



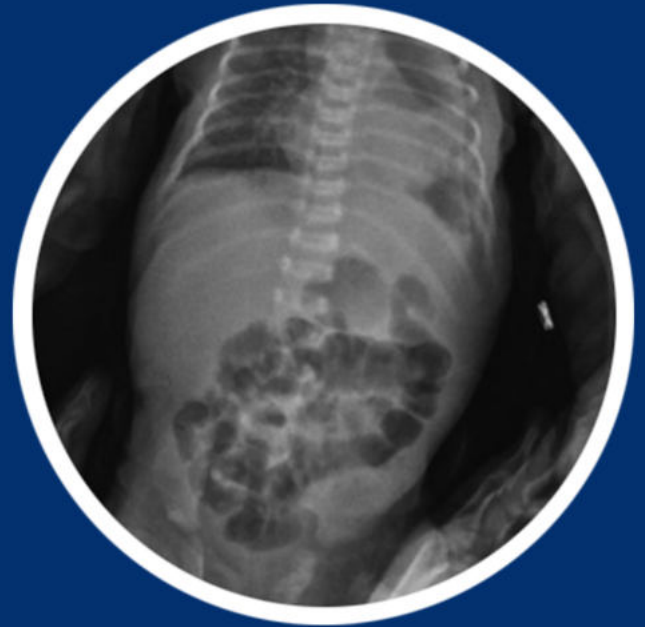
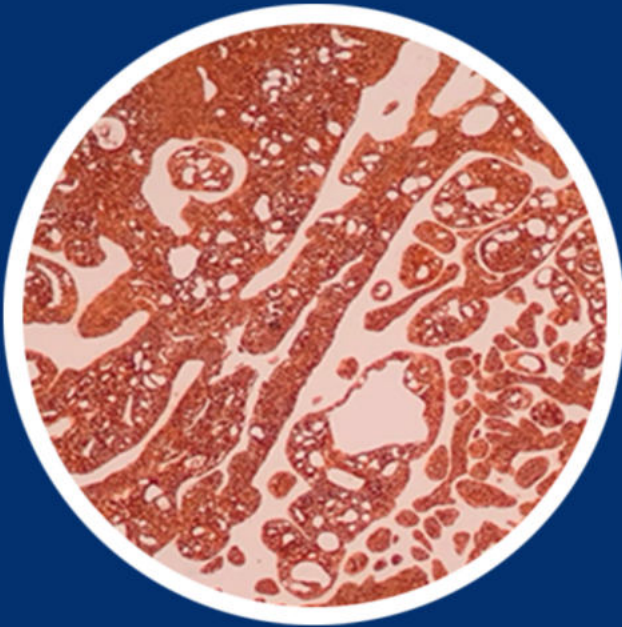
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# Hepatitis B seroprevalence in hematological oncology patients

Melda Türken<sup>1</sup>, Şükran Köse<sup>1</sup>, Ayşe Özkan Acar<sup>1</sup>, Cengiz Ceylan<sup>2</sup>, Hazal Albayrak<sup>1</sup>, Eren Arkalı<sup>1</sup>

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## ABSTRACT

**Objectives:** Hepatitis B virus (HBV) infection is still a serious public health problem today. Many factors such as frequent blood transfusion, hemodialysis, sexual contact, sterilization in surgical procedures, etc. are involved in the transmission of hepatitis B virus. In our study, HBV seroprevalence was evaluated retrospectively in order to provide vaccination of anti-HBs negative patients and to determine HBV prophylaxis in patients with hematological malignancy.

**Methods:** A total of 499 patients were included in the study. HBsAg, anti-HBs, anti-HBc IgG, anti-HDV, HBV DNA values were measured by ELISA (enzyme-linked immunosorbent assay, Liason, Diasorin, Italy) with Real-Time PCR (Cobas-Tagman, Roche Switzerland) and recorded. The obtained data were evaluated by SPSS for Windows 15.00 statistical program. A value of  $p < 0.05$  was accepted as a statistical significance value.

**Results:** HBsAg positivity was found to be 3.4% (n = 17) in all patients. Appropriate treatment regimens were started to patients with HBsAg (+). There were 166 (33.3%) patients with anti-HBs (+). One hundred nineteen (23.8%) patients had anti-HBc IgG (+), 40 (33.6%) of them were started a prophylactic treatment regimen. Anti-HBs positivity were reported after vaccination in 48 (16.8%) patients. Occult hepatitis have not been detected in patients with anti-HBc IgG positivity.

**Conclusions:** As a result of this study, anti-HBs negative patients with hematological malignancies were vaccinated. Patients and physicians should be informed about vaccination and hepatitis serology controls of hematological malignancy and other immunosuppressed patients. Sensitivity in this context should be increased in terms of prophylactic treatments.

**Keywords:** Hepatitis B virus, seroprevalence, hematological malignancy, oncology

Hepatitis B virus (HBV) infection is still a serious public health problem today. The prevalence of HBV in Turkey, an area where HBV infection is moderately endemic, is reported to be 2-7%, depending on the region [1]. In recent years there has been a decrease in carrier rates because of hepatitis B vaccina-

tion becoming more and more common [2].

Many factors such as frequent blood transfusion, hemodialysis, sexual contact, sterilization in surgical procedures, etc. are involved in the transmission of HBV [3]. Immunosuppressive therapies (such as steroids, cytotoxic chemotherapies) and frequent

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blood transfusions causing hematologic oncology patients to be involved in the risk group [4]. Due to the presence of viral hepatitis-associated exacerbations during the given immunosuppressive treatments HBsAg, anti-HBs, anti-HBc IgG screening especially important in this patient groups. HBV DNA testing should be performed in the case of HBsAg and / or anti-HBc IgG positivity [5]. Entecavir or tenofovir should be chosen for prophylactic treatment. Prophylactic treatment should be initiated as soon as possible in the HbsAg-positive patient (one week before or concurrent with immunosuppressive treatment, if possible) without losing time. Antiviral treatment should be continued for at least 12 months after immunosuppressive treatment, In case of HBsAg negative and anti-HBc positivity, prophylactic treatment is recommended if there is HBV DNA positivity. Patients in the low-risk group due to risky reactivation should be monitored at 3-month intervals with HBV DNA control [6]. Therefore, screening for viral hepatitis in all hematologic oncology patients is important.

In our study, it was aimed to determine the seroprevalence of HBV in patients who were diagnosed with hematologic malignancy in our hospital, to determine the patients who were anti-HBs negative but not yet vaccinated and to be vaccinated and to identify the patients who started treatment as prophylactic.

## METHODS

We retrospectively reviewed our patients who were diagnosed with hematological cancer in the hematology clinic of İzmir Tepecik Training and Research Hospital between 2007-2016. Ethics committee approval was taken from the local ethics committee in İzmir Tepecik Training and Research Hospital. Patients were examined retrospectively from patient records and files for demographic characteristics, hematologic cancer diagnosis, which treatment was initiated if treatment or prophylaxis

were given. Those who were younger than 18 years of age and were pregnant were not included in the study. A total of 499 patients were included in the study. Anti-HBc IgG, anti-HDV, HBV DNA values were measured by ELISA (enzyme-linked immunosorbent assay, Liason, Diasorin, Italy) with Real-Time PCR (Cobas-Tagman, Roche Switzerland) and recorded.

## Statistical Analysis

The obtained datas were evaluated by SPSS for Windows 15.00 statistical program. A value of  $p < 0.05$  was accepted as a statistical significance value. The mean age of the case groups was shown as mean  $\pm$  SD. The Kolmogorov-Smirnov test was used to assess whether the datas were fit to normal distribution. Mann-Whitney U test was used to compare the continuous variables of independent groups that did not show normal distribution. Group comparisons of nonparametric continuous variables belonging to more than 2 groups were made by Kruskal Wallis test.

## RESULTS

A total of 499 patients with hematologic malignancy were included in the study. One hundred ninety-two (38.4%) patients were female. The mean age of the patients was  $61.02 \pm 16.25$  years (Table 1). The most common hematological malignancies were multiple myeloma (MM), chronic lymphoblastic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL), acute lymphoblastic leukemia (ALL), Myelofibrosis, Hairy Cell Leukemia (HCL) respectively (Table 2).

HBsAg positivity was found to be 3.4% ( $n = 17$ ) in all patients (Table 2). Appropriate treatment regimens were started to patients with HBsAg (+). There were 166 (33.3%) patients with anti-HBs (+). One hundred nineteen (23.8%) patients had anti-HBc

**Table 1. Distribution of patients by gender and age**

Gender	n	Mean	Standard Deviation	Group median value
Female	192	63.04	15.15	64.38
Male	307	59.76	16.80	62.33
Total	499	61.02	16.25	63.26

**Table 2. Hepatitis B serological rate**

Diagnosis	HBsAg positive	HBsAg negative	Anti-HBsAg positive	Anti-HBsAg negative	Anti-HBc IgG positive	Anti-HBc IgG negative	Total
MM, n (%)	5 (4.3)	112 (95.7)	40 (32.4)	77 (65.8)	33 (28.2)	84 (71.8)	117 (100)
CML, n (%)	0 (0)	73 (100)	21 (28.8)	52 (71.2)	17(23.3)	56 (76.7)	73 (100)
CLL, n (%)	3 (3.1)	94 (96.9)	28 (28.9)	69 (71.1)	24 (24.7)	73 (75.3)	97 (100)
AML, n (%)	4 (4.7)	82 (95.3)	43 (50)	43 (50)	28 (32.6)	58 (67.4)	86 (100)
ALL, n (%)	1 (8.3)	11 (91.7)	4 (33.3)	8 (66.7)	1 (8.3)	11 (91.7)	12 (100)
NHL, n (%)	1 (1.5)	65 (98.5)	18 (27.3)	48 /72.4)	8 (12.1)	58 (90.9)	66 (100)
HL, n (%)	2 (5.9)	32 (94.1)	6 (17.6)	28 (82.4)	3 (8.9)	31 (91.1)	34 (100)
Myelofibrosis, n (%)	1 (12.5)	7 (87.5)	3 (37.5)	5 (62.5)	4 (50)	4 (50)	8 (100)
HCL, n (%)	0 (0)	6 (100)	3 (50)	3 (50)	1 (16.7)	5 (83.3)	6 (100)
Total, n (%)	17 (3.4)	482 (96.6)	166 (33.3)	333 (66.7)	119 (23.8)	380 (76.1)	499 (100)

MM = multiple myeloma, CLL = chronic lymphoblastic leukemia, AML = acute myeloid leukemia, CML = chronic myeloid leukemia (CML), NHL = non-Hodgkin's lymphoma, HL = Hodgkin's lymphoma, ALL = acute lymphoblastic leukemia, HCL = Hairy Cell Leukemia

IgG (+), 40 (33.6%) of them were started a prophylactic treatment regimen. Anti-HBsAg positivity were reported after vaccination in 48 (16.8%) patients. Occult hepatitis has not been detected in anti-HBc IgG positive patients.

## DISCUSSION

Natural course of hepatitis B infection is determined by the interaction between virus replication and the immune response of the host. HBV continues its existence in the HBV infected patients even if the serologic markers become negative. Therefore, there is a risk of HBV reactivation with immunosuppressive treatment in individuals who are infected with HBV. HBV reactivation may lead to the delay of treatment of primary disease and even to the ceasing of chemotherapy in these patients [7].

Turkey is among the moderately endemic (2-7%) regions due to hepatitis B infection [1]. While these rates vary according to the regions, the frequency of the HbsAg in the studies conducted in our country is found between 0.8-5.7%. According to the data obtained from the Turkish Red Crescent Blood Center, HbsAg positivity was found to be 0.6% in 2012 [8]. When HBsAg positivity is evaluated in our country, Eastern and Southeastern Anatolia Region is found to

be have higher rates than other regions [9].

In a study performed by Sardaş *et al.* [10], HBsAg of 16% and anti-HBs of 54% positivity were detected in hematology-oncology patients who received frequent transfusions. In control groups who never received transfusions HBsAg positivity of 4% and anti-HBs positivity of 22% were detected. It was concluded that patients who received frequent blood transfusions were under a higher risk of hepatitis B infection [10].

In a study by Sarı *et al.* [11], two groups were separated as transfusion and non-transfusion patients with hematological malignancy, and a third control group with no additional disease was established. HBsAg seropositivity in the first group was found to be 22.8%, in the second group 5.7% and in the third group 9%. In this study HBsAg seropositivity was found to be higher in patients with hematological malignancy and received frequent blood transfusion ( $p < 0.05$ ) [11]. Köse *et al.* [12] worked with 448 oncology patients in İzmir Tepecik Training and Research Hospital between 2006 and 2007. In their study, 19 (4.2%) HBsAg positivity was detected in the patients.

In some studies, it has been suggested that hepatitis B and C viruses may be associated with various lymphoid neoplasms because these viruses that can multiply in the lymphoid tissue. In the study



conducted by Yalçıntaş Arslan *et al.* [13], 165 non-cancer patients with 164 NHL were taken and 15 (9.1%) patients were found to be HbsAg positive and 3 (1.8%) patients were Anti-HCV positive. According to the control group, HbsAg positivity was high and anti-HCV positivity was found to be similar in patients with NHL [13]. In our study, HbsAg positivity was found to be 1.5% (1/67) in patients with NHL, but no significant difference was found compared to other groups ( $p > 0.05$ ).

In some studies, the relationship between MM and HBV and HCV infection was investigated. In a study by Huang *et al.* [14] 299 patients with MM and 299 patients with acute leukemia (AL) were evaluated. HbsAg positivity was found to be 19.4% in the MM-diagnosed group and 12% in the AL-diagnosed group and there was a significant difference between the two groups in terms of HbsAg positivity ( $p = 0014$ ) [14]. In our study, 5 (4.3%) HbsAg positivity was detected in 117 patients with MM. However, when compared with the other groups, there was no significant difference in HbsAg positivity in patients with MM ( $p > 0.05$ ).

Occult HBV infection is characterized by HBsAg negativity with persistence of low HBV DNA levels, regardless of Anti-Hbc IgG positivity. Immunosuppressive therapies are also likely to cause reactivation in this patient group [15]. The risk of reactivation by immunosuppression is much less than that of the HbsAg positive patient group. In one study with 204 HbsAg negative serum samples taken before cancer chemotherapy from cancer patients, isolated anti-Hbc IgG positivity was detected in 11 (5.4%) patients. HBV DNA positivity was detected in 9 (81%) of these 11 patients. Patients with hematologic malignancy had a higher incidence of occult hepatitis B compared to the group with solid organ malignancy. There was no significant difference between anti-Hbc IgG positivity and frequent blood transfusions, familial hepatitis and biochemical parameters (AST, ALT) ( $p > 0.05$ ) [16].

Although there are many seroprevalence studies with patients in the risk group such as transfusion, dialysis etc. there is a limited number of seroprevalence studies performed with hematological oncology patients [4]. The prophylactic treatment regimens initiated for the disease are lamivudine, tenofovir, entecavir. In our study, it was found that

many patients who were diagnosed as anti-HBsAg negative 333 (66.7%) were not vaccinated.

## CONCLUSION

As a result of this study, vaccination of Anti-HBsAg negative patients were provided with hematology clinic. In conclusion, patients and physicians should be informed about vaccination and hepatitis serology controls of hematological malignancy and other immunosuppressed patients. Sensitivity in this context should be increased in terms of prophylactic treatments.

### Ethical Statement

All procedures performed in studies involving human participant 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 of comparable ethical standards.

### Conflict of interest

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

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# Red cell distribution width and red cell distribution width to total serum calcium ratio as predictors of mortality in acute pancreatitis: a retrospective cohort study

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## ABSTRACT

**Objectives:** We investigated whether red cell distribution width (RDW) and red cell distribution width to total serum calcium (RDW-to-TSC) ratio were associated with mortality in patients with acute pancreatitis (AP).

**Methods:** A total of 170 patients who present emergency department between January 2017 and June 2018 with a diagnosis of AP were enrolled in this study. A comparison between survivor and nonsurvivor AP patients was performed. Diagnosis of AP were defined according to the revised Atlanta classification 2012. Variables evaluated included demographics, hospital stay, laboratorial parameters and mortality.

**Results:** AP related in-hospital mortality was 6.4%. Red cell distribution (RDW), white blood cell (WBC), platelet, serum glucose and RDW-to-TSC ratio were associated with AP mortality. RDW-to-TSC ratio had the highest AUC (0.88; 95% CI, 0.756-1.0), followed by RDW, serum glucose, platelet and WBC (AUC = 0.801, 0.797, 0.743 and 0.687 respectively). A cut-off value of RDW-to-TSC ratio >2.0, the highest sensitivity (91.9%), specificity (81.1%), PPV (82.9%), and NPV (91.1%) were achieved for prediction of AP related in-hospital mortality in approximately 86.5% of the cases ( $p < 0.001$ ).

**Conclusions:** RDW and TSC are simple, inexpensive routine serum parameters, provided in a complete blood count test and available at admission. This study showed that RDW > 16.6 and RDW-to-TSC > 2.0 were very good predictors for AP mortality.

**Keywords:** Acute pancreatitis, mortality, red cell distribution width, serum calcium

Acute pancreatitis (AP) is the sudden onset severe inflammation of pancreas and is one of the most frequent gastrointestinal causes of hospital admission [1]. The severity of the disease is varies from a mild, self-limiting form to a serious disease with severe local and systemic complications that may lead to multiorgan failure and death [2]. Several single and

multiparameter predictors for assessing severity of the disease has been described [3, 4]. However, most of them are complex and cannot be applied early enough.

The Ranson score, the Bedside Index for Severity in Acute Pancreatitis score, the Acute Physiologic Assessment and Chronic Health Evaluation II score and the Glasgow-Imrie criteria are currently in wide use

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[4]. Whereas, simplified serum markers such as C-reactive protein (CRP), blood urea nitrogen (BUN) procalcitonin, interleukin-6, interleukin-8, , have been applied to estimate the prognosis or severity of the AP, but their results have been inconsistent and unrelated to the severity of the disease [5]. Until now, a single serum marker cannot predict the severity or mortality in AP during admission. Red cell distribution width (RDW) is a routine parameter of the complete blood count (CBC) test, described as simple, easy, inexpensive and quantitative that measures size variability of erythrocytes [6]. RDW has been associated with inflammatory markers such as CRP and fibrinogen. Recent studies have shown that RDW is a strong and independent prognostic marker in many conditions such as cardiovascular diseases, pulmonary diseases and even cancer [7-9]. In addition to these conditions, RDW was also evaluated as a predictor of mortality in AP [10, 11].

RDW value and its association with other serum makers such as total serum calcium (TSC) has rarely been investigated as a potential biomarker of AP. Therefore, the aim of the study is to assess RDW and RDW-to-TSC ratio predictability for AP mortality.

## METHODS

This retrospective study was approved by local ethical committee and all procedures were performed according to Helsinki declaration. A total of 170 patients who present emergency department (ED) between January 2017 and June 2018 with a diagnosis of AP were enrolled in this study. The diagnosis of AP requires 2 of the following 3 criteria : upper abdominal pain of acute onset; serum amylase or lipase elevation  $\geq 3$  times the upper limit of normal; and characteristic findings of AP on contrast-enhanced computed tomography or abdominal ultrasonography [12]. We excluded patients with age < 18 years, abdominal trauma, lack of enough data, chronic pancreatitis, malignancy, pregnant patients,, having additional diseases affecting hematologic system (including lymphoma, leukemia, and bone marrow malignancies), or other chronic inflammatory diseases (including tuberculosis, Henoch-Schönlein purpura) and any autoimmune disorders and patients with time from onset of disease to presentation in the ED greater

than 24 h. At the time of hospital admission, a thorough medical history and physical examination were taken. Blood samples were also collected for laboratory investigations within 1 h of admission and included: CBC including white blood cells (WBC), neutrophil count, lymphocyte count, PDW, platelets (PLT) and RDW, liver function tests, BUN, TSC, serum glucose (only for non-diabetic patients), lactate dehydrogenase (LDH) and additionally, RDW-to-TSC ratio and RDW-to-platelets ratio were evaluated. The primary end point was in-hospital mortality.

## Statistical Analysis

Statistical analysis was carried out using social package for social sciences version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Normality of data distribution was assessed with Kolmogorov-Smirnov or Shapiro-Wilk test. Continuous data were expressed as mean and standard deviation (SD) or median and interquartile range based on the normality of distribution. Groups were compared using Student's t-test or Mann-Whitney test. Categorical variables were expressed as frequency and percentage and compared using X2-test or Fisher's exact test. All significant factors on univariate analysis were considered for inclusion in multiple regression analysis to predict AP-related in-hospital mortality. The receiver operating characteristic (ROC) curves were plotted to measure and compare the performance of different parameters to determine mortality risk and to select the best cut-off point at which sensitivity, specificity, positive (PPV) and negative (NPV) predictive value, positive and negative likelihood ratio (+LR,-LR) were calculated. All tests were two-tailed and statistical significance was assessed at  $< 0.05$ .

## RESULTS

### Characteristics of the Studied Patients

A total of 170 patients with a diagnosed of AP was taken to the present study. There were 104 (61.2%) male and 66 (38.8%) female with a male to female ratio of 1.57:1. The median age was 58 years and their age ranged from 24 to 98 years with a mean of  $57.68 \pm 16.2$ . There were 11 patients (6.4%) in the nonsurvivor group and 159 patients (93.6%) in survivor group. The mean hospital stay was  $8.3 \pm 9.2$

**Table 1. Summary of the demographic and clinical characteristics of the patients**

Parameter	Nonsurvivors (n = 11)	Survivors (n = 159)	<i>p</i> value
Age ( years)	62.6 ± 17.6	57.3 ± 16.1	0.335
Gender (M/F), n (%)	6/5 (54.5/45.5)	98/61 (61.6/38.4)	0.752
WBC count (10 <sup>3</sup> /mm <sup>3</sup> )	15.2 ± 5.5	12.1 ± 4.8	<b>0.038</b>
Neutrophil count (10 <sup>3</sup> /mm <sup>3</sup> )	12.4 ± 5.1	9.5 ± 5.1	0.057
Lymphocyte count (×10 <sup>3</sup> /mm <sup>3</sup> )	1.4 ± 0.6	1.5 ± 0.9	0.559
Platelet count (×10 <sup>3</sup> /mm <sup>3</sup> )	331.7 ± 100.5	253.4 ± 99.6	<b>0.007</b>
RDW (%)	18.8 ± 4.7	14.8 ± 1.8	<b>0.001</b>
Serum amylase (U/L)	212 (46-1104)	303.5 (29-3982)	0.323
Serum lipase (U/L)	496 (121-3710)	555 (14-9692)	0.713
AST (U/L)	120 (21-346)	57 (11-1768)	0.175
ALT (U/L)	46 (15-120)	45 (16-1056)	0.713
Total serum calcium (mg/dl)	7.9 ± 1.1	8.7 ± 0.8	<b>0.021</b>
Serum glucose (mg/dl)	238.8 ± 91.1	146.1 ± 62.9	<b>0.001</b>
Lactate dehydrogenase (U/L)	312 (155-427)	270 (121-2502)	0.653
RDW-to-TSC ratio	2.3 ± 0.5	1.7 ± 0.3	<b>&lt; 0.001</b>
RDW-to-platelets ratio	0.06±0.04	0.23 ± 1.96	0.504
Hospitalisation day (days)	14.7 ± 10.4	7.8 ± 9.1	<b>0.019</b>

Data are shown as mean ± standard deviation or median (range) or number (%). ALT = alanine aminotransferase, AST = aspartate aminotransferase, WBC = white blood cell, RDW = red cell distribution width, RDW-to-TSC ratio = red cell distribution width to total serum calcium ratio

days. Demographic, clinical, and laboratory characteristics of survivors and non-survivors were summarized in Table 1. All the non-survivors where the levels of WBC, platelet, RDW, serum glucose and RDW-to-TSC ratio were significantly increased, while the levels of calcium was significantly decreased (*p* < 0.05).

### Risk Factors Analysis for AP Related In-Hospital Mortality

To define the factors which can predict AP related in-hospital mortality, multiple regression analysis was applied using the significant variables from the univariate analysis (*p* < 0.05) (Table 1). It was found that increased WBC, PLT, RDW, serum glucose and

**Table 2. Multiple regression analysis of risk factors affected acute pancreatitis related in-hospital mortality**

Variables	Odds ratio	<i>p</i> value
WBC count (×10 <sup>3</sup> /mm <sup>3</sup> )	1.1 (0.9-1.17)	0.028
Platelet count (×10 <sup>3</sup> /mm <sup>3</sup> )	1.2 (0.8-1.69)	0.012
RDW (%)	1.1 (1-1.2)	0.031
Total serum calcium (mg/dl)	0.9 (0.7-1)	0.447
Serum glucose (mg/dl)	1.2 (0.9-1.4)	0.001
RDW-to-TSC ratio	0.9 (0.8-1.1)	<b>&lt; 0.001</b>

WBC = white blood cell, RDW = red cell distribution width, RDW-to-TSC ratio = red cell distribution width to total serum calcium ratio

**Table 3. Diagnostic models for prediction of acute pancreatitis related in-hospital mortality**

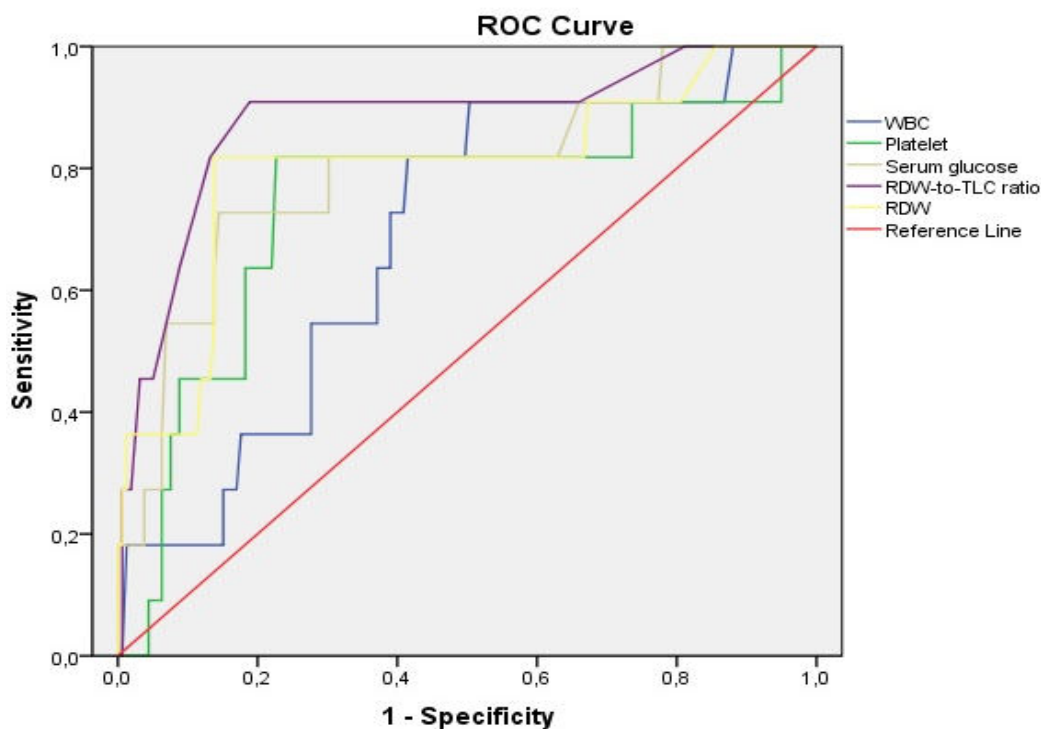
		B	SE	Wald	Sig.	Exp (B)	95% CI EXP (B) Lower-Upper		Percentage
Step 1	RDW	0.044	0.007	6.068	< 0.001	0.424	0.030	0.059	64
	Constant	0.396	0.112	3.554	< 0.001				
	RDW	0.023	0.012	1.931	0.055	0.222	-0.001	0.047	
Step 2	RDW-to-TSC	0.161	0.073	2.200	0.029	0.253	0.017	0.305	82.2
	Constant	0.432	0.111	3.875	< 0.001		0.212	0.652	

RDW = red cell distribution width, RDW-to-TSC ratio = red cell distribution width to total serum calcium ratio

RDW-to-TSC ratio were independent predictors of AP mortality ( $p < 0.05$ ) (Table 2). Moreover, using multiple regression analysis with forward LR method to construct a model with higher accuracy for prediction of AP related in-hospital mortality, a combined use of RDW and RDW-to-TSC ratio offered an accuracy of 82.2% (Table 3).

**Diagnostic Performance of Parameters for Prediction of AP Related In-Hospital Mortality**

We investigated the effectiveness of WBC, PLT, RDW, serum glucose and RDW-to-TSC ratio in prediction of AP related in-hospital mortality using ROC analysis. It was found that the area under curve (AUC) values of these variables were statistically



**Fig. 1.** Area under the receiver operating characteristic curve (AUC) of WBC, platelet, RDW, serum glucose and RDW-to-TSC ratio to predict AP-related in-hospital mortality. RDW-to-TSC ratio had the highest AUC in predicting mortality (AUC = 0.88) followed by RDW, serum glucose, platelet and WBC (AUC = 0.801, 0.797, 0.743 and 0.687 respectively). AP = Acute pancreatitis, RDW = Red cell distribution width, WBC = white blood cell, RDW-to-TSC = Red cell distribution width-to-total serum calcium.



**Table 4. Diagnostic accuracy of prognostic parameters to predict acute pancreatitis related in-hospital mortality with the best predictive cut-offs**

	AUC 95% CI	SEN (%)	SPE (%)	PPV (%)	NPV (%)	+LR	-LR	Accuracy (%)	p value
WBC ( $> 12.5 \times 10^3$ )	0.687 (0.541-0.833)	72.7	60.7	65.1	69.3	1.87	0.44	67	<b>0.038</b>
Platelets ( $299.5 \times 10^3$ )	0.743 (0.569-0.917)	81.8	77.4	78.1	81.1	3.57	0.23	73.5	<b>0.007</b>
RDW ( $> 16.6\%$ )	0.801 (0.640-0.963)	81.8	87.2	86.4	82.8	6.31	0.21	84.5	<b>0.001</b>
Serum glucose ( $> 151\text{mg/dl}$ )	0.797 (0.643-0.950)	83.6	70.7	74.3	81.6	2.9	0.23	77.5	<b>0.001</b>
RDW-to-TSC ratio ( $> 2.0$ )	0.88 (0.756-1)	91.9	81.1	82.9	91.1	4.84	0.1	86.5	<b>&lt; 0.001</b>

AUC = area under the curve, SEN = sensitivity, SPE = specificity, PPV = positive predictive value, NPV = negative predictive value, +LR = positive likelihood ratio, -LR = negative likelihood ratio, WBC = white blood cell, RDW = red cell distribution width, RDW-to-TSC ratio = red cell distribution width to total serum calcium ratio

significant to predict AP mortality (Figure 1). RDW-to-TSC ratio had the highest AUC (0.88; 95% CI, 0.756-1.0), followed by RDW, serum glucose, platelet and WBC (AUC = 0.801, 0.797, 0.743 and 0.687 respectively). Moreover, at a cut-off value of RDW-to-TSC ratio  $> 2.0$ , the highest sensitivity (91.9%), specificity (81.1%), PPV (82.9%), and NPV (91.1%) were achieved for prediction of AP related in-hospital mortality in approximately 86.5% of the cases ( $p < 0.001$ ) (Table 4).

## DISCUSSION

Recent studies have shown that RDW is a remarkable prognostic marker to determine the mortality risk in a wide range of clinical manifestations such as elderly patients with or without age-related diseases intensive care unit patients, cardiovascular diseases, and even those with acute dyspnea and pneumonia [13-15]. RDW has been recognized as a remarkable prognostic marker to determine the risk of mortality in many cases other than the AP, reflecting the state of inflammation [13,14]. In a recent study, RDW was independently associated with AP mortality [10]. The present study showed that RDW at admission and in the first 24 h

were good predictors of AP mortality with AUC  $> 0.801$ , which was higher than in other works with a reported AUC of 0.74-0.82 [10, 16]. Furthermore, we found an independent relationship with RDW-to-TSC ratio and AP mortality with AUC of 0.88. In our study, the RDW to TSC ratio and RDW were superior to prognostic scores in determining AP mortality. In a study also investigated the effect of RDW-platelet ratio on AP mortality with an AUC of 0.783 [17]. In contrast, our work showed that it was not an independent prognostic factor for mortality ( $p = 0.504$ ).

In the present study, the diagnostic accuracy of RDW (AUC  $> 0.801$ ) was significantly higher than the other studied risk factors with 81.8% sensitivity, 87.2% specificity, 86.4% PPV and 82.8% NPV at cut-off  $> 16.6\%$ . This was comparable to the findings of Şenol *et al* [10], who reported RDW having AUC of 0.817 with 47.6% sensitivity, 96.3% specificity, 79.9% PPV and 87.6% NPV at cut off  $> 14.8$  in predicting fatal outcome in AP patients. In prior studies found that serum glucose level on admission was significantly higher among AP non-survivors. This was consistent with previous studies showing that serum glucose was considered as a satisfactory predictor of mortality in AP. Our findings revealed that at a cut off value  $> 151\text{mg/dl}$ ; it had an AUC of 0.797,

83.6% sensitivity, 70.7% specificity, 74.3% PPV and 81.6% NPV in predicting AP-related in-hospital mortality.

In the past, RDW has been widely used in the differential diagnosis of anemia. In recent years, RDW has been associated with systemic inflammation because of high oxidative stress that contributes to reducing red blood cell (RBC) and RDW elevation and increasing the release of large premature RBCs in the peripheral circulation and modifying RBC membrane glycoproteins and ion channels contributes to the change of RBC morphology [18, 19]. For this reason, RDW reflects systemic inflammation and thus, can be used to estimate the severity of the AP. Although TSC did not represent a good marker for AP mortality in our study, RDW-to-TSC ratio has been shown to be a good indicator of AP mortality.

