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European Journal of Therapeutics (Eur J Ther) is the double-blind peer-reviewed, open access, international publication organ of the Gaziantep University School of Medicine. The journal is a quarterly publication, published on March, June, September, and December. The journal publishes content in English.

European Journal of Therapeutics aims to contribute to the international literature by publishing original clinical and experimental research articles, short communication, review articles, technical notes, and letters to the editor in the fields of medical sciences. The journal's target audience includes researchers, physicians and healthcare professionals who are interested or working in in all medical disciplines.

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Review Article	5000	250	50	6	10 or total of 20 images
Short Communication	1500	200	20	5	1 or total of 5 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

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Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/ cid.htm.

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Contents

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Original Article

N-Terminal-pro-Brain Natriuretic Peptide Is Increased and Closely Related with Osteoporosis in Patients with Newly Diagnosed Primary Hyperparathyroidism

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ABSTRACT

Objective: The aim of this study is to determine the prevalence of osteopenia and osteoporosis in newly diagnosed primary hyperparathyroidism patients and to evaluate the relationship between the presence of osteoporosis and the primary hyperparathyroidism routine laboratory parameters including N-terminal-pro-brain natriuretic peptide.

Methods: This prospective study included 94 patients (mean age: 59.7 ± 11.7 years, female/male: 78/16) who have been diagnosed with primary hyperparathyroidism. For all patients participating in this study, laboratory tests were performed (routine tests and tests for diagnosing hyperparathyroidism), and dual-energy x-ray absorptiometry inspections were also performed. The participants of the study were divided into 3 groups according to T score in dual-energy X-ray absorptiometry as normal (group I or T score >−1), patients with osteopenia (group II or T score between −1 and −2.5), and the patients with osteoporosis (group III or T score ≤ −2.5).

Results: Notable level increase of blood urea nitrogen and N-terminal-pro-brain natriuretic peptide from group I to group III is seen in the results. In logistic regression analysis, it was found that levels of N-terminal-pro-brain natriuretic peptide and urine calcium independently determined the patients for osteoporosis ($P < .05$). According to the analysis, it was found that increasing levels of urine calcium (per 10 mg/day) and N-terminal-pro-brain natriuretic peptide (per 10 pg/mL) increase the risk of osteoporosis by 8.6% and 9.1% for patients, respectively. When we took N-terminal-pro-brain natriuretic peptide and urine calcium cut-off values as 200 pg/mL and 300 mg/day, respectively, it determines patients for osteoporosis with 82.6% sensitivity and 73.2% specificity, and 73.9% sensitivity and 63.4% specificity, respectively. N-terminal-pro-brain natriuretic peptide and urinary calcium levels were independently associated with T score in dual-energy X-ray absorptiometry.

Conclusion: The primary outcome of this study is N-terminal-pro-brain natriuretic peptide levels are significantly increased in newly diagnosed primary hyperparathyroidism patients and are independently associated with osteoporosis presence. In addition, apart from N-terminal-pro-brain natriuretic peptide level, urine calcium level is also independently associated with osteoporosis presence, in our study.

Keywords: Primary hyperparathyroidism, osteoporosis, NT-proBNP

INTRODUCTION

Primary hyperparathyroidism (pHPT) is frequently asymptomatic until the time of diagnosis.[1] During this asymptomatic period, bone loss and osteopenia or osteoporosis may occur.[2-4] For this reason, the diagnosis of pHPT should be kept in mind in cases of bone loss, which were detected by chance at relatively young ages. In addition, bone mineral density (BMD) should be measured as soon as possible after the diagnosis of pHPT. Dual-energy X-ray absorptiometry (DEXA) technique is recommended for BMD measurement in current guidelines.[5]

Bone mineral density score in patients with pHPT is associated with disease severity and a T score of <−2.5 as determined by DEXA is a surgical criterion.[5] Although there is a correlation between BMD and biochemical parameters related to pHPT in univariate analyses, this relationship has been reported to be not independent.[6-10] Bone mineral density measurement and degree can only be done with x-ray DEXA examination. However, if a biochemical marker is associated with osteoporosis, it may be important for patients with pHPT, especially at follow-up and osteoporosis.

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It has been shown that there is an increase in brain natriuretic peptide (BNP) without cardiac involvement in pHPT patients and also an increase in BNP with the presence of atherosclerotic heart disease and heart failure and cardiovascular prognosis in these patients.[11-14] Natriuretic peptides are known to be specifically synthesized from cardiac myocytes by pressure and volume excess. Natriuretic peptides have endocrine, paracrine, and autocrine effects. One of the endocrine effects is the increased endothelin-1 (ET-1) synthesis of parathyroid cells. Both atrial natriuretic peptide (ANP) and BNP cause an increase in ET-1 synthesis from parathyroid cells.[15] Endothelin-1 is known to be particularly clear in osteoporosis due to the feature of mediated vasoconstrictor tone increase of ET-1 in bone cells.[16-18] However, in the literature to the best of our knowledge, there is no study related to increased osteoporosis with increased BNP effect in pHPT patients. We hypothesized that in pHPT patients, osteoporosis or BMD reduction may be associated indirectly with BNP.

Therefore, our study aimed to determine the prevalence of osteopenia and osteoporosis in the newly diagnosed pHPT patients and to evaluate the relationship between the presence of osteoporosis and the pHPT routine laboratory parameters including N-terminal proBNP (NT-proBNP).

METHODS

The Population of the Study

This prospective study included 94 patients (mean age: 59.7 ± 11.7 years, female/male: 78/16) who have a diagnosis of pHPT. Primary hyperparathyroidism was defined as elevated or inappropriately normal intact parathyroid hormone level (PTH) level and accompanying elevated serum calcium corrected for serum albumin.[5] In this study, the patients who have inflammatory and hematological diseases, musculoskeletal diseases, vitamin D deficiency or treatment, presence of cancer, pregnancy, and renal failure were not included. The study protocol is approved by The Cukurova University by the Ministry of Health (Date: May 15, 2018 / Desicion No: 59) and written informed consent was obtained from each participant.

A detailed medical history and a complete physical examination was performed for all groups and final basal characteristics were recorded. After measuring weight and height, the body mass index (BMI) was calculated. Laboratory tests, renal ultrasound (US), and bone densitometry were performed for all patients. All patients were searched for the diagnosis of renal stone or nephrocalcinosis in renal US for surgical indications.

Main Points

- N-terminal-pro-brain natriuretic peptide (NT-proBNP) is significantly increased in newly diagnosed primary hyperparathyroidism (pHPT) patients.
- NT-proBNP is independently associated with osteoporosis presence.
- High NT-proBNP can be followed closely for osteopenia and osteoporosis.
- Our study is a first in the literature, and it needs to be supported by new studies and assessments.

According to the National Institutes of Health consensus panel, the following are considered as surgical criteria in symptomatic pHPT patients or the presence of any of them in asymptomatic pHPT patients: (i) serum calcium level elevation for 1 mg/dL, (ii) significant hypercalciuria (> 400 mg/24 h), (iii) DEXA T score <−2.5, (iv) patient under 50 years old (<50 age), and (v) decrease in creatinine clearance by more than 30%.[5]

Biochemical Measurements

Venous blood samples were collected in blood tubes from cubital veins of patients in the outpatient clinics. By using chemiluminescence immunoassay and Beckman Coulter DXI 800, serum PTH concentration and 25-hydroxyvitamin D level were measured. The reference range was accepted as 20-40 pg/mL. Complete blood count (white blood cell count, hematocrit, and platelet counts) was measured using a Beckman Coulter DXH 800 within 5 minutes after sample ingestion. Serum glucose, Hemoglobin A1c, blood urea nitrogen (BUN), creatinine, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP), high sensitive C-reactive protein (hs-CRP), NT-proBNP, uric acid, serum calcium, serum phosphorus, and urine calcium levels were measured using an automated chemistry analyzer (Abbott Aeroset, Minn, USA) and using appropriate commercial kits (Abbott). By using the most commonly used formula in clinical practice, the corrected serum calcium levels were calculated (if serum albumin level was lower than 4 mg/dL: corrected calcium = measured total calcium (mg/dL) + 0.8 (4.0 − serum albumin [g/dL])).[19]

Measurement of Bone Densitometry by Dual-Energy x-Ray Absorptiometry

For all measurements, participants wore light clothes and removed all metal and plastic artifacts. A stadiometer and BMI-calibrated electronic scales were used for measuring the height, and the nearest millimeter was recorded. The BMD analysis of all patients in the anterior–posterior and lateral lumbar vertebrae (L1-L4) was performed with DEXA (Lunar iDXA, GE, Madison, Wis, USA).

One lumbar spine (L1eL4) scan and one total hip scan on both the iDXA and the Prodigy within 24 hours were performed for each participant. All lumbar spine scans were performed by elevating the legs and opening the intervertebral spaces to allow clear visualization of the vertebra. This positioning was assisted with the GE-Lunar spine positioning. For total hip scans, the GE-dual femur positioning device was used for allowing both legs to be abducted and inwardly rotated 25°. Scans were analyzed using Encore software versions 12.5 (Prodigy) and 13.5 (iDXA). The same experienced densitometry specialist performed the analysis of each scan, manually for the lumbar spine for the consistent placing of the intervertebral spaces and with the auto analysis used for the total hip. The specialists systematically monitored the point typing and bone edge profiles, and they acquired BMD (mean value of a pixel-by-pixel measurement of the BMD within a defined bone area), bone mineral content (a derived quantity obtained by multiplying BMD by bone area), and bone area data from each scan. The BMD T scores, BMD Z-scores, percentage tissue fat, and thickness parameters were also recorded.

Statistical Analysis

Variables were divided into 2 groups categorically and continuously. Kolmogorov–Smirnov test was used to assess whether continuous variables were suitable for normal distribution. Continuous variables were expressed as mean ± standard deviation (mean ± SD). Categorical variables are given in numbers and percentages. Continuous variables were compared by one-way analysis of variance (ANOVA) or Kruskal–Wallis one-way ANOVA test. For data with normal distribution, Scheffe and Games-Howell tests were used for multiple comparisons of groups with respect to homogeneity of variances. For non-normal distributed data, Bonferroni-adjusted Mann–Whitney *U* test was used for multiple comparisons of groups. The specialists used chi-square test for comparing the categorical variables for this study and performed multivariate logistic regression analysis with univariate analysis of $P < .05$ parameters for determining the patients with osteoporosis independently. A receiver operator characteristic (ROC) curve analysis was performed for re-evaluating the markers that are independent of detecting osteoporosis and for determining the limit value of these markers. As a measure of the accuracy of the test, the value of the area under the curve has been used. Pearson's correlation method was used, and univariate correlation analysis was performed for determining the DEXA-related parameters. A statistically significant parameter was included in a multivariate model, and linear regression analysis was performed with these parameters. Independent indicators affecting DEXA T scores were determined. For statistical significance, $P < .05$ was accepted. For all analyses, Statistical Package for the Social Sciences version 20.0 (IBM SPSS Corp.; Armonk, NY, USA).

RESULTS

The study has 3 groups of participants according to T score in DEXA as follows: normal (group I or T score >−1), patients with osteopenia (group II or T score between −1 and −2.5), and patients with osteoporosis (group III or T score ≤ −2.5). In this study, 23 of the pHPT patients (24.5%) who had osteoporosis were without any fractures in study groups.

Demographic and Laboratory Findings of Primary Hyperparathyroidism Patients According to T Scores in Dual-Energy X-Ray Absorptiometry

There was no statistical difference between groups for age and gender. In group I, group II, and group III, surgical treatment was applied in 45%, 63%, and 91% of patients, respectively, and this difference was significant between the groups. White blood cell, hematocrit, and platelet count were different between groups, and these parameters were significantly lower in group III than group II. Group III had the highest levels of creatinine, ALP, serum and urine calcium, and PTH, and there was statistical significance between group III and the other 2 groups (Table 1). High sensitive C-reactive protein level was significantly higher in group II and group III compared to group I (Table 1). Blood urea nitrogen and NT-proBNP levels increased significantly from group I to group III (Table 1). It was determined that BUN and NT-proBNP levels were statistically different between all study groups (Table 1). Other laboratory findings were similar among the groups (Table 1). In addition, 47.9% of all pHPT patients included in the study were found to have a value of NT-proBNP above 125 pg/mL.

Multivariate Logistic Regression Analysis for the Detection of Patients with T Score in Dual-Energy X-Ray Absorptiometry Score ≤−2.5

In multivariate logistic regression analysis, it was found that levels of NT-proBNP and urine calcium independently determined the patients for osteoporosis ($P < .05$ and Table 2). According to this analysis, it was found that increasing levels of urine calcium (per 10 mg/day) and NT-proBNP (per 10 pg/mL) increase the risk of osteoporosis by 8.6% and 9.1% for patients, respectively (Table 2).

Receiver Operating Characteristic Curve Analysis for the Detection of Patients with T Score in Dual-Energy X-Ray Absorptiometry Score ≤−2.5

In the ROC analysis, the area under the curve values were 0.867 and 0.728 for NT-proBNP and urine calcium, respectively ($P < .05$, Table 3 and Figure 1).When the NT-proBNP and urine calcium cut-off values were taken as 200 pg/mL and 300 mg/day, respectively, it determines patients for osteoporosis with 82.6% sensitivity and 73.2% specificity, and 73.9% sensitivity and 63.4% specificity, respectively (Table 3).

Parameters Associated with T Score in Dual-Energy X-Ray Absorptiometry

Correlation analysis was performed between T score in DEXA and other demographic and laboratory parameters (Table 4). Parameters that correlated significantly with T score in DEXA were used, and linear regression analysis was performed (Table 4). The levels of NT-proBNP and urinary calcium were found independently associated with T score in DEXA (Table 4). The relationship between T score in DEXA and NT-proBNP, and T score in DEXA and the urinary calcium level is shown in Figures 2 and 3.

DISCUSSION

The primary outcome of this study is that NT-proBNP levels are significantly increased in newly diagnosed pHPT patients and are independently associated with osteoporosis presence. In addition, apart from NT-proBNP, urine calcium level is also independently associated with osteoporosis presence, in our study. Both of these findings are not available in the literature as much as we have investigated.

Leere et al[6] evaluated the relationship between BMD and age, sex, BMI, and biochemical parameters, serum calcium, vitamin D, ALP, creatinine, PTH, and phosphorus levels, in a recent study involving 563 patients with pHPT. With some of these parameters being associated in the univariate analysis, it is reported that this relationship is not significant in multivariate analysis. Similar findings have been found in previous studies.[7-10] However, if a biochemical marker could be associated with osteoporosis, it may be important for patients with pHPT, especially those on follow-up and with osteoporosis. For this reason, BMD measurement and grading can only be evaluated by DEXA examination in patients with pHPT. However, DEXA is a radiation-related study. For this reason, it is important to obtain knowledge about

Table 1. Demographic and Laboratory Findings of pHPT Patients According to T Scores in DEXA

Variable	Group I, *n* = 20	Group II, *n* = 51	Group III, *n* = 23	*P*
Age (years)	57.1 ± 9.1	59.3 ± 11.5	62.9 ± 13.8	.256
Gender (female)	14	43	21	.069
Surgery, n (%)	9 (45)	32 (63)	21 (91.3)	**.001**
White blood cell (μL)	6.84 ± 1.22	7.04 ± 1.30 ¥	6.05 ± 1.31	**.010**
Hematocrit (%)	41.9 ± 3.6 α	41.5 ± 3.11 ¥	38.4 ± 3.84	**.001**
Platelet (K/mm^3)	242 ± 61	275 ± 49 ¥	232 ± 78	**.009**
Glucose (mg/dL)	106.5 ± 26.0	115.4 ± 33.6	103.8 ± 47.2	.373
HbA1c (%)	5.78 ± 0.29	6.16 ± 0.94	5.89 ± 1.49	.294
Blood urea nitrogen (mg/dL)	27.7 ± 7.3 α, β	32.2 ± 10.2 ¥	42.5 ± 24.7	**.001**
Creatinine (mg/dL)	0.65 ± 0.26 α	0.73 ± 0.27 ¥	1.32 ± 1.49	**.005**
Total protein (g/dL)	6.97 ± 0.32	7.09 ± 0.43	6.92 ± 0.35	.170
Serum albumin (g/dL)	4.38 ± 0.25	4.18 ± 0.43	4.12 ± 0.25	**.046**
Aspartate aminotransferase (U/L)	20.8 ± 10.6	25.1 ± 16.2	19.8 ± 10.7	.289
Alanine aminotransferase (U/L)	17.6 ± 8.6	22.6 ± 13.2	16.8 ± 9.7	.254
Alkaline phosphatase (U/L)	98.7 ± 46 α	107 ± 36 ¥	155 ± 117	**.008**
hs-CRP (mg/L)	0.28 ± 0.22 α, β	0.50 ± 0.39	0.54 ± 0.36	**.032**
NT-proBNP (pg/mL)	80 ± 27 α, β	170 ± 102 ¥	453 ± 381	**<.001**
Uric acid (mg/dL)	5.02 ± 1.09	5.51 ± 1.25	5.18 ± 1.31	.271
Serum calcium (mg/dL)	11.2 ± 0.81 α	11.3 ± 0.75 ¥	10.7 ± 0.89	**.016**
Urine calcium (mg/day)	244 ± 127 α	299 ± 198 ¥	400 ± 154	**.013**
Serum phosphorus (mg/dL)	2.60 ± 0.52	2.83 ± 0.56	2.84 ± 0.62	.255
Parathyroid hormone (pg/mL)	198 ± 101 α	242 ± 173 ¥	519 ± 513	**<.001**
25(OH) Vit D (ng/mL)	18.6 ± 8.4	19.8 ± 10.3	18.1 ± 12.7	.790

The values were shown as mean ± standard deviation or n (%). Bold values mean they are statistically significant.
Group I, normal DEXA score group; Group II, osteopenia group; Group III, osteoporosis group; 25(OH) Vit D, 25-hydroxyvitamin D; DEXA, dual x-ray absorptiometry; hs-CRP, high sensitive C-reactive protein; NT-proBNP, N terminal pro-brain natriuretic peptide.
α, significant association between group I and group III ($P < .05$).
β, significant association between group I and group II ($P < .05$).
¥, significant association between group II and group III ($P < .05$).

bone microarchitecture with a simple biochemical parameter. In our study, in accordance with previous studies, the presence of osteoporosis was found to be associated with the white blood cell, hematocrit and platelet counts, BUN and creatinine, ALP, serum and urine calcium, PTH, hs-CRP, and NT-proBNP levels in univariate analysis. In multivariate analysis, however, only urine calcium and NT-proBNP levels were independently associated with osteoporosis presence. This finding is consistent with the literature, and as far as we have investigated, there were no data on the association between urine calcium and NT-proBNP and osteoporosis in previous studies.

As with our results in our study, vitamin D serum levels were decreased due to increased vitamin D turnover in pHPT patients with active disease.[20] Although it has been reported that there is a relationship between vitamin D levels and BMD,[8,10] there are also reports that there is no significant association in 2 recent studies.[6,21] In our study, vitamin D levels were low in all BMD

Table 2. Variable Regression Analysis for the Detection of pHPT Patients with Osteoporosis

Variable	Odds Ratio	95% CI	*P*
Urine calcium (10 mg/day)	1.086	1.030-1.145	.002
NT-proBNP (10 pg/mL)	1.091	1.029-1.156	.003

NT-proBNP, N terminal pro-brain natriuretic peptide; pHPT, primary hyperparathyroidism.

Table 3. ROC Analysis for the Detection of pHPT Patients with Osteoporosis

Variable	AUROC Curve	*P*	Cut–Off	Sensitivity (%)	Specificity (%)
Urine calcium	0.728 (0.616–0.840)	.001	300 mg/day	73.9	63.4
NT–proBNP	0.867 (0.789–0.945)	<.001	200 pg/mL	82.6	73.2

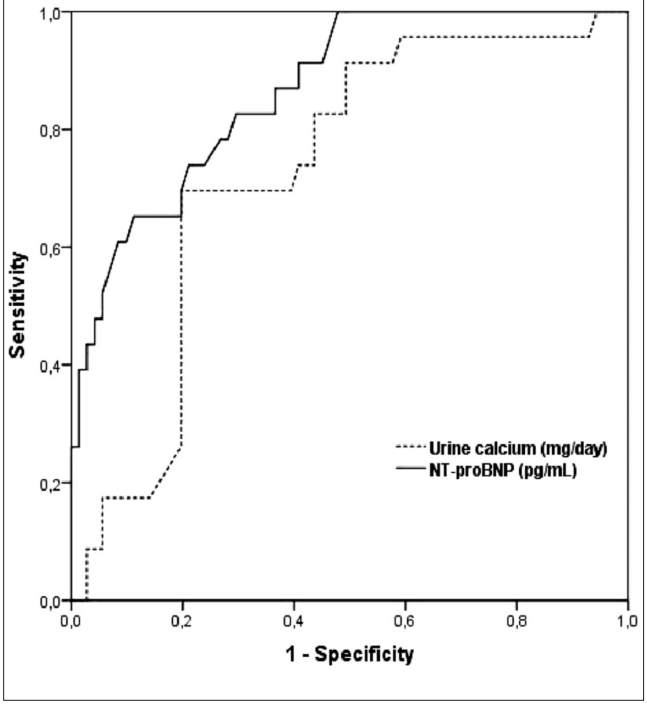
NT–proBNP: N terminal pro–brain natriuretic peptide; AUROC, area under the receiver operating characteristic curve.

groups in accordance with previous data and there was no significant relationship between the groups. So, there was no relation between vitamin D level and the presence of osteoporosis.

Natriuretic peptide follow-up is usually done in cardiac diseases. However, one of the most important problems in pHPT patients is the increased incidence of coronary artery disease, heart failure, and cardiovascular events, and therefore, several studies have been conducted on the use of BNP in pHPT patients.[11-14] The levels of NT-proBNP were shown to be above the normal reference value by 20% in patients with mild PHPT without cardiac involvement.[11] Several studies have reported that BNP increases with cardiac involvement.[12-14] In our study, the majority of patients with pHPT were serious and underwent surgical treatment, and NT-proBNP levels were found to be higher than the reference value of 125 pg/mL in 47.9% of all patients.

Several studies in pHPT patients have reported a close association between current bone disease and Left ventricular dysfunction.[22-25] However, the relationship between existing bone diseases and NT-proBNP has not been addressed in these studies. If the NT-proBNP assessment was done, the result of our study could be even more meaningful. One study that has been undertaken before the clinical use of natriuretic peptides has shown that ANP and BNP affect rat parathyroid cells by ET-1 synthesis in addition to PTH.[15] It is known that ET-1 is the most potent vasoconstrictor and also increases the osteoclastic activity and leads to osteopenia by changing intrauterine vascular tonus and causing a feeding problem.[16-18] For these reasons, it is a peptide associated with osteoporosis. We did not measure ET-1 levels in our study, but we concluded that the independent association between increased NT-proBNP and BMD is probably related with such a physio-pathological system. Our study was the first study that evaluated the relationship between NT-proBNP and osteoporosis and found that there was a meaningful relationship. In addition, we believe that our study should be considered as a preliminary study and more accurate data should be obtained by studies in which both NT-proBNP and ET-1 levels are measured and the presence of osteoporosis is evaluated together.

Figure 1. The receiver operator characteristic curve of values for N-terminal-pro-brain natriuretic peptide and urinary calcium levels for determining patients to be osteoporosis for primary hyperparathyroidism.



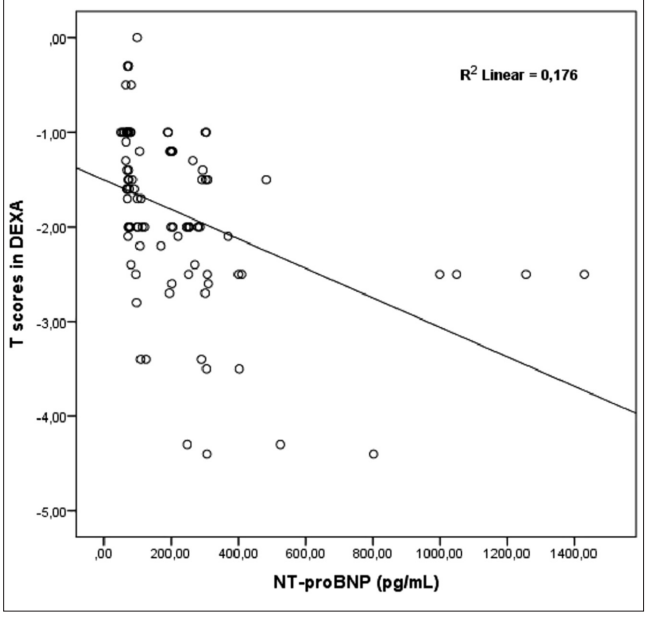
In accordance with the National Institutes of Health consensus panel, the following are considered as surgical criteria in symptomatic pHPT patients or the presence of any of them in asymptomatic pHPT patients: (i) serum calcium level elevation for 1 mg/dL, (ii) significant hypercalciuria (> 400 mg/24 h), (iii) DEXA T score <−2.5, (iv) patient under 50 years old (<50 years old), and (v) decrease in creatinine clearance by more than 30%.[5] Because of this reason, urine calcium level is used as a follow-up parameter in pHPT patients. Our study showed that there was an

Table 4. The Parameters Associated with T Scores in DEXA and Linear Regression Analysis for Parameters Significantly Correlated with T Scores in DEXA

	Univariate analysis		Multivariate analysis	
Variable	*P*	*r*	*P*	β
NT–proBNP (pg/mL)	<.001	0.418	<.001	0.361
Urine calcium (mg/day)	<.001	0.390	<.001	0.327
Parathyroid hormone (pg/ml)	.041	0.211	.733	0.042
Alkaline phosphatase (u/L)	.049	0.171	.756	0.033

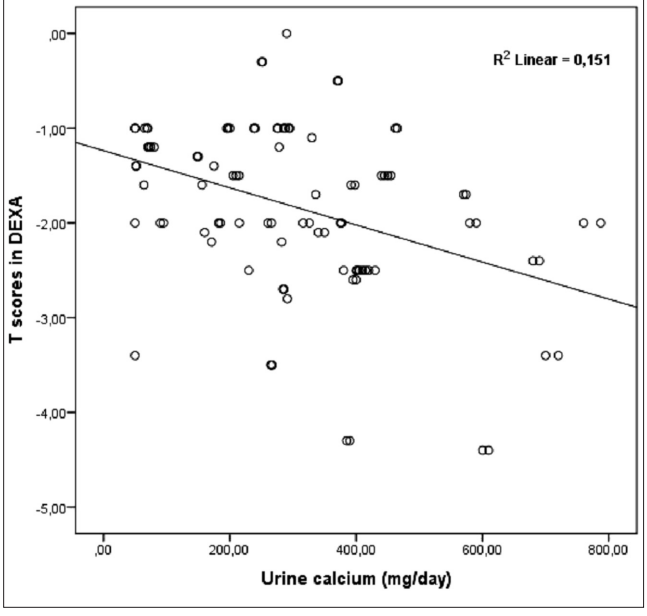
DEXA, dual x–ray absorptiometry; NT–proBNP: N terminal pro–brain natriuretic peptide.
* RAdjusted2=0.378.

Figure 2. There was a significant correlation between N-terminal-pro-brain natriuretic peptide levels and T scores in dual x-ray absorptiometry in patients with primary hyperparathyroidism.



independent relationship between urinary calcium levels and DEXA T score, and urinary calcium levels also independently identified patients with osteoporosis. As far as we investigate, a close and independent relationship between urinary calcium level and BMD has not been previously shown in patients with pHPT. However, there is no significant and independent relationship between serum calcium, PTH, and vitamin D levels and the presence of osteoporosis, but the presence of a significant and independent relationship with urinary calcium level did not make sense to us and we could not explain this relationship physio-pathologically. There is information in the literature that osteoporosis is common in patients with calcium nephrolithiasis, although it is not independently associated with urine calcium level.[22]

Figure 3. There was a significant correlation between urinary calcium levels and T scores in dual x-ray absorptiometry in patients with primary hyperparathyroidism.



In the first instance including the number of patients, there are some major limitations in our study. In conclusion, of patients' not following up there were no data on the treatment efficacy for BMD. Additionally, since the demonstration of the relationship between NT-proBNP and osteoporosis in pHPT patients is the first in the literature, there is a need to properly present data with a study involving more patients. To clarify the effect of bone density on pHPT patients in our study, the control group was not included, so studies with a control group should also be done. It has been reported that heart failure prevalence is high in patients with pHPT and high NT-proBNP.[13,14] However, in our study, we did not conduct any heart failure or cardiac evaluation.

CONCLUSION

The level of NT-proBNP is significantly increased in pHPT patients and is more useful than the other laboratory tests with the urine calcium level indicating bone involvement in patients newly diagnosed with pHPT. However, it is not a parameter to take the place of DEXA, which is a routine examination for bone involvement in pHPT patients. However, measuring a higher NT-proBNP level (>125 pg/mL) at the initial assessment and diagnostic stage of the patient may give a preliminary indication that this patient may have bone involvement or osteoporosis. Especially patients with high NT-proBNP can be followed closely for osteopenia and osteoporosis. In conclusion, NT-proBNP is an inexpensive, simple, reproducible, and objective parameter for the detection of bone involvement in addition to cardiovascular disease and cardiac involvement, in patients with newly diagnosed pHPT in early stages. However, because our study is a first in the literature, it needs to be supported by new studies and assessments.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Cukurova University by the Ministry of Health (Date: May 15, 2018 / Desicion No: 59).

Informed Consent: Written informed consent was taken from all of the participants.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A.D., B.S.A., H.E.S.; Design – M.A.D., B.S.A., H.E.S.; Supervision – B.S.A., H.E.S.; Resources – B.S.A., H.E.S.; Materials – M.A.D., B.S.A., H.E.S.; Data Collection and/or Processing – M.A.D., B.S.A., H.E.S.; Analysis and/or Interpretation – M.A.D., H.E.S.; Literature Search - M.A.D., H.E.S.; Writing Manuscript – M.A.D., H.E.S.; Critical Review – M.A.D., B.S.A., H.E.S.

Declaration of Interests: The authors have no conflicts of interest to declare.

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Original Article

Effectiveness of De Ritis (AST/ALT) Ratio in Predicting Biochemical Recurrence in Patients Underwent Radical Prostatectomy for Localized Prostate Cancer

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ABSTRACT

Objective: Several studies have shown that the De Ritis (aspartate aminotransferase/alanine aminotransferase) ratio is a prognostic biochemical biomarker in many cancers, including urological cancers. Biochemical recurrence is a well-known indicator of the biological aggressiveness of the tumor. In our study, we aimed to evaluate the predictability of the De Ritis ratio for biochemical recurrence in patients who underwent radical prostatectomy due to localized prostate cancer.

Methods: This study included 198 patients who underwent radical prostatectomy for localized prostate cancer between 2008 and 2015 in our clinic. Preoperative data of the patients included age, prostate-specific antigen level, post-biopsy Gleason score, De Ritis ratio, neutrophil/lymphocyte ratio, and platelet count. Among the postoperative data, the Gleason score, extracapsular invasion, positive surgical margin, seminal vesicle invasion, perineural invasion, lymph node invasion, and pathological tumor stage data were evaluated retrospectively. The relationship of these parameters was examined in patients who developed biochemical recurrence during the follow-up period.

Results: The mean follow-up period of the patients was 56.7 ± 23.6 months and biochemical recurrence occurred in 10.1% of all patients. In the receiver-operating characteristic analysis, the cut-off value for biochemical recurrence was 1.184, and the patients with a ratio below this value were grouped as the low De Ritis group and the patients with higher rates were grouped as the high De Ritis group. According to multivariate logistic regression analysis, high De Ritis ratio, Gleason score >8 of radical prostatectomy specimen, positive surgical margin, and the presence of seminal vesicle invasion were detected as independent risk factors for biochemical recurrence after radical prostatectomy.

Conclusion: De Ritis ratio is an independent risk factor for predicting biochemical recurrence in patients who underwent radical prostatectomy due to localized prostate cancer.

Keywords: Biochemical recurrence, De Ritis ratio, prostate cancer

INTRODUCTION

Prostate cancer is the second most common cancer in men after lung cancer and represents 15% of all cancer cases.[1] Radical prostatectomy and radiotherapy are the first options for the treatment of localized prostate cancer, and definitive treatment can be provided with both treatment methods. Unfortunately, biochemical recurrence (BCR) can be seen after radical prostatectomy, which is one of these treatment methods, and its frequency varies between 19% and 35% in a 10-year follow-up.[2] A great number of studies in the current literature have examined the factors that are associated with the risk of BCR after radical prostatectomy with interest. Fundamentally, high Gleason score, advanced stage tumor, and high basal serum prostate-specific antigen (PSA) levels are among the factors that are associated with BCR.[3]

The ratio of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), known as the De Ritis rate, was mainly utilized as an indicator for liver function.[4] Studies have implied that this ratio is an important prognostic factor for colorectal cancer, lung cancer, breast cancer, and pancreatic cancer, as well as urological cancers such as bladder cancer, upper urinary tract cancer, and testicular cancer.[5,6] Since the presence of active proliferation, active oxidative stress, and increased aerobic glycolysis in cancer cells causes this rate to increase, it can be thought that this ratio may indicate the biological behavior of cancer.[5]

In our study, we aimed to evaluate the predictability of the preoperative De Ritis ratio of patients who underwent radical prostatectomy due to localized prostate cancer and BCR recurrence in a 10-year follow-up.

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METHODS

This study was performed in accordance with the principles of Helsinki Declaration and Ethics committee approval was received for this study from the ethics committee of Ankara City Hospital No.1 Clinical Research Ethics Committee (Date: March 24, 2021, Decision No: E2-21-303).

The data of 244 patients who underwent radical prostatectomy for prostate cancer in our clinic between 2008 and 2015 were retrospectively analyzed. All of the middle-high-risk patients included in our study were scanned for metastases with whole-body bone scintigraphy and abdominopelvic computed tomography, and patients with metastases were not included in the study. Forty-six patients with known liver disease, insufficient data, not being followed up, who underwent neoadjuvant hormone therapy, and who passed away during follow-up were excluded from the study.

This study included 198 patients. The diagnosis of prostate cancer in all patients was made by a transrectal ultrasound-guided biopsy performed due to elevated PSA levels and/or abnormal rectal examination. The life expectancy of the patients was over 10 years. All patients diagnosed with localized prostate cancer as a result of the biopsy were operated on by open retropubic radical prostatectomy procedure and extended pelvic lymph node dissection was performed in all patients.

The preoperative data of the patients, which are age, serum PSA level, transrectal ultrasound-guided biopsy's Gleason score, preoperative De Ritis ratio, neutrophil/lymphocyte ratio (NLR), and platelet count, were evaluated 1-2 days before the operation. Postoperative surgical material was examined and the data of Gleason score, extracapsular invasion, surgical margin positivity, seminal vesicle invasion, perineural invasion, lymph node positivity, and pathological tumor stage (according to the American United Cancer Committee Tumor, Node, Metastasis classification) were evaluated. In the follow-up of patients after radical prostatectomy, serum PSA levels were tested every 3-6 months. A serum PSA level of >0.2 ng/mL in 2 consecutive measurements was defined as BCR.

Statistical Analysis

The coding and statistical analysis of the data were executed on the computer, accessing the Statistical Package for the Social Sciences version 22.0 (IBM SPSS Corp.; Armonk, NY, USA) package program. While descriptive statistics data for continuous variables were expressed with the average, categorical variables were expressed in terms of frequency and percentage. Mann–Whitney *U* test, Chi-square test, and Fisher's exact test were used to evaluate continuous and categorical variables. Receiver-operating characteristic (ROC) analysis was used to determine the estimated value of the De Ritis ratio that can be used to predict BCR. Univariate and multivariate logistic regression analyses were used to evaluate independent risk factors for BCR. A *P*-value of <.05 was considered statistically significant for all analyses.

