg/dL	0.92 (0.74-1.2)	0.98 (0.62-1.8)	1.17 (0.96-1.95)	**0.003**
GFR (mL/min/1.73 m^2)	69.2 ± 26.5	68.2±26.2	48.4 ± 23.1	**< 0.001**
Na, mEq/L	138.8 ± 4.5	137 ± 9.3	136.5 ± 5.2	0.296
K, mEq/L	4.3 ± 0.6	4.3 ± 0.5	4.4 ± 0.6	0.687
Ca, mg/dL	9 ± 0.6	9.2 ± 0.7	8.9 ± 0.7	0.066
TC, mg/dL	174 ± 49	176.2 ± 63.9	151.1 ± 37.6	0.107
LDL, mg/dl	105.2 ± 37.1	106.7± 48.2	85.4 ± 30.4	0.062
HDL, mg/dL	43 ± 11.7	40 ± 13.2	39.6 ± 12	0.596
TG, mg/dL	140 (111-189.5)	171 (87-249)	120 (92-165)	0.153
AST, IU/L	21 (17-28)	18.5 (15-24)	21 (17-32)	0.351
ALT, IU/L	16 (12-22.75)	19 (13.5-24.5)	16 (9-25)	0.449
INR	1.28 (1.1-1.8)	1.26 (1.14-2)	1.2 (1.1-2.1)	0.924
TSH, mIU/L	1.49 (0.8-2.4)	1.6 (0.84-2.9)	1.26 (0.54-2)	0.36
Blood digoxin level, ng/mL	0.5 (0.31-0.7)	1 (0.89-1.1)	1.95 (1.54-2.84)	**< 0.001**
Concomitant medications				
ASA	3 (10.3)	8 (30.8)	12 (26.1)	0.151
Clopidogrel	5 (17.2)	3 (11.1)	4 (8.7)	0.531
Ticagrelor	1 (3.4)	0	0	0.281
Warfarin	8 (27.6)	7 (25.9)	12 (26.1)	0.987
NOAC	15 (51.7)	12 (44.4)	22 (47.8)	0.861
ACEi	15 (51.7)	13 (48.1)	17 (37)	0.403
ARB	3 (10.3)	4 (14.8)	5 (10.9)	0.846
BB	18 (62.1)	23 (85.2)	30 (65.2)	0.117
CCB	13 (44.8)	5 (18.5)	14 (30.4)	0.104
MRA	12 (41.4)	15 (55.6)	19 (41.3)	0.444
ARNI	0 (0)	2 (7.4)	2 (4.3)	0.354
Ivabradine	1 (3.4)	2 (7.4)	1 (2.2)	0.532
Amiodaron	1 (3.4)	7 (25.9)	4 (8.7)	**0.023**
SGLT-2i	1 (3.4)	3 (11.1)	2 (4.3)	0.399
Furosemide	18 (62.1)	21 (77.8)	34 (73.9)	0.383
Thiazide	10 (34.5)	6 (22.2)	11 (23.9)	0.506
Statin	5 (17.2)	3 (11.1)	6 (13)	0.788

Data are shown as mean±standard deviation or n (%) or mean (minimum-maximum). HT = hypertension, DM = diabetes mellitus, HL = hyperlipidemia, CABG = coronary artery by-pass grefting, PCI = percutaneous coronary intervention, CKD = chronic kidney disease, AF = atrial fibrilation, HF = heart failure, LVEF = left ventricular ejection fraction, NYHA = New York Heart Association, EHRA = European Heart Rhythm Association, Hgb = haemoglobin, WBC = white blood cell, PLT = platelet, GFR = glomerular filtration rate, TC = total cholesterol, LDL = low density lipoprotein, HDL = high density lipoprotein, TG = triglyceride, AST = aspartate aminotransferase, ALT = alanine aminotransferease, INR = international normalized ratio, TSH = thyroid stimulating hormone, ASA = acetylsalicylic acid, NOAC = novel oral anticoagulants, ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, MRA = mineralocorticoid receptor antagonist, ARNI = angiontensin receptor neprilysin inhibitor, BB = beta-blocker, CCB = calcium channel blocker, SGLT2i = sodium/glucose cotransporter-2 inhibitors, HR = heart rate, ECG = electrocardiogram, min = minute, ms = milisecond

[a]$p < 0.05$ *b*etween <0.8 ng/mL and < 1.2 ng/mL groups, *$p < 0.05$ between 0.8-1.2 ng/mL and > 1.2 ng/mL groups

0.023).

Consequently, when the results of the study were evaluated, there was no statistically significant difference in frontal QRS-T angle in digoxin users compared to non-users (120° [55.5°-155.5°] vs. 106° [32°-163°]), (p = 0.833). In addition, there was no statistical significance between blood level and frontal QRS-T angle in digoxin users (109.5° [60.25°-154.25°] for < 0.8 ng/mL, 136.5° [48.5°-158.5°] for 0.8-1.2 ng/mL, 117° [34°-154°] for 1.2 ng/mL) (p = 0.773) (Figs. 1, 2 and 3).

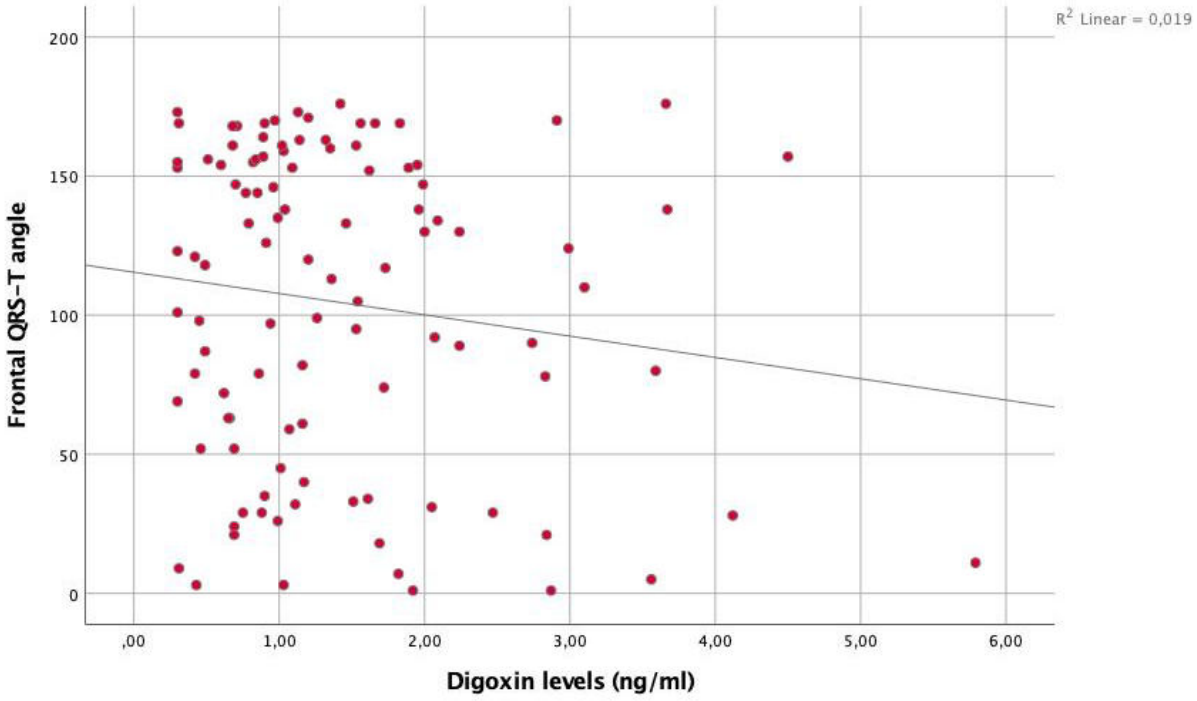

Fig. 1. Graphical representation of the relationship between digoxin level and frontal QRS-T angle.

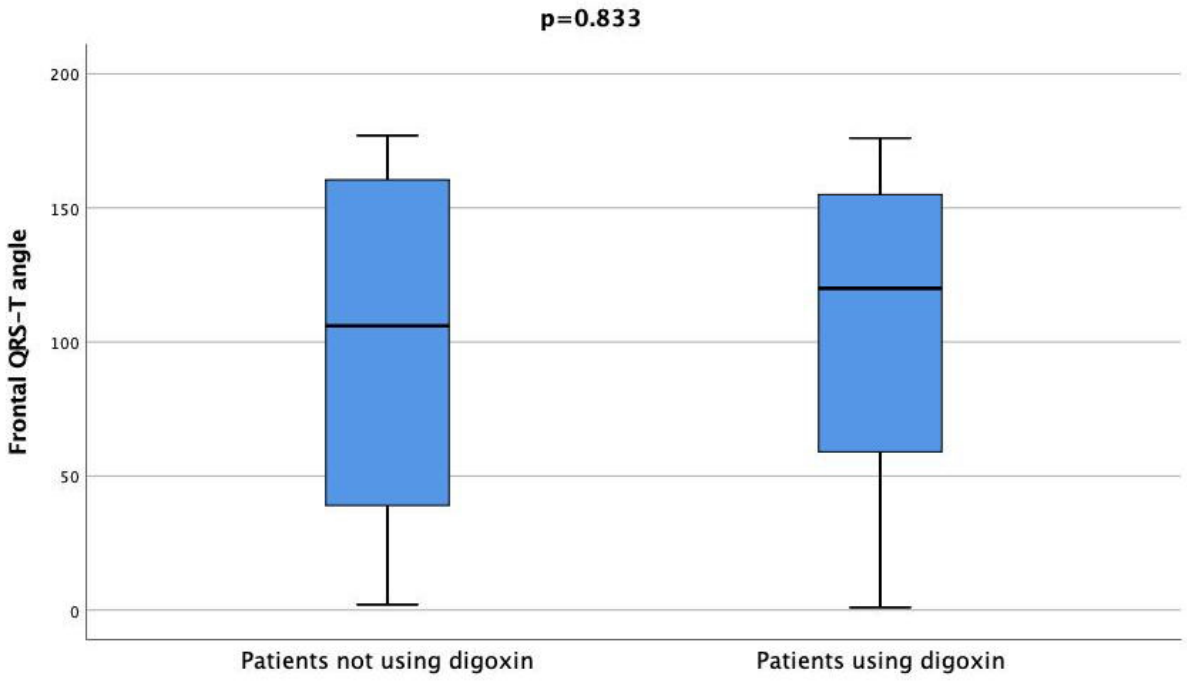

Fig. 2. Frontal QRS-T angle in digoxin and non-digoxin users.

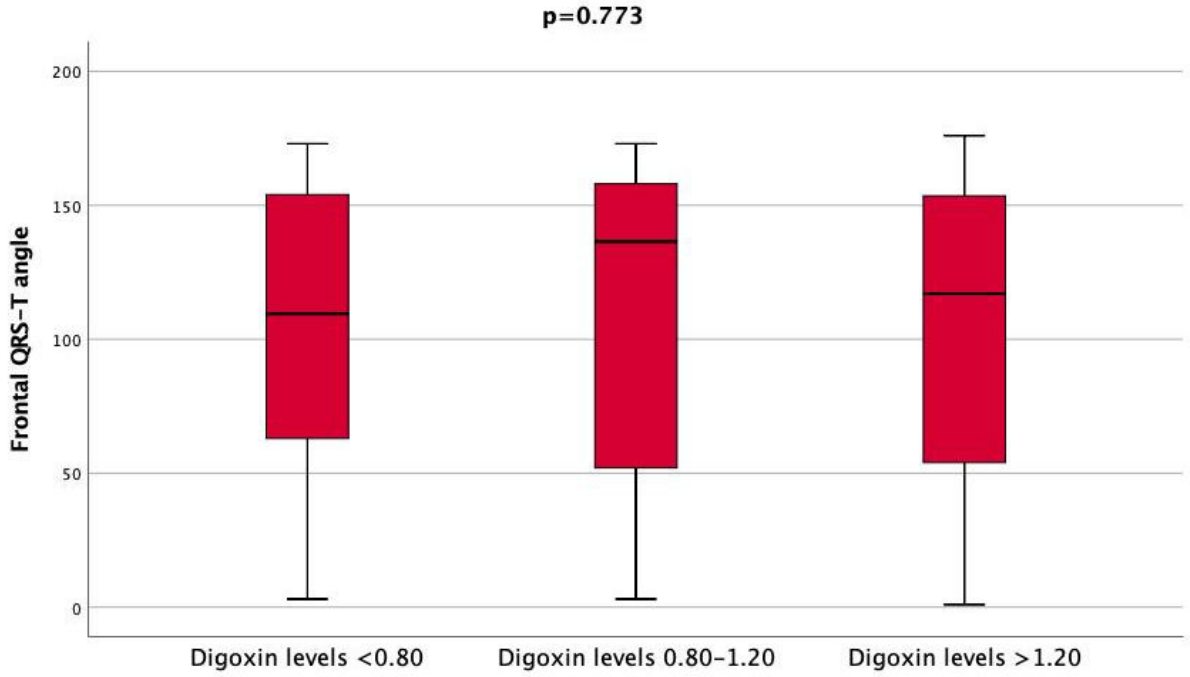

Fig. 3. Frontal QRS-T angle according to blood digoxin levels.

DISCUSSION

In our study, no significant relationship was found between frontal QRS-T angle and serum digoxin levels. Also, there was no significant relationship between drug blood levels and frontal QRS-T angle values in individuals using digoxin.

The spatial QRS-T angle can be defined as the angle between ventricular depolarization and repolarization directions and has been shown to predict cardiac death in various studies [3]. However, the spatial QRS-T angle is difficult to measure in clinical practice and requires computer-assisted electrocardiographic analysis software [4]. On the contrary, the frontal QRS-T axis is a parameter that can be obtained from a superficial 12-lead electrocardiogram (ECG) and easily calculated from automatically calculated measurements on the ECG paper and correlates well with the spatial QRS-T wave [5]. We preferred to calculate the planar frontal QRS-T angle, which we considered a more practical method in our study. Various studies have suggested different numbers regarding the cut-off values of the frontal QRS-T angle. In one study, in patients with ischemic heart disease and ICD, those with a spatial QRS-T angle less than 100°, no ventricular arrhythmia event was observed at 2-year follow-up, and its frequency was only 2% during subsequent follow-up [6]. In another study involving the middle-aged general population, a frontal QRS-T angle of ≥ 100° was found to increase the risk of arrhythmic death [3]. It has been stated that a wide QRS-T angle reflects structural abnormalities that affect depolarization or regional pathophysiological changes in ionic channels that change the repolarization order [3]. In our study, the frontal QRS-T angle was found to be above 100 degrees in both digoxin users and non-users, and those who used digoxin and had different blood levels. The lack of difference in frontal QRS-T angle in digoxin users may be associated with the presence of clinical conditions such as concomitant hypertension, diabetes mellitus, coronary artery disease, and the use of beta-blockers, amiodarone, and similar antiarrhythmic drugs. Therefore, the effect of digoxin on frontal QRS-T angle may not have been observed.

Digoxin is a drug with negative chronotropic and dromotropic, positive inotropic effects. It also inhibits the sodium-potassium pump (-ATPase) Na+/K+, increasing the availability of calcium for contractile components or myofibrils. Digoxin increases cardiac vagal activity. The inhibition of the Na-K ATPase pump increases the intracellular Na+ concentration and facilitates the entry of Ca2+ into the cell, thereby increasing cardiac inotropy [7-9]. In other words, cardiac action potential duration will be shortened in the

ventricles, and excitation-contraction coupling in the sarcomere will result in negative chronotropism and positive inotropism due to increased intracellular calcium [10]. In case of intoxication, it acts on phase 4 of the action potential [11], leading to delayed afterdepolarization and increased automaticity and/or ectopic activity. As a result, atrial and ventricular tachyarrhythmias may occur [12]. The 'reverse tic' pattern in V4-V6 is the most commonly identified ECG change and can, in fact, be described as the appearance of a biphasic T wave with initial negative and terminal positive bias. These changes are due to its effect in reducing the ventricular refractory period and cause secondary repolarization abnormalities affecting the ST segment, T and U wave. As a result, the QT interval can be detected as shortened [13]. Therefore, it can be thought that the frontal QRS-T angle, which can be defined as an indicator of heterogeneity between ventricular depolarization and repolarization [14, 15], may be abnormal in digoxin users. In our study, no statistically significant difference was found between the control group and the group using digoxin in terms of frontal QRS-T. In addition, the corrected QT interval was found to be significantly longer in the group using digoxin compared to those not using digoxin, but it was within normal limits. In the evaluation made according to digoxin level, the corrected QT interval was longer in the 0.8-1.2 ng/mL group than in the > 1.2 ng/mL group. Also, the QT interval was found to be longer in the digoxin level > 1.2 ng/mL group compared to the < 0.8 ng/mL group.

In a randomized, double-blind DIG study with 6800 patients with ejection fraction < 45%, in normal sinus rhythm were divided into 2 groups with and without digoxin. As a result of approximately 40 months of follow-up, there was no decrease in total mortality, but a statistically significant decrease was found in hospitalization. In addition, there was a trend for a lower risk of death due to worsening heart failure in the digoxin group compared with the placebo group [16]. In a retrospective cohort study of patients with AF, the use of digoxin was associated with an increased risk of mortality after multivariate adjustment [17]. In a subanalysis of the AFFIRM trial, which included more than 4000 AF patients at high risk of stroke, digoxin was associated with a significant increase in mortality even after controlling for comorbidities and trend scores, regardless of gender and the presence or absence of underlying HF [18]. In another study, patients with a serum digoxin concentration ≥ 1.2 ng/mL had a 56% increased risk of mortality (adjusted HR: 1.56; 95% CI: 1.20 to 2.04) compared to those not using digoxin. When analyzed as a continuous variable, it was associated with a 19% higher adjusted hazard of death for each 0.5 ng/mL increase in serum digoxin (p = 0.0010) [19]. For patient safety, it has been suggested that target serum concentrations of 0.5-0.9 ng/mL, which is below the "therapeutic" range [10]. According to one study, a serum digoxin concentration of 0.5-0.9 ng/mL reduces mortality and hospitalizations in all heart failure patients, including those with preserved systolic function and high blood digoxin levels. Digoxin reduces hospitalization for heart failure, but has no effect on mortality or all-cause hospitalization. Higher blood digoxin levels were associated with increased crude all-cause mortality in patients with heart failure (0.5-0.8 ng/mL, 29.9%; 0.9-1.1 ng/mL, 38.8%, and ≥ 1.2 ng/mL, 48.0%) [20, 21]. The patient population in our study included patients with ischemic-non-ischemic heart failure and heart failure with preserved-low EF. There was no significant difference in the frontal QRS-T angles of the patients who were divided into 3 groups according to their blood digoxin levels, as < 0.8 ng/mL, 0.8-1.2 ng/mL, and > 1.2 ng/mL. In addition, there was no significant finding to predict serum digoxin levels in terms of frontal QRS-T.

In one study, it was shown that the frontal QRS-T angle remained stable until middle age, and then showed a rapid increase with a straight-line correlation [22]. In our study, no significant age-related difference was observed in the frontal QRS-T angle between the control group and digoxin group, and between the groups using digoxin. This may be related to the fact that various comorbidities and antiarrhythmic drugs affected the frontal QRS-T angle value, although there were differences in age between the control group and the groups using digoxin.

Digoxin is mostly eliminated unchanged by the kidneys. 30-50% of the daily dose is excreted within 24 hours, and enterohepatic circulation is negligible [23]. The elimination half-life is 1.5-2 days, but may be prolonged up to 4-6 days in anuric patients [21, 24, 25]. There is also an increased risk of overall mortality with digoxin therapy in end-stage renal disease requiring hemodialysis, again with the safest serum levels

<0.9 ng/mL [10, 26]. In our study, significant impairment in kidney functions (creatinine, urea, and GFR) in the group with blood levels > 1.2 ng/mL may also be associated with the decrease in digoxin renal clearance.