### Limitations

There are several limitations for this study. First, our study is retrospective nature. Second, the sample size of this study is relatively small and the results are only from one center. Third, one of the most important limitations of our study is the lack of evaluation of comorbid diseases such as cardiovascular disease that may have an impact on mortality. Another limitation is that blood parameters before and during hospitalization of patients have not been studied. Further studies are needed with larger patient populations with multiple centers to more accurately assess RDW as a determinant of mortality.

### CONCLUSION

In conclusion, WBC, platelet, RDW, serum glucose and RDW-to-TSC ratio can predict AP related in-hospital mortality, where, RDW and RDW-to-TSC ratio have the higher prognostic accuracy. RDW > 16.6 and RDW-to-TSC > 2.0 were very-good predictors for AP mortality. Further prospective and multicenter studies are needed to more accurately evaluate the effect of high RDW as a marker for mortality in AP.

### Authorship declaration

All authors listed meet the authorship criteria

according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

### Conflict of interest

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

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# Increased mean platelet volume in patients with vestibular migraine

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## ABSTRACT

**Objectives:** The purpose of this study was to investigate relation between mean platelet volume, platelet distribution width, platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio and vestibular migraine.

**Methods:** This study was planned in prospective manner and conducted in Ankara Polatlı State Hospital between April 2017 and May 2018. Thirty patients diagnosed with vestibular migraine and thirty healthy, age and sex-matched subjects were enrolled to the study. Mean platelet volume, platelet distribution width, platelet count, neutrophil count and lymphocyte count in blood samples were measured.

**Results:** The mean age of the patients with vestibular migraine was  $39.90 \pm 7.16$  and the study group consisted of 11 males and 19 females. The mean platelet volume and platelet distribution width values in patients with vestibular migraine were significantly higher than the control group ( $p < 0.001$ ). There were no difference on mean neutrophil/lymphocyte ratio and platelet/lymphocyte ratio values between patient group and control group.

**Conclusions:** We found a positive relation of increased levels of mean platelet volume and platelet distribution width with vestibular migraine. Higher mean platelet volume is associated with atherosclerosis and thromboembolism. Vascular pathologies are blamed for both headache syndromes like migraine and vertiginous syndromes. This theory supports our study but pathophysiological mechanism is not clear.

**Keywords:** Hematologic parameters, mean platelet volume, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, vestibular migraine

Vestibular migraine is an important clinical syndrome characterised by migraine type headaches accompanied with vertigo. Relation between migraine and vertigo is a complex issue and not well understood. Migraine and vertigo are common problems and a patient may have both of them by chance [1]. Thirty eight percent of migraine patients have vertigo [2-3].

Also many vertiginous pathologies are associated with migraine epidemiologically. Benign paroxysmal positional vertigo, Meniere disease, motion sickness and cerebellar disorders are associated with migraine [1]. So differentiation of vestibular migraine from these pathologies is a clinical challenge. Vestibular migraine is one of the frequent causes of both headache and ver-

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tigo. Migraine prevalence is 16% and vertigo prevalence is 7% [1]. According to these values expected comorbidity of vertigo and migraine is 1.1% but Neuhauser *et al.* [4] showed vestibular migraine prevalence as 3.2%. In the view of such information many authors think vestibular migraine as a specific clinical entity rather than coincidental coexistence of vertigo and migraine.

Diagnosis of vestibular migraine is a challenge because pathophysiology is not well understood and there is no specific examination finding, biological marker or laboratory test [2]. Detailed and careful history is important and high index of suspicion is needed for clinician. Because of difficulties about diagnosis important part of patients are misdiagnosed [3].

Mean platelet volume (MPV) is average size of platelets and included in complete blood count. MPV gives information about platelet production rate. If platelet production accelerates in bone marrow MPV gets higher. Enlarged platelets have higher activity and produce vasoactive substances, so high MPV levels are associated with vasospasm and thrombosis [5]. Increased levels of MPV, Platelet Distribution Width (PDW), Platelet-to-Lymphocyte Ratio (PLR) and Neutrophil-to-Lymphocyte Ratio (NLR) are also associated with inflammatory pathologies and vascular pathologies such as atherosclerosis, thromboembolism [6].

There are many theories for explanation of pathophysiology of vestibular migraine. One of these theories is reversible vasospasm of the internal auditory

artery or its branches. This vasospasm causes the ischemia of the labyrinth and then resulting in cochleovestibular dysfunction and vertigo experienced in vestibular migraine [7-9].

MPV, PDW, PLR and NLR values in vestibular migraine have not been investigated yet and we aimed to show if there is a relation of these parameters with vestibular migraine.

## METHODS

This study was approved by the local ethics committee for non-invasive research and conforms to the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients at the beginning of the study.

This study was planned in prospective manner and conducted in Ankara Polatlı State Hospital between April 2017 and May 2018. Thirty patients diagnosed with definite vestibular migraine according to the modified Neuhauser criteria by Lempert *et al.* [10] (Table 1) and thirty healthy, age and sex-matched subjects were enrolled to the study. Patients were diagnosed with vestibular migraine by neurology specialist and referred to otolaryngology clinic. All patients underwent a detailed otolaryngologic and neurologic examination. Brain and ear magnetic resonance imaging with using gadolinium as a contrast medium were obtained from all patients.

Patients were excluded from the study if they had

**Table 1. Vestibular migraine diagnostic criteria [10]**

A. At least 5 episodes with vestibular symptoms of moderate or severe intensity, lasting 5 minutes to 72 hours
B. Current or previous history of migraine with or without aura
C. One or more migraine features with at least 50% of the vestibular episodes
1. headache with at least two of the following four characteristics:
a) one sided location
b) pulsating quality
c) moderate or severe pain intensity
d) aggravation by routine physical activity
2. photophobia and phonophobia
3. visual aura
D. Not better accounted for by another vestibular or ICHD diagnosis

acute inflammation, infection, pneumonia, chronic obstructive pulmonary disease (COPD), asthma, the diagnosis of acute or chronic pulmonary thromboembolism, coronary artery disease, congestive heart failure, renal and hepatic dysfunction, hematological diseases, cancer, auto-immune diseases, diabetes mellitus, systemic hypertension, obstructive sleep apnea, connective tissue diseases, inflammatory bowel diseases, the use of antithrombotic agents, and smoking history.

**Biochemical analyses**

MPV, PDW, platelet count, neutrophil count and lymphocyte count in blood samples were measured. Venous blood samples were obtained from antecubital vein at the time of vertigo attack and collected into tubes containing ethylenediaminetetraacetic acid (EDTA) at 9 am. following an overnight fast. To avoid platelet swelling measurements were done shorter than 30 minutes after sampling. An automated blood cell counter was used for these measurements (Horiba ABX Pentra DX 120).

**Statistical Analysis**

Continuous data were summarized as mean±standard deviation, and categorical data were as frequency and percentage. Independent sample t test was used to compare groups for continuous variables. Relation between categorical variables were analyzed by Chi-Square test. Statistical analyses were

done with SPSS v.22.0 for Windows statistical package, and statistical significance level was considered as 0.05.

**RESULTS**

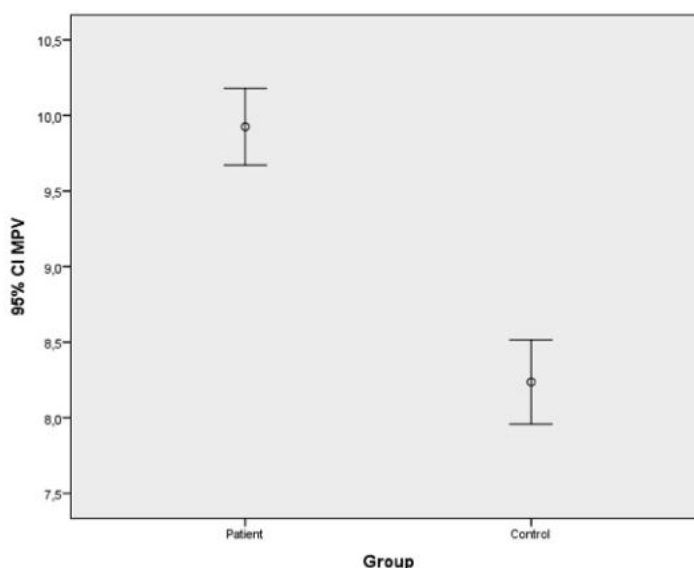
The mean age of the patients with vestibular migraine were  $39.90 \pm 7.16$  years and  $37.93 \pm 7.62$  years for the control group. The study group consisted of 11 males and 19 females and the control group consisted of 17 males and 13 females. There was no significant difference between age or gender in two  $p = 0.307$  and  $p = 0.121$ , respectively) (Table 2).

The mean MPV values were  $9.93 \pm 0.68$  fl in vestibular migraine patients and  $8.24 \pm 0.75$  fl in the control group. The mean MPV values in patients with vestibular migraine were significantly higher than the

**Table 2. Demographic data of the study and the control groups**

	Study group	Control group	p value
Age	$39.90 \pm 7.16$	$37.93 \pm 7.62$	0.307
Sex			
Female	19 (63.3%)	13 (43.3%)	0.121
Male	11 (36.7%)	17 (56.7%)	

The data are expressed in mean ± standard deviation or in numbers and percentages.



**Fig. 1. The mean MPV values of the patients with migrainous vertigo and the control group.**

**Table 3. Comparison of the mean MPV, PDW values and platelet counts of the study and the control groups**

	Study group	Control group	p value
MPV (fl)	9.93 ± 0.68	8.24 ± 0.75	< <b>0.001*</b>
PDW (%)	18.67 ± 1.53	14.08 ± 2.06	< <b>0.001*</b>
Platelet count (10 <sup>3</sup> /mm <sup>3</sup> )	274.83 ± 64.58	306.00 ± 106.32	0.175

The data are expressed in mean ± standard deviation. MPV = mean platelet volume, PDW = platelet distribution width.

\* $p < 0.05$  value was regarded as significant while the significant differences between the groups are shown in bold.

control group ( $p < 0.001$ ) (Fig. 1). The mean PDW values were 18.67% ± 1.53% in vestibular migraine patients and 14.08% ± 2.06% in control group. The mean PDW values in patients with vestibular migraine were significantly higher than the controls ( $p < 0.001$ ). The mean values of platelet count of the patients and the control group were 274.83 ± 64.58 (10<sup>3</sup>/mm<sup>3</sup>) and

306.00 ± 106.32 (10<sup>3</sup>/mm<sup>3</sup>), respectively. There were no difference on platelet count levels between groups ( $p = 0.175$ ) (Table 3).

The mean NLR values were 2.19 ± 0.86 in the patient group and 2.15 ± 1.52 in the control group. The mean PLR values were 138.24 ± 50.83 in the patient group and 127.39 ± 45.31 in the control group. There

**Table 4. Comparison of the mean NLR and PLR ratio values of the study and the control groups**

	Study group	Control group	p value
NLR	2.19 ± 0.86	2.15 ± 1.52	0.902
PLR	138.24 ± 50.83	127.39 ± 45.31	0.387

The data are expressed in mean ± standard deviation. NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio

were no difference on NLR and PLR between groups ( $p = 0.902$  and  $p = 0.387$ , respectively) (Table 4).

## DISCUSSION

Pathogenesis of vestibular migraine is not well understood but there are various etiopathogenetic theories. According to cortical spreading theory cortical depolarisation wave produce vertigo [8]. Some neuropeptides are released asymmetricly and influence vestibular centers. This neuropeptid stimulation may cause vertigo at the time of migraine attack [11]. Another explanation is based on internal auditory artery spasm –may also be caused by serotonin- at the time migraine attack [7-9].

MPV and platelet count are calculated in complete blood count test routinely and MPV is unnoticed by

the clinicians most of the time. MPV and platelet count levels give information about vasospasm and thrombotic function [6]. Main finding of our study was that MPV levels were significantly higher in vestibular migraine patients than the control group.

Relation of MPV and migraine or other headache syndromes is not investigated yet and there is only single study in literature which investigated possible relation of MPV and vertigo. Celikbilek *et al.* [12] showed MPV levels significantly higher in benign paroxysmal vertigo patients than in controls.

Some authors studied MPV values in patients with inner ear diseases and showed controversial results. Kemal *et al.* [13] and Sarıkaya *et al.* [14] reported that MPV values were significantly higher in tinnitus patients than the control group. But Beyan and Beyan [15] reported that there was no relation between high levels of MPV values with tinnitus. Recent study by

Ulusoy *et al.* [16] showed that MPV values were significantly higher in tinnitus patients than the control group. MPV values were studied also in sudden sensorineural hearing loss (SSHL) patients and results were controversial like tinnitus patients. Ulu *et al.* [17] and Sagit *et al.* [18] found high levels of MPV were associated with SSHL significantly. Durmus *et al.* [19] also reported that MPV values were significantly higher in SSHL patients than the control group [19]. In other two studies by Karli *et al.* [20] and Kum *et al.* [21] mean MPV values of SSHL patients were higher than the control group but differences were not significant. Ozturk *et al.* [22] also found no difference between SSHL and control group MPV values.

This is the first study which investigates relation of mean platelet volume with vestibular migraine. In our study we found a positive relation of increased levels of MPV with vestibular migraine. But mechanism of this positive relation is not clear. We know that higher MPV is associated with vasospasm and thromboembolism [23]. Vascular pathologies are blamed for both headache syndromes like migraine and vertiginous syndromes. This theory supports our study but pathophysiological mechanism is not understood yet.

## CONCLUSION

MPV can be considered as a practical and valuable parameter for assessment of vestibular migraine. And further studies are needed for explanation of relation between MPV values and vestibular migraine.

### Conflict of interest

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

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# Adolescent pregnancies: a 10-year single-center experience

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## ABSTRACT

**Objectives:** To investigate adolescent pregnancy rate in last decade and emphasize the perinatal outcomes.

**Methods:** Our study was a retrospective investigation of all women with singleton pregnancies who gave birth at > 20 weeks gestation aged 13-16 years old (early aged adolescent pregnancy group, n = 107), aged 17-19 years old (late aged adolescent pregnancy group, n = 991) and aged 20-35 years old (control group, n = 1,098) at Ordu University School of Medicine, Training and Research Hospital Obstetrics and Gynecology Clinic between January 2008 and January 2018. The variables used to determine the perinatal outcomes were low birth weight (birth weight < 2,500 g), macrosomic fetus (birth weight > 4,000 g) and stillbirth (delivery of infant > 20 weeks gestation without cardiac activity) prevalences were investigated and compared between groups.

**Results:** Adolescent birth ratio to all births was 4.4% (1,098/24,560). Low birth weight rate was higher in the late aged adolescent group ( $p < 0.001$ ). Cesarean section rate was significantly lower in the adolescent age group (40.2% in early aged and 7.2% in late aged) whereas rate was 56.3% in the control group. We attributed this to the high parity in the control group and the surplus of the old cesarean section indication ( $p < 0.001$ ).

**Conclusions:** Adolescent pregnancy, especially late aged adolescents were found to be closely related with low birth weight but there was no significant difference with respect to stillbirth rate in adult age group. Skilled antenatal, childbirth and postnatal care is very necessary to reduce low birth weight and therefore perinatal mortality.

**Keywords:** Adolescent pregnancy, low birth weight, public health concern

Adolescence is a transformative process between childhood and adulthood with biological, psychological and social changes. This is a very special period that influences the adolescent's and their newborn's future health and life [1, 2]. The World Health Organization (WHO) identifies adolescence as the period between 10-19 years. Secondary sexual characteristics, reproductive ability, mental processes, identity and independence develops in this period. According to the WHO report adolescents account for

30% of the world population and 95% of adolescents lives in developing countries [3-5].

Adolescent pregnancy, typically defined as a pregnancy in a female within the ages of 13-19. This is not new but the most important global health concern among the adolescence girls that occurs in high, middle and low income countries. Additionally poverty, lack of education and employment increases the incidence of adolescent pregnancy. Although 90% of births occur within marriage, for many adolescents

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pregnancy is neither planned nor wanted. According to the WHO fact sheet updated February 2018; every year 23 million adolescent girl become pregnant and 18.5 million girls give birth each year in developing regions. Almost 4 million girls undergo unsafe abortions every year. The report also emphasized that the global adolescent birth rate has declined from 6.5% in 1990 to 4.7% in 2015. Despite this overall progress, because the global population of adolescents continues to grow, projections indicate the number of adolescent pregnancies will increase globally by 2030, with the greatest proportional increases in Africa [5].

No doubt pregnancy is the most important life event for women and necessitates physical, mental and socioeconomic adaptations. Yet the adolescent girls' adaptation capacity is not enough so lots of problems may occur. Complications during pregnancy and childbirth are the second leading cause of death for adolescent girls globally [6].

In our study we aimed to investigate adolescent pregnancy rate in last decade and emphasize the perinatal outcomes.

**METHODS**

This study was conducted after receiving approval from the ethics committee of Ordu University School of Medicine, Training and Research Hospital.

Our study was a retrospective investigation of all women with singleton pregnancies who gave birth at ≥ 20 weeks gestation aged 13-16 years old (early aged adolescent pregnancy group, n =107), aged 17-19 years old (late aged adolescent pregnancy group, n = 991) and aged 20-35 years old (control group, n = 1,098) at Ordu University School of Medicine, Training and Research Hospital, Obstetrics and Gynecology Clinic between January 2008 and January 2018. The control group was randomly formed aged 20-35 years old women from 24,560 births in the last

decade. Pregnant women ≥ 35 years old were excluded from the study for not to affect the pregnancy risks of control group. Maternal age was defined as the completed age of the pregnant at the time of delivery.

Parameters such as age, parity, fetal sex, fetal birth weight, birth pattern (cesarean and vaginal delivery) were examined from hospital registry system and file scanning. The variables used to determine the perinatal outcomes were low birth weight (birth weight ≤ 2,500 g), macrosomic fetus (birth weight ≥ 4,000 g) and stillbirth (delivery of infant ≥ 20 weeks gestation without cardiac activity) prevalences were investigated and compared between groups.

**Statistical Analysis**

Statistical analyses were performed with the SPSS 20.0 program package. Mann Whitney-U test or independent samples-t test were used to compare continuous variables, chi square test was used to compare categorical variables.

**RESULTS**

The records of 24,560 patients who delivered between January 2008 and January 2018 were reviewed. Adolescent birth ratio to all births was 4.4% (1,098/24,560). The mean age of adolescent pregnancies (under 19 years old) was 17.9 ± 1.1 years (range; 13-19 years). Singleton pregnancies who gave birth at ≥ 20 weeks gestation were seperated three groups; aged 13-16 years old (early aged adolescent pregnancy group, n = 107), aged 17-19 years old (late aged adolescent pregnancy group, n = 991) and aged 20-35 years old (control group, n = 1,098) (p < 0.001). Comparison of mean age among the groups were given in Table 1.

There was a significant difference between the groups according to parity. The average of parity was 1.22 (range; 1-3) (early aged) and 1.25 (range; 1-4)

**Table 1. Mean age and standart deviation of groups**

Parity of groups	All Adolescents	Early aged Adolescent	Late aged Adolescent	Control	p value
Mean age	17.9	15.57	18.19	26.42	< 0.001
Standart deviation	1.1	0.72	0.78	4.25	



**Table 2. Mean parity and range of parity of groups**

Parity of groups	All Adolescents	Early aged Adolescent	Late aged Adolescent	Control	p value
Mean parity	1.24	1.22	1.25	2.18	< 0.001
Range of parity	1-4	1-3	1-4	1-7	

(late aged) in the adolescent pregnancy groups whereas 2.18 (range; 1-7) in control group ( $p < 0.001$ ). Comparison of parity average among the groups were given in Table 2.

Birth weight was significantly lower in the adolescent age group. The average of birth weight were  $3,201 \pm 445$  g (early aged) and  $3,158 \pm 500$  g (late aged) in the adolescent groups whereas  $3,312 \pm 523$  g in control group. The birth weight of the late aged adolescent group was lower than the birth weight of the early aged adolescent group ( $p < 0.001$ ).

Low birth weight incidence was significantly higher in the adolescent age group. The rates were 4.7% in early aged groups, 7.4% in late aged groups and 5.8% in control groups. Low birth weight rate was higher in the late aged adolescent group ( $p < 0.001$ ). Macrosomic birth weight incidence was significantly lower in the adolescent age group. The rates were 6.5%, 3% and 8.9% in groups. Macrosomic birth weight rate was higher in the early aged adolescent group ( $p < 0.001$ ).

Cesarean section rate was significantly lower in the adolescent age group (40.2% in early group and 37.2% in late groups) whereas rate was 56.3% in the

control group. We attributed this to the high parity in the control group and the surplus of the old cesarean section indication ( $p < 0.001$ ).

There was no significant difference between the fetal sexes. The male fetus rate was 51.4% in early groups, 48.8% in late groups and 53.2% in control groups. Late aged adolescent group has a bit female dominance whereas the other groups have male dominance ( $p = 0.139$ ).

There was no significant difference between the stillbirth rates (0.1% vs.0.5%). There was no stillbirth in the early aged adolescent group ( $p = 0.209$ ).

Comparison of fetal sexes and perinatal outcomes (low birth weight, macrosomic fetus, stillbirth, (delivery of infant  $\geq 20$  weeks gestation without cardiac activity) prevalences among the groups were given in Table 3.

## DISCUSSION

According to the WHO report adolescents account for 30% of the world population and 95% of adolescents lives in developing countries. Because of

**Table 3. Mean birth weight, low/macrosomic birth ratios, cesarean ratio, still birth ratio, fetal sex among the groups.**

Other parameters	All Adolescents	Early aged Adolescent	Late aged Adolescent	Control	p value
Mean birth weight (gr)	3162 + 495	3201 + 445	3158 + 500	3312 + 523	< 0.001
Low birth ratio (%)	7.1	4.7	7.4	5.8	< 0.001
Macrosomic birth ratio (%)	3.3	6.5	3	8.9	< 0.001
Cesarean ratio (%)	37.5	40.2	37.2	56.3	< 0.001
Still birth ratio (%)	0.9	0	1	0.5	0.209
Fetal sex (male) (%)	49.1	51.4	48.8	53.2	0.139

this situation the adolescent population is important and the problems of this group are affecting the whole world population. Among adolescent population adolescent pregnancy is the most important public health concern with complications during pregnancy and childbirth serving as the second leading cause of mortality in the adolescent age group worldwide that can not be ignored because of the effects of whole world population [3-5].

Adolescent pregnancy is associated with many factors. Early marriages for cultural reasons, lack of information on contraception methods, inadequate information at school and family, low sociocultural and socioeconomic level, concern to approve collective adulthood, the effects of westernization and urbanization, ethnic factors and low education level are the related factors [7]. Prevention of adolescent pregnancies must be the first-line approach. According to a research (School-Based Teen Pregnancy Prevention Programme) combination of educational and contraceptive interventions seem to reduce adolescent pregnancy [8].

In the last 25 years the global adolescent birth rate has declined from 6.5% to 4.7%. But the global adolescent population continues to grow rapidly. Because of this reason projections indicate the number of adolescent pregnancies will increase globally (especially in Africa) by 2030 [9].

Adolescent pregnancies differ between recipient countries and even within the same country. According to the Turkey Demographic and Health Survey data adolescent birth rate has declined remarkably from 10.2% in 1993 to 4.6% in 2013 [10]. In several Turkish studies, the Adolescent birth rates are lower than literature; 1.3% (Tokat area), 1.3% (Van area), 2.9% (Eskisehir area) [11-13].

Adolescent pregnancies are high risk pregnancies and lead to many socioeconomic problems. These problems include emotional lability/depression, loneliness, social isolation, poverty, low-income/unemployment, financial dependence to partner, homelessness, domestic violence, familial/social stigmatization/boycott and inability to go to school and work [14]. According to a recent study from Mersin area in Turkey, approximately one-fifth adolescents were either illiterate or had dropped out of the primary school and all pregnant adolescents were housewives with a low economic status [15].

Therefore pregnant adolescents need social support from their friends and families. Pregnant adolescents and their families should be informed about the importance of social support and the ways to mobilize social support sources [15].

Adolescent pregnant face higher risks of maternal-fetal complications as indicated in the systematic review from Brazil. Although pre-eclampsia/eclampsia, HELLP syndrome, abortion, prolonged rupture of membranes, urinary tract infection, heart and thyroid disease, placenta previa, prolonged labor, breech presentation is more common in adolescent pregnant, with a predominance of articles emphasizing prematurity, low birth weight, and perinatal mortality [16]. Additionally prenatal care is mostly inadequate among adolescent pregnant and it is known that regular prenatal visits can decrease the complications of pregnancy in this age group. Skilled antenatal, childbirth and postnatal care is very necessary to reduce low birth weight and therefore perinatal mortality [17].

Additionally many previous studies have shown that smoking, drug addiction and alcohol misuse are also more common among adolescent girls that increases the risk of maternal-fetal complications [18].

Low birth weight is one of the most determined parameter that associated with adolescent pregnancies [19, 20]. There is a consensus among the Turkey's data; almost all of the researchers have detected relation between adolescent pregnancy and low birth weight [21-26]. In the recent study we have founded a significant relation between low birth weight and adolescent pregnancy (3,201 - 3,158 vs 3,312) like the literature.

Increasing cesarean rate is a major problem in all over the world. For many years there was the belief that cesarean was more common in adolescent pregnancies. According to some studies, this excess was attributed to the incomplete development of bone pelvis in adolescents and the excess of cephalopelvic distosia indication. But recently most of studies have showed that the cesarean ratio of adolescents is lower than the adult population [27, 28]. When we look the Turkey data; Ergen *et al.* [21], Taner *et al.* [22], Melekoğlu *et al.* [23] and Seçkin *et al.* [24] have founded lower cesarean rates in adolescents, whereas Keskin *et al.* [25] and Akdemir *et al.* [26] and have founded higher cesarean rates. In our study, lower

cesarean rate was similar to the general literature (40.2% - 37.2% vs 56.3%).

Adolescent pregnancy has further long term consequences for mothers and newborns. Adolescent mothers have higher risks of negative mental health outcomes. Almost half of adolescent mothers experienced moderate to severe depression during the first postpartum year. Teenage mothers are less likely to complete their education and are more likely to be unqualified [29]. Newborns of adolescent mothers are also disadvantaged that they have a higher infant mortality and are less likely to be breast fed [30]. According to the United Kingdom report adolescent mothers were more likely to have a stillbirth and neonatal death than adult mothers [31]. According to our study, higher stillbirth risk among late aged adolescent pregnant exists yet it was not significant.

Adequate contraception after childbearing is essential for all adolescent mothers. Approximately 25% of adolescent mothers have a second child within two years. Additionally the repeat pregnancy is associated with increase in adverse pregnancy outcomes [32]. Interventions to reduce the incidence of adolescent pregnancy is the most important issue. The National Campaigns advocates to reduce adolescent pregnancy by focusing on sexual education in family and school, counseling contraception options, providing youthfriendly clinical services, implementing communitywide programs, and providing national support for programs [33, 34]. The American Congress of Obstetricians and Gynecologists recommends LARC (Long-acting reversible contraceptives) - include intrauterine devices (IUDs) and the etonogestrel implants - be offered as first-line contraceptive options for all adolescents [35].

On the other hand sexual violence is widespread and especially affects adolescents. According to WHO data 20 % of adolescent girls around the world exposed to sexual abuse including incest. Regulation of adolescent protection laws is very important in this regard [36].

Adolescent who decided to continue pregnancy should be referred as soon as possible to a clinic where comprehensive pregnancy counselling is available. All risks about continuing the pregnancy should be discussed. Optimal antenatal care must be given to improve perinatal outcomes [37].

## CONCLUSION

The prevalence of adolescent pregnancy for 10 years was 4.4% in our study. Although the cesarean rate of adolescent pregnancies seems to be less than that of the adult age group, 37.5% is higher than the acceptable primary cesarean rate. Policies to reduce cesarean rates should be supported. Adolescent pregnancy, especially late aged adolescents were found to be closely related with low birth weight but there was no significant difference with respect to stillbirth rate in adult age group. Finally, the level of awareness about contraceptive methods should be increased among the adolescent population and if the continuation of pregnancy is planned, it should be considered as high-risk pregnancy and close follow-up should be provided.

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# The additional diagnostic value of NLR and PLR for CA-125 in the differential diagnosis of endometrioma and benign ovarian cysts in women of reproductive age: a retrospective case-control study

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## ABSTRACT

**Objectives:** Aim of this study is to investigate the diagnostic value of neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) for cancer antigen-125 (CA-125) in a differential diagnosis of endometrioma from benign ovarian cysts.

**Methods:** In this retrospective study, the medical data of a total of 213 patients aged < 40 years who underwent laparoscopic or laparotomic surgery for ovarian cysts between April 2015 and June 2018 were analyzed. The patients were divided into two groups, as those with endometriomas and those with other benign ovarian cysts, all which had been confirmed histopathologically. Data on age, body mass index (BMI), preoperative US findings, complete blood count analysis results, follicle-stimulating hormone (FSH) levels, and the presence of dysmenorrhea, dyspareunia and chronic pelvic pain were recorded.

**Results:** NLR, PLR and CA-125 were increased in the patients with endometrioma, although increases in the NLR and PLR alone or combined did not contribute to the sensitivity or specificity of CA-125.

**Conclusions:** The results of our study suggest that NLR and PLR did not contribute to the diagnostic value of CA-125 in the preoperative differential diagnosis of endometriomas or other benign ovarian cysts. In addition, CA-125 was not associated with clinical symptoms, although a relationship was identified between NLR and dysmenorrhea, and between PLR and lesion size, which may be the research focus of further studies.

**Keywords:** Endometrioma, benign ovarian cyst, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, CA-125

Endometrioma is a type of benign ovarian cyst that is characterized by the presence and growth of functioning endometrial tissue in the ovaries [1]. Although it is known to be detrimental to fertility, the underlying pathophysiology is still unclear [2]. Sev-

eral mechanisms have been proposed to date, including tubo-ovarian distortion, increased oxidative stress due to the toxic content of the endometrioma, increased fibrosis, loss of cortical stroma, smooth muscle cell metaplasia and vascularization defects, all of

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which have been blamed for decreased follicular maturation and impaired oocyte quality [3].

In a differential diagnosis of endometriomas from benign ovarian cysts, a definite diagnosis is always based on pathological examination [4]. In recent years, however, there has been growing evidence indicating that surgical interventions into ovarian endometriomas may reduce ovarian reserves, which leads to reduced number of surgery in infertile patients. Furthermore, surgery is delayed in the majority of patients, except for symptomatic ones or those requiring anatomical reconstruction [5]. Nonetheless, the differential diagnosis of endometriomas from benign ovarian cysts is of utmost importance in infertile patients, irrespective of the surgical intervention. Patients should be informed preoperatively about the possible surgery-related complications of endometriosis and the potential decline in ovarian reserve, and those with endometrioma/endometriosis should be also informed about the low response rates to non-surgical infertility treatments [6]. The transvaginal ultrasound (US) is a useful diagnostic tool, while certain biomarkers in the peripheral blood can be used in atypical cases [7].

There have been several reports on the predictive, diagnostic and prognostic value of serum, plasma and a number of urinary biomarkers that are used either alone or in combination in the diagnosis of endometriosis. These include many cytokines, antibodies, cell populations, immunological factors, glycoproteins, cell adhesions, growth factors, proteomics, hormones, angiogenesis factors and apoptotic factors [8, 9]. Despite this, there is as yet no available biomarker or panel of biomarkers for endometriosis that meet the following criteria of the 2010 Biomarkers Definition Working Group of the National Institutes of Health: “*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention*” [10]. The tumor marker cancer antigen-125 (CA-125) is used most frequently in endometriosis, although it has been associated with limited diagnostic performance [11, 12].

In the present study, we investigate the diagnostic value of two simple systemic inflammatory response (SIR) parameters –neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) for CA-125 in a differential diagnosis of endometrioma.

## METHODS

In this retrospective study, the medical data of a total of 213 patients aged < 40 years who underwent laparoscopic or laparotomic surgery for ovarian cysts between April 2015 and June 2018 were analyzed. The patients were divided into two groups, as those with endometriomas and those with other benign ovarian cysts, all which had been confirmed histopathologically. Data on age, body mass index (BMI), preoperative US findings, complete blood count analysis results, follicle-stimulating hormone (FSH) levels, and the presence of dysmenorrhea, dyspareunia and chronic pelvic pain were recorded. Patients who were pre- or intraoperatively diagnosed with pelvic-systemic infection, preoperative cyst rupture, pregnant, or receiving steroid or estrogen and/or progesterone were excluded from the study. Written informed consent was obtained from each patient, and the study protocol was approved by the Local Ethics Committee, and was conducted in accordance with the principles of the Declaration of Helsinki.

The demographic characteristics of the patients, clinical data, complete blood count test results, CA-125, NLR and PLR results of the two groups were compared, and the factors that could affect NLR, PLR and CA-125 were analyzed. Sensitivity, specificity and cut-off values were calculated for PLR, NLR and CA-125, both alone and in combination. Complete blood count parameters were measured using the Coulter LH-780 hematology blood analyzer (Beckman Coulter Inc, Brea, California) and CA-125 levels were measured using an electrochemiluminescence immunoassay method (Roche Elecsys kits; Roche Diagnostics, Mannheim, Germany), and concentrations were expressed as U/mL.

