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Evaluation of ANCA in vasculitis and non-vasculitis diseases

ANCA'nın vaskülit ve vaskülit dışı hastalıklarda değerlendirilmesi

Sedef Zeliha Öner, Melek Demir, Burhan Özkan, Ergun Mete, İlknur Kaleli, Ahmet Çalışkan, Çağrı Ergin

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Abstract

Purpose: The present study aims to evaluate the existence of anti-neutrophil cytoplasmic antibody (ANCA) in vasculitis and non-vasculitis diseases.

Materials and methods: Over five years, the results of 5107 serum samples submitted to the Medical Microbiology Laboratory for ANCA evaluation were retrospectively analyzed. The existence of ANCA was studied using the preparations containing ethanol-fixed and formalin-fixed granulocyte substratum by indirect immunofluorescence (IIF) testing method; and myeloperoxidase (MPO) and Proteinase-3 (PR-3) antigens in ANCA-positive samples were studied with the ELISA method.

Results: 422 (8.3%) of the samples were considered ANCA-positive. The mean age of ANCA-positive patients was found significantly high from ANCA-negative patients (p=0.0001). ANCA-positivity was 6.7% in men and 9.4% in women. A statistically significant difference was detected between women and men in terms of ANCA positivity (p=0.0001). 62 (19.9%) of the 312 patients diagnosed with vasculitis and 360 (7.5%) of the 4795 patients with non-vasculitis were ANCA-positive. The age of ANCA-associated vasculitis (AAV) patients was statistically high compared to non-AAV patients (p=0.0001). ANCA-positivity was found 16.7% in patients with IgA vasculitis, 18.6% in leukocytoclastic vasculitis, 56.9% in rheumatoid arthritis, 46.9% in systemic lupus erythematosus, 18.6% in interstitial pulmonary disease, 7.7% multiple sclerosis, 10.2% in chronic renal failure, and 5.1% in cerebrovascular accident.

Conclusion: In vasculitis cases, ANCA positivity rate was higher than in non-vasculitis diseases. In non-vasculitis diseases, the target antigen MPO-ANCA and PR3-ANCA positivity was rare compared to vasculitis cases. Among ANCA-positive patients, the most common non-vasculitis diseases included connective tissue disease, chronic renal failure and interstitial pulmonary disease.

Keywords: Anti-neutrophil cytoplasmic antibody, MPO-ANCA, PR3-ANCA, vasculitis, connective tissue disease.

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Öz

Amaç: Bu çalışma, vaskülit ve vaskülit dışı hastalıklarda anti-nötrofil sitoplazmik antikorun (ANCA) varlığını değerlendirmeyi amaçlamaktadır.

Gereç ve yöntem: Beş yılı aşkın bir sürede ANCA değerlendirmesi için Tıbbi Mikrobiyoloji Laboratuvarına gönderilen 5107 serum örneğinin sonuçları retrospektif olarak incelendi. ANCA'nın varlığı, etanolle fikse edilmiş ve formalinle fikse edilmiş granülosit substrat içeren preparatlar kullanılarak indirekt immünofloresan (IIF) test yöntemiyle araştırıldı; ANCA pozitif örneklerde miyeloperoksidaz (MPO) ve Proteinaz-3 (PR-3) antijenleri ELISA yöntemi ile çalışıldı.

Bulgular: Örneklerin 422'si (%8,3) ANCA-pozitif olarak değerlendirildi. ANCA pozitifliği erkeklerde %6,7 ve kadınlarda %9,4 idi. ANCA pozitifliği açısından kadın ve erkekler arasında istatistiksel olarak anlamlı fark saptandı (p=0,0001). ANCA pozitif hastaların ortalama yaşı ANCA negatif hastalardan anlamlı olarak yüksek bulundu (p=0,0001). Vaskülit tanısı alan 312 hastanın 62'si (%19,9) ve vasküliti olmayan 4795 hastanın 360'ı (%7,5) ANCA pozitifti. AAV hastalarının yaşı, AAV olmayan hastalara göre istatistiksel olarak yüksekti (p=0,0001).

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ANCA pozitifliği IgA vaskülitlerinde %16,7, lökositoklastik vaskülitlerde %18,6, romatoid artritte %56,9, sistemik lupus eritematozusta %46,9, interstisyel akciğer hastalığında %18,6, multipl sklerozda %7,7, kronik böbrek yetmezliğinde %10,2 ve serebrovasküler olayda %5,1olarak bulunmuştur.
Sonuç: Vaskülit olgularında ANCA pozitiflik oranı, vaskülit dışı hastalıklara göre daha yüksekti. Vaskülit dışı hastalıklarda hedef antijen MPO-ANCA ve PR3-ANCA pozitifliği vaskülit vakalarına göre nadirdi. ANCA-pozitif hastalar arasında en yaygın vaskülit dışı hastalıklar bağ dokusu hastalığı, kronik böbrek yetmezliği ve interstisyel akciğer hastalığıydı.

Anahtar kelimeler: Anti-nötrofil sitoplazmik antikor, MPO-ANCA, PR3-ANCA, vaskülit, bağ dokusu hastalığı.

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Introduction

Antineutrophil cytoplasmic antibodies (ANCA) are a group of autoantibodies that are predominantly formed against proteins expressed in cytoplasmic granules of neutrophils. It is recognized as a laboratory test that is commonly used in the diagnosis of patients with suspected vasculitis [1]. ANCA-associated vasculitis (AAV) is characterized by inflammation of small- to medium-sized blood vessels. It includes a variety of clinicopathologies, including polyangiitis granulomatosis (GPA), microscopic polyangiitis (MPA), polyangiitis eosinophilic granulomatosis (EGPA) and vasculitis associated with renal-limited ANCA [2].

Anti-neutrophil cytoplasmic antibodies are not a specific indicator for AAV, because these autoantibodies can also be detected in different diseases [1]. They are thought to have clinical, pathogenic and/or diagnostic significance in certain diseases as well as ANCA-associated vasculitis [3].

The presence of ANCA can be screened with indirect immunofluorescence (IIF) [4]. It is reported as two main patterns, namely the cytoplasmic pattern (C-ANCA) and the perinuclear pattern (P-ANCA) [3]. Proteinase-3 (PR-3) is the main target antigen of C-ANCA, while myeloperoxidase (MPO) is the P-ANCA's primary target antigen. In addition, P-ANCA's target antigens may include elastase, cathepsin G, lactoferrin and lysozyme [4]. It is recommended to look for antigen-specific ANCA targeting PR-3 and MPO in any patients suggesting ANCA-associated vasculitis, and for ANCA in patients with anti-glomerular basal membrane disease, idiopathic interstitial pneumonia and nephritis-associated infective endocarditis [3]. The present study aimed to evaluate ANCA positivity in vasculitis and non-vasculitis diseases.

Materials and methods

The results of 5107 serum samples sent to Medical Microbiology laboratory between 01/01/2015 and 31/12/2019 for ANCA evaluation were retrospectively analyzed. Repeated samples of the same patient were excluded. The existence of ANCA was investigated using preparations that contained ethanol-fixed and formalin-fixed granulocyte substrate (Euroimmun, Lübeck, Germany) by the indirect immunofluorescence (IIF) testing method in accordance with the manufacturer's recommendations (in 1/10 serum dilations). The results were evaluated as C-ANCA and P-ANCA. The MPO antigens (AESKULISA® MPO, Aesku Diagnostics, Wendelsheim, Germany) and the PR3 antigens (AESKULISA® PR3 sensitive, Aesku Diagnostics, Wendelsheim, Germany) of the positive samples were studied with ELISA method.

The demographic data of the patients were obtained from the hospital's information system. Study was conducted with the approval of the ethics board for Pamukkale University Non-Interventional Clinical Research Ethics Committee.

Statistical analyses

The statistical analysis of data was performed with SPSS 25.0 package program. Categoric variables were expressed in numbers and percentages and continuous variables were expressed in mean ± standard deviation, minimum and maximum values. The differences between the categorical variables were examined with the Chi-square analysis and the Mann-Whitney U-test was used to perform cross-group comparisons.

Results

In total, 5107 serum samples were evaluated within the scope of the study. 2904 (56.9%) of the samples were taken from female patients. The mean age was 47.7±20.28 years (min: 3, max: 94). 422 (8.3%) of the samples were considered ANCA-positive. The mean age for positive samples was 53.54±21.39 (min:3, max:92), and 47.17±20.1 for negative samples (min:3, max:94). The mean age of ANCA-positive patients was found significantly different from ANCA-negative patients (p=0.0001)

ANCA-positivity was 6.7% in men and 9.4% in women. A statistically significant difference was detected between women and men in terms of ANCA positivity (p=0.0001). P-ANCA positivity was found in 314 (6.2%) patients, and C-ANCA positivity in 108 (2.1%).

In 62 (19.9%) of the 312 patients diagnosed with vasculitis and 360 (7.5%) of the 4795 patients with non-vasculitis diseases, ANCA-positivity was detected (Table 1, 2).

ANCA-associated vasculitis was classified as follows: GPA, MPA, renal-localized ANCA and ANCA-associated vasculitis [2] (Table 1). An evaluation of 422 ANCA-positive patients showed that 27 (6.4%) patients with ANCA-positive vasculitis had ANCA-associated vasculitis, 15 (3.6%) had IgA vasculitis (HSP) and 8 (1.9%) had leukocytoclastic vasculitis.

Table 1. ANCA evaluation in vasculitides

Vasculitis	ANCA n (%)		Positive ANCA n (%)		MPO positive n (number of tests)	PR3 positive n (number of tests)
	Negative	Positive	P ANCA	C ANCA		
Vasculitis (undiagnosed) N=49	45 (91.8)	4 (8.2)	2 (50)	2 (50)	0 (4)	1 (4)
ANCA-associated vasculitis (undiagnosed) N=6	1 (16.7)	5 (83.3)	4 (80)	1 (20)	5 (5)	0 (5)
GPA* N=17	0 (0)	17 (100)	6 (35.3)	11 (64.7)	5 (16)	11 (16)
MPA N=4**	2 (50)	2 (50)	2 (100)	0 (0)	2 (2)	0 (2)
Renal-limited ANCA associated vasculitis N=6	3 (50)	3 (50)	2 (66.7)	1 (33.3)	2 (3)	1 (3)
Leukocytoclastic vasculitis N=43	35 (81.4)	8 (18.6)	5 (62.5)	3 (37.5)	0 (6)	0 (6)
IgA vasculitis N=90	75 (83.3)	15 (16.7)	10 (66.7)	5 (33.3)	0 (10)	0 (9)
Urticarial vasculitis N=6	5 (83.3)	1 (16.7)	1 (100)	0 (0)	0 (1)	0 (0)
Takayasu N=20	18 (90)	2 (10)	2 (100)	0 (0)	1 (2)	0 (2)
Behcet's disease N=71	66 (93)	5 (7)	3 (60)	2 (40)	1 (5)	0 (2)
Total N=312	250 (80.1)	62 (19.9)	37 (59.7)	25 40.3)	16 (54)	13 (48)

*GPA: Granulomatosis with Polyangiitis, **MPA: Microscopic Polyangiitis

Table 2. ANCA evaluation in non-vasculitis diseases

Non-vasculitis	ANCA n (%)		Positive ANCA n (%)		MPO positive n (number of tests)	PR3 positive n (number of tests)
	Negative	Positive	P ANCA	C ANCA		
Connective tissue disease (undiagnosed) N=146	136 (93.2)	10 (6.8)	7 (70)	3 (30)	1 (6)	0 (5)
Rheumatoid Arthritis N=144	62 (43.1)	82 (56.9)	66 (80.5)	16 (19.5)	3 (56)	2 (47)
Systemic Lupus Erythematosus N=64	34 (53.1)	30 (46.9)	29 (96.7)	1 (3.3)	1 (22)	0 (19)
Scleroderma N=29	25 (86.2)	4 (13.8)	2 (50)	2 (50)	0 (4)	0 (4)
Sjogren N=71	64 (90.1)	7 (9.9)	6 (85.7)	1 (14.3)	2 (5)	0 (4)
Familial Mediterranean Fever N=63	61 (96.8)	2 (3.2)	1 (50)	1 (50)	1 (2)	0 (1)
Sarcoidosis N=12	10 (83.3)	2 (16.7)	1 (50)	1 (50)	0 (2)	0 (2)
Interstitial lung disease N=113	92 (81.4)	21 (18.6)	18 (85.7)	3 (14.3)	2 (14)	1 (12)
Infection N=126	111 (88.1)	15 (11.9)	14 (93.3)	1 (6.7)	2 (13)	0 (10)
Malignancy N=116	105 (90.5)	11 (9.5)	7 (63.6)	4 (36.4)	0 (7)	1 (6)
Inflammatory bowel disease (undiagnosed) N=12	9 (75)	3 (25)	2 (66.7)	1 (33.3)	0 (1)	0 (1)
Ulcerative colitis N=10	7 (70)	3 (30)	1 (33.3)	2 (66.7)	0 (1)	1 (1)
Liver disease (undiagnosed) N=16	14 (87.5)	2 (12.5)	1 (50)	1 (50)	0 (2)	0 (2)
Autoimmune hepatitis N=1	0 (0)	1 (100)	0 (0)	1 (100)	0 (1)	0 (1)
Primary sclerosing cholangitis N=1	0 (0)	1 (100)	1 (100)	0 (0)	0 (1)	0 (0)
Demyelinating disease (undiagnosed) N=194	183 (94.3)	11 (5.7)	7 (63.6)	4 (36.4)	0 (10)	1 (10)
Multiple Sclerosis N=247	228 (92.3)	19 (7.7)	13 (68.4)	6 (31.6)	1 (15)	1 (13)
Chronic renal failure N=383	344 (89.8)	39 (0.2)	33 (84.6)	6 (15.4)	2 (39)	1 (34)
Cerebrovascular accident N=393	373 (94.9)	20 (5.1)	12 (60)	8 (40)	0 (19)	2 (16)
Other (unclassifiable diseases) N=2653	2577 (97.1)	77 (2.9)	56 (72.7)	21 (27.3)	5 (56)	0 (49)
Total N=4795	4435 (92.5)	360 (7.5)	277 (76.9)	83 (23.1)	20 (276)	10 (238)

Connective tissue disease was grouped into rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), scleroderma, Sjogren, Familial Mediterranean Fever (FMF), sarcoidosis and unidentified. Malignity diagnoses included myelodysplastic syndrome, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (KLL), chronic myeloid leukemia (KML), non-Hodgkin lymphoma, multiple myeloma, central nervous system lymphoma, Ewing sarcoma, Kaposi sarcoma, thymoma, dermal malignant neoplasm, pancreatic adenocarcinoma, pulmonary, renal, stomach, colon, bladder, ovary, vulva, cervical and prostate cancer (Table 2). When 422 ANCA-positive patients are evaluated, 137 (32.5%) of ANCA-positive non-vasculitis cases were followed-up with connective tissue disease, 21 (5.0%) with interstitial pulmonary disease, 19 (4.5%) with multiple sclerosis (MS), 39 (9.2%) with chronic renal failure (CRF), 20 (4.7%) with cerebrovascular accident, 15 (3.6%) with infection and 11 (2.6%) with malignancy.