Main Points

After radical prostatectomy for localized prostate cancer, the following points were observed:

- De Ritis ratio cut-off value to predict the biochemical recurrence was 1.184.
- High De Ritis ratio was detected as an independent risk factor for biochemical recurrence.
- Gleason score >8 of radical prostatectomy specimen, positive surgical margin, and the presence of seminal vesicle invasion are other risk factors for biochemical recurrence.

RESULTS

The mean follow-up period of the patients was 56.7 ± 23.6 months. Biochemical recurrence occurred in 21 patients (10.1%). The ROC analysis was performed using the Youden index (Figure 1), the De Ritis ratio cut-off value was found to be 1.184 to predict the BCR. The patients with a ratio below this value were grouped as the low De Ritis group and the patients above it as the high De Ritis group.

According to the streaming, the relationship between the De Ritis ratio and the clinical and pathological characteristics of the patients is shown in Table 1.

Among the patient groups divided according to low and high rates of De Ritis, there was no statistically significant difference between age, preoperative serum PSA level, Gleason score of ultrasound-guided transrectal biopsy, Gleason score of radical prostatectomy specimen, extracapsular invasion, surgical margin positivity, the presence of seminal vesicle invasion, the

Figure 1. Evaluation of De Ritis ratio by ROC analysis in predicting biochemical recurrence. The cut-off point is 1.184 (AUC = 0.673, *P* = .01, 95% CI (0.557-0.789)). AUC, area under curve; ROC, receiver-operating characteristic.

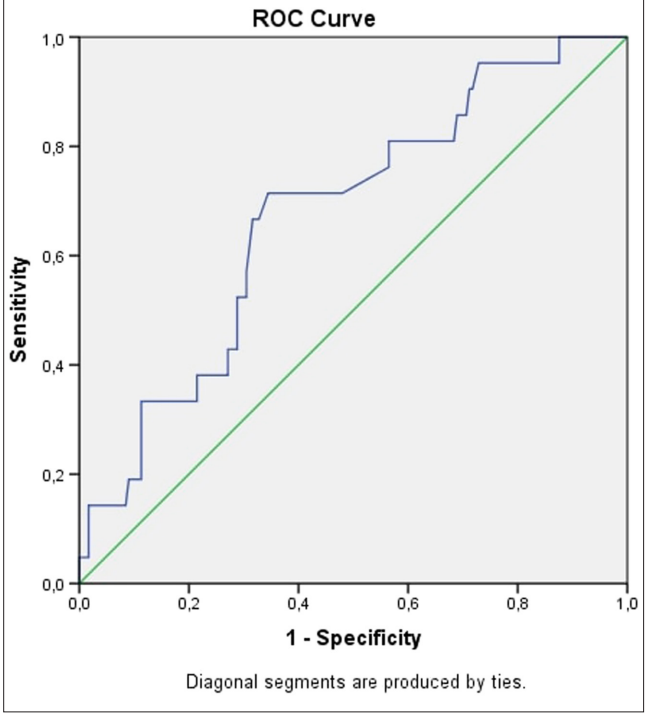

Table 1. Clinical and Pathological Characteristics of Patient Groups Separated According to De Ritis Rate

	De Ritis Ratio			
Parameters	**Total (n = 198)**	**Low (n = 128, 64.7%)**	**High (n = 70, 35.3%)**	***P***
Age, mean ± SD	62.8 ± 6.7	62.3 ± 6.8	63.6 ± 6.5	.208*
PSA, mean ± SD	10.5 ± 7.1	10.1 ± 7.1	11.2 ± 7.2	.101*
PSA, n (%)				
<10 ng/mL	130 (65.7)	90 (70.3)	40 (57.1)	.062**
≥10 ng/mL	68 (34.3)	38 (29.7)	30 (42.9)	
Post-biopsy Gleason Score, n (%)				
6	141 (71.2)	92 (71.9)	49 (70)	.622**
7	28 (14.2)	16 (12.5)	12 (17.1)	
≥ 8	29 (14.6)	20 (15.6)	9 (12.9)	
Postoperative Gleason score, n (%)				
6	113 (57.1)	77 (60.1)	36 (51.4)	.493**
7	57 (28.8)	34 (26.6)	23 (32.9)	
≥ 8	28 (14.1)	17 (13.3)	11 (15.7)	
Extracapsular invasion, n (%)				
No	159 (80.3)	105 (%82)	54 (77.1)	.408**
Yes	39 (19.7)	23 (%18)	16 (22.9)	
Surgical margin, n (%)				
No	133 (67.2)	87 (68)	46 (65.7)	.747**
Yes	65 (32.8)	41 (32)	24 (34.3)	
Seminal vesicle invasion, n (%)				
No	171 (86.4)	110 (85.9)	61 (87.1)	.813**
Yes	27 (13.6)	18 (14.1)	9 (12.9)	
Neurovascular invasion, n (%)				
No	60 (30.3)	32 (25)	26 (37.1)	.053**
Yes	138 (69.7)	96 (75)	44 (62.9)	
Lymph node invasion, n (%)				
No	186 (93.9)	120 (93.8)	66 (94.3)	.574***
Yes	12 (6.1)	8 (6.2)	4 (5.7)	
Pathological tumor stage, n (%)				
T2a	44 (22.2)	28 (24.5)	16 (22.9)	.987**
T2b	34 (17.2)	22 (16.3)	12 (17.1)	
T2c	71 (35.9)	47 (40.8)	24 (34.3)	
T3	49 (24.7)	31 (18.4)	18 (25.7)	
NLR, mean ± SD	2.5±1.3	2.6±1.4	2.4±1.1	.469*

*Mann-Whitney *U* test; **Chi-square test; ***Fisher's exact test.
PSA, prostate-specific antigen; SD, standard deviation; NLR, neutrophil/lymphocyte ratio.

Table 2. Identifying Risk Factors for Biochemical Recurrence

	Univariate		Multivariate	
Parameters	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
De Ritis ratio (high)	4.321 (1.653–11.298)	**.003**	5.84 (1.889–18.054)	**.002**
Age (continue)	0.986 (0.923–1.053)	.673		
PSA (≥10)	1.865 (0.749–4.643)	.18		
Post–biopsy Gleason score (≥ 8)	3.523 (1.281–9.684)	**.015**	0.691 (0.096–4.983)	.714
Postoperative Gleason score (≥8)	8.03 (2.998–21.511)	**<.001**	11.882 (3.229–43.726)	**<.001**
Extracapsular invasion (yes)	1.314 (0.45–3.838)	.617		
Surgical margin (positive)	4.941 (1.885–12.954)	**.001**	4.409 (1.364–14.255)	**.013**
Seminal vesicle invasion (yes)	1.575 (0.487–5.096)	**.448**	5.938 (1.013–34.792)	**.048**
Neurovascular invasion (yes)	4.2 (0.942–18.728)	.06		
Lymph node invasion (yes)	1.758 (0.358–8.626)	.487		
Pathological tumor stage (T3)	2.041 (0.791–5.265)	.14		
NLR (continue)	0.972 (0.676–1.395)	.879		
Platelets (continue)	1.001 (0.994–1.007)	.782		

PSA, prostate–specific antigen; NLR, neutrophil/lymphocyte ratio.

presence of perineural invasion, lymph node positivity, pathological tumor stage, and NLR detected ($P > .05$).

According to the multivariate logistic regression analysis performed to establish the risk factors for BCR, high De Ritis rate (OR = 5.84; 95% CI = 1.889-18.054; $P = .002$), Gleason score >8 after radical prostatectomy (OR =11.882; 95% CI = 3.229-43.726; $P < .001$), surgical margin positivity (OR = 4.409; 95% CI = 1.364-14.255; $P = .013$), and the presence of seminal vesicle invasion (OR = 5.938; 95% CI = 1.013-34.792; $P = .048$) were determined as independent risk factors (Table 2).

DISCUSSION

In our study, the high rate of De Ritis was found to be an independent risk factor for BCR in patients who underwent radical prostatectomy for localized prostate cancer. However, it has been shown that there is no relationship between the clinical and pathological features of the patients. Aspartate aminotransferase and ALT are among the most used serum biomarkers in our daily practice. It can be used to predict BCR as an easily accessible, fast, and inexpensive way.[7]

Aspartate transaminase and ALT are mainly used as important parameters in the diagnosis of liver-specific diseases and in their follow-up after treatment.[8] Generally, ALT is specific to the liver, while AST is expressed from different tissues such as brain, muscle, and kidney.[9] The De Ritis rate is defined as the ratio of the activities of AST and ALT in serum.[10] Throughout the years, it has been considered that this rate may change by various mechanisms in malignancies. Among these mechanisms, AST, which is also expressed from organs other than ALT, is more in cancer cells with high proliferation.[11] Yet again, Warburg et al[12] Showed that cancer cells use glycolysis more than normal cells, which is a fast energy resource and in which AST plays an important role. While tissue damage and metabolic changes due to rapidly proliferating cancer cells cause an increase in AST level in peripheral blood, ALT level does not change much.[13]

There are several studies in the literature examining the prognostic value of De Ritis rate in urological cancers. In a meta-analysis in which Hu et al[7] included 8 studies and 3949 patients, it was shown that high preoperative serum De Ritis rate negatively affected overall survival and cancer-specific survival in bladder and upper urinary tract cancers. Bezan et al[8] evaluated 698 non-metastatic renal cell cancer cases and showed that the De Ritis rate >1.26 was a negative prognostic factor for metastasis-free survival and overall survival. In another meta-analysis in which 8565 patients were evaluated, the rate of De Ritis was revealed to be a predictive factor for overall survival, progression-free survival, and cancer-specific survival for upper urinary tract cancer, bladder cancer, and renal cell carcinoma.[13]

The first study to assess the importance of AST and ALT levels in localized prostate cancer was managed in 2010. In this study, it was reported that a high rate of De Ritis was associated with a high Gleason score but was insufficient in predicting BCR.[14] However, the inclusion of low-grade and well-differentiated cancer patients in this study may have been effective in the emergence of this result. In another study held in 2017, the relationship between BCR and De Ritis rate after radical prostatectomy in localized prostate cancer was examined. The higher rate of De Ritis >1.325 has been shown to be associated with

a higher Gleason score after biopsy and radical prostatectomy, higher pathological tumor stage and more seminal vesicle invasion, positive surgical margin, and lymph node infiltration. Again, in this study, pathological tumor stage, Gleason score of radical prostatectomy specimen, and De Ritis rate were shown among the factors that are associated with BCR risk.[15]

In a study by Quhal et al[16] in which 214 patients who underwent salvage radical prostatectomy due to BCR after definitive radiotherapy were evaluated, the cut-off value for the De Ritis rate was considered as 1.35 and was not found to be related to the clinicopathological features of cancer. In addition, it was found that 1.8 times more BCR was observed in patients with high preoperative serum De Ritis rate, as well as 1.7 times more in those with high postoperative De Ritis rate.

In our study, similar to the results of Quhal et al[16], there was no relationship between the clinicopathological features of prostate cancer and the rate of De Ritis, while it was shown that 5.84 times higher BCR was found in patients with a De Ritis rate of >1.184. We presume that this result may be related to the inclusion of a lower number of patients in both studies compared to the others.

Neutrophil/lymphocyte ratio is also another hematological parameter whose prognostic significance has been studied in localized prostate cancer. Lee et al[17] has shown in another study that 1.36 times higher BCR was observed in patients who underwent radical prostatectomy due to localized prostate cancer with an NLR >2.5. In another study, it was shown to be an independent risk factor for poor prognosis.[18] In our study, NLR and platelet count were not found to be risk factors for BCR.

Performing retrospectively, having a low number of patients is the limitation of our study. Although patients with liver disease were not included in the study, other factors affecting liver enzymes, such as the patients' medication, could not be evaluated properly. Open retropubic radical prostatectomy was performed in all patients, yet the use of laparoscopic and robotic techniques could not be interpreted. Nonetheless, we think that our study will contribute to the literature due to the limited number of studies examining the relationship between the pathological and prognostic features of prostate cancer and the De Ritis ratio in the literature.

CONCLUSION

Although the high rate of De Ritis is not associated with the clinicopathological features of prostate cancer, it is an independent risk factor for BCR after radical prostatectomy due to localized prostate cancer, together with the Gleason score, surgical margin positivity, and the presence of seminal vesicle invasion. Preoperative serum De Ritis rate, which is an easy-to-examine method, can be used to predict BCR.

Ethics Committee Approval: This Ethics committee approval was received for this study from the ethics committee of Ankara City Hospital No.1 Clinical Research Ethics Committee (Date: March 24, 2021, Decision No: E2-21-303).

Informed Consent: Not applicable.

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Original Article

Evaluation of Upper Extremity Movement, Pain Intensity, and Respiratory Functions in Patients Who Received Thoracotomy Sparing the Serratus Anterior Muscle

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ABSTRACT

Objective: To evaluate upper extremity movement, pain intensity, and respiratory functions in preoperative and postoperative periods in patients undergoing thoracotomy sparing the serratus anterior muscle (TSSAM).

Methods: Forty-three patients (25 male and 18 female) were included in this prospective observational cohort type study. In the preoperative period and on postoperative days 1, 2, 3, and 5, ipsilateral shoulder range of motion was evaluated by a goniometer, pain intensity was evaluated by a visual analog scale (VAS), and respiratory functions were evaluated by spirometry.

Results: When compared with preoperative values, shoulder flexion and abduction angle, forced expiratory volume in 1 second (FEV_1), and functional vital capacity (FVC) decreased on postoperative day 1, while VAS significantly increased ($P < .05$). Shoulder flexion and abduction angle, FEV_1, and FVC significantly increased and VAS significantly decreased on postoperative days 2, 3, and 5 compared to postoperative day 1 ($P < .05$). However, they could not reach preoperative values on postoperative day 5 ($P < .05$). On postoperative day 1, while there was a correlation between pain and flexion ($r = -0.438$; $P = .003$) and abduction ($r = -0.503$; $P = .001$) angles, no correlation was found between pain and FEV_1 ($r = -0.189$; $P = .225$) and FVC ($r = 0.009$; $P = .953$). There was no correlation between pain and flexion, abduction, FEV_1, and FVC on postoperative days 2,3, and 5 ($P > .05$).

Conclusions: Patients undergoing the TSSAM had less upper extremity range of motion and respiratory functions and more pain intensity in the early postoperative period than in the preoperative period. It was observed that pain and flexion and abduction angles were negatively correlated on postoperative day 1. In the postoperative period, they should be taken into account in the design/development of rehabilitation programs.

Keywords: Respiratory function tests, thoracotomy, range of motion, pain

INTRODUCTION

Standard posterolateral thoracotomy (SPLT) is used for most general thoracic surgical procedures. This incision involves the incision of the latissimus dorsi and serratus anterior muscles and provides an excellent view of the entire chest cavity, which, however, causes increased blood loss, impaired pulmonary function, postoperative chest pain, and limitation of shoulder movement.[1,2] In order to minimize these disadvantages, muscle-sparing thoracotomy (MST) in which the latissimus dorsi and serratus anterior muscles are not cut, or thoracotomy sparing the serratus anterior muscle (TSSAM), in which only the latissimus dorsi muscle is cut, is preferred.[3] The serratus anterior muscle can be preferred as an alternative approach due to various advantages such as providing a wide view through posterolateral

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incision, combining muscle-sparing advantages, simple and fast application, facilitating thoracotomy closure, and the possibility of using in emergency surgical conditions.[4]

Thoracotomy may cause decreased shoulder range of motion and chronic pain. Upper extremity range of motion limitations in patients undergoing thoracotomy is thought to be due to complete denervation of the serratus anterior and latissimus dorsi muscles. Because the serratus anterior muscle and the trapezius muscle together provide the rotation of the scapula, which is necessary for shoulder abduction and flexion.[5] It has been stated that the latissimus dorsi is the most effective depressor of the humeral head, and it has been stated that it helps to hold the scapula against the thorax during upper extremity movements, by attaching the latissimus dorsi to the inferior angle of the scapula.[6] In addition, pain can be effective in these limitations.[7,8] Postoperative pain is caused by patient positioning during thoracotomy, intercostal nerve and vessel injuries during retraction, major muscle cuts, costa retraction, and chest tube placement.[8-11] Studies evaluating upper extremity range of motion and pain have generally been performed in patients with MST and SPLT.[12,13] It was reported that preserving the serratus anterior muscle increases shoulder mobility.[14] However, postoperative evaluations are needed in patients undergoing TSSAM. It is also important to determine the relationship between pain and range of motion.

It has been reported that a painful incision may cause a decrease in lung volume and respiratory functions by increasing muscle tone during inspiration. This may cause increased secretion, atelectasis, and respiratory tract infections.[15,16] Postoperative pulmonary complications are an important cause of morbidity after thoracotomy, leading to a prolonged hospital stay and increased health care costs.[17] It was stated that it is unclear whether sparing the serratus anterior muscle will enhance the recovery of postoperative respiratory function.[12] In this context, it is necessary to provide evidence to determine postoperative respiratory functions in patients undergoing TSSAM and to better predict its relationship with pain.

The aim of the study was to evaluate upper extremity movement, pain intensity, and respiratory functions in patients undergoing TSSAM on postoperative days 1, 2, 3, and 5. The hypothesis investigated was as follows: In the early postoperative period, upper extremity range of motion and respiratory functions decrease in patients undergoing TSSAM, the intensity of pain increases, and these parameters cannot reach preoperative values.

Main Points

- It was found that joint range of motion, pain, and respiratory functions were negatively affected in the early postoperative period compared to the preoperative period in patients undergoing the serratus anterior muscle (TSSAM).
- Pain on postoperative day 1 was negatively related to flexion and abduction angles.
- Joint range of motion, pain, and respiratory functions should be considered in rehabilitation programs to be applied in patients with TSSAM.

METHODS

Study Design

This prospective observational cohort type study was carried out in accordance with the rules of the Declaration of Helsinki. It was conducted at the Department of Thoracic Surgery of Gaziantep University The study was approved by the Ethics Committee of Gaziantep University (Date: October 13, 2014, Decision number: 2014/312). This study was conducted between 2014 and 2015.

Patients

Patients aged between 15 and 73 years, volunteering to participate in the study were assessed at the thoracic surgery clinic. Patients with pathology, such as tumor and tendinitis, which would cause limitation of shoulder movement on the incision side, those with an incision outside of TSSAM, those who received other incisions addition to TSSAM, or those with extended incisions such as chest wall resection were not included in the study. All patients were informed about the study and signed consent forms were obtained.

Procedures

After the patients were monitored for vascular access, arterial blood pressure, heart rhythm, and urine output, they were placed under general anesthesia. The cardiovascular and respiratory systems became available for monitoring and manipulation. The patients were placed on the operating table in the lateral decubitus position with the operated side up. A pillow was placed under the chest to increase the gap between the ribs and armpit support to prevent injury to the brachial plexus. While the upper leg was in full extension, the lower leg was kept slightly flexed. The arms were placed in flexion on the arm boards. The knee was supported for peroneal nerve damage. In addition, sternum and hip stabilizers were used. The incision site was covered with sterile drapes to prevent bacterial migration. The patients were intubated with a double-lumen endotracheal tube.[18]

The incision, which started approximately 4 cm below the nipple and at the level of the anterior axillary line, continued 1 cm below the lower end of the scapula. The incision proceeding posteriorly from the medial of the scapula was terminated after 3-4 cm over the lower end of the scapula. After the subcutaneous tissue was passed, the latissimus dorsi, serratus anterior sheath and, if necessary, the lower part of the trapezius was cut a little to reach the intercostal space. A thorax retractor was placed in the appropriate intercostal space, and the ribs were stretched enough (8-10 cm) to perform the procedure. At the end of the procedure, the thoracotomy incision was closed in the same way in each patient.[14,18]

Evaluations

The patients' age, gender, body mass index, diagnosis, and operation types were evaluated in the preoperative period. Upper extremity joint range of motion, pain intensity, and respiratory

functions were evaluated both in the preoperative period and on postoperative days 1, 2, 3, and 5. In addition, all patients routinely performed the exercises (toe climbing on the wall in addition to breathing exercises) given in the department of thoracic surgery. All assessments were performed by the same physiotherapist.

Shoulder Range of Motion

The flexion and abduction angles of the ipsilateral shoulder were measured with a goniometer. The normal range of motion of the shoulder is 0-180° for flexion and abduction.[19]

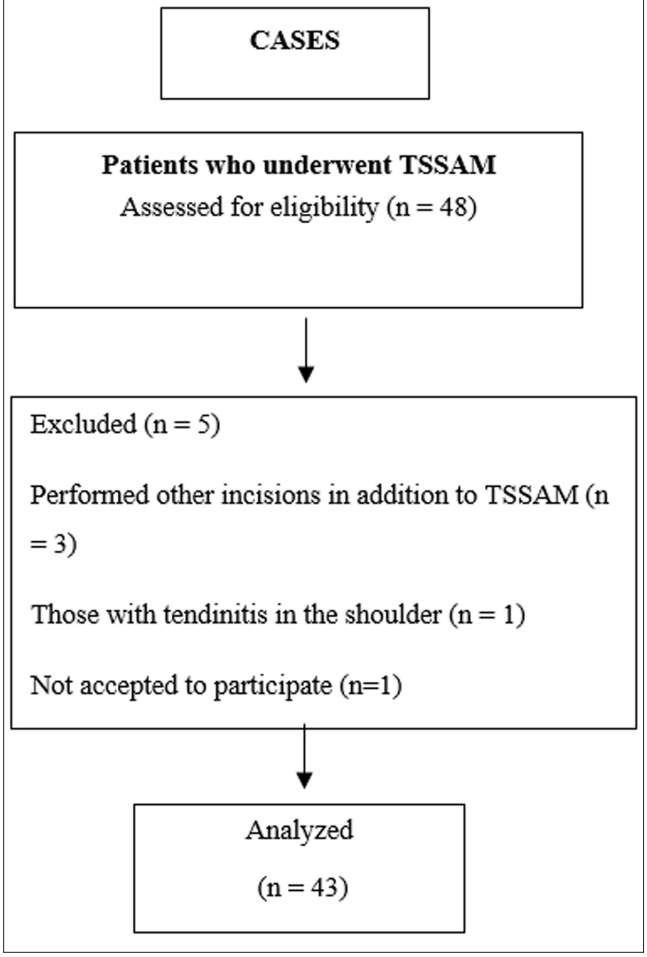
Pain Intensity

Pain intensity of the patients was evaluated with a visual analog scale (VAS). The VAS is a 10-cm horizontal line, where 0 represents no pain and 10 represents excruciating pain. All patients were asked to indicate the pain intensity they perceived on the horizontal line.[20]

Respiratory Functions

The respiratory functions of the patients were evaluated with a portable spirometer (MIR Spirobank Hand-Held Spirometer, Rome, Italy) in an upright sitting position. During the measurements, the American Thoracic Society and European Respiratory Society criteria were followed.[21] To prevent air leakage, patients wore a nose clip. First, a forced inspiration and then a forced expiration were performed. The best of 3 measurements was recorded. The volume of air exhaled in the first second of forced expiration (FEV_1) and forced vital capacity (FVC) values was recorded.[21]

Figure 1. The flowchart diagram for the patients.



Sample Size and Statistical Analyses

The effect size was calculated as 0.45 in the power analysis performed considering previous studies[12,22] on the parameters to be investigated in patients who underwent TSSAM. According to the power analysis made before the study, it was observed that at least 41 individuals should be included when the power is 80%, the error level is 0.05, and when the hypothesis is determined as bidirectional. This analysis was carried out with a statistical power analysis program (G*Power, Version 3.1.9.2, Franz Faul, Universität Kiel, German).

One-way repeated-measures analysis of variance was used to compare the measurements obtained at different times. As introductory statistics, mean ± standard deviation values for numerical variables and number and percentage values for categorical variables are given. IBM Statistical Package for the Social Sciences Statistics for Windows, Version 21.0 (Armonk, NY, USA) was used in analyses. Any $P < .05$ was considered statistically significant.

Table 1. Demographic and Clinic Characteristics of the Patients.

	Patients (n = 43)
Age (years, X ± SD)	**52.07 ± 14.46**
BMI (kg/m², X ± SD)	**28.81 ± 6.49**
Diagnosis (n, %)	
Lung cancer	22 (51.2)
Hydatid cyst	8 (18.6)
Mesothelioma	4 (9.3)
Bullous disease of the lung	1 (2.3)
Aspergilloma	1 (2.3)
Bronchiectasis	1 (2.3)
Malignant solitary fibrosis tumor	1 (2.3)
Mediastinal mass	1 (2.3)
Osteosarcoma	1 (2.3)
Prostate cancer	1 (2.3)
Renal cell carcinoma	1 (2.3)
Solitary pulmonary nodule	1 (2.3)
Procedure (n, %)	
Wedge resection	14 (32.6)
Lobectomy	10 (23.3)
Cystotomy + Capitonnage	6 (14)
Decortication	1 (2.3)
Pleural mass excision	4 (9.3)
Pneumonectomy	4 (9.3)
Exploration	3 (7)
Mediastinal mass excision	1 (2.3)

X, mean, SD, standard deviation; BMI, body mass index.

Table 2. Range of Motion on Operative Side, Visual Analog Scale, Pulmonary Function Tests, Values for Preoperative and Postoperative Days 1, 2, 3, and 5

Values	Preop (X ± SD)	Postop First (X ± SD)	Postop Second (X ± SD)	Postop Third (X ± SD)	Postop Fifth (X ± SD)	p1	ITI-p
Shoulder ROM Flexion (0–180°)	175.18 ± 4.86	127.74 ± 13.84	140.69 ± 10.97	154.79 ± 10.99	164.83 ± 10.46	**0.001***	**p2:0.001,* p3:0.001* p4:0.001,* p5:0.001* p6:0.001,* p7:0.001* p8:0.001,* p9:0.001* p_{10}:0.001,* p_{11}:0.001***
Abduction (0–180°)	177.32 ± 3.93	137.27 ± 15.41	150.32 ± 11.37	164.55 ± 10.45	171.88 ± 7.61	**0.001***	**p2:0.001*, p3:0.001* p4:0.001*, p5:0.001* p6:0.001*, p7:0.001* p8:0.001*, p9:0.001* p_{10}:0.001*, p_{11}:0.001***
VAS (cm)	1.58 ± 1.19	8.53 ± 1.12	6.13 ± 1.31	4.88 ± 1.29	2.53 ± 0.90	**0.001***	**p2:0.001,* p3:0.001* p4:0.001,* p5:0.001* p6:0.001,* p7:0.001* p8:0.001,* p9:0.001* p_{10}:0.001,* p_{11}:0.001***
Pulmonary function FEV_1 (L)	2.47 ± 0.71	0.82 ± 0.24	1.05 ± 0.29	1.36 ± 0.25	1.61 ± 0.31	***0.001***	**p2:0.001,* p3:0.001* p4:0.001,* p5:0.001* p6:0.001,* p7:0.001* p8:0.001,* p9:0.001* p_{10}:0.001,* p_{11}:0.001***
FVC (L)	3.39 ± 0.89	1.23 ± 0.24	1.59 ± 0.31	2.00 ± 0.36	2.33 ± 0.41	**0.001***	**p2:0.001,* p3:0.001* p4:0.001,* p5:0.001* p6:0.001,* p7:0.001* p8:0.001,* p9:0.001* p_{10}:0.001,* p_{11}:0.001***

*$P < .05$.

ITI-p; inter-time interaction, sub-group comparisons; p1, difference between times; p2, comparison of the preop and postop day 1; p3, comparison of the preop and postop day 2; p4, comparison of the preop and postop day 3; p5, comparison of the preop and postop day 5; p6, comparison of the postop day 1 and postop day 2; p7, comparison of the postop day 1 and postop day 3; p8, comparison of the postop day 1 and postop day 5; p9, comparison of the postop day 2 and postop day 3; p10, comparison of the postop day 2 and postop day 5; p11, comparison of the postop day 3 and postop day 5; X, mean; SD, standard deviation; preop, Preoperative; Postop, postoperative; ROM, range of motion; VAS, visual analog scale; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

RESULTS

Forty-eight patients were evaluated for the study. In total, 43 patients met the inclusion criteria (Figure 1). Demographic and clinical characteristics of the patients (25 male (58.1%), 18 female (41.9%)) are shown in Table 1. Twenty-three patients (53.5%) who underwent TSSAM had right thoracotomy and 20 (46.5%) had left thoracotomy (Table 1).

Shoulder flexion and abduction angle and FEV_1 and FVC values decreased significantly on postoperative day 1 in patients compared to preoperative values who underwent TSSAM ($P < .05$). There was a significant increase every day on postoperative days 2-5 compared to postoperative day 1 ($P < .05$). However, these values could not reach preoperative values even on postoperative day 5 ($P < .05$) (Table 2).

Pain intensity increased significantly on postoperative day 1 compared to the preoperative values ($P < .05$). There was a significant decrease in pain intensity on postoperative days 2-5 compared to postoperative day 1 ($P < .05$). However, even on postoperative day 5, the pain intensity could not reach the preoperative value ($P < .05$) (Table 2). The variation between days in shoulder flexion and abduction angle, pain, and FEV_1 and FVC values is shown in Figures 2-6.

In patients who underwent TSSAM, there was a moderate negative correlation on postoperative day 1 between pain and flexion ($r = -0.438$; $P = .003$) and abduction ($r = -0.503$; $P = .001$) angles. On the other hand, no significant correlation was found between pain and FEV_1 ($r = -0.189$; $P = .225$) and FVC ($r = 0.009$; $P = .953$) scores. At postoperative days 2-5, no correlation was found between pain and flexion angle ($r = -0.145$, $P = .354$; $r = -0.136$, $P = .386$; $r = -0.011$, $P = .946$), abduction angle ($r = -0.108$, $P = .491$; $r = -0.044$, $P = .778$; $r = 0.030$, $P = .849$, FEV_1 value ($r = -0.095$, $P = .544$; $r = -0.111$, $P = .477$; $r = 0.031$, $P = .845$), and the FVC value ($r = -0.041$, $P = .795$; $r = 0.053$, $P = .734$; $r = 0.062$, $P = .692$).

Figure 2. Demonstration of changes in flexion angle between preoperative values and values from postoperative days 1, 2, 3, and 5.

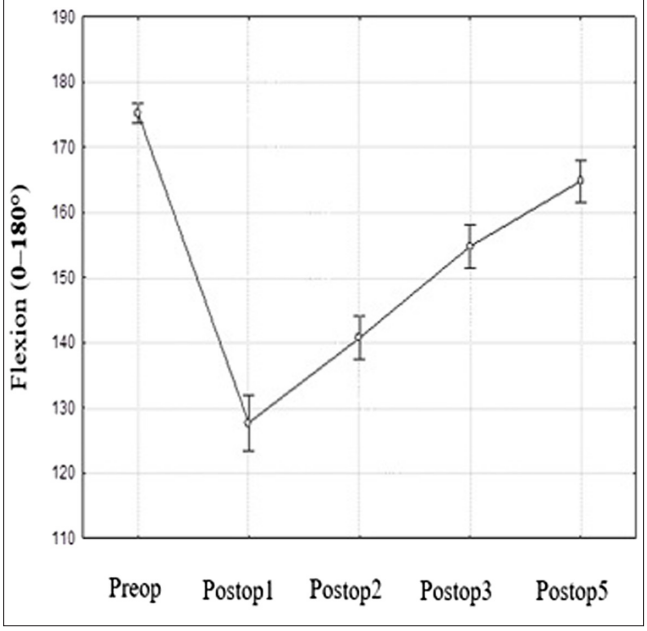


Figure 3. Demonstration of changes in abduction angle between preoperative values and values from postoperative days 1, 2, 3, and 5.

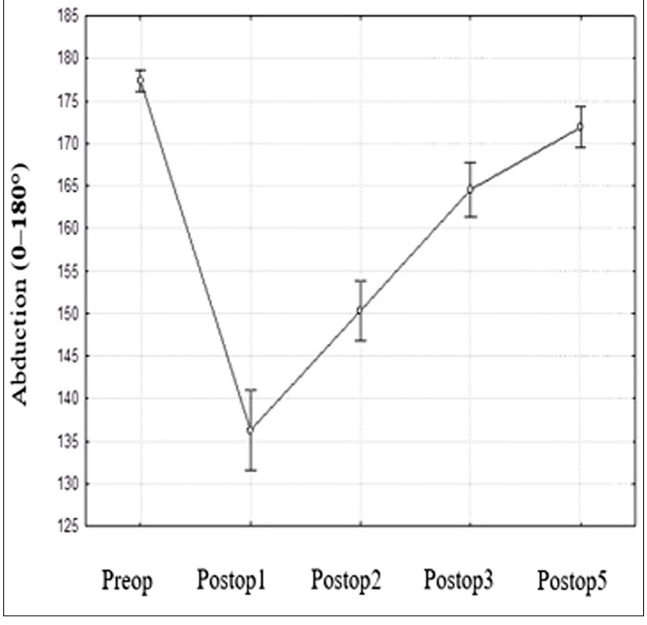


Figure 4. Demonstration of changes in VAS between preoperative values and values from postoperative days 1, 2, 3, and 5. VAS, visual analog scale.

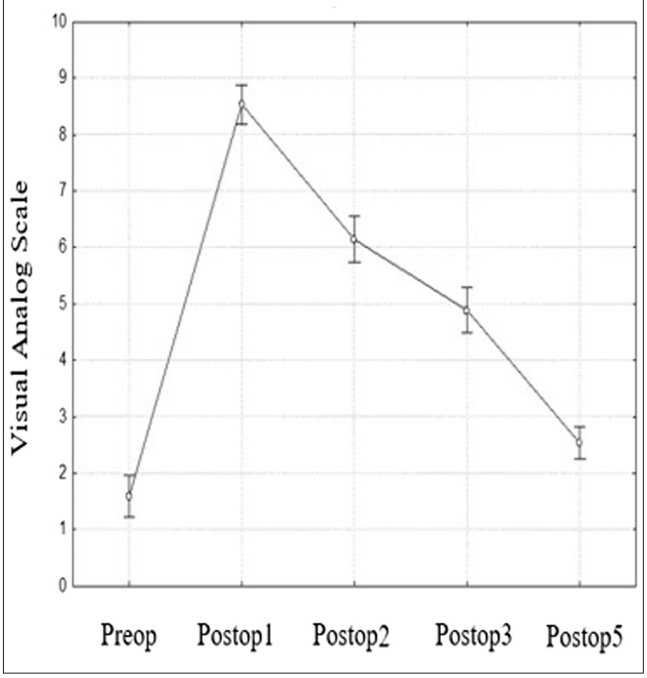
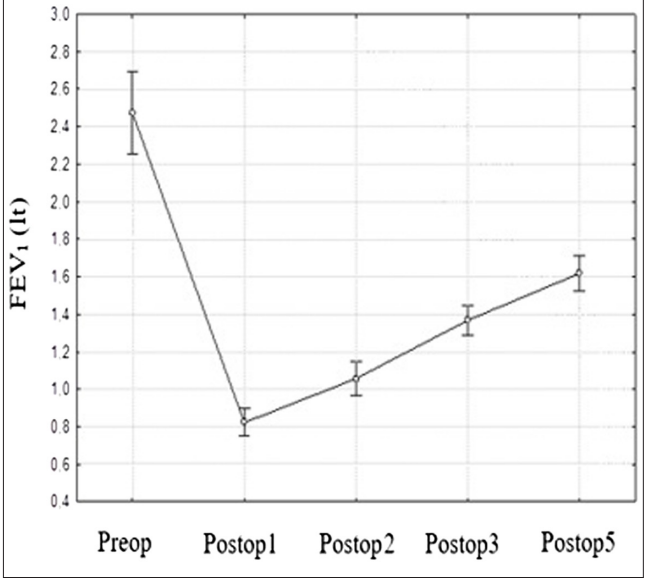


Figure 5. Demonstration of changes in FEV_1 between preoperative values and values from postoperative days 1, 2, 3, and 5. FEV_1, forced expiratory volume in 1 second.