Limitations

There are several limitations to our study. First, the number of patients included in the study is limited. In addition, the number of patients in the control group was insufficient, although clinical and demographic characteristics were similar in both groups. Different results may be obtained with studies to be conducted in larger populations. Secondly, drugs used together with digoxin, some of which are antiarrhythmic, may have caused different values in frontal QRS-T angle. The third is the use of planar frontal QRS-T angle instead of the spatial frontal QRS-T angle, which is stated to be superior in some publications. Finally, studies involving the values and comparisons obtained before and after a certain period of starting the drug in cases where digoxin is planned to be used may reveal different results.

CONCLUSION

In our study, no significant difference was found in frontal QRS-T angles in digoxin users compared to non-users. In the evaluation of frontal QRS-T angles according to blood digoxin levels, no statistically significant correlation was found between blood digoxin levels.

Authors' Contribution

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

Conflict of interest

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

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A prospective observational study on cystic stump closure during laparoscopic cholecystectomy: silk sutures (intra-corporeal ligation) or locking clips (Hem-o-Lok®)

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ABSTRACT

Objectives: The present study compares the clinicopathological features and outcomes of two cystic stump closure techniques (locking clips and intra-corporeal ligation with silk sutures) during laparoscopic cholecystectomy.

Methods: This study was conducted in a tertiary health centre as a prospective observational study between October 5, 2021, and February 15, 2022. For randomisation, double rows of silk sutures were used to close the cystic stump in patients on the odd-numbered day of the month (Group 1). In contrast, a single-row locking clip (Hem-o-Lok®) (Group 2) was used to close the cystic stump in patients on the even-numbered day of the month. The study was terminated when at least 50 patients were reached in both groups. The clinicopathological differences and cholecystectomy outcomes were compared between the groups.

Results: This observational study included 114 patients. Of the study cohort, 87 (76.3%) were female, and the mean age was 46.54 ± 14.74 years. There were 64 (56.1%) patients in Group 1 and 50 (43.9%) patients in Group 2. Preoperative ERCP requirement was higher in Group 1 (15.6% vs 4%, $p = 0.045$), while the mean operation room time was longer in Group 2 ($p = 0.015$). Morbidity rates were similar in the two groups (3.1% vs 2%) ($p > 0.05$).

Conclusions: Cystic stump closure is the essential step of laparoscopic cholecystectomy. According to the present study's results, silk sutures (intra-corporeal ligation) and locking clips (Hem-o-lok®) are materials that can be used safely to close a cystic stump.

Keywords: Cholecystectomy, clips, cystic duct, laparoscopy, morbidity, suture

Cholecystectomy due to cholelithiasis is one of the most frequently performed operations in general surgery. This operation can be achieved with open surgery and laparoscopic surgery. Laparoscopic surgery is the standard gold surgery today. The most crucial step is the successful and non-leak closure of the cystic stump orifice. Many techniques have been described in the literature to close the cystic orifice. These methods can be reduced to two main headings: clip closure (locking or non-locking) and clipless closure. The most common clips used during laparoscopic cholecystectomy are metallic clips (non-locking). Alterna-

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tive clips, called locking clips, are Hem-o-Lok® (Teleflex, Wayne, United States), Click'aV® (Grena, Nottingham, United Kingdom) and Lapro-Clip® (Medtronic, Minneapolis, United States) [1, 2]. Locking clips differ from metallic ones as they are made of polymers, are usually absorbable, and are designed to lock in place with comparable locking pressure. Therefore, they are thought to provide a more secure closure.

Clipless closure techniques have become popular due to technological developments. With the introduction of vessel sealing devices such as the Harmonic scalpel® (Ethicon, Somerville, United States) and LigaSureTM (Medtronic, Minneapolis, United States), their feasibility and outcome in closing the cystic duct during laparoscopic cholecystectomy are of interest. On the other hand, simple sutures such as silk and polydioxanone (PDS) and Endoloop® (Ethicon, Somerville, United States) are materials used for clipless closure.

Regardless of the material used, the primary purpose is to close the stump without complications. In cases where the cystic stump is not closed correctly, stump leakage with an incidence of 0.3% to 3% can be seen [3]. In a systematic review with 38 studies that included 47,491 patients, the rate of BL ranged from 0% and 4%, with an odds ratio of 0.17 (95% CI: 0.03-0.93) for overall BL for locking vs non-locking clips [4]. In addition, complications such as surgical site infection, hematoma, abscess, and postoperative bleeding can also be seen during cholecystectomy, as in any surgery [5].

This prospective observational study compared the clinicopathological features and outcomes of two cystic stump closure techniques (locking clips and intracorporeal ligation with silk sutures) during laparoscopic cholecystectomy.

METHODS

This observational study was started as a prospective study in Erzurum Regional Training and Research Hospital General Surgery Clinic after the ethics committee's approval (Decision no: 2021/17-243). Patients who underwent laparoscopic cholecystectomy for cholelithiasis between October 5, 2021, and February 15, 2022, were included in the study. On the other hand, patients in the pediatric age group (0-18 years old) and patients diagnosed and treated in different centres and admitted to our centre were not included in the study. Patients who underwent endoscopic retrograde cholangiopancreatography (ERCP) were operated on in the same hospitalisation after liver

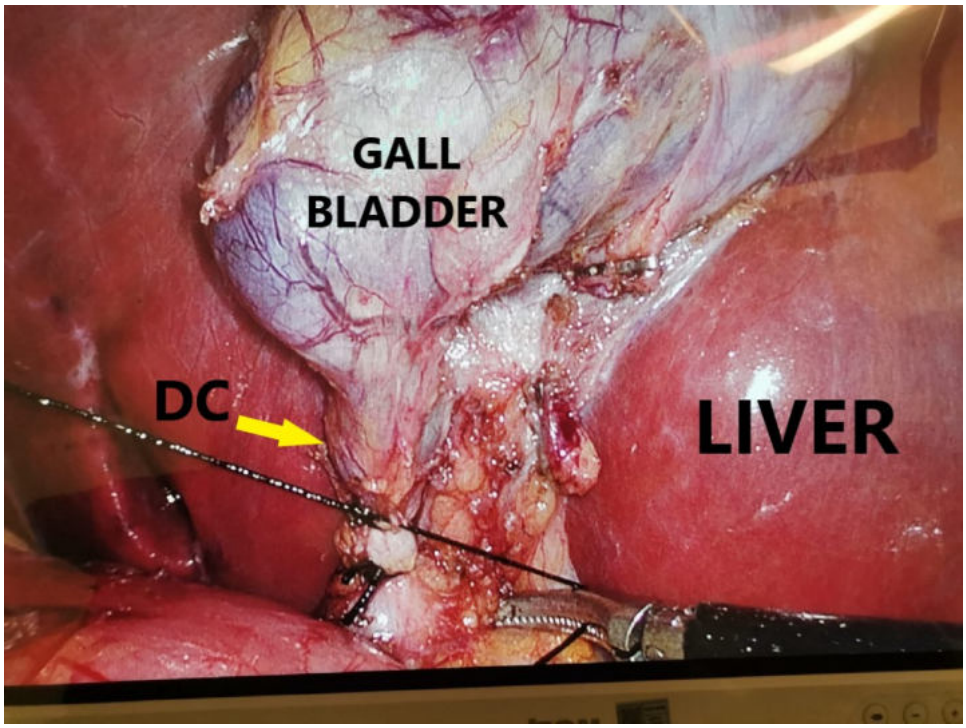

Fig. 1. Intraoperative view of the closure of the cystic duct with silk sutures. DC = ductus cysticus.

function tests returned to normal. None of the patients needed ERCP in the postoperative period.

Checked Parameters

The age and gender of the patients, indication for cholecystectomy (acute or elective), preoperative endoscopic retrograde cholangiopancreatography (ERCP) requirement, materials used to close the cystic duct, surgery time, morbidity and mortality status, length of hospital stay, and pathological evaluation of cholecystectomy specimens were searched. Hospital records, consultation and operation notes, and clinical charts of the patients were evaluated to collect the researched parameters. Patients who developed complications in the postoperative 30 days were assessed as morbidity. Due to the lack of mortality, mortality was not considered a postoperative outcome.

The patients were divided into groups according to the closing material used during cholecystectomy: Group 1= Closure with silk sutures (intra-corporeal ligation) and Group 2= Closure with locking clips (Hem-o-Lok®). The clinicopathological and outcome differences between the groups were evaluated.

Patient Randomization

Silk sutures were used to close the cystic stump in patients operated on the odd-numbered day of the month (Group 1), and locking clips (Hem-o-Lok®) were used to close the cystic stump on the even-numbered day of the month (Group 2). The study was terminated when at least 50 patients were reached in both groups. All patients were given detailed information before the operation, and an informed consent form was obtained from all patients.

Operation Technique

The same well-trained surgical team performed all operations. Hasson technique was applied to each patient during the first trocar entrance. All procedures were performed by laparoscopy with four trocars: a 10- or 12-mm trocar inserted supraumbilical (first trocar), a 10- or 12-mm trocar from the xiphoidal region, and two 5 mm trocars inserted from the right para-rectal area. CO_2 insertion was made only through the first trocar in all cases. After creating pneumoperitoneum, all cholecystectomies were performed according to the critical view of the safety principle. Cystic artery and cystic duct were exposed by dissection of Calot's triangle. According to randomisation, the cystic duct stump was closed with a double row of 2/0 silk sutures (intra-corporeal ligation) (Fig. 1) or a single row of locking clips (Hem-o-lok®) (Fig. 2). Cholecystectomy material was taken out of the abdomen from the xiphoidal port.

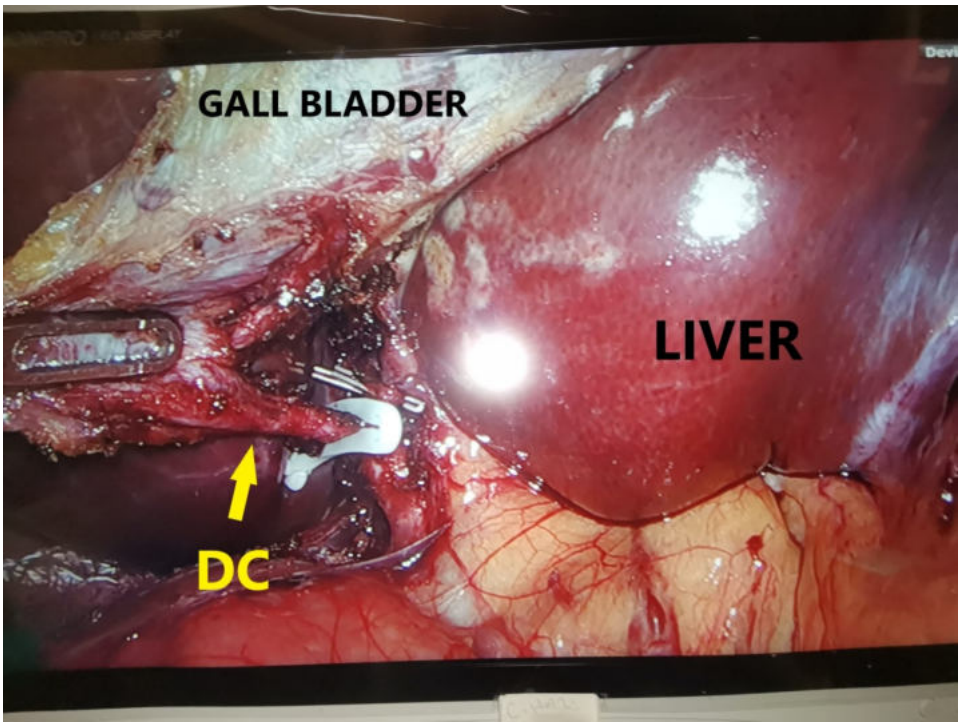

Fig. 2. Intraoperative view of the cystic duct closure with a locking clip. DC = ductus cysticus.

Statistical Analysis

Statistical evaluation was made with SPSS v22.0 (IBM, Armonk, NY, USA). Quantitative variables were expressed as mean ± standard deviation (SD), median, minimum-maximum, interquartile range and interval. Qualitative variables were reported as numbers and percentages. The normality distribution of quantitative variables was checked with the Shapiro-Wilk test. According to Shapiro-Wilk test results, quantitative variables were compared with independent samples t-test or Mann-Whitney U test. Chi-Square tests (Pearson and Fisher's exact) were used to compare qualitative variables. A p-value below 0.05 was considered statistically significant.

RESULTS

This observational study included 114 patients. Of the study cohort, 87 (76.3%) were female, and the mean age was 46.54 ± 14.74 years, with a range of 19-83 years. The clinicopathological features of the patients are shown in Table 1.

There were 64 (56.1%) patients in Group 1 and 50 (43.9%) patients in Group 2. Both groups' demographic features (mean age and gender distribution) were similar. 58 (50.9%) patients underwent laparoscopic cholecystectomy due to cholecystitis. The remaining patients were operated on under elective conditions. The rate of patients operated on for cholecystitis was similar in both groups (46.9% vs 56%, $p = 0.334$).

Cholecystectomy was performed in 12 (10.5%) patients after endoscopic retrograde cholangiopancreatography (ERCP). The rate of preoperative ERCP requirement was higher in Group 1 (15.6% vs 4%, $p = 0.045$). Intraoperative complications were seen in 5 (4.4%) patients: 3 of 5 had liver bed bleeding, and 2 of 5 had trocar site haemorrhage. All liver bed haemorrhages were controlled during laparoscopy. While 2 of these haemorrhages were controlled by energy devices, the bleeding was stopped with liver bed suturing in the remaining case. Trocar site haemorrhages were controlled with simple sutures and energy devices. Intraoperative complication rates were similar in the two groups (4.7% vs 4%). However, operation room time was lower in Group 1 ($p = 0.015$).

The morbidity rate of the study was 2.6% (n = 3), and 2 of the three patients were in Group 1. All postoperative complications were port site infections and treated with daily cleaning and drainage. Morbidity rates were similar in the two groups (3.1% vs 2%) ($p = 1.000$). A comparison of clinicopathological variables is shown in Table 2.

DISCUSSION

Laparoscopic cholecystectomy is a minimally invasive surgical procedure that removes a diseased gallblad-

Table 1. Clinicopathological data of all patients

Parameters	n (%) or mean ± SD (range)
Age (years)	46.54 ± 14.74 (19-83)
Gender	
Female	87 (76.3)
Male	27 (23.7)
Preoperative diagnoses	
Acute Cholecystitis	58 (50.9)
Cholelithiasis	56 (49.1)
Preoperative ERCP	
Yes	12 (10.5)
No	102 (89.5)
Intraoperative complication	
Yes	5 (4.4%
No	109 (95.6)
Cystic stump closure	
Silk suture (Group 1)	64 (56.1)
Locking clips (Group 2)	50 (43.9)
Operation room time (min)	42.75 ± 15.05 (20-115)
Morbidity	
Yes	3 (2.6)
No	111 (97.4)
Hospital stay (days)	1.91 ± 1.2 (1-10)
Pathology	
Acute cholecystitis	34 (29.8)
Chronic cholecystitis	80 (70.2)

ERCP = endoscopic retrograde cholangiopancreatography, SD = standard deviation

Table 2. Comparison of the cystic duct closure techniques

Parameters	Group 1 (n = 64)	Group 2 (n = 50)	*p* value
Age (years)	45.48 ± 15.56	47.90 ± 13.65	0.388*
Gender, n (%)			0.709**
Female	48 (55.2)	39 (44.8)	
Male	16 (59.3)	11 (40.7)	
Preoperative diagnosis, n (%)			0.334**
Cholelithiasis	34 (60.7)	28 (48.3)	
Acute cholecystitis	30 (51.7)	22 (39.3)	
Preoperative ERCP, n (%)			**0.045****
Yes	10 (15.6)	2 (4)	
No	54 (84.4)	48 (96)	
Intraoperative complication, n (%)			1.000***
Yes	3 (4.7)	2 (4)	
No	61 (95.3)	48 (96)	
Operation room time (min), (median/IQR)	39 (IQR=12)	43.50 (IQR=20)	**0.015****
Morbidity, n (%)			1.000**
Yes	2 (3.1)	1 (2)	
No	62 (96.9)	49 (98)	
Hospital stay (days), (median/IQR)	2 (IQR=1)	2 (IQR=1)	0.641****
Pathology, n (%)			0.707**
Acute cholecystitis	20 (31.3)	14 (28)	
Chronic cholecystitis	44 (68.8)	36 (72)	

Data are shown as mean±standard deviation or n (%) or median/IQR. ERCP = endoscopic retrograde cholangiopancreatography, IQR = Interquartile range

*Independent samples t-test, **Pearson chi-square test, ***Fisher's exact test, ****Mann Whitney U test

der. Since the early 1990s, laparoscopy has primarily replaced the open technique for cholecystectomy. Laparoscopic cholecystectomy is currently indicated for treating acute or chronic cholecystitis, symptomatic cholelithiasis, calculous cholecystitis, gallstone pancreatitis, biliary dyskinesia, and gallbladder masses or polyps [6-8].

Cystic stump closure is the essential step of laparoscopic cholecystectomy. Closing can be done with many techniques. The most common technique is to close the stump with simple (non-locking) metallic clips. Alternatives are locking clips or ligatures [1, 2]. Locking clips differ from metallic ones as they are made of polymers, are usually absorbable, and are designed to lock in place with comparable locking pressure. Therefore, they are thought to provide a more secure closure.

Regardless of the material used, the primary purpose is to close the stump without complications. In cases where the cystic stump is not closed correctly, stump leakage with an incidence of 0.3% to 3% can be seen [3]. In addition, complications such as surgical site infection, hematoma, abscess, and postoperative bleeding can also be seen during cholecystectomy, as in any surgery [5]. In one meta-analysis, the 30-day complication rate of cholecystectomy ranged from 4-31% [9]. The morbidity rate of the present study was found to be 2.6%, which is lower than the literature.

The problem of biliary leakage has been evaluated in both retrospective and prospective studies. However, very few studies compare intracorporeal ligation with silk suture and Hem-o-Lok® clip closure, and our

study stands out in terms of the number of cases. In a systematic review of 38 studies, the overall incidence of biliary leakage ranged from 0% to 4% [4]. According to the study by Singal *et al*. [10], when silk suture ligation (80 patients) and ligaclips closure (80 patients) of the cystic stump were compared, no postoperative complications, including bile leakage, were observed in the silk suture group. However, in a randomised controlled study with 105 patients comparing tied knots with Hem-o-Lok® clips, the incidence of bile leakage was 3.8% in the tied knots group, with no difference in postoperative complications [11]. In a comparative randomised controlled trial comparing intracorporeal ligation and ligaclips with 120 patients, the mean operative time was longer in the intracorporeal ligation group, with no difference in postoperative complications [12].In a comparative study using different materials (intracorporeal knotting using 2/0 vicryl suture and titanium clips), there was no difference between the groups in terms of preoperative clinical presentation, preoperative ERCP requirement, mean operation room time, and postoperative complications [13]. In a non-comparative pilot study of 80 patients, silk sutures were used to close the cystic duct, and no postoperative complications and bile leakage were observed [14]. Talebpour *et al*. [15] showed that ligation by suturing is safer with 0% bleeding and biliary leakage rates. The present study's overall morbidity rate was 2.6% without bile leakage. However, unlike the literature, the preoperative ERCP requirement rate was higher, and the mean operation time was shorter in the intracorporeal ligation group. In contrast, the preoperative clinical presentation rate (cholelithiasis/cholecystitis) was similar to the literature. While it is expected that the cholecystectomy time will be shorter in patients using locking clips, silk suture ligation time has reached similar times when the learning curve is gone. In addition, new studies with more cases are needed, as the severity of the acute cholecystitis attack, the difficulty of dissection, and the duration of removal of the cholecystectomy specimen from the abdominal cavity may affect the operation time.