AAV prevalence was found at 0.6% in 5107 patients subject to ANCA evaluation. 18 of AAV-diagnosed patients were women (54.5%) and 15 were men (45.5%). The age of AAV patients was statistically high compared to non-AAV patients (p=0.0001; non-AAV patients: 47.6±20.28, min-max: 3-94; AAV patients: 61.1±16.02 min-max: 21-86).

PR3-ANCA (68.8%) and MPO-ANCA (31.2%) were detected in GPA; MPO-ANCA (100%) in MPA; and MPO-ANCA (66.7%) and PR3-ANCA (33.3%) were detected in ANCA-associated vasculitis patients (Table 1).

ANCA-positivity was found at 16.7% for patients with IgA vasculitis, 18.6% for leukocytoclastic vasculitis, 56.9% for RA, 46.9% for SLE, 18.6% for interstitial pulmonary disease, 7.7% for MS, 10.2% for KBY, and 5.1% for SVO (Table 1, 2).

In vasculitis patients, P-ANCA was detected positive in 37 (59.7%) and C-ANCA in 25 (40.3%) patients; and in non-vasculitis patients, P-ANCA was found positive in 277 (76.9%) and C-ANCA in 83 (23.1%) patients. When the PR3-ANCA, MPO-ANCA patterns were evaluated for ANCA-positive patients MPO-ANCA was found in 16 of 62 vasculitis patients, PR3-ANCA in 13 vasculitis patients; further, of 360 patients with non-vasculitis diseases, 20 presented MPO-ANCA and 10 presented PR3-ANCA pattern (Table 1, 2).

Discussion

As seen in our study and previous ones, ANCA-positivity can be detected in ANCA-associated vasculitis and other vasculitis forms [5-8]. In ANCA-associated vasculitis, PR3-ANCA and MPO-ANCA positivity define different conditions. Patients with GPA are predominantly PR3-ANCA positive, whereas patients with MPA are predominantly MPO-ANCA positive. It is possible to classify patients with AAV according to the specificity of ANCA. In addition, ANCA's specificity predicts induction treatment responses, recurrence risk and differences in long-term prognosis [9]. In our study, PR3-ANCA was predominantly positive in patients with GPA, MPO-ANCA was predominantly positive in patients with MPA and in patients with vasculitis associated with renal-localized vasculitis.

Leukocytoclastic vasculitis (LCV) is a histopathological definition of a common small vessel vasculitis (SVV) form that can be found in various types of vasculitis that affect the skin and internal organs. Autoantibodies (anti-nuclear antibodies and ANCA) are routinely studied among the diagnostic tests [10]. In a study of the prognostic factors of the LVC, only 21% of patients were ANCA-positive, and P-ANCA pattern was found in all positive patients. Forty percent of these patients presented systemic involvement. In LVC, P-ANCA is not identified as a marker for systemic vasculitis [5]. In our study, only 18.6% of patients with LCV diagnosis were ANCA-positive. Respectively, 62.5% and 37.5% of ANCA-positive patients presented P-ANCA and C-ANCA patterns.

In a study that explores the clinical importance of ANCA positivity in patients with IgA vasculitis (Henoch-Schoenlein purpura), ANCA positivity was reported to be 5.8%. All ANCA-positive patients presented the MPO-ANCA profile. Pulmonary and nerve involvement were observed at a higher rate in ANCA-positive patients. There was no significant difference in terms of renal involvement [6]. In our study, HSP patients presented a higher rate of ANCA positivity. MPO and PR3 target antigens were not detected.

ANCA positivity is seen in non-vasculitis diseases as well. Some of these diseases include connective tissue diseases, idiopathic interstitial pneumonia, autoimmune liver diseases, inflammatory bowel diseases, anti-glomerular basal membrane (GBM) disease, infections, and malignancy [3]. In our study, ANCA-positivity was detected in non-vasculitis diseases as well.

In rheumatoid arthritis (RA) P-ANCA positivity varies between 16% and 50%. On the other hand, MPO-ANCA positivity is rarely found with antigen-specific immunoassay. The clinical value of P-ANCA is not definitive [3]. In our study, P-ANCA positivity was found at such a high rate of 85%. However, the MPO-ANCA pattern was detected in a small number.

In a study where the importance of ANCA is investigated in systemic lupus erythematosus patients, ANCA prevalence was reported as 29.6% compared to IIF. P-ANCA (89%) was detected as the most common pattern. Two patients showed positivity to MPO and PR3. No significant correlation was detected between P-ANCA/C-ANCA positivity and clinic [11]. In another study involving 74 SLE patients in Colombia, 60 (81.1%) of patients were reported ANCA-positive by the IIF method. Only one patient presented specificity for PR3-ANCA with ELISA [12]. In our study, ANCA prevalence was found in 64 SLE patients at 46.9%. Like in the two studies, MPO and PR3 were detected rarely in our study.

Interstitial pulmonary diseases (ILD) include a heterogeneous pulmonary disease group. An underlying connective tissue disease or vasculitis associated with RA or ANCA can cause ILD. The pulmonary findings of these diseases may be prior to the systemic onset. As clinical, radiological and broncho-alveolar lavage data for patients with interstitial lung disease appear similar in ANCA-positive and ANCA-negative patients at diagnosis, a systemic screening for ANCA seems reasonable. A P-ANCA screening can be useful [13]. In our study, the P-ANCA pattern was seen at a high rate (85.7%) in ANCA-positive patients diagnosed with ILD.

Different studies conducted with multiple sclerosis patients report ANCA-positive results [14-16]. In a study conducted with 176 MS patients whose autoantibody distribution was evaluated, only four patients presented ANCA positivity, and ANCA positivity was not associated with the clinic [14]. A total of 117 MS patients were evaluated in a study that explored the importance of ANCA in patients with the idiopathic inflammatory-demyelinating disease (IIDD); one patient was reported with the C-ANCA pattern, and two patients with the P-ANCA pattern [15]. The research shows a high rate of ANCA-positivity according to the MS form. 46.2% of 13 Japanese patients with optical-spinal MS forms were reported to be P-ANCA-positive [16]. In our study with 247 MS-diagnosed patients, 19 were ANCA-positive. Thirteen of them were P-ANCA and 6 of them C-ANCA. ANCA positivity was detected at a higher rate than the previous studies [14, 15]. Since the study is retrospective, it was not possible to evaluate which form of MS the patients were.

Chronic renal failure etiology is very diverse. In a recent study that researched ANCA positivity in non-vasculitis diseases associated with ANCA, chronic renal disease has been reported to be the most common disease group [17]. In the present study, chronic renal failure was found as the most common disease among non-vasculitis diseases after connective tissue diseases.

Studies evaluating ANCA usually focus on a single disease, with the exception of ANCA-related vasculitis. There are a limited number of studies in which many diseases were evaluated simultaneously. In diseases other than ANCA-related vasculitis, ANCA positivity was most frequently observed in unspecified vasculitis among rheumatic and autoimmune diseases, when cardiovascular and cerebrovascular diseases were evaluated, most frequently in atherosclerotic heart disease, and in diseases related to liver, kidney and lungs, it was most frequently observed in chronic kidney disease [17]. In another study evaluating the test and diagnostic indications in patients with ANCA positivity, the cause could not be determined in 23% of AAV, 25% of unspecified vasculitis, 48% of inflammatory condition and 4% of patients [18].

The present study has considered the diagnoses of diseases discussed as part of the 2020 international consensus about ANCA testing, and many diseases were evaluated in

the same study concerning the ANCA testing. The limitations of the present study include, above all, its retrospective design, followed by its single-centered character.

As a result, cases of vasculitis were detected ANCA-positive at a higher rate than non-vasculitis diseases. P-ANCA was the most common pattern observed in both groups. In non-vasculitis diseases, the target antigen MPO-ANCA and PR3-ANCA positivity were rare compared to vasculitis cases. Among the ANCA-positive patients, the most common non-vasculitis disease was connective tissue disease, chronic renal failure, and interstitial pulmonary disease. ANCA testing can be used to support diagnosis and to assist in differential diagnosis in vasculitis and non-vasculitis diseases.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

S.Z.O., B.O. and M.D. constructed the main idea and hypothesis of the study. S.Z.O., B.O. and M.D. developed the theory and arranged/ edited the material and method section. S.Z.O., B.O., M.D. and C.E. has/have done the evaluation of the data in the Results section. Discussion section of the article written by S.Z.O.

S.Z.O., B.O., M.D., C.E., I.K., E.M. and A.C. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Comparison of the effectiveness of various valsalva maneuvers in the management of stable narrow and wide QRS complex tachycardia in the emergency department

Acil serviste stabil dar ve geniş QRS kompleksli taşikardi tedavisinde çeşitli valsalva manevralarının etkinliğinin karşılaştırılması

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Abstract

Purpose: Narrow and wide QRS complex tachycardias are among the causes of arrhythmia in patients frequently admitted to the emergency department (ED). The first recommended step in the management of patients with a stable narrow and wide QRS tachycardia is a vagal maneuver. Althougah there are many maneuvers that can provide vagal stimulation, the most effective vagal maneuver is still unknown. This study aims at determining the most effective vagal maneuver by comparing the four most commonly used maneuvers.

Materials and methods: One hundred and thirty-two patients were included in this study; they were randomly and equally distributed into four groups. Carotid sinus massage (CSM) was applied to the first group, the REVERT method was applied to the second group (a 10-cc injector was used), a modified REVERT method was applied to the third group (a 60-cc injector was used), and the abeslang (wooden tongue depressor) method was applied to the fourth group. During these four applications, the patients' vital signs were monitored, and preparations were made for any emergency.

Results: Of the 132 patients included in the study, 61 (46.2%) were males, and 71 (53.8%) were females. The average age of the patients was 56.3 years. One hundred and twenty-six had narrow QRS tachycardias, and 6 had stable wide QRS tachycardias. The patients were randomly distributed into 4 groups. In the 1st group, 1 patient (3%) returned to a normal rhythm with the CSM method. In the 2nd group, 3 patients (9.1%) returned to a normal rhythm with the REVERT method. Nine patients (27.3%) in the 3rd group returned to a normal rhythm with the modified REVERT method. 6 patients (18.2%) in the 4th group returned to normal rhythm with the abeslang method. None of the stable wide QRS tachycardias responded to vagal stimulation methods.

Conclusion: The modified REVERT and abeslang methods are preferred primarily in patients entering the ED with the complaint of a stable narrow or wide QRS tachycardia in the case that a vagal maneuver is to be applied. On the other hand, a vagal maneuver is not effective in the management of a stable wide QRS tachycardia.

Keywords: Emergency department, narrow QRS tachycardia, wide QRS tachycardia, vagal maneuvers, valsalva maneuver.

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Öz

Amaç: Dar ve geniş QRS kompleksli taşikrdilerin acil servise sık başvuru aritimi nedenlerindendir. Stabil dar ve geniş QRS taşikardisi olan hastaların tedavisinde önerilen ilk basamak vagal manevradır. Vagal stimülasyonu sağlayabilecek pek çok manevra olmasına rağmen en etkili vagal manevra hala bilinmemektedir. Bu çalışma en sık kullanılan dört manevrayı karşılaştırarak en etkili vagal manevrayı belirlemeyi amaçlamaktadır.

Gereç ve yöntem: Bu çalışmaya dahil edilen 132 hasta rastgele ve eşit olarak dört gruba dağıtıldılar. Birinci gruba karotis sinüs masajı (KSM), ikinci gruba REVERT yöntemi (10 cc'lik enjektör kullanıldı), üçüncü gruba modifiye REVERT yöntemi (60 cc'lik enjektör kullanıldı), ve dördüncü gruba abeslang (tahta dil bastırıcı) yöntemi uygulandı. Bu dört uygulama sırasında hastaların yaşamsal bulguları izlendi ve herhangi bir acil durum için hazırlık yapıldı.

Bulgular: Çalışmaya alınan 132 hastanın 61'i (%46,2) erkek, 71'i (%53,8) kadındı. Hastaların yaş ortalaması 56,3 idi. Hastaların 126'sında dar QRS taşikardileri ve 6'sında stabil geniş QRS taşikardileri vardı. Hastalar rastgele 4 gruba dağıtıldı. 1. grupta 1 hasta (%3) KSM yöntemi ile normal ritme döndü. 2. grupta ise 3 hasta (%9,1) REVERT yöntemi ile normal ritmine döndü. 3. Gruptaki 9 hasta (%27,3) modifiye REVERT yöntemi ile normal ritme döndü. 4. gruptaki 6 hasta (%18,2) abeslang yöntemi ile normal ritme döndü. Stabil geniş QRS taşikardilerinin hiçbiri vagal stimülasyon yöntemlerine yanıt vermedi.