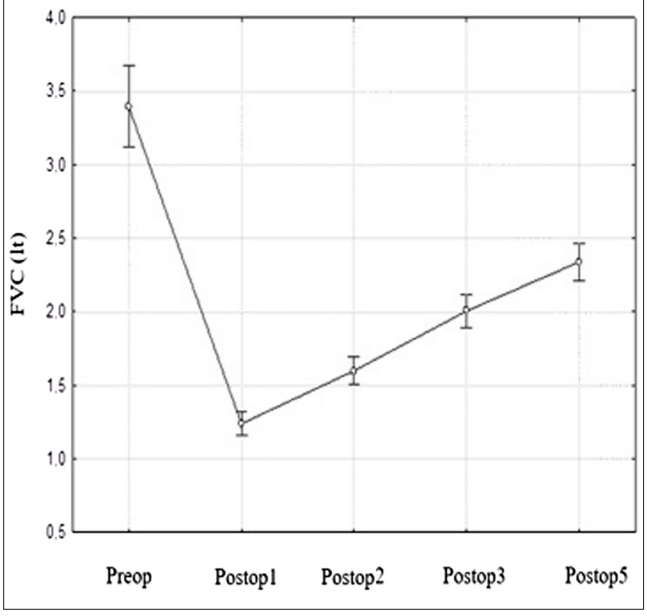


DISCUSSION

The following were observed in the present study: (i) On postoperative day 1, shoulder joint range of motion and respiratory function decreased, while pain intensity increased. (ii) On postoperative days 2-5, the shoulder joint range of motion and respiratory functions increased, while the pain intensity decreased compared to postoperative day 1. (iii) Even on postoperative day 5, none of the parameters reached the preoperative value. (iv) There was a moderate and negative correlation between the intensity of pain and range of motion on postoperative day 1.

Thoracotomy affects shoulder mobility, but muscle-sparing approaches have been reported to facilitate shoulder mobility.[14] Studies have generally focused on differences between shoulder

Figure 6. Demonstration of changes FVC between preoperative values and values from postoperative days 1, 2, 3, and 5. FVC, functional vital capacity.



joint range of motion in various thoracotomy approaches.[13,23] In the study of Öztürk et al.[23] in the comparison of the patients who underwent MST or TSSAM, it was reported that shoulder flexion and abduction were less restricted in those who underwent MST on postoperative days 1, 2, and 7. In the study of Çobanoğlu et al.[13] flexion and abduction angles were quite different than preoperative values on postoperative day 7 in the TSSAM group. In addition, the following results were obtained when thoracotomy types were compared in the same study: It was found that the shoulder abduction angle was similar in the TSSAM and MST group on postoperative day 7, and the abduction angle in both groups was significantly higher compared to SPLT. It was stated that the flexion angle was higher in the MST group compared to TSSAM on postoperative day 7, and was lowest in the SPLT group. In the study of Akçalı et al.[12] flexion and abduction angles approached the preoperative values in postoperative week 2 in patients who underwent MST. Therefore, they stated that the shoulder joint range of motion recovered in 2 weeks in the MST group. In addition, in this study in which thoracotomy types were compared, it was stated that the shoulder joint range of motion was less affected in patients who underwent MST than those who underwent SPLT. Athanassiadi et al[24] examined 2 groups who underwent MST or SPLT and found that flexion and abduction angles could not reach preoperative values in week 1, but returned to preoperative values later in the first month. In our study, it was found that flexion and abduction angles decreased in the early postoperative period. The decrease in joint range of motion may be caused by the incision of an important and large muscle group, such as the latissimus dorsi, even if the serratus anterior muscle is preserved; it may occur due to the tension on the intercostal nerves by spacing the intercostal space by means of retractors used during entry into the thorax; or, it may develop due to rib injuries that may occur at this angle (injury due to compression in the periosteum, or rib cracks/fractures).[12,13] Flexion and abduction angles increased significantly on postoperative days 2, 3, and 5, in this order. Although these results are similar with the ones reported in the literature,[12,13] flexion and abduction angles of the patients showed a faster recovery in a short period of 5 days compared to other studies. This may be due to the effects of developing surgical techniques, correct positioning, and exercises performed for the shoulder joint beginning with postoperative day 1. In cases in whom TSSAM is applied, it is important that the rehabilitation program is applied comprehensively by physiotherapists to increase the range of motion of the upper extremity in the early postoperative period.

Pain after thoracotomy is an important risk factor for morbidity. Therefore, surgeons should be aware of the effects of the techniques they have developed on pain.[25] Çobanoğlu et al[13] reported that pain intensity decreased from an average of 8-1.2 on postoperative day 7 in groups who underwent TSSAM and MST. Akçalı et al[12] reported that the postoperative day 8 pain level was 2.03 in the group who underwent MST. There are studies reporting that TSSAM and MST are less painful than SPLT and that patients who underwent TSSAM and MST have less VAS value and they need lower narcotic analgesics in the early postoperative period.[24,26] In their meta-analysis, Uzzaman et al[27] reported that pain scores were the same in patients who underwent SPLT and MST on postoperative day 1, but they were lower in patients who underwent MST on postoperative day 7 compared to those who underwent SPLT. Similar to the studies in the literature, in this study, it was determined that pain decreased to 2.53, a significant decrease, on postoperative day 5. Excessive pain in the early period may be due to the cut of the skin and pleura and retraction of the costae. The outcome that pain approaches preoperative values in the following days may be related to the fact that the retraction of the costae and the presence of chest drains are less problematic and that epidural analgesia is more effective. Physiotherapy agents can be used in the early postoperative period in order to reduce the intensity of pain in patients who underwent TSSAM.[28]

Thoracotomy causes volume loss due to impaired lung compliance and lung resection. Therefore, postoperative respiratory dysfunction is inevitable. Differences between thoracotomy types may be related to the incision of the latissimus dorsi and serratus anterior muscles, which are weak respiratory muscles. Uzzaman et al[27] stated in their meta-analysis that there may be a significant difference in pulmonary function tests in SPLT and MST groups. However, due to the limited number of studies performed in postoperative week 1, they could not evaluate respiratory functions. Ponn et al[29] stated that MST may result in better long-term pulmonary function, but the differences with other thoracotomy types are small and do not provide a significant clinical difference in the patient. Cobanoglu et al[13] found that pulmonary functions were significantly better in the MST group than in the TSSAM and SPLT groups on postoperative days 3 and 7. Hazelrigg et al[23] found that pulmonary functions reached the preoperative value approximately 1 month later. According to Miyoshi et al.[30] FEV_1 and FVC values approached 60% and 70% of the preoperative values, respectively, at an average of postoperative 9 and 26 days. In our study, it was found that although respiratory function parameters

decreased in the early postoperative period and increased within days, they did not reach the preoperative values. Although these results are compatible with the studies in the literature, the reason for not reaching the preoperative values may be the incision of the latissimus dorsi muscle. In addition, the most important parameter affecting respiratory functions is the surgical procedures that require parenchymal loss (wedge resection, lobectomy, and pneumonectomy) in a significant portion of patients. Due to low respiratory functions in the postoperative period, a pulmonary rehabilitation program should be applied more intensively beginning with the early period.

A correlation was found between pain and range of motion only on postoperative day 1. The lack of correlation between pain and range of motion on other days may be due to the early mobilization and exercises for the shoulder joint routinely given to the patients by the surgeons and accompanying physiotherapists. If there is no relationship between pain and respiratory functions, it may be that the pain improves in a shorter time and the respiratory functions need a longer time to recover.

The first limitation of the study is that it involves a short-term evaluation. Future studies should also consider long-term evaluations. Because in the long-term evaluation, positive results can be seen regarding the postoperative values reaching the preoperative values. Second, there was no control group in the study. Further studies should include a control group. Third, there are variations in surgical procedures performed in patients undergoing thoracotomy.

CONCLUSION

In conclusion, in this study, it was observed that the shoulder joint range of motion and respiratory functions decreased and the intensity of pain increased in the early postoperative period in patients who underwent TSSAM. On postoperative day 1, pain and upper extremity range of motion were found to be negatively correlated. The findings of the study suggested that these parameters should be taken into account in the evaluation of patients who underwent TSSAM and in the physiotherapy methods to be used in the treatment.

Ethics Committee Approval: The study was approved by the Gaziantep University Ethics Committee (Date: October 13, 2014, Decision number: 2014/312).

Informed Consent: Informed consent was obtained from all patients participating in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.K., A.C., S.G., M.Ş.; Design – Y.K., A.C., A.U., S.G., M.Ş.; Supervision – Y.K., A.C., A.U., S.K., S.G., M.Ş.; Resources – Y.K., A.U., S.G., M.Ş.; Materials – Y.K., A.C., S.G., M.Ş.; Data Collection and/or Processing – Y.K., S.G., M.Ş.; Analysis and/or Interpretation – Y.K., A.C., A.U., S.K.; Literature Search – Y.K., A.C., A.U., Writing Manuscript – Y.K., A.C., A.U.; S.K., S.G., M.Ş.; Critical Review – Y.K., S.K., S.G., M.Ş.

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Original Article

Percutaneous Retrieval of Embolized Catheter Fragments from Preterm Newborn to Adult: A Single-Center Experience for 10 Years

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ABSTRACT

Objective: Umbilical vein catheterization and central venous catheters are frequently used. We aimed to present our experiences in 15 of 16 patients with embolized catheters who were successfully retrieved by percutaneous intervention.

Methods: During 10 years, 16 patients with embolized umbilical vein catheters, port catheters, central venous catheters, catheter fragments, and guide wires were examined. Demographic characteristics of the patients, catheter indications, embolized catheter types, localizations and lengths of a catheter, durations of flora, entry points during retrieval of embolized catheters, snare's features used, grasping location of the embolized catheter, and additional procedures were examined retrospectively.

Results: Of the 16 patients, 7 were girls; their ages were between 11 days and 39 years; 14 of the patients were children. Their weights were between 1.3 kg and 65 kg. The umbilical vein catheter in 5 patients, the port catheter in 7 patients, a double-lumen central venous catheter in 1 patient, the distal part of the fragmented sheath in 1 patient, and the guide wire in 2 patients were embolized.

Conclusions: The procedures of umbilical vein catheterization, peripheral central vein catheterization, and port catheterization are safe in experienced hands. Rarely, those catheters may break and embolize. As soon as it is diagnosed, embolized catheters should be removed to prevent complications. Since the retrieval of embolized catheters by percutaneous transcatheter route is safe and successful, it should be used as the first choice.

Keywords: Children, embolized catheter, transcatheter removal, umbilical vein catheterization

INTRODUCTION

Umbilical vein catheterization (UVC) is a frequently used procedure especially in low birth weight premature infants in neonatal intensive care units.[1,2] Central venous catheters (CVC) are frequently used in parenteral nutrition and for monitoring hemodynamic parameters. Those procedures have also complications as in other interventional procedures; the main ones are infection, embolization of catheter, and arrhythmias.[1-3]

It has been reported that its incidence is between 0.2% and 4.2% in a systematic review and it may occur even years after implantation.[3] Since the number of cases in children is low, the incidence is not known. Retrieving these embolized catheters is very important because embolized fragments can lead to serious consequences such as arrhythmias, myocardial damage, thrombosis, infection, perforation, and death.[1-3] In this article, we aimed to present our experiences in 16 patients with cardiac or noncardiac foreign body embolization.

METHODS

Sixteen patients who were attempted to percutaneously retrieve embolized umbilical vein catheters, port catheters (PCs), CVC, catheter fragments, and guide wires between 2011 and 2021 in the Clinic of Pediatric Cardiology were included in the study. Ethics committee approval was received for this study from the ethics committee of Gaziantep University (Date: June 30, 2021, Decision number: 2021/170). Five patients, who have prematurity, had embolized UVC. Seven of them, who are oncologic patients, had embolized PC. A peripheral central catheter was inserted into the patient due to a wide burn on the body and the need for long-term intensive care treatment. One of the patients had a central venous catheter inserted due to gunshot wounds and the need for a long-term intensive care period. The latter 2 patients had embolized guide wire. One of the patients had an embolized double-lumen central catheter used for dialysis due to chronic renal failure. The balloon was applied to the last patient due to valvular and infundibular stenosis, and the distal part of the sheath was embolized while the sheath was removed.

The removal of the embolized catheters was performed under sedation. Depending on the position of the embolized part and the patient's condition, the femoral vein, subclavian vein, umbilical vein, and hepatic vein were used for intervention.

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While embolized catheters were retrieving, 10 mm Amplatz Goose Neck snare type (Plymouth, MN, USA) in 3 patients, Dotter intravascular retrieval basket catheter (Cook, Bloomington, USA) in 1 patient, and multisnare triple-loop snare in other patients (Merit Medical, South Jordan, USA) were used. Modified loop snare techniques were performed for embolized catheters that could not be retrieved by the standard method.

RESULTS

Of the 16 patients, 7 were girls, their ages were between 11 days and 39 years. Fourteen of the patients were children. Their weights were between 1.3 kg and 65 kg. Demographic characteristics of the patients, catheter indications, embolized catheter types, localizations and lengths of a catheter, durations of flora, entry points during retrieval of embolized catheters, snare's features used, grasping location of the embolized catheter, and additional procedures are shown in Table 1. Embolized catheters were successfully retrieved in 15 of 16 patients, no complications were seen.

Umbilical Vein Catheter Embolizations

The embolized umbilical vein catheter was seen in 5 of our patients. There were no clinical findings, the diagnosis was made by chest radiography, and confirmed by echocardiography (ECHO). The embolization sites were vena cava inferior (VCI) in 2 patients, extending from the right atrium to the pulmonary vein through the patent foramen ovale in 1 patient, and extending from the ductus venosus to the VCI in another patient.

The left subclavian vein was used while the embolized catheter was retrieved from the right atrium-pulmonary vein. In order to retrieve the embolized catheter with a snare, the cut pigtail and floppy wire were taken a tour around the catheter. The distal end of the wire was caught and pulled with another catheter (Figure 1). The distal part of the umbilical catheter extended from the umbilical vein- the ductus venosus to the VCI in another patient. The VCI was entered from the femoral vein with a 4F catheter. In addition, the umbilical vein was entered with an umbilical catheter, the embolized catheter was pushed from the rear and advanced to the VCI and right atrium-vena cava superior (RA-VCS). Later, an embolized catheter was caught in VCS and retrieved with a snare from the femoral vein (Figure 2). The embolized catheter was in the VCI in 1 of the patient and caused adhesion there. First, a piece of the catheter was removed with a snare. Then, the snare was advanced again and the other piece was removed. After the injection of the contrast substance, it has been observed that there is a narrowing in the VCI. The balloon was applied to the VCI and it has been seen that the lumen is opened in control injection (Figure 3). Embolized umbilical catheters were successfully retrieved in 4 of our patients, and, the embolized umbilical catheter could not be removed in 1 patient because it was stuck horizontally in the ductus venosus. Entry locations during the retrieval of embolized catheters, from which end they were caught, catheter lengths, and processing times are shown in Table 1. There were no complications during and after the procedure.

Main Points

- Umbilical vein catheterization and central venous catheters are frequently used procedures especially in parenteral nutrition and for monitoring hemodynamic parameters.
- Those procedures have also complications as in other interventional procedures; the main ones are infection, embolization of catheter, arrhythmias, myocardial damage, thrombosis perforation, and death.
- According to our knowledge, it is the largest series in the literature. Percutaneous retrieval of intravascular foreign bodies is a reliable, successful, effective, and first-choice method in experienced hands, and should be considered as the first step of treatment due to the high success rate and minimal risk of the procedure.

Port Catheter Embolizations

Port catheter embolization was seen in 7 patients. Embolization localizations are shown in Table 1. The PC was disconnected in 6 patients from the connection sites. While the PC was surgically removed, the distal part of 1 cm PC was embolized in one of our patients. The embolized PC extended from the right pulmonary artery branch to the left upper lobe of the lung in 1 patient, and the left end was immobile. When the tip of the catheter could not be caught with the multipurpose and pigtail catheter, the mobile end of the embolized catheter in the right pulmonary artery was caught with the vertebral catheter and removed (Figure 4). The duration of embolization was different in each patient. The embolized PC was noticed 1.5 years after embolization in one of our patients (it is not known when it was inserted, it was also noticed in the chest radiography 1.5 years ago). In 2 of our patients, it was embolized 1.5 months after the insertion of the PC. It was embolized in other 2 of our patients 10 months after the insertion of PC. In the last patient, while the PC was surgically removed, its 1 cm distal part was ruptured and embolized. Embolized catheters were confirmed by chest radiographs and ECHO. Entry locations during the retrieval of embolized catheters, from which end they were caught, catheter lengths, and processing times are shown in Table 1.

Non-catheter Guide Wire and Similar Foreign Body Embolizations

A peripheral central catheter was inserted into one of our patients due to a wide burn on the body and the need for long-term intensive care treatment, and she had dyspnea and orthopnea for 10 days. A long guide wire was seen in the iliac vein extending to the superior vena cava in the chest radiography. All peripheral venous interventional sites (left femoral vein, left and right subclavian vein, left and right internal jugular vein) were thrombosed, and collateral circulation was developed. Therefore, an embolized long guide wire was retrieved with transhepatic intervention and a vascular plaque was placed in the liver parenchyma (Figure 5).[4]

Another patient, who has embolized the long guide wire, was followed up in the intensive care unit due to trauma.

In another patient, a double-lumen catheter was inserted due to chronic renal failure for hemodialysis, and then it was embolized.

Table 1. Demographic Characteristics of Patients and Percutaneous Retrieval Techniques

Case	Age	Sex	Weight	Diagnosis	Embolization Catheter	Localization of Embolization	Catheter Length	Entry Point	Sheath	Snare Type	Grabbing Site	Duration of Flora (minutes)
1	0.56 m/o	M	1.3 kg	PM +İCH	UVC	RA-PFO-PV	10 cm	SV	5F	Multisnare	Distal end	3.2
2	0.63 m/o	F	1.5 kg	PM	UVC	DV-KC-VCI	7 cm	FV/UV	4F	Multisnare	Distal end	3.6
3	2.2 m/o	M	2.5 kg	PM +NEC	UVC	VCI	3.1 cm	FV	4F	Multisnare	Proximal end	5.1
4	0.36 m/o	F	1.9 kg	PM	UVC	VCI	13 cm	FV	4F	Multisnare	Distal end	2.4
5	0.33 m/o	F	2.2 kg	PM +ESCOBAR	UVC	L-DV	8 cm	FV	4F		Unsuccessful	
6	6 y/o	M	30 kg	ALL	PC	RA-RV	10 cm	FV	5F	Gooseneck	Proximal end	4.8
7	4.5 m/o	M	5.5 kg	ALL	PC	RA	8 cm	FV	6F	Gooseneck	Distal end	1.7
8	39 y/o	M	65 kg	ALL	PC	RV-RPA	28 cm	FV	7F	Multisnare	Proximal end	2
9	5.5 y/o	M	16 kg	ALL	PC	RPA-LPA	9.5 cm	FV	6F	Multisnare	Distal end	2.1
10	11.7 y/o	F	40 kg	ALL	PC	VCS	1.5 cm	FV	5F	Gooseneck	Proximal end	1.9
11	10.6 y/o	M	35 kg	ALL	PC	HV-RV	15 cm	FV	6F	Multisnare	Proximal end	0.7
12	18 y/o	F	50 kg	SARCOMA	PC	VCS-RV	6 cm	FV	7F	Multisnare	Distal end	4.1
13	13 y/o	F	43 kg	BURNED	GW	VCI-RA-RV-VCS	50 cm	HV	5F	Multisnare	Distal end	4.7
14	39 y/o	M	65 kg	TRAUMA	GW	FV	50 cm	FV	7F	Multisnare	Proximal end	3
15	16 y/o	M	45 kg	CKD	DLC	VCI-RA	15 m	FV	14F	Multisnare	Proximal end	5.9
16	7 y/o	F	24 kg	PS	Fragmented sheath FV	FV	8.5 cm	FV	5F	Multisnare	Distal end	3.1

m/o, months old; y/o, years old; PM, prematurity; İCH, intracranial hemorrhage; NEC, necrotizing enterocolitis; ALL, acute lymphoblastic leukemia; CKD, chronic kidney disease; PS, pulmonary stenosis; UVC, umbilical vein catheter; PC, port catheter; GW, guide wire; RA, right atrium; PFO, patent foramen ovale; PV, pulmonary vein; DV, ductus venosus; VCI, vena cava inferior; L, liver; RV, right ventricle; RPA, right pulmonary artery; LPA, left pulmonary artery; VCS, vena cava superior; FV, femoral vein; HV, hepatic vein; SV, subclavian vein; UV, umbilical vein.

Figure 1. (A) Angiographic images of an embolized catheter in RA-PFO-PV. (B) In order to retrieve the embolized catheter by a snare, the cut pigtail and floppy wire were taken a tour around the catheter. (C) The embolized catheter is snared. (D) Pulling back of the catheter. RA, right atrium; PFO, patent foramen ovale; PV, pulmonary vein.

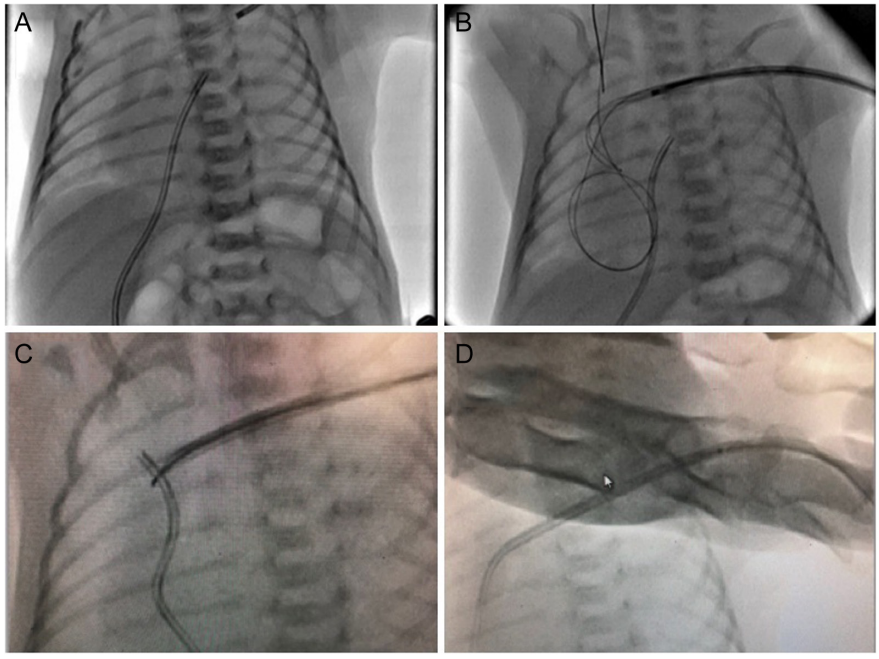


Figure 2. (A) Angiographic images of the distal part of the umbilical catheter extending from the umbilical vein- the ductus venosus to the VCI, embolized catheter advanced from the rear by an umbilical catheter inserted into the umbilical vein. (B) Embolized catheter is grabbed in VCS. (C) Pulling back of the embolized catheter. (D) Withdrawal from the femoral vein into the sheath. VCI, vena cava inferior; VCS, vena cava superior.

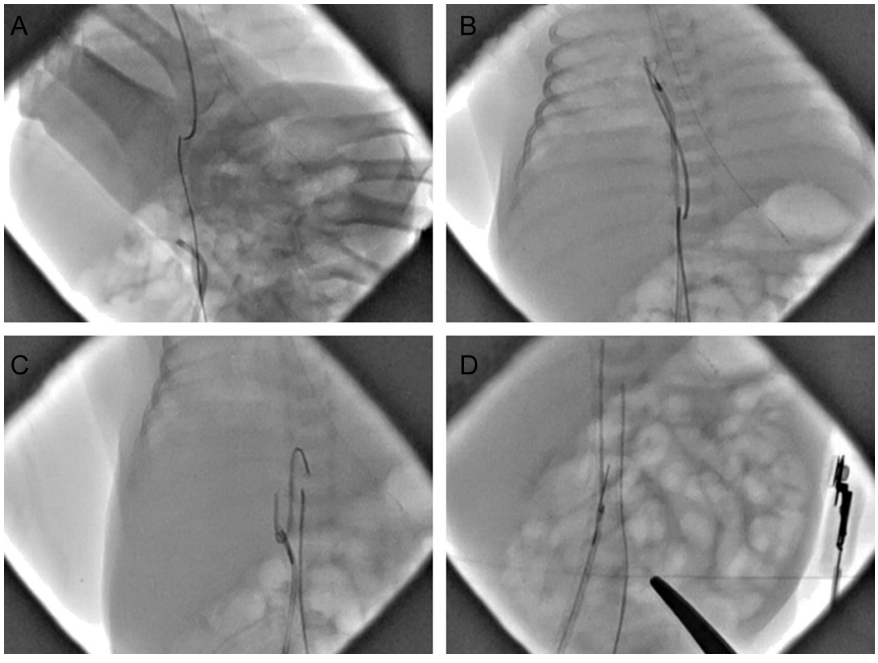
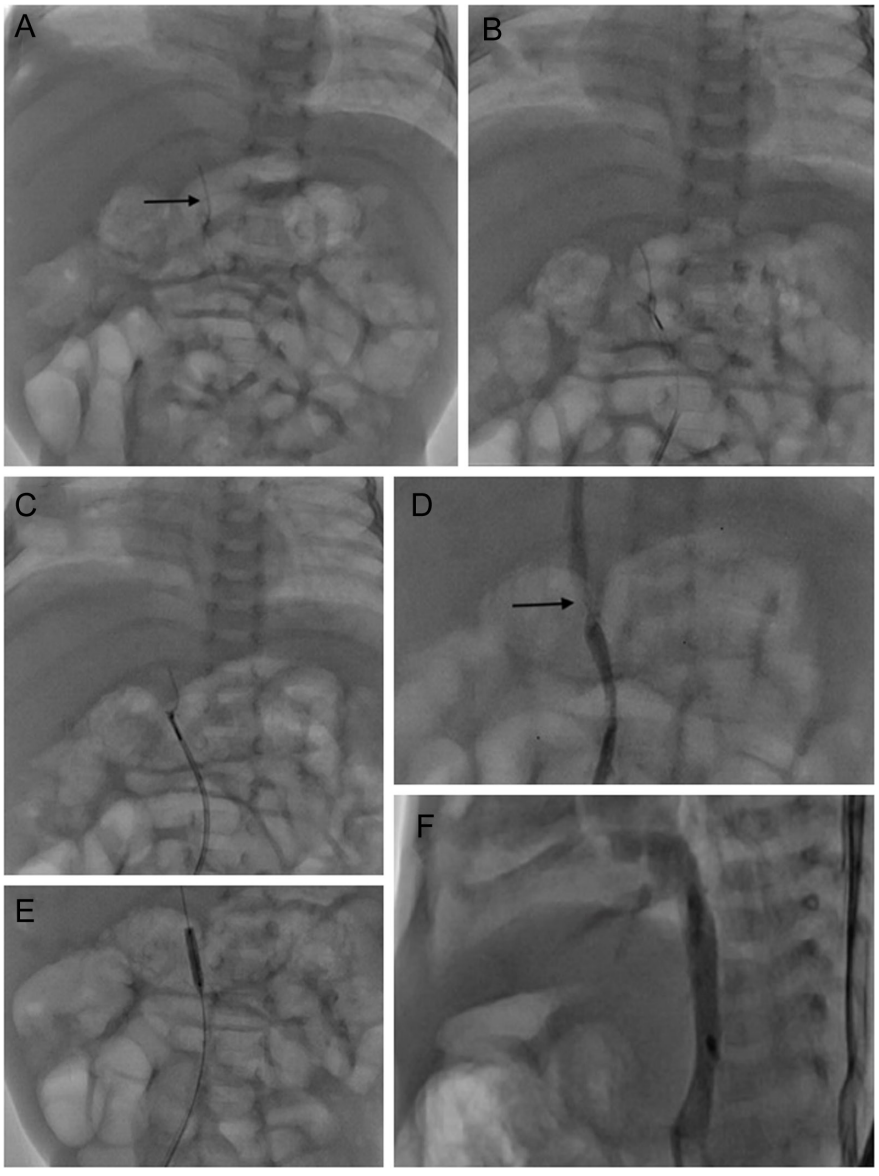


Figure 3. (A) Image shows an embolized catheter in the VCI. (B) Grabbing the proximal end of the embolized catheter by a snare. (C) Withdrawal into the catheter. (D) Narrowing in VCI. (E) Balloon application to VCI. (F) The image shows the opening of the lumen in the control injection. VCI, vena cava inferior.



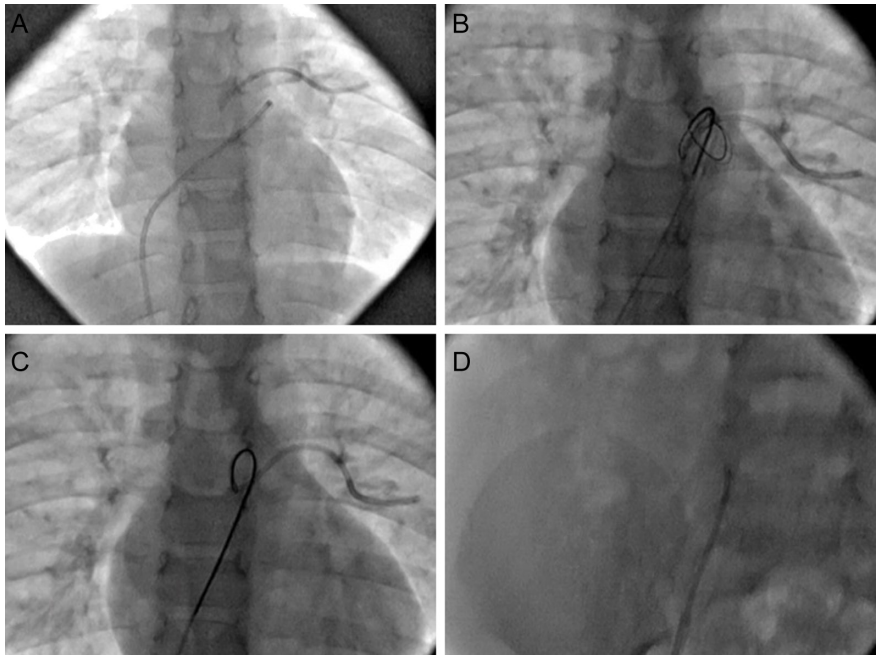
Pulmonary balloon valvuloplasty was applied to another patient due to valvular and infundibular stenosis. At the end of the procedure, while the 8F sheath was withdrawn, the 8.5 cm intravascular part of the sheath remained in the femoral vein. Then, the puncture needle was inserted into the sheath and the hydrophilic guide was advanced, 5F sheath was placed, 13F Mullins long sheath was placed in the left femoral vein, then the sheath was caught in the right atrium with a basket catheter and retrieved (Figure 6).[5] All procedures were successful and no complications were observed during follow-up.

DISCUSSION

Umbilical vein catheterization is a frequently used procedure, especially in low birth weight premature infants for parenteral nutrition, drug administration, blood sampling. It is usually simple and safe, but may rarely cause complications such as infection, thrombosis, arrhythmias, embolizations, and catheter fractures. Serious clinical findings ranging from arrhythmia to sudden death may occur in patients with catheter fractures and embolization.[1,2] Placement, care, and retrieval of UVC should be done by experts. The most common causes of embolization are the movement of the patient, inappropriate use of scissors or scalpels during these procedures. Embolization of UVC is rare in the literature, there are limited case reports.[1,2,6-8]

The number of cases is quite high in our study. According to our knowledge, it is the largest series in the literature. We think that the reason for the high incidence of UVC embolizations in our

Figure 4. (A) The embolized port catheter extending from the right pulmonary artery branch to the left upper lobe of the lung. (B) The mobile end of the embolized catheter in the right pulmonary artery is grabbed by a snare. (C) Pulling back into the catheter. (D) Withdrawing from the femoral vein into the sheath.



series is the high number of referrals from other hospitals as our center is a reference center.

The presence of foreign bodies in premature babies predisposes a risk for thrombus formation. Therefore, it is recommended to retrieve embolized catheters immediately at the time of diagnosis, rather than starting heparin due to the risk of intracranial bleeding.[7]

The embolization time of the patients except one was uncertain and there were no symptoms. Embolization occurred 4 days ago in 1 patient and there were no symptoms. It has been reported that the patient is asymptomatic although a period of 2 months passed after embolization.[8]

It has been reported that 1 premature patient deteriorates 11 days after being extubated and then re-intubation is performed.[6] After detailed re-evaluation of the patient, it has been determined that the umbilical vein catheter is embolized from the hepatic vein into the right upper pulmonary vein on chest radiography. The embolized catheter was removed successfully percutaneously. Then, the general condition of the patient improved and he was discharged 5 days later.

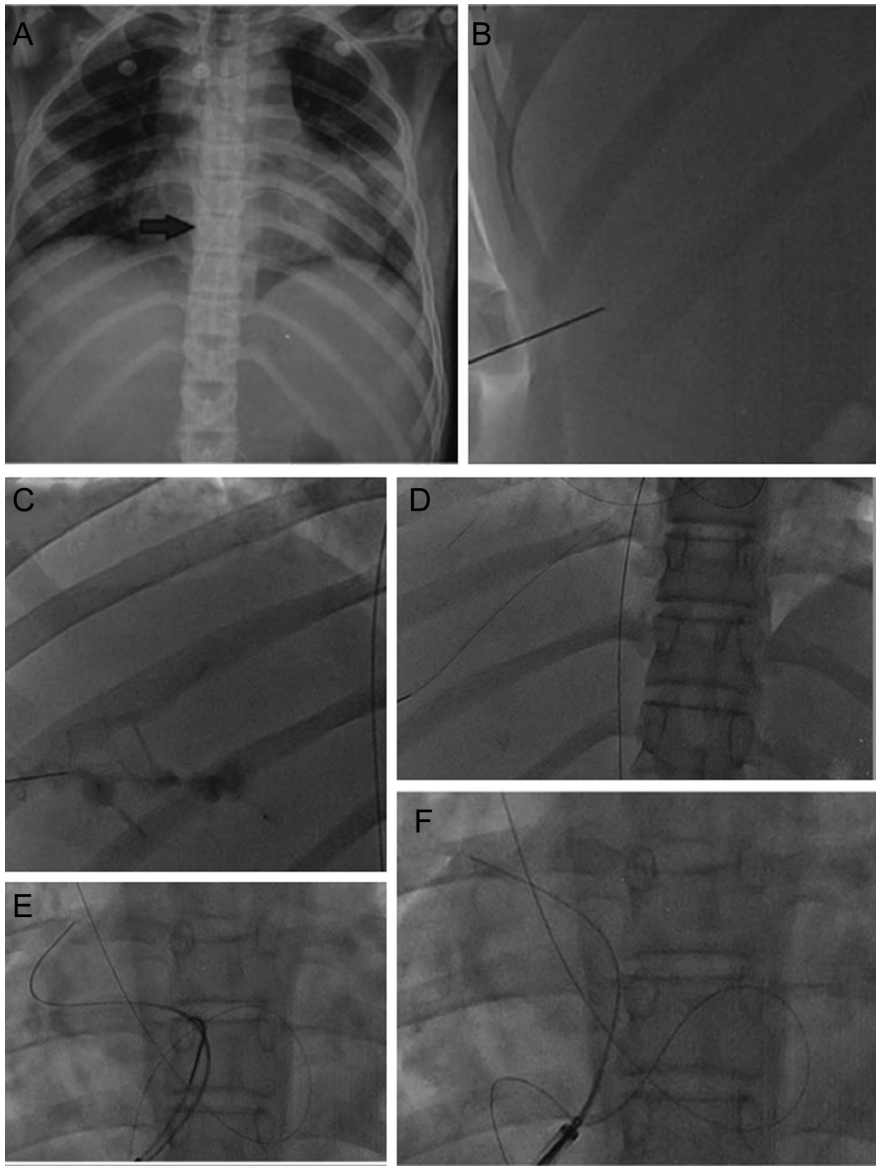
Central venous catheters are frequently used in parenteral nutrition and for monitoring hemodynamic parameters. These procedures have also complications as in other interventional procedures. The main complications are infection, catheter embolization, and arrhythmias. Embolization mechanisms of PC differ. In the study of Surov et al.[3] embolizations were mostly noticed due to pinch-off syndrome. The other reasons they detected were catheter damage, distal and proximal catheter fractures, disconnection of the port reservoir from the port cannula during catheter replacement or removal, respectively. The reasons for the embolizations of PC are incorrect locking of the PC connection, strong injection, pulling of the extravascular part of the catheter by the chest wall soft tissues.