Limitations

A significant shortcoming of our study is that the cystic closure times were not evaluated. In addition, new studies excluding patients who underwent ERCP and patients who underwent surgery for acute cholecystitis to make the study more homogeneous will fill the lack of literature and provide more robust data. In addition, longer period outcomes should be evaluated to compare the two closure techniques.

CONCLUSION

Silk sutures (intra-corporeal ligation) and locking clips (Hem-o-Lok®) are materials that can be used to close a cystic stump. Closing the cystic stump with a silk suture, more commonly used in patients who underwent ERCP in the preoperative period, is advantageous and reliable since it shortens the operation time without increasing morbidity.

Authors' Contribution

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

Conflict of interest

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

Financing

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The European Research Journal 2022;8(5):702-709 Original Article

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Is Glasgow prognostic score a predictor of mortality in infective endocarditis?

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ABSTRACT

Objectives: The inflammation-based Glasgow prognostic score (GPS), which comprises elevated serum C-reactive protein (CRP) and decreased albumin concentration, is the most valid inflammatory risk score in cancer. New prognostic markers are needed to predict high-risk infective endocarditis (IE) patients. In the present study, we investigated the in-hospital mortality estimation of GPS in infective endocarditis patients.

Methods: The retrospectively designed study included 53 IE patients diagnosed according to Duke criteria. Demographic and clinical data of the patients were recorded and GPS levels were measured. Patients were divided into two groups according to in-hospital mortality outcomes. Glasgow prognostic score was rated as 0, 1, or 2 points based on serum albumin and C-reactive protein levels.

Results: The nonsurvivor group was older and the number of patients with kidney failure or diabetes was higher in this group. Glasgow prognostic score was higher in the nonsurvivor group, while albumin levels were lower. Thirty-four patients died during intensive care unit follow-up, and the mean follow-up period was 24.1 ± 18.6 days. ROC analysis showed that the Glasgow prognostic score had a sensitivity of 82.4% and a specificity of 36.8% at a cut-off value of ≥ 1.5 in predicting in-hospital mortality. Chronic renal failure (OR: 6.720; 95% CI: 1.907-23.684; $p = 0.003$) and age (OR: 1.040; 95% CI: 1.001-1.081; $p = 0.044$) were the independent variables of the mortality prediction in univariate logistic regression analysis. In multivariate logistic regression analysis, only chronic renal failure (OR: 0.153; 95% CI: 0.036-0.653; $p = 0.011$) was found to be a significant predictor of mortality. Kaplan–Meier survival analysis revealed that long-term survival was reduced in patients with a high GPS (Log-rank: $p = 0.003$).

Conclusions: Glasgow prognostic score level is associated with increased in-hospital mortality in IE patients. Chronic renal failure and GPS are the independent predictors of mortality.

Keywords: Glasgow prognostic score, infective endocarditis, mortality

Infective endocarditis (IE) is a rare condition with an annual incidence rate of 3 to 10 cases per 100,000 population [1]. Although rare, IE is associated with increased morbidity and mortality and is the most common life-threatening infectious syndrome after sepsis, pneumonia, and intra-abdominal abscess [2]. Fever, embolic stroke and heart failure are the most common symptoms; however, nonspecificity of the symptoms makes the diagnosis of the disease difficult. Therefore, diagnostic criteria should include clinical signs, imaging and laboratory results, and the modified Duke criteria are the most frequently used diagnostic

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tool [3, 4]. Despite early diagnosis and intensive treatment modalities, the morbidity and mortality rates of IE remain high. IE responsible for 10% to 24% of in-hospital short-term mortality [5-8].

Clinical, microbiological and echocardiographic features such as white blood cell count, serum albumin level, left heart involvement, presence of comorbid disease, mental status, heart failure, causative microorganism were found to be predictors of mortality in IE patients [9-11]. C-reactive protein (CRP) level is a potent predictor of short-term adverse events and a useful marker of early risk identification. Besides that, a low serum albumin level (< 30 gLl) increases the mortality risk approximately five times [9]. New prognostic markers are needed to predict high-risk IE patients.

Studies have shown that inflammation plays a vital role in the etiopathogenesis of cardiovascular diseases [12-15]. Inflammation plays a crucial role in cancer pathogenesis [16, 17]. The Glasgow prognostic score (GPS), obtained using CRP and albumin values, is a practical tool for determining the prognosis of various cancer types. In the present study, we investigated the impact of GPS on in-hospital mortality in IE patients.

METHODS

Study Population

A total of 53 patients diagnosed with IE according to Duke criteria between 2012-2022 were included in this retrospective study. The study protocol was reviewed and approved by the institutional ethics committee under the principles of the Declaration of Helsinki. Patients younger than 18 years of age, whose serum albumin or CRP levels were not analyzed, who received systemic steroid therapy, who had malignancy or chronic inflammatory disease or who had chronic liver disease were excluded from the study.

Biochemical analysis

The GPS was calculated as follows: 0 points were given to patients with CRP (≤ 10 mg/L) and albumin (≥ 3.5 mg/dL) levels within the normal range. One point was given to patients with abnormal CRP or abnormal albumin levels. Patients with both high CRP (> 10 mg/L) and hypoalbuminemia (< 3.5 mg/dL) were given 2 points [18]. Body mass index was calculated using the formula: Body mass index = body weight / (body height)^2. Blood culture positivity and accompanying microorganism were recorded.

Clinical Follow-up

Patients underwent transthoracic and (or) transesophageal echocardiography (TEE) (Epiq 7, Koninklijke Philips N.V., Amsterdam, Netherlands); intracardiac complications such as vegetation, perivalvular abscess, leaflet perforation, and paravalvular regurgitation were evaluated. Clinical complications such as acute heart failure, acute renal failure, peripheral embolism, need for surgery, and death were recorded. In the present study, IE patients were divided into two groups as non-survivor (group 1) and survivor (group 2) to determine the in-hospital mortality rates.

Statistical Analysis

Statistical analyses were performed using the SPSS 22.0 statistics package (SPSS Inc, Chicago, Ill, USA). Categorical variables were expressed as percentages. The Chi-square test and Fisher's exact tests were used for categorical variables. Normally distributed data were reported as mean ± standard deviation after being analyzed with Kolmogorov-Smirnov test, while non-normally distributed continuous variables were presented as median. Student's t-test was used for comparing normally distributed data, Mann-Whitney U test was used for comparing non-normally distributed data. Univariate and multivariate logistic regression analyses were used to determine the independent predictors of in-hospital mortality. Receiver operating characteristics (ROC) analysis was performed to determine the optimal cut-off value of Glasgow's prognostic score to predict mortality. Kaplan-Meier survival curve constructed for low and high Glasgow prognostic score groups. A p - value < 0.05 was considered statistically significant.

RESULTS

Fifty-three patients diagnosed with IE, according to Duke's criteria, were included in our study. Of these 53 patients, 20 (37.7%) patients were female and the mean age was 66.2 ± 16 years. The main demographic, laboratory and clinical variables of the groups are summarized in Table 1 and Table 2. When we exam-

Table 1. Demographic and laboratory variables of the patients

	All patients (n = 53)	Non-survivors (Group 1) (n = 34)	Survivors (Group 2) (n = 19)	*p value*
Age (years)	66.2 ± 16	69.6 ± 15.1	60.1 ± 16.1	0.03
Gender (female), n (%)	20 (37.7)	15 (44.1)	5 (26.3)	0.2
Height (cm)	167.5 ± 7.5	168 ± 8.21	166.6 ± 6.13	0.52
Weight(kg)	75.9 ± 13.4	77 ± 14.2	74.1 ± 12.1	0.46
Hospitalization time (days)	26.9 ± 18.8	24.1 ± 18.6	32 ± 18.6	0.14
Diabetes mellitus, n (%)	18 (34)	15 (44.1)	3 (15.7)	0.03
Hypertension, n (%)	39 (73.6)	28 (82.4)	11 (57.9)	**0.05**
Coronary artery disease, n (%)	22 (42.3)	14 (41.2)	8 (44.4)	0.82
Congestive heart failure, n (%)	8 (15.1)	6 (17.6)	2 (10.5)	0.48
Atrial fibrillation, n (%)	5 (9.4)	4 (11.1)	1 (9.4)	0.43
Chronic renal failure, n (%)	29 (54.7)	24 (70.6)	5 (26.3)	**0.002**
COPD, n (%)	3 (5.6)	1 (2.9)	2 (10.5)	0.25
Angina, n (%)	12 (22.6)	8 (23.5)	4 (21)	0.83
Dyspnea, n (%)	25 (47.2)	17 (50)	8 (42.1)	0.58
Syncope, n (%)	4 (7.54)	2 (5.8)	2 (10.5)	0.53
Fever, n (%)	50 (94.3)	32 (94.1)	18 (94.7)	0.92
Cerebrovascular disease, n (%)	16 (30.2)	13 (38.2)	3 (15.7)	0.15
Electrocardiographic evaluation, n (%)				
Atrioventricular block	1 (1.88)	1 (2.94)	0 (0)	0.45
Atrial fibrillation	5 (9.4)	4 (11.7)	1 (5.2)	0.43
Hemoglobin (g/dL)	8.5 ± 2	8.36 ± 1.8	9.85 ± 2.27	**0.01**
White blood cell×10^3/mm^3	18.2 ± 9.5	21.3 ± 9.4	12.7 ± 6.7	**0.001**
Neutrophil (10^9/L)	15.4 ± 9	18.1 ± 9.6	10.5 ± 5	**< 0.001**
Bazophil (10^9/L)	0.7 ± 0.1	0.8 ± 0.1	0.5 ± 0.02	0.26
Lymphocyte 10^9/L	2 ± 1	1.8 ± 0.7	1.74 ± 1.2	0.07
Monocyte (10^9/L)	0.9 ± 0.6	0.8 ± 0.46	0.99 ± 0.82	0.58
Thrombocyte (×10^3/mm^3)	157.7 ± 500	158 ± 118	157 ± 168	0.96
Mean platelet volume (fL)	10.8 ± 1.2	11.1 ± 1.9	10.3 ± 1.9	0.16
C-reactive protein (mg/L)	44.1 ± 30	47.8 ± 71.6	37.4 ± 70.5	0.61
Red cell distribution width (%)	49.6 ± 4.2	49.6 ± 6.9	49.5 ± 6.5	0.06
Blood urea nitrogen (mg/dL)	125 ± 34	154 ± 77.9	72.2 ± 44.4	**< 0.001**
Creatinine (mg/dL) (min-max)	2.7 (0.5-13.5)	3.8 (0.6-13.5)	1 (0.5-11.6)	**0.003**
Sodium (mmol/L)	134.3 ± 7.43	133.4 ± 8.2	136.1 ± 5.5	0.2
Potassium (mmol/L)	5.17 ± 1	5.4 ± 1.1	4.73 ± 0.5	**0.006**
Calcium (mg/dL)	8 ± 0.73	7.9 ± 0.74	8.33 ± 0.66	0.058
Alanine aminotransferase (U/L) (min-max)	36 (6-1518)	30.5 (6-1518)	40 (9-200)	0.65
Aspartate aminotransferase (U/L) (min-max)	40 (10-6366)	65 (10-6366)	32 (12-202)	0.07
Albumin (g/dL)	2.7 ± 0.6	2.46 ± 0.5	3.14 ± 0.5	**< 0.001**
Glasgow prognostic score	2 (0-2)	2 (1-2)	1 (0-2)	**< 0.001**

Table 2. **Clinical variables of patients according to survival**

	All patients (n = 53)	Non-survivors (Group 1) (n = 34)	Survivors (Group 2) (n = 19)	*p value*
Mitral valve, n (%)				
Native	32 (60.4)	20 (58.2)	12 (63.2)	0.75
Prosthesis	1 (1.88)	0 (0)	1 (5.26)	0.17
Aortic valve, n (%)				
Native	15 (28.3)	9 (26.5)	6 (31.6)	0.69
Prosthesis	6 (11.3)	6 (17.6)	0 (0)	0.052
sPAP (mmHg)	34.3 ± 12.7	34.4 ± 13	34.1 ± 12.6	0.94
Ejection fraction (%)	56.7 ± 6.9	55.9 ± 7.68	58.1 ± 5.1	0.22
TEE evaluation, n (%)				
Aortic vegetation	21 (39.6)	15 (44.1)	6 (31.6)	0.37
Mitral vegetation	33 (62.3)	20 (58.8)	13 (68.4)	0.48
Tricuspid vegetation	2 (3.7)	2 (5.8)	0 (0)	0.28
Abscess	2 (3.7)	2 (5.8)	0 (0)	0.28
Fistula	1 (1.88)	1 (2.9)	0 (0)	0.45
Perforation	2 (3.7)	1 (2.9)	1 (5.2)	0.67
Pseudoaneurysm	3 (5.6)	3 (8.8)	0 (0)	0.18
Paravalvular leakage	3 (5.6)	3 (8.8)	0 (0)	0.18
Prosthetic valve dehiscence	1 (1.88)	1 (2.9)	0 (0)	0.45
Vegetation size (cm)				
Aortic valve	0.39 ± 0.59	0.45 ± 0.62	0.28 ± 0.52	0.33
Mitral valve	0.73 ± 0.7	0.7 ± 0.75	0.77 ± 0.62	0.75
Septic emboli, n (%)	16 (30.2)	13 (38.2)	3 (15.7)	0.08
Blood culture positivity, n (%)	53 (100)	34 (100)	19 (100)	0.16
Microorganism, n (%)				
Streptococci	12 (22.6)	6 (17.6)	6 (31.5)	0.16
Brucella	0 (0)	0 (0)	0 (0)	0.16
Candida	1 (1.8)	0 (0)	1 (5.2)	0.16
Corynebacterium striatum	0 (0)	0 (0)	0 (0)	0.16
Escherichia coli	1 (1.8)	0 (0)	1 (5.2)	0.16
Enterococcus faecalis	10 (18.8)	7 (20.6)	3 (15.7)	0.16
Staphylococcus aureus	20 (37.7)	16 (47)	4 (21)	0.16
Methicillin-resistant *S. aureus*	8 (15)	8 (23.5)	0 (0)	0.16
Methicillin-susceptible *S. aureus*	13 (24.5)	9 (26.5)	4 (21)	0.16
Coagulase negative staphylococcus	8 (15)	6 (17.6)	2 (10.5)	0.16
Serratia marcescens	0 (0)	0 (0)	0 (0)	0.16
Stenotrophomonas maltophilia	0 (0)	0 (0)	0 (0)	0.16
Surgery, n (%)	17 (32.1)	8 (23.5)	9 (47.4)	0.07

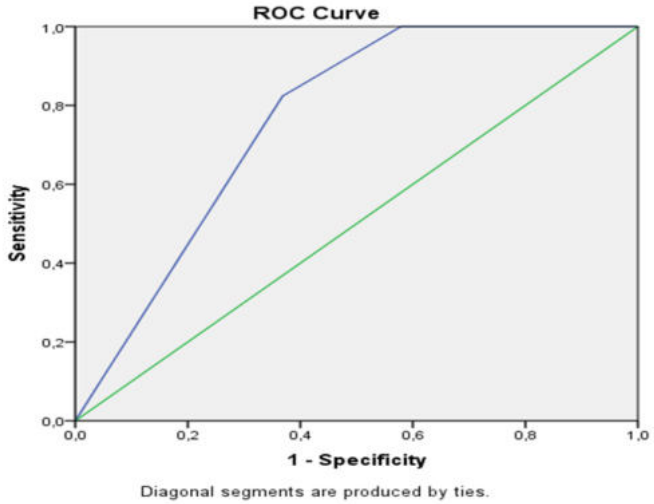

Fig. 1. Optimal cut-off value of Glasgow prognostic score found in roc curve analysis to predict in-hospital mortality

ined the basic laboratory and demographic characteristics of the patients, the non-survivor group was older (mean age group 1: 69.6 ± 15.1 vs. group 2: 60.1 ± 16.1 years; $p = 0.03$), and the number of patients with kidney failure (group 1: 24 (70.6%) vs. group 2: 5 (26.3%); $p = 0.002$) and diabetes (group 1: 15 (44.1%) vs. group 2: 3 (15.7%); $p = 0.03$) was higher in this group. When the groups were examined in terms of laboratory parameters, hemoglobin levels were lower in the non-survivor group, while the numbers of white blood cells, neutrophils, creatin, and blood urea nitrogen were higher. GPS and potassium levels were higher in the non-survivor group, while albumin levels were lower in this group. The two groups were similar in terms of other demographic and laboratory parameters. Vegetation sizes were similar between the two groups in both mitral valve and aortic valve IE. Vegetation on the native mitral valve was observed in 32 (60.4%) patients and vegetation on the prosthetic mitral valve was observed in one patient. Fifteen patients had vegetation on the native aortic valve and six patients had vegetation on the prosthetic aortic valve. The two groups were similar in terms of microorganisms grown in blood cultures, complications after IE and surgical need after complications. Thirty-four patients died during their intensive care follow-up, and the mean follow-up period was 24.1 ± 18.6 days. In the ROC analysis, the GPS had a sensitivity of 82.4% and a specificity of 36.8% at a cut-off value of ≥ 1.5 in estimating in-hospital mortality. The area under the

Table 3. Results of roc curve analysis to predict in-hospital mortality

Variable	AUC (95%)	Cut-off	*p value*	Sensitivity (%)	Specificity (%)
Glasgow prognostic score	0.765 (0.616-0.913)	≥ 1.5	**0.002**	82.4	36.8

Table 4. Univariate and multivariate logistic regression analysis of independent predictors of mortality

	Univariate analysis		Multivariate analysis	
Variables	**OR (95% CI)**	***p value***	**OR (95% CI)**	***p value***
Age	1.040 (1.001-1.081)	**0.044**	1,014 (0.969-1.062)	0.540
Hypertension	3.394 (0.955-12.057)	0.059	0.348 (0.077-1.579)	0.171
Chronic renal failure	6.720 (1.907-23.684)	**0.003**	0.153 (0.036-0.653)	**0.011**
Heart failure	1.821 (0.329-10.071)	0.492	0.659 (0.095-4.573)	0.673
Perforation	0.545 (0.032-9.249)	0.675	2.199 (0.090-53.782)	0.629

curve was 0.765 (95% CI: 0.616-0.913; p = 0.002) (Fig. 1) (Table 3). In Univariate logistic regression analysis, chronic renal failure (OR: 6.720; 95% CI: 1.907-23.684; p = 0.003), age (OR: 1.040; 95% CI: 1.001-1.081; p = 0.044) were independent variables in predicting mortality. In the multivariate logistic regression analysis, only chronic renal failure (OR: 0.153; 95% CI: 0.036-0.653; p = 0.011) was found to be a predictor of mortality (Table 4). Kaplan-Meier survival analysis also revealed that long-term survival was significantly reduced in patients with a high GPS level (Log-rank: p = 0.003) (Fig. 2).

DISCUSSION

To the best of our knowledge, the present study is the first to show an association between GPS and in-hospital mortality in IE patients. We demonstrated that long-term survival was reduced in patients with a high GPS. In addition, chronic renal failure was found to be an independent predictor of mortality.