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Sonuç: Acil servise stabil dar/geniş QRS taşikardi şikayetiyle başvuran hastalara vagal manevranın uygulanacağı durumda öncelikle modifiye REVERT ve abeslang yönteminin tercih edilmesini önerir. Öte yandan, stabil geniş QRS taşikardi yönetiminde vagal manevranın etkin olmadığı saptanmıştır.

Anahtar kelimeler: Acil servis, dar QRS taşikardi, geniş QRS taşikardi, vagal manevralar, valsalva manevrası.

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Introduction

Narrow QRS complex tachycardias (NCTs) and wide QRS complex tachycardias (WCTs) are among the causes of arrhythmia in patients frequently admitted to the emergency department (ED) [1, 2]. Although such tachycardias frequently occur in patients with normal anatomies and/or cardiac functions and rarely represent life-threatening conditions, they are common sources of morbidity and mortality [2, 3]. Narrow QRS complex tachycardias are rapid heart rhythms with a QRS duration of 120 ms or less; wide QRS complex tachycardias are rapid heart rhythms with a QRS duration of 120 ms or more. Narrow QRS complex tachycardias originate on or within the His bundle. Wide QRS complex tachycardias may be ventricular tachycardias, supraventricular tachycardias with a bundle branch block, or an accessory pathway.

Correctly diagnosing NCTs is an arduous exercise because NCTs and WCTs can sometimes have similar presentations on an electrocardiography (ECG). Two studies have suggested that medical house staff and attending physicians diagnose NCTs incorrectly in approximately 40% of cases. A study by Mehta et al. [4] demonstrated that junior doctors operating in an accident and emergency department had difficulties distinguishing paroxysmal supraventricular tachycardia through fast atrial fibrillation and atrial flutter. Other studies have confirmed this challenge, underlining the difficulties encountered in distinguishing paroxysmal supraventricular tachycardia from other NCTs [5-7]. In addition to making incorrect diagnoses because of a lack of specific knowledge of NCTs, physicians are often misled by their excessive trust of frequently incorrect computer-generated interpretations of supraventricular tachycardias. Since the distinction between NCTs and WCTs cannot be always made, the European Society of Cardiology (ESC) has published recommendations for this issue. According to the ESC-2019 guideline for the management of supraventricular tachycardia, the first recommended procedure for NCTs and WCTs is the vagal maneuver. There are many methods that provide vagal stimulation, but the most commonly practiced method is the valsalva maneuver. This study compares the effectiveness of carotid sinus massage (CSM), the REVERT method (10 cc injector), the modified REVERT method (60 cc injector), and uvula/pharyngeal stimulation with an abeslang stick (wooden tongue depressor).

Materials and methods

Study design

This study was carried out in the ED of Denizli Servergazi state hospital in Turkey, which admits approximately 190.000 patients annually. This study focuses on patients who were admitted to the hospital between March 17, 2022 and August 17, 2022 with symptoms of a stable NCT or WCT who were older than 17 years of age. In total, 132 patients agreed to participate in the study, and they were randomly and equally distributed into four groups. Carotid sinus massage was applied to the first group. The REVERT method was applied to the second group (a 10-cc injector was used). The modified REVERT method was applied to the third group (a 60-cc injector was used). Finally, the abeslang method was applied to the fourth group (Figure 1). A 60-cc syringe was used in the modified REVERT method because it is easier for patients to hold and allows for intrathoracic pressure to increase with more blowing. During the application of these four methods, patients' vital signs were monitored, and preparations were made for any emergency.

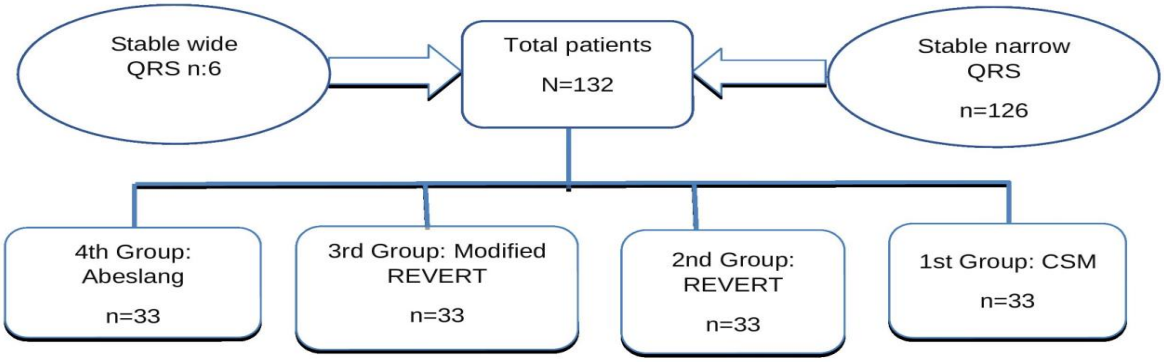

Figure 1. Distribution of patients participating in the study

Inclusion criteria

- Patients with symptoms of stable narrow/wide QRS complex tachycardia (palpitations, dyspnea, diaphoresis, angina, etc.)
- Patients with a heart rate of 150 beats per minute (bpm) or greater

Exclusion criteria

- Pregnant patients
- Patients with a history of transient ischemic attack (TIA) or stroke
- Patients with unilateral carotid artery stenosis
- Patients with carotid artery murmur

Data collection

Patients who entered the ED with symptomatic NCTs or WCTs were immediately monitored. After providing detailed information about their health status and the health history of their relatives, patients were also informed about the study. Only those patients who agreed to participate in the study and provided their consent were included. The type of vagal maneuver (SCM, REVERT, m.REVERT, abeslang) used on each patient was chosen based on a lottery; either the patient or their relatives drew their lots. In the drawing, a total of 132 cards were divided equally into four groups (SCM, REVERT, m.REVERT, abeslang). After the application of the vagal maneuver, the result was recorded. Patients who did not respond to the vagal maneuver were given medical treatment. All patients included in the study were in generally good health condition after treatment, and no mortality was detected.

Compliance with standards

This study was approved by Pamukkale University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee and Denizli provincial health directorate in Turkey.

Statistical analysis

Patient data was recorded using the SPSS 22.0 Windows software package (IBM Corp. Armonk, NY). Descriptive statistics were presented as percentages and median values. Fisher's exact test or Pearson's chi-square test analyzed the relationships between categorical variables. The Mann-Whitney U test evaluated variables that did not fit into the normal distribution.

Results

Of the 132 patients included in the study, 61 (46.2%) were males, and 71 (53.8%) were females; the average age of patients was 56.3 years (Table 1). Of these patients, 126 had stable NCTs and six had stable WCTs. The patients included in the study were randomly divided into four groups. In the first group, one patient (3%) returned to a normal rhythm with the CSM method. Three patients (9.1%) in the second group returned to a normal rhythm with the REVERT method. Nine patients (27.3%) in the third group returned to a normal rhythm with the modified REVERT method. Six patients

(18.2%) in the fourth group returned to a normal rhythm with the abeslang method massage (Adjusted residual values were: 2.4, 0.7, -1.0 and -2.1 respectively) (Table 2). None of the stable WCTs responded to vagal stimulation methods. While 125 (94.7%) of the 132 patients included in the study were discharged within 24 hours, seven patients (3.6%) were hospitalized for further diagnosis and treatment. While 66% (n=87) of the patients stated that they had been diagnosed with a cardiac arrhythmia before, 45 patients (34%) stated that they had no history of arrhythmia. Patients who did not respond to the vagal maneuver were given medical treatment. No adverse events were detected during the study period. The inclusion criteria for patients in this study included the criterion of "stability of the patients." All patients included in this study were in generally good health condition after treatment, and no mortality was detected.

Table 1. Demographic characteristics of the patients included in this study

Demographic Characteristics	Total	*p*
Average age/year	56.3	-
Male n (%)	61 (46.2)	0.608
Female n (%)	71 (53.8)	

Table 2. Effectiveness of the various vagal maneuvers applied in the management of stable tachycardias

Groups	Group analysis	Resolved rhythm/ return to normal	Unresolved rhythm/failure to return to normal	Total
1st Group	N (%)	1 (3.0)	32 (97.0)	33 (100)
	Adjusted residual	-2.1	2.1	-
2nd Group	N (%)	3 (9.1)	30 (90.9)	33 (100)
	Adjusted residual	-1.0	1,0	-
3rd Group	N (%)	9 (27.3)	24 (72.7)	33 (100)
	Adjusted residual	2.4	-2.4	-
4th Group	N (%)	6 (18.2)	27 (81.8)	33 (100)
	Adjusted residual	0.7	-0.7	-
Total	N (%)	19 (14.4)	113 (85.6)	132 (100)

Pearson's chi-squared test was used

Discussion

Wide and narrow QRS tachycardias are frequently encountered in the ED. While some of these cases may be symptomatic and self-terminating, others lead to severe outcomes. According to the ESC's guideline for the management of supraventricular tachycardia (SVT) (ESC 2019), the first recommended procedure for stable NCTs and WCTs is the vagal maneuver [1]. With vagal maneuvers, the main goal is to provide vagal stimulation. The autonomic nervous system consists of two parts: the sympathetic and the parasympathetic systems. The entire autonomic nervous system regulates visceral functions. For example, in the thorax and abdomen, the vagus nerve is the dominating nerve in terms of parasympathetic nervous system activity. This parasympathetic response can be created with various physical maneuvers, which can be used both diagnostically, such as in the case of carotid sinus hypersensitivity, and therapeutically, such as in the case of the termination of SVT. Through the provision of vagal stimulation, parasympathetic (vagal) stimulation in the heart causes the local release of acetylcholine, thus reducing the rate of impulse generation in the sinus node, slowing the conduction rate in the atrioventricular (AV) node, and prolonging the refractory period.

There are many types of maneuvers to provide vagal stimulation, but CSM is the most commonly used approach. In addition to the lack of clear information about the effectiveness of CSM, it is not recommended in patients with a history of transient ischemic attack and stroke, unilateral carotid artery stenosis, or carotid artery murmur. Although the choice of vagal maneuver in SVT patients depends on the clinical scenario and the patient's ability to successfully perform the maneuvers, there is no clear indication as to which maneuver should be preferred first.

Although the effectiveness of these maneuvers has been frequently compared in pairs in many studies, there have not been enough studies that explore multiple maneuvers and consider whether the effects of the maneuvers are permanent. This study conducted a clinical comparison of four different vagal maneuvers in terms of their ease of use in the ED. Of the 132 patients included in our study, 61 (46.2%) were males, and 71 (53.8%) were females. There was no statistical difference between the two groups (p=0.608). The average age of the patients was 56.3. In the electrophysiological study conducted by Brembilla et al. [2] where the relationship between Paroxysmal Supraventricular Tachycardia (PSVT) and age was examined, most patients were found to be between 50 and 60 years of age. However, at the end of the study, the authors state that age had no effect on the mechanism of tachycardia in patients with PSVT [2].

In our study, modified REVERT was found to be the most effective vagal maneuver method to return stable NCTs and WCTs to a normal rhythm, followed by the abeslang method and then the REVERT method. The least effective maneuver was carotid massage (Adjusted residual values were: 2.4, 0.7, -1.0 and -2.1 respectively). In a study on spontaneous PSVT patients conducted by Ornato et al. [3], the success rate of CSM was found to be 11.8%. The study also concluded that it was much more difficult to return spontaneously developing SVTs to a normal rhythm with CSM. Mehta et al. [4] and Wen et al. [5] have argued that the Valsalva maneuver, such as REVERT, is more effective than carotid massage. In a randomized controlled study in an ED, Lim et al. [6] achieved a success rate of 10.5% with CSM and a 19.4% success with the valsalva maneuver, which includes REVERT in the initial treatment. In a randomized controlled study conducted by Appelboam et al. [7], the response to the REVERT method was found to be around 43%. These results suggest that the REVERT method is superior to the carotid sinus massage method. Unfortunately, no study on the modified REVERT and abeslang methods could be found, except for one by Nicolas and Collins. Conducted in 2015, their study compared the benefit-harm situation by examining previous articles about the clinical use of CSM. In their conclusion, they express their opinion in favor of the use of safer and more effective alternative maneuvers due to the potential devastating side effects [8].

Although vagal maneuvers are primarily effective in the case of SVT, they are sometimes recommended to be used to differentiate SVT from stable ventricular tachycardias. In our study, no WCTs returned to a normal rhythm with vagal maneuvers [1, 9].

With regard to the limitations of this study, the effect of vagal maneuvers on WCTs could not be clearly determined due to the small number of patients with stable WCTs included in the study. It should be noted, however, that some of the patients with symptomatic tachycardia did not consent to participate in any study before they were treated and recovered because of anxiety and death guard. Another limitation of our study was its short duration; as a result, other modified vagal maneuvers could not be studied.

This study recommends that the modified REVERT and abeslang methods should be preferred primarily in patients visiting the ED with the complaint of stable NCTs or WCTs in the case that a vagal maneuver is to be applied. We do not recommend carotid massage, especially in the elderly and patients with a history of TIA/CVO, unilateral carotid artery stenosis, and carotid artery murmur due to its high complication rate and low effectiveness. Additionally, this study, which has found that vagal maneuvers are not effective in the management of stable WCTs, suggests that researchers conduct further studies on this subject with larger samples.

Conflict of interest: No conflict of interest was declared by the authors.

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Ethics committee approval: This study was approved by Pamukkale University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (date: 15.11.2022, number: E-60116787-020-287527) and Denizli provincial health directorate in Turkey.

Authors' contributions to the article

Authors' contributions to the article R.B. constructed the main idea and hypothesis of the study. R.B. and M.O. developed the theory and arranged/edited the material and method section. R.B. has done the evaluation of the data in the results section. Discussion section of the article was written by R.B., M.S. and M.O., R.B. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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The relationship between COPD attack applications and air pollution in the emergency department

Acil serviste KOAH atağı başvuruları ile hava kirliliği arasındaki ilişki

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Abstract

Purpose: We aimed to investigate the relationship between air pollution, temperature and COPD attacks in two different centers.