Disconnection of the port reservoir and cannula part and embolization was observed in 6 of our patients (Figure 4). While the reservoir of the PC was surgically removed, the distal part of 1 cm PC was ruptured and embolized in one of our patients. Another cause of catheter fracture is catheter fatigue. Catheters that remain in the body for a long time become fragile and fractures are more commonly seen. Embolization was not noticed in one of the patients, because the PC was not used for a long time, and the patient was also asymptomatic. The cause of embolization in this patient may be increased fragility due to catheter fatigue. Therefore, unused PCs should be removed as soon as possible to prevent complications.

Duration of embolization is very different for each patient. After implantation, the duration of catheter embolization is difficult to determine. An embolized catheter is often noticed in the chest radiography taken incidentally. Therefore, sometimes it may remain unnoticed for a long time. To evaluate the risk of embolization, the PC should be examined regularly by the chest x-ray after insertion.[9,10]

In our study, the most common embolization site is the right ventricle, mostly extending from the right atrium to the pulmonary

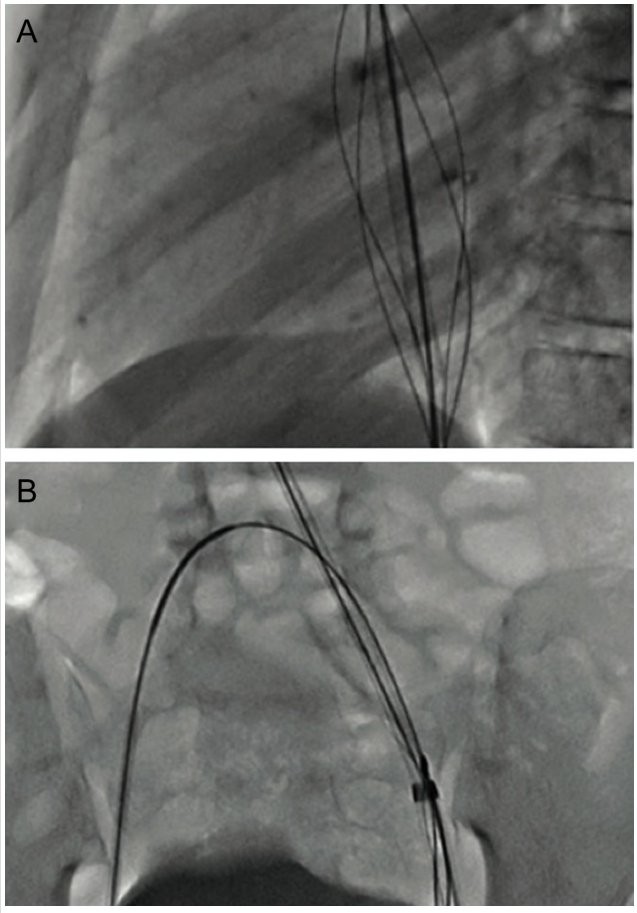
Figure 5. (A) The embolized guide wire is observed in this chest x-ray from the midpoint of the superior caval vein to the iliac vein, with a loop in the heart. (B) Entrance to the liver with the Chiba needle. (C) The manual contrast medium injection through the Chiba needle reveals the blood flow to the heart with the hepatic vein and the portal venous flow spread in the portal area. (D) The coronary guide wire inside the Chiba needle is advanced from the hepatic vein to the inferior caval vein and right atrium. (E and F) The embolized guide wire (arrow), which is trapped between the 2 right Judkins catheters and the hydrophilic guide wire, is advanced inside the long sheath.



artery. In the study conducted by Surov et al.[3] catheter embolizations were mostly in the pulmonary artery. The most common association was the right ventricle and the pulmonary artery, and catheter parts were long enough to cover several regions. It has been reported that localizations of PC embolization are in different parts of the cardiovascular system such as the superior vena cava, right atrium, right ventricle, or pulmonary artery in 7 patients.[9] In the study conducted by Pazinato et al.[11] the embolization site was pulmonary artery in 44.1% of the patients. The localization of the embolized catheter fragments in the cardiovascular system varies depending on the length and stiffness of the embolized substance, and the flow pattern of the reservoir or vessel. Most fragments embolize to the superior vena cava, right ventricle, and/or pulmonary artery. Flexible long catheter fragments inserted into the subclavian vein usually go to the right ventricular trabeculum and settle there. Shorter pieces of catheter go into the smaller branches of the pulmonary artery and are more difficult to retrieve.[9]

After venous catheter embolization, mortality and morbidity depend on the localization of the embolized parts. The highest

Figure 6. (A) Opened basket catheter attempt to snare the sheath with a coaxial position in the right atrium. (B) The whole system was slowly withdrawn from the opposite femoral vein.



mortality is seen when catheter fragments are compressed in the right ventricle. Less mortality in the pulmonary artery and the least mortality are seen in the vena cava or peripheral veins.[3] There was no mortality in our study. We were successful in 15 of 16 patients. Our success rate was 93.7%. The reason for the failure in 1 patient was that the umbilical catheter is stuck horizontally in the ductus venosus. Despite repeated attempts through the venous system, the embolized catheter could not be removed because there was no free tip.

The major limitation of our study was that it is retrospective. In contrast to that, the number of our patients was high since our hospital is the reference center of the region. We think that this will increase the impact of the study.

CONCLUSION

As a result, embolized catheters are difficult to diagnose and may remain unnoticed for a long time. It should be removed at the time of diagnosis, and embolized catheters can be retrieved with loop snare and its modification in most cases. Percutaneous retrieval of intravascular foreign bodies is a reliable, successful, effective, and first-choice method in experienced hands, and should be considered as the first step of treatment due to the high success rate and minimal risk of the procedure. Percutaneous intervention should be tried even in delayed cases before referring it to surgery.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (Date: June 30, 2021, Decision number: no: 2021/170).

Informed Consent: N/A.

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Original Article

Hashimoto's Thyroiditis is Not a Negative Contributor to Papillary Thyroid Cancer

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ABSTRACT

Objective: Hashimoto's thyroiditis is the most common autoimmune thyroid disease. Papillary thyroid cancer is the most common thyroid cancer. Whether Hashimoto's thyroiditis is a predisposing factor for papillary thyroid cancer remains unclear. In this study, the frequency of papillary thyroid cancer was investigated in patients with Hashimoto's thyroiditis who underwent total thyroidectomy.

Methods: In this study, 534 patients were screened retrospectively. Preoperative thyroid function tests, anti-thyroid antibodies, ultrasonography findings, fine-needle aspiration biopsies, and thyroidectomy pathology results were examined. According to the pathology, 139 patients had Hashimoto's thyroiditis. Patients with Hashimoto's thyroiditis (group 1) and non-Hashimoto's thyroiditis (group 2) were compared.

Results: Papillary thyroid cancer was found in 70 patients (50.4%) in group 1 and 156 patients (39.5%) in group 2 ($P = .026$). The odds ratio was 1.59 (95% CI; 1.07-2.34). There was no difference for sex, age, tumor size, microcarcinoma–macrocarcinoma distribution, subtype, number of tumor focus, lymph node metastasis, vessel invasion, and extrathyroidal spread.

Conclusion: We found more papillary thyroid cancer and less capsular invasion in patients with Hashimoto's thyroiditis, but we did not find any differences between groups in terms of age, gender, tumor size, vascular invasion, and metastasis. According to these findings, Hashimoto's thyroiditis may be a risk factor for papillary thyroid cancer.

Keywords: Hashimoto's thyroiditis, risk, papillary

INTRODUCTION

Hashimoto's thyroiditis (HT) is the most common autoimmune thyroid disease. Therefore, it is the most prevalent cause of hypothyroidism.[1] Hashimoto's thyroiditis leads to diffuse lymphocytic infiltration, fibrosis, and parenchymal atrophy in the thyroid gland.[2] Hashimoto's thyroiditis is a histopathologic diagnosis, but it can also be diagnosed by physical examination and laboratory tests. It affects 0.3-1.5 people per 1000 people and women constitute the majority of them.[3]

According to the Surveillance, Epidemiology, and End Results database, the prevalence of thyroid cancer in 2002 was 8.7/100 000 people. Papillary thyroid cancer (PTC) is the most common subtype and its prevalence is 7.7/100 000 according to the same database.[3] High-resolution thyroid ultrasonography (USG) and ultrasound-guided fine-needle aspiration biopsy are becoming more common and histopathological evaluation can be performed in more detail. These may be the underlying causes of PTC increase.[4]

The relationship between HT and PTC was first discussed in 1955. Since then, a number of studies have been conducted trying to show the relationship between these 2 diseases, but this is still not clear. Studies have shown that the co-occurrence of HT to PTC is associated with a better prognosis. These studies have given importance to the subject.[5] The aim of this study was to determine whether HT is a predisposing factor for PTC.

METHODS

Exclusion criteria: Patients younger than 18 years old who underwent subtotal thyroidectomy or lobectomy were not included. Patients whose histopathology revealed a malignancy other than well-differentiated thyroid cancer, who had preoperative hyperthyroidism, and/or who had no preoperative thyroid USG were excluded. Between July 2014 and July 2016, 850 patients who underwent bilateral total thyroidectomy by the general surgery department were retrospectively analyzed.

The patients were divided into 2 groups as with and without HT according to histopathology. Demographic characteristics, preoperative thyroid function tests, anti-thyroid antibodies, thyroid USG findings, thyroid fine-needle aspiration biopsies, and bilateral total thyroidectomy pathology results were evaluated.

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We could not detect all patients' anti-thyroid antibodies, so we did not evaluate them. In addition, tumor size, tumor number, the presence of invasion, extrathyroidal spread, and lymphatic metastasis were obtained from the hospital database.

Laboratory

Serum thyroid-stimulating hormone (TSH) (normal range: 0.34-5.6 mIU/mL), free thyroxine (normal range: 0.61-1.2 ng/dL) and free triiodothyronine (normal range: 2.5-4.2 pg/mL), anti-thyroid peroxidase antibody (normal range: 0-9 IU/mL), and anti-thyroglobulin antibody (normal range: 0-4 IU/mL) levels were determined. Not all autoantibodies were available in all patients so they were not studied. It was found that these measurements were studied with the chemiluminescence immunoassay method in UniCel DxI 800 (Beckmann-Coulter, Brea, Calif, USA) device.

Thyroid Fine-Needle Aspiration Biopsy

Thyroid fine-needle aspiration biopsies (TFNAB) were examined. The results had been reported according to Bethesda system[6] as non-diagnostic (I), benign (II), unspecified atypia (AUS)/indeterminate follicular lesion (AUFL) (III), follicular neoplasm (FN) or suspicious for FN (IV), suspicious for malignancy (V), and malignant (VI).

Ethics Committee Approval: The local ethics committee approved this study from Ankara Education and Research Hospital. (Date: October 27, 2015, Decision number: 617/2015) All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki eclaration and its later amendments or comparable ethical standards.

Indications for Thyroidectomy

Thyroid fine-needle aspiration biopsies with AUS/AUFL, FN/suspicious for FN, suspicious for malignancy, malignant, and TFNABs with 2 times non-diagnosed were the causes for total thyroidectomy. Also, patients with multinodular goiter, giant thyroid nodule (>4 cm), and primary hyperparathyroidism + multinodular goiter underwent total thyroidectomy.

Histopathological Diagnosis

Histopathological diagnosis of HT was based on total thyroidectomy material. For the diagnosis of HT, infiltration of the gland by lymphocytes and plasma cells and destruction of follicles due to this infiltration are required. Some epithelial cells become eosinophilic granular cytoplasm and are called askanazy or hurtle cells. The diagnosis of HT was confirmed by examining the thyroid parenchyma according to the above criteria, independent of tumor tissue.[7]

Main Points

- According to our study, Hashimoto's thyroiditis is a predisposing factor for papillary thyroid cancer.
- Capsule invasion and vascular invasion are less likely in papillary thyroid cancer in people with Hashimoto's thyroiditis.
- Hashimoto's thyroiditis does not lead to more severe papillary thyroid cancer.

Statistical Analysis

Statistical analyses were made using Statistical Package for the Social Sciences version 22.0. (IBM SPSS Corp.; Armonk, NY, USA). Numerical variables were summarized with mean ± standard deviation or median (min-max). Categorical variables were indicated by number and percentage. The chi-square test or Fisher's exact test was used to determine whether there was any difference between the groups in terms of categorical variables. The Kolmogorov–Smirnov test was used to determine whether the normal variables showed normal distribution, and the homogeneity of the variance was examined by Levene's test. Differences between 2 independent groups in terms of numerical variables, the differences between the 2 independent groups were approached as follows: When the parametric test assumptions are met, the t-test used for independent groups. In the absence of parametric test assumptions, Mann–Whitney U test was used. Post hoc analysis was used to compare more than 2 groups. The significance level was taken as $P < .05$.

RESULTS

This study included 534 patients who met the study criteria. The group of patients with HT (group 1) consisted of 139, and the group without HT (group 2) consisted of 395 patients (Table 1). Hashimoto's thyroiditis was diagnosed according to thyroidectomy results.

Around 94.2% (131) of the group 1 were female, while 82.8% (327) of the group 2 were female ($P = .001$). There was no difference in the mean age between the 2 groups ($P = .574$) (Table 1).

Thyroid-stimulating hormone level, thyroid status, thyroid volume, thyroid parenchymal echogenicity, the number of nodules, and suspicious nodules were significantly different between the groups ($P < .001$).

The sonographically suspicious nodules were 201 in group 1 and 750 in group 2. Their location, echogenicity, the presence of microcalcification and macrocalcification, marginal arrangement, and the ratio of anterior–posterior diameter to transverse diameter of the nodule were not different between the groups. But there was a significant difference in terms of nodule volume and the presence of cystic content ($P = .03$) and nodule biopsy results ($P < .001$) (Table 2).

There was a difference between the groups in terms of total thyroidectomy indications ($P < .001$). Post hoc analysis showed that the difference was caused by the indication groups of multinodular goiter (MNG), TFNAB:AUS/AUFL, and suspicious for malignancy (Table 3).

Papillary thyroid cancer was detected in 70 patients of group 1 and 156 patients of group 2 ($P = .026$). The odds ratio was 1.59 (95% CI; 1.07-2.34). There were no differences between the groups in terms of age and gender distributions, tumor diameter, tumor subtype, number of focus, microcarcinoma–macrocarcinoma

Table 1. Demographic, Biochemical, and Ultrasonographic Features of Patients

		HT Group (Group 1) (n=139)	Non-HT Group (Group 2) (n=395)	*P*
Gender	Female, n (%)	131 (94.2)	327 (82.8)	.001*
	Male, n (%)	8 (5.8)	68 (17.2)	
Age		48.0 ± 12.5	47.3 ± 12.3	.574
TSH		1.84 (0.35-17.9)	1.12 (0.13-14.2)	<.001*
Thyroid status	Euthyroid, n (%)	129 (92.8)	393 (99.5)	<.001*
	Hypothyroid, n (%)	10 (7.2)	2 (0.5)	
Presence of MNG, n (%)		103 (74.1)	339 (85.8)	.002*
TSH	Normal, n (%)	129 (92.8)	393 (99.5)	<.001*
	High, n (%)	10 (7.2)	2 (0.5)	
Number of patients with autoantibodies viewed, n (%)		64 (46)	176 (44.5)	.68
Thyroid right lobe volume, mL		9609.6 (594.9-60 889.9)	11 771 (611.5-312 000)	.018*
Thyroid left lobe volume, mL		8255.5 (0-172 380)	10 978.2 (2051.1-182 520)	<.001*
Parenchymal echogenicity	Homogeneous, n (%)	3 (2.2)	92 (23.3)	<.001*
	Mild heterogeneous, n (%)	13 (9.4)	127 (32.2)	
	Mild to moderate heterogeneous, n (%)	15 (10.8)	16 (4.1)	
	Moderate heterogeneous, n (%)	64 (46)	147 (%)	
	Moderate to advanced heterogeneous, n (%)	16 (11.5)	2 (0.5)	
	Advanced heterogeneous, n (%)	28 (20.1)	11 (2.8)	
Number of nodules per patient, n		2 (0-14)	3 (1-16)	<.001*
Pathological-looking number of nodules per patient, n		1 (0-5)	2 (0-5)	<.001*

HT, Hashimoto's thyroiditis; TSH, thyroid-stimulating hormone; MNG, multinodular goiter.

distribution, lymphatic metastasis, and extrathyroidal spread. Vascular invasion of the tumor was not detected in the HT group. The mean preoperative TSH level and hypothyroidism tendency were more pronounced in group 1 as expected. There were significant differences between the groups in terms of thyroid volume, MNG presence, parenchymal echogenicity, the number of nodules, and the number of suspicious nodules. As a result, while PTCs were more in group 1, the capsular invasion of the tumor was more prominent in group 2, no difference was observed in other features (Table 4).

DISCUSSION

Since the contribution of chronic inflammation to tumor development it has been shown in many tissues that chronic inflammation leads to tumor development. From this point of view, it has been investigated in many studies whether autoimmune thyroiditis also causes thyroid cancer. In some studies, HT has been shown to be a risk factor for PTC, while others have not shown it to be a risk factor. These studies are retrospective or prospective studies based on total thyroidectomy or TFNAB results.[1] Since HT was diagnosed primarily with histopathology, in our study, patients with and without HT were retrospectively evaluated based on bilateral total thyroidectomy.

In our study, patients who underwent bilateral total thyroidectomy were divided into 2 groups based on histopathology as those with and without HT. Similar to the literature, prominent female gender dominance, hypothyroidism tendency, low volume of the thyroid gland, and low parenchymal echogenicity in patients with HT were detected.[8-10] Pathologic ultrasonographic findings such as hypoechogenicity, solid structure, and the presence of microcalcifications in malignant nodules developing on the basis of nodular HT are similar to the general population.[11,12] In our study, although the number of sonographically suspicious nodules was higher in the non-HT group (Table 1), in the detailed analysis of these nodules, no significant difference was found between the 2 groups except that the cystic content and nodule volume were lower in the HT group (Table 2).

Table 2. Characteristics of Sonographically Suspicious Nodules

Nodule		HT Group (Group 1) (n=201)	Non-HT Group (Group 2) (n=750)	*P*
Volume, mL		1091.7 (12-35 490)	1582.6 (22.1-114 400)	.030*
Location, n (%)	Right	108 (53.7)	373 (49.7)	
	Left	82 (40.8)	329 (43.9)	.587
	Isthmus	11 (5.5)	48 (6.4)	
Echogenicity, n (%)	Isoechoic	38 (18.9)	129 (17.2)	
	Hypoechoic	51 (25.4)	149 (19.9)	
	Iso-hypoechoic	21 (10.4)	79 (10.5)	.102
	Mixed	83 (41.3)	380 (50.7)	
	Hyperechoic	5 (2.5)	9 (1.2)	
	Iso-hyperechoic	3 (1.5)	4 (0.5)	
Ratio of anterior-posterior diameter to transverse diameter, n (%)	<1	192 (95.5)	696 (92.8)	.223
	>1	9 (4.5)	54 (7.2)	
Edge layout, n (%)	Regular margin	173 (86.1)	673 (89.7)	.141
	Irregular margin	28 (13.9)	77 (10.3)	
Central vascularity, n (%)	No	177 (88.1)	645 (86)	.449
	Yes	24 (11.9)	105 (14)	
Cystic content, n (%)	No	176 (87.6)	609 (81.2)	.035*
	Yes	25 (12.4)	141 (18.8)	
Microcalcification, n (%)	No	154 (76.6)	579 (77.2)	.861
	Yes	47 (23.4)	171 (22.8)	
Macrocalcification, n (%)	No	170 (84.6)	652 (86.9)	.386
	Yes	31 (15.4)	98 (13.1)	

HT, Hashimoto's thyroiditis.

Table 3. Indications for Total Thyroidectomy

Indication	HT Group (Group 1) (n=139)	Non-HT Group (Group 2) (n=395)	*P*
MNG, n (%)	**19 (13.7)**	**136 (34.4)**	
TFNAB, n (%)	93 (66.9)	177 (44.8)	
Malignant	16 (11.5)	32 (8.1)	
AUS/AUFL (1 or 2 times)	**47 (33.8)**	**83 (21)**	
suspicious for malignancy	**12 (8.6)**	**8 (2)**	<.001
ND (2 times)	14 (10.1)	39 (9.9)	
FN/suspicious for FN	4 (2.9)	15 (3.8)	
Giant nodule, n (%)	19 (13.7)	74 (18.7)	
MNG + parathyroid lesion, n (%)	7 (5.7)	8 (2)	

MNG, multinodular goiter; TFNAB, thyroid fine-needle aspiration biopsy, AUS, unspecified atypia; AUFL, indeterminate follicular lesion; ND, non-diagnosed; FN, follicular neoplasia.

In patients with nodules requiring fine-needle aspiration biopsy in the general population, the risk of thyroid cancer was 9-13%.[13,14] Which nodules require biopsy is decided based on ultrasonographic appearance. The risk is determined according to the presence of microcalcification, hypoechoic structure, edge irregularity, central vascularity, solid component, and irregular and thick halo. In the presence of a cystic component, spongiform appearance, hyperechogenicity, fine and regular halo, comet artifact, and the likelihood of the nodule being a benign nodule increase, thus biopsy is not necessary.[15-17] Simply, a biopsy is recommended for intermediate-high suspicion nodules with a size of >1 cm. But, non-risky nodules other than size are recommended to follow without biopsy.[18] The association of benign and malignant nodules in HT is well known, however, specific guidelines for the approach to these nodules are not available today, they are approached as in the general population.[15] In studies conducted, an approach similar to the general population seems to be sufficient for HT.[11] In our study, the nodules with suspicious appearance in the HT group were evaluated based on the current guidelines and were found to have lower volume and less cystic content compared to the non-HT group. In the general population, cystic content is less in malignant nodules, but as the volume increases, the risk of malignancy increases.[19] In our study, since all of these nodules did not undergo fine-needle aspiration biopsy in all groups, these differences could not be evaluated.

Indications for total thyroidectomy can be grouped under 3 main headings: malignant thyroid nodule/nodules, non-toxic nodular/multinodular goiter, and hyperthyroidism. The most common cause is malignant nodule/nodules and the most common benign cause is non-toxic multinodular goiter. Thyroid cancer risk is the most common cause of total thyroidectomy in HT.[20,21] In our study, bilateral total thyroidectomy indications were different between the groups. We did post hoc analysis to see where the difference stems from; this difference was due to MNG, AUS/AUFL, and suspicion for malignancy groups. The most common operation indication was biopsy results in both groups. There was a significant difference between the groups with a detailed examination of the biopsy results; HT patients were most frequently operated on due to the biopsy result of the AUS/AUFL.

The issue of whether HT caused thyroid cancer since 1955 is constantly discussed. Some studies supported this thesis and some argued that there was no such relationship. Since the development of neoplasia on the basis of chronic inflammation is already known, the chronic inflammatory process present in HT may be causing cancer development. The inflammatory process can lead to DNA damage through reactive oxygen mediators resulting in cancer development. Helper and cytotoxic T lymphocytes, and high amounts of anti-thyroid antibodies lead to papillary cell damage by administering this inflammatory response. On the other hand, the presence of accompanying HT in cases with thyroid cancer may be an incidental condition.[1] According to data from a large number of meta-analyses, PTC is seen 3 times more in HT patients. The incidence of HT with PTC varies between 0.5% and 58% in various populations and this association has increased significantly in the last 20 years. Of course, both the increase in the frequency of autoimmune diseases, the spread of USG and biopsy, and the increase of awareness are important factors in this increase.[21] As in the literature, in our study, PTC was more common in HT patients (50.35% vs. 39.49%, $P = .026$) and the odds ratio was detected as 1.59.

According to the literature, HT and PTC are seen more often, are more focused, and are smaller (usually <1 cm) in younger women.[22-24] In our study, PTC was found to be more common in females but there was no difference between the groups in terms of gender and age distribution. The mean tumor size was <1 cm in both groups. Papillary thyroid cancer was found as a single focus in more than 50% of patients in both groups, but no statistically significant difference was observed ($P = .432$, Table 4).

One of the hypotheses to explain the causal relationship between HT and PTC is the high level of TSH. High levels of TSH contribute to the development of papillary cancer by inducing follicular epithelial proliferation in patients with hypothyroidism.[25] In our study, the mean TSH level was higher in group 1 and the tendency to hypothyroidism was higher ($P < .001$).

There are studies showing that PTCs accompanied by HT have a better prognosis. In the study performed by Ahn et al.[26] HT and PTC showed smaller tumor size, single focus, less lymph node metastasis, and better survival. Also, in a more recent study by Borowczyk27, chronic lymphocytic thyroiditis decreases the stage of differentiated thyroid cancer. There are several hypotheses to explain why HT has a better prognosis for PTC. In HT, follicular cells express Fas and Fas ligand, which induce Fas-related apoptosis and cause destruction in thyroid tissue. Because PTC originates from follicular cells, the immune response caused by the common antigen may cause the destruction of tumor cells.[28] The BRAF mutation is also lower in PTC with HT, which is associated with a good prognosis.[29] In our study, the vascular invasion was not seen in group 1, while it was seen in 7 patients of group 2, but the statistical comparison was not possible because of scarcity. The capsular invasion was found to be low in the HT group close to significance ($P = .061$). Lymphatic metastasis and extrathyroidal spread were detected in both groups but no difference was found.

CONCLUSION

Papillary thyroid cancer was found to be significantly higher in HT patients. Papillary thyroid cancer was more common in females in both groups and age distribution was similar. Hence, different effects of HT on age and gender were not detected. In the HT group, the tumor was less prone to capsular invasion and did not perform vascular invasion, which was a favorable result. The effect of HT on tumor size and the number of foci were not observed. Finally, since our study is a cross-sectional study, it is currently not possible to tell the effect of HT on PTC prognosis; however, long-term follow-up is predicted to reach a meaningful outcome.

Limitations

We could not detect all patients' anti-thyroid antibodies so we did not evaluate them. We could not tell if there is any relationship between thyroid autoantibodies and PTC. Unfortunately,

Table 4. Comparison of Papillary Carcinomas Detected in the Resected Thyroid Tissue of the 2 Groups

			Group 1 (HT)	Group 2(non-HT)	*P*
Number, n (%)			70 (50.35)	156 (39.49)	.026*
gender, n (%)	Female		65 (92.9)	133 (85.3)	.166
	Male		5 (7.1)	23 (14.7)	
age			46.8 ± 12.4	46.1 ± 12.8	.705
Preoperative TSH			1.99 (0.36–17.9)	1.22 (0.13–14.2)	<.001
Thyroid function status, n (%)	Euthyroid		62 (88.6)	155 (99.4)	.001*
	Hypothyroid		8 (11.4)	1 (0.5)	
MNG presence, n (%)			51 (72.9)	133 (85.3)	.042*
Right thyroid lobe volume, mL			7920.1 (1560.0–60 889.9)	9684.5 (611.5–140 874.2)	.028*
Left thyroid lobe volume, mL			7271.7 (338.3–172 380)	8616.9 (2355.0–88 058.9)	.044*
Parenchymal echogenicity,	Homogeneous, n		1 (1.4)	36 (23.1)	<.001*
	Heterogeneous		69 (98.6)	120 (76.9)	
Nodule number, n			2 (1–11)	3 (1–16)	.001*
Suspicious nodule number, n			1 (0–5)	1 (0–5)	<.001*
Tumor diameter, mm			9 (0.6–45)	9 (0.4–68)	.899
Diameter group, n (%)	Microcarcinoma		47 (67.1)	97 (62.2)	.473
	Macrocarcinoma		23 (32.9)	59 (37.8)	
Tumor subtype, n (%)	Classic		60 (85.7)	132 (84.6)	.093
	Follicular		7 (10)	23 (14.7)	
	Tall cell		0 (0)	1 (0.6)	
	Hurtle cell		2 (2,85)	0 (0)	
	Oncocytic cell		1 (1,42)	0 (0)	
Capsular invasion, n (%)			2 (2.9)	18 (11.5)	.061
Vascular invasion, n (%)			–	7 (4.5)	.102
Extrathyroidal spread, n (%)			4 (5.7)	17 (10.9)	.321
Lymphatic metastasis, n (%)			10 (14.3)	20 (12.8)	.930
Focus number, n (%)		Single focus	36 (51.4)	89 (57.1)	.432
		>1 focus	34 (48.6)	67 (42.9)	

HT, Hashimoto's thyroiditis; TSH, thyroid-stimulating hormone; MNG, multinodular goiter.

the biopsy of every nodule with sonographically suspicious appearance was not done.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara Education and Research Hospital. (Date: October 27, 2015, Decision number: 617/2015).

Informed Consent: The written informed consent obtained form all the participants.

Peer-review: Externally peer-reviewed.

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Original Article

The Prognostic Importance of Neutrophil–to–Lymphocyte Ratio and Platelet–to–Lymphocyte Ratio in Adult Patients with Sepsis Who Underwent Hemoperfusion in General Intensive Care Unit

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ABSTRACT

Objective: Sepsis is a major cause of mortality and morbidity in the intensive care units. The goal of this study is to investigate whether changes in the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio are a prognostic marker for patients with sepsis (according to sepsis stages, and patient's and disease's characteristics) who have been followed up in the intensive care unit and who have received HA330 resin-directed hemadsorption column for sepsis.

Methods: The study included a group of 100 (male [healed: 19, exitus: 42], female [cured: 29, exitus: 10]) sepsis patients who were followed up in the intensive care unit between December 2019 and December 2021 and who received HA-330 sepsis adsorption column.

Results: Although a strong positive correlation was found between the neutrophil-to-lymphocyte ratio and the baseline platelet-to-lymphocyte ratio values ($r = 0.725$ and $P = .001$), a weak positive correlation was found between the baseline neutrophil-to-lymphocyte ratio and the comorbidity values ($r = 0.253$ and $P = .001$). In addition, the period found for hemoperfusion in those who healed was statistically significantly higher in exitus patients ($P = .001$). It was noted that the improvement in repeated neutrophil-to-lymphocyte ratio measurements in the healing and death observations was identical ($P > .05$). The repeated neutrophil-to-lymphocyte ratio measurement values were found to be statistically significantly different for those with healing ($P = .014$). In addition, repeated neutrophil-to-lymphocyte ratio measurement values were found to be statistically significantly different from those with exitus ($P = .001$). It was observed that the change of repeated platelet-to-lymphocyte ratio measurements in the observations with healing and death was statistically significant ($P < .05$).

Conclusion: It is thought that it may be a cheap and useful biomarker in the prognosis of patients who are followed up in the intensive care unit and are treated with HA-330 sepsis adsorption column since the rate of neutrophils and lymphocytes in patients with hematological healing and death differs greatly.

Keywords: Sepsis, hemoperfusion, neutrophils, lymphocytes, blood platelets, ratio, intensive care units

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INTRODUCTION

Sepsis is characterized by a life-threatening organ dysfunction and manifested by an irregular host response to the severe infection. It is a syndrome that includes physiological, pathological, and biochemical abnormalities, the pathobiology of which has not been fully elucidated, hence becoming an important public health problem.[1,2] The septic shock, on the other hand, is a subset of sepsis in which vasopressors are needed to maintain mean arterial pressure (MAP) above 65 mmHg and above 2 mmol/L despite adequate fluid resuscitation and to harbor the cellular metabolism disorders and acute circulatory abnormalities.[3] Both clinical conditions have high mortality rates although several criteria have been tried to be developed to diagnose sepsis. The Systemic Inflammatory Response Syndrome (SIRS) criteria had been an accepted scoring system used in the diagnosis of sepsis. Today, sepsis is currently diagnosed by 2 or more point-infected organ dysfunction scored by the Sequential Organ Failure Assessment.[2,4-9]

Failure to make an early diagnosis and treatment of sepsis may result in multiple organ failure and death. The main treatment method to reduce the mortality rates is to recognize the infection center causing the sepsis, to initiate an effective focal antibiotic therapy, and to provide a hemodynamic support in this process.[4,10-13]

Since the clinical signs and symptoms of sepsis are nonspecific and often variable, the search for a rapid test is ongoing for the diagnosis and assessment of sepsis severity.[14-19] In this respect, several parameters are considered useful since they have the ability to show the absence or presence or the severity of the sepsis, to follow the patient clinically, to be used for classification purposes, and to predict the outcome of sepsis.[20] Two of these parameters namely the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are readily available and have intensively been studied and evaluated in the demonstration of inflammation and sepsis due to being time- and cost-effective.[21-22]

The purpose of the study is to investigate whether variations in the NLR and PLR are prognostic markers in the patients diagnosed with sepsis (according to the sepsis stages, and patient's and disease's characteristics) who are followed up in the intensive care unit (ICU) and undergoing treatment using "HA-330 Sepsis Adsorption Column" and whether there is a relationship between NLR and PLR and the hematological parameters in these septic patients.

Main Points

- In intensive care units, sepsis is a major cause of mortality and morbidity.
- They have led to the development of different modalities of care to control them. Extracorporeal treatment approaches such as hemoperfusion have also become increasingly interesting in this context.
- Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio are shown as low-cost, effective, and easily applicable markers in inflammatory and infectious processes in patients with sepsis.

METHODS

This research is designed for the Biruni University Medical Faculty Department General Intensive Care as a single-center study assessing demographic and hematological parameters. The research sample included 100 (male [healed: 19, exitus: 42], female [healed: 29, exitus: 10) sepsis patients who were followed up in the ICU between December 2019 and December 2021 and applied the HA-330 Sepsis Adsorption Column (Hemoadsorption device HA-330, Jafron Biomedical Co., Ltd., China), in accordance with the study patient's inclusion criteria.

Patient inclusion criteria in the study were as follows:

- A retrospective consent form was obtained from the patients who were hospitalized in our hospital due to the diagnosis of sepsis and benefited from the treatment, or from the relatives of deceased patients hospitalized in our hospital due to the diagnosis of sepsis.
- Those who were general intensive care patients, of 18 years old and over, who met the sepsis criteria, and underwent hemoperfusion (which is an extracorporeal blood purification method consisting of passing an anticoagulated whole blood through a device, usually a column containing the adsorbent particles, to remove the cytokines in the blood of septic patients).

While diagnosing patients with sepsis, the following criteria in the guidelines of the American Intensive Care Association were discussed[23]:

- Sepsis: certain/possible infection + ≥2 SIRS criteria, and
- SIRS: systolic blood pressure (SBP) <90 mmHg or Mean Arterial Pressure (MAP) <65 mmHg or lactate >2 mmol/L (after initial fluid loading), international normalized ratio (INR) >1.5 or activated partial thromboplastin time (APTT) >60 hour, bilirubin >34 µmol/L, urine output during 2 hours <0.5 mL/kg/h, creatinine >2 mg/dL, platelets <100 × 109 L, and patients who do not have SpO_2 <90% in room air and who received HA-330 hemoadsorption.