Inflammation plays an essential role in the etiopathogenesis of cardiovascular diseases. Increased inflammatory markers are associated with poor prognosis in cardiovascular diseases [13, 18-20]. Inflammatory markers correlate with the mortality rate in various cancer types [21, 22]. The present study showed that GPS based on serum CRP and albumin levels had prognostic value in IE as in cancer patients. The elevation of inflammatory markers, such as leukocyte and neutrophil counts together with the GPS in the nonsurvivor group, supports this result.

Albumin is one of the variables in the GPS. Albumin, synthesized by hepatocytes, is the most abundant plasma protein. In addition to its role in osmotic pressure, it is also a good indicator of nutritional status and has antioxidant and anti-inflammatory properties [23, 24]. Therefore, lower albumin levels may cause increased inflammation and oxidative stress. Albumin is also an acute phase reactant protein; during the inflammatory process, albumin synthesis decreases due to decreased synthesis in hepatocytes, increased leakage into the interstitial space and catabolism [16]. Hypoalbuminemia, which occurs due to increased inflammation, is a powerful predictor of mortality in cardiovascular disease [17]. Hypoalbuminemia is associated with coagulation factors and a prothrombotic state [25]. Low albumin levels might be associated with a poor prognosis in IE patients.

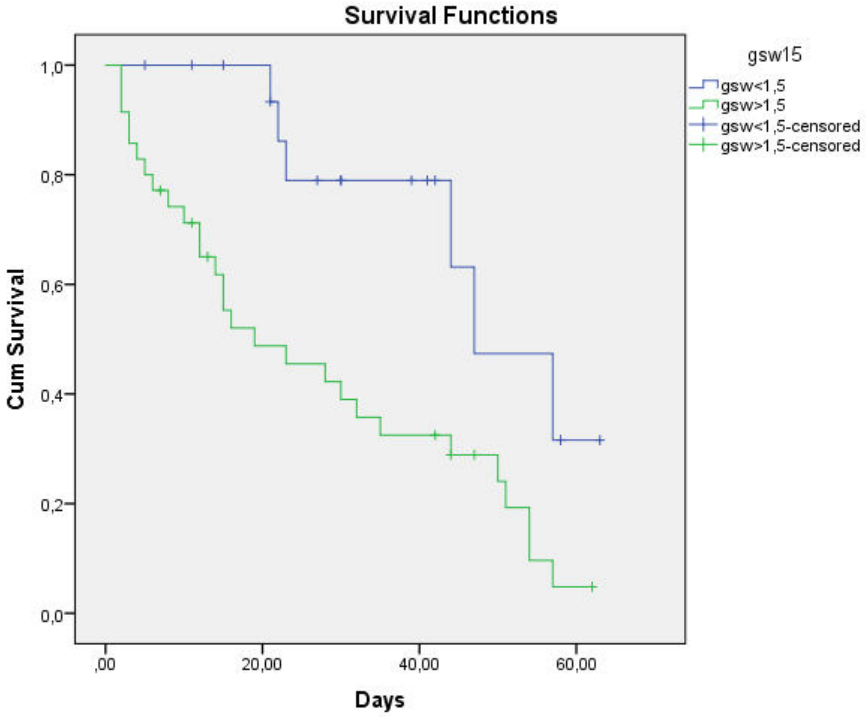

Fig. 2. Kaplan-Meier survival curve for low and high Glasgow prognostic score (GPS) groups. In-hospital mortality occured more often in patients with a higher GPS. While the mean life expectancy was 26.8 days in the group with GPS > 1.5, it was 47.9 days in the group with GPS < 1.5 (p = 0.003).

The prevalence of chronic kidney disease increases every year and the number of patients undergoing dialysis accelerates accordingly. Such patients are more susceptible to complications such as malnutrition, cardiovascular events, anemia and infections compared to healthy individuals [26]. As renal function declines with age, renal clearance gets lower in elderly patients. In the current study, the high rate of renal failure in the nonsurvivor group may be attributed to the fact that they were not under an adequate antimicrobial treatment due to avoidance of nephrotoxicity or drug side effects.

GPS is a specific index of systemic inflammation and malnutrition calculated by the combination of CRP and albumin. GPS predicts mortality in various types of cancer and can identify critically ill patients in many diseases [27-30]. As expected, low hemoglobin, presence of DM and advanced age, which are associated with adverse outcomes in infectious diseases, were more common in the high GPS group of the current study.

Limitations

The study is a retrospective, single-center research, and its sample size is relatively small. Retrospective nature of the study might have caused some factors that affected the results to be overlooked. The present study should be supported by multicenter, long-term prospective studies investigating the use of GPS in predicting prognosis in IE patients.

CONCLUSION

A high GPS is an independent indicator of in-hospital mortality in IE patients. GPS , determined using albumin and CRP levels, is a simple and practical index for predicting the prognosis in hospitalized patients with IE.

Authors' Contribution

Study Conception: NE, MAŞ; Study Design: MAŞ, NE; Supervision: EE, AGÖ; Funding: AGÖ; Materials: MAŞ; Data Collection and/or Processing: MAŞ, NE; Statistical Analysis and/or Data Interpretation: CA; Literature Review: CA; Manuscript Preparation: NE, CA and Critical Review: AGÖ.

Conflict of interest

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

Financing

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The European Research Journal 2022;8(5):710-715 Original Article

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Clinicopathological characteristics and management of patients with early readmission to our surgical oncology clinic

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ABSTRACT

Objectives: This study aimed to discuss the frequency of early readmission to the hospital after discharge in our oncology clinic, clinicopathological features, and management of these patients in light of current literature.

Methods: The medical records of 237 early readmitted patients within 30 days of discharge in our clinic were retrospectively reviewed. The patients were categorized according to their first diagnosis, Eastern Cooperative Oncology Group (ECOG) performance status, demographic, clinicopathological characteristics, readmission reasons, first treatment type, postoperative complications, the time of application after discharge and the type of treatment after admission

Results: The mean age of the patients was 58.45 years, 57.4% were female, and the mean readmission time after discharge was 11.54 days. The most common primary diagnosis was gastric cancer (35.9%), and the most common emergency pathology requiring hospitalization was ileus-subileus (45.1%). After readmission, 42.6% of the patients received medical treatment. 60% of the readmitted patients had postoperative complications before discharge. Patients who had postoperative complications during the first hospitalization were more likely to have major or minor interventions after readmission ($p < 0.01$). Admission with a diagnosis of bowel obstruction was associated with the probability of major intervention ($p < 0.01$). Patients with an ECOG performance score of ≥ 2 was more frequently administered medical treatment ($p = 0.001$). Patients admitted with the diagnosis of anastomotic leak/abscess had a higher probability of having postoperative complications ($p = 0.001$).

Conclusions: Readmissions are a concern for all healthcare providers, including comprehensive cancer centers. Recent health policies strive to reduce preventable admissions. Hence, we believe focusing on postoperative complications, and palliative care services is necessary.

Keywords: Surgical oncology, patient readmission, complication, palliative care

Unplanned readmissions in the first 30 days after discharge are a major medical problem and have been accepted as a quality indicator in recent years. Readmissions are associated with reduced quality of life, increased morbidity, and increased costs. Therefore, it is vital to evaluate the frequency of readmissions and predisposing factors, identify high-risk patients, and prevent readmissions by reducing costs and overwork. Readmission is a complex phenomenon formed by the combination of several different factors,

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such as the patient's characteristics, the inadequacy of the care given to the patient, and the characteristics of the health system. Each factor has a different effect on the readmission rate, depending on the type of disease or procedure examined, the duration, and the population in which the study was performed. Despite all its adverse impacts, it is not possible to eliminate readmissions. Hence, such an approach is also logically wrong. In this context, for policies to reduce readmissions to be successful, the factors causing readmissions and the types of readmissions must be determined [1].

Early rehospitalizations were suggested to reflect poor quality of care [2]. However, some authors suggest that some readmissions are necessary after complex treatments, such as major oncologic operations, to manage treatment-related complications appropriately [3, 4].

Studies focused on surgery on readmissions included different patient groups, used different datasets, and reported varying readmission rates ranging from 5.6% to 37.3%. Although there are significant differences between US hospitals, it was reported that approximately one in seven patients who were discharged after undergoing a major surgical procedure were readmitted to the hospital within 30 days [5]. The reasons for readmitting surgical patients to the hospital soon after discharge are likely different from those for returning patients receiving medical treatment. While patients receiving medical treatment may readmit for reasons such as inadequate social support, inability to access primary health care services, or worsening general health status, surgical patients are more likely to return due to complications from surgery [5].

Cancer and complications of medical treatments and surgical procedures can cause various problems after discharge. Hospitalized oncology patients are usually of advanced age and have locally advanced or metastatic disease. Malnutrition, significant symptom burden, dependence to some extent, and various comorbidities often leading to polypharmacy accompany these patients.

In this study, the frequency of early hospital readmission after discharge in cancer patients of our surgical oncology clinic, clinicopathological features, and the management of these patients will be discussed in light of the current literature.

METHODS

The medical records of 258 early readmitted patients within 30 days after discharge in our clinic between January 2015 and May 2021 were collected and reviewed retrospectively over the electronic data system. Due to the lack of data, 21 patients were excluded, and 237 consecutive patients who were either hospitalized or transferred were included in the study. Patients were documented according to their demographic and clinical characteristics and categorized by initial diagnosis, gender, age, ECOG performance status (PS) (0-4) at time of hospitalization, reasons for readmission, type of initial treatment, presence of postoperative complications, time of admission after discharge, and type of post-admission treatment. Also, the admissions were categorized according to four weeks of the month per the admission time. The admissions between 08:00 and 17:00 were classified as working hours and other hours as duty hours. The hours between 17:00 on Friday and 08:00 on Monday were considered weekends and the remaining weekdays. Stomach, colon, rectum, pancreas, oesophagus, and urogynecological malignancies can be counted among the initial diagnoses of the patients. The main reasons for hospitalization include partial and/or complete bowel obstruction, intra-abdominal ascites, anastomotic leak, abscess, bleeding, and evaluation for emergency feeding route (for jejunostomy, gastrostomy, and PEG). Patients were classified as major surgeries (e.g., gastrectomy, colectomy, pancreaticoduodenectomy, esophagectomy) with organ resections and reconstructions, and minor palliative interventions (e.g., diagnostic laparoscopy, biopsy), according to the type of the first operation.

Moreover, according to the treatments administered after hospitalization, patients were categorized into having major interventions (e.g., bridectomy, organ resections, and anastomosis), medical treatment, and minor interventions (e.g., paracentesis, PEG, stenting, jejunostomy, abscess drainage) with or without radiology and/or endoscopy guidance.

Approval of the Local Research Ethics Committee of our tertiary hospital was obtained before initiating the study (Ankara University Faculty of Medicine, Cebeci Hospital Turkey, Decision number: İ5-350-21, Date: 02-07-2021).

Statistical Analysis

SPSS 24.0 Windows program was used in the analysis of the data. Descriptive statistics were presented as mean±standard deviation values for the variables with normal distribution, median (min. max) for variables with non-normal distribution, and the number of cases and (%) for nominal variables. Categorical variables were analyzed with the Pearson Chi-Square or Fisher Exact test. The results were considered statistically significant for $p < 0.05$.

RESULTS

The number of patients included in the study was 237. The mean age of the patients was 58.45 ± 14.2 years (20-86), and 136 (57.4%) of the patients were female. The mean duration of admissions after discharge was 11.54 ± 9.2 (1-30) days. The most common primary diagnosis was gastric cancer (35.9%), and the most common emergency pathology requiring hospitalization was ileus-subileus (Table 1). 86.1% of the patients (n = 204) had major surgeries, including related organ resections and reconstructions, and the remaining patients had only diagnostic and or minor palliative interventions due to non-resectable and inoperable disease. After readmission, 42.6% (n = 101) of the patients were treated with medical treatment, 35.4% (n = 84) with major surgical intervention, and 21.9% (n = 52) with minor surgical interventions. Most patients were admitted during working hours (82.7%) and on weekdays (84.8%). At the rate of 60% (n = 142), most patients had postoperative complications before discharge. The highest number of readmissions (36.7%, n = 87) was made in the first week of the one month after discharge (Table 2). The patients had the most common ECOG 2 performance score (33%, n = 78) in the distribution according to their performance scores at first admission (Table 2). The most common reasons for consultation according to their diagnosis are shown in Table 3.

No significant correlation was determined in the analysis of the distribution of complications according to their diagnosis with the chi-square test ($p = 0.72$). Having post-operative complications during the first hospitalization was significantly associated with the probability of major or minor intervention after readmission ($p < 0.01$). Indeed, readmission with a diagnosis of bowel obstruction was associated with the probability of major intervention ($p < 0.01$). Pre-discharge ECOG performance score of ≥ 2 was associated with the probability of receiving medical treatment without any intervention after readmission ($p = 0.001$). Patients readmitted with anastomotic

Table 1. Primary diagnosis of patients and reasons for readmission

	Number (n)	Percentage (%)
Malignity localization		
Stomach	85	35.9
Colon	51	21.5
Pancreas	45	19
Rectum	32	13.5
Small intestine	6	2.5
Esophagus	9	3.8
Gynecological	9	3.8
Reason for readmission		
Intestinal obstruction	107	45.1
Intra-abdominal ascites	29	12.2
Hemorrhage	18	7.6
Anastomotic leak/Abscess	72	30.4
Emergency nutrition and palliative	11	4.6
TOTAL	237	100

Table 2. Post-discharge admission times and pre-discharge ECOG performances of patients

	n (%)
Hospitalization time	
1st week	87 (36.7)
2nd week	74 (31.2)
3rd week	25 (10.5)
4th week	51 (21.5)
ECOG performance	
0	40 (16.9)
1	100 (42.2)
2	78 (32.9)
3	19 (8)
4	-

Table 3. The most common reasons for consultation according to diagnosis

The diagnosis	The most common reasons	n (%)
Stomach Ca	Ileus/Subileus	18 (30.5)
Colon Ca	Ileus/Subileus	26 (72.2)
Pancreas Ca	Ileus/Subileus	4 (33.3)
Rectum Ca	Local Nüx/ascites	8 (57.4)
Small intestine Ca	Ileus/Subileus	2 (50)
Esophagus Ca	Anastomotic leak	3 (60)
Gynecological Ca	Ileus/Subileus	16 (72.29
Other Ca	Metastasis	8 (24.2)

leak/abscess diagnosis were associated with the possibility of postoperative complications ($p = 0.001$).

DISCUSSION

Our study provides information about readmissions to a surgical oncology clinic. The recent interest of insurance companies in preventable hospitalizations has increased the interest in this issue. Studies have revealed that at least 20% of hospitalizations are preventable [4, 6]. The common denominator in most literature studies is postoperative complications [7-10]. Similarly, patients with pancreatic resection, postoperative wound infections, pancreatic fistulas, and delayed gastric emptying have been reported to be associated with an increased risk of readmission [11-13]. It has also been reported that the possibility of being African American and having a lower socioeconomic status is correlated to higher readmission rates [7]. Reducing readmission rates will reduce the burden on health expenditures [14]. Besides, through the excellent use of hospital resources, it will be possible to treat another patient who needs care [15]. We have little information on this subject due to the limited number of reports from surgical oncology departments.

The mean age of the patients included in our study was relatively high (59.5 years), as they were oncological patients, and the male/female ratio was 2/5. It is known that the rate of readmission in elderly patients is high [7]. Although there are conflicting studies, the female gender has also been reported as a risk factor for readmission [7, 10].

In the study of Güven *et al*. [16], based on the data of the medical oncology departments, the rate of readmission of cancer patients was reported as 22.7%, and the main determinants as advanced disease stage, polypharmacy, and hospitalization through the emergency department. In our study, all of our patients were readmitted through the emergency service, and 15% of the readmitted patients had advanced non-resectable diseases.

Readmissions are major concerns for healthcare providers and the insurance system. Most unplanned readmissions to cancer hospitals are related to disease progression, new diagnoses, and complications of procedures. Some readmissions may not be avoided. After taking the major complications into account, it was reported that BMI >30 and preoperative weight loss increase the risk of readmission [10]. As can be seen, it is almost impossible to change some factors preoperatively. Risk factors for readmission after complex oncological procedures are high, but postoperative complications trigger readmissions in these patients. Specifically, it is assumed that postoperative complications, in particular, increase a patient's chances of returning to the hospital. Taking appropriate steps to minimize postoperative complications will reduce early readmissions [14]. Most of our patients (60%) readmitted to the hospital in our study consisted of patients who had complications in the postoperative period. Thus, patients experiencing postoperative complications are likely to have a more complex discharge plan that includes wound care instructions, antibiotic regimens, and/or rehabilitation therapy, and each of these may lead to readmission if not appropriately administered after discharge from the hospital [14]. Our study classified the performance status of oncological patients with the ECOG scale. This scale ranges from 0 to 4; "0" indicates a fully functional and asymptomatic patient, and "4" indicates a bedridden status (17).

Most patients (59%) had ECOG performance scores of ≤ 2 before discharge, and these patients were also more likely to undergo surgical intervention after hospitalization. This is probably due to the compelling orientation towards palliative and medical treatments in patients with poor performance scores. Although 58% of the patients had major or minor surgical interventions after hospitalization, it should not be ignored that there are still preventable readmissions. Prevent-

able readmission rates will increase through the on-site management of postoperative complications and revision of discharge criteria. On the other hand, most patients given medical treatment are admitted because of the difficulties in palliative care services and or issues in access. We believe that providing on-site and appropriate palliative services would reduce the intensity in oncology departments. The use of total parenteral nutrition (TPN) at discharge has been associated with potentially preventable readmission [4]. Home TPN applications to patients should be evaluated in this context.

Most readmissions are related to complications and disease progression and may not be preventable. However, adequate symptom management, discharge planning, or medication adjustments at discharge can potentially prevent at least 20% of readmissions [4, 18].

The majority of hospitalized patients (85%) had gastric, pancreatic, and colorectal malignancies (Table 1). We could not determine a relationship between primary diagnoses and the presence of complications. Most (75%) reasons for readmission were due to partial and/or complete intestinal obstruction and anastomotic leak/abscess. The probability of admission due to anastomotic leak/abscess was high in those who had postoperative complications. Also, patients readmitted with a diagnosis of intestinal obstruction were more likely to be treated with a major intervention.

Most patients were readmitted during working hours (83%) and on weekdays (85%). This condition may be due to the ease of access of these patients to emergency health services and an effective emergency consultation system.

CONCLUSION

Identifying factors associated with potentially avoidable readmissions is crucial to make any discharge decision that will ultimately result in fewer readmissions and better outcomes. We are optimistic that identifying risk factors for readmission, improving discharge and follow-up practices, and expanding patient education will lead to reduced readmissions in oncology departments. Minimizing readmissions in complex cancer patients is challenging. Larger multi-agency datasets are needed to set a reasonable standard for expected readmission rates.

Authors' Contribution

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

Conflict of interest

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

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The European Research Journal 2022;8(5):716-721 Original Article

DOI: 10.18621/eurj.1137995 *Infectious Diseases*

Candida strains and resistance patterns identified in a tertiary hospital

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ABSTRACT

Objectives: *Candida* species are the most common fungal infectious agents. *Candida* species are important with their increasing frequency in hospital-acquired infectious agents. The issue of antibiotic resistance, which frequently encountered in bacterial agents, is unfortunately also valid in fungal infections. In the present study, we aimed to determine the resistance of *Candida* species in our hospital including 1350 patient beds, the materials and units of production, and their sensitivity to antifungal drugs, in particular fluconazole.