## Statistical Analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows, version 11.5 software (SPSS Inc., Chicago, IL, USA). Descriptive data was expressed as mean  $\pm$  standard deviation (SD) and median (min-max) for quantitative variables, and in number and frequency (%) for qualitative variables. To analyze the statistically significant differences within the quantitative variables between the groups, the Student

t-test was used for normally distributed data and the Mann-Whitney U-test was used for non-normally distributed data. Chi-square and Fisher's exact tests were used to analyze qualitative variables. A Linear regression analysis was carried out to evaluate the effects of independent variables on the quantitative

variables. As the optimum method for the estimation of the cut-off value for quantitative variables, receiver operating characteristic (ROC) curves were used, and sensitivity and specificity were calculated. A *p* value of 0.05 was considered statistically significant.

**Table 1. Demographic and laboratory data of patients**

Variable	Study Population (n = 213)				p value
	Endometrioma (n = 128)		Other benign ovarian cysts (n = 85)		
	Mean ± SD	Median (Min-Max)	Mean ± SD	Median (Min-Max)	
Age(year)	30.30 ± 4.41	30.50 (19-39)	29.14 ± 4.84	29 (20-39)	0.073 <sup>a</sup>
BMI, (kg/m <sup>2</sup> )	25.15 ± 4.16	24.53 (17.72-37.80)	26.15 ± 4.28	26.22 (18.50-36.92)	0.075 <sup>b</sup>
Gravida (n)	0.11 ± 0.46	0 (0-4)	0.19 ± 0.87	0 (0-7)	0.879 <sup>b</sup>
Leukocyte count (/mm <sup>3</sup> )	7245.31 ± 1916.03	7050 (4100-13100)	7238.82 ± 1808.52	7100 (3700-12600)	0.793 <sup>b</sup>
Lymphocyte count (/mm <sup>3</sup> )	2.12 ± 0.55	2.06 (1.12-3.66)	2.42±0.75	2.27 (1.10-4.90)	<b>0.006<sup>b</sup></b>
Neutrophil count (/mm <sup>3</sup> )	4.46 ± 1.45	4.21 (1.68-9.30)	3.88 ± 1.24	3.80 (1.50-6.92)	<b>0.005<sup>b</sup></b>
Platelet count (/mm <sup>3</sup> )	273500 ± 71218.09	260000 (145000-544000)	269647.06 ± 72307.50	263000 (111000-469000)	0.773 <sup>b</sup>
MPV, fL	9.81 ± 1.88	9.70 (7-25)	9.60±1.39	9.80 (6.10-11.90)	0.935 <sup>b</sup>
RDW, %	14.09 ± 1.62	13.75 (11.30-20.20)	13.23 ± 1.12	13.10 (11-16.70)	<b>&lt; 0.001<sup>b</sup></b>
Hemoglobin, (gr/dL)	12.70 ± 1.34	12.75 (8.70-16.10)	13.18 ± 1.11	13.30 (10.20-15.70)	<b>0.005<sup>b</sup></b>
CA-125, U/mL	50.59 ± 46.63	38 (3.70-286)	15.75±12.46	11.50 (3.80-72.50)	<b>&lt; 0.001<sup>b</sup></b>
Lesion size (cm)	4.63 ± 2.67	4 (1-15)	5.94 ± 2.73	6 (1-15)	<b>&lt; 0.001<sup>b</sup></b>
Baseline FSH (mIU/mL)	7.05 ± 2.91	6.69 (0.97-19)	5.83 ± 2.22	5.86 (0.55-12.25)	<b>0.002<sup>b</sup></b>
NLR	2.21 ± 0.80	2.20 (0.62-3.91)	1.66 ± 0.47	1.57 (0.72-3.13)	<b>&lt; 0.001<sup>b</sup></b>
PLR	135060.57 ± 42215.70	136059.13 (58965.52-281645.57)	118352.10 ± 36820.63	120000 (46682.46-232727.27)	<b>0.007<sup>b</sup></b>

<sup>a</sup>Student's t-test, <sup>b</sup>Mann-Whitney U-test. SD = standard deviation, BMI = body mass index, MPV = mean platelet volume, RDW = red blood cell distribution width, FSH = follicle-stimulating hormone, NLR = neutrophil/lymphocyte ratio, PLR = platelet/lymphocyte ratio

## RESULTS

Of a total of 213 patients, 128 had an endometrioma and 85 patients had a benign ovarian cyst. Based on a pathological examination results of the cysts, 52 (61.25) were found to be dermoid cysts, 14 (16.5%) were benign serous cystadenomas, seven (8.2%) were mucinous cystadenomas, six (7.1%) were follicular cysts, four (4.7%) were hemorrhagic cysts and two (2.3%) were corpus luteum cysts. There was no significant difference in the age, BMI, gravid or parity between the groups. The demographic and laboratory data of the patients is presented in Table 1, while the clinical characteristics are shown in Table 2.

A linear regression analysis was carried out to analyze the differences in NLR, PLR and CA-125 between the groups, to evaluate whether age, BMI or smoking affected these values, and to identify any relationship between these variables and the clinical symptoms (Tables 3, 4 and 5 respectively). The analysis revealed that NLR was associated with dysmenorrhea ( $p = 0.013$ ) and PLR was associated with the lesion size ( $p = 0.018$ ), while no correlation

was found between the CA-125 and clinical symptoms of the patients. There was a statistically significant correlation between endometriomas and benign cysts in terms of all three variables ( $p < 0.001$ ,  $p = 0.003$  and  $p < 0.001$  for NLR, PLR and CA-125, respectively).

In the multivariate linear regression model, CA-125 was not found to be statistically significant (Table 5).

The sensitivity, specificity and cut-off values of NLR, PLR and CA-125, both alone and combined, were calculated for endometrioma and benign cysts (Table 6, Fig. 1).

## DISCUSSION

In the present study, we investigated the diagnostic value of NLR and PLR for CA-125 in a differential diagnosis of endometrioma from benign ovarian cysts, and found that NLR, PLR and CA-125 were increased in the patients with endometrioma, although increases in the NLR and PLR alone or combined did

**Table 2. Clinical characteristics of patients**

Variable	Study Population (n = 213)					p value
	Endometrioma (n = 128)		Other benign ovarian cysts (n = 85)			
		n	%	n	%	
Smoking	No	103	80.5	71	83.5	0.572 <sup>a</sup>
	Yes	25	19.5	14	16.5	
Parity	No	122	95.3	82	96.5	1.000 <sup>b</sup>
	Yes	6	4.7	3	3.5	
Chronic pelvic pain	No	106	82.8	72	84.7	0.715 <sup>a</sup>
	Yes	22	17.2	13	15.3	
Dyspareunia	No	123	96.1	82	96.5	1.000 <sup>b</sup>
	Yes	5	3.9	3	3.5	
Dysmenorrhea	No	108	84.4	79	92.9	0.061 <sup>a</sup>
	Yes	20	15.6	6	7.1	
Affected ovary	Right	42	32.8	37	43.5	< 0.001 <sup>a*</sup>
	Left	53	41.4	44	51.8	
	Bilateral	33	25.8	4	4.7	

<sup>a</sup>Chi-square test, <sup>b</sup>Fisher's exact test. \* $p < 0.05$  statistically significant



**Table 3. Multivariate linear regression analysis of NLR**

Independent variables	$\beta$	SE	R <sup>2</sup>	p value	95% CI	
					Lower limit	Upper limit
Group	-0.552	0.097	0.134	< 0.001	-0.742	-0.361
Lesion size	0.001	0.018	0.001	0.990	-0.037	0.036
Chronic pelvic pain	0.234	0.136	0.014	0.087	-0.035	0.503
Dysmenorrhea	0.385	0.153	0.029	0.013*	0.084	0.687
Age, year	-0.003	0.011	0.001	0.762	-0.025	0.018
BMI, kg/m <sup>2</sup>	-0.020	0.012	0.013	0.102	-0.043	0.004
Smoking	-0.048	0.131	0.001	0.715	-0.307	0.211

$\beta$  = Beta (regression) coefficient, SE = standard error, CI = confidence interval. \* $p < 0.05$  statistically significant. NLR=2.692–0.527 and Group + 0.284 for dysmenorrhea ( $p < 0.001$  and  $p = 0.049$ , respectively). These variables included in the analysis explain the change in the NLR values by 14.9%.

**Table 4. Multivariate linear regression analysis of PLR**

Independent variables	$\beta$	SE	R <sup>2</sup>	p value	95% CI	
					Lower limit	Upper limit
Group	- 16708.471	5618.408	0.040	<b>0.003*</b>	- 27783.873	-5633.069
Lesion size	2390.928	1005.726	0.026	<b>0.018*</b>	408.370	4373.487
Chronic pelvic pain	12960.257	7526.085	0.014	0.087	-1875.694	27796.207
Dysmenorrhea	16115.738	8506.951	0.017	0.060	-653.764	32885.241
Age, year	-262.484	610.793	0.001	0.668	-1466.522	941.554
BMI, kg/m <sup>2</sup>	140.834	665.950	0.001	0.833	-1171.934	1453.603
Smoking	7030.723	7245.526	0.004	0.333	-7252.171	21313.616

$\beta$  = Beta (regression) coefficient, SE = standard error, CI = confidence interval. \* $p < 0.05$  statistically significant. PLR=140966.612–20978.644 and Group + 3258.942 for lesion size ( $p < 0.001$  and  $p = 0.001$ , respectively). These variables included in the analysis explain the change in the PLR values by 8.6%.

**Table 5. Multivariate linear regression analysis of CA-125**

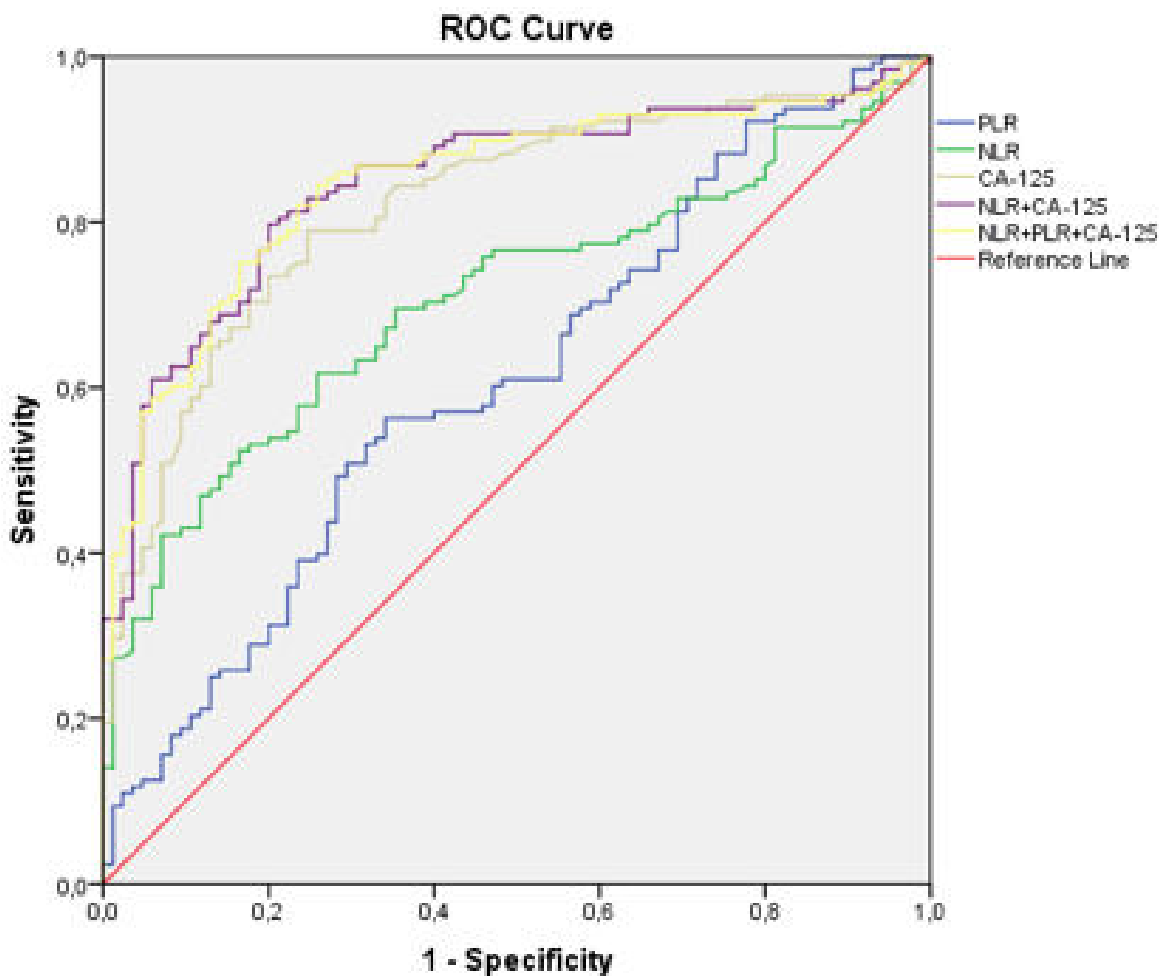
Independent variables	$\beta$	SE	R <sup>2</sup>	p value	95% CI	
					Lower limit	Upper limit
Group	-34.839	5.180	0.177	< <b>0.001</b>	-45.050	-24.629
Lesion size	-0.803	1.013	0.003	0.429	-2.800	1.194
Chronic pelvic pain	5.160	7.535	0.002	0.494	-9.693	20.013
Dysmenorrhea	8.222	8.520	0.004	0.336	-8.573	25.018
Age, year	0.853	0.605	0.009	0.160	-0.341	2.046
BMI, kg/m <sup>2</sup>	-1.186	0.658	0.015	0.073	-2.483	0.110
Smoking	-0.071	7.228	0.001	0.992	-14.319	14.176

$\beta$  = Beta (regression) coefficient, SE = standard error, CI = confidence interval

**Table 6.** Sensitivity, specificity, and cut-off values of NLR, PLR, and CA-125 alone and combined

	AUC (95% CI)	SE	Sensitivity , %	Specificity, %	Cut-off value	p value
<b>PLR</b>	0.608 (0.531-0.685)	0.039	57.0	60.0	125992.19	<b>0.007</b>
<b>NLR</b>	0.707 (0.638-0.775)	0.035	67.2	65.9	1.73	<b>&lt; 0.001</b>
<b>CA-125</b>	0.820 (0.763-0.877)	0.029	78.9	75.3	17.71	<b>&lt; 0.001</b>
<b>NLR + CA-125</b>	0.847 (0.795-0.900)	0.027	79.7	80.0	33.66	<b>&lt; 0.001</b>
<b>NLR + PLR + CA-125</b>	0.847 (0.794-0.899)	0.027	78.1	78.8	3913755.25	<b>&lt; 0.001</b>

AUC = area under curve, CI = confidence interval, SE = standard error, PLR = platelet/lymphocyte ratio, NLR = neutrophil/lymphocyte ratio



**Fig. 1.** ROC curve of sensitivity and specificity of NLR, PLR, and CA-125 alone and combined. ROC = receiver operating characteristic, PLR = platelet/lymphocyte ratio, NLR = neutrophil/lymphocyte ratio.

not contribute to the sensitivity or specificity of CA-125.

The neutrophil/lymphocyte ratio is used as a marker of subclinical inflammation, and it can be also used as a prognostic indicator in patients with systemic inflammatory response syndrome and such other diseases as coronary heart disease, myocardial infarction, lung cancer, ovarian cancer and colorectal cancer [13]. It is also used to monitor the progress of endometriosis, which is a chronic inflammatory disease [14]. In a 2008 study, Cho *et al.* [15] recommended the use of NLR as a diagnostic marker in patients with endometriosis, reporting an NLR sensitivity and specificity of around 60 percent; however, NLR combined with CA-125 increased sensitivity, but decreased specificity. Similarly, Sayan *et al.* [16] showed that the sensitivity of NLR combined with CA-125 was higher when compared to NLR alone, while specificity tended to decline. In the aforementioned study, the authors reported further that NLR and CA-125 values increased as the stage of endometriosis advanced. Consistent with previous studies, Yang *et al.* [17] found that the diagnostic value of NLR combined with CA-125 increased in patients with advanced endometriosis. In contrast, Kim *et al.* [18] found no correlation between NLR combined with CA-125 and the stage of endometriosis, while Yavuzcan *et al.* [19] reported no relationship between NLR and CA-125 values and the stage of endometriosis in patients with advanced endometriosis. In the present study, NLR alone had relatively low sensitivity and specificity when compared to CA-125 in patients with endometrioma. In addition, no contribution of NLR alone to the diagnostic value of CA-125 was identified in the present study, with the specificity of CA-125 increasing from 75 to 80 percent when NLR was combined with CA-125. Concurring with our findings, in a 2017 study including patients with endometrioma CA-125 showed a limited diagnostic value in non-infected endometriomas, while a slight increase was achieved with the addition of NLR [20]. In infected endometriomas, all marker values increased significantly, which was also the primary outcome of the study.

Platelet/lymphocyte ratio has been investigated extensively as an inflammatory and immunological marker in the differential diagnosis of adnexal masses.

As the lymphocyte ratio decreases in case of malignancies, NLR and PLR, when combined with CA-125, are considered useful markers in a differential diagnosis [21]. Topcu *et al.* [22] reported PLR combined with CA-125 as being a strong predictive marker in malignant adnexal masses, while NLR was not a useful predictor in such cases. Lymphocyte activity results in alterations in endometriosis, and many abnormalities can be seen, including reduced activity of cytotoxic T cells and natural killer cells, cytokine secretion by helper T cells, and autoantibody production by B lymphocytes [23]. On the other hand, Yavuzcan *et al.* [19] found no diagnostic value of PLR in the differential diagnosis of advanced endometriomas and benign adnexal masses. Similarly, Yang *et al.* [24] reported that the diagnostic value of PLR alone or combined with CA-125 was lower than for CA-125 alone in the diagnosis of endometriosis. Tokmak *et al.* [25] reported that combination of NLR and CA-125 improved diagnostic accuracy than CA-125 alone. Consistent with previous studies, in the present study, we observed that the diagnostic value of PLR was lower than NLR and CA-125 in endometrioma cases, and PLR, even when combined with NLR and CA-125, did not show superiority to CA-125.

In the present study, we also analyzed the relationship between the NLR, PLR and CA-125 and age, BMI, smoking and clinical symptoms, and identified a relationship between NLR and dysmenorrhea and between PLR and lesion size, but no relationship between CA-125 and clinical symptoms. On the other hand, in a previous study on dysmenorrhea, no relationship between dysmenorrhea and NLR or PLR was established [26], and in another study, no association was found between CA-125 and pelvic pain or dysmenorrhea [27]. While NLR was found to be positively correlated with age in a study, no relationship between CA-125 and NLR and the stage of endometriosis was found [18]. Jiang *et al.* [28], on the other hand, suggested that CA-125 combined with NLR and PLR may be a predictor of adenomyosis-related dense pelvic adhesions. Based on all these findings, it can be concluded that the relationships between NLR, PLR and CA-125 and the clinical symptoms of endometriosis are still unclear, and remain to be elucidated.

## Limitations

There are some limitations to this study that should be noted, among which, its retrospective nature and the exclusion of intraoperative signs from the analysis can be considered the main ones. Accordingly, we recommend further studies investigating the relationship between NLR, PLR and CA-125 and pelvic inflammatory disease symptoms, adhesions and stage of endometriosis.

## CONCLUSION

In conclusion, the results of our study suggest that NLR and PLR did not contribute to the diagnostic value of CA-125 in the preoperative differential diagnosis of endometrioma or other benign ovarian cysts. In addition, CA-125 was not associated with clinical symptoms, although a relationship was identified between NLR and dysmenorrhea, and between PLR and lesion size, which may be the research focus of further studies.

### Conflict of interest

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

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# Prevalence of joint hypermobility, hypermobility spectrum disorder and hypermobile Ehlers-Danlos syndrome in a university population: an observational study

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## ABSTRACT

**Objectives:** To investigate the prevalence of joint hypermobility classes, hypermobility spectrum disorders, hypermobile Ehlers-Danlos syndrome and their relations with sex in a university population. They are notable topics in rehabilitation, since they represent the basis for some secondary disability conditions.

**Methods:** Three hundred and thirty-five students met the inclusion criteria (university students who are not disabled, without known disease, aged 18-25 years). Joint hypermobility were classified as generalized, peripheral and localized asymptomatic or hypermobility spectrum disorders. Hypermobile Ehlers-Danlos syndrome was defined according to; 1) Brighton criteria with cut-off Beighton scoring  $\geq 4/9$ , 2) Villefranche criteria with cut-off Beighton scoring  $\geq 5/9$ , and 3) The 2017 International Classification of Ehlers-Danlos syndrome.

**Results:** In total, 77.3% (n = 259) of participants had any class of joint hypermobility and 25.9% (n = 87) of them had generalized class. Asymptomatic joint hypermobility and hypermobility spectrum disorders prevalence in a university population were found to be 38.8% and 38.5%, respectively. Generalized, peripheral, localized asymptomatic joint hypermobility and hypermobility spectrum disorders were found; 13.1%, 4.2%, 21.5%, and 12.8%, 7.5%, 18.2%, respectively. Prevalence of hypermobile Ehlers-Danlos syndrome according to three classifications were found to be; 19.4%, 15.2%, and 1.2%, respectively.

**Conclusions:** The most common classes are localized asymptomatic joint hypermobility in women and localized hypermobility spectrum disorders in men. Awareness of the prevalence of joint hypermobility, hypermobility spectrum disorders and hypermobile Ehlers-Danlos syndrome in healthy young population may contribute to prevention of disability.

**Keywords:** Joint laxity, musculoskeletal complications, chronic pain, myopia, dental braces, soft tissue problems

Joint hypermobility is a condition in which a joint has a range of motion greater than normal [1]. Joint hypermobility, hypermobility spectrum disorders, and hypermobile Ehlers-Danlos syndrome (EDS) are notable topics in rehabilitation, since they represent the basis for some secondary disability condi-

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tions [2, 3]. The 2017 International Classification has been published for update the EDS nosology and develop best practice clinical guidelines on diagnosis of EDS recently [4]. Although hypermobile EDS is defined as benign, it is a structural predisposition leading to a decline in the quality of life due to multi-systemic manifestations [5-9].

Shepert *et al.* [10] published their longitudinal cohort study describing the natural history and the prognosis in 101 children with joint hypermobility syndrome or hypermobility-type EDS according to the Brighton and Villefranche criteria. Pain, fatigue, and psychological distress had a significant impact on disability that affect patients with generalized joint hypermobility, joint hypermobility syndrome or hypermobility-type EDS considerably [3, 10, 11]. They reported functional impairment as predictive for decreased quality of life over time. Awareness of the prevalence of joint hypermobility, hypermobility spectrum disorders and hypermobile EDS in healthy young university population may contribute recognition, management and prevention of disability. It is important to investigate the situation in university students, because they form a young and dynamic group in terms of future labor force.

Prevalence of generalized joint hypermobility varying from 2% to 57% in different populations [12, 13]. In Turkey, studies report it in healthy subjects between 12.4 and 22 % for women and 6.1-7.7 % for men of varying ages [14-17]. Reasons of variations are different ages or Beighton scoring cut-offs, and lack of consistency in clinical assessment methods. In addition, there is no information about prevalence of localized, peripheral classes of joint hypermobility, hypermobility spectrum disorders and hypermobile EDS according to the old and new criteria.

Our purposes in this research in a university population are; (i) to investigate the prevalence of new defined classes of asymptomatic joint hypermobility (generalized, peripheral and localized classes) and hypermobility spectrum disorders, (ii) to investigate the prevalence of hypermobile EDS, defined according to Brighton, Villefranche and the 2017 International Classification criteria in order to allow comparison with previous studies, and (iii) to investigate relationship between the Beighton scoring, the Brighton criterion and sex.

## METHODS

### Participants

We conducted an observational (a cross-sectional) study between 2016 and 2017. Trakya University students aged 18-25 years were invited to participate. Our Physical Therapy and Rehabilitation Department is an integrated department of faculty that provides education for a population of 346 residents of Turkey.

A total of 346 students (all of Physical Therapy and Rehabilitation students in Trakya University Faculty of Health Sciences) were targeted. Students voluntarily agreed to participate in the study and informed consent was obtained from each student. Inclusion criteria were; university students who are not disabled, without known disease, and aged 18-25 years. Exclusion criteria were; students with known disease, inappropriate age, and unwilling to participate). Eleven (3.1%) students were not eligible to inclusion criteria (4 students with known disease, 3 students with inappropriate age and 4 students declined to participate). The local ethics committee approved the conduction of the research (BAEK 2016/255).

### Procedures

Demographic data of participants, including age, height, and weight, were recorded. Beighton scoring was performed face-to-face with each participant by the same physiatrist [18, 19]. Asymptomatic and symptomatic joint hypermobility (generalized, peripheral, and, localized), were classified according to Beighton scoring, anatomic location of hypermobile joints and accompanying musculoskeletal problems [20]. hypermobile EDS was diagnosed according to three classifications; 1) Brighton criteria with cut-off Beighton scoring  $\geq 4/9$  for generalized joint hypermobility [19], 2) Villefranche criteria with cut-off Beighton scoring  $\geq 5/9$  for generalized joint hypermobility [21], and 3) The 2017 International Classification of EDS [4].

### Beighton Scoring

For the Beighton scoring, the following items were evaluated; 1) Placement of hands flat on the floor without bending the knees, 2) Hyperextension of the elbow to  $\geq 10^\circ$ , 3) Hyperextension of the knee to  $\geq 10^\circ$ , 4) Opposition of the thumb to the volar aspect of

the ipsilateral forearm, and 5) Passive dorsiflexion of the fifth metacarpophalangeal joint to  $\geq 90^\circ$  [18]. The first item for Beighton scoring was calculated as one point; the other items as one points for right and left side. The results were summarized arithmetically, with one score given for each positive item. Joint hypermobility was classified as generalized, peripheral, and localized according to defined Beighton score and anatomic localization of affected joint [20]. Participants with hypermobility in only only hands and/ or feet without involvement of large and axial joints were classified as peripheral joint hypermobility. Involvement of one or fewer than five joints were defined as localized joint hypermobility [20]. Generalized joint hypermobility was defined in participants firstly as a Beighton scoring of  $\geq 4/9$  (for Brighton criteria) and secondly with Beighton scoring  $\geq 5/9$  (for Villefranche and the 2017 International Classification) [19, 21]. Brighton criteria [21] and hypermobile EDS criteria described in the 2017

International Classification [4] were asked, and recorded.

### Brighton Criteria

The presence of one of the following criteria was deemed sufficient for hypermobile EDS diagnosis [19]: 1) the presence of any of the four major criteria, 2) one major criterion + two minor criteria, 3) four minor criteria, and 4) two minor criteria [19]. As major criteria, a Beighton scoring of  $\geq 4/9$  and arthralgia existing over 3 months in four joints were interrogated. In addition, minor criteria (having a Beighton scoring of 1-3/9, arthralgia in 1-3 joint, ossicular dislocations, soft tissue problems with  $\geq 3$  lesions (e.g., epicondylitis, tenosynovitis, and bursitis), Marfan-like appearance, striae of skin, eyelid laxity and hernia, prolapse, and varicose vein history) were questioned [18, 19]. Positivity of the one of following evaluations were enough for decision of Marfanoid habitus; arm span/height ratio  $> 1.05$ ,

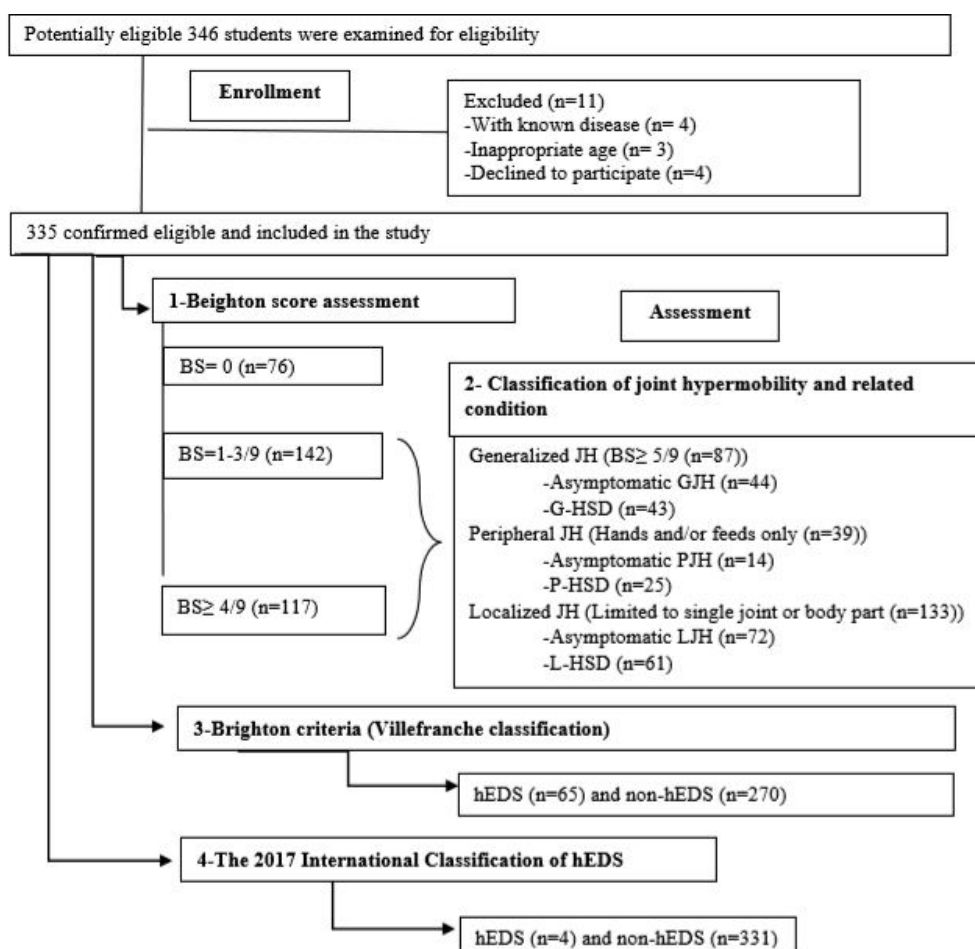


Fig. 1. Flow diagram of study

positive Steinberg sign, and the positive wrist sign and arachnodactilia (bilateral positivity of Steinberg or wrist sign) [4, 18].

### Hypermobile EDS Diagnosis According to the 2017 International Classification

A diagnosis of hypermobile EDS was assigned when criteria 1, 2 and 3 simultaneously existed in each participant; (i) Criterion 1 were generalized joint hypermobility with cut-off point Beighton scoring  $\geq 5/9$ ; (ii) Criterion 2 were accepted when at least two of features A, B and C were present in participants. However, feature B, (positive family history of hypermobile EDS) was accepted enough for the diagnosis. Features A was accepted when at least 5 meets of a 12 systemic features of a connective tissue as soft, mild hyperextensible skin, stria without a history of a significant gain or loss of weight, bilateral

piezogenic papules of heel, atrophic scarring, abdominal hernia, genitourinary prolapse, dental crowding, arachnodactyly, prolonged arm span, mild mitral valve prolapse, aortic root dilatation were present in participants [4]. At least one existence of following three musculoskeletal complications were accepted as Feature C; 1) musculoskeletal pain with daily recurrence in at least two limbs, 2) chronic widespread pain. These complications must be present at least for 3 months. 3) At least 3 non-traumatic dislocations in the same joint or more in 2 different joints happening at different times or non-traumatic joint instability at least 2 sites with medical confirmation. (iii) Criterion 3 were accepted when all of following conditions were met; 1) Lack of extraordinary skin fragility, 2) Exclusion of further connective tissue disorders, and 3) Exclusion of another diseases with joint hypermobility [4].

**Table 1. Participant’s demographics and prevalences of joint hypermobility classes, hypermobility spectrum disorders, and hypermobile Ehlers-Danlos syndrome.**

	Female Mean (SD)	Male Mean (SD)	Total Mean (SD)				
Age	20.2 (1.6)	21.2 (1.8)	20.5 (1.7)				
Body mass index	20.9 (2.4)	22.6 (2.3)	21.5 (2.5)				
Beighton score (median)	3.0	2.0	2.0				
Classes	n (%)	n (%)	n (%)	OR	95% CI	LR	LR <i>p</i> value
Asymptomatic Joint Hypermobility							
Generalized	40 (18.1)	4 (3.5)	44 (13.1)	0.165	0.057-0.472	16.906	< <b>0.001</b>
Peripheral	5 (2.3)	9 (7.9)	14 (4.2)	3.703	1.211-11.324	5.564	<b>0.018</b>
Localized	48 (21.7)	24 (21.1)	72 (21.5)	0.961	0.553-1.670	0.020	0.888
Hypermobility spectrum disorder							
Generalized	33 (14.9)	10 (8.8)	43 (12.8)	0.548	0.260-1.156	2.696	0.101
Peripheral	19 (8.6)	6 (5.3)	25 (7.5)	0.591	0.229-1.523	1.278	0.258
Localized	33 (14.9)	28 (24.6)	61 (18.2)	1.855	1.055-3.262	4.528	0.033*
Beighton score of 0/9							
	43 (19.4)	33 (29.0)	76 (22.6)	0.593	0.351-1.002	3.766	0.052

OR odds ratio with 95% confidence interval (CI), LR likelihood ratio and *p* value for the LR

**Table 2. Prevalence of the generalized joint hypermobility and hypermobile Ehlers-Danlos syndrome according to different criteria**

	Female N (%)	Male N (%)	Total N (%)	OR	95% CI	LR	LR <i>p</i> value
Beighton scoring							
≥ 4/9	92 (41.6)	25 (21.9)	117 (34.9)	0.394	0.235-0.661	13.400	< 0.001
≥ 5/9	73 (33.0)	14 (12.3)	87 (25.9)	0.284	0.152-0.531	18.407	< 0.001
Joint hypermobility syndrome According to Brighton criteria with cut-off Beighton score ≥ 4/9							
	53 (24.0)	12 (10.5)	65 (19.4)	2.687	1.368-5.257	9.443	0.002
Hypermobile Ehlers-Danlos syndrome According to Villefranche criteria with cut-off Beighton score ≥ 5/9							
	42 (19.0)	9 (7.9)	51 (15.2)	0.365	0.171-0.780	7.893	0.005
Hypermobile Ehlers-Danlos syndrome According to the 2017 International Classification							
	4 (1.8)	0 (0.0)	4 (1.2)	1.525	1.411-1.649	3.353	0.067

OR odds ratio with 95% confidence interval (CI), LR likelihood ratio and *p* value for the LR

Furthermore, participants were questioned for history of the use of dental braces. When accompanying symptoms not meets hypermobile EDS criteria, the joint hypermobility was defined as component of hypermobility spectrum disorder [20]. All the examinations and measurements were performed by the same physiatrist (the author).