Materials and methods: Two centers, Bandırma and Van, were included in the study. In addition, temperature grouping was also done. Air pollution and temperature values were obtained from official sites. COPD data were scanned retrospectively from hospital information management systems.

Results: In the first 3 months (Group 1) included in the study, the PM10 value, the number of COPD treatment in the emergency department (ED) and the number of COPD hospitalizations in the ED were also found to be high in Van (p=0.05, p=0.05 and p=0.034, respectively). In the last 3 months (Group 2) period included in the study, it was observed that the mean temperature was lower in Van, and the rate of hospitalizations and hospitalizations due to COPD were higher in Van (p=0.05, p=0.05, and p=0.05, respectively). In the correlation analysis, a strong positive correlation was found between PM10 value and COPD treatment and hospitalization for COPD in Group 1 (r;0.986, p<0.001 and r;0.885, p=0.019, respectively). In Group 2, a strong negative correlation was found between the decrease in air temperatures and COPD treatment in the ED, hospitalization due to COPD and hospitalization rates (r;-0.905, p=0.013, r;-0.966, p=0.002 ve r;-0.867, p=0.025, respectively).

Conclusion: COPD attacks are associated with temperature and air pollution. For COPD attacks in ED, possible increases in intensity can be estimated by closely monitoring air pollution parameters as well as temperature.

Keywords: Chronic obstructed pulmonary disease, COPD attack, air pollution, temperature, emergency medicine.

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Öz

Amaç: Çalışmamızda hava kirliliği, sıcaklık ve KOAH atakları arasındaki ilişkiyi iki farklı merkezde araştırmayı amaçladık.

Gereç ve yöntem: Bandırma ve Van olmak üzere iki merkez çalışmaya dâhil edildi. Ayrıca sıcaklık gruplandırması da yapılmıştır. Hava kirliliği ve sıcaklık değerleri resmi sitelerden alınmıştır. KOAH verileri geriye dönük olarak hastane bilgi yönetim sistemlerinden tarandı.

Bulgular: Çalışmaya alınan ilk 3 aylık dönemde (Grup 1) de Van' daki hava kirliliği ölçütü olan PM10 değeri Bandırma'ya göre daha yüksek saptanmıştır (p=0,05). Benzer şekilde acil serviste COPD tedavisi ve acil servisten COPD yatış sayıları da Van'da yüksek saptanmıştır (sırasıyla p=0,05 ve p=0,034). Çalışmaya alınan son 3 aylık periyodda sıcaklık ortalamasının Van' da daha düşük ve acil servisten COPD nedenli yatışların ve yatış oranının daha fazla olduğu gözlenmiştir (sırasıyla p=0,05, p=0,05 ve p=0,05). Yapılan korelasyon analizinde Grup 1' de PM10 değeri arttıkça acil serviste COPD tedavisi alma ve COPD nedenli yatışta kuvvetli bir pozitif korelasyon saptanmıştır (sırasıyla r;0,986, p<0,001 ve r;0,885, p=0,019). Grup 2' de hava sıcaklıklarının düşmesi ile acil serviste COPD tedavisi alma, COPD nedenli yatışta ve yatış oranlarında kuvvetli bir negatif korelasyon saptanmıştır (sırasıyla r;-0,905, p=0,013, r;-0,966, p=0,002 ve r;-0,867, p=0,025).

Sonuç: KOAH atakları sıcaklık ve hava kirliliği ile ilişkilidir. Acil servislerde KOAH atakları için sıcaklık yanı sıra hava kirliliği parametreleri yakından takip edilerek yoğunluktaki olası artışlar tahmin edilebilir.

Anahtar kelimeler: Kronik obstrüktif akciğer hastalığı, KOAH atağı, hava kirliliği, sıcaklık, acil tıp.

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Introduction

Chronic obstructive pulmonary disease (COPD); It is characterized by progressive limitation of airflow that is completely irreversible. It is an important disease in both high-income and low-income countries worldwide. [1]. Several factors for the occurrence of COPD are well documented, such as genetics, smoking, occupational exposure, and chronic asthma, but the nature of exacerbations is not fully elucidated, although various aetiologies have been suggested for COPD exacerbations. [2, 3]. Although the role of air pollution in the development of COPD is still controversial in current studies, adverse effects of air pollution, particularly particulate matter (PM) and nitrogen oxides (NOx) on COPD risk, have been demonstrated [4-7]. Particularly, particulate matter with an aerodynamic diameter of less than 2.5 μm ($PM_{2.5}$) is an important risk factor for COPD [8]. Although regular measurements of each parameter are made at air measurement stations, PM_{10} values are usually measured. One important variable in COPD exacerbations is seasonal variation. Wise et al. [9] in the Tiotropium Safety and Performance In Respimat (TIOSPIR) study conducted by them, it was observed that exacerbations peaked in December, whereas severe attacks peaked in January, regardless of severity.

In our study, it was aimed to compare the relationship between air pollution, temperature and COPD attacks in two different centers between August and January.

Materials and methods

The study was approved by the health science Non-Interventional Research Ethics Committee of Bandırma Onyedi Eylül University.

Two centers, Bandırma and Van, were included in the study to better evaluate conditions such as air pollution and temperature difference. Both centers had a tertiary education and research hospital. Only adult emergency service (≥18 years old) applications were evaluated in the study. PM10 values of the air pollution data of the centers between August 2022 and January 2023 were taken from the air quality monitoring system of The Ministry of Environment, Urbanization and Climate (http://www.havaizleme.gov.tr) [10]. Air temperature measurements were obtained from the website of the General Directorate of Meteorology at https://www.mgm.gov.tr and from the website https://tr.weatherspark.com as the monthly average temperature [11, 12]. The parameters presented below were obtained from the hospital information management system retrospectively monthly.

- Number of admissions to the emergency department (ED)

- Number of patients treated for COPD in the ED

- Number of patients hospitalized with COPD from the ED

Statistical analysis

SPSS 22 program was used for statistical analysis. The normal distribution of data was tested with the Kolmagorov-Smirnow / Shapiro-Wilks test. Number, percentage, mean, median, standard deviation, and minimum-maximum expressions were used for descriptive statistics. Mann Whitney-U tests were used to compare the mean between 2 independent groups. Pearson correlation analyses or Spearman's correlation analyzes were used in the correlation between centers, air pollution parameters, temperature averages, number of admissions to the ED, number of patients treated for COPD in the ED, number of patients hospitalized with COPD from the ED, and rate of hospitalization from the ED. $P<0.05$ was accepted for statistical significance.

Results

The total number of ED admissions, the number of COPD patients treated in the ED, the number of patients hospitalized with COPD from the ED, and hospitalization rates were evaluated in both centers over 6 months. In Bandırma, the total number of applications to the ED 6 months period was 140141 people, the number of patients with COPD treated in the ED was 46 people, and the number of hospitalizations from the ED due to COPD was 15 people. Similar parameters were determined for Van as 96532 people, 87 people, and 48 people, respectively. In addition to ED information, basic information about air temperature and air pollution parameters is shown in Table 1.

Table 1. Basic information of the centers

Centers	Months	Air Pollution Parameter	Average Monthly Temperature (°C)	Number of admissions to the ED	Number of patients treated for COPD in the ED	Number of patients hospitalized with COPD from the ED	Hospitalization rate from the ED (%)
		PM_{10}					
Bandirma	August	32.90	24.0	20568	3	1	33.33
	September	31.29	20.5	20006	2	1	50.00
	October	36.51	16.0	21096	4	1	25.00
	November	46.17	10.5	23535	9	3	33.33
	December	49.04	7.5	29037	17	5	29.41
	January	45.46	6.0	25899	11	4	36.36
Van	August	44.52	21.0	16552	8	3	37.50
	September	43.58	17.0	15252	7	5	71.43
	October	44.99	11.0	16302	9	5	55.56
	November	49.61	5.0	15712	15	8	53.33
	December	46.25	1.0	18007	23	12	52.17
	January	53.07	-3.0	14707	25	15	60.00

ED; Emergency department, COPD; Chronic obstructive pulmonary disease, PM_{10}; Particulate Matter, SO_2; Sulfur Dioxide, NO_2; Nitrogen Dioxide

According to the seasonal grouping of Group 1 (August-September-October) and Group 2 (November-December-January), the comparison of air pollution parameters, temperature variables, and emergency parameters of the centers is shown in Table 2. In the first 3 months period (Group 1) included in the study, the PM_{10} value, which is the measure of air pollution in Van, was found to be statistically significantly higher than in Bandırma (p=0.05). Similarly, the number of COPD treatments in the ED and the number of COPD hospitalizations in the ED were also found to be high in Van (p=0.05 and p=0.034, respectively). No statistically significant difference was found between Bandırma and Van between PM_{10} values in the last 3 months period (Group 2) included in the study (p=0.127). It was observed that the mean temperature in Van was lower than in Bandırma, and the number of hospitalizations due to COPD from the ED and hospitalization rate was higher in Van than in Bandırma (p=0.05, p=0.05, and p=0.05, respectively).

In seasonal grouping, correlation analysis between institutional variation, PM_{10} value, average air temperature, number of admissions to the ED, number of patients treated for COPD in the ED, number of patients hospitalized with COPD from the ED and hospitalization rates in the results; In the first 3 months of the study (Group 1), a strong correlation was found between the PM_{10} value and the institutional relationship in favor of Van province (rho;0.878, p=0.021). As the PM_{10} value increased, a strong positive correlation was found between the number of patients treated for COPD in the ED and the number of patients hospitalized with COPD from the ED (r;0.986, p<0.001 and r;0.885, p=0.019, respectively). A similar relationship was not observed for air temperature averages (Table 3). In the last 3 months of the study (Group 2), no statistically significant relationship was observed between the PM_{10} value and the centers (p=0.135). It was observed that the air temperature decreased in Van as the center (rho;-0.878, p=0.021). Similarly, a strong negative correlation was found between the decrease in air temperatures and the number of patients treated for COPD in the ED, the number of patients hospitalized with COPD from the ED, and the hospitalization rate (r;-0.905, p=0.013, r;-0.966, p=0.002 and r;-0.867, p=0.025, respectively).

Table 2. Comparison of air pollution parameters, temperature variables, and emergency parameters of the centers according to the grouping of the months

		Group 1 (August, September, October)		*p*	Group 2 (November, December, January)		*p*
		Bandirma	**Van**		**Bandırma**	**Van**	
Air Pollution Parameters	**PM_{10} [median (min.; max.)]**	32.9 (31.29; 36.51)	44.52 (43.58; 44.99)	0.05	46.17 (45.46; 49.04)	49.61 (46.25; 53.07)	0.127
Average Monthly Temperature (°C) [median (min.; max.)]		20.5 (16; 24)	17 (11; 21)	0.513	7.5 (6; 10.5)	1 (-3; 5)	0.05
Number of admissions to the ED [median (min.; max.)]		20568 (20006; 21096)	16302 (15252; 16552)	0.05	25899 (23535; 29037)	15712 (14707; 18007)	0.05
Number of patients treated for COPD in the ED [median (min.; max.)]		3 (2; 4)	8 (7; 9)	0.05	11 (9; 17)	23 (15; 25)	0.127
Number of patients hospitalized with COPD from the ED [median (min.; max.)]		1 (1; 1)	5 (3, 5)	0.034	4 (3;5)	12 (8; 15)	0.05
Hospitalization rate from the ED (%) [median (min.; max.)]		33.33 (25; 50)	55.55 (37.50; 71.43)	0.127	33.33 (29.41; 36.36)	53.33 (52.17; 60)	0.05

ED; Emergency department. COPD; Chronic obstructive pulmonary disease, PM_{10}; Particulate Matter, Min.; Minimum, Max.; Maximum

Table 3. Results of correlation analysis groups and parameters

			Centers	Air Pollution Parameter PM_{10}	Average Monthly Temperature (°C)	Number of patients treated for COPD in the ED	Number of patients hospitalized with COPD from the ED	Hospitalization rate from the ED (%)
Gruop 1	Centers	Correlation Coefficient	1.000	0.878	-0.293	0.878	0.949	0.683
		Sig. (2-tailed)	-	0.021	0.573	0.021	0.004	0.135
		N	6	6	6	6	6	6
	Air Pollution Parameter PM_{10}	Correlation Coefficient	0.878	1.000	-0.572	0.986	0.885	0.433
		Sig. (2-tailed)	0.021	-	0.235	<0.001	0.019	0.391
		N	6	6	6	6	6	6
	Average Monthly Temperature (°C)	Correlation Coefficient	-0.293	-0.572	1.000	-0.595	-0.633	-0.365
		Sig. (2-tailed)	0.573	0.235	-	0.213	0.177	0.477
		N	6	6	6	6	6	6
Group 2	Centers	Correlation Coefficient	1.000	0.683	-0.878	0.683	-0.878	-0.878
		Sig. (2-tailed)	-	0.135	0.021	0.135	0.021	0.021
		N	6	6	6	6	6	6
	Air Pollution Parameter PM_{10}	Correlation Coefficient	0.683	1.000	-0.614	0.621	0.649	0.578
		Sig. (2-tailed)	0.135	-	0.195	0.188	0.164	0.229
		N	6	6	6	6	6	6
	Average Monthly Temperature (°C)	Correlation Coefficient	-0.878	-0.614	1.000	-0.905	-0.966	-0.867
		Sig. (2-tailed)	0.021	0.195	-	0.013	0.002	0.025
		N	6	6	6	6	6	6

ED; Emergency department. COPD; Chronic obstructive pulmonary disease, PM_{10}; Particulate Matter, Min; Minimum, Max; Maximum

Discussion

In our study, there is a positive relationship with air pollution and a negative relationship with air temperatures between COPD treatment in the emergency room and the number of COPD hospitalizations in the emergency room. COPD is a life-threatening progressive lung disease that obstructs airflow from the lung, predisposing to exacerbations and serious illness [13]. COPD attacks, one of the most common causes of hospitalization, pose the greatest burden on the health system in developed countries [14]. Environmental factors and air pollution are thought to be the second most common cause of COPD attacks after infections. In studies conducted in the USA and Europe, it has been shown that the rate of admission to the hospital for COPD attacks increases when the number of respirable particles (<10μm) and the amount of "ozone" in the air increase [15]. Although epidemiological data show that increased air pollution is associated with a slight increase in COPD attacks and hospital admissions, its mechanism is not well known [16]. As a mechanism, air pollution particles induce proinflammatory immune responses in multiple immune cell classes by affecting different types of immune cells such as macrophages, inflammatory neutrophils, as well as mucosal irritation [17]. The clinical effects of air pollution, particularly the known association between high ambient pollution and attacks of asthma and COPD, are consistent with these identified immunological mechanisms [17].