Patient exclusion criteria from the study:

- Patients under 18 years of age and over 80 years of age and patients with bleeding diathesis;
- Those with neurodegenerative diseases.

Study Protocol

Hemoadsorption device HA-330 (Jafron Biomedical Co., Ltd., China) in combination with HP/Continuous renal replacement therapies (CRRT) were initiated. The patient had acute renal failure, and the patient had a history of diabetes and high blood pressure. Before the onset of HP CRRT, the patient has oliguria (urine 400 cm^3 in 24 hours). After HA-330, the patient's urine output reached 1100 cm^3 in 24

hours. Continuous renal replacement therapies mode used was as follows: continuous venovenous hemofiltration predilution and post-dilution every 2 hour, blood flow: 200-250 mL/min, substitution flow: 25 cm^3/kg, ultrafiltration (UF):20 ml/h, heparinization: 10 U/kg/h, patient's weight: 70 kg. The HP cartridge was added to the CRRT circuit simultaneously with the start of CRRT, and HP and CRRT were started simultaneously. After 6 hours, the HP cartridge was removed from the CRRT circuit, and CRRT was continued. After 20 hours, the second HP cartridge was added to the CRRT circuit, and it was used for 6 hours and then removed. The fluid balance was maintained neutral.

Data Collection Method

Data on sepsis patients as a sample of the study were first collected from patients who were followed up by the responsible investigators working in the ICU in compliance with the sepsis criteria and the laboratory tests and examinations were obtained before and after the HA-330 Hemoadsorption Sepsis Column application. Patients' hematological parameters, NLR/PLR ratios, were determined using hemogram values. Furthermore, patient's demographic information (age, gender, disease information, risk factors, etc.) was collected from patient records and regularly reported in the Microsoft Office Excel file.

Statistical Analysis

Statistical Package for the Social Sciences version 24.0 (IBM SPSS Corp.; Armonk, NY, USA) used in the statistical analysis of the data obtained during the research. The Kolmogorov–Smirnov test tested the suitability of the data to normal distribution, the Student's *t*-test was used to compare normally distributed features in 2 independent groups, and the Mann–Whitney *U* test was used to compare non-normally distributed features in 2 independent groups. With the Friedman test and post hoc test, the features that did not display a normal distribution at repeated times were corrected and then were examined with the Wilcoxon test. In repeated measurements, the variations between repetitive measurements in the prognosis and exit groups were explored by the 2-way analysis of variance (ANOVA) test. Using the chi-square test, the relationship of categorical features with groups was tested. Mean ± standard deviation, median for numerical variables, and number and percent values were given for categorical variables as descriptive statistics. The $P < .05$ value was accepted as significant in the statistical analysis.

Ethical Statement

All authors declare that the study was conducted in accordance with the World Medical Association Helsinki "Ethical Principles for Medical Research Containing Human Subjects." All patients were given full information about the study procedures before providing written consent. Besides, an informed consent form was obtained from patients who participated in this clinical investigation. In addition, the approval of the study by the Ethics Committee was obtained from the Biruni University Clinical Research Ethics Committee (Date: January 27, 2019, Decision no: 2019/27-14).

Table 1. Distribution of Demographic and Clinical Characteristics of the Patients

Parameters	Median	Mean ± SD	Min–max
Age	69.5	67.39 ± 15.62	20–103
Hemoperfusion duration (days)	7	5.41 ± 2.01	1–7
Intensive care unit duration (days)	14	28.73 ± 38.35	2–270
Comorbidity number	2	2.01 ± 1.05	0–4
Male, n (%)	61 (61)		
Comorbidity 1	76 (76)		
Comorbidity 2	67 (67)		
Comorbidity 3	43 (43)		
Comorbidity 4	15 (15)		
Exitus/healed	71/29 (71/29)		

n=100.
SD, standard deviation.

RESULTS

The distribution of demographic and clinical characteristics of the patients included in the study is given in Table 1.

The results of comparing the hemogram parameters of the patients included in the study according to the hemoperfusion duration and the duration of stay in the ICU are given in Table 2.

According to Table 2, a strong positive correlation was found between the initial values of NLR and PLR ($r = 0.725$ and $P = .001$). A weak significant positive correlation was found between the NLR baseline value and the number of comorbidities ($r = .253$ and $P = .001$).

The relationship between prognosis and parameters is given in Table 3.

The hemoperfusion time observed in those who healed was statistically significantly higher than the hemoperfusion time observed in those who were dead ($P = .001$) according to Table 3. It was found that in those who were healing and those who died ($P > .05$), comorbidity distributions were similar.

Based on the results of the 2-way repeated ANOVA analysis, the change in the repeated NLR measurements in the healing and death observations was similar ($P > .05$). The change of NLR repeat measurements, in other words, is independent of the status of healing and death. Again, there was no statistically significant difference between the cure and death groups in repeat measurements of NLR and PLR ($P > .05$). The repeated NLR measurement values were found to be statistically significantly different for those with healing ($P = .014$). The NLR value calculated at T5 and T7 was found to have decreased significantly compared to the initial value ($P < .05$). Repeated NLR measurement values

Table 2. Results of Comparison of Hemogram Parameters of Patients According to Hemoperfusion Duration and Intensive Care Unit Duration

Parameters		NLR (Start)	PLR (Start)	Hemoperfusion Duration/ Day	Intensive Care Unit Duration	Comorbidity Number
NLR (start)	R	1	0.716**	−0.051	−0.129	0.247*
	P		.000	.617	.200	.013
	n	100	100	100	100	100
PLR (start)	R			0.069	0.047	0.032
	P			.496	.646	.749
	n			100	100	100
NLR (avg.)	R		0.741	−0.090	−0.196	0.135
	P		.001	.374	.051	.180
	n		100	100	100	100
PLR (avg.)	R			0.004	−0.072	0.069
	P			.971	.474	.463
	n			100	100	100
NLR (7 repetition)	R		0.7	0.032	−0.363	0.256
	P		.001	.881	.008	.067
	n		52	52	52	52
PLR (7 repetition)	R			0.003	−0.152	0.161
	P			.988	.282	.255
	n			52	52	52
Hemoperfusion duration (day)	R				0.440**	0.073
	P				.001	.470
	n				100	100
Intensive care unit duration (day)	R					−0.144
	P					.153
	n					100

*$P < .05$; **$P < .001$.
R, correlation coefficient; *P*, significance value; n, number of cases; NLR, neutrophil–to–lymphocyte ratio; PLR, platelet–to–lymphocyte ratio.

were reported to be statistically significantly different from those with exitus ($P = .001$). Compared to the initial value, NLR values calculated at T5, T6, and T7 were found to be significantly lower ($P < .05$).

Based on the results of the 2-way repeated ANOVA analysis, the variance of repeated PLR measurements in the healing and death observations was shown to be statistically significantly different ($P < .05$).

DISCUSSION

The ICU is a multidisciplinary facility that provides a wide range of profiles of patients.[7,8] Over time, the population of patients treated in the ICU has also changed. Intensive care patients are now more advanced elderly and patients with complicated comorbidities due to the rise in the elderly population. Advances in malignancy therapies, developments in surgery and other diagnostic techniques, increasing expectations of the society and many other factors accompany this. Intensive care unit patients are patients who are at high risk of mortality.[9,11,13,14] Infection, respiratory distress, and other organ failure are the most important risk factors that increase mortality. An ideal prognosis method should be used to evaluate all these patient groups.[15,19] In addition, the reason for hospitalization and the presence of chronic systemic disease may also affect the prognosis of the patient.[12] Based on this, in the study, it was investigated whether

Table 3. The Relationship Between Prognosis and Parameters

	Healed	Exitus	
Parameters	**Mean ± SD (M)**	**Mean ± SD (M)**	***P***
Age	67.59 ± 1 5.40 (68)	68.13 ± 15.75 (72)	[1].200
Intensive care unit duration	42.07 ± 56.48 (20)	23.28 ± 26.47 (13)	[2].096
Hemoperfusion duration (day)	6.55 ± 1.06 (7)	4.94 ± 2.12 (5)	[2].001
Com number	1.90 ± 1.05 (2)	2.06 ± 1.05 (2)	[2].511
Gender (n: %)			
Male	19 (65.5)	42(59.2)	[3].554
Female	29 (40.8)	10 (34.5)	
Comorbidity (n%)			
Comorbidity 1	19 (65.5)	57 (80.3)	[3].117
Comorbidity 2	18 (62.1)	49 (69.0)	[3].503
Comorbidity 3	12 (41.4)	31 (43.7)	[3].834
Comorbidity 4	6 (20.7)	9 (112.7)	[3].309
NLR			
T1	22.28 ± 33.05 (8.8)	20.15 ± 28.25 (11.4)	[2].630
T2	18.81 ± 27.46 (9.1)	19.59 ± 19.02 (14.7)	[2].300
T3	15.99 ± 25.84 (5.8)	17.41 ± 19.26 (10.7)	[2].162
T4	11.29 ± 13.17 (7.1)	16.4 ± 19.96 (8.1)	[2].351
T5	*10.94 ± 13.01 (7.6)	*14.08 ± 13.23 (8.4)	[2].189
T6	9.17 ± 6.98 (7.6)	*15.48 ± 18.97 (6.7)	[2].711
T7	*9.69 ± 8.31 (6.6)	*15.4 ± 20.6 (6.4)	[2].876
	[4]0.014	[4]0.001	
PLR			
T1	45.44 ± 81.86 (22.5)	39.99 ± 46.25 (24.9)	[2].945
T2	25.08 ± 17.25 (18.2)	31.64 ± 30.99 (21.3)	[2].904
T3	25.5 ± 23.81 (19.4)	26.6 ± 37.76 (14.9)	[2].459
T4	*17.17 ± 12.94 (13.9)	*23.73 ± 29.77 (12.4)	[2].794
T5	*16.58 ± 14.42 (12.1)	*18.5 ± 20.34 (12.2)	[2].972
T6	*17.96 ± 13.52 (16.1)	*21.77 ± 29.53 (9.6)	[2].118
T7	*20.66 ± 18.09 (15.4)	*21.4 ± 31.95 (9.1)	[2].099
	[4]0.001	[4]0.001	

[1]Student's *t*–test; [2]Mann–Whitney *U* test; [3]Chi–square (Yates) correction; [4]Friedman test. *According to the initial value, it shows statistical significance according to the Friedman post hoc test ($P < .05$).
M, median; SD, standard deviation; NLR, neutrophil–to–lymphocyte ratio; PLR, platelet–to–lymphocyte ratio.

NLR and PLR changes were a prognostic marker in patients with sepsis (according to sepsis stages and characteristics of patient and disease) who were followed up in the ICU and underwent HA-330 Sepsis Adsorption Column.

In order to determine the severity of the disease during their clinical and practical treatments, promising new treatments, researchers and physicians have led patients to need to find new parameters.[24] Thus, different prognostic models have been developed to

determine the prognosis in patients and to use current treatment methods rationally. These are used in multiple ICUs and to conduct quality control at different times in the same ICU.[25] Furthermore, these parameters are used extensively for some scientific studies. In developed countries, intensive care costs account for 20-30% of hospital costs.[26] Due to the high cost of ICUs and the emotional conditions of patients and their families, the evaluation of the prognosis of these patients and the proper utilization of ICU in the last 2 decades have become an important issue.[27,28]

For clinicians today, early detection of infections is still an important issue. In general, for any suspected infection, the use of antibiotics is not recommended, as serious problems may occur with increasing bacterial resistance to antibiotics. Thus, in diagnosis, biomarkers unique to bacterial infections may be useful.[29]

Hemoperfusion with neutral microporous resin column is a blood purification technology within the scope of research, which has excellent efficacy in hemoperfusion rhythm diseases applied to septic patients, and which is of great importance in the early prevention and clearance of inflammatory mediators in the treatment of critical diseases.[30] Hemoperfusion can cleanse inflammatory mediators specifically and effectively and regulate the immunity of the body. Thus, by allowing the damaged organs to recover rapidly and the patient's symptoms to disappear, it offers excellent efficacy in the treatment of essential diseases.[31-32] Studies have shown that HA330 adsorption therapy can support the recovery of organ functions in patients with sepsis.[33-35]

In most sepsis studies, age was found to be above 60 years.[36] The reduction in physiological ability and response to factors that cause stress is an expected outcome in elderly patients with the lifetime accumulation of molecular and cellular damage as a result of aging, and this increases the risk of elderly patients to become critically ill.[37] In a study of patients undergoing and admitted to intensive care, the number of elderly patients and the number of patients with sepsis increased from year to year. There are also opinions arguing that severe sepsis is a disease specific to old age. Most elderly patients who were discharged were found to have cognitive or functional sequelae and it was reported that these elderly patients had a higher risk of being critically ill.[38] The average age of sepsis patients was found to be 69.5 years in this study.

Neutrophils and lymphocytes are one of the main cellular components of the defense system against infection.[39] Depending on the stage of sepsis, the patient's immunological status and the etiology of the infection, and the number of white blood cells may change during sepsis.[40] The clinician should be reminded of an infection by the increased neutrophil count and the reduced lymphocyte count. One of the basic inflammation biomarkers that can be measured in routine hematological tests is NLR. A useful index in the diagnosis of sepsis and many diseases in adult patients has been found to be NLR.[41]

The fact that repeated NLR measurement values in those with healing and those with exitus are statistically significantly different in the study indicates that NLR is an important parameter for patients with sepsis. In the research by Lorente et al.[42] the NLR value was found to be higher than the survivor in the diagnosis of sepsis, and this outcome was correlated with mortality. In the study, the time observed for hemoperfusion in those who recovered was statistically significantly greater than the time observed for hemoperfusion in exitus-patients.

In the study, it was observed that the change of repeated PLR measurements in the observations with healing and death was statistically significant ($P < .05$). Duman et al[43] found no statistically significant difference in PLR between patients with sepsis, septic shock, and severe sepsis ($P = .737$). However, similar to our study, Zencir et al[44] found PLR was significantly higher in the group with in-hospital mortality in infective endocarditis ($P = .008$).

Therefore, although PLR is shown as a low-cost, easily applicable marker in inflammatory and infectious processes, we believe that further studies are needed to determine how much PLR will benefit the clinician in terms of showing mortality.[45-47]

Limitation

The limitations of our study are that it was retrospective, conducted on a limited number of intensive care patients, and absolute lymphocyte or absolute neutrophil values were not included in the evaluation.

CONCLUSION

In this study, because of the large difference in the rate of neutrophils and lymphocytes in patients with sepsis who are followed up in the ICU and undergoing HA-330 Sepsis Adsorption Column, and in patients who are healing from hematological parameters and those who are dead, it is suggested that it could be a cheap and useful biomarker in patient prognosis. When the results of the study were analyzed, NLR and PLR parameters were found to be the parameters that can be used for early diagnosis, follow-up, treatment, and prognosis of patients hospitalized in ICUs, which are similar to previous studies.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Biruni University Clinical Research Ethics Committee (Date: January 27, 2019, Decision no: 2019/27-14).

Informed Consent: Informed consent form was obtained from the patients and/or their relatives.

Peer-review: Externally peer-reviewed.

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Original Article

Investigation of the Antioxidant, Antimicrobial, and Cytotoxic Activities of Endemic *Marrubium rotundifolium* Boiss

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ABSTRACT

Objective: *Marrubium L.* genus (Lamiaceae), which has 40 taxa in the world, is represented by 25 taxa in Turkey. *Marrubium rotundifolium* Boiss. is an endemic species and is distributed in Aegean. This study was aimed to determine the antioxidant, antimicrobial, and cytotoxic effects of hexane, ethyl acetate, and methanol extracts of the endemic *Marrubium rotundifolium* Boiss.

Methods: Ultrasonic-assisted extraction was applied to aerial parts of the plant. The Folin-Ciocalteu and aluminum chloride/potassium acetate methods determine the extract's total phenolic and flavonoid contents. The 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical decolorinaztion assay was used to determine the extracts' Trolox equivalent antioxidant capacity. The deoxyribose assay was carried out for determining the extracts' OH^- radical scavenging activity, and 2,2-diphenyl-1-picrylhydrazyl was used for radical scavenging assay. 3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide method was used to determine the in vitro cytotoxicity of plant extracts on 3 cancer (Caco-2, SH-SY5Y, and PC-3) and 2 non-cancer cell lines (NIH-3T3 and HK-2). The antimicrobial activity was examined through the microdilution method.

Results: Phenolic contents of 2.62 ± 0.16 gallic acid equivalents µg/mL were observed in the methanol extract, while the highest flavonoid content was determined in *n*-hexane extract (168.63 ± 2.76 QE µg/mL). In the $OH^{\cdot -}$, $ABTS^{\cdot +}$, and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity studies, *M. rotundifolium* methanol extract showed higher IC_{50} values at lower doses (0.277 ± 0.024, 3.21 ± 0.081, 0.033 ± 0.001 mg/mL, respectively). Concerning the cytotoxic activity, only methanol extract showed inhibition on PC-3 cells (IC_{50}: 0.173 ± 0.018 µg/mL). Minimum inhibitory concentrations of the extracts against Gram-positive bacteria were lower than Gram-negative bacteria and yeast strains.

Conclusion: This study is the first detailed study that examines antioxidant and cytotoxic properties of endemic *M. rotundifolium*.

Keywords: Marrubium, cytotoxicity, antioxidant, antimicrobial, extract

INTRODUCTION

Reactive oxygen species (ROS) are regularly produced as intermediate or end products in physiologic reactions concerning oxygen in the cell. Although ROS plays a crucial role in several vital processes (e.g., redox homeostasis, gene expression, signal transduction, enzymatic reaction, and regulation of the immune response), their uncontrolled presence in the cell poses distress linked to the high oxidant reactivity.[1,2]

Nowadays, it is well-known that ROS and oxidative stress play an essential role in the pathophysiology of various diseases like chronic inflammation, neurodegenerative, and cardiovascular diseases. Although the oxygen-free radical hypothesis has been known for over 50 years, the role of oxidative stress in the progress of diseases has been identified in the last 2 decades. Afterward, studies on antioxidants for preventing and treating these diseases have gained significant importance.[1-3]

Plant extracts are well-known sources shown to have antioxidant capacities due to the phytochemicals included. Antioxidant activities have been associated primarily with the phenolic and flavonoids contents of plants. As the most commonly applied synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole are being questioned about safety, natural antioxidants take more consideration by researchers.[3]

Marrubium L. genus (Lamiaceae), 40 taxa globally, is represented by 25 taxa in Turkey. Studies show that this genus is rich in phenolic and flavonoid compounds, labdane-type diterpenes, and

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lignans.[4-6] In addition, there are many pieces of research on the antioxidant, antimicrobial, anti-inflammatory, and cytotoxic activities of different *Marrubium* species.[6,7] *Marrubium rotundifolium* Boiss. is an endemic species that is distributed in Aegean region of Turkey and is known as "kalartopu."[8] It is perennial, and cauline leaves are broadly elliptical or ovate. It has been reported that the infusion prepared from the whole plant has been used as carminative and for treating intestinal spasms, dyspepsia, cold, and flu in traditional medicine.[9] However, the research on the phytochemistry and bioactivity of this species is very limited.

This study intended to ascertain the antioxidant, antibacterial, and cytotoxic effects of hexane, ethyl acetate, and methanol extracts of the endemic *M. rotundifolium* Boiss.

METHODS

Plant Materials

The aerial parts of the plant were collected from Bozdag, Izmir, in June 2016. Plats were identified by Prof. Sura Baykan. Voucher specimens were stored in the Herbarium of Ege University, Faculty of Pharmacy (IZEF: 6052).

Extraction

Aerial parts of *M. rotundifolium* (826 g) were air-dried, grinded, and then extracted with *n*-hexane, ethyl acetate, and methanol (3× 1L), by turns using an ultrasonic water bath for 24 hours, and filtered. Finally, the extracts were evaporated to dryness with a rotary evaporator at 40°C. The quantity of obtained *n*-hexane, ethyl acetate, and methanol extracts for the plant was 9.247, 10.133, and 69.652 g, respectively.

Determination of Total Phenolic Content

In order to determine the total phenolic content (TPC) of extracts, 0.5 mL of extract (0.1 mg/mL), 5 mL of Folin-Ciocalteu (10%) reagent, and 4 mL of Na_2CO_3 (1 M) were mixed in test tubes.[10] The tubes were incubated for 15 minutes at 45°C. The sample's absorbance values were measured at 765 nm by a spectrophotometer. The standard curve was established with the following gallic acid concentrations: 25, 50, 100, 150, 200, and 250 mg/mL. The TPCs of the extracts were expressed in mg gallic acid equivalents (GAE) per gram of dry weight.

Determination of Total Flavonoid Content

In order to determine the total flavonoid content (TFC) in extracts, 0.1 mL of $AlCl_3$ (10%), 0.1 mL of CH_3CO_2K (1 M), 1.5 mL methanol, 0.5 mL of extract (1 mg/mL), and 2.8 mL distilled water were added to test tubes, in the same order. Tubes were mixed and incubated for 30 minutes. Then sample's absorbance was measured at 415 nm.[11] The standard curve was established with the various quercetin concentrations (2.5, 25, 50, 75, and 100 µg/mL). Results were represented as µg quercetin equivalent (QE) per mg dry weight.

Main Points

- Antioxidant, antimicrobial, and cytotoxic effects of methanol, ethyl acetate, and hexane extracts of the endemic *Marrubium rotundifolium* Boiss. were investigated.
- The highest total phenolic content and antioxidant activities were observed in methanol extracts.
- Weak cytotoxic activity was determined on cancerous cell lines.

Assay for Trolox Equivalent Antioxidant Capacity

The Trolox equivalent antioxidant capacity (TEAC) of extracts was determined by the decolorization of ABTS radical.[12] Firstly, $ABTS^{+\cdot}$ radical was prepared by mixing 1 part of $K_2S_2O_8$ (2.45 mM) with 2 parts of ABTS (7 mM) and kept in the dark for 16 hours. Then, the mixture was diluted with phosphate buffer (5 mM) until it reached an absorbance value of 0.70 ± 0.02 at 734 nm. Next, 200 µL of the diluted radical solution was mixed with 2 µL of various concentrations of extracts in a 96-well plate. The absorbance was determined at 734 nm in a microplate reader (Varioskan Multimode Flash, Thermo Scientific, Vantaa, Finland) for 0-6 minutes. Finally, the percentages of radical cation inhibition were calculated using the following equation (Eq 1):

$$ABTS^{+\cdot}\ \text{Inhibition}\% = [(A_{ABTS^{+\cdot}} - A_{6min})/A_{ABTS^{+\cdot}}] \times 100 \quad \text{(Eq 1)},$$

where $A_{ABTS^{+\cdot}}$ is the absorbance of the $ABTS^{+\cdot}$ at 734 nm and A_{6min} is the absorbance after adding the formulations to the $ABTS^{+\cdot}$.

The "Trolox standard curve" was prepared for the experiment using different concentrations of Trolox solution (0.25-2.5 µmol/mL). The tested formulations' absorbance was compared to that of the Trolox standard curve, and the antioxidant value of extracts was expressed as the half-maximal effective concentration (EC_{50}) value, the concentration of antioxidants that causes a 50% decrease in the radical absorbance.

Determination of 2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was tested according to Wang et al[13] with minor modifications. Different concentrations of extracts (0, 62.5, 125, 250, 500, and 1000 µg/mL) and ascorbic acid as standards (2, 4, 8, 12, 16, 20 µg/mL) were diluted in ethanol. In a clear 96-well plate, $DPPH^{\cdot}$ solution (100 µL of 200 mmol/L) was mixed with 100 µL of extract or standard. Then the plate was incubated for 30 minutes, and the optical density value of the residual $DPPH^{\cdot}$ was determined at 517 nm wavelength in the microplate reader spectrophotometer. The inhibition percentage of radical was calculated using the following equation (Eq 2).

$$\%\ DPPH^{\cdot}\ \text{inhibition} = [(A_b - A_a)/A_b] \times 100 \quad \text{(Eq 2)},$$

where A_a is the absorbance of samples or standards and A_b is the absorbance of DPPH radical : ethanol (1 : 1) solution. Results were represented as EC_{50} value for $DPPH^{\cdot}$ scavenging.

Determination of Hydroxyl (OH^-) Radical Scavenging Activity

The hydroxyl (OH^-) radical scavenging activity of the extracts was determined by deoxyribose assay.[14] To perform the test, 100 µL of each solutions of 2-deoxy-D-ribose (3.36 mM), H_2O_2 (1 mM), $FeCl_3$ (1 mM), EDTA (1 mM), and ascorbic acid (0.1 mM) were taken and mixed with the extract samples at concentrations (0,

20, 60, 100, and 500 µg/mL). Then, 1.6 mL of phosphate buffer (20 mM, pH 7.4) was added. After 1 hour of incubation at 37°C, 1 mL of thiobarbituric acid (1% in 0.025 M NaOH) and 1 mL of trichloroacetic acid (2.8%) were added to the reaction mixture and incubated for an additional 30 minutes at 100°C. Finally, the tubes were cooled. The absorbance of the samples was measured spectrophotometrically at 532 nm. The results were represented as % inhibition of deoxyribose oxidation that was calculated using the following equation (Eq 3):

$$\text{Inhibition } \% = [(A_c - A_S)/A_c] \times 100 \text{ (Eq 3)},$$

where A_c is the absorbance of the control (α-tocopherol) and A_S is the absorbance of the sample. Results were represented as EC_{50} value for OH^- radical scavenging.

Cell Culture and Cytotoxicity Assay

The potential cytotoxic activity of extracts was tested against human colorectal adenocarcinoma (Caco-2), prostate adenocarcinoma (PC-3), neuroblastoma (SH-SY5Y), kidney tubular epithelial (HK-2), and embryonic mouse fibroblast (NIH-3T3) cell lines by colorimetric 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) reagent.[15] All immortalized cell lines were obtained from American Type Culture Collection (ATCC, USA).

Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 2 mM L-glutamine supplemented at 37°C in a humidified atmosphere of 5% CO_2 air.

In order to perform cytotoxicity assays, cells were plated into 96-well plates at a density of 5×10^3 cells/well. Plates were incubated overnight. Then, the cells were treated with various concentrations of extracts (0, 0.313, 0.625, 12.5, and 50 µg/mL) for 48 hours. At the end of the incubation, the medium was replaced with 200 µL MTT (5 mg/mL) solution in DMEM. Cells were incubated for another 4 hours. Finally, DMEM was removed and 100 µL dimethyl sulfoxide (DMSO) was added to wells. The absorbance of evaluated blue formazan solution in wells was measured with a microplate reader at 570 and 620 nm wavelengths. Dimethyl sulfoxide-treated cells were used as controls.

The cell viability was calculated and revealed as a percentage (%) of control. The median inhibitory concentration (IC_{50}) was determined in GraphPad Prism 5 statistical software.

Antimicrobial Activity

The minimum inhibitory concentration (MIC) of the samples was investigated with microdilution method.[16] *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* Refik Saydam Culture Collection (RSKK) 02021, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* RSKK 04059, *Candida albicans* ATCC 90028, and *Candida parapsilosis* RSKK 04057 strains were used in antimicrobial activity experiments.

Serial dilutions of the extracts were performed using 96-well microplates including broth media in order to adjust the concentrations between 1 and 2048 µg/mL. Plates were incubated at 37°C for 24 hours after the addition of bacterial and fungal suspensions. The lowest concentration that inhibited the growth of the microorganisms was defined as MIC value.

Ciprofloxacin and fluconazole were used as reference molecules in the assays. The quality control ranges of the results were assessed according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST).[17,18]

Statistical Analysis

Each experiment was done in triplicate. The EC_{50} and IC_{50} values were determined with GraphPad Prism 5 software (San Diego, Calif, USA). Statistical comparisons were performed using analysis of variance followed by Tukey's post hoc test. A P value < 0.05 was recognized as statistically significant.

RESULTS

Determination of Total Phenolic Content and Total Flavonoid Content

The TPCs and TFCs of the methanol, *n*-hexane, and ethyl acetate extracts obtained from the aerial parts of the *M. rotundifolium* were determined by Folin-Ciocalteu and $AlCl_3/CH_3CO_2K$ methods, respectively. Obtained data are represented in Table 1.

As a result of the determination of TPC in the obtained extracts, only the phenolic content of the methanol extract (2.62 ± 1.6 GAE µg/mg) was determined among the 3 extract samples. There was no detected phenolic content in the other 2 extract samples.

As a result of the determination of TFC experiments performed with the same samples, the TFC of ethanol and ethyl acetate extracts were 70.76 ± 1.32 and 142.58 ± 3.45 µg QE per mg of extract, respectively. The highest TFC was found in the *n*-hexane extract (168.63 ± 2.76 µg QE per mg of extract).

Determination of Antioxidant Activities

Antioxidant activities of methanol, *n*-hexane, and ethyl acetate extracts of the *M. rotundifolium* were evaluated by TEAC, $DPPH^{\cdot}$, and OH^- radical scavenging activity. Obtained data are represented as the half-maximal effective concentration (EC_{50}) in Table 2.

Table 1. Total Phenolic and Flavonoid contents of *Marrubium rotundifolium* extracts

	Total Phenolic Content (GAE µg/mg)	Total Flavonoid Content (QE µg/mg)
Methanol	2.62 ± 0.16[a]	70.76 ± 1.32
Ethyl acetate	–	142.58 ± 3.45[b]
n–Hexane	–	168.63 ± 2.78[b,c]

Data are expressed as mean ± standard deviation. Distinct letters (a–c) indicate significant differences ($P < .05$) between extracts.
GAE, gallic acid equivalent; QE, quercetin equivalent.
[a] $P < .01$ methanol versus ethyl acetate and *n*–hexane extracts.
[b] $P < .01$ *n*–hexane and ethyl acetate versus methanol extract.
[c] $P < .01$ *n*–hexane versus methanol extracts.

Table 2. Values of Different Antioxidant Activity Assays of *Marrubium rotundifolium* Extracts (EC_{50}, mg extracts/mL)

	TEAC	DPPH Radical Scavenging Activity	OH−˙Radical Scavenging Activity
Methanol	3.21 ± 0.081[a]	0.033 ± 0.001[a]	0.277 ± 0.024[a]
Ethyl acetate	NA	0.099 ±0.001[b]	0.832 ± 0.032[b]
n–Hexane	NA	NA	1.179 ± 0.132

Data are expressed as mean ± standard deviation. Distinct letters (a–c) indicate significant differences ($P < .05$) between extracts.
TEAC, Trolox equivalent antioxidant capacity; DPPH, 2,2–diphenyl–1–picrylhydrazyl; OH, hydroxyl.
[a] $P < .01$ methanol vs. ethyl acetate and *n*–hexane extracts.
[b] $P < .01$ ethyl acetate vs. *n*–hexane methanol extract.

As can be seen from the results in Table 2, similar to TPC results, only the methanol extract has a significant TEAC value (EC_{50}: 3.21 ± 0.081 mg extract/mL) among the 3 extract samples.

Moreover, as a result of the determination of the DPPH assay, the EC_{50} value of methanol extract (0.033 ± 0.001 mg extract/mL) was found significantly higher than ethyl acetate extract (0.099 ± 0.001 mg extract/mL). Nevertheless, no DPPH radical scavenging activity was observed in the ethyl acetate extract (Table 2).

In the $OH^{\cdot -}$ radical scavenging activity, the lowest EC_{50} value was determined in the methanol extract (0.277 ± 0.024 mg/mL), while the EC_{50} values of ethyl acetate and *n*-hexane extract were 0.832 ± 0.032 and 1.179 ± 0.132 mg/mL, respectively.

Antimicrobial Activity

The results of the microdilution method are presented in Table 3. Dimethyl sulfoxide was ineffective against bacterial and fungal strains at studied concentrations, and the MIC values of the ciprofloxacin and fluconazole were within the limit according to the EUCAST criteria. According to the results of the microdilution method, ethyl acetate extract inhibited the growth of *S. aureus* and *E. faecalis* at 128 µg/mL concentrations and *n*-hexane extract inhibited the growth of *E. faecalis* and *B. subtilis* at similar concentrations. Moreover, 128 µg/mL methanol extract also inhibited the growth of *B. subtilis*. Additionally, the MIC values of ethyl acetate and n-hexane extract were similar as 256 µg/mL against *S. aureus*. Ethyl acetate extract also inhibited the growth of Gram-negative bacteria and yeast strains at the concentration of 512 µg/mL.

Determination of Cytotoxic Activities

The cytotoxic effects of the aerial parts of *M. rotundifolium* methanol, *n*-hexane, and ethyl acetate extracts were tested against 3 cancerous (Caco-2, PC-3, and SH-SY5Y) and 2 non-cancerous (NIH-3T3 and HK-2) cell lines. For this, cells were treated with increasing concentrations (5-100 µg/mL) of *M. rotundifolium* extracts for 48 hours. The potential cytotoxic activity of extracts on cell line were determined by MTT assay (Table 4).

In the cytotoxicity studies performed on cancerous cells, it was determined that methanol extract of *M. rotundifolium* (100 µg/mL) caused a significant decrease in PC-3 cell viability compared to the control cells (40.37 ± 1.32%, IC_{50}: 0.173 ± 0.018 µg/mL) ($P < .01$). However, it was determined that the other extracts did not have a significant cytotoxic effect on any of the tested cancerous cell lines at the tested concentrations ($IC_{50} > 200$ µg/mL).

It was determined that the application of methanol, *n*-hexane, and ethyl acetate extracts led to a significant decrease in NIH-3T3 cell viability compared to the control (40.90 ± 0.361%, 44.50 ± 1.04%, and 46.99 ± 0.652%, respectively) ($P < .01$). In addition, as a result of the calculations made with the data obtained, the IC_{50} values of the *n*-hexane and ethyl acetate extracts

Table 3. Minimum Inhibitory Concentration Values of *Marrubium rotundifolium* Extracts (µg/mL)

		Extracts		
Strains		**Methanol**	**Ethyl Acetate**	***n*–Hexane**
Gram (+) bacteria	*Staphylococcus aureus*	128	256	256
	Enterococcus faecalis	128	256	128
	Bacillus subtilis	256	128	128
Gram (–) bacteria	*Escherichia coli*	512	1024	1024
	Salmonella enterica	512	512	1024
	Pseudomonas aeruginosa	512	1024	1024
Fungi	*Candida albicans*	512	1024	1024
	Candida parapsilosis	512	512	512

Table 4. Cytotoxic activities of *Marrubium rotundifolium* Extracts (IC_{50}, µg/mL)

Extract	Caco-2	PC-3	SH-SY5Y	NIH-3T3	HK-2
Methanol	0.778 ± 0.048	0.173 ± 0.018	NA	0.750 ±0.003	0.119 ±0.002
Ethyl acetate	0.262 ± 0.009	NA	NA	0.139 ± 0.001	0.156 ±0.011
n-hexane	0.388 ± 0.031	0.243 ± 0.033	NA	0.145 ± 0.009	0.159 ±0.017

Values are represented as mean ± standard deviation.
NA, not active at 100 µg/mL concentration.

were determined as 0.145 ± 0.009 and 0.139 ± 0.001 µg/mL, respectively.

In HK-2 cells, the treatment with 100 µg/mL of *M. rotundifolium* methanol, *n*-hexane, and ethyl acetate extracts for 48 hours caused a significant decrease in cell viability compared to control cells by 47.85 ± 0.724%, 40.54 ± 1.356% and 43.67 ± 0.872%, respectively ($P < .01$). As a result of the calculations made with the findings obtained, the IC_{50} values of the methanol, *n*-hexane, and ethyl acetate extracts were determined as 0.119 ± 0.002, 0.159 ± 0.017 and 0.156 ± 0.011 µg/mL, respectively.