**Statistical Analysis**

Statistical significance is defined by *p* value of < 0.05. The data of the study were evaluated by IBM SPSS version 20.0 statistical software (Released 2011. IBM Corp., Armonk, NY, USA). Normal distribution of data was examined using Kolmogorov-Smirnov tests. Frequencies and percentages were used for categorical data as descriptive statistics. Median (min-max) values and arithmetic means with standard deviations were used for quantitative data. To evaluate the relationship between sex and Beighton scoring, the participants were divided into groups according to their scores: 0 score, 1-3 score, and ≥ 4 score. The distribution of these groups by sex was then examined. The Pearson chi-square test was used in the analysis of qualitative data. The association between age and Beighton scoring was examined using the Spearman correlation analysis. Likelihood ratios were computed

for prevalence of joint hypermobility classes, hypermobility spectrum disorders, hypermobile Ehlers-Danlos syndrome, and Beighton score, with Chi-Square tests.

**RESULTS**

In total, 335 university students with age between 18 and 25 years, of whom 221 were female and 114 were male, were analyzed in the study (Fig. 1). Demographic data of participants and prevalences in a university population of joint hypermobility classes, hypermobility spectrum disorders, and hypermobile Ehlers-Danlos syndrome were showed in Table 1. In total, 77.3% (n = 259) of participants had any class of joint hypermobility and 25.9% (n = 87) of them had generalized joint hypermobility. Hypermobility spectrum disorders and asymptomatic joint hypermobility were found 38.5% (n = 129) and 38.8% (n = 130), respectively. We found negative correlation between age and Beighton scoring (Spearman’s rho; *r*: -0.332, *p* < 0.001).

The most common classes of joint hypermobility are localized asymptomatic (mainly in women) and localized hypermobility spectrum disorders (mainly in

**Table 3. The relationship between sex and Brighton criteria**

Female n (%)	Male n (%)	Total n (%)	OR	95% CI	LR	LR p value
Major and minor criteria of Brighton						
Arthralgia for longer than 3 months in 4 or more joints						
10 (4.5)	8 (7.0)	18 (5.4)	1.592	0.611-4.153	0.885	0.347
Arthralgia $\geq$ 3 months in 1-3 joints or back pain $\geq$ 3 months						
30 (13.6)	9 (7.9)	39 (11.6)	0.546	0.250-1.193	2.498	0.114
Dislocation/subluxation in $\geq$ 1 joint or in 1 joint $\geq$ occasion						
35 (15.8)	21 (18.4)	56 (16.7)	1.200	0.662-2.177	0.356	0.551
Soft tissue problems $\geq$ 3 lesions						
57 (25.8)	45 (39.5)	102 (30.4)	1.876	1.159-3.037	6.518	<b>0.011</b>
Marfanoid habitus						
12 (5.4)	15 (13.2)	27 (8.1)	2.639	1.191-5.849	5.718	<b>0.017</b>
Stria, papyraceous scarring						
145 (65.6)	32 (28.1)	177 (52.8)	0.205	0.125-0.335	43.523	<b>&lt; 0.001</b>
Drooping eyelids, antimongoloid slanting eyes, or myopia						
93 (42.1)	43 (37.7)	136 (40.6)	0.834	0.524-1.325	0.596	0.440
Varicose veins, hernia, or uterine/rectal prolapse						
8 (3.6)	5 (4.4)	13 (3.9)	1.221	0.390-3.822	0.116	0.733
Total						
221 (100.0)	114 (100.0)	335 (100.0)				

OR odds ratio with 95% confidence interval (CI), LR likelihood ratio and *p* value for the LR

men) (Table 1). Nearly half of joint hypermobility participants have hypermobility spectrum disorders. Generalized joint hypermobility and hypermobile EDS were significantly higher in females (Table 2). Given the Beighton scoring and sex relation, the highest prevalence distributions were 41.6% (*n* = 92) with  $\geq$  4 points in females and 49.1% with 1-3 points in males (Pearson  $\chi^2$  = 13.191, *p* = 0.001). The prevalence of skin striae was significantly higher in females, while soft tissue problems and Marfanoid habitus was significantly higher among male

participants (*p* < 0.05) (Table 3). The relationship between sex and the questioned 2017 International Classification Criteria is shown in Table 4.

When participants were asked about dental braces statistically significant difference was found between generalized joint hypermobility or hypermobile EDS and participants with a Beighton scoring of 0/9. The prevalences of participants with dental braces in the hypermobile EDS and non-hypermobile EDS groups were 44% (*n* = 22) and 35.7% (*n* = 94), respectively (Pearson  $\chi^2$ ; *p* = 0.268).



**Table 4. The relationship between sex and the 2017 International Classification Criteria**

	Female n (%)	Male n (%)	Total n (%)	OR	95% CI	LR	LR p value
Criterion 1	73 (33.0)	14 (12.3)	87 (25.9)	3.523	1.885-6.586	18.407	< 0.001
Criterion 2	5 (2.3)	1 (0.9)	6 (1.8)	2.616	0.302-22.660	0.923	0.337
Features A	10 (4.5)	4 (3.5)	14 (4.2)	1.303	0.400-4.251	0.199	0.655
Features B	0 (0.0)	0 (0.0)	0 (0.0)				
Features C	101 (45.7)	62 (54.4)	172 (51.3)	0.706	0.448-1.111	2.277	0.132
Criterion 3	221 (100.0)	114 (100.0)	335 (100.0)				

OR odds ratio with 95% confidence interval (CI), LR likelihood ratio and *p* value for the LR

## DISCUSSION

The present study contributes to existing literature by obtaining that 3/4 of a university population had any class of joint hypermobility and 1/4 of them had generalized joint hypermobility, which is much higher than populations with different ages in the previous studies from Turkey [14-17, 22] and in line with the one study from America [23] (Table 5). Our low prevalence of peripheral joint hypermobility supported the view that this class, which is mainly non-pathological and affects small joints, is more common in children [20]. Generalized joint hypermobility, which is the presence of hypermobility simultaneously at upper, lower limbs, and axial skeleton, took second place in frequency ranking. Localized joint hypermobility, which is mainly in a single small or large joint, took first place in the ranking of frequency.

Joint hypermobility is usually accepted as non-symptomatic feature. Despite that, almost 50% of our participants with joint hypermobility were defined as hypermobility spectrum disorders when questioned. However, the frequency of hypermobility spectrum disorders was not reported before in literature due to its new definition [20]. None of studies from Turkey have reported hypermobile EDS (joint hypermobility syndrome or benign joint hypermobility syndrome) prevalence before. However, our results are in line with the findings of Russek and Errico and higher than the those found in two studies with a limited number of participants [24, 25] (Table 5). We found prevalence

of hypermobile EDS according to different classifications in the same participants group at a decreasing prevalence; 19.4%, 15.2%, and 1.2%, respectively. Main reason for this may be the lack of features B in our populations, information about the syndrome in young people's families (they were unaware of the existence of the syndrome), therefore, decision were made only in positivity of both features A and C. The another reason may be the fact that with the 2017 International Classification criteria, the joint hypermobility and secondary musculoskeletal problems were not adequate for the definition of a genetic syndrome, unless the interference of at least one different tissue or structure [20].

Most hypermobile and hypermobile EDS were in female gender, which is also in line with the proposed association between sex and joint laxity [14, 16, 17, 23, 26, 27]. We found asymptomatic localized joint hypermobility in females, and localized hypermobility spectrum disorders in males at the highest prevalence. Arthralgia and back pain has been reported as the most common complaint in participants with generalized joint hypermobility [16]. In fact, stria in females (65.6%) and soft tissue problems in males (39.5%) were the most prevalent criteria for definition of joint hypermobility syndrome. These results are consistent with the view that the most frequent detected Brighton criteria for joint hypermobility syndrome are firstly dermal and secondly eye problems [28, 29]. Although the relationship of joint pain and generalized joint hypermobility is still controversial in literature [14, 16,



**Table 5. Prevalences in literature**

Year, Authors	Evaluation	Participants count	Ages of population (years)	Beighton cut-off point	Definition	Females (%)	Males (%)	All (%)
2005, Seckin <i>et al.</i> [22]	Beighton	961	13-19	4/9	GJH	16.2	7.2	11.7
2005, Yildirim <i>et al.</i> [23]	Beighton	857	6-16	6/9	GJH	19.9	7.7	13.0
2006, Ofluoglu <i>et al.</i> [21]	Beighton	58 females	21-61	4/9	GJH	22	Nk	Nk
2013, Russek and Errico [31]	Brighton	290 female College athletes	Adolescent and College age	Nk	JHS	17.9	Nk	Nk
2015, Barcak <i>et al.</i> [20]	Beighton	437	11-18	5/9	GJH	12.4	6.1	9.1
2015, Sopper <i>et al.</i> [32]	Beighton	27 elite-level net ballers	14-26	4/9	GJH	Nk	Nk	63
	Brighton	27 elite-level net ballers	14-26		JHS	Nk	Nk	14.8
2016, Russek and Errico [26]	Beighton	267	17-26	4/9	GJH	51.0	27.4	40.1
	Beighton	267	17-26	5/9	GJH	36.7	13.7	26.2
	Brighton	267	17-26		JHS	24.5	13.7	19.5
Current study, Tuna	Beighton	335	18-25	4/9	GJH	41.6	21.9	34.9
	Beighton	335	18-25	5/9	GJH	33.0	12.3	25.9
	Brighton	335	18-25	4/9	JHS	24.0	10.5	19.4
	the 2017 IC	335	18-25		HSD	38.4	38.7	38.5
	the 2017 IC	335	18-25		hEDS	1.8	0	1.2

Nk = not known, GJH = generalized joint hypermobility, PJH = peripheral joint hypermobility, LJH = localized joint hypermobility, HSD = hypermobility spectrum disorder, hEDS = hypermobile Ehlers-Danlos syndrome, IC = International Classification

30], our low percentages of arthralgia can be due to the inclusion criteria (self-defined healthy in mean of non-painly). Based on our study, more than half of the participants with generalized joint hypermobility were “not healthy” when the Brighton criteria were applied. So as Albayrak *et al.* [30] stated; “symptoms other than pain may be overlooked during the examination of benign joint hypermobility syndrome patients unless they are specifically questioned”.

### Limitations

The present study has some limitations. Although, participants were questioned for history of use of dental braces with medical confirmation, first, the data of dental braces information is self-reported. It may not reflect the exact incidence of these. Second, there are only 18-25 year old participants in the study, which makes it difficult to extrapolate the results to entire populations. However, to the best of our knowledge,

this is the first study to analyze the prevalence in a university population of classified joint hypermobility as generalized, peripheral, and localized. In addition, to analyze the prevalence of hypermobility spectrum disorders and hypermobile EDS diagnosed according to three classifications in the same population.

## CONCLUSION

In conclusion, joint hypermobility is quite common and nearly half of participants with joint hypermobility have hypermobility spectrum disorders. The difference between old and new classification results raised the needs of new researches in the area. We want to urge colleagues to consider carefully researching this group of patients in order to create opportunity for timely identify and resolve multisystemic complaints. We think that prevention of disability and awareness of the the multisystemic nature of syndroms starts with definition of generalized joint hypermobility and associated syndromes.

### *Conflict of interest*

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

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# Comparison of the effects of hyperbaric oxygen and extracorporeal shock wave therapy on healing in osteotendinous junctional injuries: an experimental study of the rabbit model

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## ABSTRACT

**Objectives:** To biomechanically and histopathologically compare the effects on healing in the osteotendinous region of the treatment methods of hyperbaric oxygen (HBO) and extracorporeal shock wave (ESW) on an injury created at the osteotendinous junction in an experimental rabbit model.

**Methods:** The study included 36 New Zealand rabbits. The patellar tendon tibial osteotendinous junction was cut and then repaired. Group 1 was evaluated as the control group, with no further intervention. In Group 2, a single session of ESW was applied at 14 hz/750 Impulse/10 mins to an area 1×1 cm<sup>2</sup> in the repaired area under fluoroscopy guidance. The animals in Group 3 were applied with 100% oxygen as HBO at 2.4 ATA/110 min for 7 days. At the end of 6 weeks, 6 animals from each group were applied with a tension test and 6 animals were processed for histopathological examination.

**Results:** In Group 1, the forces at the start of the separation of the osteotendinous junction, at a 2 mm separation and at full rupture were lower than those of Group 2 and Group 3 ( $p = 0.001$  and  $p = 0.001$ , respectively). The highest separation force values were determined in Group 3 ( $p = 0.001$ ). Histopathologically, a statistically significant difference was determined in respect of the fibrocartilage tissue formation, and fusion with new bone tissue between Group 1 and Group 2 and between Group 1 and Group 3 ( $p < 0.05$ ). No statistically significant difference was determined between Group 2 and Group 3 ( $p = 0.310$ ).

**Conclusions:** It was observed that the application of HBO and ESW improved biomechanical resistance and healing in the injuries in the osteotendinous junction by increasing the bone-tendon fusion in this area. The application of HBO and ESW increased biomechanical resistance compared to the control group. In the histopathological evaluation, the ESW group was better than the control group and the HBO group was evaluated as the best.

**Keywords:** Tendon, hyperbaric, oxygen, shock wave, healing

Some studies have reported that the results are not pleasing after surgical interventions of the osteotendinous junction injuries. Healing of the osteotendinous junction is depend on both the bone and the surrounding soft tissues [1]. Osteotendinous junction injuries are require an aggressive multidisciplinary

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physical therapy and rehabilitation program because of the complex anatomic structure of this region. Failure rates have been reported at 20%-25% after rotator cuff repair in the shoulder, and at 10%-22% after reconstruction with tendon autograft in anterior cruciate ligament ruptures [2, 3]. For effective and successful treatment, in addition to early surgical intervention; biological or supplementary treatments are needed to start with an early rehabilitation program that cannot be achieved in routine clinical practice generally.

Biological treatments such as Transforming Growth Factor-beta (TGF- $\beta$ ), Epithelial Growth Factor combined with TGF, Recombinant Human Bone Morphogenic Protein (rhBMP-2), Bone Growth Factor, Granulocyte Colony Stimulating Factor, Periosteal Cells and Autologous Mesenchymal Stem Cells have not routinely applied because of the high costs and difficulties of their acquisition [4, 5-8]. However, supplementary treatments such as Hyperbaric Oxygen Treatment (HBO), Extracorporeal Shock Wave (ESW), Electric Stimulation and Low-intensity Pulsed Ultrasound Stimulation (LIPUS) are currently used in chronic musculoskeletal system diseases [9-13].

Hyperbaric oxygen (HBO) therapy is the inhalation of 100% oxygen at a pressure of at least 1.4 atmospheres absolute (ATA), 140 kPa, in the hyperbaric chamber [1-8]. HBO was first used in decompression sickness [14]. Subsequently, with understanding of the physiological and biomechanical effects, HBO has been widely used in the supplementary treatment for gas embolism, carbon monoxide poisoning, diabetic foot ulcers, peripheral vascular diseases, thermal burns, skin grafts and flaps, purpura fulminans, osteomyelitis, osteoradionecrosis, cerebral and retinal oedema [15-18].

In the literature, efficacy of ESW has been investigated by both experimental and clinical studies, as a result started to use in chronic musculoskeletal system disorders such as tennis elbow, adhesive capsulitis, heel spur, patellar tendinopathy, Achilles tendinitis, delayed bone union, osteochondritis dissecans and avascular necrosis of the femoral head [19-28].

The aim of this study was examined and compared the effects of HBO and ESW biomechanically and histopathologically as a supplementary treatment methods that help to healing of osteotendinous injury on an experimental rabbit model after surgical intervention.

## METHODS

Approval for this experimental study was granted by the Animal Experiments Local Ethics Committee. Number of animals was decided with power analysis and a total of 36 adult, female, New Zealand White (*Oryctolagus cuniculus* L) rabbits weighting mean 3.10 kg (range, 2.85-3.36) and with a mean age of 6 months with simple randomisation separated into 3 groups as 12 as control group (Group 1), ESW group (Group 2) and HBO group (Group 3) and included in the study.

### Surgical Procedure

Under general anaesthesia, the rabbits in supine position were performed to 5 centimeter (cm) longitudinal incision on the patellar tendon. After exposure of the patellar tendon and the proximal tibia, with 45 degree angle to tibia, a full layer cut was made with a lancet at the bone-tendon junction. Then, immediately below the cut, a tunnel was opened vertical to the tibia long axis, using a 2 mm drill. The bone junction tendon was anatomically repaired by passing 3.0 prolene sutures through the created tunnel with the Krakow method. To prevent breakage, a quadriceps tenotomy was made to eliminate the extensor power of the quadriceps tendon over the repaired osteotendinous junction. After ensuring the stability of the repaired osteotendinous junction, the layers were anatomically closed [6].

A polyethylene long-leg brace was applied with the hip joint in 30° flexion, the knee joint in full extension and the ankle free, and thus immobilisation was provided for 6 weeks. In Group 1, as the control group, the repair was made and no additional treatment method was applied. In Group 2, sedation was applied and with an EMD/E 1000 ESW scopy device, a single session of ESW was applied at 14 Hertz (Hz)/750 Impulse/1 centimeter square (cm<sup>2</sup>)/10 minutes (min) to the osteotendinous junction.

The animals in Group 3 were placed in a pressure chamber (Hiperteks Neoks Arbe Chamber) and were applied with 100% oxygen at 2.4 ATA/110 mins for 1 session each day throughout 7 days.

### Biomechanical and Histopathological Evaluation

At the end of 6 weeks, all animals were sacrificed by same surgical team and samples were separated



randomly into 2 equal subgroups for biomechanical and histopathological evaluations. Entering over the old incision, the cut was extended proximally and distally and an osteotomy was applied with an osteotome to the centre of the femur and tibia diaphysis. Soft tissues were removed from the patella-patellar tendon-tibia proximal third and the samples were placed in saline and kept at 4°C until the biomechanical tests were applied on the same day.

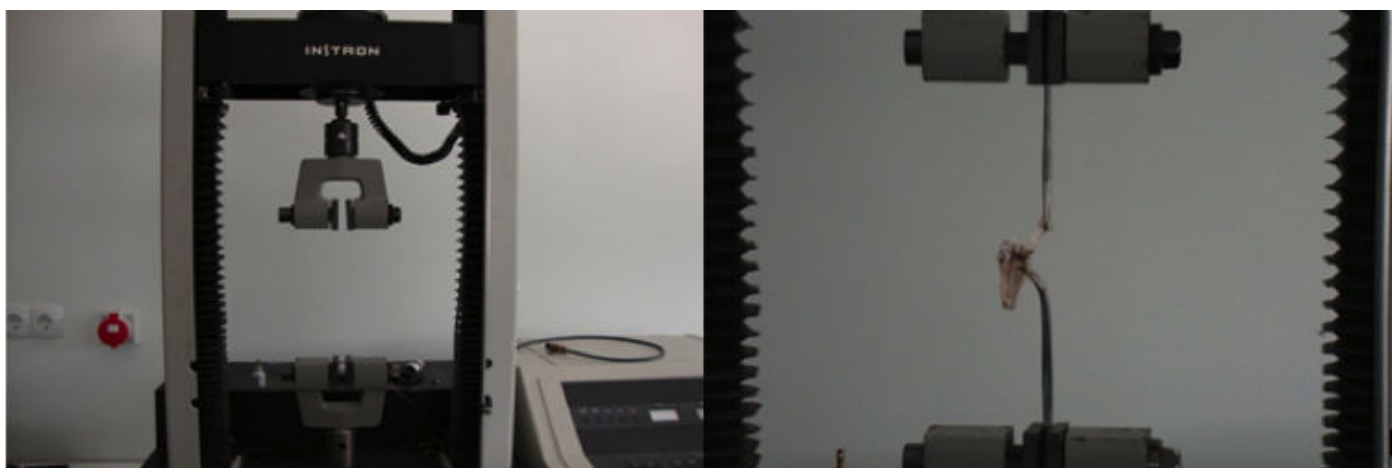
The materials taken for histopathological examination were placed in a sterile dishes in 10% formaldehyde solution and stored until tested. The whole material was evaluated macroscopically in respect of rupture and separation and the results were recorded. For the biomechanical evaluations, tension tests were applied using an Instron 4301 test device in the Physics Laboratory of the Textile Engineering Department of Uludag University (Fig.1).

The tension test procedure was started at a fixed rate of 25mm/min and was continued. The tension forces (Newton units/kN) were determined at the moment when separation started, when there was a 2 mm separation, and at the moment of complete rupture, and these values were recorded. Histopathological examinations were conducted in the Pathology Laboratory of Uludag University Hospital. After removal of the sutures in the osteotendinous junction area, a longitudinal cut was made from 2 different points and routine follow-up procedures were applied to the two corresponding pieces separately. After the routine procedures, the pieces were embedded in paraffin blocks and sections were cut 4-

5 microns in thickness. These sections were stained with haematoxyline and eosin and evaluated by a pathology specialist blinded to the study. In the histopathological examination, the fibrocartilage tissue, new bone formation and tendon-bone tissue fusion in the healed area of the osteotendinous junction were evaluated using the histological scoring system described by Weng *et al.* [29]. Each parameter was scored with points.

### Statistical Analysis

Statistical analyzes were carried out with SPSS v.16.0 software (SPSS Inc., IBM Corporation, Armonk, New York, USA) and power analysis was carried out with G-Power v.3.1.9.1 software [Franz Faul, Universitat Kiel, Germany]. For this study, alfa error was accepted as %5 and  $p < 0.05$  values were accepted as statistically significant. As there were a total of 3 paired comparisons, Bonferroni correction was applied because of the group analysis, the p value was calculated as  $0.05/3 = 0.017$ . Before the study, power analysis was performed. Due to similar studies at the literature statistical power ( $1-\beta$ ) was accepted %80 and total sample size was determined as 36 animals. Demographic datas of rabbits as age and weight were evaluated with the Chi-square test. Because of small sample groups, results were accepted to non-normally distribute so median, minimum and maximum values are determined and non-parametric datas were compared with Kruskal-Wallis and Mann Whitney U-tests.



**Fig. 1.** Application of the tension test with the Universal Instron 4301 test device.



## RESULTS

A significant difference was observed with comparisons of the force at the start of the separation of the healing area of the osteotendinous junction, the force creating the 2 mm separation and the force providing full rupture ( $p = 0.001$ ) (Table 1).

In Group 1; the separation at the healing area of osteotendinous junction was started first, biomechanical resistance to 2 mm separation and full rupture were significantly lower than Group 2 and Group 3 ( $p = 0.001$  and  $p = 0.001$ , respectively). The highest biomechanical resistance to separation were determined in Group 3 ( $p = 0.001$ ) (Table 1).

The fibrovascular tissue, collagen bundles, fibrocartilage tissue and new bone formation were examined in the materials taken from the osteotendinous junction of the other half of the subjects. Fibrovascular tissue and new bone formation were more evident in the treatment groups compared to the control group (Fig. 2a, 2b and 2c). Collagen alignment was more regular and mature in the treatment groups than in the control group. Osteotendinous fusion, fibrocartilage tissue formation, new bone evaluation formation and total histological score were evaluated (Fig. 3a, 3b and 3c). Except fibrocartilage tissue formation ( $p = 0.049$ ); in histological evaluations, there was a statistically significant difference between control group and treatment groups but no difference between treatment groups. (Table 1).

## DISCUSSION

Although osteotendinous junction injuries are often encountered, because of the complex anatomic

structure of the region, the expected successful results cannot always be obtained even if early and optimum surgical treatment has been applied [2, 7]. As postoperative immobilisation of the extremity is required and no consensus has yet been reached in literature on the treatment method, there is still not a full understanding of the biomechanical function and histological properties of the anatomic structure of the osteotendinous region and this has rendered treatment of injuries in this region complicated [1, 2].

Similarly, in a rabbit model of delayed osteotendinous junction injuries, Qin *et al.* [12] reported that in the group applied with ESW, there was more bone formation in the healing area of the patella-tendon junction at 8 weeks (293.4%) and 12 weeks (185.8%) compared with control group and histologically collagen bundles were more mature and fibrocartilage tissue was thicker and more regular. Wang *et al.* [13] reported that ESW accelerated neovascularisation in the Achilles tendon-bone junction in rabbits and this effect lasted for up to 12 weeks. In those studies, an increase was determined in angiogenic markers in the tissue such as VEGF and endothelial nitric oxide (eNOS).

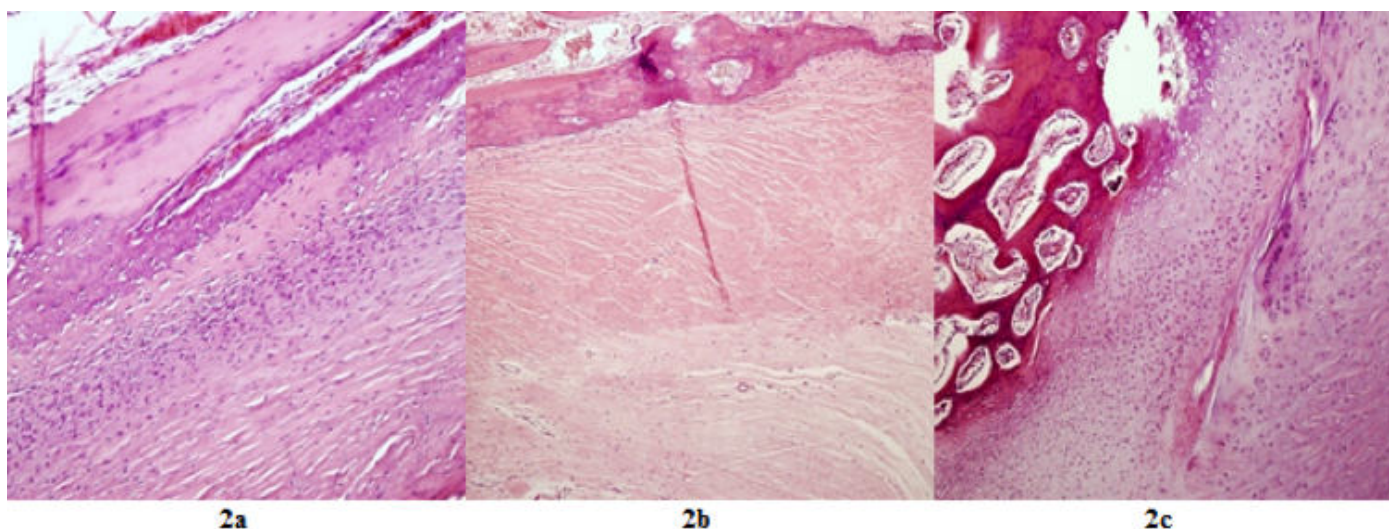
In an Achilles tendon partial rupture model in rats, Orhan *et al.* [30] compared subjects applied with ESW (500 shock/15 kV) with a control group and reported that in the ESW group, neovascularisation was increased, there were fewer adhesions and a mechanically stronger tissue was obtained.

In a patellar tendinitis model created in rabbits by Hsu *et al.* [31], the application of ESW at 0.29Mj/mm<sup>2</sup> energy was seen to increase collagen synthesis in the healing tissue, accelerated neovascularisation and increased tensile strength of the tissue. Similar to these findings in literature, accelerated collagen synthesis

**Table 1. Biomechanical and histological evaluations**

	Initial Sep. <sup>3</sup>	2mm Sep. <sup>4</sup>	Full Sep. <sup>5</sup>	OF <sup>6</sup>	FTF <sup>7</sup>	NBF <sup>8</sup>	TS <sup>9</sup>
K-W <sup>1</sup>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.016</b>	<b>0.049</b>	<b>0.016</b>	<b>0.014</b>
MW-U <sup>2</sup>							
Group 1-2	<b>0.002</b>	<b>0.002</b>	<b>0.002</b>	<b>0.015</b>	0.093	<b>0.014</b>	0.093
Group 1-3	<b>0.002</b>	<b>0.002</b>	<b>0.002</b>	<b>0.015</b>	<b>0.026</b>	<b>0.009</b>	<b>0.002</b>
Group 2-3	<b>0.002</b>	<b>0.002</b>	<b>0.002</b>	0.310	0.818	0.240	0.310

<sup>1</sup>Kruskal-Wallis Test, <sup>2</sup>Mann Whitney-U Test, <sup>3</sup>Initial Separation (k/N), <sup>4</sup>2mm Separation (k/N), <sup>5</sup>Full Separation (k/N), <sup>6</sup>Osteotendinous Fusion, <sup>7</sup>Fibrocartilage Tissue Formation, <sup>8</sup>New Bone Formation, <sup>9</sup>Total Score



**Fig. 2 (a, b and c).** Histopathological examination images of the fibrovascular tissue, collagen bundles, fibrocartilage tissue and new bone formation of the groups.

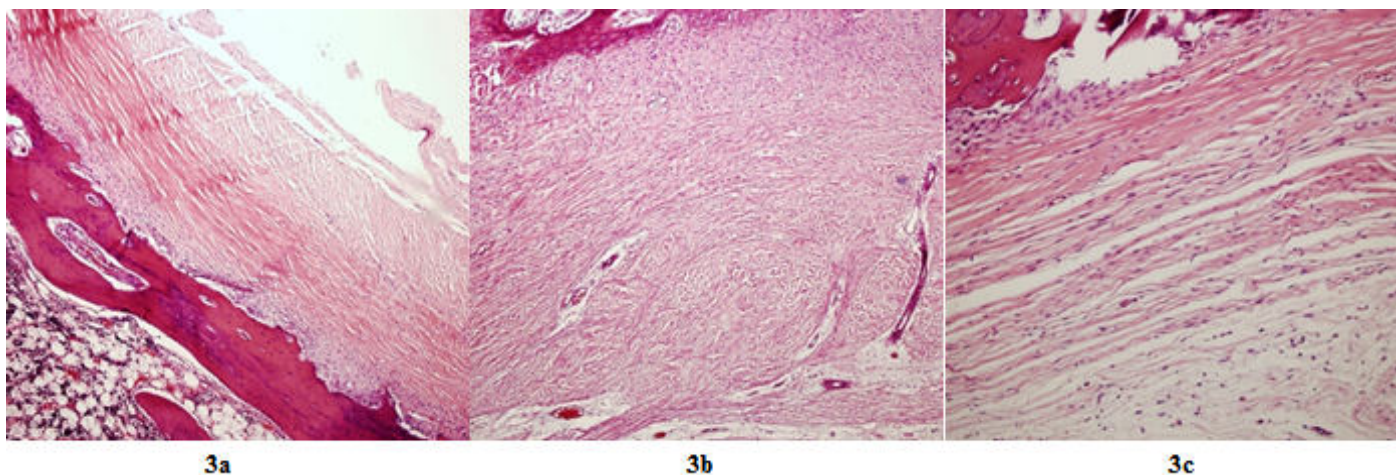
and maturation; osteotendinous fusion and new bone formation were observed significantly higher in the group 2 (Table 1 and 2) ( $p = 0.015$  and  $p = 0.014$ ). As a result of these histological changes, the osteotendinous fusion was increased and biomechanical strength of the tendon was increased.

In the group 3 as the other supplementary treatment method, the biomechanical resistance was determined to be statistically significantly greater than other groups ( $p = 0.002$ ). The results of the histological evaluation showed a similar statistically significant higher osteotendinous fusion and new bone formation but no significant difference found at

fibrocartilage tissue formation ( $p = 0.049$ ).

In the literature, several studies were researched to effects of HBO with biological and biomechanical datas and concluded that, HBO treatment was accelerated the fibroblast proliferation, increased the structural protein synthesis, inhibit to TGF- $\beta$ 1 synthesis, inhibited the metalloproteinase effects as a result inhibited the degradation also tendon-bone healing and biomechanical strength of tendon was significantly increased [29, 32, 33].

In the current study, the histopathological and biomechanical findings of the animals applied with supplementary treatments were found statistically



**Fig. 3 (a, b and c).** Histopathological evaluation images of the collagen alignment and maturation and the formation of fibrocartilage tissue of the groups

**Table 2. Biomechanical results**

		Initial Separation (k/N)	2 mm Separation (k/N)	Full Separation (k/N)
Group 1	n	6	6	6
	Median	173.50	193.00	205.50
	Minimum	168	188	200
	Maximum	179	199	209
Group 2	n	6	6	6
	Median	244.50	261.50	296
	Minimum	236	252	288
	Maximum	252	266	300
Group 3	n	6	6	6
	Media	291	326	345
	Minimum	280	310	330
	Maximum	296	338	352
Kruskal-Wallis	<b>p value</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>

significant to compared with group1 except fibrocartilage tissue formation (Table 1 and 2). Osteotendinous fusion, new bone formation and biomechanical strenght were observed significantly increased to compared with group 1 ( $p < 0.05$ ). Comparison between group 2 and 3; the biomechanical strength was significantly increased in group 3 ( $p = 0.002$ )but there were no significant difference in histopathological findings ( $p > 0.05$ ).