Air pollution; can be defined as the presence of pollutants such as particulate matter (PM), sulfur dioxide (SO_2), and nitrogen oxides (NOx) in the outdoor air we breathe at levels that will have negative effects on the environment and health [18]. According to the World Health Organization (WHO) studies, upper limits have been determined for the annual average density values of these gases that cause air pollution. WHO has stated that the annual average value of 40 μg/m3 for NO_2, the ratio of SO_2 concentration in the air to 500 μg/m3 for 10 minutes on average, and that for PM_{10} this value exceeds the annual average of 25 μg/m3, the ambient air is polluted and inhaled [19]. Shehu et al. [14] showed that there is a relationship between COPD and environmental pollution. Wang et al. [20] showed in their study that long-term exposure to air pollution, including $PM_{2.5}$, PM_{10}, NOx, and NO_2, was positively associated with the risk of COPD. The proven effects of air pollution include chronic obstructive pulmonary disease and exacerbations of pre-existing obstructive airway disease, as well as lung cancer [20, 21].

The relationship between air pollution and COPD attacks is also stated in the literature. In studies conducted in İzmir and Eskişehir, an increased relationship was found between PM and SO2 levels and increased nasal resistance, and increased COPD emergency hospital admissions [22, 23]. In the study conducted by Fişekçi et al. [24] from Denizli, a correlation was observed between the average SO_2 and PM of the previous week and emergency hospital admissions due to COPD. In the same study, it was stated that the relative risk ratio in emergency admissions due to COPD increased with the increase in daily SO_2 and PM amounts [24]. In our study, we observed that the number of patients treated for COPD in the ED increased with air pollution (PM_{10}) in group 1. Jenkins et al. [25] In the TORCH (TOwards a Revolution in COPD Health) study, it is stated that while attacks are seen in 9% of patients in the northern hemisphere between November and February, this rate drops to 5% between June and August. Similarly, Wise et al. [9] reported that COPD exacerbations peaked in December in the TIOSPIR study. This may explain the difference between air pollution and the number of patients admitted to the emergency department for COPD treatment between the centers (Bandırma and Van) in August, September, and October in our study. Extremely cold temperatures, together with increases in mortality and morbidity in people with COPD, have an impact on lung function and COPD attack risk [26]. A large study using national health insurance registry data in Taiwan found a 0.8% increase in COPD episodes for every 1°C decrease in mean daily temperature [27]. This pathogenesis is explained in the literature by considering mucus hypersecretion as a potential mediator of the cold response of COPD, in addition to the bronchoconstriction and inflammation that may occur with cold exposure [28, 29]. The difference in the number of patients treated for COPD in the ED, number of patients hospitalized with COPD from the ED, and hospitalization rates, which includes

November, December, and January, in which air pollution between centers was observed in our study, supports this situation in the literature. In addition, the negative correlation between temperature and the number of patients treated for COPD in the ED, the number of patients hospitalized with COPD from the ED, and hospitalization rates in the same seasonal period supports this situation.

As a result, COPD attacks are associated with seasonal temperature and air pollution. Although seasonal changes can be taken into account in the provision of emergency services, possible increases in intensity can be predicted by closely monitoring air pollution parameters. There is a need for large randomized controlled multicenter studies on this subject.

Conflict of interest: The authors declare that there is no conflict of interest.

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Ethics committee approval: The study was approved by the health science Non-Interventional Research Ethics Committee of Bandırma Onyedi Eylül University (The approval date: 23.02.2023 and number is 310)

Authors' contributions to the article

H.Y.B constructed the main idea and hypothesis of the study. H.Y.B developed the theory and arranged/edited the material and method section. H.Y.B and H.N.C have done the evaluation of the data in the Results section. Discussion section of the article written by H.Y.B.

H.Y.B and H.N.C reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Analytical investigation of demographic, laboratory, and clinical characteristics of patients with microbial keratitis

Mikrobiyal keratitli hastaların demografik, laboratuvar ve klinik özelliklerinin analitik olarak incelenmesi

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Abstract

Purpose: In this study, we investigated epidemiological properties, clinical findings, risk factors, direct microscopy, and culture results in patients diagnosed with microbial keratitis.

Materials and methods: We examined the hospital records of patients with microbial keratitis between March 2016 and March 2021, retrospectively. Also, clinical findings, risk factors, microbiological results, empirical treatment and, treatment responses were evaluated.

Results: 42 eyes of 42 patients whose mean age was 57.8 (range 18-70 years) were included in the study. Gram-positive and gram-negative bacteria were found on stained microscopic examination in 12 patients (28.5%). In total, microbial growth was detected in the culture of 7 patients (16.6%), while growth was not detected in 35 patients (83.4%). No etiological factor was detected in 27 patients (64.4%). The complaints at admission were pain in 24 patients (57.6%), redness in 12 patients (28.8%), and both redness and pain in 6 patients (14.1%). While the visual acuity of 18 cases was preserved after the treatment, 24 cases (88.8%) achieved 1 or more line with the treatment. While the mean visual acuity was 0.79±1.1 (0-3.1) logMAR before treatment, it increased to 0.69±1.1 (0-3.1) logMAR after treatment (p=0.006).

Conclusion: The keratitis is a common cause of unilateral blindness. Early diagnosis and treatment of keratitis is a significant role in the prognosis. The success of the therapy can be provided by starting empirical antimicrobial therapy by taking into consideration of the regional risk factors and common pathogens.

Keywords: Etiology, keratitis, treatment.

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Öz

Amaç: Bu çalışmada mikrobiyal keratit tanısı alan hastalarda epidemiyolojik özellikler, klinik bulgular, risk faktörleri, direkt mikroskopi ve kültür sonuçlarını inceledik.

Gereç ve yöntem: Mart 2016-Mart 2021 tarihleri arasında mikrobiyal keratit tanısı alan hastaların hastane kayıtları retrospektif olarak incelendi. Ayrıca klinik bulgular, risk faktörleri, mikrobiyolojik sonuçlar, ampirik tedavi ve tedaviye yanıt değerlendirildi.

Bulgular: Yaş ortalaması 57,8 (dağılım 18-70) olan 42 hastanın 42 gözü çalışmaya dahil edildi. Boyalı mikroskopik incelemede 12 hastada (%28,5) gram-pozitif ve gram-negatif bakteriler saptandı. Toplamda 7 hastada kültürde üreme saptandı (%16,6), 35 hastada (%83,4) büyüme saptanmazken, 27 hastada (%64,4) etyolojik özellik saptanmadı, 24 hastada (%57,6) başvuru yakınmaları ağrı, 12 hastada (%28,8) kızarıklık şeklindeydi ve 6 hastada (%14,1) kızarıklık ve ağrı şikayetleri vardı. 18 olgunun tedavi sonrası görme düzeyleri korunurken, olguların 24'ünde (%88,8) tedavi ile 1 sıra ve üzeri görme artışı sağlandı. Tedavi öncesi ortalama görme keskinliği 0,79±1,1 (0-3,1) logMAR iken tedavi sonrası 0,69±1,1 (0-3,1) logMAR'a yükseldi (p=0,006).

Sonuç: Keratit, tek taraflı körlüğün yaygın bir nedenidir. Keratitte erken tanı ve tedavi prognoz üzerinde önemli rol oynar. Bölgesel risk faktörleri ve sık görülen patojenler dikkate alınarak ampirik antimikrobiyal tedaviye başlanması tedavinin başarısı sağlayabilir.

Anahtar kelimeler: Etiyoloji, keratit, tedavi.

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Introduction

Microbial keratitis, (MK) characterized by infiltration in the epithelial and stromal layers of the cornea, is one of the leading causes of unilateral blindness all over the world [1]. Contact lens wear, surgical or nonsurgical trauma, previous corneal disease, and ocular surface problems are some of the predisposing risk factors for microbial keratitis, and the condition can be caused by a variety of bacteria [2]. The most common cause of microbial keratitis in developed countries is incorrect contact lens use, while ocular trauma takes the first place in the etiology in developing countries [3].

For efficient diagnosis, care, and prevention of microbial keratitis, it is vital to determine the incidence, microbiological agent diversity, and predisposing factors [4]. Before the culture and antibiotic sensitivity results are obtained, the clinician must decide on the antibiotic regimen and immediately start the treatment. While making this decision, the patient's demographics, risk factor profile, and local microbial distribution model are important. Geographic and climatic conditions generate regional variances in the pattern of microbiological isolates, necessitating local epidemiological research [5].

Our aim in this study is to determine the demographic characteristics of the patients we have followed up in our clinic for the last 5 years with the diagnosis of keratitis, to get an idea about the etiology of keratitis in our region, to evaluate the causative microorganisms, to present the empirical treatment protocols we have applied and the responses we have received to the treatment.

Materials and methods

Fourty-two eyes of 42 patients hospitalized in our ward with the diagnosis of keratitis from March 2016 and March 2021 were included in this retrospective study. The ethics committee approval of Recep Tayyip Erdogan University was obtained for the study and the Helsinki Declaration rules were followed. Patients who were hospitalized for less than 3 days in make microbiological evaluations were excluded from the study. The patient's age, gender, complaints, visual acuity before and after treatment, ocular examination findings including biomicroscopy, intraocular pressure measurement, fundoscopy and ocular ultrasonography, and treatment protocols were documented from their charts. After being admitted to our service, they were re-examined in our service and their consent was obtained for corneal scraping.

Before corneal scraping, one drop of topical anesthetic (0.5% proparacaine-Alcaine®) was instilled into the keratitis eye. Some irrigation was done with physiological saline. Scraping samples were taken from the edges of the lesion with the help of a sterile scalpel under the guidance of a slit lamp, and the slide was spread, then inoculated on blood agar, chocolate agar, thiogluconate medium and sabouraud agar. It was sent to the microbiology laboratory for stained microscopic examination and culture antibiogram. Empirical topical augmented treatments were started hourly, without waiting for the laboratory results, and changes were made in the treatment protocols according to the laboratory results. Dose adjustments were made according to the response to the treatment and the toxic reaction caused by the side effects of the treatment. Considering the clinical findings, subconjunctival treatment was also applied in some unresponsive cases with hypopyon. Topical steroid treatment was also added to the patients who had no epithelial defect and regression in their clinical findings in the post-discharge controls. Examination findings were noted every day. Reduction in the area and depth of infiltration, regularization of borders, and decrease in anterior chamber reaction, hypopyon, and pain were accepted as clinical improvement findings.

SPSS 20 package program was used for statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and numerical measurements were summarized as mean (median and minimum-maximum where necessary). The statistical significance level was accepted as $p \leq 0.05$.

Results

The mean age was 57.8 years (range 18-70), 50% were female and 50% were male. In 12 (28.5%) patients, a positive finding was found in the stained microscopic examination. In 9 of them, gr (+) cocci were detected and only leukocytes were found in 3 patients. While no factor could be seen in direct examination in three patients, growth was detected in the

culture. Pathogen was detected in both direct examination and culture in 3 patients. Pathogen was detected in direct examination in nine patients, but there was no growth in culture. In 71.5% of the patients, no findings were found in both the stained microscopic examination and the culture. Pseudomonas aeruginosa was grown in the culture of two of the 12 patients with Gram-positive cocci, and Streptococcus Pneumonia was grown in the others. In the cultures of 3 patients whose direct examination was negative, pseudomonas aeruginosa grew again. In total, growth was detected in culture in 7 patients (16.6%), while growth was not detected in 35 patients (83.4%). The results are shown in Table 1.

Table 1. Cases with microbiological findings

	Culture -	Culture +	Total
Direct view +	9	3	12
Direct view -	6	24	30
Total	15	27	42

There were predisposing factors that could cause keratitis in 15 patients (35.6%). Herbal trauma in 9 patients, no antibiotic use following foreign body removal in 4 patients, and unhygienic contact lens use in 2 patients were noted. No etiological feature was detected in 27 patients (64.4%). The complaints at admission were pain in 24 patients (57.6%), redness in 12 patients (28.8%), and both redness and pain in 6 patients (14.1%). At the first admission, the visual level was hand movements in 5 cases, finger counting from 1 meter (mps) – 5 mps in 6 cases, 0.1-0.5 in 16 cases, and full vision in 15 cases. While the visual levels 15 cases were preserved after the treatment, 24 cases (88.8%) achieved 1 or more line with the treatment. Visual acuity did not change in 3 cases (11.2%). While the mean visual acuity was 0.79±1.1 (0-3.1) logMAR before treatment, it increased to 0.69±1.1 (0-3.1) logMAR after treatment (p=0.006).

Vancomycin (50 mg/ml) and amikacin (50 mg/ml) combination were given to 57.14% of patients (28 patients), and 33.3% (8 patients) to vancomycin + amikacin + amphotericin B (0.15 mg/ml) combination, 4.76% (2 patients) fluconazole (0.04 mg/ml) + moxifloxacin; Topical fortified treatments were started empirically in 4.76% (2 patients) of amphotericin B + moxifloxacin + gentamicin (14 mg/ml) and 4.76% (2 patients) with vancomycin + ceftazidime (50 mg/ml). Systemic antibiotic therapy was not given because the ocular transmission was low. When the clinical response to the given treatment was evaluated, 80.9% of the patients (34 patients) benefited from the treatment. No clinical response was obtained in 8 patients (19.1%).