Methods: Yeast growths, colony morphology, germ tube formation and VITEK 2 Compact automated identification system detected in the samples evaluated in the central laboratory of our hospital between February 1, 2020 and December 31, 2020 were typed. Antifungal susceptibilities, especially fluconazole, caspofungin, and amphotericin B susceptibilities, were determined by an automated system.

Results: In total, 2446 within the *Candida* growing sample was determined as 49% *Candida albicans*, 26.9% *Candida parapsilosis*, %17.9 *Candida tropicalis*, *Candida glabrata* and *Candida krusei* were observed in 2.3%. Although the distribution of *Candida* species in other intensive care units and services was comparable to the general incidence, *C. albicans* was detected in 38%, *C. parapsilosis* 30% and *C. tropicalis* 27% in surgical intensive care units. Moreover, *C. tropicalis* was the dominant species in the neonatal intensive care unit (75%)

Conclusions: In the present study, *C. albicans* was the most common candida species, and *C. parapsilosis* was the second most frequently reproduced species. It has been suggested that resistance patterns differ between species and between wards, therefore species identification and susceptibility analysis are important, and these should be taken into account when starting empirical, preemptive and antifungal treatment.

Keywords: *Candida*, fungal infection, antifungal agents

Candida species are the most commonly cultivated fungal strains. They are natural flora elements of the gastrointestinal tract, skin and urogenital system in humans. They can be isolated from various clinical specimens, especially in hospitalized immunocompromised individuals, and cause many different infections focus. *Candida glabrata*, *Candida tropicalis Candida parapsilosis, Candida krusei*, most commonly *Candida albicans*, are found in human flora. *C. albicans* is major source for fungemia engendered by *candida*, and the mortality rate due to this candidemia is reported as 38-49% [1-5].

The circumstances such as body pH changes, immunosuppressive treatments, cancer precipitate an increase in the frequency of candida infections. *Candida* species shift their resistance and susceptibility to antifungal drugs. It is also stated that the frequency of *Candida* species diverges in various age and risk groups. For instance, while *C. parapsilosis* is spotted in 30% of candidemia cases in newborns, it is detected

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in 10-15% of adults, and *C. glabrata* is more common in elderly and in patients with malignancy [6-8].

Although the number of *Candida* species exceeds 150, those isolated as pathogens in humans are 15. Although *C. albicans* is still the most frequently isolated species, it is observed that different species are gradually taking its place in different regions. Increased central catheter use and increased fluconazole prophylaxis are expressed as the main reasons for this. In recent years, while *C. albicans* infections have decreased gradually in continental United States and many European territories, it has been mentioned that the frequency of *C. parapsilosis* or *C. tropicalis* disorders is surging at other locations where they are replaced by *C. glabrata* infections [9-12].

It is obvious that the delay in initiating appropriate antifungal therapy is a factor that augments the mortality rate, particularly candidemia, and therefore, knowing the species and antimycotic susceptibilities of candida agents seems essential for choosing appropriate treatment, especially for empirical therapy. In the current era, when antifungal resistance has become a problem similar to antibiotic resistance, knowing the predominantly culture species in the region and the unit and their resistance has gained importance in rapid and effective treatment [6, 13, 14].

By the present investigation, the aim was to specify the sensitivity status of *Candida strains* and antifungal drugs extracted from cultures of clinical cases in different age groups from different clinics sent to microbiology laboratory in Bursa City Hospital.

METHODS

The replications detected from different materials in the central laboratory of Bursa City Hospital between 01.02.2020 and 31.12.2020 were included in the present study, identification was made with the VITEC 2 Compact (Biomerieux, France) system and identification was accomplished at species level. In addition, the susceptibility of these species to Fluconazole, Amphotericin B and Caspofungin was investigated. Germ tube test and microscopic appearance of *Candida* species in Corn Flour-Tween 80 Agar medium were explored.

The included subjects were the hospitalized patients with *Candida* growth in any of their samples. Exclusion criteria were as follows: *Candida* growths in the cultures of outpatients, growth in the culture without *Candida* typing or resistance patterning and growths of the same *Candida* species detected in repeat cultures of the same patient.

The study was approved by the University of Health Sciences, Bursa Yüksek Ihtisas Training and Research Hospital Clinical Research Ethics Committee (Date: 10.08.2022 and Decision No: 2011-KAEK-25 2022/08-04).

Statistical Analysis

Descriptive statistical methodologies were implemented to display the properties of the data collected for the study.

RESULTS

Over 2446 *Candida-growing* samples, *C. albicans* was detected in 1199 (49%), *C. parapsilosis* identified in 658 (26.9%), C. tropicalis in 438 (17.9%), *C. glabrata* and *C. krusei* were observed in 2.3% (Table 1). The most frequently isolated clinics in the hospital were followed as anesthesia intensive care, surgical intensive care, internal medicine intensive care and COVID-19 intensive care (21.8%, 21%, 16.2% and 16.1%; respectively) (Table 1).

Although the frequency of *Candida* strains in other critical treatment units and services was comparable to the general incidence, *C. albicans* was detected in 38%, *C. parapsilosis* 30% and *C. tropicalis* 27% in surgical intensive care units. Moreover, *C. tropicalis* was the dominant species within the infantile critical care facility (75%) (Table 1).

Of the 2446 isolated *Candida* strains, 64% were obtained from blood, 14% from urine, 12% from respiratory, 5% from peritoneum, 3% from wound and 2% from CSF samples (Table 2). Considering the antifungal resistance rates, fluconazole resistance was disclosed as 6% for *C. albicans*, 14.2% for *C. glabrata*, 66.6% for *C. krusei*, 53.5% for *C. parapsilosis*, 4.1% for *C. tropicalis* and total fluconazole resistance rate was 22% (Table 3).

Caspofungin resistance rate was 1.5% in *C. albicans*, 66.6% in *C. glabrata*, 3.1% in *C. parapsilosis*, 2.7% in *C. tropicalis*. No resistance was found against *C. krusei*. The total Caspofungin resistance ratio was

Table 1. The incidence of *Candida* strains in the clinics

	Anesthesia ICU	COVID Service	COVID ICU	Surgery service	Surgery ICU	Pediatric ICU	Internal medicine service	Internal medicine ICU	Infant ICU	n (%)
C. albicans	264 (49.5)	72 (83.7)	180 (45.6)	173 (61)	196 (38)	45 (60.1)	101 (70.6)	162 (41)	6 (25)	**1199 (49)**
C. dubliniensis	4	0	8 (2)	0	0	0	0	0	0	**12 (0.5)**
C. glabrata	3	4 (4.6)	23 (5.8)	4 (1.4)	13 (2.6)	0	3	7 (1.7)	0	**57 (2.3)**
C. guilliermondii	0	0	0	0	0	6 (8)	0	0	0	**6 (0.2)**
C. krusei	23 (5)	0	11 (2.8)	12 (4.2)	8 (1.5)	0	0	2 (.5)	0	**56 (2.3)**
C. lipolytica	0	0	8 (2)	0	0	0	0	0	0	**8 (0.3)**
C. lusitaniae	0	0	4 (1)	0	0	0	0	4 (1)	0	**8 (0.3)**
C. parapsilosis	150 (28)	10 (11)	100 (25)	52 (18)	158 (30)	23 (31.1)	15 (10.5)	150 (37.8)	0	**658 (26.9)**
C. pelliculosa	0	0	0	0	0	0	0	4 (1)	0	**4 (0.19)**
C. tropicalis	89 (17)	0	60 (15)	42 (14.9)	138 (27)	0	24 (16.7)	67 (17)	18 (75)	**438 (17.9)**
Total, n (%)	**533 (21.8)**	**86 (3.5)**	**394 (16.1)**	**283 (11.6)**	**513 (21)**	**74 (3)**	**143 (5.8)**	**396 (16.2)**	**24 (1)**	**2446**

ICU = intensive care unite

Table 2. Materials from which *Candida* samples were obtained

Location of sample taken	n	%
Blood	1564	64
Urine	342	14
Respiratory tract	294	12
Peritoneum	123	5
Wound	74	3
CSF	49	2
Total	2446	100

CSF = Cerebrospinal fluid

2.2% (Table 4).

Amphotericin resistance ratio was 4% for *C. albicans* and *C. tropicalis*, 3.1% for *C. parapsilosis*. No resistance was detected in *C. glabrata* and *C. krusei*. Total Amphotericin resistance percent was 3.4% (Table 5).

DISCUSSION

Candida species are the most frequently isolated species among fungal species. They are the elements of natural flora in the gastrointestinal tract, skin and urogenital system within humans. They can be isolated from miscellaneous clinical archetypes, especially

Table 3. **Flucanazole sensitivity rates**

Fluconazole sensitivity	Sensitive (n)	Resistant n (%)	Total
Candida albicans	187	12 (6)	199
Candida glabrata	17	3 (15)	20
Candida krusei	4	8 (66.6)	12
Candida parapsilos	124	68 (53.5)	192
Candida tropicalis	69	3 (4.1)	72
	401	**94 (19)**	**495**

Table 4. **Caspofungin sensitivity rates**

Caspofungin sensitivity	Sensitive (n)	Resistant n (%)	Total
Candida albicans	**196**	**3 (1.5)**	**199**
Candida glabrata	**6**	**2 (25)**	**8**
Candida krusei	**5**	**0**	**5**
Candida parapsilos	**125**	**4 (3.1)**	**129**
Candida tropicalis	**71**	**2 (2.7)**	**73**
	405	**9 (2.2)**	**414**

Table 5. **Amphotericin B sensitivity rates**

Amphotericin B sensitivity	Sensitive (n)	Resistant n (%)	Total
Candida albicans	192	8 (4%)	200
Candida glabrata	17	0	17
Candida krusei	12	0	12
Candida parapsilos	125	4 (3.1%)	129
Candida tropicalis	72	3 (4%)	75
	418	**15 (3.4%)**	**433**

from hospitalized immunocompromised individuals, along with cause many different infections focus[1-3]. *Candida* genus yeasts, which constitute approximately 96% of opportunistic mycoses, are amid the most prevalent underlying factors of serious infective disorders such as candidemia as well as superficial mycoses [3, 6, 9].

In the last few years, there has been a significant increase in the frequency of candidemia in patients of intensive care units is reported, in cancer patients and in neutropenic cases. *Candida* species are responsible for 8-15% of infections within circulatory system and progresses with a mortality rate of 38% [8].

In a study conducted in Taiwan, 36-fold increase was detected in the rate of candidemia, from 1981 to 2000 [15]. The most consistently cultivated strain is *C. albicans*, while the ratio of *C. albicans* varies between 50-85% in the studies [16, 17]. Compatible with previous studies, in the present investigation, the most frequent candida species determined has been *C. albicans* (49%).

The strains out of *C. albicans* such as *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis*, which are known to respond more difficult to antifungal treatment, are increasing day by day. As a matter of fact, in the current study 51% of the isolated species were non-albicans *Candida* [18].

Species identification is crucial for empirical treat-

ment, where nowadays *Candida* infections are increasing rapidly. Adiloglu *et al*. [19] reported *C. glabrata* as 13% and *C. tropicalis* and *C. parapsilosis* as 3% in their studies. Gultekin *et al*. [20] determined the species distribution as 23% *C. parapsilosis*, 14% *C. tropicalis*, and 12% *C. glabrata*. In our study, *C. parapsilosis* was detected in 26.9%, *C. tropicalis* in 17.9%, *C. glabrata* and *C. krusei* in 2.3% of the specimens. Consistent with other studies, *C. parapsilosis*, *C. glabrata* and *C. tropicalis* were found to be the most repeatedly segregated non-albicans candida strains [16, 17, 21].

By this study, in which the data of our center were evaluated, *C. albicans* was the most often *Candida* species, and *C. parapsilosis* was the second most prevalent *Candida* species and these results suggested that it should be taken into account when initiating antifungal therapy. Considering the increase in *Candida* infections and the relative increase in the rate of non-albicans species resistant to antifungals and since empirical treatment should be started as soon as possible, the incidence of the subjects in our hospital, the clinics they are isolated from, and the resistance patterns are of vital importance in initiating appropriate empirical treatment.

Zer *et al*. [22] found that fluconazole resistance was 23% in *C. albicans* strains. Fluconazole resistance was found to be lower (6%) in our study. However, fluconazole resistance was quite high in *C. parapsilosis* and *C. krusei* (53.5% and 66.6%; respectively). Resistance development in *C. albicans* strains against amphotericin B is quite low [19, 23]. In our study, amphotericin B resistance was less than 5% for all *Candida* species.

Caspofungin resistance was found as high as 25% in *C. glabrata*, while it was low (< 5%) in *C. albicans* and other strains. The resistance for Amphotericin B and Caspofungin were low (< 5%) in *C. parapsilosis* and *C. tropicalis* strains that we isolated most frequently after *C. albicans*. However, *C. parapsilosis* showed significant fluconazole resistance (53.5%), while *C. tropicalis* had resistance less than 5%.

CONCLUSION

The widespread use of antibiotics and antifungals causes detection of fungal infections and antifungal resistant strains more frequently. The fact that *Candida* species and their antifungal susceptibility differ in different clinics regionally and even within the hospital is of great importance because of the necessity to initiate accurate empirical antifungal treatment until the species and the sensitivity are determined, in cases of urgency.

Authors' Contribution

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

Conflict of interest

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The European Research Journal 2022;8(5):722-731 Original Article

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Investigation of remission with ultrasound in patients with rheumatoid arthritis according to different clinical remission criteria

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ABSTRACT

Objectives: To investigate remission with ultrasound (US) in patients with Rheumatoid arthritis (RA) according to different clinical remission criteria.

Methods: A total of 105 patients with RA who were in remission for at least 6 months according to disease activity score in the 28 joints using C-reactive protein (DAS28-CRP) were included in the study. US remission rates were analyzed according to different remission criteria [DAS28-CRP, DAS28 using erythrocyte sedimentation rate (DAS28-ESR), clinical disease activity index (CDAI), simplified DAI (SDAI), and the 2011 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Boolean remission criteria]. US remission was determined as power doppler (PD) US score = 0.

Results: Remission rates achieved for each remission criteria were 100%, 82.9%, 55.2%, 58.1% and 42.9% and US remission rates were 57.1%, 57.5%, 53.4%, 55.7%, 57.7% for DAS28 CRP, DAS 28 ESR, CDAI, SDAI, 2011 ACR/EULAR remission criteria, respectively. When the patients compared for the US findings between remission and non-remission patients according to the different clinical remission criteria, no difference was found ($p > 0.05$).

Conclusions: This study shows that clinical remission criterias are not sensitive enough to accurately detect remission and there was no increase in the US remission rates as per the stricter remission criteria. Using US in addition to the clinical criteria would prove to be more useful in evaluating remission.

Keywords: Clinical remission, ultrasonographic remission, rheumatoid arthritis, subclinical synovitis, DAS28 CRP, remission criteria

Rheumatoid arthritis (RA) is a chronic inflammatory disease that can cause bone erosions and joint motion limitation. The treatment of RA aims to suppress inflammation by achieving low disease activity and/or complete remission [1, 2]. Clinical remission is defined as the absence of significant signs and symptoms of inflammatory disease activity and the elimination of any signs of systemic inflammation. The definition of clinical remission in RA is developed by evaluating composite scores of disease activity. These composite scores include the following: disease activity score in the 28 joints using erythrocyte sedimentation rate (DAS28-ESR), the DAS28 using C-reactive protein (DAS28-CRP), clinical disease activity

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index (CDAI), simplified DAI (SDAI), and the 2011 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Boolean remission criteria [3-5]. Reported remission rates depend on the criteria that are used to define remission and may vary in relation with each other [6]. Using the combination of tumor necrosis alpha inhibitor (TNFi) and conventional synthetic disease-modifying drugs (csDMARDs) is predicted to increase clinical remission rates and provide greater control of radiographic progression [7-9]. However, despite achieving the goal of remission, patients who receive only csDMARD and patients who receive both TNFi + csDMARD, experience progressive structural and functional damage. Because although the patients are in clinical remission, subclinical synovitis may persist that can only be detected radiologically. Therefore, the validity of these criteria is controversial. Besides, with stricter remission criteria, higher rates of US remission are expected. In recent years, many attempts have been made to redefine the concept of remission in RA. Studies show that ultrasound (US) is more sensitive than clinical findings in detecting inflammation and can be used to define remission [10-16]. Synovial hypertrophy (SH) and power Doppler (PD) signals are used in the detection of subclinical synovitis using US. Since PD shows synovial vascularity, it reflects active inflammation better [17-19]. The detection of subclinical synovitis in patients in remission is very important for the prognosis of RA and has been emphasized to be the most important predictor of radiographic damage [20-22].

The issues such as which clinical remission criteria are better to reflect true remission, which remission criteria should be used, and whether US findings should be added to the definition of remission are controversial. Therefore, in this study, it was aimed to investigate remission with US according to different clinical remission criteria and to determine which clinical remission criteria is more effective in predicting US remission in RA patients who receive csDMARD alone and combination of TNFi + csDMARD.

METHODS

Study Design and Patient Selection

A total of 105 patients with RA were included in the study. These patients were in remission for at least 6 months, and 55 of these patients received a combination of TNFi + csDMARD and 50 of them received csDMARD alone. Demographic data such as age, gender, smoking habit, and current medication usage were collected from the patients. The study received ethics approval from the local ethics committee of the Uludag University School of Medicine on June, 07 2016 (approval number: 2016-11/27), and written informed consent was obtained from the patients. The study was conducted in Rheumatology outpatient clinic of Uludag University Faculty of Medicine.

Inclusion Criteria

Patients who met the following five criteria were included in the study: 1) diagnosed with RA according to the 1987 revised ACR and/or 2010 ACR/EULAR criteria, 2) >18 years old, 3) in remission according to DAS28-CRP (DAS 28-CRP < 2.6), 4) no swollen joint 5) no disease exacerbation in the last 6 months, 6) achieved stability of treatment in the last 6 months and did not require a change of treatment.

Patients using stable doses of nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids (< 7.5 mg prednisolone or equivalent taken orally every day) were included in the study.

Exclusion Criteria

Patients with RA who were younger than 18 years of age, did not receive treatment, required a change of treatment in the last 6 months, and were administered intra-articular steroid injections into the wrist or other joints during an examination within the last 6 months were not included in the study.

Clinical and Laboratory Evaluation

Clinical and physical assessments were performed. This included the tender joint count (TJC), swollen joint count (SJC), the physicians' [physician global assessment (PhGA)] and patients' [patient global assessment (PtGA)] visual analog scale (VAS) scores (0-10), and Health Assessment Questionnaire (HAQ) scores. All these parameters were evaluated by a rheumatologist. ESR, CRP, rheumatoid factor (RF), and anticyclic citrullinated peptide (anti-CCP) levels were measured; DAS28-ESR, DAS28-CRP, CDAI, SDAI values were calculated for each patient; and the 2011 ACR/EULAR Boolean remission criteria were

evaluated.

Ultrasonographic Evaluation

With respect to the US examination of the joints, SH and PD scores were evaluated according to the definitions of the Outcome Measures in Rheumatology Clinical Trials and using a standard methodology to assess synovial vascularity [23]. US was performed by an experienced rheumatologist (SE).

On the same day, after clinical examination and patient evaluation, seven joints including the 2nd and 3rd metacarpophalangeal (MCP) joint, 2nd and 3rd proximal interphalangeal joint (PIP), wrist (radiocarpal and intercarpal joints), the 2nd and 5th metatarsophalangeal (MTP) joints were evaluated bilaterally using US [24]. That is, a total of 14 joints were evaluated. SH was evaluated using US, and PD was performed with the 6-18 MHz multifrequency linear-probe MyLab60 (ESAOTE, Genova, Italy) ultrasound machine. PD pulse repetition frequency was set to 750 Hz. The Doppler color gain setting was reduced until the artifacts under the bone cortex disappeared.