## CONCLUSION

In conclusion, the results of this study concluded that the supplementary treatments were accelerated to healing of osteotendinous junction injuries with increased new boneformation, osteotendinous fusion and biomechanical strength. Comparison between ESW and HBO, except the biomechanical strenght was significantly increased in HBO; there were significant difference. In the light of these results,

ESW and HBO treatments can be considered beneficial in the early postoperative healing of osteotendinous junction injuries and as increased strenght of the bone-junction tendon, could make a positive contribution to the rehabilitation process and have a positive effect on outcomes. Also further well-designed randomise clinical trials are required.

### Conflict of interest

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

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# The management of Bochdalek congenital diaphragmatic hernia: a single center experience and review of the literature

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## ABSTRACT

**Objectives:** Congenital diaphragmatic hernia (CDH) is a rare congenital anomaly of the diaphragm with pulmonary hypoplasia and persistent pulmonary hypertension and has serious consequences. Despite recent diagnosis and treatment strategies, CDH is still a challenging condition. We aim to present our clinical experiences of CDH, and review of the literature.

**Methods:** Data of CDH patients who were operated in our clinic between January 2010 and September 2018 were obtained from the patient's chart. The clinical course and results of the patients with Bochdalek type CDH were reviewed.

**Results:** We performed diaphragmatic closure in 16 patients with Bochdalek CDH during study period. Fourteen (82%) cases were diagnosed antenatally. In 5 (29%) patients, preoperative pulmonary hypertension developed and nitric oxide was administered. Twelve of the sixteen patients (75%) underwent primary repair and 4 of them underwent prosthetic patch. Three patients were repaired thoroscopically. Five (31%) patients died due to severe persistent pulmonary hypertension on postoperative period. There was no recurrence in our patients who were followed-up for a mean of 27 months.

**Conclusion:** In the management of Bochdalek CDH, the clinical success has been increasing in parallel with the important developments with the application of new treatment modalities in the neonatal intensive care units and new surgical techniques in recent years.

**Keywords:** Bochdalek hernia, congenital diaphragmatic hernia, newborn, pulmonary hypertension

Congenital diaphragmatic hernias (CDH) occur in approximately 2500-3000 births and they are congenital anomalies. Diaphragm begins to develop at the fourth weeks of the pregnancy, and completes the formation in about 20 weeks. It has been found that some genetic and environmental factors play a role in the pathogenesis, but whose exact pathogenesis is still not understood well. Anatomically, CDH can be clas-

sified as posterolateral (Bochdalek, 70-75%), anterior (Morgagni, 23-28%) or central (2-7%) CDH caused by defects. Bochdalek hernias are the most common type of congenital diaphragmatic hernia resulting from a failure of pleuroperitoneal membrane closure in utero, characterized by the protrusion of intra-abdominal contents into the thoracic cavity through the posterolateral diaphragmatic defect. In 85% of the cases,

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Bochdalek CDH is seen on the left side, in 13% right and in 2% bilaterally [1-3].

In Bochdalek CDH patients, pulmonary hypoplasia and pulmonary hypertension (PH) are the major determinants of survival in the neonatal period and predictors of long-term morbidity. Because of pulmonary hypoplasia and PH which are the results of preventing the development of terminal bronchioles, alveoli and pulmonary vessels, critical respiratory insufficiency arises immediately after birth. Although many pharmacological treatments and also many current applications such as “gentle ventilation”, high frequency oscillatory ventilation (HFOV) and extracorporeal membrane oxygenation (ECMO) are now used, the mortality and morbidity rates are still high in Bochdalek CDH patients. Additionally, there are still ongoing discussions on some aspects of this disorder such as the best method of surgical repair, the choice of patch to be used in place of lacking diaphragm and optimal time of surgery [2, 4].

In this study, we aimed to retrospectively review the patients who underwent surgery for Bochdalek CDH, and to present our approaches in the light of the literature.

## METHODS

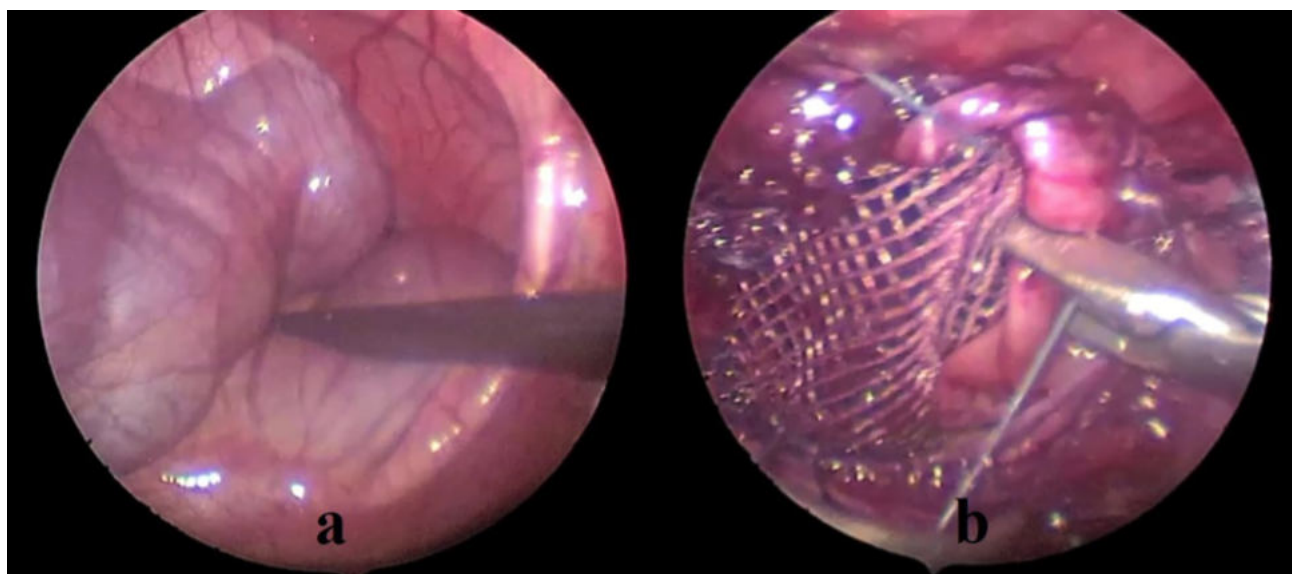
Between January 2010 and September 2018, data of newborns who were operated in our clinic because of Bochdalek CDH have been reviewed retrospectively. The cases have been evaluated from the antenatal findings, gestational ages, birth weights, ventilator requirements and parameters, preoperative data, additional medical treatments, operation times, operative pattern, patch requirement, duration of the mechanical ventilation, complications, additional surgical procedures and mortality.

In our hospital, neonatal intensive care unit (NICU) is managed by neonatologists. Pre- and post-operative care of the patients with Bochdalek CDH are implemented in this unit. Although the patients with Bochdalek CDH are managed by different neonatologists, standard approaches are carried out. The management of Bochdalek CDH patients in our institution is briefly: postnatally, after initial resuscitation with avoidance of the bag mask ventilation and subsequent barotrauma, a nasogastric

tube is placed, a suitable vascular access is inserted, and the case with Bochdalek CDH is transferred to the NICU. The patient is monitored for pre- and postductal O<sub>2</sub> saturation. Arterial blood gas and initial CBC, basic metabolic parameters, and coagulation profile is measured. A chest X-ray and abdominal X-ray (Fig. 1), ultrasonography (US), and echocardiography to investigate accompanying organ anomalies, and to assess the degree of pulmonary hypertension, ductal patency and ventricular function are studied routinely. Intravenous fluid, electrolyte and nutrition are administered. Analgesics and sedatives are also administered to facilitate optimal ventilation especially in neonates with severe pulmonary hypertension. Synchronized conventional ventilation (SIMV) with tidal volume monitoring is chosen to allow permissive hypercapnia as initial ventilatory strategy. If needed, high frequency oscillatory ventilation (HFOV) which is a lung protective strategy is used to reduce ventilator induced lung injury. If PH persists, pulmonary vasodilator therapy is started, with



**Fig. 1.** The chest X-Ray showing the large diaphragmatic hernia.



**Fig. 2.** Intraoperative photos showing the diaphragmatic defect which is too large to close with thoracoscopic primary repair (a), and using prosthetic patch to repair diaphragmatic hernia in same patient (b).

inhaled NO as the first choice and/or Sildenafil a phosphodiesterase-5 inhibitor. ECMO in the treatment of neonates with Bochdalek CDH is not currently used in our institution.

For surgical repair, laparotomy was mostly performed with the transverse incision of the classical left upper quadrant. After gently pulling the organs into the abdomen, the defect margins were primarily closed with U-shaped suturing with nonabsorbable sutures. If the diaphragmatic edges were not developed, the defect was closed by using either of the muscle flaps prepared from the near structures or synthetic patch in very large defects. Finally, the organs were checked for other anomalies and the intestinal malrotation before the abdomen was closed. If the abdominal cavity is not sufficient, only skin closure can be done, but it was not needed in our cases.

In the thoracoscopic repairing, the optic port under the scapula and two work ports from the laterals of this point were placed, herniated organs pushed to the abdomen and the defect was closed with either primary U-shaped sutures or synthetic mesh (Fig. 2).

**RESULTS**

During the 8-year study period, data of 17 patients were found and restorative surgeries were performed in 16 patients for Bochdalek CDH. The antenatal and postnatal data of the cases are presented in Table 1. Fourteen out of 17 cases (82%) were diagnosed antenatally, and the mean gestational age for diagnosis was 20 weeks (min-max: 16- 32). In one case, the right Bochdalek CDH was detected (6%), and this case was being followed conservatively since the liver prevented intestinal herniation. Due to the development of PH, NO was initiated in 5 patients (29%) preoperatively. A patient with very low birth weight and bilateral severe pulmonary hypoplasia died before surgery. Patients were taken to the operation approximately in postpartum 30th hours. The records of the operations and postnatal follow-up are summarized in Table 2. Primary repair was made with laparotomy in 12 patients. Also, in one infant, primary repair was made by forming flaps from intercostal and

**Table 1. Antenatal and postnatal clinical data of patients with CDH**

Gender (F/M)	10/7
Antenatal diagnosis (%)	14 (82%)
Mean gestational age at diagnosis	20 weeks (16-32 weeks)
Mean gestational age at birth	35 weeks (30-41 weeks)
Mean birth weight	2810 gr (1100-4400 gr)

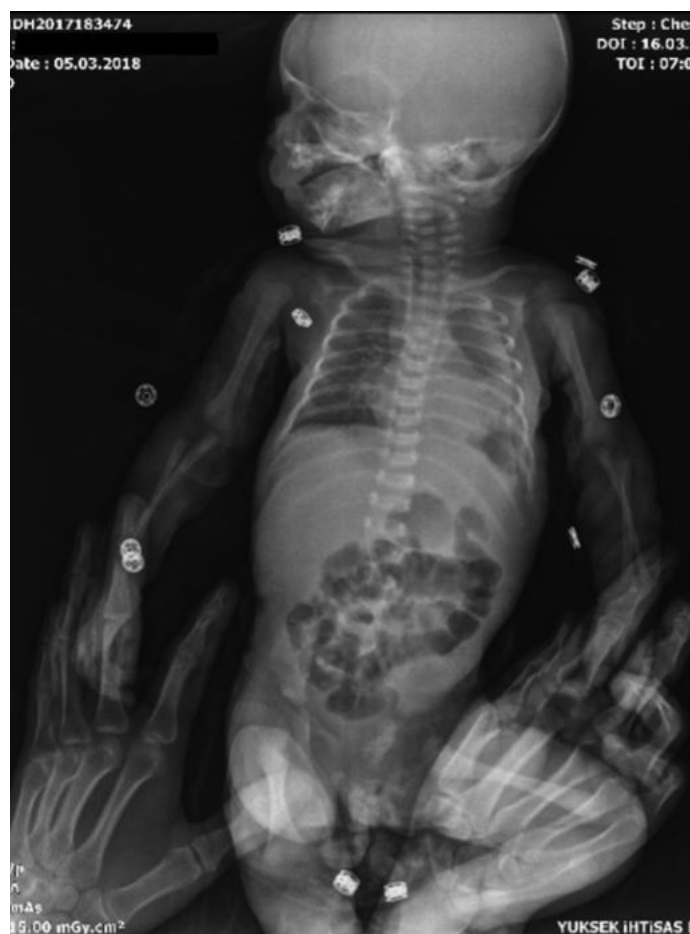
CDH = Congenital diaphragmatic hernia, F = Female, M = Male

latissimusdorsi muscle flaps. Synthetic patch was required in four patients. Mostly, propylene mesh was used as the patch. Besides propylene mesh, polytetrafluoroethylene (PTFE), polyglactin and propylene mixture meshes were also used as the patch. Three cases were approached with thoracoscopic method, primary closure of the defect was achieved in two patients, and propylene mesh was implanted in one. The mean duration of the mechanical ventilation was 5.5 days. Because of persistent PH, NO was continued or started in 7 patients postoperatively.

**Table 2. Surgical and postoperative follow-up symptoms of patients with CDH**

The timing of surgery after birth	30 <sup>th</sup> hour (24 <sup>th</sup> -52 <sup>nd</sup> hour)
Operation	16
Laparotomy	
Primary	10
Prosthetic	3
Thoracoscopic	
Primary	2
Prosthetic	1
Hernia content	
Liver	9
Spleen	13
Stomach	11
Small bowel	17
Colon	14
Additional intraabdominal anomaly	Malrotation (n = 2)
Mean duration on ventilator support	5.5 days (2-13 days)
Long term morbidity	GER (n = 2) Pectus excavatum (n = 3)
Additional surgery	Fundoplication (n = 2)
Mortality	31%

CDH = Congenital diaphragmatic hernia, GER = gastroesophageal reflux



**Fig. 3. Postoperative chest X-Ray showed restoration of the normal contour of the left hemidiaphragm in the patient who underwent thoracoscopic repair.**

Sildenafil was initiated in five patients.

Five patients died due to postnatal early period persistent PH and respiratory insufficiency (31%). Mean follow-up period was 27 months (1-84 months). Hernia recurrence was not seen in any patient (Fig. 3). However, later on, two patients were re-operated with the cause of severe gastroesophageal reflux (GER).

## DISCUSSION

In recent years, despite the important developments in surgery and anesthesia of newborn, the management of Bochdalek CDH patients is still a major problem. There are significant differences between clinics in the use of antenatal steroids, ventilation modes, methods used in PH treatment, the usage of ECMO, and timing of surgical repair [4].

These are the important issues striving to be standardized. The findings detected on the antenatal US and fetal magnetic resonance imaging (MRI) has begun to be used as prognostic criteria in Bochdalek CDH. It has been suggested that a prediction about prognosis can be made according to observed/expected lung-to-head ratio, total lung volume, liver herniation grade, and appearance of the stomach over four chambers of the heart in US and fetal MRI. The reason for the use of lung-head ratio is an indirect evaluation of the contralateral lung volume and therefore the possibility of pulmonary hypoplasia. The degree of volumetric intrathoracic liver herniation using fetal MRI indicates that the liver is better on lower position at predicting postnatal survival than the above [5]. In our series, Bochdalek CDH was detected in the antenatal US follow-up of 14 patients. However fetal MRI was not performed in our patients. It is obvious that there is a need to establish the standards in the antenatal screening of Bochdalek CDH.

Many studies have been also done on the prediction of the survival rates of newborns with Bochdalek CDH postnatally. Prognosis is determined by defect size, patch requirement, pulmonary hypoplasia, pulmonary vascular structure and PH grade. Recently, a model has been tried to be used to estimate the probability of survival based on various parameters such as low birth weight, low Apgar score, severe PH, major cardiac anomaly and chromosome anomalies [6]. This model seems that it could be implemented with data that could easily be found in the clinical setting. We did not have sufficient data on the postnatal and preoperative pulmonary morphology in our series.

In order to reduce pulmonary vascular resistance and right-to-left shunt preoperatively, alkalosis ( $\text{pH} > 7.55$ ;  $\text{PCO}_2 < 20$  mmHg), which was attempted to be induced by chemical and ventilator settings, led to barotrauma and later neurological sequel. Thereupon, "permissive hypercapnia" is defined. Thus, it has been proposed that HFOV, which is the most appropriate ventilator mode for optimal ventilation, should be administered initially, as it is more appropriate than conventional mechanic ventilation strategies [7]. The most frequently used ventilator mode was SIMV as traditional mechanical ventilation mode in our patients on preoperative period. HFOV administration was initiated in the patients in the early period if required.

PH is a pathological condition of the pulmonary vascular structure and results in pathophysiological pulmonary circulation. PH generally affects oxygenation, ventilation, and/or cardiac function. In patients with Bochdalek CDH, PH is caused by vascular thickening of the medial and adventitial layers of pulmonary vascular structures, a hypoplastic vascular bed with diminishing branching, and pulmonary artery/arteriole which have the inappropriate response to physiological and pharmacological signals. As a result of the external compression of the herniated organs into the lung, despite the high oxygen supply, the  $\text{O}_2$  saturation cannot be increased and carbon dioxide retention occurs [8]. On the other hand, it was shown that mechanisms such as retinoic acid pathway, NO pathway, endothelin pathway, and vascular endothelial growth factor contributes to the formation and/or progression of the PH in the patients with Bochdalek CDH. It has been shown that nitrophen, a retinal dehydrogenase inhibitor, forms Bochdalek CDH experimentally. It was also seen that Bochdalek CDH risk increased in infants of mothers who have received less than 800 mg of vitamin A during pregnancy [9]. NO pathway plays a role in angiogenesis, lung development and vasorelaxation in the human fetal lung. NO is spread to smooth muscle cells from endothelia and decrease the cytosolic calcium depending on c-GMP, and induces pulmonary vasodilation. In fact, endothelial NO synthase, an enzyme responsible for the expression of NO in the vascular endothelium, both increased and decreased in animal and human Bochdalek CDH studies. For this reason, despite the responsibility for PH in patients with Bochdalek CDH is still not understood completely, but NO continues to be used in PH management. In the etiology of PH, besides endothelin-1, which is highly detected in Bochdalek CDH patients, the disorder of balance ETA (vasoconstriction) and ETB (vasodilatation) which primary receptors of endothelin-1, have been responsible for increased pulmonary vascular pressure and exacerbation [10]. Lastly, vascular endothelial growth factor has been experimentally found at different levels in nitrophen-induced Bochdalek CDH. It has been found to stimulate pulmonary vasculogenesis/angiogenesis which is critical in embryological pulmonary development and growth.



On the other hand, human postmortem studies have shown that this factor has been found to be increased, and it has been estimated that it is probably due to a response to the stimulation of angiogenesis in the hypoplastic vascular structure [8,11]. In the examination of our patients who were included in our study, the intake of vitamin A was not given details from history. However, we know that pregnant women followed by obstetricians routinely receive multivitamin pills. Although studies on endothelin and vascular growth factor levels were not measured, preoperative and postoperative NO treatments were applied to three patients. ECMO, which is a subject in itself in PH management, is seen as the last step treatment of PH, and is an invasive approach. Additionally, besides NO inhalation, many drugs such as sildenafil, milrinone, prostanoids, prostoglandin E1, bosentan and MgSO<sub>4</sub>, are applied in PH management. Sildenafil use is recommended to be stored in refractive PH cases. And, it is claimed that it connects with the better results. On the other hand, in 2015, updated CDH EURO Consortium in order to provide postnatal treatment is standardized in European countries has been proposed in consensus that, however, if there is no or an insufficient response to inhaled NO, intravenous prostacyclin, intravenous sildenafil or medication involving the endothelin pathway should be considered [12]. Sildenafil was begun to use in five patients in our series.

There is no consensus on the timing of surgery for the patients with Bochdalek CDH. Some centers promote early surgery (first 24 hours). Over the time, this approach has largely changed from early surgical intervention to management and control of pulmonary hypertension before surgical repair [1]. The European working group suggests that surgical repair must be performed after physiologic respiratory and cardiac stabilization [12]. We also operated our cases at the postnatal 30<sup>th</sup> hour.

Standard open surgery in Bochdalek CDH patients is performed using a subcostal incision; the diaphragm is repaired by using with interrupted non-absorbable sutures after returning the herniated organs to the abdomen from thoracic cavity. In the majority of cases (60-70%), primary closure of the diaphragm can be achieved. Although it is known to be unsuccessful in half, in cases where the diaphragm never develops or the defect is too large to close the defect, muscle flap

techniques, a synthetic or biosynthetic prosthetic patch may be required [13]. The muscle flaps have the advantage of repairing the diaphragm without tension, but are often impractical for primary repair. Abdominal compartment syndrome is rare after Bochdalek CDH repair. But, if there is not enough abdominal cavity, the closure of the abdominal fascia can be delayed [14]. After Silen and his colleagues performed the first thoracoscopic approach in 1995, minimally invasive surgery (MIS) arose as a safe and feasible alternative to open surgery [15]. The benefits of MIS include reduced postoperative pain and a good cosmetic appearance. Beside these benefits, MIS provides reduction of organs within a wide field of view through thoracic cavity. The disadvantages of MIS are limitation of diagnosing and treatment of other intraabdominal pathologies such as malrotation, narrow space for diaphragm repair, and physiological sensitivity of the newborn [13]. In our series, the diaphragmatic defects were closed with primary sutures in 12 patients (75%), and two of whom were thoracoscopic. The intercostal and latissimusdorsi muscle flaps were used to close the defect in one. In four cases, prosthetic material was needed to close the large diaphragmatic defect, and the most common propylene mesh patch was used in these patients. The patch was placed thoracoscopically on one of these patients. The malrotation anomaly was detected and Ladd Procedure performed in two patients who operated by laparotomy. The abdominal compartment was not seen in any patient. None of the patients developed recurrence.

Mortality rates for Bochdalek CDH patients vary in the literature. While survival rates were 90% in single-center studies, the general mortality rate in multicenter studies ranged from 15 to 70% [1, 2, 5, 6]. This difference makes it difficult to accurately assess the effect of recent treatment progress on survival outcomes. In our series, the overall survival rate is 69%. This shows that it is compatible with the literature. The success of surgical treatment may be related to patient density. In the hospital complex which includes our center, there are big birth clinic and the most equipped newborn intensive care unit of the region. These provide that the patients are gathered at our center.

Surviving Bochdalek CDH patients in the long term expose to the risk of many diseases. These



chronic diseases include chronic pulmonary diseases such as restrictive pulmonary defects, reactive airway; gastrointestinal morbidity such as GER (45-90%); musculoskeletal abnormalities such as chest deformity and scoliosis; as well as growth retardation, neurological disorder and hearing loss [2]. The average time spent on the ventilator was 3 days in our series. Any sequel depend to ventilator was not seen. However, severe GER developed in 2 patients. Antireflux surgery was done to these patients in the early period. In 3 patients, pectusexcavatum is available; however, operation will be decided during the adolescence period of these patients. We did not have any patient with neurological developmental delay on neurologic follow-up.

## CONCLUSION

Bochdalek CDH management has shown significant improvement in the recent years. The survival rates after surgical repairs are increasing with the application of new treatment modalities with the advances in the NICU.

### *Conflict of interest*

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# The opinion of sports science professionals for the benefit of statistics: an international web-based survey

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## ABSTRACT

**Objectives:** The present study, an international web-based survey, was focused on four aims: to obtain sports science professional's self-reported statistical knowledge levels and how this knowledge varies by research area, to investigate and specify when statistics courses should be taught in sports science education and to identify the key statistical methods relevant to sports science education.

**Methods:** In the present study, sports science professional data obtained by a web-based survey. Participants were selected randomly from the PubMed (www.ncbi.nlm.nih.gov) database for the years 2010-2018 using the keywords "school of sports science, faculty of sports science, sports faculty" by screening the sports science journals. Therefore, the participants were determined by searching the keywords in the corresponding or the first author's address information of the articles.

**Results:** The results indicate that while sports science professionals emphasize statistics education and the participants also believe that taking a statistics course is useful for their occupation. A statistics education should also emphasize the necessity of statistical consultation.

**Conclusions:** Our study provides information regarding self-reported levels of statistical knowledge of sports science professionals by research area and academic position, and provides guidance regarding the ideal semester for administering a statistics course.

**Keywords:** Statistics course, statistics knowledge, sports science education, sports science professionals, web-based survey

The acceptance of the results obtained from scientific research depends on the correct interpretation of the findings obtained by appropriate statistical analysis. In the generalizability process of the results; if statistics is considered as a tool, this tool must be used correctly and consciously. The fact that every step of scientific research from planning to the interpretation of results includes statistics, this tool leads to the integration of itself into various disci-

plines. Since scientific journals are aware of this fact and do not want to compromise on the statistical quality of the work they publish, they also include statistics or biostatistics editors in the editorial team. This importance given by scientific journals to the statistics requires the authors and readers should have at least a basic knowledge of statistics to be able to publish their work and benefit from the studies published in these journals that want to maintain this quality [1]. Many

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studies in the literature evaluated the importance given to statistics according to various disciplines. While some of these studies [2-6] examined the importance of statistics regarding the authors who submitted their studies to the journals, some of them [7-13] focused on the statistical errors in the articles published in scientific journals and aimed to emphasize the importance given to the statistics regarding journals. In addition to this statistics-based press, scientific journals sometimes try to fulfill their mission by publishing articles aimed at informing their readers about basic statistical concepts and sometimes informing them about the advanced statistical methods specific to their disciplines. Despite these positive initiatives, the effort and pressure to increase the number of publications produced in the academic environment, unfortunately, sometimes violate the scientific framework based on the concepts of rigor, repeatability, and transparency [14].

By applying statistical methods to various disciplines, statistical methods specific to the fields have emerged, and thus, the science of statistics has been integrated into many fields. One of the integrated fields of statistics science is sports sciences. As a result of this integration Casals and Finch [15], mentioned the fields of specializations that are specific to statistics and sports science as sabermetrics, Moneyball, sports analyst, and sports biostatistician. As in every discipline, the place of statistics in sports sciences is also important for the view of selecting the appropriate statistical procedure, to analyzing data, to the presentation of results. Moreover, this importance is directly proportional to the statistics education administered at the undergraduate and graduate level and the need for statistics in the publication stage of studies in scientific journals. For this reason, our study focused on sports science professionals with a PhD. degree. Therefore, participants just with a bachelor's or master's degree excluded from the evaluation.

The present study, an international web-based survey, was focused on four aims: to obtain sports-science researchers' self-reported statistical knowledge levels and how this knowledge varies by research area, to investigate and specify when statistics courses should be taught in sports science education and to identify the key statistical methods relevant to this education process.

## METHODS

In the present study, sports science professional data obtained by a web-based survey. Participants were selected randomly from the PubMed ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) database for the years 2010-2018 using the keywords “*school of sports science, faculty of sports science, sports faculty*” by screening the sports science journals. Therefore, the participants were determined by searching the keywords in the corresponding or the first author's address information of the articles. After the identification of the participants, they were also confirmed to be sports science professionals from their institutional web page or their previous studies. The participants were invited to participate in the survey via e-mail, and the respondents were directed to the survey at SurveyMonkey (<https://www.surveymonkey.com>).

In the first part of the survey, subjects were asked whether a statistics or biostatistics course would be useful for their future careers (from “completely disagree: 1”; to “completely agree: 5”), at which semester or semesters should statistics or biostatistics be administered, and how much importance they placed on statistics (from “not important: 0”; to “very important: 10”). In the second section of the survey, the subjects were asked which statistical methods, tests and techniques they knew, out of 54 methods and techniques which referenced based on our previous studies [2-5]. Only self-reported general knowledge about the procedures was assessed. In the questionnaire, methods, tests and techniques were grouped as “general statistics knowledge”. Subgroup statistical methods, tests and techniques were classified as follows: “parametric tests”, “non-parametric tests”, “multivariate methods”, “sampling methods” and “survival analysis methods”. The self-reported statistics knowledge of each participant was converted to a ratio by dividing the number of methods, tests, and techniques that the participant knew by the total number of methods, tests and techniques in that subject group.

## Statistical Analysis

In this study, the Shapiro-Wilk normality test was applied to determine whether the variables were

normally distributed. For comparison, Analysis of Variance (ANOVA), Kruskal-Wallis test, Independent samples t-test and Mann-Whitney U test were applied using a significance level of  $\alpha=0.05$ . Data were presented with median and interquartile range (IQR) which is equal to the difference between the 25<sup>th</sup> and 75<sup>th</sup> percentile value and also supported with mean  $\pm$  standard deviation values. The relation between categorical variables was examined using correlation analysis, and Spearman correlation coefficient was computed. Statistical analyses performed by using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

## RESULTS

Of the 2089 e-mail invitations sent, 123 were rejected by the server due to e-mail addresses being either incorrectly spelled or no longer valid, leaving an estimated 1,966 e-mail recipients. Those who responded with the intention of participating numbered 166, reflecting a response rate of 8.44%. Additionally, of 166 respondents, 29 were excluded from the study due to their failure to complete the survey.

Participants mean age was  $24.80 \pm 8.98$  years (range: 26 to 70 years). The majority of participants were male ( $n = 102, 74.48\%$ ). Socio-demographic characteristics of the participants presented in Table 1.

**Table 1. Socio-demographic features of the participants**

Age	
Mean $\pm$ Std. Deviation	24.80 $\pm$ 8.98
Minimum: Maximum	26:70
Gender, n (%)	
Female	35 (25.55%)
Male	102 (74.45%)
Academic Rank, n (%)	
Professor	27 (19.71%)
Associate Professor	36 (26.28%)
Assistant Professor	46 (33.58%)
Lecturer	28 (20.44%)
Academic, n (%)	
Academic	109 (79.56%)
Non- Academic	28 (20.44%)

A total of 137 sports science researchers from five continents and 38 countries participated in our study (Table 2).

Of the total 137 participants, 109 (79.56%) were academic staff, and 28 (20.44%) were not. All of 137 participants had a PhD. degree. Nearly 41% of the participants ( $n = 55$ ) stated that they had administered a statistics course in postgraduate education. The second most chosen option was determined by those ( $n = 52, 37.96\%$ ) who administered the course at both the

**Table 2. Distribution of the participants according to continents and countries**

Continent	Country
Africa n = 3 (2.19%)	Egypt (1), South Africa (1), Uganda (1)
America n = 20 (14.60%)	Brazil (12), Portugal (3), Mexico (2), Peru (1), Trinidad and Tobago (1), United States (1)
Asia n = 50 (36.50%)	Turkey (28), Iran (10), Japan (5), Taiwan (1), Austria (1), China (1), Kuwait (1), Republic of Korea (1), Thailand (1), Turkmenistan (1)
Europe n = 63 (45.99%)	Greece (15), Spain (12), Italy (11), Croatia (4), France (3), United Kingdom (3), Germany (2), Sweden (2), Switzerland (2), Czech Republic (1), Hungary (1), Ireland (1), Lithuania (1), Luxembourg (1), Netherlands (1), Poland (1), Romania (1), Macedonia (1)
Oceania n = 1 (0.73%)	Australia (1)

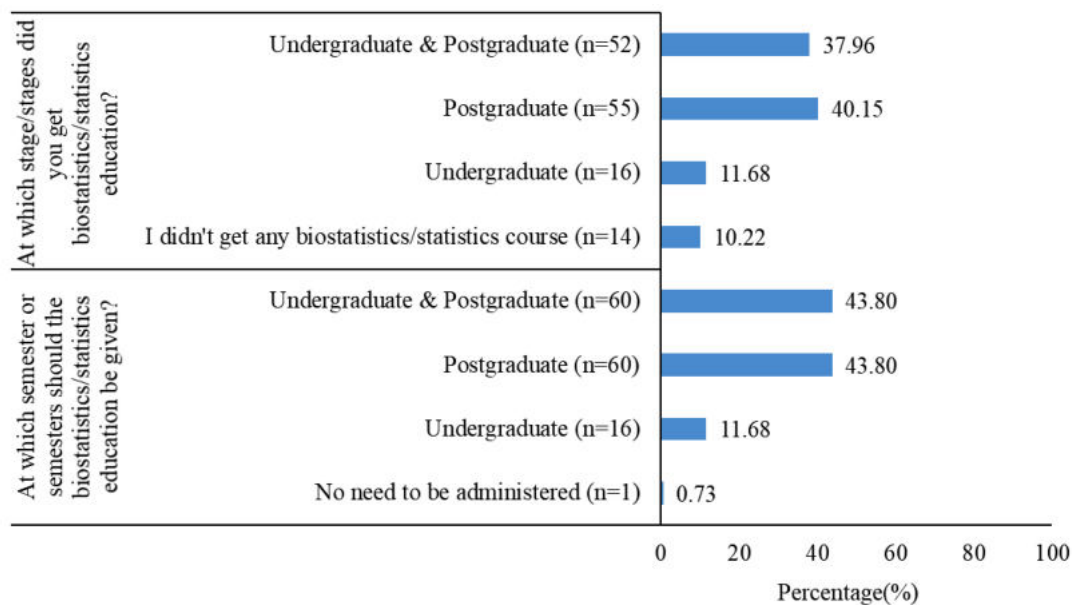


Fig. 1. Percentage of when the participants enrolled a biostatistics course and preferred time line.