Discussion

Microbial keratitis is still one of the leading causes of unilateral blindness. Although there are predisposing factors such as ocular trauma and contact lens use, there are some microorganisms that can penetrate through the intact cornea [6]. Microorganisms and etiological factors causing keratitis may vary according to geographical regions [7]. Effective treatment of keratitis is possible by accurately determining the causative microorganism and initiating appropriate empirical treatment.

Rize; It is a province of the Eastern Black Sea Region, which has a climate with cool summers, mild winters, and rainy seasons. Tea production in the region is a source of livelihood. Our aim in this study; is to examine the keratitis patients in our region, to determine the epidemiological features and the factors that predispose to keratitis, to determine the importance of culture and gram staining in the detection of common microorganisms, and to examine the effectiveness of empirical treatment.

In our study, there were 42 patients and the mean age was 57.8. The numbers of men and women were equal. There is no clear distinction regarding gender in the literature. While the rate is in favor of women in Madurai, it is in favor of men in Praguay and Nepal [8, 9]. Although there was no gender difference in our study, it can be explained by the fact that males are more common in some publications, considering predisposing factors such as ocular trauma and that they take a more active role in activities such as agriculture and animal husbandry.

In the etiology of keratitis, contact lens use is the most common etiology in developed societies, while trauma is shown in developing countries [3]. Keratitis occurs due to contact lens misusage such as sleeping with the lens, taking a bath, swimming in the sea-pool, not changing

the lens on time and not renewing the solution in the lens case. In the study of Lam et al. [10], the use of contact lenses was determined as a risk factor in 26.4% of the patients. Culture positivity in patients using contact lenses was found to be 36%; P. aeruginosa grew in 20.3% of these [10]. P. aeruginosa overgrowth in our patients with a history of contact lens use.

Crosslinking treatment, which is one of the important causes of keratitis in developed countries, is a widely used treatment method recently to stop the progression of keratoconus. Crosslinking is a treatment method that stops the progression of keratoconus by activating riboflavin with UV-A, causing an increase in the collagen cross-links of the cornea, hardening and an increase in its biomechanical strength [11]. Common complications; It was reported as 7.6% sterile corneal infiltrates, 2.9% vision loss, 2.8% central corneal scar. A rare case of microbial keratitis has been reported [12] While the absence of epithelium is a ready-made risk factor for keratitis, the fact that topical antibiotic drops are not used regularly can be considered to predispose to the development of keratitis.

According to our results; in 12 (28.5%) patients, a finding was found in the stained microscopic examination. In 9 of them, gr (+) cocci and only leukocytes were found in 3 patients. While no factor could be seen in direct examination in three patients, growth was detected in the culture. Pathogen was detected in both direct examination and culture in six patients. Pathogen was detected in direct examination in six patients, but there was no growth in culture. In 71.5% of the patients, no findings were found in both the stained microscopic examination and the culture.

In the study of Tewari et al. [13], it was observed that 37% of the patients had direct examination (-) culture (-), and 4% had direct examination (+) culture (-). When we look at other studies in the literature, reproduction rates in culture are reported to be between 35% and 68% [14, 15].

Gram (+) cocci constitute the most common type of microorganism produced in keratitis [16]. The most common Gram-positive bacterium was reported as S epidermidis in some studies, S pneumonia in some, and S aureus in some [17]. In our study, Pseudomonas and S. pneumonia were the agents produced from those with growth.

Our culture reproduction rate was found to be lower than the literature data. The reason for the low growth rate in culture may be the treatments that the patients received in other centers before applying. Co-ordination with the microbiology department can also help to increase the growth rate from the culture.

Although our culture growth rate was low, it was observed that our success rate was high with empirical treatment and there was a significant increase in visual acuity after the treatment, in line with the studies in the literature. We attribute the high success rate in the treatment to the hospitalization of the patients, the rapid initiation of broad-spectrum antibiotic therapy, and close monitoring until clinical improvement is observed.

The limited number of our patients, the short follow-up period of the cases, and the lack of follow-up in all cases were the limitations of our study.

Early diagnosis and initiation of treatment for keratitis, which is one of the most serious diseases of the eye that can lead to blindness, are of great importance in terms of prognosis. Initiation of empirical treatment for microorganisms that may be causative without waiting for laboratory results will positively affect the visual prognosis. In case of unresponsiveness to empirical treatment, working in coordination with the laboratory unit and switching to treatment for the causative pathogen will both prevent antibiotic resistance and provide effective treatment.

In conclusion, the keratitis is a common cause of unilateral blindness. Early diagnosis and treatment of the keratitis is a significant role on the prognosis. The success of the therapy can be provided starting empirical antimicrobial therapy by taking into consideration of the regional risk factors and common pathogens. On the other hand, direct microscopy and culture-antibiogram provide serious support in cases where the treatment response is not available.

Conflict of interest: No conflicts of interest was declared by the authors.

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Authors' contributions to the article

I.B. constructed the main idea and hypothesis of the study. F.S. and IB and developed the theory and arranged/edited the material and method section. F.S. has evaluated the data in the results section. Discussion section of the article was written by F.S., F.U. and S.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Efficiency and reliability of serum hepcidin level measurement in evaluation of disease activation in patients with ulcerative colitis

Ülseratif kolitli hastalarda serum hepsidin düzeyi ölçümünün hastalık aktivasyonunu değerlendirmedeki etkinliği ve güvenirliği

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Abstract

Purpose: The study is to examine the relationship between hepcidin and inflammation markers and disease severity.

Materials and methods: Hepcidin levels are determined in 108 Ulcerative Colitis patients and 56 control subjects, a total of 164 subjects, equally active and in remission. Correlations between hepcidin levels and Age, Gender, ESR, CRP, WBC Hb levels are determined in the data. The relationship between hepcidin level of active UC patients and Age, Gender, ESR, CRP, WBC, Hb, Truelove-Witts score and Mayo score is evaluated by regression analysis.

Results: ESR, CRP and Hepcidin levels in the Active Ulcerative Colitis group are statistically significantly higher than in the remission and control groups; ESR, CRP, Hepcidin levels are found to be statistically significantly higher in the entire ulcerative colitis group compared to the control group.

In addition, a statistically significant positive correlation is found between the hepcidin level and the Truelove Witts score and the Mayo score in the AUC group (p<0.05).In the regression analysis perform to determine the factors affecting hepcidin level in ulcerative colitis patients, increase in Age (B=0.143, p<0.05), increase in Truelove Witts Score (B=5.224, p<0.001) increased Hepcidin level, whereas increase in Erythrocyte Sedimentation Rate decreased hepcidin level (B=-0.160, p<0.05) is determined.

Conclusion: It is promising that serum hepcidin level can enter into clinical use as a marker that can be used to evaluate not only disease activation but also the severity of activation.

Keywords: Ulcerative colitis, hepcidin, inflammation markers.

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Öz

Amaç: Çalışmada hepsidin ile inflamasyon markerları ve hastalık şiddeti arasındaki ilişkinin irdelenmesi amaçlanmıştır.

Gereç ve yöntem: Bu çalışmada toplam 164 kişi katılmış olup eşit sayıda aktif ve remisyonda olmak üzere 108 Ülseratif Kolit hastası ile 56 kontrol kişisinin hepsidin düzeyleri belirlenmiştir. Elde edilen verilerde hepsidin düzeyleri ile yaş, cinsiyet, ESH, CRP, WBC Hb düzeyleri arasındaki korelasyonlar tespit edilmiş, aktif ÜK hastalarının hepsidin düzeyi ile yaş, cinsiyet, ESH, CRP, WBC, Hb, Truelove-Witts skoru ve Mayo skoru arasındaki ilişki regresyon analizi ile değerlendirilmiştir.

Bulgular: Aktif Ülseratif Kolit grubunda ESH, CRP ve Hepsidin düzeyleri remisyon ve kontrol grubuna göre istatistiksel olarak anlamlı düzeyde yüksek; tüm ülseratif kolit grubunda, ESH, CRP, Hepsidin düzeylerinin kontrol grubuna göre istatistiksel açıdan anlamlı düzeyde yüksek saptanmıştır. Ayrıca AÜK grubunda, hepsidin düzeyi ile Truelove Witts skoru ve mayo skoru arasında istatistiksel olarak anlamlı düzeyde pozitif yönde bir korelasyon bulunmuştur (p<0,05) ve ülseratif kolit hastalarında hepsidin düzeyini etki eden faktörleri saptamak için yapılan regresyon analizinde yaş artışının (B=0,143, p<0,05), Truelove Witts Skoru'nun artışının (B=5,224, p<0,001) hepsidin düzeyini arttırdığı; Eritrosit Sedimentasyon Hızının yükselmesinin ise hepsidin düzeyini azalttığı (B=-0,160, p<0,05) tespit edilmiştir.

Sonuç: Serum hepsidin düzeyinin yalnızca hastalık aktivasyonunu değil aynı zamanda aktivasyon şiddetinin de değerlendirilmesinde kullanılabilecek bir marker olarak klinik kullanıma girebileceği konusunda umut vadetmektedir.

Anahtar kelimeler: Ülseratif kolit, hepsidin, inflamasyon markerları.

Kaşıkçı EE, Çelik M, Akpınar K. Ülseratif kolitli hastalarda serum hepsidin düzeyi ölçümünün hastalık aktivasyonunu değerlendirmedeki etkinliği ve güvenirliği. Pam Tıp Derg 2024;17:33-39.

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Introduction

Ulcerative colitis (UC) is a chronic, recurrent disease whose inflammation is limited to the colonic mucosa, generally starting from the rectum and progressing proximally, progressing in attacks and remissions [1]. The symptoms of UC mainly include abdominal pain, diarrhea, mucus, pus and blood in the stool, and extraintestinal symptoms. In addition, UC may present with nonspecific symptoms such as fever, loss of appetite and weight, fatigue, and primary amenorrhea. The cause of the disease is seen as an abnormal immune response against intestinal flora [2]. Inflammatory bowel diseases such as UC and Crohn's Disease are characterized by the interaction of inflammatory and cytotoxic mechanisms. The Truelove-Witts classification and Mayo Scoring System are frequently used to evaluate disease activity, predict prognosis, and plan treatment in UC. In addition, clinical, laboratory, endoscopic, histopathological and radiological findings are used together in the evaluation process of patients with IBD. The most frequently used laboratory parameters in the evaluations are Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), Cytokine levels, WBC (leukocyte), and Hemoglobin (Hb), Fecal biological markers (Calprotectin, S100, Lactoferrin).

Hepcidin molecule is a recently discovered low molecular weight hepatic peptide and plays an important role in iron metabolism. It is known that this molecule is affected by inflammation, iron stores, hypoxia, and anemia [3, 4]. Hepcidin is also a type 2 acute phase protein and is thought to be a primary regulator of iron homeostasis [5].

Studies have shown that hepcidin levels increase significantly in inflammatory disease [6]. Inflammation and infection increase hepcidin synthesis. Patients with sepsis, inflammatory bowel disease, myeloma, burns, and C reactive protein (CRP) levels >10 mg/dL exhibit significantly elevated hepcidin levels [7, 8]. The role of hepcidin in host defense and iron homeostasis also increases the interest in examining its relationship with UC disease. One study in patients with Ulcerative Colitis (UC), hepcidin levels were found to be reduced. This reduction was more pronounced in UC patients with anemia. It is suggested that utilizing hepcidin levels as a guide might be beneficial for the administration of oral iron supplements in patients with UC and other chronic inflammatory diseases [9].

In this study, it was aimed to evaluate the relationship between hepcidin and inflammation markers and disease severity. It was thought that the follow-up of hepcidin levels would be useful in the routine follow-up of UC patients, in predicting the severity of the disease, and in evaluating the response to treatment, supported by more comprehensive studies.

Material and method

This study was carried out in Pamukkale University Faculty of Medicine, Department of Gastroenterology between January 2018 and July 2019. A total of 108 patients who were active (54) and in remission (54) were followed up in the study, whose histopathology confirmed UC by colonoscopy and whose hepcidin levels complied with the exclusion criteria. In the control group, 56 healthy volunteers without any known disease in the anamnesis were included in the study. Patients (except for UC) and control group patients with any known systemic disease, findings suggestive of active infection, a history of malignancy, or a ferritin level above normal were not included in the study.

The Anamnesis, Age, Gender, Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), leukocyte (WBC), hemoglobin (Hb), and levels of all participants were recorded and their averages were calculated (Table 1). Then, the relationship between Hepcidin level and Age, ESR, CRP, WBC, and Hb levels in all cases was evaluated by correlation analysis (Table 2). After this evaluation, a comparison was made between the 3 groups in terms of Age, Gender, ESR, CRP, WBC, Hb, and Hepcidin levels (Table 3). Age, Gender, ESR, CRP, WBC, Hb, and Hepcidin levels were compared between all UC patients (group 1 and group 2) and the control group (Table 4). In addition, the relationship between Hepcidin level and Age, ESR, CRP, WBC, and Hb levels in the whole UC group was evaluated by correlation analysis (Table 5). Finally, the Truelove-Witts score of all active UC patients was calculated and the shortened endoscopic Mayo Activation Score was determined by colonoscopy, the relationship between them was evaluated (Table 6) and a regression analysis was performed between the scores (Table 7).