Each joint was semiquantitatively scored from 0 to 3 for B-mode SH and synovial PD signal. SH scoring was as follows: 0 = no synovial hypertrophy, 1 = mild, 2 = moderate, and 3 = severe; PD scoring was as follows: 0 = normal/minimal vascularity, 1 = mild hyperemia, 2 = moderate hyperemia, and 3 = distinct hyperemia. US scores were expressed as the sum of the scores obtained per joint for all the joints of each patient [23].

The Definition of Remission

Clinical Remission

Remission criteria were determined to be DAS28-CRP < 2.6, DAS28-ESR < 2.6, CDAI < 2.8, SDAI ≤ 3.3, and 2011 ACR/EULAR remission criteria (At any time point, a patient must satisfy all of the following: TJC≤ 1, SJC ≤ 1, CRP ≤ 1 mg/dl and PhGA ≤ 1 (on a 0-10 scale) or Index-based definition at any time point, a patient must have SDAI ≤ 3.3) [5, 25].

Ultrasonographic Remission

There are several different ultrasonographic remission criteria such as strict remission (all SH and PD = 0), a less strict remission (all SH and PD ≤ 1), and remission criteria based solely on PD absence (PD = 0). In the study remission criteria based solely on PD absence (PD = 0) was used as US remission criteria.

Statistical Analysis

The statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp.) statistical analysis package program. The Kolmogorov–Smirnov test was used to test whether the data were normally distributed. For descriptive variables that did not fit the normal distribution, median (minimum–maximum) values were given. For the comparison of two independent groups, the independent samples t-test was used for the variables that conformed to the normal distribution and the Mann–Whitney U test was used for the variables that did not conform to the normal distribution. The chi-square test was used for qualitative variables that did not fit the normal distribution. A univariable logistic regression was conducted to investigate factors associated with the imaging outcomes. The significance level was set at $p = 0.05$.

RESULTS

Demographic Data and Drugs Used

The demographic data, remission criteria, using drugs, laboratory and US findings of RA patients who receive only csDMARD and both TNFi + csDMARD are shown in Table 1. The median disease duration was 10 years and the remission duration was 12 months. The disease duration in the combination group was found to be significantly higher than that in the csDMARD group ($p = 0.035$). There was no difference between the two groups in terms of the use of methotrexate (MTX) (p = 1.00), leflunomide (LEF) ($p = 0.416$), sulfasalazine (SLZ) ($p = 0.824$), and hydroxychloroquine (HCQ) ($p = 0.846$) (Table 1) With regard to the distribution of TNFi use in the combination group, 23.6% (n = 13) of the patients were using adalimumab, 21.8% (n = 12) were using golimumab, 21.8% (n = 12) were using certolizumab, 20% (n = 11) were using etanercept, and 12.7% (n = 7) were using infliximab.

Remission Rates Achieved for Each Remission Criteria

Remission rates achieved for each remission criteria were 82.9% (n = 87) for DAS-28 ESR, 55.2% (n = 58) for CDAI, 58.1% (n = 61) for SDAI, 42.9% (n

Table 1. Demographic data, remission criteria, drugs, laboratory and ultrasonographic datas of the patients

	All patients (n = 105)	TNFi and csDMARD combination group (n = 55)	csDMARD group (n = 50)	*p* value
Demographic data				
Age (years) †	55 (14-85)	56.00 (23-76)	50.00 (19-85)	0.426
Gender (F), n (%)	83 (79)	45 (81.8)	38 (76)	0.483
Smoking habit, n (%)	47 (44.8)	21 (38.2)	26 (52)	0.173
Disease duration (years) †	10 (1-38)	7.00 (1-13)	2.00 (1-8)	**0.035**
Disease duration (> 5 years), n (%)	81 (77.1)	47 (85.5)	34 (68)	**0.029**
Remission duration (months) †	12.00 (6-60)	8.00 (6-20)	7.50 (6-12)	0.371
Remission duration (> 12 months), n, (%)	47 (44.8)	21 (38.2)	26 (52)	0.173
Remission criteria				
DAS 28-CRP †	1.87 (1.35-2.57)	1.86 (1.46-2.56)	1.88 (1.35-2.57)	0.508
DAS 28-CRP < 2.6, n (%)	105 (100)	55 (100)	50 (100)	1.000
DAS 28-ESR †	2.00 (0.80-.3.5)	2.30 (0.80-3.50)	1.95 (0.80-2.90)	0.104
DAS 28-ESR < 2.6, n (%)	87 (82.9)	40 (72.7)	47 (94)	**0.004**
CDAI †	2.50 (0-7)	2.50 (0-5.50)	2.50 (0-7.00)	0.925
CDAI ≤ 2.8, n (%)	58 (55.2)	31 (56.4)	27 (54)	0.698
SDAI †	3.05 (0-7)	3 (0.30-5.8)	3.15 (0.60-7.30)	0.946
SDAI < 3.3, n (%)	61 (58.1)	34 (61.8)	27 (54)	0.436
ACR/EULAR Remission †	2.30 (0.3-5.3)	2.30 (0.30-4.80)	2.30 (0.30-5.30)	0.475
ACR/EULAR Remission, n (%)	45 (42.9)	27 (49.1)	18 (36)	0.236
TJC, 28 joints†	0 (0-.2)	0 (0-2)	0 (0-2)	0.805
HAQ†	0 (0-0.9)	0 (0-0.9)	0 (0-0.9)	0.861
PtGA VAS (0–10) †	1.5 (0-3)	1.5 (0-3.00)	1.5 (0-3)	0.416
PhGA VAS (0–10) †	1.00 (0-2)	1.00 (0-1.50)	0.50 (0-2)	0.328
Drugs used				
Glucocorticoid usage, n (%)	41 (39)	23 (41.8)	18 (36)	0.556
MTX usage, n (%)	71 (67.6)	37 (67.3)	34 (68)	1.000
LEF usage, n (%)	36 (34.3)	21 (38.2)	15 (30)	0.416
SLZ usage, n (%)	26 (24.8)	13 (23.6)	13 (26)	0.824
HCQ usage, n (%)	45 (42.9)	23 (41.8)	22 (44)	0.846
Laboratory tests				
ESR (mm/h) †	13 (2-526)	14.00 (2-56)	13.00 (2-28)	0.188
CRP (mg/dL) †	0.30 (0.10-1)	0.30 (0.10-1)	0.40 (0.10-0.9)	0.059
RF (IU/ml) †	45 (4-2180)	39.00 (9.50-1280)	45 (4-2180)	0.887
Anti-CCP (IU/ml) †	109.3 (3-1520)	99.00 (3.00-1520)	145.5 (3-1398)	0.526
Ultrasound results				
Total_SH †	4 (0-17)	4 (0-14)	2.50 (0-17)	**0.048**
Total_PD †	0 (0-11)	0 (0-11)	0 (0-8)	0.578
Total_SH_PD †	5 (0-20)	5 (0-19)	4 (0-20)	0.122
SH score = 0, n (%)	28 (26.7)	9 (16.4)	19 (38)	**0.015**
SH score ≥ 2, n (%)	67 (63.8)	41 (74.5)	26 (52)	**0.025**
PD score = 0, n (%)	60 (57.1)	30 (54.5)	30 (60)	0.693
PD score ≥ 1, n, (%)	45 (42.9)	25 (45.5)	20 (40)	0.693
PD score ≥ 2, n (%)	36 (34.3)	20 (36.7)	16 (32)	0.684
SH score = 0, PD score = 0, n (%)	23 (21.9)	8 (14.5)	15 (30)	0.063

Values are presented as median (minimum–maximum) and percentage. Comparison between groups via Mann–Whitney U test and the Pearson's chi-square test with a value of $p < 0.05$ was considered significant. † Median (minimum–maximum). F = female, TNFi = tumor necrosis alpha inhibitör, csDMARD = conventional synthetic disease-modifying antirheumatic drugs, DAS28-CRP = disease activity score in the 28 joints using C-reactive protein, DAS28-ESR = DAS28 using erythrocyte sedimentation rate, CDAI = clinical disease activity index, SDAI = simplified DAI, ACR/EULAR remission = 2011 American College of Rheumatology/European League Against Rheumatism Boolean remission criteria, TJC = tender joint count, HAQ = Health Assessment Questionnaire, PtGA = patient global assessment, PhGA = physician global assessment, VAS = visual analog scale, MTX = methotrexate, LEF = leflunomide, SLZ = sulfasalazine, HCQ = hydroxychloroquine, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, RF = rheumatoid factor, Anti-CCP = anti-cyclic citrullinated peptide, SH = synovial hypertrophy, PD = power Doppler

= 45) for 2011 ACR/EULAR Boolean remission criteria. The proportion of patients meeting all the remission criteria was 10.5% (n = 11) (Table 1).

The US Remission Rates According to Different Clinical Remission Criteria

US remission rates according to all the clinical remission criteria were 57.1% (n = 60), 57.5% (n = 50), 53.4% (n = 31), 55.7% (n = 34), 57.7% (n = 26) for DAS28 CRP, DAS 28 ESR, CDAI, SDAI, ACR/EULAR remission criteria, respectively (Table 2).

When the remission rates in the combination and csDMARD groups were compared according to different clinical remission criteria, no significant difference was observed between the two groups according to DAS28-CRP, DAS28-ESR, CDAI, SDAI, and 2011 ACR/EULAR Boolean remission criteria ($p = 0.693$, $p = 0.828$, $p = 0.795$, $p = 0.796$, $p = 0.435$, respectively) (Table 2).

Comparison of the Patients With Remission and Non-Remission According to Different Clinical Remission Criteria

The disease duration, remission duration, TJC, ESR, and CRP values of patients in remission and non-remission and the SH and PD scores determined by the US were compared according to all the remission criteria (Table 3). No differences were found between remission and non-remission patients according to the different clinical remission criteria in US findings ($p > 0.05$).

The Relationship between Ultrasonographic Score and Other Findings

The relationship between PD-SH scores and the disease duration, remission duration, ESR, CRP, RF, anti-CCP, TJC, DAS28-CRP, DAS28-ESR, HAQ, CDAI, SDAI, 2011 ACR/EULAR Boolean remission criteria, PhGA, and PtGA were evaluated via logistic regression. There was a positive correlation both between PD and SH score and disease and remission duration. No correlation was found between US scores and the other parameters (Table 4).

DISCUSSION

This study investigated US remission using different definitions of clinical remission criteria. US remission rates according to all clinical remission criteria were between %53.4-%57.7. There was no increase in US remission rates as per the stricter remission criteria. This suggests that current criteria may lack the sensitivity necessary for accurate remission assessment. Using US in addition to the clinical criteria would prove to be more useful in evaluating remission.

In many studies, it has been reported that some of the patients with RA in remission still have subclinical synovitis and the frequency of the synovitis varies significantly between 50% and 95% as per SH and between 15% to 60% as per PD scores [13, 26, 27].

Table 2. The US remission (PD = 0) rates of the patients according to different clinical remission criterias

Clinical remission criteria	All patients n (%)	TNFi + csDMARD combination group n (%)	csDMARD group n (%)	*p* value
DAS28-CRP (< 2.6)	60/105 (57.1)	30/55 (54.5)	30/50 (60)	0.693
DAS28-ESR (< 2.6)	50/87 (57.5)	22/40 (55)	28/47 (59.6)	0.828
CDAI (< 2.8)	31/58 (53.4)	17/31 (54.8)	16/27 (59.3)	0.795
SDAI (≤ 3.3)	34/61 (55.7)	18/34 (52.9)	16/27 (59.3)	0.796
2011 ACR/EULAR remission criteria	26/45 (57.7)	16/27 (59.3)	10/18 (55.7)	0.435

Values are presented as numbers and percentages. TNFi = tumor necrosis alpha inhibitör, csDMARD = conventional synthetic disease-modifying antirheumatic drugs, DAS28-CRP = disease activity score in the 28 joints using C-reactive protein, DAS28-ESR = DAS28 using erythrocyte sedimentation rate, CDAI = clinical disease activity index, SDAI = simplified DAI, 2011 ACR/EULAR remission criteria = 2011 American College of Rheumatology/European League Against Rheumatism Boolean remission criteria

Table 3. Comparison of the patients with remission and non-remission according to different clinical remission criteria

DAS28-ESR remission criteria			
Variables	**Yes (n = 88)**	**No (n = 17)**	***p* value**
Disease duration (years) †	10 (1-38)	13 (1-37)	0.516
Remission duration (months) †	12 (6-60)	11 (6-36)	0.150
TJC †	0 (0-2)	1 (0-2)	0.001
ESR †	11 (2-32)	35 (18-56)	< 0.001
CRP †	0.30 (0.10-0.90)	0.40 (0.10-1)	0.204
Total SH score †	4 (0-17)	2 (0-14)	0.538
Total PD score †	0 (0-11)	0 (0-6)	0.772
Total_SH and PD score †	5(0-20)	3 (0-18)	0.618
SH score = 0 and PD score = 0, n (%)‡	20 (22.7)	3(17.6)	0.637
SH score = 0, n (%)‡	25 (28.4)	3(17.6)	0.358
SH score ≥ 1, n (%)‡	63 (71.6)	14 (82.4)	0.933
PD score = 0, n (%)‡	51 (58)	9 (52.9)	0.702
PD score ≥ 1, n (%)‡	37 (42)	8 (47.1)	0.644
CDAI remission criteria			
Variables	**Yes (n = 58)**	**No (n = 47)**	***p* value**
Disease duration†	9.5 (1-38)	11 (1-37)	0.155
Remission duration†	12(6-40)	12 (6-60)	0.557
TJC†	0 (0-1)	0 (0-2)	0.002
ESR†	13 (2-56)	13 (2-56)	0.454
CRP†	0.30 (0.10-1.00)	0.31 (0.1-0.9)	0.997
Total SH score†	4 (0-17)	4 (0-14)	0.830
Total PD score†	0 (0-11)	0 (0-7)	0.783
Total_SH and PD score†	4 (0-20)	5 (0-18)	0.579
SH score = 0 and PD score = 0, n (%)‡	14 (24.1)	9 (19.1)	0.572
SH score = 0, n (%)‡	16 (27.6)	12 (25.5)	0.813
SH score ≥ 1, n (%)‡	42 (72.4)	35 (74.5)	0.680
PD score = 0, n (%)‡	33 (56.9)	27 (57.4)	0.955
PD score ≥ 1, n (%)‡	25 (43.1)	20 (42.6)	0.962
SDAI remission criteria			
Variables	**Yes (n = 61)**	**No (n = 44)**	***p* value**
Disease duration†	9 (1-38)	11.5 (1-37)	0.107
Remission duration†	12 (6-40)	12.5 (6-60)	0.458
TJC†	0 (0-1)	0 (0-2)	0.001
ESR†	13 (2-56)	14 (2-56)	0.351
CRP†	0.30 (0.10-1.00)	0.40 (0.1-0.9)	0.312
Total SH score†	4 (0-17)	3.5 (0-14)	0.976
Total PD score†	0 (0-11)	0 (0-7)	0.974
Total_SH and PD score†	4 (0-20)	5 (0-18)	0.754
SH score = 0 and PD score = 0, n (%)‡	14 (24.1)	8 (18.2)	0.433
SH score = 0, n (%)‡	17 (27.9)	11 (25)	0.743
SH score ≥ 1, n (%)‡	44 (72.1)	33 (75)	0.704
PD score = 0, n (%)‡	34 (55.7)	26 (59.1)	0.732
PD score ≥ 1, n (%)‡	27 (44.3)	18 (40.9)	0.651
2011 ACR/EULAR remission criteria			
Variable	**Yes (n = 45)**	**No (n = 60)**	***p* value**
Disease duration†	10 (1-38)	11 (1-37)	0.349
Remission duration†	12 (6-40)	12.5 (6-60)	0.423
TJC†	0 (0-1)	0 (0-2)	0.019
ESR†	13 (2-56)	13.5 (2-56)	0.437
CRP†	0.30 (0.10-1.00)	0.40 (0.1-0.9)	0.216
Total SH score†	4 (0-17)	3.5 (0-14)	0.663
Total PD score†	0 (0-11)	0 (0-7)	0.968
Total_SH and PD score†	4 (0-20)	5 (0-18)	0.881
SH score = 0 and PD score = 0, n (%)‡	9 (20)	14 (23.3)	0.683
SH score = 0, n (%)‡	10 (22.2)	18 (30)	0.372
SH score ≥ 1, n (%)‡	35 (77.8)	42 (70)	0.907
PD score = 0, n (%)‡	26 (57.8)	34 (56.7)	0.909
PD score ≥ 1, n (%)‡	19 (42.2)	26 (43.3)	0.812

†Values are presented as median (minimum–maximum). ‡ Values are in numbers and percentages. Comparison between groups via Mann–Whitney U test and the Pearson's chi-square test with a value of $p < 0.05$ was considered significant. DAS28-CRP = disease activity score in the 28 joints using C-reactive protein, DAS28-ESR = DAS28 using erythrocyte sedimentation rate, CDAI = clinical disease activity index, SDAI = simplified DAI, ACR/EULAR remission = 2011 American College of Rheumatology/European League Against Rheumatism Boolean remission criteria, TJC = tender joint count, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, RF = rheumatoid factor, Anti-CCP = anticyclic citrullinated peptide, SH = synovial hypertrophy, PD = power Doppler

Table 4. The evaluation of the relationship between PD-SH scores and the disease duration, remission duration, different remission criteria, and laboratory values of the patients

All patients (n = 105)				
	PD score OR (CI)	***p* value**	**SH score OR (CI)**	***p* value**
Disease duration	1.068 (1.012-1.127)	**0.016**	1.312 (1.174-1.467)	**< 0.001***
Remission duration	1.086 (1.030-1.145)	**0.002**	1.099 (1.031-1.172)	**0.004***
DAS28-CRP	0.926 (0.296-2.901)	0.895	0.699 (0.217-2.247)	0.547
DAS28-ESR	0.544 (0.273-1.085)	0.084	1.088 (0.548-2.158)	0.809
CDAI	1.052 (0.808-1.369)	0.708	0.999 (0.762-1.310)	0.994
SDAI	1.028 (0.790-1.337)	0.837	0.980 (0.747-1.284)	0.883
2011 ACR/EULAR remission	1.035 (0.739-1.450)	0.840	0.941 (0.665-1.331)	0.731
PhGA VAS (0–10)	1.059 (0.484-2.318)	0.887	1.160 (0.517-2.602)	0.719
PtGA VAS (0–10)	1.028 (0.670-1.557)	0.901	1.007 (0.648-1.564)	0.977
HAQ	1.024 (0.309-3.388)	0.969	1.213 (0.351-4.192)	0.761
TJC	1.259 (0.632-2.510)	0.513	0.871 (0.431-1.761)	0.700
ESR	0.983 (0.946-1.022)	0.387	1.016 (0.980-1.053)	0.395
CRP	0.911 (0.076-1.098)	0.941	0.403 (0.064-2.551)	0.335
RF	1.000 (0.999-1.002)	0.449	1.001 (0.999-1.002)	0.288
Anti-CCP	1.001 (1.000-1.002)	0.291	1.000 (0.999-1.001)	0.883

Logistic regression was used for the table above. OR = odds ratio, CI = confidence interval, *p value is significant, DAS28-CRP = disease activity score in the 28 joints using C-reactive protein, DAS28-ESR = DAS28 using erythrocyte sedimentation rate, CDAI = clinical disease activity index, SDAI = simplified DAS, ACR/EULAR remission = 2011 American College of Rheumatology/European League Against Rheumatism Boolean remission criteria, PhGA = physician global assessment, PtGA = patient global assessment, VAS = visual analog scale, HAQ = Health Assessment Questionnaire, TJC = tender joint count, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, RF = Rheumatoid factor, anti-CCP = Anti-cyclic citrullinated peptide

Consistent with other studies, ultrasonographic synovitis was found in 52%-74% of patients who were in remission of them as per SH and in 40%-45% of them as per PD in this study. In the literature, US remission rates vary according to different remission criteria but mostly vary between 35-58% [6, 28]. US remission rates in this study were found to be similar to other studies between patients who received only csDMARDs and those who received a combination of csDMARD and TNFi [15, 16]. These results explain why there is a high probability of relapse when the drug is discontinued or its dose is reduced, even if a patient has achieved clinical remission.