**Table 3. Descriptive values and comparisons of whether enrolling in a statistical/biostatistics course are useful for one’s occupation and the importance placed on biostatistics in sports science according to academic staff and research area**

Academic Statue	Do you agree with the idea of taking statistics course of a sports science researcher is useful for his/her occupation? (min-max:1-5)	What is the importance of statistics in sports sciences? (min-max:0-10)
Academic Staff (n = 109)	5 (1) 4.35 ± 1.04	10 (2) 9.01 ± 1.29
Non-academic Staff (n = 28)	4.50 (1) 4.21 ± 0.99	8.50 (2) 8.43 ± 1.83
<i>p</i> -value	0.299 <sup>a</sup>	0.061 <sup>a</sup>
Research Area		
Exercise Physiology (n = 60)	5 (1) 4.23 ± 1.11	10 (2) 8.95 ± 1.55
Physical Performance (n = 39)	5 (1) 4.41 ± 0.88	9 (2) 8.85 ± 1.39
Physical Education (n = 23)	5 (1) 4.22 ± 1.04	9 (2) 8.78 ± 1.41
Department of Coaching and Psychology (n = 6)	5 (1.75) 4.17 ± 1.60	8.50 (2.25) 8.33 ± 1.21
<i>p</i> -value	0.904 <sup>b</sup>	0.460 <sup>b</sup>

Data were presented as Median (Interquartile range) and Mean ± Std. Deviation. <sup>a</sup> = Mann Whitney U test, <sup>b</sup> = Kruskal Wallis test; Trainer Education (n = 4), Sports Management (n = 3), Recreation (n = 2) due to insufficient sample size these groups were excluded from the analysis.



**Table 4. The comparisons of the level of self-reported statistics knowledge possessed by academic status.**

Academic Statute	Univariate Methods (%)	Multivariate Methods (%)	Parametric Tests (%)	Non-parametric Tests (%)	Survival Analysis Methods (%)	Sampling Methods (%)	General Statistics (%)
Academic Staff (n = 109)	56.27 ± 18.41	16.67 (33.33)	100 (14.29)	42.86 (25)	0 (0)	0 (4.17)	29.63 (21.30)
Non-academic Staff (n = 28)	55.61 ± 19.70	16.67 (25)	85.71 (28.57)	42.86 (28.57)	0 (25)	0 (8.33)	31.48 (26.39)
<b>p-value</b>	0.868 <sup>a</sup>	0.378 <sup>b</sup>	0.469 <sup>b</sup>	0.533 <sup>b</sup>	0.446 <sup>b</sup>	0.562 <sup>b</sup>	0.759 <sup>b</sup>

Data were presented as Median (Interquartile range) and Mean ± Std.Deviation. <sup>a</sup> = Independent Samples test, <sup>b</sup> = Mann Whitney U test

undergraduate and graduate level. The distribution of the responses of the remaining participants as follows: 11.68% (n = 16) took a biostatistics course only during undergraduate education, and the remaining 10.22% (n = 14) stated that they never took a statistics or biostatistics course (Fig. 1).

Two of the preferences of the period in which the course should be taken were surprisingly the same and the highest ratio with 43.80% (n = 60). The rest of the preferred opinions were as follows: 11.68% (n = 16) of the participants preferred that the course is administered only at the undergraduate level, and 0.73% (n = 1) stated that there was no need to administer the course (Fig. 1).

It was determined that SPSS is the most preferred statistical software for statistical analysis (Fig. 2). The three most-preferred statistical software are as follows: SPSS (65.96%), STATISTICA (9.22%) and LISREL&GraphPad (3.55%).

Academic participants which include staff personnel who hold an academic rank with titles such as professor, associate professor, assistant professor, instructor, lecturer, or the equivalent of any of these academic ranks and non-academic participants think that the statistics course is very important for them and that administering the course will benefit the profession in the future (Table 3). There was no difference in responses between academic and non-academic staff. Furthermore, all participants also agreed on the importance of statistics with the median point 9 (IQR: 2). Regardless of whether they were academic staff or not, there was also no difference between participant's responses to the importance of statistics and its usefulness, even when examined only in the research area of sports science (Table 3).

There is no difference between the academic and the non-academic staff according to the self-reported information level (Table 4). Moreover, there is also no difference between the research areas according to the self-reported knowledge levels (Table 5).

There is a relationship between the number of studies that participants place in the first order and their statistical knowledge levels. With the increase in the level of knowledge of the participants about general statistics, multivariate methods and parametric tests, the number of publications they are in the first order are increasing (Table 6).

**Table 5. The comparisons of the level of self-reported statistics knowledge possessed by research area.**

	Univariate Methods (%)	Multivariate Methods (%)	Parametric Tests (%)	Non-parametric Tests (%)	Survival Analysis Methods (%)	Sampling Methods (%)	General Statistics (%)
Exercise Physiology (n = 60)	56.03 ± 17.91	16.67 (25) 23.33 ± 22.64	100 (14.29) 88.81 ± 15.80	39.64 ± 22.95	0 (0) 15.00 ± 30.95	0 (8.33) 12.78±27.42	30.56 (22.22) 34.57 ± 18.02
Physical Performance (n = 39)	56.41 ± 19.53	25 (33.33) 29.49 ± 29.11	100 (14.29) 86.81 ± 19.50	41.21 ± 23.72	0(0) 14.53 ± 32.26	0 (0) 9.62 ± 25.90	29.63 (18.52) 35.52 ± 21.60
Physical Education (n = 23)	55.07 ± 18.65	25 (25) 30.07 ± 24.71	85.71 (42.86) 77.02 ± 28.81	44.41 ± 15.37	0 (0) 4.35 ± 15.26	0 (0) 9.06 ± 23.69	31.48 (22.22) 33.57 ± 17.15
Department of Coaching and Psychology (n = 6)	61.11 ± 15.77	37.50 (29.17) 40.28 ± 23.22	85.71 (17.86) 88.10 ± 10.75	47.62 ± 19.52	0 (33.33) 11.11 ± 17.21	4.17 (29.17) 15.28 ± 26.04	36.11 (26.39) 41.36 ± 17.12
<b>p-value</b>	0.914 <sup>a</sup>	0.179 <sup>b</sup>	0.482 <sup>b</sup>	0.728 <sup>a</sup>	0.442 <sup>b</sup>	0.308 <sup>b</sup>	0.728 <sup>b</sup>

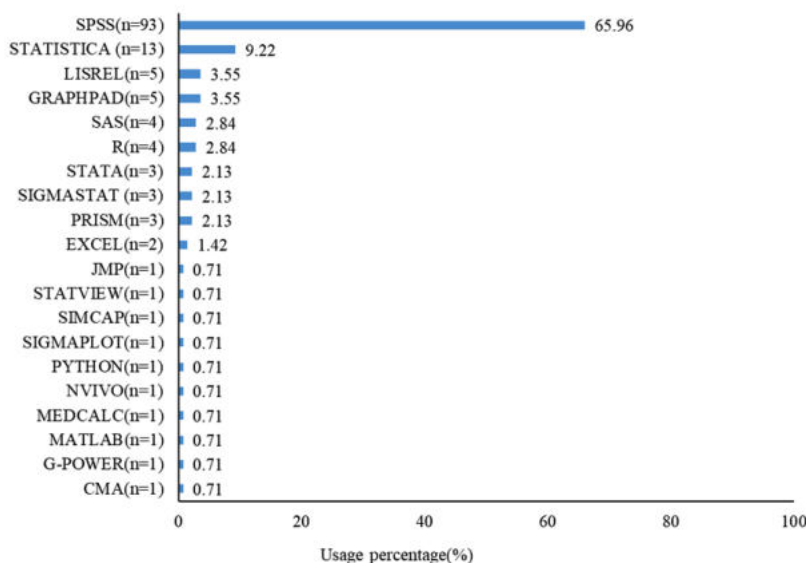
Data were presented as Median (Interquartile range) and Mean ± Std. Deviation, <sup>a</sup> = One-Way ANOVA test, <sup>b</sup> = Kruskal Wallis test; Trainer Education (n = 4), Sports Management (n = 3), Recreation (n = 2) due to insufficient sample size these groups were excluded from the analysis.

## DISCUSSION

The science of statistics is constantly evolving, and the products that it creates as a result of its cooperation with different disciplines make statistics popular. Understanding statistical concepts and skills are very important for those who read and publish scientific articles [1, 16]. A statistician who is proficient in instruments and uses in statistics science can use these features to specialize in his field.

As in every branch where statistics act together, statistics are of great importance for sports sciences. In the present study, it is aimed to examine whether this importance has changed according to the sub-branches of sports sciences and whether the participants are academic staff or not. Similar to our previous studies [2-6], it is seen that statistics (to the biostatistics in the sports sciences undergraduate and graduate education curriculum) are also given importance in sports sciences. However, this emphasis on statistics did not differ according to whether the participants were academic staff or not. Beyond the difference between academic and non-academic staff members, regarding the importance of statistics course and the role in career advancement, there was also no difference among research areas of sports science professionals. There is a clear distinction between the participants according to their opinions about when the course should be given. Almost half of the participants stated that they took the course only during the undergraduate term; the other group, who had a similar rate, stated that they took the course both in undergraduate and postgraduate periods. Since the present study is also multinational, it is likely that the undergraduate and graduate curricula that include the statistical course vary from country to country. Also, this finding is consistent with previous studies [2-5]. As in medicine, veterinary, dentistry and nursing; it is not surprising to see that the importance of statistics is also given in sports sciences.

Ten percent of the participants in our study stated that they did not take the course of statistics throughout their education. This can be interpreted that the remaining participants experience at least one statistical software during the application in the application of statistics or biostatistics course. With the experience of our previous studies, researchers



**Fig. 2.** Usage percentage of preferred statistical software by sports science researchers.

tend to prefer statistical software with the user interface in their work. As in our previous studies [2-5], SPSS is the software that the participants prefer in statistical analysis. In our study, as in our previous studies, participants highlighted the SPSS program as a tool to transfer their statistical skills to their scientific research.

It is known that biostatistics or statistics courses are considered a cautious course by professionals, especially in health sciences, and they do not have the necessary importance in postgraduate education [17, 18]. The fact that the required importance is not given to the statistics course and that the required interest is not shown, causes a lack of basic level of statistics to be felt especially in the case of an academic career.

Emphasizing the importance of statistics by the researchers involved in our previous studies may be a potential consequence of this lack. Also, it should be kept in mind that the deficiency felt against the statistics can be explained by the general lack of knowledge, but also by the inadequacy of specific techniques. In our study, the participants were asked to indicate which of the subtitles included in the statistical techniques, and the awareness levels about the statistics were tried to be measured. There was no difference between academic and non-academic sports science professionals regarding self-reported knowledge of statistics subjects.

Moreover, there was no difference in the level of knowledge by a sports science professional's research

**Table 6.** The relationship between the participants' knowledge of statistical methods and their number of published articles

(n = 137)	Number of total published articles (listed as the first author)	
Statistics subjects	r <sub>s</sub>	p-value
General Statistics	0.249	<b>0.003</b>
Multivariate Methods	0.269	<b>0.002</b>
Parametric Tests	0.249	<b>0.003</b>
Non-parametric Tests	0.160	0.062
Survival Analysis Methods	0.087	0.312
Sampling Methods	0.070	0.415

r: Spearman Correlation Coefficient

areas. A noteworthy finding is that both academic and non-academic staff have high levels of self-reported knowledge about parametric tests. To use parametric tests, some assumptions such as normality or homogeneity of variances should be provided. In contrast to the parametric tests, the relatively low level of self-reported knowledge of the participants about nonparametric tests necessitates the questioning of their ability to distinguish between parametric and non-parametric tests. The use of nonparametric tests where parametric tests should be applied will result in loss of power and questioning of the findings obtained from the analyzes [19-21]. The highest level of knowledge of the participants after parametric tests is about univariate analysis methods.

Moreover, it is seen that the participants know nonparametric tests even though below average. When Table 4 and Table 5 are examined, it is seen that the level of general self-reported statistical knowledge of the participants is low. Another finding that did not surprise us in our study and was confirmed by our previous studies [2-4] is that the level of knowledge about sampling is quite low. The finding that sport science professionals are almost completely unaware of sampling techniques is somewhat unsettling because sampling is the first important topic that a researcher considers during the planning stage of the study. Researchers hope that the data collected from given samples and its interpretation will accurately reflect the conditions found in the general population or group [1]. For this reason, to make a consistent, efficient and unbiased prediction at the end of a study, it is important to apply sampling techniques accurately [3].

When we investigated the relationship between sports science professionals' self-reported knowledge level of statistical methods and the number of articles they published, there was a significant relationship between the participants' knowledge of univariate methods, multivariate methods and general statistical knowledge with the number of articles they published. This finding indicates that the number of first name publications of the participants increased with the increase in the level of knowledge in the related titles. Considering the level of awareness of the participants about statistics, it is recommended that scientific studies should be carried out with an expert from the planning stage to the reporting stage. It is important to

remember that the design of each study and the characteristics of the data obtained may be different and specific to a particular study, so each study may require different statistical methods with which the researchers may be unfamiliar [3]. In this aspect, collaboration of statisticians or biostatisticians is essential.

### Limitations

One of the main limitations of this study is the low response rate (< 10%). The low response rate is not surprising, given that response rates to surveys have dramatically declined over time, due to the proliferation of junk mail, the rapid growth and ease of large-scale surveys, and resulting complaints that people feel "bombarded" with Internet-based surveys in the face of increasing demands on their time [22]. However, our response rate of 8.44% is similar to that of web-based studies in previous research aimed at nursing professionals (5.07%) [5], academic veterinarians (4.38%) [2], primary care physicians (5.7%) [23], dental physicians (9.1%) [3], and a group of urologists (9.3%) [24]. When similar studies are considered, our response rate is acceptable.

### CONCLUSION

The present study is significant regarding its international scope, intent and originality due to the uniqueness of this scope. Our study provides information regarding self-reported levels of statistical knowledge of sports science professionals by research area and academic position and provides guidance regarding the ideal semester for administering a statistics course. This study can also contribute to revising higher education sports science curricula by including frequently used statistical methods as a part of sports science research to enable professionals to understand current research and contribute to its ongoing discussion.

#### *Conflict of interest*

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

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# Early bacterial identification and carbapenemase detection from positive blood culture by mass spectrometry and Blue-Carba test

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## ABSTRACT

**Objectives:** In this study, we evaluated a rapid and simple protocol for direct identification of microorganisms with Matrix-Assisted Laser Desorption/Ionization Time of Flight, Mass Spectrometry (MALDI-TOF MS) after a short incubation in solid medium (3 to 5h).

**Methods:** We have examined a total of 1101 positive blood culture bottles from 782 patients.

**Results:** We obtained a correct identification in 1037 (94.18%); 190 (98.44%) were *Staphylococcus aureus*, and 386 (98.44%) *Enterobacteriaceae*. Both are the most frequent etiological agents of sepsis. A total of 1004 bottles were monomicrobial (96.81%) and 33 (3.18%), polymicrobial. In the latter we identified at least one species. Two hundred eighty (27.00%) organism isolated were considered skin contaminant. Carbapenemase tests were performed with Blue-Carba test in 140 patinas from Gram negative bacilli, we have detected earlier 27 of 29 positive (93.10%).

**Conclusions:** Mass spectrometry by MALDI-TOF MS is very useful to quickly identify the microbial agent and Blue-Carba contribute to adapt the antibiotic therapy to obtain a correct clinical management of the patient with bacteremia.

**Keywords:** MALDI-TOF MS, rapid identification, blood culture, carbapenemase, short incubation

Sepsis is associated with high mortality rate in adults and children worldwide. Blood cultures continue to be the gold standard for the etiological diagnostic. Considering the emerging resistance mechanisms and the diverse spectrum of organisms, the rapid microbiological identification and the fast resistance mechanisms detection are crucial for optimal management of these infections [1]. Also, it is important to know the local resistance patterns to optimize the empirical treatment. We carry out this work for giving a quick response that allows adequate management of the bacteremic patient, and a decrease in mor-

bidity and mortality associated with these serious infections. Techniques to decrease turnaround time to identify the causative agents are imperative for the reduction of mortality due to sepsis. A standard protocol for microbial identification is based on phenotypic aspect and biochemical patterns. Complete identification is routinely achieved within 18 to 48h, but may require more time for fastidious or atypical organisms. Introduction of Matrix-Assisted Laser Desorption/Ionization Time of Flight, Mass Spectrometry (MALDI-TOF MS) changed the general workflow in the clinical microbiology laboratories, had increased speed and

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accuracy of microbiological results. In a few minutes, MALDI-TOF MS can allow the analysis of proteins, mainly ribosomal type, for the exact identification of bacteria, mycobacteria and yeasts through the creation of a mass spectrum specific for each microorganism. The mass spectra of test isolates are sequentially compared with those in a reference database for identification. Depending on the MALDI-TOF MS score, the genus and species identification for an organism may be accurate. Individual mass peaks are used for microorganism identification and provide valuable information for the fingerprinting of bacteria. This technology generates fast and reliable identifications, and has the ability to analyze a large number of isolates simultaneously [2-4].

MALDI-TOF MS could be applied directly to blood cultures. In prospective studies, using different protocols such as Sepsityper (Bruker, Germany) or in-house procedures for bacterial extraction followed by lysis centrifugation and washing, it is possible to identify almost 72 to 80% of the microorganisms of positive blood cultures [2, 5, 6].

The marked increase in the incidence of infections due to antibiotic-resistant Gram-negative bacilli in recent years is of great concern, as patients infected by those isolates might initially receive antibiotics that are inactive against the pathogens [1, 7].

The objective of this study is to validate the bacterial identification from patinas obtained from short solid incubation of positive blood cultures, compared to overnight incubation and the rapid carbapenemase detection in Gram-negative bacilli that has clinical and epidemiological implications.

## METHODS

Between 6/1/2014 and 12/31/2016 from 7 am to 2 pm, except weekends, a total of 1101 positive blood culture bottles, aerobic plus/F, anaerobic plus/F and pediatric plus/F, from BACTEC FX (Becton Dickinson, USA) were included. A total of 50 µl of broth was taken from the positive bottles and inoculated onto Columbia sheep blood agar plate (bio-Merieux, Marcy-l'Étoile, France) and incubated in a 5% CO<sub>2</sub> environment at 35° C. Simultaneously, the Gram stain was done and if structures compatible with anaerobic bacteria were seen, we added other plate in

anaerobic condition of incubation. Three hours later, the presence of bacterial growth as patina was controlled and, if it was positive, the MALDI-TOF MS (Bruker Daltonic, Germany) typing was performed; otherwise, the plaque was reincubated and it was observed after 2 h. If the grow was visible, it was proceeded to study. The processing of the patina consisted in transferring an amount of the bacterial patina to a spot of the reusable plate with a wooden stick. One µl of 100% formic acid (Fluka, Germany), and then 1 µl of alpha-cyano-4-hydroxycinnamic acid matrix solution (HCCA, Bruker Daltonik, Bremen, Germany) was added. Calibration was performed using a BTS calibrator following the instructions given in the procedure manual; data was entered according to the software.

Criteria for successful identification were achieved according to manufacturer and international literature [8-10]. The Maldi Biotyper Software version 3.1 (Bruker Daltonik) was used to process the data. The results were compared with the routine identification procedures, including MALDI-TOF MS from overnight incubation in all cases of preliminary acceptable identification.

To classify in true bacteremia or contamination we rely on medical records according to the criteria of the CDC/NHSN [11].

One hundred and forty gram negative bacilli patinas of hospitalized patients were tested against the Blue-Carba Test (BCT) [7, 12]. This method is based on the hydrolysis of imipenem in a bromothymol blue solution, in order to detect the presence of bacteria producing carbapenemases. The phenotypic confirmation of the presence of carbapenemases was made through the sensitivity profile and the use of different inhibitors (APB, EDTA, DCM-Brit) [13-15]. The carbapenemases isolates, other than *Klebsiella pneumoniae* carrying KPC, were confirmed molecularly by PCR / DNA sequencing.

## RESULTS

The results are summarized in Table 1. From 1101 positive blood cultures bottles; a valid identification was obtained in 1037 (94.18%). Two hundred and eighty patinas (27%) were considered skin contaminants. A total of 1004 bottles were

**Table 1. Results of the bottles studied**

Bottles studied	1101	100,00%
Positive Identification	1037	94.18%
No Score	64	5.82%
Polymicrobials	33	3.18%
Monomicrobials	1004	96.81%
Contaminated	280	27.00%

monomicrobial (96.81%) and 33 (3.18%), polimicrobial. In the latter we identified at least one species.

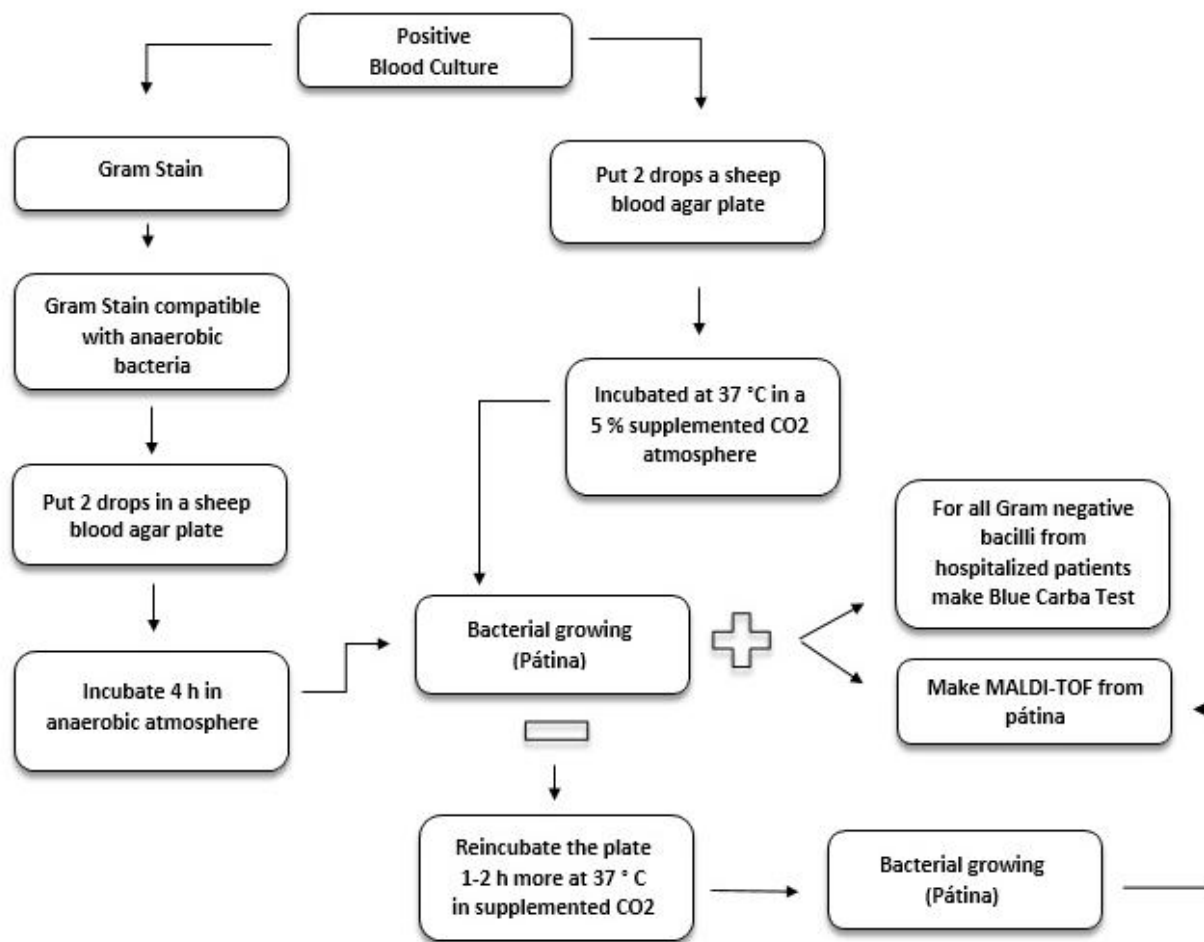
Table 2, shows the groups of microorganisms by gender and species, followed by the total number of these and their percentage of positivity identification, and also the average score of each group of them.

From 1037 blood cultures bottles, were identified

563 Gram-positive cocci, 453 Gram-negative bacilli, and 21 Gram-positive bacilli. Within this last category, 11 were anaerobes; 7, belonged to the genus *Corynebacterium*; 3 were included in the other categories (*Bacillus cereus* and *Lactococcus lactis*). The remaining 64 (5.81%) patinas showed no results. No yeasts results were obtained between 3 and 5 hours of incubation of the solid media. Whichever the reason was, we did not achieve reliable results or visible patinas.

Of all the patinas studied, more reliable scores were observed in the Gram-negative bacilli, and *Staphylococcus aureus* since these showed an average score of 2.00. On the other hand, Gram-positive cocci showed an average score of 1.80 (between coagulase negative staphylococci and streptococci).

Table 3 shows the bottles in which microorganisms were considered contaminant. Out of the 140 patinas tested by BCT, 29 were positive, and no false positive ones were obtained. There were two



**Fig. 1. Workflow diagram.**

**Table 2. MALDI-TOF MS identification results at 3 and 5 h-incubation time on solid medium at genus and species levels with average score and % of identification**

Microorganisms	Number	% of isolates	Id.	No Id.	Average score	% of Id.
<b>Gram-positive cocci</b>	<b>610</b>		<b>563</b>	<b>47</b>		<b>92.29</b>
<i>Staphylococcus aureus</i>	193		190	3	2.01	98.44
<i>Coagulase Negative Staphylococcus (CNS)</i>	302		262	40		86.75
<i>Staphylococcus capitis</i>	10		8	2	1.86	
<i>Staphylococcus caprae</i>	1		1			
<i>Staphylococcus cohnii</i>	2		2		1.7	
<i>Staphylococcus epidermidis</i>	188		162	26	1.83	86.17
<i>Staphylococcus haemolyticus</i>	38		35	3	1.79	
<i>Staphylococcus hominis</i>	48		36	12	1.92	
<i>Staphylococcus saprophyticus</i>	1		1		1.82	
<i>Staphylococcus lugdunensis</i>	4		4		2.33	
<i>Staphylococcus simulans</i>	3		3		1.99	
<i>Staphylococcus schleiferi</i>	1		1		1.68	
<i>Staphylococcus xylosus</i>	3		3		1.98	
<i>Staphylococcus pseudintermedius</i>	2		2		1.98	
<i>Staphylococcus pettenkoferi</i>	1		1		1.75	
<b>Enterococcus spp.</b>	<b>49</b>		<b>48</b>	<b>1</b>		<b>97.96</b>
<i>Enterococcus faecalis</i>	35		34	1	2.05	97.14
<i>Enterococcus faecium</i>	13		13		1.9	
<i>Enterococcus casseliflavus</i>	1		1		2.24	
<b>Streptococcus spp.</b>	<b>52</b>		<b>51</b>	<b>1</b>		<b>98.07</b>
<i>Streptococcus pneumoniae</i>	20		19	1	2.17	
<i>Streptococcus anginosus</i>	4		4		1.92	
<i>Streptococcus oralis</i>	6		6		1.92	
<i>Streptococcus constellatus</i>	1		1		2.14	
<i>Streptococcus gallolyticus</i>	2		2		1.91	
<i>Streptococcus gordonii</i>	1		1		2.04	
<i>Streptococcus lutetiensis</i>	1		1		2.18	
<i>Streptococcus parasanguinis</i>	3		3		1.95	
<i>Streptococcus salivarius</i>	7		7		1.99	
<i>Streptococcus agalactiae</i>	3		3		2.31	
<i>Streptococcus pyogenes</i>	4		4		2.42	
<b>Other Gram-positive cocci</b>	<b>14</b>		<b>12</b>			
<i>Aerococcus viridans</i>	6		4	2	1.73	
<i>Micrococcus luteus</i>	5		5		1.85	
<i>Rothia mucilaginosa</i>	3		3		1.91	
<b>Gram-negative bacilli</b>	<b>461</b>		<b>453</b>	<b>8</b>		<b>98.26</b>
<i>Enterobacteriaceae</i>	386		380	6		98.44
<i>Citrobacter freundii</i>	3		3		2.19	
<i>Citrobacter koseri</i>	1		1		1.98	
<i>Enterobacter cloacae</i>	24		23	1	2.07	
<i>Enterobacter kobei</i>	2		2		2.23	
<i>Enterobacter aerogenes</i>	6		6		2.02	
<i>Enterobacter asburiae</i>	2		2		2.13	
<i>Escherichia coli</i>	129		128	1	2.13	99.22

Table 2 continued.

<i>Klebsiella pneumoniae</i>	160	156	4	2.12	97.50
<i>Klebsiella oxytoca</i>	4	4		2.07	
<i>Khuyvera ascorbata</i>	1	1		1.98	
<i>Leclercia adecarboxylata</i>	3	3		1.99	
<i>Morganella morganii</i>	5	5		2.29	
<i>Proteus mirabilis</i>	17	17		2.10	
<i>Providencia stuartii</i>	3	3		2.13	
<i>Raoultella ornithinolytica</i>	8	8		2.13	
<i>Salmonella sp</i>	5	5		1.94	
<i>Serratia marcescens</i>	12	12		2.08	
<i>Aeromonas caviae</i>	1	1		2.10	
<b>Non fermenters Gram-negative bacilli</b>	<b>67</b>	<b>66</b>			<b>98.50</b>
<i>Acinetobacter baumannii</i>	31	31		1.94	
<i>Acinetobacter nosocomialis</i>	3	3		1.92	
<i>Acinetobacter pittii</i>	3	3		2.12	
<i>Achromobacter xylosoxidans</i>	1		1	no peaks	
<i>Burkholderia vietnamiensis</i>	1	1		2.05	
<i>Comamonas kerstersii</i>	1	1		1.84	
<i>Pseudomonas aeruginosa</i>	15	15		2.09	
<i>Pseudomonas oryzihabitans</i>	3	3		1.84	
<i>Pseudomonas putida_Group</i>	5	5		1.93	
<i>Stenotrophomonas maltophilia</i>	4	4		1.98	
<b>Gram negative cocobacilli</b>	<b>6</b>	<b>6</b>			
<i>Haemophilus influenzae</i>	3	3		1.79	
<i>Neisseria meningitidis</i>	3	3		1.75	
<b>Other gram negative bacilli</b>	<b>1</b>		<b>1</b>		
<i>Capnocytophaga sputigena</i>	1		1	no peaks	
<b>Gram positive bacilli</b>	<b>12</b>	<b>10</b>	<b>2</b>		
<i>Corynebacterium jeikeium</i>	1	1		1.98	
<i>Corynebacterium striatum</i>	4	4		1.75	
<i>Corynebacterium imitans</i>	2	2		1.99	
<i>Bacillus cereus</i>	2	2		2.11	
<i>Brevibacterium ravenespurgense</i>	1		1	no peaks	
<i>Lactococcus lactis</i>	1	1		2.33	
<i>Listeria monocytogenes</i>	1		1	no peaks	
<b>Anaerobes</b>	<b>13</b>	<b>11</b>	<b>2</b>		<b>84.61</b>
<i>Clostridium perfringens</i>	6	6		2.04	
<i>Fusobacterium mortiferum</i>	1	1		2.16	
<i>Peptostreptococcus anaerobius</i>	1	1		1.95	
<i>Propionibacterium acnes</i>	5	3	2	1.76	
<b>Yeasts</b>	<b>5</b>		<b>5</b>		
<i>Candida albicans</i>	3		3	no peaks	
<i>Candida tropicalis</i>	1		1	no peaks	
<i>Candida parapsilosis</i>	1		1	no peaks	
	<b>1101</b>		<b>1037</b>		

Id = Identification

false negative patinas corresponding to 2 *Enterobacter cloacae* with OXA 163. The carbapenemases detected by the BCT were 24 KPC from 22 *Klebsiella pneumoniae*, one *Escherichia coli*, one *Serratia*

*marcescens*; 3 MBL from two *Acinetobacter baumannii* with NDM and one *Pseudomonas putida* with VIM. The average turnaround time of BCT for the detection of KPC was 10 minutes and 1.30 h for



**Table 3. Microorganisms that were consider contaminants**

Microorganisms	Number	% of isolates	Id	No Id	Average score	% of identification
<i>Staphylococcus capitis</i>	10		8	2	1.86	
<i>Staphylococcus caprae</i>	1		1		1.7	
<i>Staphylococcus cohnii</i>	2		2		1.7	
<i>Staphylococcus epidermidis</i>	143		130	13	1.83	90.9
<i>Staphylococcus haemolyticus</i>	36		32	4	1.79	88.8
<i>Staphylococcus hominis</i>	44		36	12	1.92	81.8
<i>Staphylococcus saprophyticus</i>	1		1		1.82	
<i>Staphylococcus lugdunensis</i>	4		4		2.33	
<i>Staphylococcus simulans</i>	3		3		1.99	
<i>Staphylococcus schleiferi</i>	1		1		1.68	
<i>Staphylococcus xylosus</i>	3		3		1.98	
<i>Staphylococcus pseudintermedius</i>	2		2		1.98	
<i>Staphylococcus pettenkoferi</i>	1		1		1.75	
<i>Streptococcus salivarius</i>	7		7		1.99	
<i>Aerococcus viridans</i>	6		4	2	1.73	
<i>Micrococcus luteus</i>	5		5		1.85	
<i>Rothia mucilaginosa</i>	3		3		1.91	
<i>Bacillus cereus</i>	2		2		2.11	
<i>Brevibacterium ravensturnense</i>	1			1	no peaks	
<i>Propionibacterium acnes</i>	5		3	2	1.76	
Total	280		248			88.8

Id = Identification

MBL. In 27 (93.10%) microorganisms we have detectet earlier the carbapenemase resistance. The specificity and sensitivity of BCT were 100% and 93.55%, respectively, linking molecular method, and the positive and negative predictive values were respectively 100% and 98.23% comparing with molecular routine. These results are showed in Table 4. Based on the data obtained, we propose the flow chart shown in Fig. 1.