DISCUSSION

This research investigates the antioxidant capacities and potential cytotoxic effects of different polarity extracts of *M. rotundifolium*. Our study is the first report that investigates the antioxidant capacity of the plant in detail.

In previous reports concerning the antioxidant activities of *Marrubium* species, the DPPH radical scavenging activity test was widely used among the antioxidant activity assays. Also, it is reported many times that methanol/ethanol extracts have more antioxidant activity than essential oils and other apolar extract types such as ethyl acetate, chloroform, and acetone extracts.[19-21] Similarly, in our $OH^{\cdot -}$, ABTS, and DPPH radical scavenging effect studies, *M. rotundifolium* methanol extract showed higher IC_{50} values at lower doses (0.277 ± 0.024, 3.21 ± 0.081, 0.033 ± 0.001 mg/mL, respectively). The radical scavenging effects are thought to be due to the high phenolic content in methanol extracts. Five different extracts (acetone, methanol, petroleum ether, ethyl acetate, and sodium hydrogen carbonate) were prepared from the aerial parts of *Marrubium peregrinum* with the study carried out by Stankovic in Serbia.[21] The activity of the extracts was expressed as percent inhibition of DPPH radicals and IC_{50} values (µg/mL). Percentage values range from 27.26% to 89.78%. The largest capacity to neutralize DPPH radicals was found for methanol extract, which neutralized 50% of free radicals at the concentration of 187.41 µg/mL.[21] Furthermore, in 2010, *n*-hexane and methanol extracts were prepared from the leaves of the *M. parviflorum* collected from Gaziantep to study the in vitro antioxidant activity by Yumrutas and Saygideger.[22] The extracts' TPC and TFC were investigated by β-carotene/linoleic acid, DPPH, ABTS, potency reduction, and metal chelation tests. Since methanol extracts of these plants contain a high amount of phenolic compounds, they exhibited more significant antioxidant activity than *n*-hexane extract. *M. parviflorum* methanol extract has been reported to have the following antioxidant potentials: DPPH: 22.72 ± 0.11 mmol TE/g dw, ABTS: 34.10 ± 1.80 mmol TE/g dw, power reduction test: 46.34 ± 2.43 mmol AAE/g dw, and metal chelation test: 11.47 ± 0.81 mmol EDTAE/g dw.[22]

According to our results obtained from determining the TPC by the Folin-Ciocalteu method, no phenolic content was observed in the *n*-hexane and ethyl acetate extract, while a phenolic content of 2.62 ± 0.16 GAE µg/mL was observed in the methanol extract. In a previous study conducted in Muğla, the TPC of the *M. globosum* subsp. *globosum* was found to be the highest in the polar subfractions (25.60 ± 0.74 µg/mL).[23] Likewise, in another study, among the petroleum ether, ethyl acetate, chloroform, butanol, and methanol extracts obtained from the leaves of the *Marrubium deserti*, the TPC was found to be higher in the methanol extract by the Folin-Ciocalteu colorimetric method.[24] As a result, when we search the studies conducted in this field with *Marrubium* species, it is generally seen that the TPC is higher in polar extracts in parallel with the results we obtained.

Determining the in vitro cytotoxicity of plant extracts is the first step toward investigating the anticancer effects of compounds derived from natural sources. With this in mind, the cytotoxic effects of methanol, ethyl acetate, and hexane extracts from the aerial parts of *M. rotundifolium* were investigated on 3 cancerous (Caco-2, SH-SY5Y, and PC-3) and 2 non-cancer cell lines (NIH-3T3 and HK-2) by the MTT method. No significant results were found in the extracts generally. Only methanol extract of *M. rotundifolium* (100 µg/mL) caused a significant decrease in PC-3 cell viability compared to the control cells (40.37 ± 1.32%, IC_{50}: 0.173 ± 0.018 µg/mL) ($P < .01$). When we look at the other studies in this field, the cytostatic effects and cell growth inhibitory activities of the methanol extract obtained from the aerial parts of the *Marrubium thessalum* against HeLa, MCF-7, FM3, and HCT-116 cancer cells lines were demonstrated by MTT assay. It has been reported to exhibit potent cytotoxic activity against cell lines.[25] In another study, the cytotoxic effects of *Marrubium crassidens* methanol extract against MCF-7 (breast cancer) cell line were demonstrated by the MTT method, too.[26] In previous studies with other *Marrubium* species, the cell lines we tested were not used.

According to the results of antimicrobial activity experiments, the extracts inhibited the growth of 3 Gram-positive bacteria at the concentration of 128 or 256 µg/mL. When the MIC values were compared, the extracts were found to be more effective against Gram-positive strains than Gram-negative bacteria and

yeasts. In parallel to our findings, Zarai et al[27] showed that the *Marrubium vulgare* essential oil had antimicrobial activity against Gram-positive bacteria including *S. aureus*, *E. faecalis*, and *B. subtilis* strains.[27] In another study, Ulukanlı and Akkaya[28] reported that the hexane extracts of *Marrubium catariifolium* and *Phlomis pungens* var. *hirta* compared to the acetone and methanol extracts exhibited antibacterial activity against Gram-positive bacteria such as *S. aureus*, *Staphylococcus epidermidis*, and *B. subtilis* except *E. faecalis*.[28] In our studies, it was demonstrated that the MIC value of the ethyl acetate extract was 128 µg/mL against *S. aureus* and *E. faecalis*. Ethyl acetate extract inhibited the growth of Gram-negative bacteria and yeast strains at 512 or 1024 µg/mL concentrations. In addition to the inhibitory effects against Gram-positive bacteria, methanol and *n*-hexane extracts also inhibited the growth of Gram-negative bacteria and yeast at 512 or 1024 µg/mL concentrations.

Although the rich traditional uses are high in a number of Anatolian *Marrubium* species, the knowledge about the phytochemical and bioactivity is still limited. Many *Marrubium* taxons are used as an antipyretic, analgesic, diuretic, and against the cold in Anatolian folk medicine.[29-31] However, all the uses are associated with the plants' antioxidant and antimicrobial potentials, and more research is needed with *Marrubium* species. Also, *M. rotundifolium* is a much districted studied plant, and further detailed phytochemical studies and more bioactivity studies should be performed to understand the causality of traditional uses of this plant.

CONCLUSION

Marrubium species attract significant attention in the world. Although the gene center is accepted as Turkey due to the high rate of endemism, and it shows a dense distribution area, scientific studies on *Marrubium* species in Anatolia are limited. This study is the first detailed study that examines antioxidant, antimicrobial, and cytotoxic properties of endemic *M. rotundifolium*. Although there is a potential for an antioxidant and antimicrobial effect in general, weak cytotoxic effects were observed. Further detailed phytochemical studies are needed to explain the causality of the activities.

Ethics Committee Approval: The manuscript does not contain any studies with human participants or animals.

Informed Consent: The manuscript does not contain any studies with human participants or animals.

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Original Article

The Impact of COVID-19 Outbreak on Electrophysiological Procedures: A Single-Center Tertiary Experience

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ABSTRACT

Objective: We aimed to investigate the impact of novel coronavirus pandemic on the number and diversity of electrophysiology procedures in a tertiary referral electrophysiology unit in Turkey.

Methods: All consecutive electrophysiology procedures were retrospectively analyzed performed in our electrophysiology department between January 2017 and March 2021. The number of procedures and the distribution of cases per month were calculated preceding and during the pandemic. The diversity of the procedures was also evaluated. We compared the number of electrophysiology procedures between pre-coronavirus disease and post-coronavirus disease period.

Results: Overall, the electrophysiology procedures were decreased by 11.1% compared to previous years ($P = .017$). The most significant difference was observed in April (−89.8%), May (−66.1%), November (−21.7%), December (−29.4%) 2020, and the first month of 2021 (−38.8%). These intervals coincided with the peak coronavirus disease incidence in our country. Atrial fibrillation and supraventricular tachycardia ablation rates significantly dropped by 22.2% ($P = .038$ and $P = .039$; respectively) throughout the coronavirus disease outbreak; however, only mild non-significant change occurred in the number of ventricular tachycardia ablations.

Conclusion: The coronavirus pandemic has significantly affected the number of electrophysiological studies in our center. It is apparent that this pandemic will be affecting our practice for a while. We need to develop contemporary measures to improve healthcare for non- coronavirus disease patients.

Keywords: Electrophysiology, COVID-19, arrhythmia, coronavirus

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was declared as a pandemic on March 11, 2020, by the World Health Organization and spread throughout the world rapidly.[1] The novel coronavirus infected more than 3 million Turkish inhabitants since its first detection in March 2020.[2] The capacity of the hospitals was overwhelmed by the increasing number of affected individuals. Most of the countries prioritized COVID-19 management and implemented extensive precautions such as social distancing and suspending elective procedures to overcome this unprecedented situation. Hospitals rearranged their in-hospital services to divert their all existing sources through this challenging period. Officials announced avoiding hospital admission in non-emergent/non-urgent conditions.

Coronavirus disease 2019 has devastating and life-threatening outcomes relating to the cardiovascular system; arrhythmias are frequently observed as a result of medications and the complications that occurred by the disease itself.[3,4] Thus, all units and cardiac electrophysiologists only performed highly essential procedures in non-COVID patients to minimize the risk of disease transmission as well as to prevent healthcare system crisis. Consequently, the overall usual patient care was hampered unwillingly. Herein, we report our experience about the impact of the COVID-19 outbreak on the number and the diversity of electrophysiological (EP) procedures conducted at a tertiary referral center in Turkey.

METHODS

Data Collection

This is a single-center observational retrospective study. We analyzed all consecutive electrophysiological procedures performed in a tertiary referral center between January 2017 and March 2021. Procedures were divided into 2 main groups: (i) procedures

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performed before the declaration of COVID-19 outbreak (pre-COVID) and (ii) procedures performed after the declaration of COVID-19 outbreak (post-COVID). The procedural distribution was also categorized into 4 quarters: (i) January 1 to March 31 (first quarter), (ii) April 1 to June 31 (second quarter), (iii) July 1 to September 30 (third quarter), and (iv) October 1 to December 31 (fourth quarter). The data were extracted from the electronic health record system of our hospital. The mean number of studies was calculated for each month and quarter preceding January 2020. These parameters were compared with the post-COVID numbers calculated in the same way.

Procedures were categorized as ventricular tachycardia (VT) ablation, atrial fibrillation (AF) ablation, and supraventricular tachycardia (SVT) ablation. Atrial fibrillation ablations performed either by cryoballoon or radiofrequency ablation were included in this subgroup. The supraventricular tachycardia ablation group consists of atrioventricular nodal reentrant tacycardia, atrioventricular reentrant tachycardia, and atrial tachycardia (AT). All procedures were also sub classified as conventional ablation procedures which were performed without 3-dimensional electroanatomic mapping systems and complex ablation procedures which were performed via 3-dimensional electroanatomic mapping systems.

Periprocedural Precautions

A detailed assessment for COVID-19 symptoms was done on all patients scheduled for the EP study. A routine polymerase chain reaction test for the novel coronavirus was obtained in whom general anesthesia was preferred (catheter ablation of VT, AF, and AT). On detecting a positive test, the procedure was postponed if the medical condition was deferrable. Periprocedural measures were undertaken to minimize the risk of transmission according to local institutional recommendations.

Statistical Analysis

All analyses were done using Statistical Package for the Social Sciences software version 25 (IBM SPSS Corp.; Armonk, NY, USA). Categorical data were depicted using percentage and frequency and numerical data were depicted using means and standard deviations. Chi-square or Fisher's test was used to compare 2 groups as appropriate. *P*-value < .05 was considered statistically significant.

Main Points

- We report the impact of the coronavirus disease (COVID) pandemic on the electrophysiology (EP) procedures performed in our EP department which showed a significant reduction compared to the pre-COVID period.
- Overall, the effect is inversely correlated with the course of the pandemic. The most significant decrease was noticed in April, May, November, and December when the COVID-19 incidence was the highest.
- The catheter ablation of atrial fibrillation and supraventricular tachycardias were markedly reduced, while no significant difference was observed in terms of ventricular tachycardia ablation compared to previous years.
- We also observed that rebooting normal activity was instantly provided when the incidence of COVID-19 was under control.

RESULTS

The average number of annual EP procedures performed in our center was 762 in the pre-COVID period. Overall, the number of procedures decreased by 11.1%. In total, 677 studies were performed during the COVID outbreak. The procedural details are depicted in Table 1. The number of AF and SVT ablations significantly reduced by 22.2% during the COVID pandemic ($P = .006$ and $P = .003$, respectively) On the other hand, there was a statistically non-significant increase in the number of VT ablation during the COVID pandemic compared to pre-COVID time (+3%, $P = .036$). April (89.8%) and May (66.1%) were the months when the most significant patient decrease was observed ($P < .001$) We performed AF ablation in 3 patients, VT ablation in 1 patient, SVT ablation in 4 patients in April and AF ablation in 9 patients, VT ablation in 5 patients, SVT ablation in 8 patients in May. Moreover another significant drop was noticed in November (21.7%) and December (29.4%) ($P = .039$ and $P = .038$, respectively). The comparison of the amount of procedural distribution throughout the pandemic is depicted in Figure 1. The patient admission continued to decrease in January 2021 where a statistically significant reduction was observed compared to both the pre-COVID period (38.8%; $P = .009$) and the previous year (58.4%; $P < .001$). We also analyzed the procedural distribution in 4 quarters to minimize the potential month-to-month variability, and we observed a reduction in the procedural rate (54.9%) during the second quarter (April-May-June) compared to previous years (Figure 2).

DISCUSSION

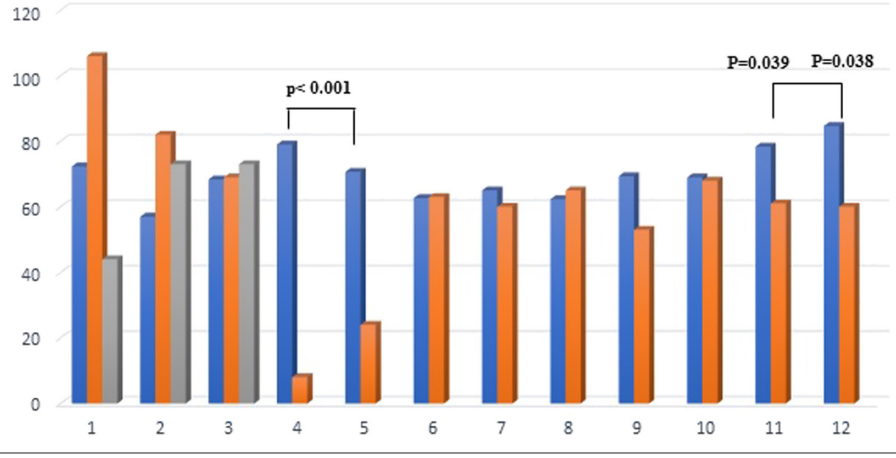
In the present study, we aimed to evaluate the effect of a novel COVID pandemic on the EP procedures performed in our department. The findings of our study can be summarized as follow: (i) the number of electrophysiological studies significantly reduced during the pandemic compared to previous years, (ii) there was 3 distinct period during which the most significant reduction occurred, April to May 2020, November to December 2020, and

Table 1. The Procedural Details Before and During COVID–19 Outbreak

	Pre–COVID (n)	Post–COVID (n)	*P*	Change (%)
Complex EP (3D mapping)	628	538	.008	−14.3
Conventional EP	134	139	.717	+3
VT ablation	96	109	.364	+13.5
AF ablation	274	213	.006	−22.2
SVT ablation	319	248	.003	−22.2
Total	762	677	.017	−11.1

EP, electrophysiology; VT, ventricular tachycardia; AF, atrial fibrillation; SVT, supraventricular tachycardia; 3D, 3 dimensional; COVID, coronavirus disease.

Figure 1. Procedural distribution pre- and post-COVID era. Blue column: pre-COVID, orange column: post-COVID (2020), gray column: post-COVID (2021). COVID, coronavirus disease.



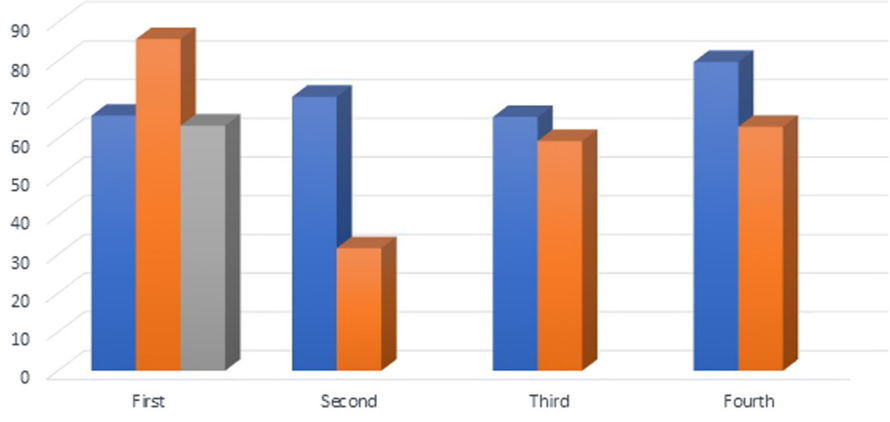
at the beginning of 2021, (iii) the rate of procedures was similar to the pre-COVID period during the remaining months of the year, and (iv) we also observed that the number of VT ablations was not affected by the outbreak whereas all other EP procedures decreased in the same period.

The current outbreak directly or indirectly affected the routine daily function of the hospitals. Several recommendation papers were published recently about the organization of EP units and appropriate patient triage during the pandemic.[5-7] Nevertheless, massive COVID-related hospital admissions occupied most of the clinics which in turn limited and hampered non-COVID patient care in clinical practice.

Previously, several clinical and survey studies all over the world demonstrated similar results at the beginning of the pandemic.[8,9] The number of cardiac device implantation and EP procedures was extremely reduced in all countries especially when the pandemic intensified. More than 50% of the reduction was observed in high-volume EP laboratories.[10,11] Non-COVID-patient care was jeopardized as the COVID incidence accelerated. Current data show us that the fluctuating course in the number of cases during the pandemic causes serious disruptions in the treatment and follow-up of patients without COVID-19. It is apparent that the physicians are trying to adapt themselves to this challenging disease. Although more than a year has passed since the onset of the pandemic, there has been an unpreventable increase in the COVID-19 incidence and mortality rates. Unfortunately, it seems that we will continue to face the current situation for a while. Thus, we need to do our best to ensure that arrhythmia patients receive adequate treatment.

In our analysis, there was 2 distinct time interval when we observed a marked reduction in the number of procedures. These intervals were correlated with the peak COVID-19 incidence in

Figure 2. Procedural distribution pre- and post-COVID era. The number of EP procedures was significantly reduced in the second quarter ($P < .001$) Blue column: pre-COVID, orange column: post-COVID (2020), gray column: post-COVID (2021). COVID, coronavirus disease.



our country. Although we tried to perform all undeferrable and essential procedures, the number of patients was inevitably influenced by the pandemic.

Interestingly, our data showed that the incidence of VT ablation did not differ from previous years. A survey study conducted in Poland among electrophysiologists showed similar results that all other EP procedures including cardiac device implantation reduced significantly in the second quarter of the year, while the number of VT ablation was found to be similar or higher in their clinical practice.[12] The finding may be due to the fact that VT ablation is more urgent than the other procedures, and unlike AF or SVT ablation, it is undeferrable. Also, this may be a coincidental presentation.

The significant reduction in the rate of EP studies is considered to be multifactorial. First, this may be attributable to the hesitance of the patients on hospital admissions to avoid COVID transmission. Besides, elective procedures were deferred by the physicians as recommended during the periods of increased COVID-19 incidence to prevent hospital overload. Moreover, we admit a considerable amount of patients from all across the country as being a referral center. Thus, general orientation in the healthcare system is also reflected in our procedural statistics. Additionally, we need to keep in mind that the electrophysiologist, as well as physicians, from other specialties were assigned to a COVID unit or were infected by the disease which also had a negative influence on our EP practice.

Another issue that needs to be addressed is the potential long-term complications of the novel COVID infection. Although acute respiratory distress is the principal manifestation in the majority of the cases, multisystemic involvement including the cardiovascular system is common. Coronavirus disease-related arrhythmias are associated with high mortality and morbidity in hospitalized patients.[13] Several mechanisms were proposed as responsible triggers for the arrhythmia development.[14] Additionally asymptomatic myocarditis is also prevalent in patients recently recovered from severe acute respiratory syndrome coronavirus-2 (SARS-CoV 2) infection which was illustrated by cardiac magnetic resonance imaging.[15] The long-term consequences of possible arrhythmic complications in individuals exposed to SARS-CoV 2 virus are unknown yet. Potentially, electrophysiologists will have to encounter such patients in the future which will increase the burden of EP units significantly. Rebooting usual patient care is of utmost importance in this setting.

Limitations

There are several limitations of our study. First, this is a single-center retrospective observational study. Multicenter studies from different regions are needed to confirm our findings. Second, we only observed a specific time interval so we are unable to make any assumption about the long-term outcome of this pandemic on arrhythmia patients. Furthermore, since our country started vaccination recently, we have no data regarding its potential favorable impact on the overall healthcare system and the organization of EP laboratories.

CONCLUSION

To our knowledge, this is the first study showing the effects of the current pandemic on an EP Unit in Turkey. Our study showed that the EP procedures were significantly affected by the outbreak. The pandemic created an unprecedented clinical scenario. The need for solid measures has emerged in the management of similar unexpected situations that we will encounter in the near future.

Ethics Committee Approval: This trial was a retrospective observational non invasive statiscal study thus we did not apply for ethical approval.

Informed Consent: This was a retropective study. No informed consent was available from the patients.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – B.S., H.Y., K.A.; Design – B.S., K.A.; Supervision – H.Y., K.A.; Resources – A.H.A.; Materials – B.S., A.H.A.; Data Collection and/or Processing – B.S., A.H.A.; Analysis and/or Interpretation – B.S., H.Y.; Literature Search – B.S., A.H.A.; Writing Manuscript – B.S., K.A.; Critical Review – B.S., A.H.A., H.Y., K.A.

Declaration of Interests: The authors have no conflicts of interest to declare.

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Original Article

Food Allergy and Philagrine Mutation in Children with Atopic Dermatitis

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ABSTRACT

Objective: To determine the frequency and type of food allergy in patients having atopic dermatitis and to show the presence of mutations genetically.

Methods: Patients diagnosed as having atopic dermatitis according to the Hanifin Rajka criteria were evaluated retrospectively. Eosinophils, total immunoglobin E, milk-specific immunoglobulin E, egg-specific immunoglobulin E, wheat-specific immunoglobulin E, and filaggrin gene mutation results were recorded. Nutrient elimination was performed for 1 month in patients who were thought to have food allergy owing to skin prick test and milk-specific immunoglobulin E results. The diagnosis was confirmed through a food loading test for the patients who benefited from the elimination.

Results: Of the 66 patients included in the study, 42 (63.63%) were male. Food allergies were detected in 40 patients (60.6%). According to the Scoring of Atopic Dermatitis index, 9 out of 16 patients aged 40 years and over had food allergy and 31 out of 50 patients aged under 40 years had food allergy. There was no significant difference between the groups ($P = .56$). All patients included in the study were examined for filaggrin. Only 1 patient with a Scoring of Atopic Dermatitis index below 40 and milk allergy was found to have p.R501 * and c.2282-2285delCAGT mutations.

Conclusion: Atopic dermatitis food allergy was found to be 60.6%. The most common improvement was egg allergy and egg elimination. There was no difference between atopic dermatitis severity and food allergy and laboratory tests. Severe atopic dermatitis was found to be 24.2%.

Keywords: Child, atopy, allergy

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease that usually starts in infancy and early childhood occurring as a result of the interaction of genes and environmental factors and can be triggered by various allergens.[1] The frequency of AD is 15-20% in children living in developed countries, whereas it is between 1% and 3% in adults, and the lifetime frequency is 17.3%.[2] It is known that AD is associated with food allergies. However, a relationship between AD and food allergy has been shown in only one-third of patients with moderate-to-severe AD.[3] Although AD has a mild clinical course in 70-84% of patients, approximately 20% of patients present with a severe clinical course.[4,5] Filaggrin (FLG) plays a critical role in epidermal barrier function. The importance of FLG mutations was first noticed in 2006 with the discovery of dysfunctional mutations in patients with ichthyosis vulgaris.[6] Studies have shown that the FLG mutation is community-specific. The R501X and 2282de14 mutations, which were found frequently, and S3247X and R2447X mutations, which were found rarely, were found in 7-10% of the Caucasian European population in studies.[7] The relationship between AD and food allergy has been brought to the agenda again because FLG mutations have been considered as a possible mechanism in the development of AD in recent years. Changes in this gene are thought to play a key role in cutaneous exposure to food allergies, by increasing the permeability of the skin to proteins.[8]

Food allergies often accompany AD, which is very common in children. It is known that genetic factors play a role. In our study, we aimed to show the frequency, type, and effect of FLG mutations in patients who were diagnosed as having AD and evaluated the severity using the Scoring of Atopic Dermatitis (SCORAD) index.

METHODS

Patients diagnosed as having AD according to the criteria of Hanifin Rajka[9] in the pediatric allergy immunology outpatient clinic between March 2018 and October 2018 were evaluated retrospectively. The study was approved by the Ethics Committee of Erzurum Regional Training and Research Hospital Clinical Research Ethics Committee (Date: November 5, 2018, Decision No: 2018/17-167). Patients' age at admission, symptom onset age, symptom frequency, spreading area of dermatitis,

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SCORAD index, food prick, inhaler prick, eosinophil count, total immunoglobin E (IgE), milk-specific immunoglobulin E (sIgE), egg sIgE, wheat sIgE, peanut sIgE, fish sIgE, food provocation tests, and FLG gene mutation results were examined and recorded from medical records. The patients were followed up and evaluated by the same allergy immunologist throughout the study. The SCORAD index was calculated according to the formula: A (prevalence)/5 + B (density) × 7/2 + C (subjective symptoms; itching severity + loss of sleep). If the score was <15, AD was considered to be mild, if it was between 15 and 40, AD was considered to be moderate, and if it was >40, AD was considered to be severe.[10] The patients were divided into 2 groups as mild-moderate and severe AD, and the groups were compared in terms of food allergy, susceptible allergen sensitivity, and laboratory results. Skin test results of eggs, sIgE milk, wheat, peanuts, and fish were examined, and those with values ≥0.35 IU/mL were considered positive. The allergen solutions of Allergopharma® (Reinbek, Germany) with standard activity and concentrations including standard food allergens belonging to milk, egg yolk, egg white, wheat, hazelnut, peanut, lentil, sesame, walnut, fish, soybean were used while evaluating skin prick tests (SPT). Skin prick tests with 12 standard aeroallergens were performed (Allergopharma) with *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria*, *Cladosporium*, *Aspergillus*, *Candida albicans*, tree pollen mixture 1, tree pollen mixture 2, grass pollen mixture, weeds, cockroach, and 10 mg/mL histamine phosphate as positive control and 0.9% saline as negative control. Those with sIgE values >0.35 IU/mL and above in blood were considered positive. Prick test result 3 mm and above from negative control was accepted as positive. Food elimination was performed for 1 month in patients who were diagnosed with AD and who were thought to have food allergy with prick test and sIgE results. The diagnosis was confirmed by performing an oral food challenge (OFC) test in patients who benefited from elimination. Oral food challenge tests were performed in the form of open provocation about 4-6 hours after fasting early in the morning while the patients were completely healthy and their eczema was completely under control. Drugs containing antihistamines that could mask early allergic reactions and develop in the OFC were discontinued for 2 weeks before the OFC. The families of the patients were informed before the OFC. All risks were explained, and the patients signed an informed consent form. Foods to be used in the OFC were prepared freshly and the test started with 0.1 mL of cow milk, 1 g of egg, wheat, lentil, sesame, and 125 mg of walnut. The doses were doubled at 15-minute intervals, and the patients were examined after each dose. The doses were increased up to 100 mL milk, 50 g eggs, 50 g wheat, and lentils in patients without reactions. In the OFC, the test was considered positive in patients with type 1 hypersensitivity reactions such as urticaria and angioedema, and anaphylaxis within the first 4-6 hours. Some of these patients had a rash only during the test, whereas some had an exacerbation in the eczema within a few days and their test was considered positive. Foods were freed in the diet if no reaction was observed.[11] Filaggrin mutation analyses were performed in the genetic diseases diagnosis center. In this study, a new-generation sequence analysis was performed for the common R501X and 2282de14 mutations. The study was planned retrospectively: 13 patients who were thought to have food allergy according to OFC, SPT, and/or food sIgE results but could not be tested for provocation were excluded from the study. Forty patients with food allergies were tested with a total of 78 different foods because some patients had more than 1 food allergy, 68 of which were positive. The families of the patients were informed about the food challenge test and signed an informed consent form. The statistical analysis of the study was performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (IBM Inc, Chicago, IL, USA) program. For descriptive statistics of the study data, continuous data are given as mean ± standard deviation, median, and minimum–maximum, and categorical variables are given as numbers and percentages. Pearson's chi-square and Fisher's exact tests were used in comparisons.

Main Points

- It is a known fact that atopic dermatitis (AD) is associated with food allergies. However, a relationship between AD and food allergy was shown in only 1/3 of patients with moderate to severe AD.
- Food allergy was detected in 40 (60.60%) of the samples. We found egg allergies to be the most common. Egg sensitivity was observed in 35 patients (53.03%) in total, and the diagnosis was confirmed by elimination and loading.
- No significant correlation was found between the severity of AD and food allergy and laboratory tests. Severe AD was found to be 24.2% (16). In our study, the rate of FLG mutations was found to be 1.51%.

RESULTS

Of the 66 patients included in the study, 42 (63.63%) were boys and 24 (36.3%) were girls. The average age at admission was 17.9 ± 19.4 (min: 4, max: 98) months. Symptom onset age was 5.5 ± 8.4 (min: 1, max: 48) months. The most common symptoms were erythema, edema/papulation, and dryness in 53 (80.3%) patients, whereas in 13 (19.6%) patients, the most common symptom was skin irritation/crusting, excoriation, and lichenification.

Table 1. Clinical Features of Patients with Atopic Dermatitis

Male/female, n (%)	**42 (63.6%)/24 (36.3%)**
Symptom onset age (months) (min–max)	5.5 ± 8.4 (1–48)
Age at admission, months (min–max)	17.9 ± 19.4 (4–98)
Average IgE, IU/mL (min–max)	81.0 ± 181.46 (1–1182)
Average eosinophil count, mm^3 (min–max)	489.6 ± 406.3 (10–2340)
Patients with food allergy	40 (60.6%)
SCORAD index average (min–max)	28.2 ± 11.2 (15–60)

IgE, immunoglobulin E; SCORAD, Scoring of Atopic Dermatitis.

Table 2. Comparison Between Patients with Mild–Moderate Atopic Dermatitis and Patients with Severe Atopic Dermatitis

	SCORAD <40	SCORAD ≥40	*P**
Total IgE average, IU/mL (min–max)	81.98 (1–980)	77.25 (15–1182)	.664
Average eosinophil count, mm^3 (min–max)	451.16 (10–2340)	610.0 (10–1500)	.241
FLG mutation, n	1	0	.758
Food allergy, n (%)	31 (62)	9 (56.2%)	.560
Inhalant allergen sensitivity, n (%)	6 (12)	3 (18.7%)	.67

n, number of patients; mild–moderate atopic dermatitis, SCORAD<40; severe atopic dermatitis, SCORAD>40.
**P*: Fisher's exact test.
IgE, immunoglobulin E; SCORAD, Scoring of Atopic Dermatitis; FLG, filaggrin.

The mean total IgE level was 81.0±181.46 (min: 1, max: 1182) IU/mL, and the mean eosinophil count was 489.6 ± 406.3 mm^3. The mean SCORAD index was 28.2 ± 11.2 (min: 15, max: 60) (Table 1). In 21 (31.8%) patients, lesions were observed on the cheek and head, in 19 patients (29.7%) on cheek + trunk + limb, in 16 patients (24.2%) on cheek + trunk, and in 10 patients (15.1%) on cheek + extremity. A SCORAD index ≥40 was considered as severe AD. In this study, the SCORAD index of 40 patients (24.24%) was ≥40 and <40 in 50 (75.75%) patients. Forty-nine (74.2%) patients with a SCORAD index of 15-40 were evaluated as having moderate AD, and 1 patient (1.51%) with a SCORAD index of <15 was considered as having mild AD. Patients with mild and moderate AD were grouped together. A comparison was made between patients with mild-moderate and severe AD. The mean total IgE and eosinophil counts were compared and no significant difference was found between the 2 groups (*P* = .664, *P* = .241, respectively) (Table 2). In 16 (24.24%) patients with a SCORAD index ≥40, 9 (56.2%) had food allergy, and in 50 (75.75%) patients with SCORAD index <40, 31 (62%) had food allergy. There was no significant difference between the groups (*P* = .56). Allergen sensitivity was detected in 9 (13.6%) patients. Inhaler allergen sensitivity was detected in 6 (12%) patients with a SCORAD index <40 and in 3 (18.7%) patients with a SCORAD index >40 (*P* = .67) (Table 2). Filaggrin was investigated in all patients included in the study and p.R501X and c.2282del4 mutations were detected in only 1 (1.51%) patient who had a SCORAD index <40 and milk allergy. There was no difference between the 2 groups (*P* = .785) (Table 2).

According to the SPT and/or food sIgE result, OFC was performed in 46 of 66 patients. Forty-six patients with food allergies underwent OFCs with different foods 78 times in total (egg: 39, milk: 30, wheat: 5, sesame: 1, lentil: 2, walnut: 1). As a result, 68 OFCs were positive in 40 patients due to multiple food allergies. Food allergy was not found in 26 (39.39%) patients. Milk and egg allergy in 18 patients (27.27%) diagnosed with food elimination and loading, milk + egg + sesame allergy in 1 (1.51%) patient, milk + egg + walnut allergy in 1 patient, egg allergy in 12 patients (18.1%), milk allergy in 4 patients (6.06%), milk + egg + wheat allergy in 2 patients (3.03%), and wheat allergy in 1 patient (1.51%) were observed. Multiple food allergies were observed in 24 patients (Table 3).

When patients who were found positive in specific IgE and/or SPTs and confirmed by food elimination and challenge tests were evaluated, egg allergy in 35 (53%) patients, milk allergy in 27 (40.9%) patients, wheat allergy in 3 (4.5%) patients, sesame allergy in 1 (1.5%), lentil allergy in 1 (1.5%), and walnut allergy in 1 (1.5%) patient were detected (Table 4). These foods were eliminated from the diet for 1 month and clinical improvement was observed. The diagnosis of food allergy was confirmed through an OFC. Food allergy was detected in 40 (60.60%) patients. Egg allergy was the most frequently encountered allergy.