Different criteria are used to evaluate remission in patients with RA. DAS28-CRP is a remission criterion that is easy to calculate; thus, it is frequently used in clinical practice. However, since this criterion can be met (< 2.6) in patients with tender/swelling joints or acute phase elevation, it may not accurately reflect the absence of inflammation. DAS28-ESR is similar to DAS28-CRP in reflecting clinical remission. SDAI and ACR/EULAR remission criteria are known as the more stringent criteria. In previous studies, only one clinical remission criterion was used to evaluate remission, and only a few studies have used and compared different remission criteria [16, 27, 29-31]. In these studies, US remission rates were different according to different remission criteria. For instance, Naredo *et al*. found that US remission rates were significantly lower in patients in remission according to DAS28 than that in patients in remission according to SDAI [30]. Peluso *et al*. found that using ACR remission criteria showed fewer US remission than those using the DAS28 remission criteria [31]. On the other

hand, Balsa *et al*. [29] did not find any significant difference between the ACR/EULAR and DAS28 remission criteria. In addition to this, when SDAI was used, US remission was found to be significantly lower compared to that when ACR and DAS28 criteria were used [29]. In the EULAR targeted therapy recommendations updated in 2019, the ACR/EULAR Boolean remission is now preferred over DAS28 remission because it is emphasized that ACR/EULAR remission criteria are more stringent and reflect remission better than other criteria [32]. Both SDAI and ACR/EULAR remission criteria are considered more stringent measures of remission as it allows for the least abnormalities of variables. In our study, although the remission rates of patients who were in remission according to DAS28 CRP were lower with SDAI and ACR/EULAR remission criteria, which are evaluated as stricter criteria, US remission rates did not change under more stringent criteria. In our study, the 2nd and 5th MTP of the foot joints were also evaluated while performing joint US examinations. The only criteria that evaluate the foot joints are the 2011 ACR/EULAR remission criteria. Therefore, it may be thought that other remission criteria may miss the evidence of disease activity and misclassify patients as in remission and US remission will be detected more frequently with the ACR/EULAR remission criteria. However, it was not as expected in our study. One possible reason for this is that, including the foot joints, the patients were in remission. The most likely explanation may be that current clinical remission criteria are largely subjective, do not take into account subclinical inflammation, and neither clinical criterion is superior to the other in demonstrating remission.

Limitations

The most important limitations of this study are that US evaluation was performed by a single physician and the study is a cross-sectional study with a lack of long-term follow-up results. An important factor that would strengthen the study is the fact that US evaluation should be carried out by at least two physicians and the reliability between the physicians should be checked. Another limitation of this study is that the dose and frequency of NSAIDs used by the patients were not recorded. NSAIDs can modify the symptoms and levels of synovitis by masking the clinical symptoms and signs.

Strengths

In previous studies, the presence of US remission was evaluated according to one or two clinical remission criteria. In this study, US remission rates were investigated according to all remission criteria.

CONCLUSION

This study underlines clinical remission criteria does not clearly indicate the presence of remission and none of the remission criteria are superior to each other to evaluate the US remission. The accurate remission in RA would not rely solely on clinical examination but may require imaging to confirm the remission.

Authors' Contribution

Study Conception: HED; Study Design: YP; Supervision: HED, YP; Funding: HED; Materials: BNC; Data Collection and/or Processing: SE, BY; Statistical Analysis and/or Data Interpretation: SE; Literature Review: BY; Manuscript Preparation: SE and Critical Review: BY, YP.

Conflict of interest

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

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Clinical update of medications associated with QT prolongation among COVID-19 patients

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ABSTRACT

In the struggle against COVID-19 pandemic, chloroquine (CQ) (a 4-aminoquinoline) and its derivative hydroxychloroquine (HCQ) have both been used as a potential form of treatment among infected patients. Originally known as an antimalarial quinolone, many countries have adopted their use as an option to treat COVID-19 patients. In humans, dose-dependent chloroquine induces QT interval prolongation. It also blocks the human ether-a-go-go-related gene (hERG), which encodes the rapidly activating delayed rectifier K+ channel. The action potential duration is then prolonged, as the eventual QTc interval of the electrocardiogram (ECG), resulting in torsade de pointes and cardiac arrhythmias that could lead to sudden death. It is yet unknown whether COVID-19 itself has any effect on the QTc interval. The current review established what is new and different from other studies involving the use of chloroquine and hydroxychloroquine among COVID-19 patients plus the corresponding QT interval prolongation in affected individuals.

Keywords: Long QT prolongation, hERG, ionic channels, drug repurposing, mobile devices, SARS-CoV-2

Up until now, there is no end in sight despite the recent vaccines produced; some are yet to get each their regulatory approval. The irony of this pandemic is the paralysis of businesses/economies that is catastrophic, fatalities, change in our ways of living and the huge price still being paid with increasing numbers of lives lost (6.14M) and 487M cases in 220 countries/areas worldwide as of March 31, 2022 [1]. The associated consequences have also dehumanized human beings, as people are left to die in self-isolation without loved ones and its sudden change in normality [2].

T he panic and the rush for measures in tackling the pandemic on a global scale have resulted, for a few cases, in the use of various drugs without proven efficacy, for instance, hydroxychloroquine, favipiravir, remdesivir, azithromycin or lopinavir/ritonavir [3]. The affinity of these medications in blocking the rapid component of the delayed rectifier current (IKr) encoding the human ether-a-go-go-related gene (hERG) as well as their propensity to prolong cardiac repolarization (QTc interval) and to cause torsades de pointes (TdP) (Fig. 1) were reported [4]. The quantification of drug-induced long QT syndrome (LQTS) through the use of six indices has been carried out by querying the U.S. Food and Drug Administration Adverse Event Reporting System (FAERS) database with particular key words, according to Michaud *et al*. [5].

As of March 31, 2022, there are 6,274 studies on COVID-19 in the World Health Organization database, available at https://clinicaltrials.gov/ct2/who_table. Based upon their modes of action, some repurposed drugs were selected for the therapeutic management of COVID-19 [6]. If one of these is administered, it

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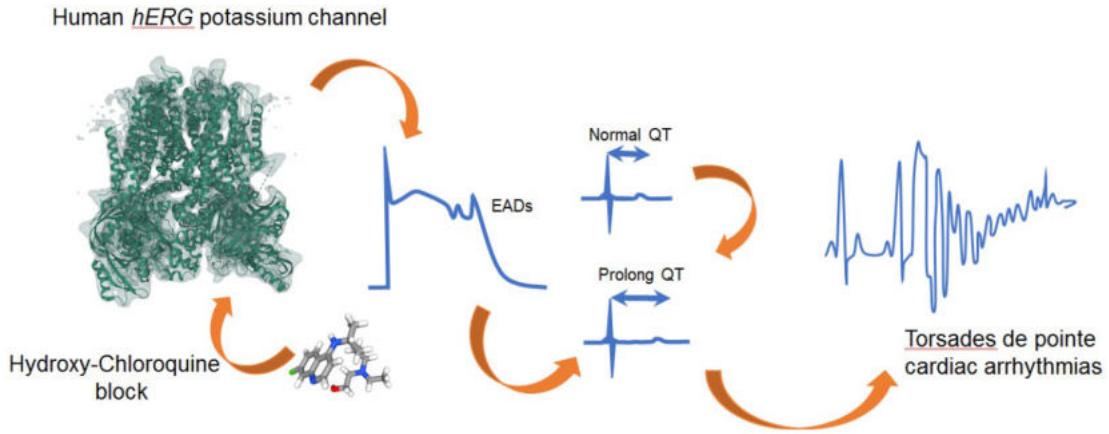

Fig. 1. Hydroxychloroquine and chloroquine have the affinity of these medications in blocking the rapid component of the delayed rectifier current encoding the human ether-a-go-go-related gene (hERG) as well as opensity to prolong cardiac repolarization (QTc interval) and to cause torsades de pointes.

could lead to serious, even fatal consequences, depending on its appropriate and safe use. Drugs such as hydroxychloroquine and chloroquine (Fig. 2) prolong the QTc interval and may result in ventricular arrhythmia and sudden cardiac arrest [7]. As a consequence, direct impacts of COVID-19 infection may include ventricular arrhythmias, harmful side effects of systemic illness and adverse events to medications applied in treating it [8].

COVID-19 was rapidly evolving right from the onset, and it is now a global battle to treat and be contained. There are variations from one population to the other with regard to prevalence of arrhythmia episodes and conduction system disorders, along with cardiovascular diseases in patients with COVID-19 [9]. The particular causes for palpitations, or even the types of arrhythmia, have not been specified in most available reports [10, 11]. Electrolyte abnormalities and hypoxia, both known to be associated with the development of acute arrhythmias are observed in the acute phase of severe COVID-19, however, it is unclear yet the exact contribution of an infection to arrhythmia in asymptomatic, mildly ill, critically ill and recovered patients [12].

Interestingly, gender was compared in terms of QT prolongation among COVID-19 patients undergoing chloroquine and hydroxychloroquine with/without azithromycin treatment. In women, it is identified as a risk factor, being one of the highest pro-arrhythmic [13-15]. It is significant, the greater one for developing the potentially fatal torsades de pointes (TdP) ventricular tachycardia, which is related to certain medications that prolong the repolarization of ventricles, being more prominent in women than men. The female gender is considered an independent risk factor for the incidence of syncope and sudden death in the inherited long QT syndrome, knowing that TdP is three times more commonly in women if compared to men [16].

According to Rosenberg *et al*. [17], the sex disproportionality in hospitalizations is precluded as being a distinct risk factor in association with the female

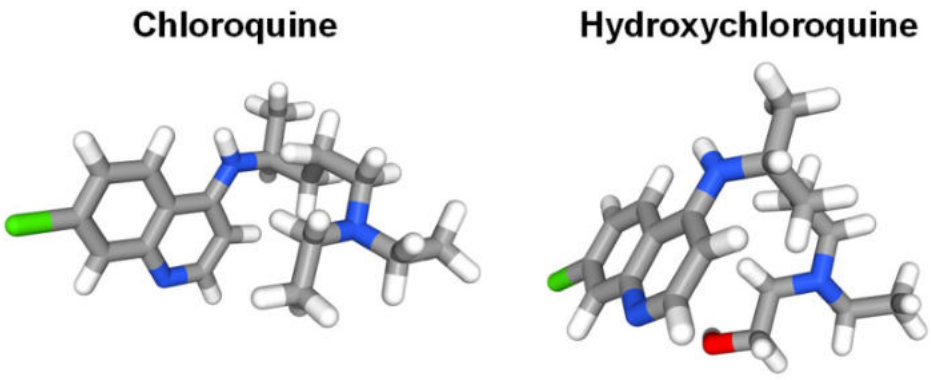

Fig. 2. Drugs such as hydroxychloroquine and chloroquine prolong the QTc interval.

COVID-19 patients, as a greater proportion that are admitted to hospitals are males. hERG channel is responsible for controlling the movement of potassium ions going out of the myocytes, and for conducting the rapid IKr component that is critical in phase 3, the repolarization phase of the cardiac action potential (AP) [18]. The higher susceptibility of women to drug-induced LQTS is believed to be attributed to the estrogen-mediated reduced repolarization [19].

Congenital LQTS is described as the hereditary form linked with mutations in several genes, the most significant being KCNQ1, KCNH2 two potassium channels, and SCN5A (specific to INa sodium current). LQTS could also be acquired, the most prevalent form having an incidence rate of 0.8 to 1.2 per million people per year [20]. Many wide-ranging pharmacological agents, from different therapeutic categories, block IKr and induce QT prolongation, they include class III antiarrhythmics and medications that are prescribed for non-cardiovascular indications like antibiotics, antidepressants, antipsychotics and antimalarial drugs (Table 1).

Pharmacodynamics and QTc-Prolonging Chloroquine/Hydroxychloroquine

The synthetic analog of quinine, namely chloroquine, is a muscle relaxant extracted from the bark of the cinchona tree (Cinchona officinalis). The synthesis of chloroquine was first conducted in 1934, and marketed as Resochin® by Bayer following the isolation of quinine in 1820. It was initially used in the West to treat malaria in 1631, in Rome. As for hydroxychloroquine, it was synthesized in 1946, and clinically introduced in 1955 under the brand name of Plaquenil®, while the introduction of chloroquine was, on the other hand, in 1947 as a prophylactic treatment of malaria [21].

Treating COVID-19 patients with the administration of hydroxychloroquine, chloroquine and/or azithromycin led to statistically significant increases in QT prolongation, as even after controlling a proportion of patients for electrolyte abnormalities [22]. The prolongation of QTc interval seemed consistent, irrespective of race or ethnicity [23].

It is perceived that chloroquine and hydroxychloroquine can have in vitro antiviral characteristics [24]. The entry and post-entry stages of SARS-CoV-2 infection are believed to be acted upon by CQ and HCQ through effects on endosomal pH and ensuing under-glycosylation of angiotensin-converting enzyme 2 (ACE2) receptors needed for viral entry. It has been hypothesized that the therapeutic effects of chloroquine and hydroxychloroquine involve (a) prevention of SARS-CoV-2 infection via inhibition of ACE2 mediated viral entries and (b) attenuation of the cytokine storm induced by the virus and experienced in severe COVID-19 cases [25].

If multiple medications are administered in combination, the risk of drug-induced sudden cardiac death (DI-SCD) could be amplified, each of which has its own QTc-prolonging and torsadogenic potential [26]. There has been scrambling for solutions and treatment options in the battle against COVID-19, such that little or no adequate time for precautionary measures.

A decision rested with the treating clinician and patient implied the risk-benefit calculus and navigating around a QTc value of 500 ms or more. Among younger COVID-19 patients (less than 40 years of age) who may have mild symptoms with above value, it could be appropriate to avoid treatment completely as the risk of arrhythmia may outweigh that of developing COVID-19-related acute respiratory distress syndrome [26]. On the other hand, patients more than 65 with a QTc of 500 ms or more, immunocompromised, underlying health conditions, and/or gradually worsening breathing problems, the potential benefit of a possible QTc-prolonging pharmacotherapy for COVID-19 may exceed the risk of arrhythmia.

For more than two decades, the inclination of using old drugs has intensified based on costs and reduction of time frames with the approval of regulatory agencies, considering that their safety profile is already known. There is no defined clarity to use the term “repurposing” here, except for the fact that off-patented drugs are being indicated as new therapeutic medications. The successes associated with repurposed drugs are few, but many have failed expectations from non-clinical investigations and observational studies [21].

By taking safety concerns into consideration, many advocates for chloroquine are based on data from patients with rheumatic diseases, but applying these facts for COVID-19 is implausible biologically. The infection process of COVID-19 and its metabolic consequences promote a pro-arrhythmic milieu, and

Table 1. QTc-prolonging drugs and arrhythmogenic adverse drug events for repurposed medications used for COVID-19.

Drug Name	VT/VF/TdP/LQTS	VT/VF/VA/VFL/VT	TdP/LQTS
Azithromycin			
Chloroquine			
Chlorpromazine	0		0
Cilostazol			
Cisapride			
Clarithromycin			
Clozapine			
Dasatinib			
Domperidone			1
Donepezil			
Droperidol			2
Escitalopram			
Halofantrine	4	1	1
Haloperidol			
Hydroxychloroquine			
Lapatinib			3
Lopinavir/Ritonavir			
Methadone			
Ondansetron			
Pentamidine	0	0	0
Pimozide	7	6	4
Propofol			
Risperidone			
Ritonavir			
Thioridazine	6	9	1
Vandetanib	1	1	1

VT = Ventricular tachycardia, VF = Ventricular fibrillation, TdP = Torsade de pointes, LQTS = Long QT syndrome, VA = Ventricular arrhythmia, VFL = Ventricular flutter (*Modified from* Michaud *et al.* [5])

added adverse events of medication could generate a devastating blow [27].

In terms of practice guidelines, the American College of Physicians do not recommend either chloroquine or hydroxychloroquine for prophylaxis or treatment. The pursuit and investment toward the use of 4-aminoquinolines in COVID-19 are not prudent and it is high time to bring to an end [27].

Portable ECG Measurements for QTc-monitoring of COVID-19 Patients

An interesting fact is that some FDA-approved mobile consumer ECG devices are capable of generating accurate QTc measurements [28]. AliveCor Inc., the manufacturer of the Kardia-Mobile 6L device that was approved for atrial fibrillation detection received in March 2020 an emergency clearance from the FDA for its use in monitoring COVID-19 patients prescribed with QTc-prolonging medications. The next-generation algorithm determines whether two or more ventricular ectopic beats are detected, if narrow complexes are noticed, and if there are QRS intervals of

120 ms or longer [29].

For COVID-19 patients who are about to be treated with drug-inducing torsade de pointes/sudden cardiac death, baseline QTc values could be obtained either through a conventional 12-lead ECG or the use of a smartphone-enabled mobile QTc meter to minimize personnel exposures [26]. Patients having QTc intervals of ≥ 500 ms (with QRS ≤ 120 ms) present an increased risk of QT prolongation and polymorphic VT [30]. The electrolyte abnormalities (for instance, hypocalcemia, hypokalemia, and/or hypomagnesemia) in such patients could be corrected with a potassium level close to 5 mEq/L.

Generally, patients with the following QTc intervals are perceived to be at low risk of significant QT prolongation and polymorphic VT [31].

–Prepubertal male/female ratio = QTc < 460 ms

–Postpubertal males = < 470 ms

–Postpubertal females = < 480 ms

There are concerns of cardiovascular toxicity associated with chloroquine and hydroxychloroquine, especially because of their relationship with electrical instability, characterized by QT interval prolongation. The mechanism relates to block the hERG potassium channel, which lengthens ventricular repolarization and duration of its AP. Throughout the course of COVID-19, malignant arrhythmia episodes that involve multifocal ventricular tachycardia/ventricular fibrillation develop and were linked with higher troponin T levels. Myocardial injury defined as troponin elevation could be because of target organ damage by hypoxemia, Takotsubo cardiomyopathy, or myocarditis, indicating that myocardial injury may play a role in the fatality of some COVID-19 infected patients [32, 33]. The characteristic features of patients treated with CQ and HCQ are shown in Table 2.

The risk of optimization and the benefit ratio when exploratory drugs are being given should be taken into consideration before resorting to electrocardiographic/QTc monitoring plus associated decisional guidance. The patients with COVID-19 who are severely ill are frequently disturbed by underlying comorbidities especially electrolyte imbalances, consequential QTc-prolonging medications and systemic inflammatory reactions resulting to torsades de pointes.

With the chaos and confusion surrounding the search for effective COVID-19 treatment, there has been a lack of systematic strategy, hence the rush toward CQ and HCQ. The repurposing of both for COVID-19 has been carried out without any formal determination of antiviral safety, effectiveness and dosing. Also, the global scientific community spirit has been collaborative but it seems to be fragmented and disoriented as well.