Table 1. Age, Gender, ESR, CRP, WBC, Hb, and Hepcidin parameters levels in all cases

All Patients (n=164)	Average ± Standart Deviation
Age (Year)	41.54±14.54
Gender (Female)	82 (50%)
ESR (mm\dL)	23.68±15.39
CRP (mg\dL)	0.80±2.01
WBC (K\µL)	7.82±2.68
Hb (g\dL)	13.16±2.13
Hepcidin (ng/L)	10.06±6.08

Table 2. Correlation analysis between Age, ESR, CRP, WBC, and Hb levels in all cases

		Age (year)	ESH (mm\dL)	CRP (mg\dL)	WBC (K\µL)	Hb (g\dL)
Hepcidin (ng/L)	**R**	0.08	0.14	0.07	0.11	-0.12
	P	>0.05	>0.05	>0.05	>0.05	>0.05

Table 3. Comparison of Age, Gender, ESR, CRP, WBC, Hb, and Hepcidin levels of the three groups included in the study

	Group 1 (n:54)	Group 2 (n:54)	Group 3 (n:56)	*P* value
Age (year)	41.51±13.41	42.11±15.78	41.01±14.58	Gp1-Gp2 *p*>0.05 Gp1-Gp3 *p*>0.05
Gender (female)	27 (50%)	27 (50%)	28 (50%)	*p*>0.05
ESH (mm\dL)	33.70±17.71	21.40±12.12	16.33±9.74	Gp1-Gp2 *p*<0.001 Gp1-Gp3 *p*<0.001
CRP (mg\dL)	1.81±3.25	0.42±0.52	0.21±0.30	Gp1-Gp2 *p*<0.001 Gp1-Gp3 *p*<0.001
WBC (K\µL)	8.34±3.15	7.87±2.77	7.28±1.94	Gp1-Gp2 *p*>0.05 Gp1-Gp3 *p*>0.05
Hb (g\dL)	12.89±1.94	13.05±2.05	13.53±2.36	Gp1-Gp2 *p*>0.05 Gp1-Gp3 *p*>0.05
Hepcidin (ng/L)	13.37±6.69	8.08±5.41	8.80±4.38	Gp1-Gp2 *p*<0.001 Gp1-Gp3 *p*<0.01

Group 1: Active Ulcerative Group, Group 2: Ulcerative Group in Remission, Group 3: Healthy Control Group

Table 4. Comparison of all UC patients (group 1 and group 2) and control group in terms of Age, Gender, ESR, CRP, WBC, Hb, and Hepcidin levels

	UC patients (n:108)	Control Group (n:56)	*P* value
Age (Year)	41.81±14.58	41.02±14,59	>0.05
Gender (Female)	54 (50%)	28 (50%)	>0.05
ESR (mm\dL)	27.56±16.32	16.33±9.75	<0.001
CRP (mg\dL)	1.12±2.43	0.21±0.30	<0.001
WBC (K\µL)	8.11±2.97	7.29±1.95	>0.05
Hb (g\dL)	12.98±1.99	13.54±2.37	<0.05
Hepcidin (ng/L)	10.73±6.74	8.81±4.38	*<0.05*

Table 5. Correlation analysis of the relationship between Hepcidin level and Age, ESR, CRP, WBC, and Hb levels in all UC patients

		Age (year)	ESR (mm\dL)	CRP (mg\dL)	WBC (K\µL)	Hb (g\dL)
Hepcidin (ng/L)	**R**	0.035	0.098	0.044	0.129	-0.158
	P	0.718	0.312	0.653	0.183	0.103

Table 6. Correlation analysis of the relationship between Hepcidin level and Age, ESR, CRP, WBC, Hb, Truelove-Witts's score, and Mayo Score in active UC patient group

		Age (year)	ESR (mm\dL)	CRP (mg\dL)	WBC (K\µL)	Hb (g\dL)	Trulove-Witt Score	MAYO Score
Hepcidin (ng/L)	***P***	0.209	-0.146	-0.117	0.024	-0.085	0.293	0.286
	R	0.130	0.291	0.401	0.861	0,542	0.032	0.036

Table 7. Regression analysis between Hepcidin level and Age, ESR, CRP, WBC, Hb, Truelove-Witts Score, and Mayo Score in the active UC group

				95% GA da B	
Independent variables	**B**	**Std. Error**	***P***	**Min.**	**Max.**
Age (year)	0.143	0.060	0.021	0.022	0.264
ESR (mm\dL)	-0.160	0.053	0.004	-0.267	-0.053
Truelove-Witts Score	5.224	1.159	<0.001	2.894	7.554

The patient group, Age, ESR, CRP, WBC, Hb, Mayo and Truelove Scores were included in the model and backward linear regression analysis was performed. R2=0.31

Measurement of hepcidin level

For the measurement of hepcidin in serum, 9-10 mL of venous blood was drawn from all cases and sent to the laboratory rapidly. The serum of the blood, which was centrifuged 3' at 5000 rpm, was divided into Eppendorf tubes into 2-3 parts. The samples were stored in a deep freezer at -80ºC until the day of analysis and all samples were studied in one go.

In the study with Human BT ELISA kits, all samples and kits collected first were brought to room temperature. After the standards and chemicals of the kits were prepared, standards and samples were placed in the wells on the plate. Then, following the steps described in the package insert, the samples were colored according to their concentrations, and the absorbance values of the wells were read by using the Kayto RT -2100c Microplate reader at 450 nanometers (nm), and the results were printed out. Concentrations were calculated using the serum absorbance values found. Values found are pg/mL units for hepcidin.

Statistical analysis

The obtained data were transferred to the SPSS v25 (Chicago, Illinois, USA) Program and statistical analyzes were made. Differences between groups were evaluated using Mann Whitney U and Kruskal Wallis tests. Spearman correlation analysis was performed to examine the interaction of the variables with other parameters and the relationship between these parameters. Regression analysis was used to evaluate the relationship of these parameters between groups $p<0.05$ was accepted as significant for all tests.

Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee.

Results

Age, Gender, levels of ESR, CRP, WBC, Hb, and Hepcidin parameters common to all participants in the study are given in Table 1, and the results of correlation analysis between Age, and ESR, CRP, WBC, and Hb parameters are given in Table 2. As a result of the correlation analysis, no statistically significant correlation was found between Hepcidin level and Age, ESR, CRP, WBC, and Hb (p>0.05) (Table 2).

There was no statistically significant difference between the three groups in terms of age, gender, WBC, and Hb (p>0.05). When evaluated in terms of ESR levels, the ESR value of the AUC group was found to be statistically significantly higher than the RUC and control group (p<0.05). In CRP values, the results of the AUC group were found to be statistically significantly higher compared to the RUC and control group (p<0.001). Finally, when the hepcidin levels were compared, it was observed that the AUC group values were statistically significantly higher than the RUC and control groups (p<0.001) (Table 3).

There was no statistically significant difference between UC patients and control group- in terms of Age and Gender (p>0.05). When compared in terms of ESR and CRP levels, it was found that the values of the UC group were statistically significantly higher than the control group (p<0.001). In hepcidin and Hb levels, the values of the UC group were found to be statistically significantly higher than the control group (p<0.05) (Table 4).

In all UC patients (Remission and Active UC), no statistically significant relationship was found between Hepcidin level and Age, ESR, CRP, WBC, and Hb (p>0.05) (Table 5).

When the relationship between hepcidin level and age, ESR, CRP, WBC, Hb, Truelove-Witts score, and Mayo Score was evaluated with correlation analysis only in AUC patient group, no statistically significant correlation was found between Hepcidin level and Age, ESR, CRP, WBC, and Hb levels (p>0.05). A statistically significant positive correlation was found between Hepcidin level and Truelove -Witts score and Endoscopic Mayo Score (p<0.05) (Table 6).

In the regression analysis performed to determine the factors affecting Hepcidin levels in active UC patients, it was found that the increase in age and (B=0.143, p<0.05) Truelove Witts Score (B=5.224, p<0.001) increased Hepcidin level; it was determined that the increase in the Erythrocyte Sedimentation Rate decreased the Hepcidin level (B=-0.160, p<0.05) (Table 7).

Discussion

Many studies have evaluated whether there is a significant relationship between serum hepcidin levels and some laboratory parameters. In liver diseases, serum hepcidin levels are generally decreased, while hepcidin levels are increased in individuals with chronic renal failure [10, 11]. Various studies have also found an association between hepcidin and Crohn's disease and ulcerative colitis in patients with IBD and anemia [12].

There are numerous studies in the literature on the hepcidin molecule. The fact that the molecule was increased in inflammatory conditions in studies suggested that this molecule can be used as an alternative or adjunct to other inflammatory parameters in UC patients with endothelial-epithelial damage. Therefore, in our study, it was aimed to show whether hepcidin level in UC patients, its relationship with inflammatory markers, whether there is a correlation with various scoring systems developed to reflect UC disease activation, and if this correlation is provided, whether it can be used as a new alternative parameter to show disease activity. Since no statistically significant difference was found in terms of age and gender in the study, we can conclude that the groups were balanced and comparable among themselves.

Several studies have shown the relationship between hepcidin and inflammation in patients with IBD, and positive correlations were found between serum - urine hepcidin levels and CRP and IL-6 levels [13]. In this study, the ESR level was found to be significantly higher in the AUC group compared to RUC and control group (Table 3). In addition, the ESR level of the entire UC group was found to be significantly higher than the control group (Table 4). In the study of Semrin et al. [14] (2006) a positive correlation between CRP and hepcidin was found. In another study, no significant correlation was

found between hepcidin and disease activity and inflammation markers in inflammatory bowel disease [15]. In our study, when the AUC, RUC, and control group were compared in terms of hepcidin levels, hepcidin levels were found to be statistically significantly higher in the AUC group (Table 3). In addition, when we compared the whole UC group (AUC and RUC) and the SC group in terms of hepcidin levels, it was found that the hepcidin level was statistically significantly higher in the whole UC group compared to the SC group (Table 4). However, in the correlation analyses performed in the whole UC group and active UC group, no statistically significant correlation was found between ESR and CRP values and hepcidin levels. This result made us think that hepcidin levels increase with inflammation, as do CRP and ESR levels in UC patients, but this increase is independent of the increase in ESR and CRP. The idea that hepcidin level measurement shows inflammation independent of CRP and ESR levels and that it may be a more effective molecule showing activation in UC patients has been strengthened. With these findings, it is thought that the evaluation of activation by looking at ESR and CRP in AUC patients will be incomplete, and hepcidin level measurement may be useful in cases where activation cannot be evaluated with ESR and CRP.

Bleeding, leukocytosis and anemia secondary to chronic inflammation are the findings in active UC. In a study, it was reported that there were significant increases in hepcidin levels in individuals with IBD, and anemia was observed in 42% of them. As a result of multivariate analyzes, it was shown that serum hepcidin levels were correlated with ferritin and disease activity, but not with anemia. It was also found that hepcidin levels were lower in the patient group compared to healthy participants, independent of anemia status [16]. In another study conducted on healthy individuals and IBDs, serum hepcidin levels were found to be significantly lower in the IBD group. Decreased innate immunity, intestinal epithelial damage, and Paneth cell loss have been reported as the cause of this condition [17]. In our study, hepcidin levels in the AUC group and all UC groups were found to be statistically significantly higher than in the other groups, and no significant leukocytosis was found. The Hb level was found to be significantly lower in the entire UC group compared to the SC group (Table 4). This finding shows that AUC patients should not be evaluated based on WBC and Hb levels alone. Since no significant difference was observed between hepcidin and Hb in the correlation analysis, it can be concluded that hepcidin levels in UC patients are affected by inflammation rather than anemia. It was observed that there was a positive correlation between the scoring systems used in the study (Table 6). In the regression analysis performed in the AUC group, a significant correlation was found between the hepcidin level and the Truelove-Witts scoring system, which is one of the most important findings of this study (Table 7).

ESR, CRP, WBC, Hb, etc. Parameters used to evaluate disease activation, such as the disease, only find a place for themselves in scoring systems. From this point of view, according to the result of our study, hepcidin was found to be superior to other parameters in reflecting disease activation alone, which other laboratory parameters could not. The fact that ESR and CRP levels were insufficient in the AUC group and did not show any statistically significant difference in scoring systems, while hepcidin was higher compared to the other groups indicates that it may be a serum marker. The reason for the variability of hepcidin levels between studies may be due to the complex pathophysiological mechanisms involved in hepcidin regulation. Because hepcidin level varies depending on many factors such as iron storage in the body, hypoxia, and inflammation.

These results suggest that serum hepcidin level may enter into clinical use as a marker that can be used to evaluate not only disease activation but also the severity of activation. Controlled studies in which hepcidin levels can be shown to decrease with treatment in active UC patients may provide useful information on whether hepcidin measurement can be used as a useful marker in the follow-up of treatment response. In this study, it was aimed to evaluate the relationship between hepcidin and inflammation markers and disease severity. It is thought that the follow-up of hepcidin levels may be useful in the routine follow-up of the patients, in the prediction of the severity of the disease, and in the evaluation of the response to the treatment, supported by more comprehensive studies.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

M.C. and E.E.K. constructed the main idea and hypothesis of the study and developed the theory and arranged/edited the material and method section. E.E.K. and K.A. has done the evaluation of the data in the results section. The discussion section of the article was written by M.C. and E.E.K. who also reviewed, corrected, approved it. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Outcomes of the surgical treatment of carpal tunnel syndrome under local anesthesia: comparison of the surgeries with and without tourniquet use

Karpal tünel sendromunun lokal anestezi ile yapılan cerrahi tedavisinin sonuçları: turnikeli ve turnikesiz yapılan ameliyatların karşılaştırılması

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Abstract

Purpose: Carpal tunnel syndrome is the most common upper limb entrapment neuropathy. The current study reviews outcomes in carpal tunnel syndrome surgeries performed with local anesthesia in a procedure room outside the operating room and compares the surgeries with and without tourniquet use.

Materials and methods: Patients who underwent carpal tunnel syndrome surgery between June 2019 and January 2023 were retrospectively analyzed. Patients were divided into two groups: with and without tourniquet use. Demographic characteristics, operative time, complications, and outcomes were compared. All patients were examined preoperatively and at postoperative month 3 using the Quick Disabilities of the Arm, Shoulder and Hand scale (QDASH) questionnaire, which measures upper extremity activity and participation restrictions.

Results: The study included 119 patients. The operative time was longer in the nontourniquet group than in the tourniquet group, with a statistically significant difference (16.75±2.39 min and 14.47±1.88 min, p<0.001). Bipolar use was higher in the nontourniquet group, with a statistically significant difference (p<0.001). The preoperative QDASH score was statistically similar in both groups (62.58±6.67 and 63.86±6.04, p=0.229). The mean postoperative QDASH score was 4.79±7.65 in the nontourniquet group and 4.24±3.86 in the tourniquet group (p=0.799).