DISCUSSION

In our study, the rate of boys (63.6%) was higher. In a study involving 110 infants with AD, it was found that 70% were male.[12] In our study, food allergy was found in 60.60% of patients with AD. In a study conducted in Korea, food allergy was found in 37.1% of infants with AD aged 0-5 months and in 38.5% of infants aged 6-11 months.[13] In genetically susceptible individuals, AD is accompanied by an IgE-mediated food allergy ranging from 30% to 60% in infancy.[14] In our study, the most common allergies were milk + eggs (31.8%), eggs (18.1%), milk (6.06%), milk + egg + wheat (3.03%), and wheat (1.51%). Egg allergy was detected in 35 (87.5%) of 40 patients in whom food allergy was confirmed through food elimination and OFC. In an international cohort study, 64% of 2184 infants with AD aged 11.8-25.4 months were reported to develop IgE-mediated food sensitivity to egg and/or cow milk and/or peanut before the age of 3 months.[15]

In our study, the mean SCORAD index was found to be 28.2 ± 11.2, and the most common sIgE and/or SPT test positivity was observed for egg. There was no difference between SCORAD index ≥40 and <40 in terms of total IgE and eosinophil counts. In a study evaluating the relationship between SCORAD index and atopy patch tests in patients with AD, the mean SCORAD index was 37.3 ± 12.13 (range: 15.1-66.0), the fresh prick test with food was found positive in 32.5%, and in the atopy patch test, the highest positivity was for egg (54.1%). In this study, no relation was observed between SCORAD index and prick test positivity, total IgE, and serum eosinophil count.[16] These results were similar to our results. In a study in which 236 patients with AD were examined, food allergen sensitization was positive in 31% of patients, and the most common food allergies were

Table 3. Food Challenging Test Results

Number of patients who underwent food provocation test, n (%)		**46 (69.6)**
Mean age of patients who underwent food provocation test (min–max)		8 (6–72)
Number of food provocation tests performed		78
Foods used in food provocation test (%)	Egg	39 (59.09)
	Milk	30 (45.4)
	Wheat	5 (7.5)
	Sesame	1 (1.51)
	Lentil	2 (3.03)
	Walnut	1 (1.50)
Number of patients with positive food provocation test result, n (%)		40 (60.6)
Distribution of patients with food allergy, n (%)	Egg + milk	18 (27.27)
	Egg + milk + lentil allergy	1 (1.51)
	Egg + milk + sesame allergy	1 (1.51)
	Egg + milk + walnut allergy	1 (1.51)
	Egg	12 (18.1)
	Milk	4 (6.06)
	Milk + egg + wheat	2 (3.03)
	Wheat	1 (1.51)

found against egg, cow's milk, and peanuts. In that study, the patients were divided into 2 groups according to the severity of AD as mild and medium-severe AD, and no difference was found between the 2 groups in terms of age at symptom onset, sex, family history of atopy, IgE level, eosinophil count, and inhalant allergen sensitization. It was found that food allergy was significantly more common in the medium-severe AD group.[17] In our study, there was no difference between the groups, and food allergy was also observed in the mild AD group.

Inhalant allergen sensitivity was detected in 9 (13.6%) patients, and SPTs with inhalant allergens gave sensitive results. In a study conducted in 64 patients with AD, foods were responsible for allergy in children aged under 2 years, food and inhalant allergens between the ages of 2-10 years, and inhalants above 10 years of age.[18]

Table 4. Foods with Positive Provocation Test

Food type	Number of patients, n = 40 (%)
Egg allergy	35 (53)
Milk allergy	27 (40.9)
Wheat allergy	3 (4.5)
Sesame allergy	1 (1.5)
Lentil allergy	1 (1.5)
Walnut allergy	1 (1.5)

In our study, p.R501X and c.2282del4 mutations were detected in only 1 (1.51%) patient who had a SCORAD index <40 and had milk allergy. There was no difference between the 2 groups. In a study, the relationship between peanut allergy and p.R501X and c.2282del4 mutations was investigated, and it was shown that there was a relationship between peanut allergy and FLG mutations.[19] In a study of 249 Japanese patients with AD, 14 FLG mutations and sensitivity to 15 different allergens were investigated and at least 1 FLG mutation was detected in 25.7% of patients. At least 1 allergen susceptibility was detected in 47.7% of patients. However, only a relationship between peanut allergy and carrying the FLG mutation was found, no relationship was found between the FLG mutation and other allergens.[20] In a study conducted in Indian children, the p.R501X mutation, which was common, was investigated and detected in 5.5% of 90 children with allergy; none of the 81 patients in the control group had these mutations.[21] Surprisingly, although p.R501X and c.2282del4 FLG mutations were common in Europe, these mutations were not found to be significant in a study conducted in the Italian population.[22] In our study, the rate of FLG mutations was found as 1.51%. The FLG mutation was detected in a patient with milk allergy in our study. The limitation of our study was that only the R501X and 2282de14 mutations were investigated, which are common among FLG mutations.

CONCLUSION

The rate of food allergy confirmed through OFCs in patients with AD was 60.6% in our study. Egg allergy was found most frequently in patients with AD. As a result of this study, it could be said that even if the SCORAD index is not high in pediatric patients with AD, it is necessary to investigate food allergy and to decide on elimination in this way. It was thought that the failure to find a relationship with the FLG mutation might be related to social differences or types of food allergy.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erzurum Regional Training and Research Hospital Clinical Research Ethics Committee (Date: November 5, 2018, Decision No: 2018/17-167).

Informed Consent: Informed consent obtained from parents of participants.

Peer-review: Externally peer-reviewed.

Declaration of Interests: The authors have no conflicts of interest to declare.

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Original Article

Laparoscopic Ovarian Drilling Improve Endometrial Receptivity by Increasing Production of Endometrial Metabolites

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ABSTRACT

Objective: Laparoscopic ovarian drilling normalizes ovulation by reducing cortical thickening, lowering androgen production, and regulating luteinizing hormone pulse frequency. On the other hand, the effect of laparoscopic ovarian drilling on the endometrium is unknown. This study was planned to investigate the changes in the functional markers of choline, creatine, lactate, and lipid metabolites of the endometrium before and after laparoscopic ovarian drilling in women with polycystic ovary syndrome.

Methods: Twenty women diagnosed with clomiphene-resistant polycystic ovary syndrome who did not ovulate successfully despite the administration of clomiphene citrate or aromatase inhibitor were included in the study. Patients were offered Assisted Reproductive Technology (ART) or laparoscopic ovarian drilling options. Patients who accepted laparoscopic ovarian drilling formed the study group. Endometrial MR spectroscopy was applied to the participants in the mid-luteal phase before laparoscopic ovarian drilling. Choline, creatine, lactate, and lipid metabolites of all patients were measured and denominated parts per million. The second MR spectroscopy was performed 2 months after the first MR spectroscopy, and the changes in endometrial metabolites after laparoscopic ovarian drilling were recorded. Twenty patients who did not have clinical and laboratory findings of polycystic ovary syndrome and were matched for age and body mass index were accepted as the control group. The patients in this group were selected from fertile women with at least 2 children. MR spectroscopy was performed in the mid-luteal phase in fertile women. The obtained results were compared within and between groups and the possible effects of laparoscopic ovarian drilling on metabolite synthesis were tried to be determined.

Results: During MR spectroscopy examination main endometrial metabolites choline, creatine, lactate, and lipid were detected in the polycystic ovary syndrome group. The most prominent metabolite peak before and after laparoscopic ovarian drilling was recorded as choline and creatine. There was a significant increase in choline and creatine peaks after laparoscopic ovarian drilling compared to the values before laparoscopic ovarian drilling. There was no significant increase in lactate and lipid signals before and after laparoscopic ovarian drilling. The choline and creatine metabolite levels of the women with polycystic ovary syndrome before laparoscopic ovarian drilling were significantly lower than those of the fertile women. The choline and creatine metabolite levels of the women with polycystic ovary syndrome after m laparoscopic ovarian drilling were similar to those of the fertile women. There was no significant difference between lactate and lipid signals before and after laparoscopic ovarian drilling.

Conclusion: Laparoscopic ovarian drilling improves polycystic ovary syndrome-related subfertility by increasing endometrial choline and creatine metabolite levels to those of fertile women.

Keywords: Laparoscopic ovarian drilling, polycystic ovary syndrome, endometrium, metabolite, subfertility

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine problem encountered in infertility practice and is one of the most difficult causes of subfertility for clinicians to treat. Although oligo-anovulation is accepted as the main culprit as a cause of subfertility, it is known that the endometrium of PCOS patients varies considerably compared to fertile patients.[1] In PCOS endometrium, both receptivity genes, steroid receptor expressions, and glucose transport are impaired compared to fertile cases.[2,3] All these reasons may lead to inadequate decidualization and inadequate implantation in PCOS patients. The nearly double increase in obstetric complications in PCOS is another proof that the endometrial environment is defective.[4] While rising insulin and insulin resistance increase luteinizing hormone (LH)-mediated androgen release, decrease hepatic synthesis of sex hormone-binding globulin.[5] Increased androgen

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levels and hyperinsulinemia contribute to subfertility by impairing IGFBP-1 synthesis in the endometrial stroma.[6] All these data are proof that not only the ovulatory process but also the endometrial environment is defective in PCOS.

Lifestyle change and weight loss are the main treatment approaches in subfertile PCOS patients.[1,5,6] Although clomiphene citrate is the first-line treatment in the medical treatment of subfertility, it is very difficult to get an ovulatory response to Clomiphene Citrate (CC) in most of the cases. In CC-resistant PCOS patients, adding low-dose gonadotropin to the treatment or a combination with insulin-sensitizing treatments can be tried in the second step. If there is no response to all these approaches, treatment with aromatase inhibitors is tried. After this stage, patients are either referred to ART, or ovarian drilling is recommended.[7]

Laparoscopic ovarian drilling (LOD) provides an ovulatory process equivalent to medical therapy in patients with clomiphene-resistant PCOS. In addition, it increases the pregnancy rates[7-9] by causing an improvement in the hormonal parameters and insulin resistance of PCOS. There is only one study investigating the effects of LOD on the endometrium.[7] The main reason for the low number of studies is the need for biopsy, which is an invasive procedure, for endometrial evaluation. MR spectroscopy is a non-invasive method used in the evaluation of the endometrium and provides clues about the function of the endometrium. It provides clear information about the current state of endometrial cells according to the metabolite density obtained.[10,11] Choline (Cho), creatine (Cr), lactate, and lipid are the main metabolites detected in the spectroscopy panel of healthy endometrial cells.[10,11] When reviewing the literature the effect of LOD on endometrial Cho, Cr, lactate, and lipid metabolites has not been studied yet. We, therefore, attempted to investigate whether LOD alters the expression levels of basic functional metabolites in the endometrium of infertile women with clomiphene-resistant PCOS.

METHODS

The participants of this case-controlled study consisted of 40 patients, 20 of whom were PCOS and 20 were fertile. Twenty women diagnosed with clomiphene-resistant polycystic ovary syndrome who did not ovulate successfully despite the administration of clomiphene citrate or aromatase inhibitor were included in the study.

Main Points

- Laparoscopic ovarian drilling normalizes ovulation by reducing cortical thickening, lowering androgen production, and regulating luteinizing hormone pulse frequency.
- Laparoscopic ovarian drilling improves polycystic ovary syndrome-related subfertility by increasing endometrial choline and creatine metabolite levels to those of fertile women.
- Mid-luteal phase MR spectroscopy can be used for the evaluation of endometrium metabolite levels.

Patient Selection

The participants of this case-controlled study consisted of 40 patients, 20 of whom were PCOS and 20 were fertile. Twenty women diagnosed with clomiphene-resistant polycystic ovary syndrome who did not ovulate successfully despite the administration of clomiphene citrate or aromatase inhibitor were included in the study. Polycystic ovary syndrome was defined according to revised Rotterdam criteria, which require 2 of the following 3 manifestations: (i) oligo-anovulation (oligomenorrhoea or amenorrhea); (ii) high concentrations of androgen in the bloodstream and/or clinical signs of hyperandrogenism; and (iii) polycystic ovaries shown by ultrasonography (more than 12 follicles measuring 2-9 mm on at least one ovary). When CC treatment fails, defined as failure to ovulate after 6 months of treatment at an appropriate dose, the patient is regarded as resistant to CC. Induction with letrozole was planned as second-line treatment in PCOS patients for whom the diagnosis of CC resistance was certain. Women with PCOS were offered ART or LOD options. Patients who accepted LOD formed the study group. Endometrial MR spectroscopy (MRS) was applied to the participants in the mid-luteal phase before LOD. Twenty patients who did not have clinical and laboratory findings of PCOS and matched for age and body mass index (BMI) were accepted as the control group. The patients in this group were selected from fertile women with at least 2 children. MR spectroscopy was performed in the mid-luteal phase in fertile women. The obtained results were compared within and between groups and the possible effects of LOD on metabolite synthesis were tried to be determined.

Women with PCOS were subjected to progesterone-induced withdrawal bleeding to determine their secretory phases. Preoperative blood samples were taken from PCOS and control subjects for complete hormonal assays and insulin analysis. Insulin resistance was calculated using the homeostasis model assessment-insulin resistance index (HOMA-IR). Hormonal parameters were re-evaluated after the second MRS. Patients who used hormonal medication in the last 6 months or had a history of ovarian surgery and patients diagnosed with hydrosalpinx were not included in the study. Those with endocrine disease, especially diabetes and hypo/hyperthyroidism, were not included in the study. Women taking antiandrogens, antidiabetics, and lipid-lowering drugs were excluded. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee of Vocational School of Beykent University (Date: May 21, 2020, Decision no: 2020/66).

Drilling Procedure

In the PCOS group, LOD was performed in the mid-luteal phase, which was calculated according to progesterone withdrawal bleeding. Laparoscopic ovarian drilling was performed with a monopolar hook and 5 perforations of 2-3 mm deep in the capsule of per ovary bilaterally. Thermal dose adjusted according to ovarian volume (60 J/cm^3 of ovarian tissue). Since there was no difference between performing 5 or 10 punctures (450-750 J) on the ovarian cortex in terms of clinical and reproductive results, each ovary was exposed to 5 punctures.[7] Electrocautery was used due to its ease of application and access to necessary equipment.

Endometrium Spectroscopy

Before ovarian drilling of the endometrium, MR spectroscopy was performed using MR imaging. T1-weighted images (500/20) and T2-WI (1600/80) with 4-mm thick sections were obtained in the axial and coronal planes. Both single and multi-voxel point-resolved spectroscopy sequences with short and long TEs were used. The metabolite ratios of the peaks were determined using Magnetic Resonance User Interface software. Although their intensities were different, the main metabolites detected before or after LOD were the same. The endometrium was first visualized using magnetic resonance imaging before the voxel was placed. Due to the importance of the voxel location on the appropriate endometrial area, the volume of interest was placed to the center of the endometrium. Choline, Cr, lactate, and lipid metabolites of all patients were measured and denominated parts per million. The second MRS was performed 2 months after the first MRS, and the changes in endometrial metabolites after LOD were recorded.[10,11]

Statistical Analysis

The data was analyzed with the Statistical Package for Social Sciences software 21.0 (IBM SPSS Corp.; Armonk, NY, USA) Normality of data was examined by the Shapiro–Wilk test. Pearson's Chisquare was used to test categorical variables. Continuous variables were analysed with Mann–Whitney U-test. Data are presented as mean ± SD. A p value <.05 was accepted statistically significant.

RESULTS

Patients in the PCOS and control groups were found to be similar in terms of mean age and BMI values. Total testosterone, LH, and insulin levels measured before LOD were significantly higher in the PCOS group compared to the control group (Table 1). Similarly, HOMA-IR values of women with PCOS before LOD were significantly higher than the control group. There was a significant decrease in serum testosterone, LH and insulin levels, and HOMA-IR value after LOD (Table 2).

Table 1. Demographic and Hormonal Characteristics of PCOS and Fertile Groups

	PCOS (n=20)	Fertile Control (n=20)	*P*
Age (years)	27.5 ± 0.13	28.6 ± 1.44	.65
BMI (kg/m^2)	24.7 ± 1.43	24.9 ± 2.33	.08
Infertility duration (years)	3.44 ± 2.03	NA	NA
Endometrial thickness (mm)	8.66 ± 2.05	9.12 ± 0.22	.86
Testosterone (ng/dL)	84.6 ± 4.14	44.5 ± 5.11	.001*
LH (mIU/mL)	11.3 ± 1.20	5.43 ± 2.11	.01*
FSH (mIU/mL)	5.08 ± 1.01	4.46 ± 1.43	.32
Insulin (mU/L)	11.4 ± 1.44	6.19 ± 2.04	.03*
HOMA–IR	3.78 ± 1.50	1.56 ± 0.66	.02*

Data are presented as means ± SD.
*$P < .05$.
BMI, body mass index; FSH, follicle–stimulating hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; HOMA–IR, homeostasis model assessment–insulin resistance index; NA, not applicable; SD, standard deviation.

Table 2. Comparison of Endometrial Metabolites and Hormonal Values Before and After Ovarian Drilling

	Before Ovarian Drilling	After Ovarian Drilling	Control	*P**
Choline	1.65 ± 0.33	2.67 ± 1.03	2.87 ± 1.09	.02
Creatine	1.10 ± 0.11	1.85 ± 1.77	1.99 ± 0.33	.03
Lactate	1.02 ± 0.02	0.98 ± 0.08	0.95 ± 0.03	.08
Lipid	0.89 ± 0.30	0.80 ± 0.32	0.81 ± 0.05	.60
Total testosterone (ng/dL)	84.6 ± 4.14	66.5 ± 5.44	44.5 ± 5.11	.01
HOMA–IR	3.78 ± 1.50	2.32 ± 1.03	1.56 ± 0.66	.03
Fasting insulin (mU/mL)	11.4 ± 1.44	8.02 ± 2.54	6.19 ± 2.04	.001
LH (mIU/mL)	11.3 ± 1.20	7.34 ± 2.05	5.43 ± 2.11	.02

Data are presented as means ± SD.
**P* values compared before and after drilling.
BMI, body mass index; FSH, follicle–stimulating hormone; LH, luteinizing hormone; HOMA–IR, homeostasis model assessment–insulin resistance index; SD, standard deviation.

During MRS examination main endometrial metabolites Cho, Cr, lactate, and lipid were detected in the PCOS group (Table 2). The most prominent metabolite peak before and after LOD was recorded as Cho and Cr. There was a significant increase in Cho and Cr peaks after LOD compared to the values before LOD. There was no significant alteration in lactate and lipid signals after LOD. The Cho and Cr metabolite levels of the women with PCOS before LOD were significantly lower than those of the fertile women. The Cho and Cr metabolite levels of the women with PCOS after LOD were similar to those of the fertile women. There was no significant difference between lactate and lipid signals before and after LOD. The decrease in insulin and testosterone levels after LOD was correlated with an increase in Cho and Cr signals. However, the current correlation did not reach statistical significance. No significant correlation was found between the other hormonal and demographic characteristics of the patients in the PCOS group and their endometrial metabolite levels.

DISCUSSION

In subfertile PCOS patients, the following treatment protocols should be tried before going to ART, and if no success is achieved, a higher-level treatment should be started; (i) lifestyle change and weight loss, (ii) ovulation induction with clomiphene citrate, (iii) low-dose gonadotropin addition to treatment in CC-resistant cases or combination with insulin-sensitizing drugs,

(iv) aromatase inhibitors or LOD, and (v) ART.[1,5-7,12] Since most researchers attribute infertility due to PCOS only to the ovulatory factor, treatment approaches have also been directed toward it. However, in the last decade, it has been reported that the endometrium of PCOS patients is defective, both morphologically and molecularly, compared to fertile controls.[2-4] It has been suggested that increased androgen and insulin levels impair decidualization in stromal cells via mitogen-activated protein kinase (MAPK) pathways and Insulin Like Growth Factor Binding Protein 1 (IGFBP-1) synthesis and cause subfertility.[6,13] It has also been reported that the synthesis of homeobox 10, the basic gene of endometrial receptivity, is lower in PCOS patients compared to healthy controls.[7,14] It has been suggested by another researcher that the reason for the decrease in the homeobox gene in PCOS patients may be increased testosterone levels.[14] However, due to the invasive nature of endometrial sampling, adequate receptivity studies could not be performed in subfertile PCOS patients. Moreover, the variability of inter and intra-observer variations of transcriptomics tests has limited their routine use.[10]

MR spectroscopy is a non-invasive imaging method that can detect the life activities of cells in living tissues at the physiological or pathological level. It has been reported that this method can be used for diagnostic or screening purposes in many tissues, especially the brain, endometrium, myometrium, and ovaries.[10,11] In the presence of normal cellular functions, the metabolites that dominate the spectrum are Cho and Cr, indicating the cell's membrane integrity and energy balance, respectively. In case of deviation from normal cellular functions, the metabolites that dominate the spectrum are lactate and lipid. In the light of these data, we non-invasively tested the change of endometrial metabolites in endometrial MR spectroscopy performed before and after ovarian drilling in subfertile PCOS patients. Laparoscopic ovarian drilling is a minimally invasive method used in subfertile women with PCOS who are resistant to clomiphene citrate administration. When LOD is applied in patients with BMI >30 and LH >10 IU/L, it improves both ovarian morphology and endocrine picture.[5,6] We performed endometrial spectroscopy before LOD in patients in the PCOS group and repeated the measurement 2 months later. We found that endometrial Cho and Cr metabolites increased significantly in control measurements. Choline and Cr levels measured before LOD were significantly lower than infertile patients. After LOD, Cho and Cr levels increased and reached the levels of fertile cases. The increase in Cho and Cr levels after LOD may have developed due to the decrease in hormonal parameters and insulin resistance. However, a positive but insignificant correlation was found between the increase in Cho and Cr and the levels of insulin and testosterone. A significant correlation may not have been found due to the low number of cases.

On the other hand, there was no change in lactate and lipid levels, which are pathological endometrial metabolites, after LOD. Lactate is an indicator of anaerobic glycolysis and its levels increase in case of hypoxia. Since lactate levels do not change in subfertile PCOS cases, it suggests that there is no pathology in endometrial oxygenation.[10,11] Similarly, the lipid metabolite is an indicator of membrane integrity and its levels did not change after LOD. This finding is important evidence that endometrial integrity is preserved in PCOS patients.

Our study showed that LOD significantly increased the levels of endometrial metabolites Cho and Cr in subfertile PCOS patients, but did not affect the levels of pathological metabolites lactate and lipid. The increase in Cho and Cr levels may have a positive effect on endometrial receptivity. However, studies comparing metabolite levels and receptivity genes are needed to make a clear comment on this issue. Despite the small number of cases, this study showed for the first time non-invasively that LOD affects the endometrium. Thanks to spectroscopy, we can have preliminary information about endometrial receptivity without biopsy.

CONCLUSIONS

In addition to ovulatory dysfunction and hyperandrogenemia, changes in endometrial metabolites occur in subfertile PCOS patients. These changes can be detected non-invasively by spectroscopy and receptivity can be increased by performing interventions for the endometrium. The fact that spectroscopy provides preliminary information about the endometrium without the need for a biopsy may enable this method to be used in the future to calculate the receptivity timing and to determine the transfer day.

Ethics Committee Approval: Ethics committee approval was received from the Vocational School of Beykent University (Date: May 21, 2020, Decision no: 2020/66).

Informed Consent: Participants were selected from patients with consent for laparoscopy

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Original Article

A Review of Pediatric Celiac Patients in Southeastern Turkey: A Single-Center Experience

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ABSTRACT

Objective: Celiac disease is an autoimmune enteropathy that primarily affects the small intestine. Celiac disease occurs with the ingestion of foods containing gluten and is characterized by malabsorption in individuals with a genetic predisposition. This study aimed to review the clinical, laboratory, radiological, and pathological findings of pediatric celiac patients who were followed in our clinic and to compare the data with previous reports in the literature.

Methods: A total of 509 patients who were diagnosed with celiac disease in the Pediatric Gastroenterology Clinic between 2010 and 2013 were included in this study. Medical records of the patients were reviewed retrospectively to collect their demographic characteristics, anthropometric and bone mineral density measurements, laboratory results, radiological imaging, endoscopic examinations, and pathology reports of the biopsy materials.

Results: Of the 509 patients enrolled in the study, 290 (57%) were females and 219 (43%) males. Among these patients, 441 (86.6%) presented with typical symptoms and 68 (13.4%) presented with atypical symptoms. A total of 479 (94.1%) patients were compliant with the gluten-free diet, whereas 30 (5.9%) patients were not. The chief complaint was growth retardation in the patient groups aged 61 to 144 months (44.6%) and >145 months (59%) and diarrhea (26.1%) in the patient group aged 0-60 months. In all patients, the most common physical examination findings at the time of presentation were normal (57.4%), and the most common comorbidities were iron deficiency anemia (35%) and osteoporosis (33%). While Marsh-Oberhuber stage 3c (52.5%) was most common in patients aged 0-60 months, Marsh-Oberhuber stage 3b was most common in patients aged 61-144 months and patients aged >145 months (51.9% and 63.8%, respectively).

Conclusions: Although growth and developmental retardation and chronic diarrhea are cautionary for celiac disease, patients may also present with extra-gastrointestinal (atypical) findings. The mainstay of celiac disease treatment is strict compliance with a gluten-free diet. Reviewing a relatively large number of cases, this study sheds some light on the current status of pediatric celiac disease patients in the southeastern part of Turkey.

Keywords: Celiac disease, child, Marsh

INTRODUCTION

Celiac disease (CD) is an autoimmune disease that results in damage to the intestinal mucosa and malabsorption after a series of immunological processes triggered by gluten intake in individuals susceptible to the gluten content in cereals such as wheat, barley, and oat. In addition to environmental factors such as gluten, immunological and genetic factors also play a role in the pathogenesis of CD. In this disease, there is a permanent intolerance to gluten, which continues for a lifetime.

According to screening studies, the prevalence of CD is increasing all over the world. Apart from the genetic background of a population, other factors are also responsible for the development of the disease. The global prevalence of CD has been estimated as 0.05-0.1%.[1] In Turkey, the prevalence of CD was found to be 0.9% in a study conducted in 1000 healthy children aged 2-18 years and 0.47% in another study conducted in 20 190 healthy children in the 7-18 age group.[2,3]

Clinical findings in CD patients include malabsorption due to damage to the intestinal mucosa and consequently growth and developmental retardation, diarrhea, abdominal distension, and fatty stools. In recent years, the frequency of occurrence of the non-classical findings, also called atypical or extra-gastrointestinal system findings, has increased.[4] These non-classical findings include short stature, delayed puberty, treatment-resistant iron deficiency anemia, aphthous stomatitis, elevated serum

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transaminases, arthritis, alopecia, dental enamel disorders, gastroesophageal reflux, and constipation.[5]

Studies reviewing the data on CD in large case series of Turkish children are limited. This study aimed to retrospectively evaluate 509 patients diagnosed with CD in Pediatric Gastroenterology Outpatient Clinic and compare them with available data in the literature.

METHODS

For this study, 509 patients who presented to the Pediatric Gastroenterology Clinic between 2010 and 2013 and were diagnosed with CD were reviewed retrospectively. The local ethics committee approved the study protocol which was implemented in accordance with the principles established in the Declaration of Helsinki Ethics committee approval was received for this study from the ethics committee of Gaziantep University (Date: November 5, 2013, Decision no: 373). All patients were diagnosed with CD as per the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) criteria.[6] The medical files of the patients were reviewed to record demographic data (age, sex), anthropometric measurements, clinical findings at the time of presentation, dietary compliance, medical history, family history of CD, physical examination findings, bone mineral density dual-energy x-ray absorptiometry (DEXA) measurements, laboratory results, pathology reports of the endoscopic examination and duodenal biopsy material, and follow-up findings in the case forms. The most common presenting complaints of the patients and current medical conditions were recorded. If any patient had multiple problems that could be associated with CD, those were also recorded as separate items. Patients with gastrointestinal symptoms such as chronic diarrhea and abdominal distension were considered as having typical CD, while those with additional extra-intestinal symptoms such as isolated short stature, headache, weakness, and pallor were considered as having atypical CD. The patients were divided into 3 age groups: 6-60 months, 61-144 months, and over 145 months. A DEXA Z-score between −1 and −2.5 SD was categorized as osteopenia, and Z-score of >−2.5 SD was categorized as osteoporosis. All patients underwent small intestine biopsy for definitive diagnosis, and histopathological evaluation was done according to Marsh criteria.

Main Points

- This study aimed to present the clinical and demographic features of pediatric patients with celiac disease (CD) followed at our center.
- Determining the risk factors for CD may be useful in the early diagnosis, prognosis, and treatment of the disease.
- In our study, patients were divided into age groups, and age groups were evaluated among themselves.
- The frequency of atypical CD increased over time; therefore, patients presenting with extra-intestinal symptoms as well as asymptomatic patients with risk factors should be screened for CD.

Statistical Analysis

The Kolmogorov–Smirnov test was used to check the goodness-of-fit of continuous variables to normal distribution. The Student's *t*-test was used to compare 2 independent groups of variables with normal distribution, and the Mann–Whitney *U* test was used for variables with non-normal distribution. For the comparison of more than 2 independent groups, Kruskal–Wallis test and Dunn's multiple comparison tests were used. The correlation between categorical variables was tested using the chi-square analysis, and the correlation between continuous variables was tested using Spearman's correlation analysis. Frequency, percentage, and mean ± standard deviation were used as descriptive statistics. The Statistical Package for the Social Sciences for Windows, version 11.5 (IBM Inc, Chicago, IL, USA) was used for statistical analysis, and a P value of $< .05$ was considered statistically significant.

RESULTS

A total of 509 patients diagnosed with CD were included in the study, of which 290 (57%) were females and 219 (43%) were males. The mean age of the patients was 129.4 ± 49.3 months, the mean age at onset of complaints was 76.5 ± 43.2 months, and the mean age at onset of gluten-free diet was 93.8 ± 46.7 months. The mean body weight of the patients was 19.4 ± 8.7 kg, the mean height was 111.1 ± 21.6 cm, and the mean body mass index was 15.0 ± 2.0 kg/m^2. Delayed bone age was detected in 28.3% of the patients. The mean tissue transglutaminase (tTG) value of the patients at baseline was 354.6 ± 71.1 IU/mL. Other laboratory parameters are presented in Table 1.

Of the patients, 441 (86.6%) presented with typical symptoms and 68 (13.4%) patients presented with atypical symptoms. Typical symptoms at presentation were also most common among the age groups. Of the patients, 479 (94.1%) were compliant with the gluten-free diet, whereas 30 (5.9%) patients were not (Table 2).

Table 3 shows presenting complaints, physical examination findings, and comorbid conditions of the patients and their distribution by age groups. Growth retardation was the most common presenting complaint in all patients (47.5%). Among age groups, the most common presenting complaint was growth retardation (44.6% and 59%) in the patient groups aged 61-144 months and >145 months, while it was diarrhea (26.1%) in the patient group aged 0-60 months. The number of patients screened due to having a sibling with CD and diagnosed with CD was 46 (9%). In all patients, the most common physical examination findings at the time of presentation were normal (57.4%), whereas pallor was the most common (21.4%) pathological physical examination finding. Pallor was also the most common pathological physical examination finding in the age groups of 0-60 months and 61-144 months (44.3% vs. 18.1%, respectively). Fatigue (21.8%) was the most common pathological physical examination finding in children over 145 months. Iron deficiency anemia (35%) and osteoporosis (33%) were the most common comorbidities. While the most common comorbidity disease was anemia in the patient groups aged 0-60 months and 61-144 months (67.2% vs. 33.1%, respectively), it was osteoporosis (51.1%) in the group

Table 1. Demographics and Laboratory Findings of the Cases

Parameters	All Patients (n = 509)
Sex, female, n (%)	290 (57%)
Age (months)	129.4 ± 49.3
Age of onset of complaint (months)	76.5 ± 43.2
Age of onset of gluten-free diet (months)	93.8 ± 46.7
Weight (kg)	19.4 ± 8.7
Height (cm)	111.1 ± 21.6
BMI (kg/m^2)	15.0 ± 2.0
Delayed bone age, n (%)	144 (28.3)
Laboratory parameters	
Hemoglobin (g/dL)	12.3 ± 1.6
Platelet count (×10^3/μL)	312 ± 95
White blood cell count (×10^3/mL)	7.5 ± 2.3
Iron (μg/dL)	62.1 ± 37.4
Iron binding capacity (μg/dL)	332 ± 82.1
Ferritin (ng/mL)	24.2 ± 29.8
AST (U/L)	32 ± 21.3
ALT (U/L)	21.9 ± 18.7
Albumin (mg/dL)	4.4 ± 0.4
Calcium (mg/dL)	9.6 ± 0.5
Phosphorus (mg/dL)	4.7 ± 0.7
Vitamin D (IU)	21.7 ± 9.6
Vitamin B12 (pg/mL)	370.7 ± 160.8
Folic acid (ng/mL)	9.2 ± 3.7
Initial tissue transglutaminase (IU/mL)	354.6 ± 71.1

BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

aged over 145 months. Considering the distribution of comorbidities by sex, osteopenia was more common in girls, whereas type 1 diabetes mellitus (DM) was more common in boys. In addition, iron deficiency anemia was more common in patients who did not adhere to the diet.

Endoscopic findings of the patients showed that 448 (88%) had duodenitis, 118 (23.2%) had endoscopic gastritis, 53 (10.4%) had esophagitis Grade A, 26 (5.1%) had endoscopic nodular gastritis, 13 (2.6%) had esophagitis Grade B, 9 (1.8%) had cardio-esophageal sphincter failure, and 5 (1%) had bile reflux. Pathology reports of small intestine biopsies were most commonly consistent with Marsh stage 3b (55.4%). Among age groups, Marsh stage 3c (52.5%) was observed most commonly in patients aged 0-60 months, while Marsh stage 3b was most prevalent in 61-144 months and over 145 months (51.9% vs. 63.8%, respectively). A statistically significant association was found between Marsh-Oberhuber stage and dietary compliance of the patients ($P = .031$). Marsh stage 3b (56.8%) was most common in patients with dietary compliance, while Marsh stage 3c (50%) was most common in patients without dietary compliance.

DISCUSSION

Celiac disease is an autoimmune enteropathy that occurs in individuals with genetic predisposition as a result of eating foods containing gluten. The ESPGHAN guidelines are taken into account in the diagnosis of CD.[6] Diagnosis is made on the basis of serology testing and small intestine biopsy. Although the symptoms of CD may occur and the diagnosis may be made at any age, studies have shown that the prevalence of CD increases with age.[7] In the current study, the age of onset of complaints was 76.5 months; however, the age of onset of gluten-free diet was 93.8 months.