When the level of interleukin-6 (IL-6) is ≥ 10 pg/mL along with QTc > 500 ms, it is advisable to administer anti-IL-6 targeted drugs such as tocilizumab and sarilumab. The risk of inflammation-driven QTc changes could be minimized through blockage of IL-6, thereby reducing the need or even withdrawal of COVID-19 repurposed pharmacological treatments. It is not a sure thing if benefits are associated with the use of chloroquine or hydroxychloroquine to treat patients with COVID-19; rather, these drugs have links to ventricular arrhythmias and show a higher risk of in-hospital death [34]. In consequence, their usage should be prevented outside clinical and other emergency randomized trials.

Despite a widespread use of both medications in treating malaria and autoimmune disorders, even for a short-term duration, the undesirable effects associated with such drugs cannot be ignored. These adverse drug reactions are involving, but are not limited to, gastrointestinal and cutaneous manifestations, hypoglycemia, neuropsychiatric effects, and drug-drug interactions to name some. The cardiac-related complications are prevalent among 85% of affected patients while other non-specific events include ventricular hypertrophy, heart failure, hypokinesia, pulmonary arterial hypertension and valvular dysfunction. The RECOVERY trial that is the world's largest for COVID-19 concluded that hydroxychloroquine had no beneficial effect in COVID-19 hospitalized patients, and stopped the enrolment to that arm of the trial immediately.

Since the inception of the pandemic, both chloroquine/hydroxychloroquine have significantly gained scientific and political attention, however, evidence from large epidemiological studies and clinical trials have shown that CQ/HCQ have no significant mortality benefits among hospitalized COVID-19 patients. There are equivocations and inconsistencies surrounding their roles during the early phases of the outbreak, resulting in unsubstantiated support benefits for outpatients with COVID-19. Additionally, cardiotoxicity

Table 2. Summary of chloroquine or hydroxychloroquine and the cardiotoxicity among COVID-19 treated patients

Drug used	Cardiac toxicity	Beta blockers	Ca^{2+}channel blockers
HCQ 1,200 mg daily for 3 days, then 800 mg daily for 2 or 3 weeks	QT prolongation Cardiac arrhythmia during therapy course	Potential interaction	Potential interaction
High-dose (12 g) or low-dose (2.7 g) HCQ for 10 days	QTc > 500 ms or ventricular arrhythmia for 28 days	Potential interaction	Potential interaction
HCQ 200 mg 3 times daily for 5 days	Not reported		
Azithromycin or HCQ	QT prolongation Cardiac arrhythmia during therapy course		
HCQ 600 mg daily Duration not reported	QT prolongation Arrhythmia		
HCQ 200 mg twice daily for at least 3 days	QT prolongation Arrhythmia		
HCQ 400 mg twice daily on day 1, then 200 mg twice daily for 4 days	QT prolongation		
Chloroquine 600 mg daily for 5 days	QT prolongation		
HCQ 200 mg twice daily for 10 days	QT prolongation		
HCQ dose and duration, not reported	QT prolongation		
HCQ 600 mg daily for 10 days	QT prolongation		
HCQ 600 mg twice daily on day 1, then 200 mg twice daily for 4 days	QT prolongation		
HCQ 400 mg twice daily on day 1, then 200 mg twice daily for 4 days	Not reported		

(*Adapted from* Tleyjeh *et al.* [41] and Levett *et al.* [47])

particularly prolongation of QTc is the major concerns raised.

Other treatment agents applied in COVID-19 beyond therapies targeting cytokines, alias the cytokine release syndrome (CRS), include antivirals, antibacterial drugs, immunomodulators, angiotensin II receptor blockers, bradykinin B2 receptor antagonists, corticosteroids, anthelmintics, antiprotozoal drugs, H2 blockers and anticoagulants. Remdesivir, which is a broad-spectrum antiviral agent, was given approval in October 2020 by the FDA in the USA for the treatment of hospitalized patients with COVID-19. These were adult and pediatric subjects (over 12 years of age and weighing at least 40 kg or more) with severe disease. The limitations and controversial efficacy of remdesivir make it difficult to be used widely in hospital patients [35].

The first oral antiviral drug molnupiravir (Lagevrio; Merck, NJ, USA) approved in the UK for the treatment of COVID-19 was on November 4, 2021, for adults who tested positive with at least a risk factor for developing severe illness [36]. Its role is still limited for moderate to severe COVID-19 patients despite the fact that it prevents SARS-COV-2 to replicate by causing multiple mutations in the genome, and led to a significant reduction in hospitalization or death among mild cases. Like all developments in repositioning, COVID-19 drug repurposing research requires to pass through three stages before it can be assumed for advancement into the product portfolio:

the recognition of candidates, the mechanistic evaluation of effects in preclinical models, and the assessment of its efficacy in phase II clinical trials.

A protease inhibitor by the name of Paxlovid (Pfizer, NY, USA) undergoing phase III trials for testing its safety and effectiveness in order to treat non-hospitalized adults having COVID-19, but who are not at risk of developing serious illness, is a nirmatrelvir and ritonavir combination. While Paxlovid is being explored in post-exposure prophylaxis among patients with previous SARS-CoV-2 infection [37], it was able to reduce hospitalization by 80% based on its clinical efficacy.

In another development, angiotensin II receptor antagonist, a sartan derivative, an antihypertensive drug is also under study for COVID-19 treatment. From the onset of the pandemic, it has been reported that coronaviruses transfer their genetic material to the host cell, binding the ACE2 receptors. The physiological effects of angiotensin II are counteracting when losartan blocks binding between ACE2 and SARS-CoV-2 through the AT1 receptor.

The glucocorticoids are indicated for COVID-19 pneumonia, except in the event of specific comorbid conditions such as COPD exacerbations, according to a general advice from World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC), however, there are still controversies surrounding the use of corticosteroids.

Recent results published in The Lancet Respiratory Medicine support benefits of Evusheld in the outpatient treatment of mild to moderate COVID-19. Results from the TACKLE Phase III outpatient treatment trial showed that AstraZeneca's Evusheld (tixagevimab and cilgavimab, formerly AZD7442) provided clinically and statistically significant protection against COVID-19, from progressing to severe disease or death for any cause compared to placebo. Earlier treatment with Evusheld in the course of the disease led to favorable outcomes [38]. TACKLE is the phase III study of AZD7442 for treatment of COVID-19, which is a randomized, double-blind, placebo-controlled, multi-centre trial assessing the safety and efficacy of a single Evusheld 600 mg IM dose compared to a placebo in the outpatient treatment of mild-to-moderate disease. The study was conducted in 95 sites in the USA, Latin America, Europe and Japan. The sample comprised of 903 participants being randomized (1:1) to receive either Evusheld (n = 452) or saline placebo (n = 451), administered in two separate consecutive intramuscular (IM) injections.

The enthusiasm surrounding the use of hydroxychloroquine as a potential therapy for COVID-19 was based on a combination of factors, such as broad availability, oral administration, and its history in treating malaria. However, as reported recently by Self *et al*. [39] and White *et al*. [40], recent findings are consistently showing hydroxychloroquine as ineffective for treating COVID-19 patients. Furthermore, the risks of chloroquine- or hydroxychloroquine-induced QT prolongation and relatively higher events of torsade de pointes, ventricular tachycardia, or cardiac arrest are likely to be encountered among COVID-19 patients under treatment with these antimalarial drugs (see Fig. 1). It is therefore not advisable to use these agents in the routine management of people affected with COVID-19. There should be adequate monitoring of those who could be treated with chloroquine or hydroxychloroquine and for other indications. QT interval prolongation and torsade de pointes that are provoked have also been implicated in occasional case reports of systematic treatment in patients with systemic lupus erythematosus [41].

There are multiple risk factors associated with COVID-19 syndrome. For instance, hypokalemia, in the range of 3.0–3.4 mol/L is common, drug-induced Ikr blockade is amplified by fever; an increase in the levels of IL-6 has been experienced in COVID-19 infection, so this could be a mechanism for prolonging the QTc interval that is linked with inflammation [42]. Some practical measures have been highlighted to be considered when using CQ or HCQ alone or along with azithromycin. These include ECG recording before initiation of treatment where possible, avoidance of concomitant non-essential drugs known to prolong QT, potassium supplementation to > 4 mmol/L, if QTc is long (> 480 ms) at the baseline, obtaining an ECG within 2–4 hours after the first dose and to refrain from further treatment if QTc is documented to be above 520 ms [43]. And noteworthy, in the meta-analysis study by Fiolet *et al*. [44] reaffirmed that hydroxychloroquine alone does not have any therapeutic effect on patients with COVID-19, but the risk of mortality is enhanced if it is combined with azithromycin.

CONCLUSION

Summarily, from all indications, the use of CQ or HCQ for treating COVID-19 infected patients has been non-beneficial and resulted in the risk of ventricular arrhythmias with a greater chance of in-hospital death among the affected individuals. Also, the combination of azithromycin and hydroxychloroquine did not increase the likelihood of survival or discharge in hospitalized patients with COVID-19. This inference tallies with European Respiratory guidelines, which indicated no clinical benefits in relation to the use of hydroxychloroquine and/or azithromycin to treat infected COVID-19 patients [45]. The optimal approach in managing COVID-19 is yet to be established and the evidence for suggesting repurposing HCQ is purely based on the reduction of in vitro viral replication whereas the clinical data are in contradiction. From the RECOVERY trial, no mortality benefit is found associated with HCQ in treating COVID-19 patients, with longer hospital stays, and higher risk of disease progression toward invasive mechanical ventilation and/or death. In a similar fashion, the WHO SOLIDARITY trial also indicated no advantage either [46]. In RECOVERY and WHO SOLIDARITY trials, HCQ was used in comparative higher doses, with the exception of REMAP-CAP trial.

Interestingly, Evusheld has marketing authorization by the European Union and had granted a conditional status by the Medicines and Healthcare Products Regulatory Agency (MHRA) in the UK for pre-exposure prophylaxis of COVID-19, likewise in the USA. Evusheld is gaining authorization for use and being supplied in many countries around the globe with regulatory filings increasing for both prevention and treatment worldwide. These are encouraging developments and an alternative solution especially among those individuals that were reluctant or did not want to be vaccinated for COVID-19.

Authors' Contribution

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

Conflict of interest

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

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The European Research Journal 2022;8(5):742-746 Case Report

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Takotsubo syndrome triggered by emotional stress: a case report

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ABSTRACT

Takotsubo syndrome is an acute cardiac syndrome characterized by transient systolic dysfunction of the left ventricle without coronary artery disease. It's etiology has not been fully explained, physical and intense emotional stress triggers this syndrome so it is also called "broken heart syndrome" or "stress cardiomyopathy". Takotsubu syndrome has been reported that approximately 2% of patients presenting with acute coronary syndrome. In this case, we presented a 65-year-old patient who had a Takotsubo syndrome attack after emotional stress, thinking that it would contribute to the literature and increase awareness of this disease.

Keywords: Takotsubo syndrome, stress cardiomyopathy, catecholamine, emotional stress, somatic stress

Takotsubo syndrome (TS) is an acute cardiac syndrome characterized by transient left ventricular systolic dysfunction without coronary artery disease [1]. It was first reported in Japan by Sato *et al*. [2] in the 1990's. Although its etiology is not fully explained, it is known that sudden and intense emotional stress triggers this syndrome [3]. This is why it is also called "broken heart syndrome" or "stress cardiomyopathy". There are somatic and emotional stress factors defined for this disease in Table 1 [4]. It is frequently observed in women and in the post-menopausal period [5].

It has been reported that approximately 2% of patients presenting with acute coronary syndrome have TS [5]. The psychiatric aspect of TS, which is encountered in cardiology and anesthesia clinics and whose treatment is regulated by these clinics, is bypassed. Here, I present a rare TS case that develops due to stress to draw attention to the psychiatric aspect of the disease.

CASE PRESENTATION

Our patient was a 65 years old female patient, a retired teacher. The patient came for a psychiatric examination at the request of her children, who were physicians. She had complaints of concern, anxiety, irritability, and sleep disturbance. Her children stated that their mother was very prissy and could not tolerate a mess at home. However the main reasons for bringing the patient to the examination, it was after the patient learned that she was diagnosed with takotsubo syndrome during a detailed cardiological examination.

When the anamnesis was deepened, it was learned that the psychiatric complaints of the patient started in her youth. She lost her father at an early age. Afterwards, she started to get angry, stress, distress, sleep disturbance, occasional palpitations, and shortness of breath. She occasionally had crying jags. She had no lack of enjoyment and unhappiness in life. She was

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Table 1. Preceding somatic or emotional stressors in the development of stress-induced cardiomyopathy [4]

Somatic stressors	Emotional stressors
Vigorous exercise	Grief (eg, death or illness of a loved one)
Pheochromocytoma	Receiving bad news (eg, being diagnosed with major illness, learning of a daughter's divorce)
Subarachnoid hemorrhage	Fear
Seizure	Relationship conflicts
Postoperative pain	Public speaking
Hyperthyroidism	Financial problems
Alcohol/opiate withdrawal	Being bullied
Invasive medical procedures	Surprise party
Exacerbation of underlying noncardiac disease	Changing residence involvement in accident
Sexual intercourse	
Administration of sympathomimetics	

also a prissy person at that time, doing thorough cleaning once a week. She did not get help because she thought that no one else could clean as she did. On the other days, she was very careful about her cleanliness and order and could not tolerate mess. She was always a stubborn person, and no one else could change what she knew right.

The patient had a heart attack. In the evening of her routine weekly cleaning, her neighbor and little child came to visit without notice. The little boy messed up the cleaned house and soiled it. The patient was very angry with this situation, and distress started first. Chest tightness, palpitations, and shortness of breath were started then. Her complaints, which she had ignored before, increased over time. Chest pain intensified, palpitations, shortness of breath increased, left arm numbness, cold sweating occurred.

They were admitted to the emergency department and, after the first laboratory examinations and ECG, she was told that she had a heart attack, and was taken to the intensive care unit. Coronary artery occlusion was not detected in the angiography performed. Echocardiography revealed left ventricular apical dyskinesia (aneurysmatic), ejection fraction 60%, and mild mitral insufficiency. The patient was treated in the intensive care unit for three days. And then, cardiac treatment was arranged, saying that heart failure developed secondary to the attack. Control angiography was recommended for subsequent follow-ups. She was examined by a different cardiologist after her discharge. Coronary artery occlusion was not observed in the patient who underwent angiography again, and no other disease was detected in the patient at that time. Considering the prior angiography and laboratory findings, it was stated that the acute coronary attack she experienced in the past was TS, a condition that may develop due to stress.

In the mental state examination, her general appearance was compatible with her age, and her self-care was complete. Her attitude during the interview was communicative, and she made eye contact. Her affect was tense, and her mood was anxious. Her concentration of attention, orientation, judgment, and abstract thinking was complete. No perceptual disorder was detected.

The patient was diagnosed with a generalized anxiety disorder and obsessive-compulsive personality disorder according to DSM 5 diagnostic criteria [6]. And sertraline 50 mg and mirtazapine 15 mg were started. Since her complaints did not regress during her follow-up, sertraline dosage was increased to 100 mg. The patient, who stated that she started to gain weight over time, and her sleep improved, mirtazapine was discontinued, and her treatment with sertraline

100 mg was continued. She was followed up in the psychiatry outpatient clinic for a year. And the patient's cardiac attack did not recur.

DISCUSSION

TS is diagnosed according to Mayo clinic diagnostic criteria. ECG findings of patients, who present with complaints of chest pain, shortness of breath, palpitations similar to acute myocardial infarction, reveal T negativity and ST elevation, and a moderate elevation in troponin levels occur [7]. Differential diagnosis from acute myocardial infarction can be made by angiography [8]. Although there is no stenosis in the coronary artery, there is temporary dyskinesia or akinesia in the apical and middle segments of the left ventricle. This condition creates the appearance of a narrow neck and apical ballooning at the end of the systole in the left ventricle. The name Takotsubo was given because the appearance of the heart in echocardiography was compared to the shape of the container the Japanese used to catch octopus [7]. Although TS is thought to have a relatively benign prognosis, ventricular fibrillation can be observed in cardiogenic shock [7].

The typical feature of TS is postmenopausal woman and emotional or somatic stress that precede the onset of cardiothoracic symptoms [4, 9]. Studies have reported the prevalence of anxiety and depression as 56% and 48% in these patients [10]. Similarly, it was observed in our patient that she had a generalized anxiety disorder and obsessive-compulsive personality traits before TS appeared. Just before the postmenopasual female patient had an acute cardiac attack, she experienced an event that caused an increase in stress. During her emergency admission, she was followed up and treated considering acute myocardial infarction. Coronary artery disease was not detected in the angiography performed for the patient. Echocardiography showed apical ballooning in the left ventricle, which is diagnostic for TS. In this respect, our patient had typical TS.

The patient has a psychiatric disorder that has existed for a long time. However, she did not accept this situation and did not receive any psychiatric support. Not sharing negative feelings after stressful events in these patients may cause exaggerated sympathetic and cardiovascular reactions [11]. And, exposure to an internal or external stimulus that overwhelms their psychological coping ways in these patients can cause somatic complaints. In other words, they can respond to a psychological threat as a physiological threat [11]. The stressful event experienced in our patient also caused an excessive sympathetic reaction physiologically.

The pathophysiology of TS is complex. It includes cardiovascular responses to endogenous catecholamines or to exogenously administered catecholamines, often spiked during acute severe stress. Therefore, catecholamine concentration is shown as a trigger factor in TS [12]. Characteristic morphological changes similar to cardiotoxic effects caused by catecholamines were also observed in cardiac biopsies taken in the acute phase of the patients [13].

The stress response in the human body is managed through both the central nervous system and the sympathetic nervous system. Somatic or emotional stress causes catecholamine release in the central nervous system. The basic anatomical structures that play a role in stress response are the limbic system, neocortex, spinal cord, reticular formation, and brainstem. Locus coeruleus serves as the central area of neurons in the sympathetic nervous system. And it can receive afferent signals from the amygdala, hypothalamus, and cingulate gyrus. It is about excitement and alertness during stress [12].

Neuroendocrine changes caused by stimulation of these structures include intense stimulation of the locus coeruleus-adrenomedullin axis and hypothalamus-pituitary-adrenocortical axis. Postganglionic fibers of the sympathetic nervous system extend along the epicardial vessels, affecting the myocardial and coronary circulation [12]. As a result, two stages are mentioned in TS cases triggered by emotional stress. The first stage begins with the increased release of epinephrine and norepinephrine initiated by the cognitive centers of the brain through the activation of the hypothalamic-pituitary-adrenal axis. In the second phase, a cardiovascular response occurs to increased catecholamines in the circulation [5].

Anxiety and chronic stress have been found to increase the likelihood of developing TS significantly [14, 15]. However, this situation is considered to serve not as

an isolated cause of TS, but to increase susceptibility to the disease. It is because resting catecholamine levels in these individuals fluctuate higher in response to stressors [5].

TS cases accompanying dissociative amnesia and acute manic episode of bipolar disorder have also been reported in the literature [16, 17]. In addition, TS cases due to the electroconvulsive therapy and psychotropic drugs, which are frequently used in the treatment of patients in psychiatry clinics, have also been reported [18, 19]. Although the event that triggers TS is often attributed to the psychiatric condition, no detailed information has been obtained about how the clinical approach to these patients will be.

CONCLUSION

This case shows that TS may be a syndrome closely related to psychiatric diseases. Although it seems necessary that the primary treatment should be regulated by cardiology and anesthesia clinics due to the presence of an acute coronary attack, it shows that the patient may need psychiatric support afterwards.

Authors' Contribution

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

Informed consent

Written informed consent was obtained from the patient for publication of this case and any accompanying images.

Conflict of interest

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

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