## DISCUSSION

The results of positive blood cultures should be quickly or promptly available to guide the treatment of critically ill patients. Conventional diagnoses are based on isolated colonies of 24h solid media. Species identification could be accelerated by the use of MALDI-TOF MS if short growth patinas in solid medium are used [16]. These results are usually not

**Table 4. Carbapenemases in 29 hospitalized patients**

Resistance mechanism	Microorganism	N
KPC	<i>Klebsiella pneumoniae</i>	22
KPC	<i>Escherichia coli</i>	1
KPC	<i>Serratia marcescens</i>	1
MLB	<i>Acinetobacter baumannii</i> (NDM)	2
MLB	<i>Pseudomonas putida</i> (VIM)	1
OXA 163	<i>Enterobacter cloacae</i> (False Negative)	2

used to initiate antibiotic therapy, but rather to assess whether the initial empirical therapy was accurate and otherwise to adjust it [17]. In general, blood culture identification techniques take one day of incubation, and although subsequent staining of Gram can be performed in several minutes, the result of this technique alone does not provide enough information to properly administer adequate antibiotic therapy. Therefore, additional testing should be performed in order to properly identify the pathogen causing the disease, leading to an increase in time to initiate appropriate antibiotic therapy [18].

Previous work has shown that the identification obtained with MALDI-TOF MS from subcultures of very few hours of incubation is concordant with that obtained with the conventional technique of identification from subcultures of 18-24h [19-23].

In order to reduce identification times, different extraction techniques have been attempted, which are performed directly from the positive blood culture bottles. So, as to obtain a sample suitable for identification with the MALDI-TOF MS, it should not be forgotten that the proper proteins of the blood can interfere with the identification [17]. These procedures take time and are laborious, and in some cases, the identification is not achieved [2]. In a study that we have done in our hospital comparing Sepsityper with an in-house procedure, we have obtained better results with the later procedure, compared to most of extraction procedures, commercially, and in-house [2, 3, 22-24].

In a study of 925 positive blood cultures bottles (representing 470 bacteremic episodes), Verroken *et al.* [25] obtained a correct identification in 727 (81.1%

of the 896 monomicrobial blood cultures), In Gram-positive cocci 85.6% and in 92.7% of the enterobacteria and in 94.1% of the non-fermenters Gram-negative bacilli; with failure being mostly observed with anaerobes and yeasts. Bazzi *et al.* [20] evaluated 4 methods of blood culture procedures, and in one of them, the pellet of the extraction tubes was cultured in blood agar plates, which were incubated during 90-180 min, obtaining the identification of 94.5% of the microorganisms. Curtoni *et al.* [4] also evaluated the growth of short-incubation patinas obtaining about 92.2% of the Gram-positive cocci; 93.1% of the Gram-negative bacilli and 66.7% of the non-fermenters. Hong *et al.* [26] analyzed 175 microbial blood cultures evaluating bacterial growth between 4-6 h, of which 50.9% were Gram-positive and 49.1% were Gram-negative bacteria. They achieved a concordance of 98.9% at the gender level compared to conventional biochemical tests. Finally, Kohlmann *et al.* [27] reached similar percentages by performing tests on short incubation subcultures.

Regarding *Enterobacteriaceae*, the percentage of identification in our study was high 98.44% and the bacterial grow as patina has been seen in 4h or less. About the non fermenting Gram-negative bacilli, it took 5 h for the observation of the patina, and the identification success was 98.5%.

With the Gram-positive cocci we obtained identification in 92.30% of the microorganisms; 86.75% in coagulase negative staphylococci (CNS) and 98.44% in *Staphylococcus aureus*. There was a great difference between CNS and *Staphylococcus aureus* identification times. In CNS the growth of the patina was much slower and we have to incubate them at least 5h. The early identification of the CNS in one of two blood cultures, allows us to foresee the possibility of skin contaminants. In our study we considered skin contaminated (280) 27.00% of the 1037 bottles. Thus we contributed to reduce time and costs and avoided the use of unnecessary antibiotic therapy.

In the polymicrobial blood culture bottles, the concomitant presence of two different bacteria could be anticipated in some instances by Gram staining. We must highlight that the correct interpretation of the Gram staining from the positive blood culture bottles has a great impact on the microorganisms' identification, in this way, we were able to obtain

anaerobic identifications in 4h since they were incubated in an anaerobic atmosphere.

The BCT for 140 blood cultures from hospitalized patients with Gram-negative bacilli allowed re-evaluating or initiating the antibiotic approach in the presence of carbapenemase-producing bacteria. As for the isolated bacteria tested, we obtained 29 carbapenemase resistant (20.71%); 27 were detected quickly with the BCT (93.10%); the majority of them came from the intensive unit therapy, which is our hospital epidemiology. We believe that the performance of MALDI-TOF MS identification after short-term subculture is directly related to the sufficient growth of microorganisms.

The quickly bacterial identification from the patinas has the advantage of performing no previous treatments on the sample; it is an easy method and greatly reduces the time and material used. It can be used together with local antibiotic resistance data in order to optimize the empiric antimicrobial treatment. BCT is an early diagnostic tool to guide an appropriate treatment in order to reduce the mortality associated with the infections caused by these multi-resistant bacteria.

## CONCLUSION

The use of patina for BCT and early identification by MALDI-TOF MS, is a powerful tool that shortens time and helps select appropriate antibiotic therapy. This benefits the patient. Limitation of the patina MALDI-TOF MS identification was also observed for mixed bloodstream infection, where only one bacterium could be identified.

In conclusion, the application of MALDI-TOF MS identification to rapid growth microorganism obtained from positive blood culture allows an early identification of the most important microorganisms growing in blood culture such as *Staphylococcus aureus* and *Eenterobacteriaceae* which is important for the management of nosocomial and community bloodstream infections. Laboratory automation and work flow optimization may play an important role in reducing the microbiology results turnaround time.

### Conflict of interest

The authors disclosed no conflict of interest during

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# Evaluation of the relationship between serum cholesterol levels and multiple sclerosis disease activity

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## ABSTRACT

**Objectives:** Multiple sclerosis (MS) is an immune-mediated, inflammatory, demyelinating, neurodegenerative disease of the central nervous system affecting young adults. Cholesterol and lipids are essential components of nerve cells and are abundant in the myelin sheath. In this study, we aimed to investigate the relationship between plasma cholesterol levels and severity of the disease and lesion burden in cranial magnetic resonance imaging.

**Methods:** A total of 70 patients (22 males and 48 females) with the diagnosis of MS were included in the study. Age, gender, duration of disease, Expanded Disability Status Scale (EDSS) scores, total number of relapses since diagnosis, current treatment and lipid levels of all participants were recorded. The patients were grouped according to EDSS scores, total number of relapses and number of lesions observed in cranial MRI and the data were compared among the groups.

**Results:** The mean age was  $38.62 \pm 9.94$  years and the duration of the disease was  $7.50 \pm 5.88$  years. The mean EDSS score was  $2.80 \pm 1.69$ . Total cholesterol, triglyceride and LDL levels were found to be significantly higher in the group with more than 3 relapses. Patients with  $> 9$  lesions were older and EDSS scores were higher. In correlation analysis; there was a significant positive correlation between total cholesterol and LDL levels and EDSS scores and disease duration.

**Conclusions:** We found that the number of relapses, disease duration, and EDSS scores were significantly correlated with cholesterol levels. The changes in plasma cholesterol levels which are easily accessible laboratory tests may provide insight into MS disease activity and progression.

**Keywords:** Multiple sclerosis, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides

Multiple sclerosis (MS) is an immune-mediated, inflammatory, demyelinating and neurodegenerative disease of the central nervous system affecting young adults. Myelin damage, loss of myelin and oligodendrocytes, and reactive astrogliosis are considered as the underlying processes in the complex pathogenesis. Clinical findings depend on the damaged area in the brain and severity of injury. Serious disability

associated with these complex pathological features and unpredictable course of disease leads to difficulties with the follow-up of the patients. Therefore, easily accessible biomarkers associated with disease activity and outcomes are needed to improve our understanding of specific disease processes in MS [1].

Cholesterol and lipids are essential components of nerve cells. In particular, myelin synthesis and stabi-

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lization are a cholesterol-dependent process. Several studies have reported that high plasma cholesterol levels were associated with clinical deterioration in dementia and MS. Thereafter, some studies have begun to investigate the travel of cholesterol between the plasma and central nervous system [2, 3]. Changes in the cholesterol levels, which are abundant in the myelin sheath, may have some consequences in the course of the disease. There is no clear information confirming this relationship.

In this study, we aimed to investigate the correlation between cholesterol levels and disease severity, recurrence of relapses and lesion burden in cranial magnetic resonance imaging (MRI) in MS patients.

## METHODS

A total of 70 patients (22 males and 48 females) who were followed-up with the diagnosis of MS according to the 2010 Mc-Donalds diagnostic criteria and admitted to our MS outpatient clinic between August 2017 and August 2019 were included in the study. Patients were between 18-65 years old and had no history of relapses and steroid treatment in the last 3 months. Patients who were diagnosed with diabetes mellitus, lipid metabolism disorder, thyroid disease, liver and renal disease and with any treatment for these diagnoses were excluded. Ethical approval (protocol number: 2011-KAEK-25 2019 / 10-11) was granted from the local ethical committee and the study was conducted according to declaration of Helsinki.

Age, gender, duration of disease, Expanded Disability Status Scale (EDSS) scores, total number of relapses since diagnosis, current treatment and lipid levels of all participants were recorded. The patients were grouped according to EDSS scores, total number of relapses and number of lesions observed in cranial MRI and data were compared among the groups.

## Statistical Analysis

SPSS 22 software was used for statistical analysis. Independent sample t-test was used for descriptive statistical methods (mean, standard deviation) and comparison of the two groups. Chi-square test was used for the comparison of parametric data. The results were evaluated at  $p < 0.05$  level of significance.

## RESULTS

The mean age, disease duration and EDSS scores of the participants were  $38.62 \pm 9.94$  years,  $7.50 \pm 5.88$  years and  $2.80 \pm 1.69$ , respectively. Twenty-seven patients had more than 3 relapses and 47 patients had  $> 9$  lesions in cranial MRI. The general characteristics of the participants are shown in Table 1.

The demographic data, total cholesterol, LDL, and triglyceride levels of the patients were compared between 2 groups (group 1:  $\leq 3$  relapses, group 2:  $> 3$  relapses). There was no significant difference between the groups in terms of age and gender, while the duration of disease was higher in patients with relapses  $> 3$  (Table 2). While the total cholesterol, triglyceride and LDL levels were found to be significantly higher in group 2 ( $p < 0.01$ ,  $p = 0.045$ , and  $p = 0.001$ , respectively), no significant differences were detected with HDL cholesterol levels between the groups ( $p = 0.573$ ). The duration of the disease was significantly longer in group 2 ( $p = 0.007$ ). No significant differences were found among the EDSS scores and cranial MRI lesion counts.

The patients were divided into two groups as  $\leq 9$

**Table 1. General characteristics of study population**

Male/ female	22/48
Age, years	$38.62 \pm 9.94$
Disease duration, years	$7.50 \pm 5.88$
Total cholesterol, mg/dL	$198.12 \pm 59.29$
Triglycerides, mg/dL	$134.16 \pm 85.45$
HDL, mg/dL	$52.17 \pm 15.31$
LDL, mg/dL	$116.69 \pm 46.65$
EDSS score	$2.8 \pm 1.69$
Number of lesions	
1-9 Lesions	23
$> 9$ Lesions	47
Number of relapses	
$\leq 3$ relapses	43
$> 3$ relapses	27

Data are shown mean  $\pm$  standard deviation or number. EDSS = Expanded Disability Status Scale, LDL = low-density lipoprotein, HDL = high-density lipoprotein

**Table 2. Comparison of data by number of relapses**

	<b>Group 1 ≤ 3 relapses n = 43</b>	<b>Group 2 &gt; 3 relapses n = 27</b>	<b>p value</b>
Male/ female	10/33	12/15	0.063
Age, years	37.20 ± 10.13	40.88 ± 9.36	0.133
EDSS	2.51 ± 1.68	3.25 ± 1.62	0.072
Disease duration, years	6.02 ± 5.36	9.85 ± 6.01	<b>0.007</b>
Number of lesions			0.133
1-9 Lesions	17	6	
> 9 Lesions	26	21	
Total cholesterol, mg/dL	177.81 ± 37.66	230.48 ± 72.59	<b>&lt; 0.001</b>
Triglycerides, mg/dL	118.02 ± 79.41	159.89 ± 89.87	<b>0.045</b>
HDL, mg/dL	51.88 ± 15.59	54.02 ± 15.06	0.573
LDL, mg/dL	102.42 ± 32.21	139.40 ± 56.81	<b>0.001</b>

Data are shown mean ± standard deviation or number. EDSS = Expanded Disability Status Scale, LDL = low-density lipoprotein, HDL = high-density lipoprotein

lesions and > 9 lesions according to the number of lesions observed on cranial MRI and the data were compared. There was no significant difference between the groups in terms of gender, duration of disease and lipid parameters, whereas patients were older (40.35 ± 10.15 years vs. 35.08 ± 8.65 years) and EDSS scores were higher in > 9 lesion group (Table 3).

Finally, in the correlation analysis, there was a significant positive correlation between total cholesterol and LDL levels and EDSS scores and disease duration (Table 4).

## DISCUSSION

In this study, we found a significant correlation between the total number of relapses and cholesterol levels. There was a positive correlation between disease duration and EDSS scores and total cholesterol and LDL levels. Relapses in MS are the result of ongoing inflammatory process and disease activation. As a result of demyelination and neurodegeneration in axons during relapses, a number of degradation products are formed in the peripheral blood. The focus of most of ongoing studies is to detect these

**Table 3. Comparison of data by number of lesions**

	<b>Lesion ≤ 9 n = 23</b>	<b>Lesion &gt; 9 n = 47</b>	<b>p value</b>
Male/ female	6/17	16/31	0.501
Age, years	35.08 ± 8.65	40.36 ± 10.15	<b>0.036</b>
EDSS	1.69 ± 0.82	3.34 ± 1.75	<b>0.000</b>
Disease duration, years	5.82 ± 5.20	8.31 ± 6.08	0.096
Total cholesterol, mg/dL	188.39 ± 66.22	202.89 ± 55.73	0.340
Triglycerides, mg/dL	116.18 ± 77.14	142.96 ± 88.70	0.221
HDL, mg/dL	53.01 ± 17.22	52.56 ± 14.48	0.908
LDL, mg/dL	111.59 ± 50.89	119.18 ± 44.79	0.527

Data are shown mean ± standard deviation or number. EDSS = Expanded Disability Status Scale, LDL = low-density lipoprotein, HDL = high-density lipoprotein

**Table 4. Evaluation of the correlation analysis between EDSS and disease duration and lipid parameters**

	<b>r</b>	<b>p value</b>
Tchol/ EDSS	0.283	<b>0.018</b>
LDL/ EDSS	0.346	<b>0.003</b>
HDL/ EDSS	0.008	0.945
Trig/ EDSS	0.130	0.285
Tchol/ Disease duration	0.293	<b>0.014</b>
LDL/ Disease duration	0.327	<b>0.006</b>
HDL/ Disease duration	- 0.067	0.582
Trig/ Disease duration	0.215	0.074

Tchol = Total cholesterol, EDSS = Expanded Disability Status Scale, LDL = low-density lipoprotein, HDL = high-density lipoprotein, Trig = Triglycerides

degradation products. Forty percent of the myelin sheath consists of water, while the remainder consists of lipids (75%) and proteins (25%). In experimental models of encephalomyelitis, increased concentrations of cholesterol esters, sulfatide and cerebroside have been shown in CNS during myelin destruction [4]. Consistent with the literature, our findings support increased cholesterol in peripheral blood as a result of increased myelin destruction due to increased number of relapses under longer disease duration.

Eighty percent of plasma 24S-OH-cholesterol which is thought to be an indicator of brain cholesterol degradation originates from the brain. Studies have shown a significant positive correlation between serum cholesterol and 24S-OH-cholesterol [5-7]. It has been shown that after 24S-OH-cholesterol was formed, it was crossed the blood-brain barrier and was transported in plasma by low-density lipoprotein (LDL) [8]. Increased plasma cholesterol levels may reflect neurodegeneration and demyelination processes in MS patients.

Researchers have found links between cholesterol metabolism, inflammatory pathways and the immune system. The best example of this is the removal of cholesterol crystals by macrophages in atherosclerosis [9]. Proinflammatory properties of lipids are thought to be effective in the pathogenesis of MS. Dyslipidemia can induce inflammation that causes

dysfunction of the endothelium by initiating upregulation of adhesion molecules and aggregation of monocytes. The passage of immune cells through the blood brain barrier via the vascular endothelium constitutes one of the most important phases of MS pathogenesis [10, 11]. LDL particles in the serum of MS patients have been shown to be more susceptible to oxidation. These particles are also thought to be proinflammatory because they have less affinity for LDL receptors [12]. MS patients have been found to have high oxidized LDL in plasma and CSF samples, and have high levels of autoantibodies against them in their sera [13-15]. HDL has an anti-inflammatory effect by inhibiting the expression of adhesion molecules in endothelial cells and by reducing the flow of monocytes. High HDL values have been found to be associated with lower injury and cell extravasation in the blood brain barrier [16, 17].

Although cholesterol metabolism in the brain is independent, inflammation and oxidative stress in the body can affect brain function and contribute to increased activity in MS. Therefore, it is not known exactly whether high lipid levels in the blood cause deterioration, or whether increased lipid levels are due to increased myelin destruction associated with increased disease activity [2].

In a study evaluating MS patients with a single relapse, a significant correlation was found between the mean number of contrast-enhancing lesions and total cholesterol and LDL cholesterol levels [18]. In another study, high LDL cholesterol levels were shown to correlate with the number of new T2 lesions and the total number of new / enlarging T2 lesions. Weinstein-Guttman *et al.* [19] demonstrated the adverse effect of lipid profile on MRI lesion activity of high-risk patient who experience their first relapse. While age and EDSS scores were significantly correlated with the number of lesions, cholesterol levels didn't show any differences in our study.

In the literature, it has been shown that EDSS score was correlated with total cholesterol levels, and the increase in total cholesterol and triglyceride levels were correlated with increased disability scores [3]. In another study with a larger sample size, it was shown that total cholesterol and triglyceride levels were lower in patients with slow progression than in those with rapid progression. Based on this, it is thought that

serum lipid profile may have an effect on disease progression. In the same study, a positive correlation was found between total cholesterol levels and EDSS score in MS patients [2]. Similarly, we found a positive correlation between EDSS score and total cholesterol and LDL levels, but no significant correlation with triglyceride and HDL levels.

### Limitations

The limited number of cases, lack of control group, unregistered body mass indices, cross-sectional examination of MRI, and the absence of distinction of emerging and contrast-enhancing lesions may be considered as limitations of our study.

### CONCLUSION

In conclusion, we found that the number of relapses, duration of disease and EDSS scores were significantly correlated significantly with cholesterol levels. Increased plasma cholesterol levels may be detected as a result of increased metabolism as a result of myelin destruction in the CNS that occurs during the relapse and long disease course. In the follow-up of MS patients, changes in plasma cholesterol levels which are an easily accessible laboratory test may provide insight into disease activity and progression. Further studies in larger samples of MS patients are needed to understand this relationship.

### Conflict of interest

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# Three cases with Klippel-Trenaunay syndrome and treatment options

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## ABSTRACT

Klippel-Trenaunay syndrome (KTS) is a rare congenital anomaly characterized by capillary malformations, soft tissue and bone hypertrophy and varicosities. The presence of at least two of the three clinical conditions is sufficient for the diagnosis of KTS. Early diagnosis and treatment are important because of possible serious complications such as deep vein thrombosis and pulmonary embolism. Venous system aplasia and/or hypoplasia might be observed in KTS. Therefore, deep venous system must be evaluated definitely before determining the treatment strategy. It's herein presented our conservative and surgical treatment applications in three KTS patients.

**Keywords:** Klippel-Trenaunay syndrome, embryonic, varicose, venous, venous insufficiency, stripping

**K**lippel-Trenaunay Syndrome (KTS) is a rare congenital anomaly characterized by capillary malformations, soft tissue and bone hypertrophy and varicosities [1]. It was first described by Klippel and Trenaunay in 1900 [2]. The presence of at least two of the three clinical conditions is sufficient for the diagnosis of KTS [3]. A form with soft tissue hypotrophy without bone pathology is defined as “atypical Klippel Trenaunay Syndrome” [4, 5]. Early diagnosis and treatment are important because of possible serious complications such as deep vein thrombosis and pulmonary embolism.

Patients with KTS may usually be treated with conservative methods. However, surgical treatment can be performed in patients with symptomatic varicose veins due to KTS, if they do not have any problem like thrombosis with their deep venous systems [6]. It's herein presented our conservative and surgical treatment applications in three KTS patients.

## CASE SERIES PRESENTATION

### Case # 1

A 26-year-old male patient was admitted to our outpatient clinic with pain, redness, and hardness in his varicose veins. He stated that he has had varicose veins since his childhood period, and that his leg thickens progressively but that the redness and stiffness has just begun. During his physical examination, it was observed that there were a large number of varicose veins especially in the lateral crural region. Erythematous and sensitive thrombotic venous segments were observed around the ankle. Reddish-pinky hyperpigmented skin lesions on the left lower extremity, extending to the back of the foot were observed (Fig. 1). When compared to the right lower extremity, the left lower extremity was hypertrophic. Colored Doppler ultrasonography (CDU) revealed multiple varicose veins under the skin, acute thrombus

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**Fig. 1.** Reddish-pinky hyperpigmented skin lesions on the left lower extremity, extending to the back of the foot. There are a large number of varicose veins especially in the lateral crural region, and erythematous and sensitive thrombotic venous segments around the ankle.

formation within the varicose veins around the ankle and lateral embryonic varicose vein (LEV). According to CDU evaluations LEV which started at the level of the ankle and joined popliteal vein had severe venous reflux. Deep venous system and the great saphenous vein (GSV) were patent and no reflux was detected. The patient was then given medical therapy for acute thrombophlebitis. Under spinal anesthesia on the 15th day after the treatment, a stripping operation was applied to the LEV below the popliteal entrance. Segmental phlebectomies were performed for the enlarged satellite venous segments. He was discharged on the 1st postoperative day with a compression sock and symptomatic treatment. During the first month follow-up, the patient stated that the pain in his lower extremity decreased significantly and the quality of his life increased.

### Case # 2

A 24-year-old female with a 24-week pregnancy

was referred to our outpatient clinic from the obstetrics and gynecology clinic. She stated that she applied many times to different hospitals since her childhood period due to a growth failure in both her left arm and left leg, birthmarks and vascular enlargement on the skin but that she could not be diagnosed. Physical examination revealed hypoplasia in the soft tissue of her upper and lower left extremities, pervasive reddish-pinky hyperpigmented skin lesions in her upper and lower left extremities, and subcutaneous varicosities in her left lower extremity. X-ray examination for bone structures could not be performed due to pregnancy, but there was no difference in length between the extremities and their functions were normal. No Valsalva maneuver was ordered to undergo due to pregnancy in CDU evaluation. Deep venous system was observed as being patent. No thrombus was observed in deep or superficial venous systems. As a result, the patient was diagnosed with 'atypical KTS' with soft tissue hypotrophy. Compression sock was recommended to prevent venous complications and polyclinic control was suggested after delivery.

### Case # 3

A 19-year-old male patient was admitted to our outpatient clinic with the complaint of increased and distinctive subcutaneous varicose veins in the left lower extremity. According to his medical history, he had been diagnosed with Klippel-Trenaunay Syndrome 3 years ago and had undergone a stripping operation of LEV in his left lower extremity together with segmental phlebectomies by a different cardiovascular surgeon. He hasn't used compression socks for almost a year.

On his physical examination, there were pervasive reddish-pinky hyperpigmented skin lesions and also pervasive subcutaneous varicose enlargements especially in the posterolateral region of his left lower extremity. When compared to the right one, the left lower extremity was hypertrophic. His deep and superficial venous systems were observed as being patent in the CDU examination. Additionally, pervasive varicose enlargements were detected in his subcutaneous tissue. No new surgical intervention was planned. Regular polyclinic controls with compression socks and symptomatic treatments were recommended.

## DISCUSSION

KTS is composed of soft tissue and bone hypertrophy, capillary hemangiomas and superficial varicose veins often located laterally [7]. Skin lesions and edema are common. Depending on the symptoms and skin attitudes, patients may consult different clinics during their whole life-course. This process, which starts with pediatric admissions in childhood period, might be followed by many different clinical admissions such as dermatology, orthopedics, plastic surgery, cardiovascular surgery. For these reasons, a multidisciplinary approach is required for the diagnosis, follow-up and treatment of the disease according to the patient's symptoms, organ involvement and his/her expectations. KTS is unilateral in 85% of patients, and 10% of patients have both upper and lower extremity involvement, as in our second case [8]. LEV is observed in 68-80% of cases. It drains into iliac vein (5%), popliteal vein (11%), great saphenous vein (14%), superficial femoral vein (17%), deep femoral vein (20%) or gluteal veins (33%) [9]. Before the operation, LEV and deep venous system have to be evaluated in detail. Venous system aplasia and hypoplasia might be observed in KTS. In a study by Redondo *et al.*, hypoplasia and aplasia in the deep venous system were detected in 22% of the patients with KTS. In this group of patients, LEV functions as a functional collateral and for that reason surgical intervention is strictly contraindicated in these patients particularly [10]. Conventional venography, magnetic resonance venography, CDU can be used for the examination of the venous system. Although it was stated that venography has an important place in the literature in patients with KTS, it is seen that CDU alone is sufficient to diagnose these cases in many different reports as seen in the report of Usta *et al.* [11]. Because in the classical definition of KTS, a triad is mentioned in which the presence of two is sufficient. This triad is a rare congenital anomaly characterized by capillary malformations, soft tissue and bone hypertrophy and varicosities [1]. Therefore, if the presence of varicose structures typical for CTS is evident by inspection and you have the opportunity to work with a radiologist whose experience you believe, CDU examination alone may be sufficient to determine the indication for the operation. Moreover, it will also protect the patient from the invasive

intervention of venography and from the nephrotoxic drug exposure. For these reasons, due to the presence of our radiologists, whose experience we are sure of, we did not require venography during the evaluation of our cases and decision-making process, and CDU evaluations were found sufficient. We believe that such an algorithm is important not only for the patient but also for cost analysis. It is evident that CDU examination alone in the presence of a well-experienced radiologist whom the vascular surgeon is sure of him/her will be much more cost effective than venography.

Treatment of CTS is conservative and symptomatic. Elastic compression stockings and venotonic agents may be used for vascular involvement of the lower extremities. Thrombophlebitic conditions that may occur as in our first case should be treated conservatively. Operation should be avoided in the period when thrombophlebitis is active. Surgical intervention can be performed in selected patients whose symptoms persist despite of conservative treatment and whose quality of life decreases [5, 12]. In our first case, LEV was present and surgical treatment was performed because of the existence of patent deep venous system and venous hypertension symptoms. In our second patient, while conservative treatment was planned because of her pregnancy, third patient was not planned to undergo a surgery, since he was previously operated. He, too was taken with medical treatment only.

Another point to be considered in the differential diagnosis is that KTS should be differentiated from Parkes Weber Syndrome characterized by capillary malformation, hypertrophic extremity, heart failure associated with hyperdynamic arteriovenous fistulas. It was stated that arteriovenous malformation presents the criterion for distinguishing Parkes Weber syndrome from Klippel-Trenaunay syndrome [13].

## CONCLUSION

In conclusion, even though KTS is a rare syndrome, it should be evaluated more carefully than classical venous insufficiency patients. The composition of soft tissue and bone hypertrophies, which are a part of its triad, should be well studied. In

particular, the condition of the deep venous system should be carefully examined. Because the current status of the deep venous system is one of the most important factors that determine the surgical indication. Most patients with KTS are treated conservatively with elastic compression socks and venoactive agents. However, surgical treatment can be performed if there is a complaint related to venous hypertension-induced pain and venous dilatation in the presence of LEV together with a patent deep venous system. CDU examination alone performed in safe hands may be sufficient to determine the indication for the surgical operation.

#### *Informed consent*

Written informed consent was obtained from the patient for publication of these cases and any accompanying images.

#### *Conflict of interest*

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

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## Squamoid eccrine ductal carcinoma: a case report

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### ABSTRACT

Squamoid eccrine ductal carcinoma (SEDC) is a subtype of malignant tumours of skin appendices, showing eccrine and apocrine differentiation. It is a rare tumour and making a histopathological diagnosis is difficult. Our patient was a 79-year-old male, presenting to our hospital with the complaint of a long-term persistent wound in the scalp. An excisional biopsy was performed due to a preliminary diagnosis of a pyogenic granuloma. However, the patient was diagnosed with squamoid eccrine ductal carcinoma. Although an excisional biopsy was performed, the tumour was present on the surgical margins. Consequently, the patient underwent an extended re-excision but the tumour was detected at the surgical margins again. We aimed to present this case as SEDC is a rare tumour.

**Keywords:** Eccrine gland, appendageal skin tumour, squamous eccrine ductal carcinoma, squamous cell carcinoma

Squamoid eccrine ductal carcinoma (SEDC) is an extremely rare cutaneous tumor [1]. SEDC is clinically important because it is a potential for locoregional aggressiveness and metastasis. In histopathological examination, the tumour is biphasic with two different components, superficial and deeper areas showing different patterns. Therefore, superficial biopsies may lead to a misdiagnosis of squamous cell carcinoma (SCC) as the biopsy specimen will most likely be composed of tissues showing a squamous differentiation [2]. Herein we report a case of 79-year-old male patient with SEDC in the scalp.

### CASE PRESENTATION

A 79-year-old male patient presented to our hospital with the complaint of a long-term persistent

wound in the scalp. His medical history revealed that the patient had diabetes mellitus, chronic obstructive pulmonary disease, and benign prostatic hyperplasia. A protuberant, red, and ulcerated lesion of 1.2 cm in diameter was present on the scalp. An excisional biopsy was performed due to a preliminary diagnosis of pyogenic granuloma. In the macroscopic examination, a protuberant lesion of 1.2×1×0.4 cm in size, red in colour, and having an ulcerated surface was observed with its base adjacent to the surgical margins. The microscopic examination revealed lobules and tumour cell islets with squamous differentiation in the superficial areas (Figs. 1 and 2). In the deeper areas, an infiltrative growth pattern and irregular cords were present. In the desmoplastic stroma, tumour cells were observed showing ductal differentiation (Fig. 3). Mitoses were observed especially in areas with squamous differentiation (Fig.

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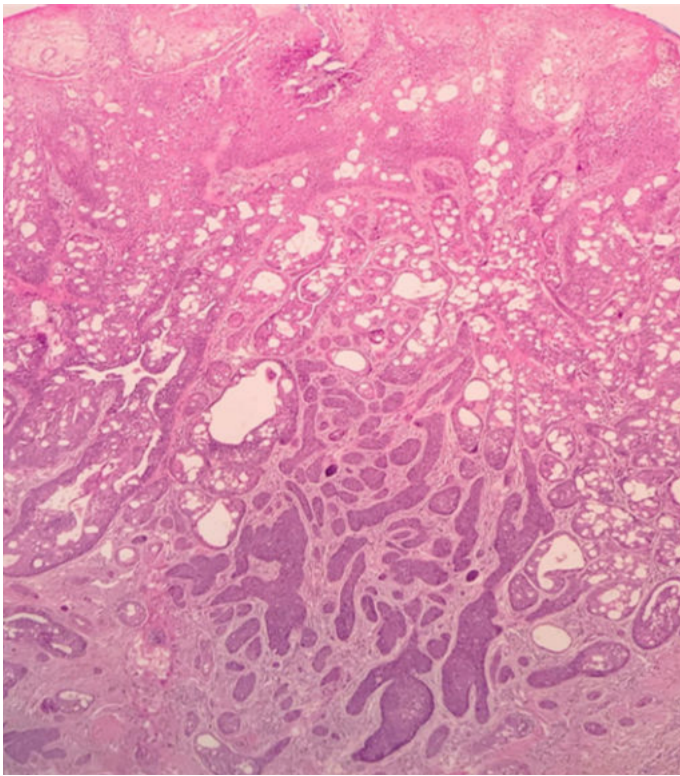
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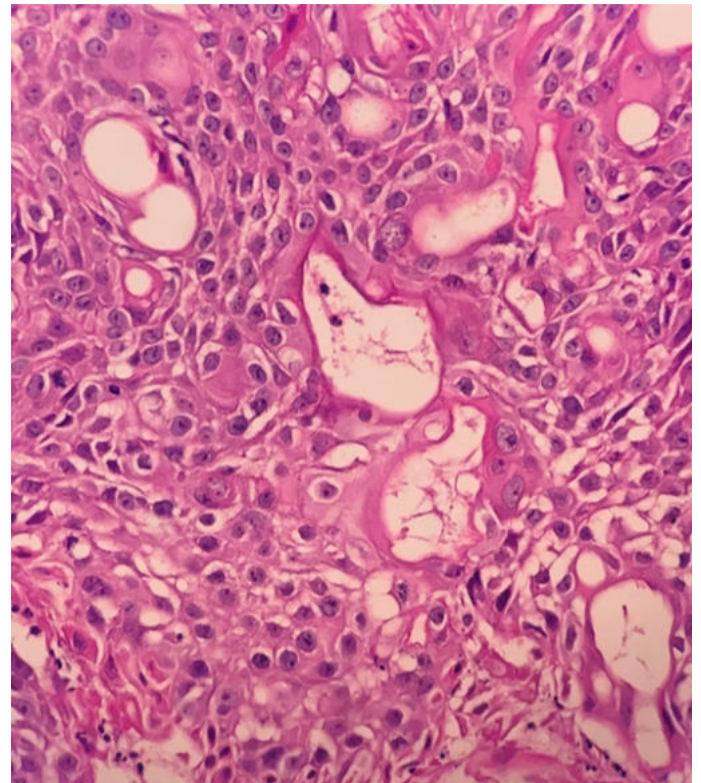
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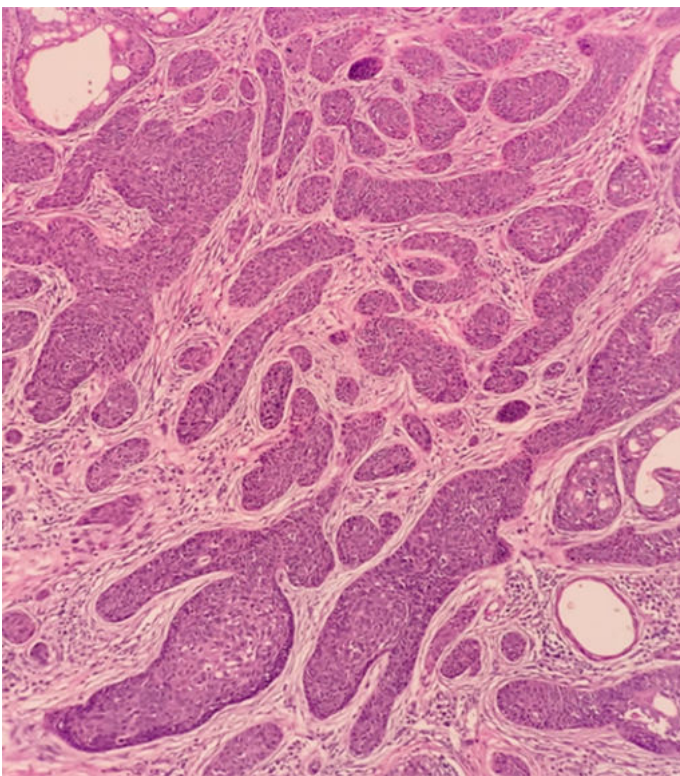




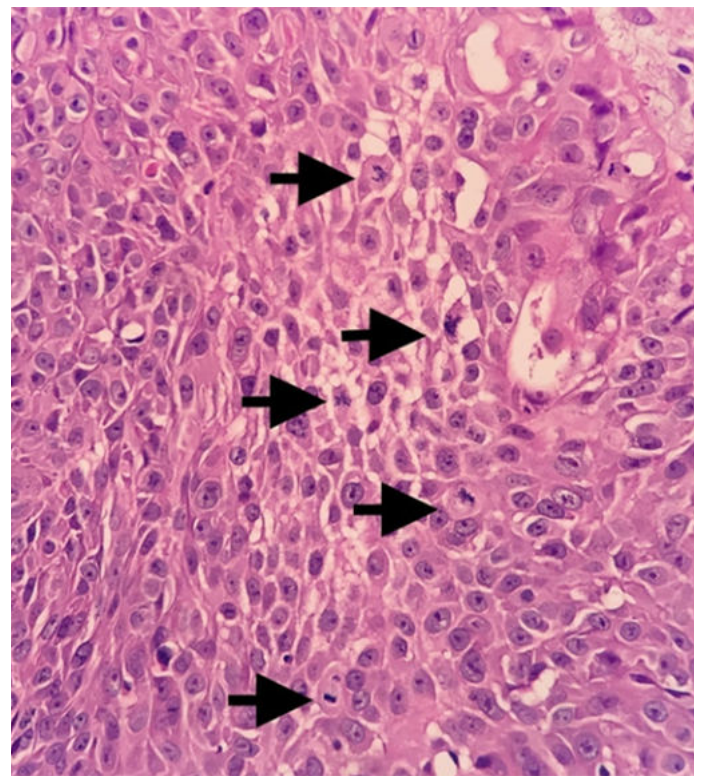
**Fig.1.** The light microscopic appearance of the lesion (Hematoxylin-eosin stain,  $\times 40$ ).