Celiac disease is among the leading causes of chronic diarrhea, especially in childhood. Classical CD symptoms include diarrhea, weight loss, and growth retardation. The most common extra-intestinal findings are iron deficiency anemia and metabolic bone disease. Other extra-intestinal findings include infertility, dermatitis herpetiformis, myocarditis, dilated cardiomyopathy, idiopathic pulmonary hemosiderosis, immunoglobulin A (IgA) nephropathy, neurological and psychiatric diseases such as depression and peripheral neuropathy due to vitamin deficiency, anemia due to iron, folate, and vitamin B12 deficiency, autoimmune diseases such as type 1 DM and autoimmune thyroiditis.[8-10]

In many published studies, the presenting complaints vary widely. In the current study, the most common presenting complaint was growth retardation in all patients, followed by abdominal pain. In a multi-center study, diarrhea was found to be the most common complaint at a rate of 51%.[11] In a study on 87 CD cases identified between 2000 and 2007, diarrhea was the most common complaint (96.3%) at the time of admission.[12] In a study by Kondolot et al[13] distributing the complaints of the patients

Table 2. Dietary Compliance and Distribution of Typical–Atypical Celiac Disease Symptoms by Sex

		All Patients	Female	Male	*P*
Dietary compliance	Yes, n (%)	479 (94.1)	276 (95.2)	203 (92.7)	.24
	No, n (%)	30 (5.9)	14 (4.8)	16 (7.3)	
Typical symptom	Yes, n (%)	441 (86.6)	253 (87.2)	188 (85.8)	.647
	No, n (%)	68 (13.4)	37 (12.8)	31 (14.2)	

Table 3. Presenting Complaints, Examination Findings, and Comorbidities by Age Group of the Cases

Parameters	All Patients (n=509)	0-60 months (n=61)	61-144 months (n=260)	>145 months (n=188)	*P*
Presenting complaint					
Growth retardation, n (%)	242 (47.5)	15 (24.6)	116 (44.6)	111 (59)	.001
Abdominal pain, n (%)	178 (35.0)	13 (21.3)	93 (35.8)	72 (38.3)	.05
Diarrhea, n (%)	133 (26.1)	34 (55.7)	64 (24.6)	35 (18.6)	.001
Abdominal distension, n (%)	119 (23.4)	32 (52.5)	70 (26.9)	17 (9)	.001
Loss of appetite, n (%)	56 (11)	9 (14.8)	31 (11.9)	16 (8.5)	.318
Sibling screening, n (%)	46 (9.0)	4 (6.6)	23 (8.8)	19 (10.1)	.694
Vomiting, n (%)	36 (7.1)	8 (13.1)	17 (6.5)	11 (5.9)	.14
Constipation, n (%)	26 (5.1)	3 (4.9)	14 (5.4)	9 (4.8)	.958
Other, n (%)	81 (15.9)	3 (4.9)	29 (11.1)	49 (65.9)	
Physical examination findings					
Normal physical examination, n (%)	292 (57.4)	19 (31.1)	160 (61.5)	113 (60.1)	.001
Pallor, n (%)	109 (21.4)	27 (44.3)	47 (18.1)	35 (18.6)	.001
Weakness, n (%)	97 (19.1)	17 (27.9)	39 (15)	41 (21.8)	.034
Abdominal distension, n (%)	82 (16.1)	24 (39.3)	41 (15.8)	17 (9)	.001
Other, n (%)	83 (16.5)	8 (13)	40 (15.5)	35 (18.5)	
Comorbidities					
Iron deficiency anemia, n (%)	178 (35)	41 (67.2)	86 (33.1)	51 (27.1)	.001
Osteoporosis, n (%)	168 (33)	5 (8.2)	67 (25.8)	96 (51.1)	.001
Osteopenia, n (%)	93 (18.3)	13 (21.3)	53 (20.4)	27 (14.4)	.214
Diabetes mellitus, n (%)	16 (3.1)	–	8 (3.1)	8 (4.3)	.253
Epilepsy, n (%)	13 (2.6)	3 (4.9)	9 (3.5)	1 (0.5)	.07
Gastroesophageal reflux, n (%)	10 (2)	–	5 (1.9)	5 (2.7)	.241
Other, n (%)	134 (14.5)	12 (16.4)	52 (19.8)	70 (26.3)	

by their ages, the complaint of diarrhea was found to decrease with age, and growth retardation was the most common presenting complaint in patients aged over 12 years. The presenting complaints in our study are comparable to those previously reported in the literature. Among age groups in our study, the most common presenting complaint was growth retardation in the patient groups aged 61-144 months and over 145 months, while it was diarrhea in the patient group aged 0-60 months. The number of patients who were screened due to having a sibling with CD and found to have CD was 46 (9%). In a study conducted by Telega et al.[4] more than half of the patients over the age of 7 years presented and were diagnosed with this form of the disease known as the atypical presentation. Balamtekin et al[14] investigated the frequency of symptoms in 220 patients with CD and found that 129 (58.6%) had gastrointestinal symptoms, 76 (34.6%) had extra-gastrointestinal symptoms, and 15 (6.8%) had silent symptoms. In our study, patients with atypical symptoms were older, with a higher mean age of onset of complaints, and a higher mean age of onset of a gluten-free diet compared to those with typical symptoms, which is in line with the data reported by Telega et al.[4] This finding suggests that the disease should be detected before the growth of the patient is adversely affected, and site-specific screening should be performed for this purpose. Early diagnosis and treatment are important for preventing growth and developmental retardation, osteoporosis, autoimmune diseases, and intestinal lymphoma that may develop in these patients.[15]

The hematologic system is an important system that is also affected in CD as with many diseases. Anemia is the most common hematological abnormality in the CD, with a prevalence at diagnosis varying between 12% and 69%, and may even be the

first clinical manifestation of subclinical/silent CD.[16] Consistent with previous reports, iron deficiency anemia was the most common comorbidity in our study. Guidelines recommend screening for CD with serological testing in patients presenting with iron deficiency anemia. Bone and skeletal systems may also be involved in CD. Due to mucosal damage in the proximal small intestine, calcium absorption is impaired and serum calcium concentration decreases. Zanchi et al[17] found osteopenia at a rate of 18% using DEXA measurements in 54 untreated children with CD. Osteoporosis is a well-known complication of untreated CD. In our study, osteoporosis was detected in 168 (33%) patients and osteopenia was detected in 93 (18.3%) patients.

Neurological complications are observed in 6-10% of celiac patients.[18] Headache, myelopathy, myopathy, dementia, perception disorders, attention deficit, and psychiatric disorders, in particular, cerebellar ataxia, peripheral neuropathy, and epilepsy have been reported in the course of CD. There are many studies reporting a higher incidence of epilepsy in children with CD compared to healthy children. Zelnik et al[19] showed that 8 (7.2%) of 111 CD children and 6 (2.8%) of 211 healthy control children had epilepsy. Dalgıç et al[20] found the prevalence of CD diagnosed with biopsy in 70 epileptic children at a rate of 1.17%, which was higher compared to the control group. In our study, the presenting complaint of 27 (5.3%) patients was headache and 13 (2.6%) patients had coexisting epilepsy.

Since CD is an autoimmune disease, it is frequently associated with diseases such as type 1 DM, thyroiditis, Sjögren's disease, sclerosing cholangitis, Addison's disease, rheumatoid arthritis, dermatitis herpetiformis, osteoporosis, primary biliary cirrhosis, Down syndrome, and selective IgA deficiency. About 5% of CD patients manifest with type 1 DM. Similarly, 5% of patients with type 1 DM have CD.[21] Therefore, individuals at risk for these diseases should be screened for CD. In our study, there were 16 patients with type 1 DM, 13 patients with epilepsy, 9 patients with thyroiditis, 5 patients with familial Mediterranean fever, 4 patients with rheumatoid arthritis, and 2 patients with dermatitis herpetiformis, suggesting the need for screening patients at risk for additional diseases, even when they are asymptomatic.

The only known treatment of CD is lifelong adherence to a gluten-free diet that requires avoidance of wheat, oat, and barley to provide symptomatic, serological, and histological remission.[22] This is the only way to achieve optimal quality of life in the light of current knowledge. Complications that may develop in the long term are prevented with early diagnosis and appropriate treatment. Problems related to malabsorption, such as growth retardation caused by the disease, resolve in the first 1-3 years, and patients reach normal weight and height percentiles for their age.[23] Adherence to a lifelong diet as a treatment method is challenging for both patients and families. Not surprisingly, the rate of non-compliance with diet is quite high. The most important factors in non-compliance with the diet include limited access to and high cost of gluten-free products, unavailability of gluten-free diet products, contamination with gluten and other prolamins in many products in the market (despite "gluten-free" label), and absence of alternative products, such as pure corn flour and rice flour. Failure to achieve a reduction in tissue transglutaminase levels after 6 months of a gluten-free diet suggests ongoing gluten intake from hidden sources. Adolescents do not often adhere to the diet. In our study, dietary compliance was lower in the adolescent age group (over 12 years old) compared to other age groups. Since the disease is silent in this age group, the adolescent may believe that the disease has improved. However, mucosal damage continues. Consequently, growth retardation and delayed sexual maturation will be observed when these patients follow a gluten-containing diet.

The present study has a number of limitations. First, this was a retrospective, single-center study. However, the sample size was relatively large, increasing the value of the study. Secondly, the study was designed as an analysis of the data from a single center collected retrospectively from a clinical registry and this may limit the generalizability of our findings.

CONCLUSION

Celiac disease causes a plethora of gastrointestinal and extra-intestinal symptoms. It should be kept in mind that although growth and developmental retardation and chronic diarrhea are cautionary for CD, patients may present with atypical extra-gastrointestinal symptoms. A multidisciplinary approach is required for the evaluation of CD patients. The mainstay of CD treatment is strict compliance with a gluten-free diet. Patients and caregivers should be trained during routine polyclinic controls to maintain compliance with treatment. Considering the number of cases, we believe that our study is an important study conducted in Turkey.

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Informed Consent: Informed Consent: Informed consent was obtained from legal guardians to participate.

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Author Contributions: Author Contributions: Concept – S.Y., Ş.H.; Design – S.Y., Ş.H.; Supervision – S.Y., Ş.H.; Resources – S.Y., H.K.; Materials – S.Y., H.K.; Data Collection and/or Processing – S.Y., H.K.; Analysis and/or Interpretation – S.Y., Ş.H.; Literature Search – S.Y., Ş.H., H.K.; Writing Manuscript – S.Y., Ş.H.; Critical Review – Ş.H., H.K.; Other – SY., Ş.H., H.K.

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Original Article

Temporal Inverted Internal Limiting Membrane Flap Technique for the Treatment of Macular Holes

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ABSTRACT

Objective: To determine the anatomic and functional outcomes of macular hole surgery with the temporal inverted flap technique.

Methods: In this retrospective study, 9 eyes of 9 patients, who were treated with pars plana vitrectomy using the temporal inverted flap technique and had at least 6 months of postoperative follow-up were enrolled. Best-corrected visual acuity and spectral-domain optical coherence tomography images were determined before and after surgery.

Results: The primary disorders were idiopathic macular holes. The mean minimum macular hole diameter was 456.7 ± 150.0 µm (221-622). In all patients, macular hole closure was performed successfully. The final U-shaped foveal contour type was achieved in 77.7% (7/9 eyes) of the patients and V-shape in 22.2% (2/9 eyes). None of the patients had W-shaped closures or flat/open-type contour. Mean best-corrected visual acuity increased from 1.47 ± 0.40 logarithm of the minimal angle of resolution to 0.8 ± 0.41 logarithm of the minimal angle of resolution at the last follow-up visit ($P < .001$).

Conclusion: Temporal inverted flap technique may be an effective method for treating macular holes with different etiologies to minimize the microsurgical trauma. Further large-scale studies are required to assess the efficacy and safety of this technique.

Keywords: Internal limiting membrane, inverted flap, macular hole, pars plana vitrectomy, temporal inverted flap

INTRODUCTION

Since the last 3 decades, pars plana vitrectomy (PPV) with internal limiting membrane (ILM) peeling has been used to treat idiopathic macular hole (MH) with a favorable success rate approaching 98%.[1-3] However, in a lower percentage of patients, surgical closure cannot be achieved, which necessitates additional interventions. In addition, suboptimal closure configuration of the MHs, causing a decrease in visual acuity, like flat-open shape is not uncommon postoperatively.[4,5] As a result, several surgical strategies have been recently developed to improve postoperative outcomes for the treatment of the aforementioned challenging situations. Previously, Michalewska et al[6] first defined the inverted ILM flap technique to treat the idiopathic large MHs and later modifications were done to treat myopic MHs, repeat MH surgery, and treat large MHs as well.[7-11]

Recently, removal of the ILM was suggested to be related to dissociated optic nerve fiber layer (DONFL) and minimal iatrogenic trauma to the retina.[12] To avoid this problem, Michalewska et al[13] reported a modification of the classical inverted ILM flap technique, known as the temporal inverted ILM flap technique, by decreasing the area of peeled ILM. This modification was found to have similar MH closure rates and visual function improvements compared with the inverted flap technique. According to this technique, ILM was peeled on the temporal side of the fovea only and inverted to the MH. With this procedure, a lower incidence of DONFL with satisfactory anatomic and functional outcomes was reported. However, there is not enough data evaluating the anatomic and functional outcomes of the temporal inverted flap technique for large idiopathic MHs and in particular, no previous report regarding the outcomes of the new technique on the different MH stages. In the light of these data, the aim of the present study is to report the outcomes of the patients who underwent PPV with temporal inverted flap technique for the treatment of MHs and to investigate the restoration of foveal anatomy by using spectral-domain optical coherence tomography (SD-OCT).

METHODS

Study Population

In this retrospective study, we reviewed the medical database of the patients with MH who were operated on by 1 experienced surgeon (YT) between January 2018 and January 2019. The patients who had PPV using the temporal inverted ILM flap technique with a follow-up duration of at least 6 months after surgery were included. Exclusion criteria were a history of

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previous vitreoretinal surgery, retinal vascular disease, or age <18 years old.

The medical records including age, gender, lens status, intraocular tamponade usage, and intraoperative and postoperative complications were reviewed for each patient. In every patient, comprehensive ophthalmological examinations including Snellen's best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, and fundoscopy were performed. Spectral-domain optical coherence tomography (Heidelberg Engineering, Heidelberg, Germany) examinations were conducted preoperatively and at each follow-up visit. Macular hole configuration, MH diameter, the presence of external limiting membrane, the success of MH closure, the reoccurrence of MH, and final foveal contour type (U-, V-, or W-type) were assessed using SD-OCT. Full-thickness MH (FTMH) was defined as the presence of a full-thickness neurosensory defect. Lamellar MH was defined as a defect with an irregular fovea contour with dehiscence of the inner from the outer layers in the fovea confirmed by SD-OCT.[14] Optical coherence tomography-based anatomic classification was used for classification into small (≤250 µm), medium (>250 µm and ≤400 µm), and large (>400 µm) based on the horizontally measured linear width at the narrowest point of the hole, as described before.[15] Macular hole closure configuration was characterized as U-shape, V-shape, W-shape (irregular), flat open, and flap closure.[4,16]

Before the procedure, informed consent was taken from every patient. The study was in compliance with the principles outlined in the Declaration of Helsinki and approved by Institutional Ethics Committee of Ankara Yıldırım Beyazıt University, (Date: January 9, 2019, Decision no: 26379996/01).

Surgical Technique

Under retrobulbar anesthesia, a standard PPV was achieved using a conventional 25-gauge 3-port system with the Constellation Vision System (Alcon Laboratories, Inc., Fort Worth, Tex, USA) with a cutting speed of 7500 cuts per minute. A non-contact visualization system (Eibos system, Moller-Wedel International, Wedel, Germany) was used for endo-ocular visualization. Phacoemulsification and intraocular lens implantation were performed simultaneously on phakic eyes according to the surgeon's discretion. Using triamcinolone-assisted visualization, the central core vitrectomy and posterior vitreous detachment were performed. Membrane Blue-Dual (DORC, Zuidland, Netherlands) was performed for staining of the ILM around the fovea to remove the ILM. The temporal inverted ILM flap technique was accomplished as described previously.[13] In brief, the ILM at the temporal side of the fovea was peeled in a circular manner at least 2-disk diameter around the MH by about 180° and was prepared as a semicircular flap. Thereafter, the inverted flap was flipped over the fovea and was placed gently over the nasal side of the fovea to cover the MH. To avoid losing the flap, the surgeon turned off the infusion line when the flap was placed to the macular area. After ensured for the proper placement of the flap on the macula surface, fluid–air exchange was performed with a backflush needle placed on the nasal part of the optic nerve. To canalize the fluid flow to the optic nerve, the globe was rotated a little to the nasal direction, keeping the backflush needle away from the flap on the optic nerve surface. At the end of the surgery, 20% sulfur hexafluoride was injected as endotamponade, and patients were requested to take a prone position for 3 days.

Main Points

- Conventional internal limiting membrane (ILM) peeling is associated with mechanical and subclinical traumatic changes to the retinal layers.
- The risk of iatrogenic trauma in the area of papillomacular bundle was lower while using the temporal inverted ILM flap technique than conventional ILM peeling.
- The temporal inverted ILM flap technique is a safe and successful procedure for treating large idiopathic full-thickness macular holes (MHs) and myopic MHs.
- It is an effective method for treating MHs not only for challenging cases but also in the case of smaller MHs to minimize the microsurgical trauma.

Statistical Analysis

Decimal acuity values were converted to logarithm of the minimal angle of resolution (logMAR) for statistical analysis. Finger counting vision was defined as 20/2000 (2.0 logMAR), and hand motion was described as 20/20000 (3.0 logMAR).[17] A paired *t*-test was used to compare the preoperative and postoperative outcomes with a significance level set at $P < .05$. All statistical analyses were performed using Statistical Package for the Social Sciences Version 22.0. (IBM SPSS Corp.; Armonk, NY, USA).

RESULTS

Basal Characteristics

In our study, 9 eyes of 9 patients were included. The mean age of the patients was 70 ± 7 years (62-85). The mean minimum MH diameter was 456.7 ± 150.0 µm (221-622). The mean base diameter was 1126.64 ± 228.9 µm (724-1603). The mean follow-up period was 6.8 ±1.5 (6-10) months. The primary diseases of the patients were idiopathic MH. According to the baseline OCT characteristic, 2 (22%) eyes were small MH, 1(11%) eyes were medium-size MH, and 6 (66%) were large MH. Cataract surgery was simultaneously conducted in 3 patients (33%). Characteristics of the 9 eyes included in the study were shown in Table 1.

Anatomic Results and Functional Outcomes

All eyes achieved complete closure of MH at the first-month follow-up. During the follow-up, there was no reopening of the MH. According to the closure patterns on SD-OCT, the final U-shaped foveal contour type was achieved in 77.7% (7/9 eyes) of the patients and V-shape in 22.2% (2/9 eyes). In none of the patients, the W-shaped closures or flat/open-type contour were detected. On postoperative SD-OCT, an ILM flap was visible in 22.2% (2/9 cases) of the patients (case 1 and case 7 in Figure 1). In addition, epiretinal membrane formation was detected in only 1 (11.1%) patient on postoperative SD-OCT (case 2 in the Figure). The mean BCVA recovered significantly from 1.47 ± 0.40 logMAR at baseline to 0.8 ± 0.41 logMAR at last visit ($P < .001$). Visual improvement was noted in all eyes (100%). Visual acuity of

Table 1. Baseline Characteristics of the Study Population

Case	Age (Years)/Sex (F/M)	Lens Status	Minimum Diameter, µm	Basal Diameter, µm	Cataract Surgery	Preoperative BCVA, Snellen (logMAR)	Postoperative BCVA, Snellen (logMAR)	Follow-up, Months
1	73/M	Pseudophakic	601	1603	No	20/400 (1.3)	20/60 (0.5)	9
2	74/M	Phakic	519	1225	Yes	20/2000 (2)	20/200 (1)	6
3	67/M	Pseudophakic	248	724	No	20/400 (1.3)	20/30 (0.2)	6
4	85/F	Pseudophakic	465	1113	No	20/2000 (2)	20/400 (1.3)	6
5	63/F	Phakic	386	1000	No	20/2000 (2)	20/400 (1.3)	7
6	62/M	Pseudophakic	439	1155	No	20/200 (1)	20/40 (0.3)	10
7	74/M	Pseudophakic	622	1115	No	20/400 (1.3)	20/200 (1)	6
8	71/M	Phakic	221	1130	Yes	20/250 (1.1)	20/80 (0.6)	6
9	69/F	Phakic	610	1069	Yes	20/400 (1.3)	20/200 (1)	6

BCVA, best-corrected visual acuity.

20/40 was achieved in 2 eyes (22%). Representative cases of MH are presented in Figure 1.

Adverse Events

The surgery was done successfully in all cases and no intraoperative and postoperative complications were recorded. A missing flap was not observed in any patient.

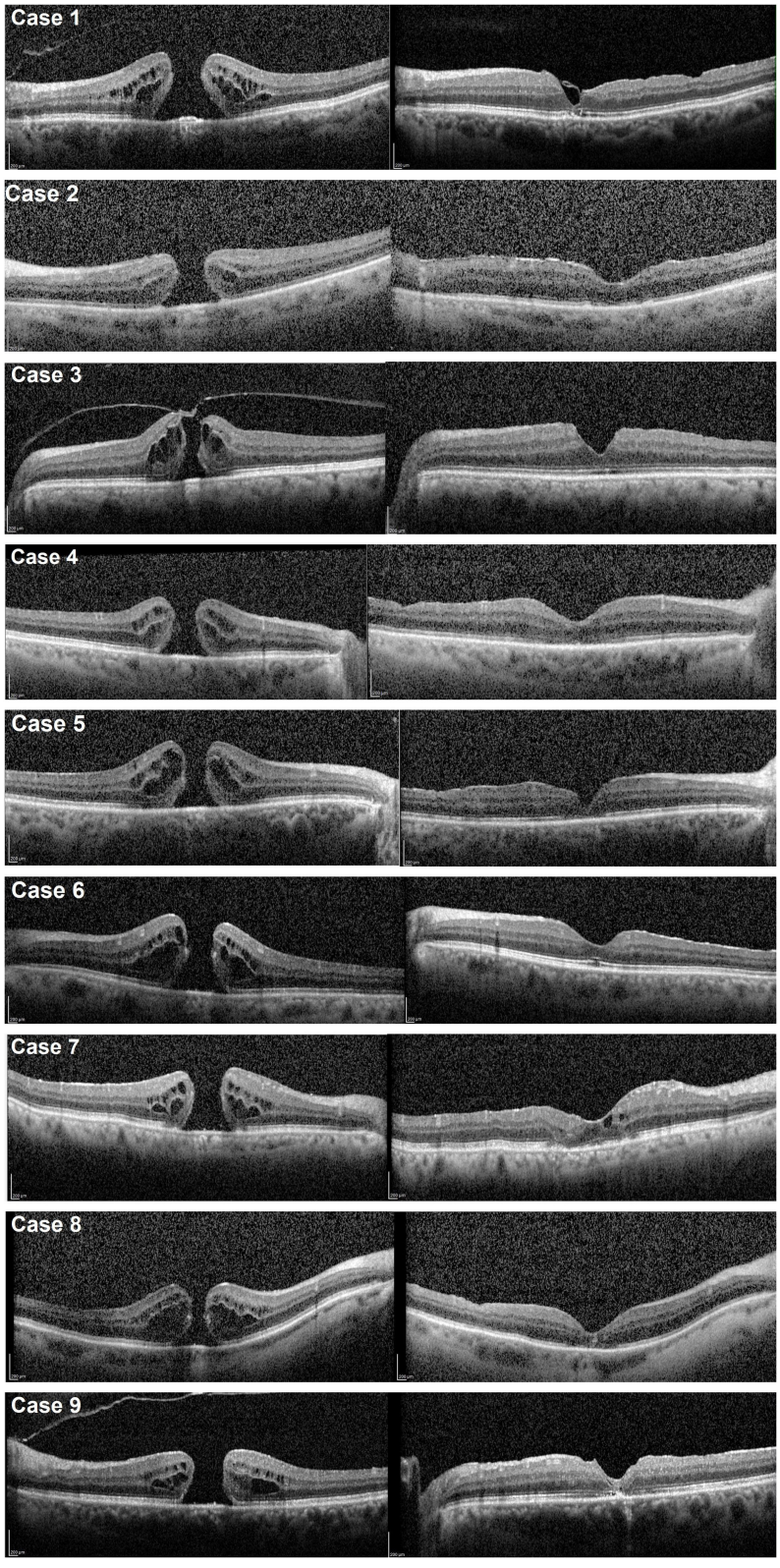
DISCUSSION

Our findings demonstrated that all 9 eyes with MH were successfully treated with PPV by the temporal inverted flap technique. Using this method, complete MH closure was accomplished in all eyes and U-type closure was demonstrated on OCT examinations of most eyes. In addition, significant improvement was also observed in visual acuity. As in line with previously reported data, our findings indicated that the temporal inverted flap technique leads to reasonable anatomical and functional outcomes in patients with MHs.

Previous studies showed that ILM peeling is associated with mechanical and subclinical traumatic changes to the retinal nerve fiber layer and a decline in the thickness of the nerve fiber layer, the ganglion cell layer, and inner retinal layer.[18,19] Using the temporal inverted ILM flap technique in which the ILM is removed from the temporal side of the macula, Michalewska et al[13] reported that the risk of iatrogenic trauma in the area of papillomacular bundle was lower than conventional ILM peeling and had a lower incidence of the DONFL appearances. The temporal inverted flap technique was used by different operators for improving surgical outcomes in challenging situations like large MHs and myopic cases.[13,20,21] However, in our study, in addition to challenging MH cases, we also enrolled small and medium-size MHs in order to minimize the risk of surgical trauma in the papillomacular bundle area as well as DONFL appearance.

The general success rate of MH closure with surgery is greater than 90%.[22] According to the study by Michalewska et al.[13] the temporal inverted flap technique had a 93% anatomical success in large MHs, which was found superior to the conventional ILM peeling technique and non-inferior to the inverted flap technique. In our experience, the MH closure rate was 100% with temporal inverted flap technique for the treatment of all MH sizes, which was also higher than the generally accepted anatomical success rate. In addition, postoperative MH closure morphology is an important determinant of the postoperative outcomes, where U-shaped closure is associated with better results.[16] In the present study, according to the anatomical closure morphology, the proportion of U-shaped closure was 77.7% and the other remaining cases had V-shaped configuration at the last follow-up visit. This percentage was higher than the U-shaped closure rate of 64% in the study by Michalewska et al[13] at the postoperative sixth month. Moreover, according to the previous reports with conventional ILM peeling techniques, flat-open type MH closure was observed in 19- 39% of idiopathic MHs,[5,16] whereas none of the patients showed flat-open appearance in our study. The higher frequency of better anatomical success could be attributed to the relatively smaller minimal diameters of MHs in

Figure 1. SD-OCT scans of the preoperative appearance and the postoperative evolution of macular hole in patients who underwent PPV with temporal inverted flap technique.
SD-OCT, spectral-domain optical coherence tomography; PPV, pars plana vitrectomy.



our study. In the present study, 36% of the patients were of small-medium-size MH and none of the patients was myopic or a recurrent case. On the other hand, either U- or V-shaped closure was observed in large MHs without any W-shaped closures or flat/open-type contour. Medium-size FTMH (250-400 microns) had a higher success rate with vitrectomy with or without ILM peeling than large MHs.[23] Furthermore, different visual outcomes have been reported depending on the stage of MH. As in line with the previous reports, in our study, the improvement in the visual acuity was observed in all patients.[24-26] These results suggested that the temporal inverted flap method was associated with favorable anatomical and functional outcomes in the case of different MH sizes.

Despite the improvement in surgical outcomes, this novel technical modification is not free of challenging conditions depending on the surgeon's skill. An important issue is preventing the ILM flap retroversion during the fluid–air exchange. Michelega et al[13] reported 7% failure after initial surgery, which was related to the spontaneous retroversion of the flap during fluid–air exchange.[13] To prevent this complication, some authors offered to use low molecular weight viscoelastic material or heavy liquids for stabilization of the flap.[27,28] In the current study, we did not observe any failure of MH closure related to destabilization of the flap on the macula. To avoid flap stabilization failure, the surgeon in our study performed some technical modifications in addition to the original technique to maintain the flap on the nasal side of the macula. First, during ILM flap invertion to the nasal side of the fovea, the fluid aspiration was turned off and then fluid–air exchange was performed. In addition, long-lasting gas was filled to the vitreous cavity to stabilize the flap for a long time. This is different from the original technique in which air was used instead of gas. Moreover, it is important to note that our results were based on the work of an experienced vitreoretinal surgeon. Our findings should be evaluated in the light of previously mentioned patients' characteristics, which include not only challenging cases but also lower-risk patients.

Our findings should be interpreted with caution because of several limitations. First, this was a retrospective study without any control group. Second, the sample size was small with a limited follow-up period. Third, the study population was heterogeneous with a limited number of cases other than large MHs. Despite these limitations, our study presented a comprehensive case series operated with temporal inverted flap technique.

CONCLUSION

Although the temporal inverted flap technique was originally performed in eyes with large MHs and MHs in myopic eyes, to the best of our knowledge, there were no reports investigating the anatomic and functional results in the case of small and medium-size MHs. Our findings demonstrated that the temporal inverted flap technique may be an effective method for treating MHs not only for challenging cases but also in the case of smaller MHs to minimize the microsurgical trauma. Further large-scale, prospective, randomized studies with long-term follow-up are needed to determine the actual benefits for temporal inverted flap technique and use in different settings.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara Yıldırım Beyazıt University (Date: January 9, 2019, Decision no: 26379996/01).

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Technical Note

A New and Practical Method for Transmission Electron Microscopy Analysis of *Proteus mirabilis*

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Keywords: Proteus mirabilis, transmission electron microscopy, protocol

INTRODUCTION

Proteus mirabilis is rod-shaped Gram-negative bacteria that have distinguishing features like swarming motility and urease activity.[1] Transmission electron microscopy (TEM) is used to examine very small structures (0.1 nm) via short-wavelength electrons.[2] Although there are various protocols for TEM analysis of *Proteus* species, the materials needed for the preparation of the samples cannot easily be found in the microbiology laboratory settings.[3] In this study, we aimed to delineate a new simplified protocol for TEM imaging of *Proteus mirabilis* isolates to the researchers working at clinical microbiology laboratories.

METHODS

Ten *Proteus mirabilis* clinical isolates were included in the study. The bacterial isolates were stored at −20°C and thawed and subcultured onto 5% sheep blood agar prior to testing. After 24 hours of incubation at 36°C, one loopful (10 μL) of bacteria from growing colonies was transferred into a tube containing 2 mL Mueller Hinton broth (MHB; Merck, Germany) and incubated at 36°C for 3 hours. Then, 1 mL of the bacterial suspension was re-suspended inside 5 mL of 2.5% glutaraldehyde solution; the suspension was kept at room temperature for 10 minutes, then centrifuged at 3000 g for 5 minutes in a 15 mL falcon tube. The supernatant on the surface was discarded, and 1 mL 2.5% glutaraldehyde solution was added to the pellet. After 20 minutes, the pellet was removed from the bottom of the tube with the help of a Pasteur pipette. Concurrently, the pellet was embedded into 96-well microplates which had been filled with freshly prepared (>45°C; about to solidify) 1 mL Mueller Hinton agar (MHA; Merck, Darmstadt, Germany). The pellet was mixed with the agar using a sterile injector needle for 5 seconds. Then, the microplate was covered and left to solidify for 2 hours at room temperature. Afterward, using the needle and a scalpel tip, the solidified agar piece was scraped from the wall of the well and placed on a sterile slide as a mold. It was divided into 1 mm^3 piece with the scalpel tip. The pieces were stored in falcon tubes containing 15 mL of phosphate buffer at 4°C, which was then examined by the TEM laboratory the next day. The post-fixation was applied to samples with 1% osmium tetroxide solution for 1 hour at 4°C. Later, dehydration of the samples was achieved by graded series of alcohol, and a transparency process was applied with propylene oxide. Subsequently, the samples were embedded in epoxy resin in embedding capsules and polymerized in a 60°C incubator for 24 hours. From the blocks obtained, sections of 70 nm thickness were taken on copper grids with an ultra-microtome, and the sections were contrasted with uranyl acetate and lead citrate. The contrasting sections were examined and photographed by TEM (Jeol® JEM1011, Japan).

DISCUSSION

Since TEM imaging is not very common in clinical microbiology laboratories, when microbiologists need to obtain electron microscopic images of bacteria, they search for different protocols and try to adapt those protocols to their studies. For microscopic examination under TEM, suspensions of bacteria must be supported on a thin film of plastic, carbon, or a combination of the two applied to the surface of an electron microscope specimen grid.[4] In our study, we tested MHB and MHA, frequently used media in microbiology laboratories, as dilution and fixation media for TEM, and the results were satisfactory. We were able to get clear and detailed TEM images of *P. mirabilis* (Figure 1 and 2). Organelles such as nucleoids, ribosomes, cell membrane, and

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Figure 1. Detailed transmission electron microscopy image of *Proteus mirabilis* bacteria.

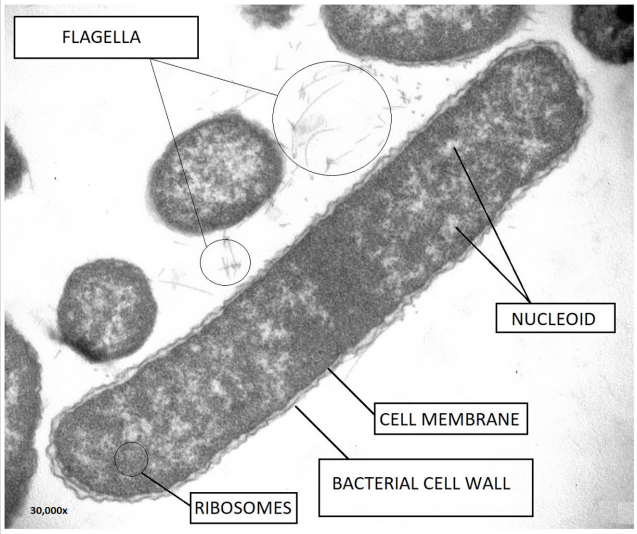
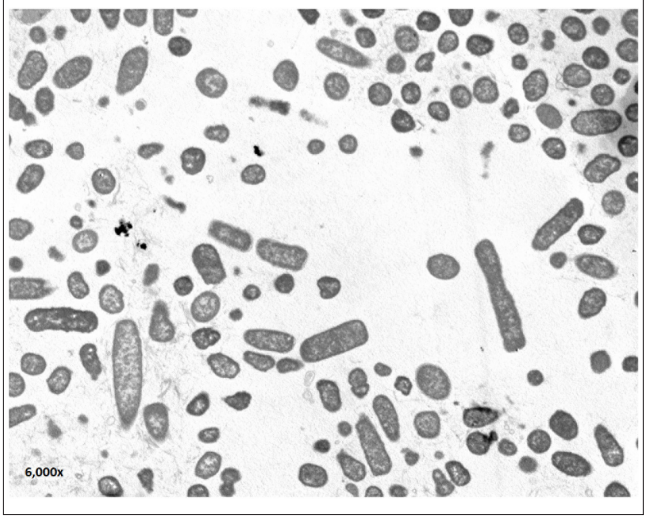


Figure 2. Transmission electron microscopy image of a group of *Proteus mirabilis* bacteria.



cell wall can be observed clearly under a microscope (Figure 1). In our protocol, *Proteus* bacteria were scattered adequately that they did not touch each other, but so close that a few of them could be observed in each frame (Figure 2). We think that this protocol can easily be repeated and applied to the other members of the order Enterobacterales using the equipment that can be found in clinical microbiology laboratory settings.

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Image

Advanced Proliferative Diabetic Retinopathy

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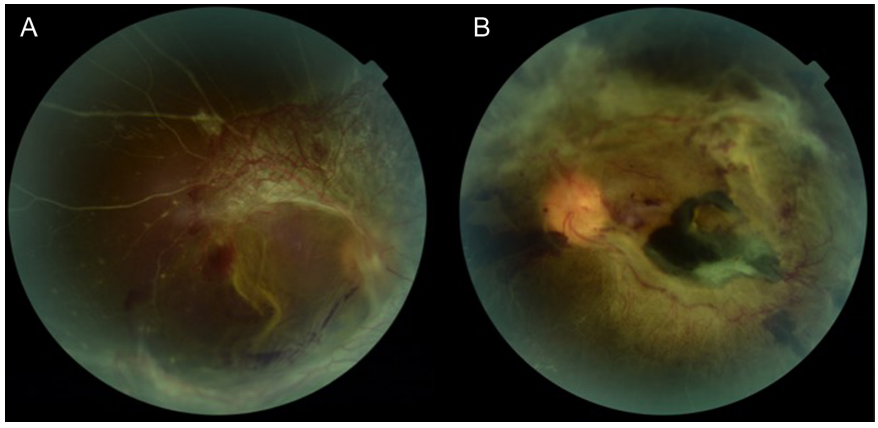
Color fundus photograph of a 31-year-old man with type 1 diabetes. Best-corrected visual acuity was 20/100 in each eye. Note the extensive fibrovascular proliferation, ghost vessels, macular dragging, preretinal hemorrhages, and tractional retinal detachment (**A:** right eye and **B:** left eye).

Keywords: Advanced proliferative diabetic retinopathy, diabetes mellitus, proliferative diabetic retinopathy, tractional retinal detachment



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