**Fig.2.** Squamous differentiation areas in the superficial part of the tumor (Hematoxylin-eosin stain,  $\times 200$ ).

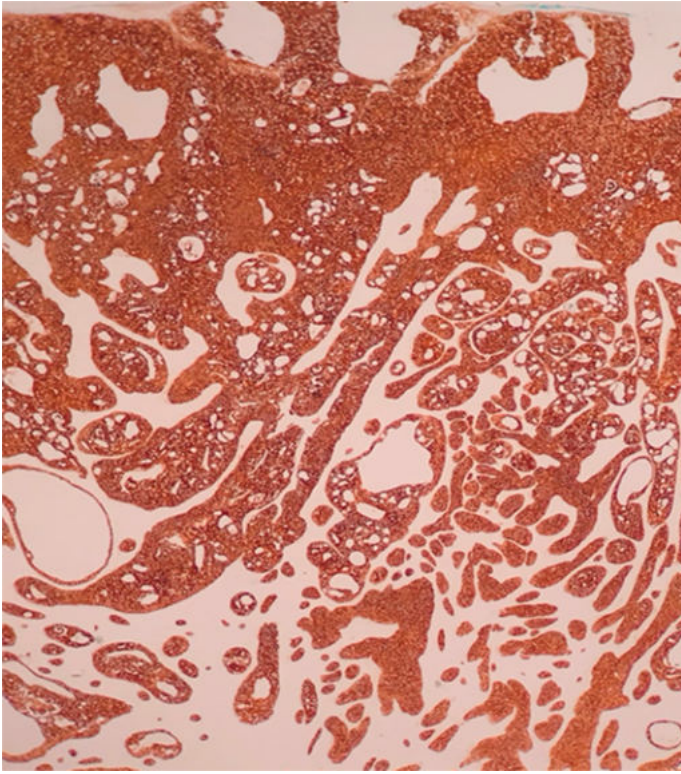


**Fig.3.** Infiltrative growth pattern and ductal differentiation areas in desmoplastic stroma in deep areas of the tumor (Hematoxylin-eosin stain,  $\times 100$ ).

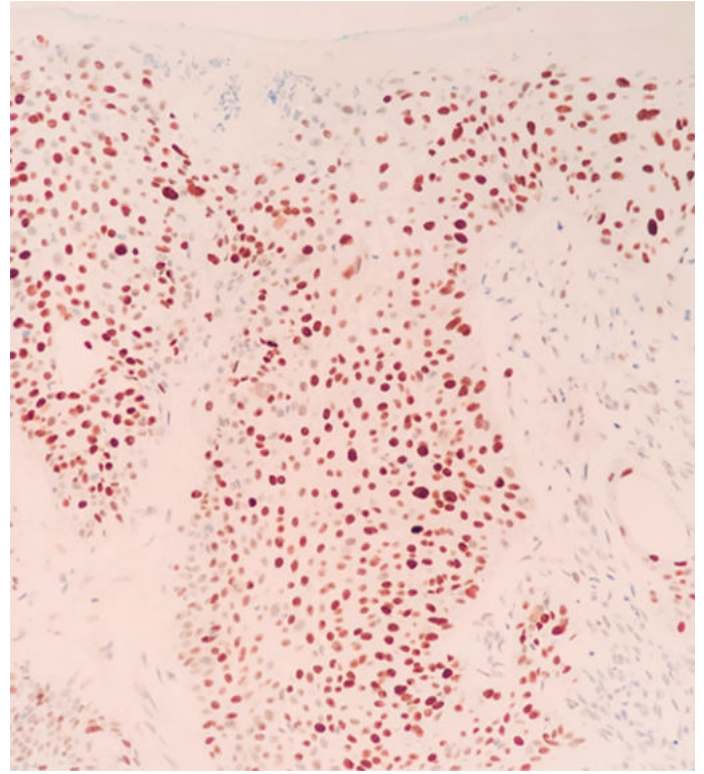


**Fig.4.** Mitosis in areas of squamous differentiation (Hematoxylin-eosin stain,  $\times 400$ ).

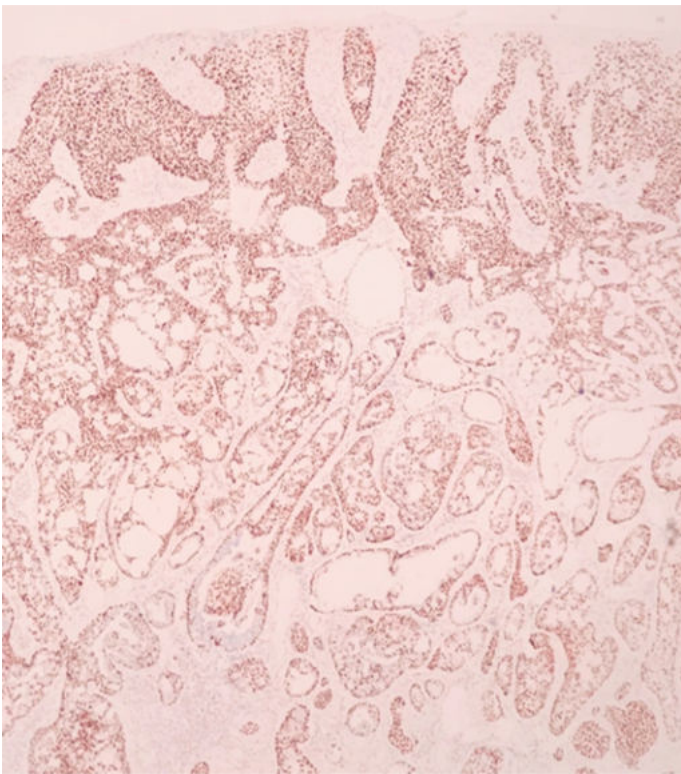




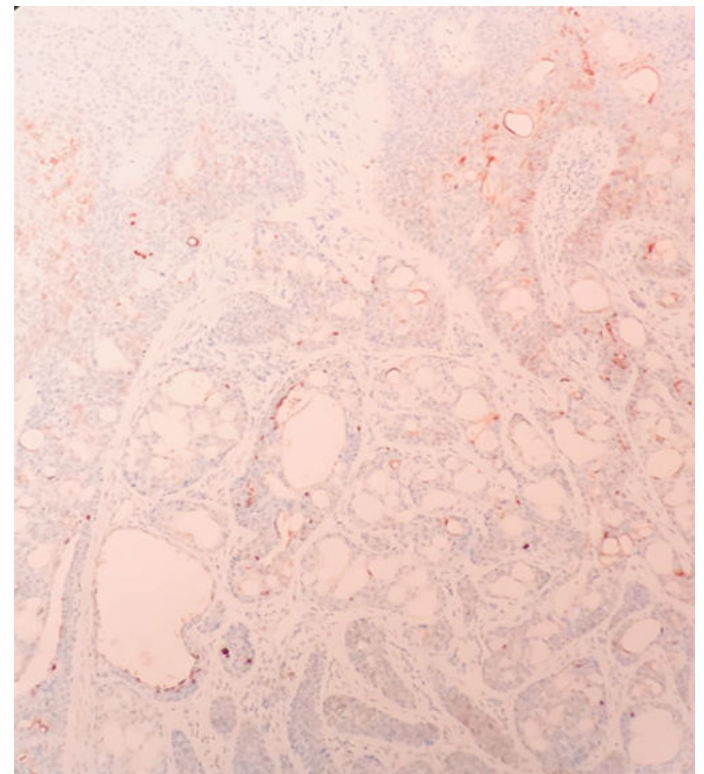
**Fig.5.** Positive reaction with immunohistochemistry CK5 / 6 (×40).



**Fig.6.** Positive reaction with immunohistochemistry p53 (×200).



**Fig.7.** Positive reaction with immunohistochemistry p63 (×40).



**Fig.8.** Positive reaction with immunohistochemistry EMA in ductal differentiation areas (×100).

4). No lymphovascular or perineural invasion was observed. In the immunohistochemical examination; a positive reaction was observed with P63, CK5/6, and P53 in the area of squamous differentiation, and another positive reaction was observed with CEA and EMA at the area of ductal differentiation (Figs. 5 to 8). Based on the histopathological and immunohistochemical findings, a diagnosis of SEDC was made. A re-excision was performed because the tumour was present in the surgical margins. The second excision confirmed the diagnosis of SEDC, however, the tumour was detected in the surgical margins again.

## DISCUSSION

SEDC is a rare subtype of malignant adnexal tumours showing eccrine and apocrine differentiation [1]. SEDC was first described by Wick and Swanos in 1991 as a sub-type of eccrine carcinoma showing squamous differentiation, which may be misdiagnosed as SCC, especially in superficial biopsies [3]. In 1997, Wong et al. used the term SEDC when they reported three patients [4]. Currently, there are 60 case reports with SEDC available in the literature.

SEDC is usually seen in the head and neck region, in males older than 80 years old, who had a medical history of sun damage to the skin. The lesions are usually in the shape of nodules or plaques, and ulcerated. The case reports in the literature define a tumour diameter in the range of 0.15-1.8 cm with a mean of 0.43 cm [1]. The tumour demonstrates an infiltrative and malignant growth pattern in the histopathological examination, extending beyond the surgical margins. There may be atypical pleomorphic cells and atypical mitoses. The tumour is biphasic with two different components, superficial and deeper areas showing different patterns. While the superficial areas mostly show a squamous differentiation, syringoma-like basaloid, angulated, and tubular structures characterised with a more infiltrative pattern are mostly observed in the deeper parts [1-8]. Therefore, superficial biopsies may lead to a misdiagnosis of SCC as the biopsy specimen will most likely be composed of tissues showing a squamous differentiation [2, 9, 10]. The differential diagnosis includes SCC, metastatic carcinoma, microcystic

carcinoma with squamous differentiation, porocarcinoma with squamous differentiation, Merkel cell carcinoma, and benign neoplasms [1, 11].

In the differential diagnosis of eccrine neoplasms, immunohistochemical tests usually involve S-100, EMA, CK, CEA, and p63. Glandular tissues typically stain positive for EMA and CEA, supporting the adnexal origin [1]. On the other hand, epithelial malignancies, including SCC, stain negative for EMA and CEA. SEDC staining negative for EMA and CEA in the squamoid differentiation areas but positive in the areas showing ductal differentiation is a critical supporting finding for making the diagnosis [1]. CK5/6 is a high molecular weight cytokeratin, expressed normally in the stratified squamous epithelium, in the myoepithelial cells of the secretory glands, and in the epithelial cells of the apocrine excretory glands. Positive staining for CK5/6 and p63 rules out a diagnosis of metastatic carcinoma [1, 2]. Although SEDC is recognized as a low-grade neoplasm, it is locally aggressive and bears a malignancy potential; therefore, it is an important clinical condition [1, 4, 12, 13]. Because SEDC is a rare tumour, less is known of its biological behaviour and optimum treatment. Currently, the largest series available in the literature has been reported in 2016. This case series with 30 patients reported a 25% rate of local metastasis and a 13% rate of metastasis, including three lymph node metastasis and a distance metastasis [14].

## CONCLUSION

In summary, we presented a patient with SEDC in this present case report. It should be recognized that there is a potential of making a misdiagnosis in these patients, especially with incisional or shave biopsies as these methods will collect tissue samples only containing areas with squamous differentiation but will exclude the parts of the lesion with ductal differentiation. These patients should be closely followed-up due to the high risk for recurrences.

### *Informed consent*

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.



### Conflict of interest

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

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## Peripheral artery disease in women

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### ABSTRACT

Peripheral artery disease (PAD) is a common disease among women and is expected to increase in the coming years. Diagnostic and therapeutic management of PAD in women is difficult due to their gender-related differences, comorbid factors, atypical symptoms, more functional disorders, the presence of advanced disease, being older age of the disease onset, smaller vessel diameter, and psychosocial factors. Especially due to the high prevalence of asymptomatic disease, late admission status and further disease on admission affect the success of the treatment negatively. By focusing more on PAD in women and more clinical studies, gender-specific differences can be identified. Hereby, there is a need to develop appropriate strategies to improve women's overall quality of life. In this review, we aimed to draw attention to the female gender with PAD, which is increasingly occurring.

**Keywords:** Peripheral artery disease, women, female gender, risk factor

Peripheral artery disease (PAD) occurs as the third most common result of systemic atherosclerosis after coronary artery disease and cerebrovascular disease. Lower limb PAD is estimated to affect about 8.5 million people in America and 202 million people worldwide over 40 years of age, which is increasingly leading to impaired functional disability and quality of life, and is a cardiovascular disease that is associated with myocardial infarction and stroke-related mortality [1]. It has been reported that major cardiovascular events, vascular interventions and re-hospital admissions are significantly higher in patients with PAD than in patients with coronary artery disease [2]. Patients with PAD have 5-6 times higher risk of morbidity or mortality than stroke and coronary artery disease [3].

The main risk factors for PAD are older people, smoking, hypertension, diabetes and dyslipidemia [4, 5]. However, gender-specific risk factors have not

been fully elucidated. In 2012, American Heart Association (AHA) highlighted that in the "Scientific Statement- A call the action: Women and Peripheral Artery Disease", the prevalence of PAD in adults over 40 years of age has increased and clinical awareness in women, focused treatment plans and research on PAD in women are needed [6]. Although it is stated that the prevalence of PAD is higher in men, recent evidence suggests that PAD is also common in women. Since PAD is an age-related disease, considering that women make up the majority of the elderly population, it can be said that PAD in women will increase rapidly in the near future. In addition, asymptomatic / subclinical disease rates are higher in women and most of them have atypical symptoms [7]. In addition, the psychological effects of the disease are more pronounced in women, and in a study, younger women under 65 years of age diagnosed with PAD have a 4-fold higher risk of depression than men [8]. Therefore, in this re-

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view, we aimed to draw attention to the female gender with PAD, which is increasingly occurring.

## PREVALENCE OF PAD IN WOMEN

The prevalence of PAD in women has not been fully elucidated. Recently, there is no study in progress on a large population [6]. In the first studies on the prevalence of PAD, the disease was reported to be more common in men than in women and was accepted. However, these studies were conducted on symptomatic patients [9, 10]. In a review, the prevalence of PAD has been reported to be between 3% and 29% among women 45 to 93 years of age, as in the male gender [9]. A recent review related to population-based studies reported that the mean prevalence of PAD is 15.6% in women and 13.4% in men [11]. Similarly, Sigvant *et al.* [12] reported contradictory results in women with a prevalence of PAD, that is similar to or higher than men. Population studies have showed a prevalence of PAD of 19.2% and 16.5% in women and men, respectively, which is not statistically significant. However, there was a significant gender-based recognition for asymptomatic PAD with a higher prevalence in women than in men (13% vs. 9%;  $p < 0.03$ ).

Compared to Western European countries, although total cholesterol levels are low in Turkey, risk factors such as increased smoking, increased frequency of obesity and metabolic syndrome, diabetes and hypertension are reported as important problems [13]. Although Turkey has a young population, the prevalence of atherosclerotic diseases and their associated mortality are highly observed. Therefore, although there is no large-scale study on PAH in Turkey, this rate is estimated to be higher [14].

## RISK FACTORS FOR PAD IN WOMEN

General risk factors for PAD are similar among men and women, including age, smoking, diabetes mellitus, hypertension, and dyslipidemia. In women with PAD, the disease manifests itself, on average, 10-20 years later than men [15]. PAD affects approximately 20-30% of women who are 70 years old or older [10, 16]. Female sex hormones, especially

estrogen, have been shown to protect women from cardiovascular disease until menopause [17]. Estrogen has been shown to participate in endothelial function, such as antioxidative defense, a reduction in cytokine-induced inflammation, and healing of vascular injury, including vasodilation and preservation of vascular tone [18]. The cardioprotective effects of estrogen can explain why women have cardiovascular disease 10-20 years after men. Pregnancy loss associated with low estrogen concentrations in postmenopausal women has been associated with a three-fold higher risk of cardiovascular disease. Additionally, inflammatory markers, which are predictors for PAD, are higher [19-21]. The difference in PAD prevalence between men and women closes with age. Postmenopausal women have similar or even higher PAD prevalence rates than men. The outcomes of hormone replacement therapy on PAD in postmenopausal women are not clear.

The Women's Health Initiative (WHI) and the Heart and Estrogen / progestin Replacement (HERS) studies found that hormone replacement therapy did not make any difference in terms of PAH and coronary artery disease [22-24]. In contrast, the Rotterdam study and another prospective cohort study reported that hormone replacement therapy was associated with a decrease in PAD prevalence [25, 26]. The Cardiovascular Health After Maternal Placental Syndrome (CHAMPS) study showed three-fold increased risk of PAD and two-fold increased risk of coronary artery and cerebrovascular disease in patients with maternal placental syndrome, including preeclampsia, gestational hypertension, placental abruption and placental infarction [27]. Although the possible cause is the underlying endothelial dysfunction, the mechanisms for this relationship are unclear.

Smoking is a greater risk factor for PAD in men, but diabetes and dysglycemia increase the risk of intermittent claudication in women 4 times [28, 29]. In patients with chronic renal failure, younger women (< 70 years; mean age 56.5) may have a higher PAD prevalence compared to paired men (1.53 times higher risk) [30]. Many studies have shown an association between obesity, C-reactive protein (CRP), osteopenia / osteoporosis and hypothyroidism, and PAD in women [31-35]. Risk factors specific to women are summarized in Table 1.

**Table 1. Risk factors for female gender-specific PAD**

Risk Factors	Comments
Menopause	Possible vasculoprotective effects
Oral contraceptives	Increased risk of venous and arterial thrombosis
Maternal placental syndrome	Preeclampsia, gestational hypertension, placental abruption and placental infarction
Depression	< Age of 65, 4 times higher risk than men
Elevated CRP	Higher levels of CRP than men
Osteopenia / Osteoporosis	Age-dependent
Hypothyroidism	This is controversial

PAD = Peripheral artery disease, CRP = C-reactive protein

## SCREENING AND SYMPTOMS

The classic symptom of PAD is intermittent claudication, but not in all cases. Up to 40% of cases may not complain about leg symptoms and may occur with atypical symptoms. Women have higher subclinical, asymptomatic and atypical PAD rates [36, 37]. The American College of Cardiology / American Heart Association (ACC / AHA) guidelines recommend screening with the ankle-brachial index (ABI) for asymptomatic individuals over 65 and 50-64 years old with diabetes and smoking [37]. In the Women's Health and Aging Study (WHAS), 35% of 933 disabled women over 65 years were found to have ABI < 0.9 and were diagnosed with PAD. In their study, they showed that 63% of patients diagnosed with PAD did not have classical exercise pain [38]. Asymptomatic PAD is common among older women. Most patients with PAD are asymptomatic or have atypical symptoms, and only 10% of patients present with classical claudication [1]. Women may be more asymptomatic or present with atypical symptoms. Atypical symptoms in women can be misinterpreted as arthritis, neuropathy or spinal stenosis, which is also evident in this population. There is a similarity with PAD severity between the level of physical activity and the measured ABI value, but this may not apply to women [39]. In a study conducted at Northwestern University, it was found that women applying for PAD evaluation had more severe disease than men with ABI test [40]. Brevetti *et al.* [41] stated that women applying to the vascular laboratory are more likely to get Fontaine stage III or IV disease than men and have

less classic symptoms of claudication than men. In a cohort study, the Walking and Leg Circulation Study (WALCS), among 460 PAD patients without critical limb ischemia, they found that the presence of atypical stress symptoms, which sometimes began to rest in 187 female patients, was twice as high as in men [42].

Women with PAD have more functional disorders than men. McDermott *et al.* [42] measured walking speed and distance, daily physical activity and performance scores in 273 PAD patients. They stated that despite similar disease severity in both genders, women experienced more impairment in lower limb function and strength, women had lower walking speeds, and achieved much shorter distances than men in a 6-minute walk. In a recent study in which PAD patients were followed up to 4 years, it was found that women with PAD had higher mobility loss and functional decline rates than men with PAD [43]. In a prospective WHAS cohort study, 847 women with low ABI (< 0.6) were predicted to have greater walking-related disorders [44]. PAD impairs the functional state more in women and women have a lower quality of life compared to men.

## DIAGNOSTIC METHODS

A complete vascular history, presence of existing risk factors, and physical examination and control of the pulses are crucial for the diagnosis of PAD. Non-invasive diagnostic tests and objective evaluations are very valuable in detecting PAD due to limitations in physical examination. The most common first

diagnostic test is resting ABI. ACC / AHA guidelines recommend screening with ABI for asymptomatic individuals over 65 and 50-64 years old with diabetes and smoking [37]. In a study of 1775 healthy individuals, ABI was found to be approximately 0.02 lower in women [45].

Physiological tests such as exercise ABI, toe-brachial index, transcutaneous oxygen pressure, or skin perfusion pressure can be performed according to the clinical picture and resting ABI result [46]. Duplex ultrasound, magnetic resonance angiography (MRA), computed tomographic angiography, or invasive angiography are useful in providing anatomical location and severity of the disease. Currently, there is no large gender difference in the diagnostic sensitivity or accuracy of these tests [46].

Difficulties in diagnosis and treatment of gender-specific women in PAD are summarized in Table 2.

## TREATMENT OF PAD IN WOMEN

### Cardiovascular risk factor modification, exercise and medical therapy

The aim of symptomatic and asymptomatic PAD treatment should be primarily to decrease the progression of ischemic vascular events and to increase the quality of life and physical functionality. 2016-AHA / ACC guidelines recommend exercise

program, smoking cessation, lipid lowering with statin therapy, blood pressure control with angiotensin receptor inhibitors, antiplatelet therapy, and aggressive diabetes management [1]. The main goals for both genders are to reduce cardiovascular morbidity and mortality and improve the quality of life of patients by improving limb-related symptoms. In addition to modification of risk factors, medical and revascularization therapy should be added to these targets for the treatment of claudication.

Although aggressive risk factor modification is the basis of treatment, the German REACH registry cohort study showed that patients with PAD alone were less likely to receive treatment with statins, angiotensin-converting enzyme inhibitors or antiplatelet agents compared to concomitant coronary artery disease patients [47]. In parallel with this study, the REACH (Reduction of Atherothrombosis for Continued Health) registry records showed that women with 8,322 PAD patients had significantly less optimal risk factor control than men [48]. This makes us think that the success of PAD treatment in women will be less than men, by taking the recommended risk factor control treatment of women with PAD less.

In a study investigating the effect of quitting smoking by Armstrong *et al.* [49], a five-year follow-up showed a marked reduction in all-cause mortality and amputation-free survival in both men and women. Sigvant *et al.* [50] reported that smoking for 10 years

**Table 2. Difficulties in diagnosis and treatment for female gender-specific in PAD**

Status	Comment
Asymptomatic and atypical symptom	Delay in diagnosis
Higher prevalence of orthopedic diseases	Arthritis and arthrosis Delay in diagnosis
Presence of progressive disease at diagnosis	Low treatment success
Diagnosis at an advanced age	Low treatment success
Insufficient aggressive risk factor modification	Lower usage of antiplatelet agents, statins, angiotensin converting enzyme inhibitors Low treatment success
Failure to benefit from exercise training	Low exercise tolerance Low treatment success
Small arterial diameter (< 7mm)	Low patency rates Low treatment success

PAD = Peripheral artery disease

in women is a risk factor for PAD, while it is 30 years in men. Ness *et al.* [51] described that smoking has a more detrimental effect on females than males in the presence of symptomatic PAD in older patients. In the light of these studies, we can say that smoking has a significantly more negative effect on PAH in women than men. In that case, stopping smoking seems to be an important element of PAH treatment compared to males.

Patients with PAD have limited exercise in their daily lives. There is evidence that a regular exercise training program is useful. It has been shown that lower limb exercise training increases the duration of claudication, increases the distance before claudication and increases the total walking distance [52]. In a meta-analysis examining 1200 people with stable leg pain, it was found that the maximum walking time improved significantly with exercise, with an overall improvement of 50% to 200% in walking ability [53]. In most randomized trials comparing supervised exercise training with pharmacological therapy or revascularization procedures, gender-specific analyzes were not performed, as female participants were less than 30% on average [6]. On the other hand, a study has shown that especially women with diabetic PAD have less responses to exercise rehabilitation programs, and this is partly due to further deterioration in calf muscle oxygen saturation during and after exercise. They also attributed this to lower leg strength, higher inflammation, higher oxidative stress level during ambulation, and lower hemoglobin saturation during insulin resistance [54].

Many studies have shown that the use of cholesterol-lowering therapy, especially statin, can not only improve symptoms of intermittent claudication but also increase walking distance from baseline [55, 56]. Although the proposal for statin use does not vary by gender, women with PAD are found to be less likely to receive statins.

Hypertension is an important risk factor for PAD and must be treated. Heart Outcomes Prevention Evaluation (HOPE) study [57] involving those with PAH and Ahimastos *et al.* [58] in their studies, they reported that in blood pressure control with ACE-I, cardiovascular mortality and morbidity decreased and painless walking distance improved. Therefore, ACE-I is recommended as the first-line treatment for

hypertensive patients with PAD. However, ACE-I use in women has some limitations such as fetal toxicity, multidrug-intolerance syndrome and breast cancer recurrence [59, 60].

Current guidelines recommend antiplatelet therapy in symptomatic PAD, but also recommend the use of antiplatelet therapy in patients with ABI < 0.9 and no symptoms. It can be administered with 75-325 mg of aspirin once a day or 75 mg of clopidogrel once daily as a safe and effective alternative [1]. This recommendation does not vary by gender.

Among the available drugs, cilostazol, a phosphodiesterase inhibitor that inhibits platelet aggregation and has arterial vasodilating properties, is the drug with the most accurate evidence as a first-line pharmacological treatment in symptomatic PAD, including intermittent claudication [7]. In randomized clinical studies, it was concluded that Cilostazol improved both painless and maximum treadmill walking distance compared to placebo at a dose of 100 mg twice daily [61]. There were no different results in the treatment of cilostazol by gender.

### Surgical Revascularization

The guidelines recommend revascularization in the presence of intermittent claudication that limits function despite optimal medical therapy. But although they have similar comorbidities with men, women have less revascularization procedures [6]. In the article "Scientific Statement- A call the action: Women and Peripheral Artery Disease" published by AHA in 2012, it was stated that women constitute only 32% of the participants in the PAD revascularization studies, and this shows that making treatment decisions is mostly with the results obtained from men [6]. Although the treatment options for both genders are similar, it was emphasized that women were recommended less surgical revascularization due to smaller vessel diameter, presence of advanced disease in diagnosis, advanced age of onset of disease, psychosocial factors and worse surgical results [6, 62, 63]. In studies conducted, it was stated that the patency rates of the graft were lower in women in lower extremity surgical revascularization [6]. Although these reports suggest that there are low patency rates, especially in women treated with surgical bypass, no significant gender effects have been identified in patients treated with endovascular procedures, surgical



bypass or a combination of these [64]. Similarly, another study reported that there were no gender differences in graft patency rates [65]. However, studies have found that female gender is an independent risk factor for postoperative wound complications (odds ratio: 1.376, 95% CI: 1.076-1.757;  $p = 0.01$ ). It has also been noted that wound complications increase the risk of limb loss, mortality, increased hospital stay, and decreased quality of life [66]. However, it has been suggested that women with lower extremity bypass are more likely to have hematoma, seroma and infection than men [67]. Despite the gender-related differences noted, the idea that revascularization option should be considered as a priority in women is strengthened because the amputation-free survival is similar in women and men [6].

### Endovascular Therapy

It is likely that women are less likely to benefit from revascularization due to anatomical, comorbidity, advanced disease, age of onset of the disease, etc. In addition, it has been shown by studies that wound complications are more common in women. All these reasons raise the question of what is the place of endovascular treatments (stent, balloon angioplasty, atherectomy) in women.

According to the TransAtlantic Inter-Society Consensus (TASC), in iliac obstructive lesions for both men and women, endovascular approach with angioplasty and/or stenting is recommended as the first treatment option today, not only for patients with TASC A and B lesions, but also for patients with TASC C and D lesions, who are particularly poor candidates for open surgical repair [6].

As with surgical revascularization, caution should be exercised in endovascular therapy due to the presence of more aggressive atherosclerotic disease and smaller arterial diameter in the female sex. In several published studies [68-70], the results of iliac artery stents have been published, especially in women, but the results were insufficient as only bare metal stents were used. Piazza *et al.* [71] in their study investigating the effect of gender on endovascular treatment for iliac lesions, they reported that primary and secondary patency rates were significantly lower in women in the long-term follow-up, although they showed similar results in men and women in the early

period. They determined that this was due to the fact that women had smaller arterial diameters and that the stent diameter was less than  $< 7$  mm as a negative predictor. In the same study, it was emphasized that open surgery may still be a viable option in female patients with a target artery diameter of  $< 7$  mm [71]. In another large patient-scale study, Ramkumar *et al.* [72] investigated the role of gender in determining the type of intervention in endovascular therapy. In this study, they found that women were less likely to undergo stenting or atherectomy than men, while they found higher rates of occlusion and re-intervention in women, especially in femoropopliteal arteries. Again in this study, they emphasized the effect of artery diameter on gender inequality in endovascular treatment.

As mentioned before, women with PAD are at an advanced age and more advanced disease stages, so any treatment in women is difficult. Matsi and Manninen [73] noted significantly higher bleeding complications (15% vs. 6%) in women undergoing percutaneous lower extremity angioplasty. In three state studies involving open surgery and endovascular treatments, a total of 372,692 patients, approximately 45% of whom were women, Vouyouka *et al.* [74] reported that women had higher mortality and morbidity rates than men after any type of vascular intervention.

### CONCLUSION

The scarcity of research studies also prevents us from fully evaluating PAD results in female gender. Diagnosis and treatment management of PAD in women is difficult due to their gender-related differences. Especially due to the high prevalence of asymptomatic disease, late admission status and further disease on admission affect the success of the treatment negatively. As a result, the risk of mortality and morbidity increases in women. By focusing more on PAD in women and more clinical studies, gender-specific differences can be identified. Hereby, there is a need to develop appropriate strategies to improve women's overall quality of life.

#### Authorship contributions

Concept-Design: KKÖ, ŞY; Data collection:

KKÖ, USS, FT; Analysis: KKÖ, FT, ŞY; Literature search: KKÖ, USS, FT; Writing: KKÖ, USS, ŞY; Critical review: ŞY.

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