Conclusions: Tourniquet use may slightly shorten the operative time and may be more effective in controlling bleeding. However, there was no significant difference between the groups regarding postoperative results. The results indicate that operating with a local anesthesia alone is an effective alternative to tourniquet use and a safe choice.

Keywords: Carpal tunnel syndrome, tourniquet, wide awake hand surgery.

Aykac B, Turkkan A. Outcomes of the surgical treatment of carpal tunnel syndrome under local anesthesia: comparison of the surgeries with and without tourniquet use. Pam Med J 2024;17:41-50.

Öz

Amaç: Karpal tünel sendromu en sık görülen üst ekstremite tuzak nöropatisidir. Bu çalışma, ameliyathane dışında bir işlem odasında lokal anestezi ile yapılan karpal tünel sendromu ameliyatlarının sonuçlarını gözden geçirmekte ve turnikeli ve turnikesiz ameliyatları karşılaştırmaktadır.

Giriş ve yöntem: Haziran 2019 ile Ocak 2023 tarihleri arasında karpal tünel sendromu ameliyatı geçiren hastalar retrospektif olarak incelendi. Hastalar turnikeli ve turnikesiz olmak üzere iki gruba ayrıldı. Demografik özellikler, operasyon süresi, komplikasyonlar ve sonuçlar karşılaştırıldı. Tüm hastalar ameliyat öncesi ve ameliyat sonrası 3. ayda, üst ekstremite aktivitesini ve katılım kısıtlamalarını ölçen Hızlı Kol, Omuz ve El Engelliliği ölçeği (QDASH) anketi kullanılarak değerlendirildi.

Bulgular: Çalışmaya 119 hasta dahil edildi. Ameliyat süresi turnike olmayan grupta istatistiksel olarak anlamlı derecede daha uzundu (16,75±2,39 dk ve 14,47±1,88 dk, p<0,001). Bipolar kullanımı turnike olmayan grupta istatistiksel olarak anlamlı derecede daha yüksekti (p<0.001). Preoperatif QDASH skoru her iki grupta istatistiksel olarak benzerdi (62,58±6,67 ve 63,86±6,04, p=0,229). Ameliyat sonrası ortalama QDASH skoru turnikesiz grupta 4,79±7,65, turnikeli grupta 4,24±3,86 idi (p=0,799).

Sonuç: Turnike kullanımı ameliyat süresini biraz kısaltabilir ve kanama kontrolünde daha etkili olabilir. Ancak ameliyat sonrası sonuçlar açısından gruplar arasında anlamlı fark yoktu. Bu, tek başına lokal anestezik ile ameliyatın turnike kullanımına güvenli ve etkili bir alternatif olduğunu düşündürmektedir.

Anahtar kelimeler: Karpal tünel sendromu, turnike, uyanık el cerrahisi.

Aykaç B, Türkkan A. Karpal tünel sendromunun lokal anestezi ile yapılan cerrahi tedavisinin sonuçları: turnikeli ve turnikesiz yapılan ameliyatların karşılaştırılması. Pam Tıp Derg 2024;17:41-50.

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Introduction

Carpal tunnel syndrome (CTS) is the most prevalent entrapment neuropathy of the upper extremity [1, 2]. Treatment of CTS includes both conservative and surgical practices. For mild-to-moderate symptoms patients may receive nonsurgical treatments [3]. However, many patients do not respond to conservative treatment, and surgical release of the carpal ligament is required if severe symptoms occur [2, 3].

To improve cost and efficacy, CTS surgery has recently been performed outside of the operating room in a smaller procedure room with patients awake [4-6]. Surgical procedures can be performed under local anesthesia (LA) and through mini incisions with or without a tourniquet.

Tourniquet devices are widely used in orthopedic procedures to provide a blood-free operating field in surgical procedures involving the extremities [7]. However, patients may experience pain, discomfort, and compression-related complications when the tourniquet is inflated [2, 7]. Therefore, some surgeons have suggested non-tourniquet procedures, stating that it is possible to control bleeding with alone local anesthetic injection [1, 8-11]. At the same time, patients can have a more comfortable perioperative period with the elimination of pain and discomfort that may occur due to the tourniquet [8, 12, 13]. It has been reported by many authors in the literature that surgeries without tourniquet do not increase the duration of surgery and complications [8, 10, 12, 14]. However, there is no consensus on which of these two approaches is superior to the other.

In this study, we retrospectively reviewed patients who underwent CTS surgery with and without tourniquet use under LA in a procedure room outside the operating room. In our study, we aimed to evaluate the effect of two different surgical procedures on the recovery status of the patients and possible complications.

Materials and methods

This retrospective examination was approved by Bursa Medicana Hospital Clinical Research Ethics Committee with the number 2023/03 and was subsequently performed by the regulations of the Declaration of Helsinki. In our study, 119 patients who underwent surgery for CTS between June 2019 and January 2023 in the Neurosurgery Clinic and the Orthopedics and Traumatology Clinic of our hospital, whose diagnosis was confirmed by electromyography (EMG) after examination, and who did not respond to conservative treatment were retrospectively studied.

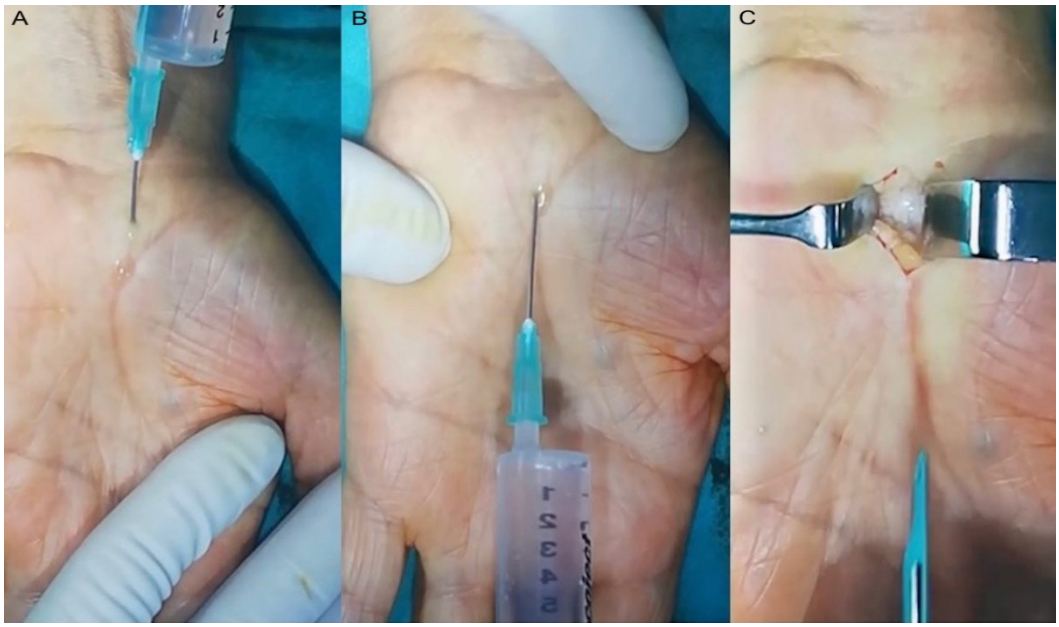
Our study included 119 patients who were given LA in a procedure room outside the operation room. The patients included in the investigation were split into two groups: those who received a tourniquet with a cuff (TY) and those who underwent surgery without a tourniquet (TN). Patients who underwent bilateral or additional hand surgery in the same session were excluded. At the same time, patients with peripheral neuropathy as well as patients who underwent surgery after fracture revision surgery and surgery under sedation and general anesthesia in the operating room were excluded from the study. Age, gender, date of surgery, use of a tourniquet, use of bipolar cautery, postoperative complications, and total follow-up time of all cases were analyzed from the patient record system.

In 71 patients in the TY group, after the upper arm was wrapped with circular cotton wool and an arm cuff was applied, the surgical field was cleaned with povidone iodine and the surgical field was covered with a sterile drape. Then, 5 mL prilocaine (2%) was injected into the incision site and 5 mL prilocaine (2%) into the carpal tunnel. At the 1st minute after LA, the surgical procedure was started after tourniquet application under 250 mmHg pressure, with the upper extremities elevated just before the surgical incision. In the TN group, 48 patients underwent the same surgical preparation and local procedures without tourniquet use (Picture 1).

At the end of both preparation phases, the patients underwent the same surgical procedure. An incision of approximately 2 cm starting from the distal part of the flexor line of the volar aspect of the wrist was made. Blunt dissection was performed up to the flexor retinaculum. When the median nerve was visualized after opening the flexor retinaculum with sharp dissection, the nerve was protected and the flexor retinaculum was loosened proximally and distally. The bleeding was checked. Bipolar cautery was not

used in any of the patients in the tourniquet-applied group, whereas bipolar cautery was used for bleeding control in 7 of the 48 patients without tourniquet application. The skin incision was primarily sutured. The wound was closed with a sterile dressing.

Wrist and finger movements were allowed in the early postoperative period. Patients in both patient groups were evaluated based on their preoperative and postoperative 3rd month Quick Disabilities of the Arm, Shoulder and Hand scale (QDASH) functional scores (Figure 1).



Picture 1. A) Local injection application to the incision site. B) Local anesthetic application into the carpal tunnel. C) Median nerve decompression with mini incision

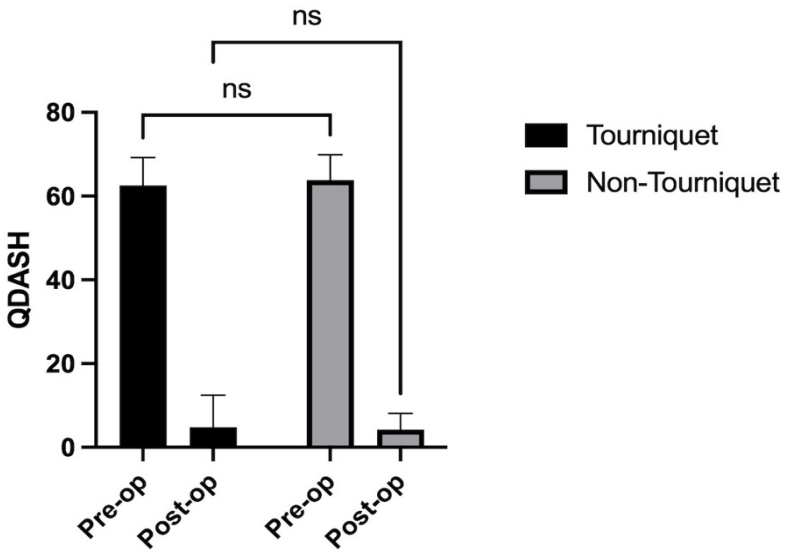


Figure 1. Comparison of preoperative and postoperative 3rd month Quick Disabilities of the Arm, Shoulder and Hand scale (QDASH) scores in tourniquet groups (TY) and non-tourniquet groups (TN) groups, ns: not statistically significant

Statistical method

The data were in the IBM SPSS Statistics Version 26 statistical package program (IBM Corp., Armonk, New York, USA). The number of units (n), percent (%), mean±standard deviation (x±sd), median (M), minimum (min) and maximum (max) values were given as descriptive statistics. The Shapiro-Wilk normality test was used to test the normal distribution of the numerical variables and based on the results, the Mann-Whitney U test was employed to compare single measurement numerical variables of the patients in the groups. Pearson Chi-square test was employed to compare categorical variables with each other. A *p* -value of <0.05 was assessed as statistically significant.

Results

In our study, 138 patients who underwent surgery for CTS between June 2019 and January 2023 were retrospectively studied. Fifteen patients who had bilateral CTS and three patients with missing information in their records were excluded from the study. A total of 119 patients were included in the study; 94 patients (79%) were male and 25 (21%) were female. The mean age was 54.56±13 years (26-88 years), and 42 patients (35.3%) were diabetic and 53 (44.5%) were current smokers. Demographic information of the patients is summarized in Table 1.

Table 1. Descriptive characteristics of the patients

Variables	**n (%)** **x±sd** **M (min-max)**
Age	
x±sd	54.6±13
M (min-max)	56 (26-88)
Gender, n (%)	
Famale	94 (79.0)
Male	25 (21.0)
QDASH Preop	
x±sd	63.4±6.4
M (min-max)	63.6 (48.7-80.0)
QDASH Postop 3rd Months	
x±sd	4.4±5.7
M (min-max)	4.5 (0-54)
QDASH Delta	
x±sd	58.9±7.4
M (min-max)	60 (17.7-73.2)
Tourniquet	
x±sd	4.5±0.6
M (min-max)	4 (3-6)
Operation Time	
x±sd	15.4±2.4
M (min-max)	15 (10-25)
Bipolar Use, n(%)	
No	112 (94.1)
Yes	7 (5.9)

x: Mean, sd: Standard Deviation, M: Median, %: Row Percent,
QDASH: Quick Disabilities of the Arm, Shoulder and Hand scale

Patients were divided into two groups: TY group (n=71) and TN group (n=47). The gender distribution of the patients was statistically similar in both groups. There was no statistical difference in age distribution between the groups (53.13±12.57 years vs. 56.69±13.46 years, p=0.101) (Table 2).

The operative time was longer in the TN group than in the TY group, and this difference was statistically significant (16.75±2.39 min and 14.47±1.88 min, p<0.001). In the TY group, the tourniquet inflation time was 4.47±0.60 min (Table 2) (Figure 2).

Bipolar use was needed in seven patients (14.6%) in the TN group for bleeding control. Bipolar use was not needed in the TY group.

Intraoperative complications were not observed in the groups. No infection was detected in either of the two groups. Only one minor complication (transient ulnar nerve palsy) was recorded in the postoperative period in the TY group and resolved spontaneously within the first 24 hours postoperatively (p=0.210) (Table 2). None of the patients required reoperation.

The mean preoperative QDASH score was 62.58±6.67 in the TN group and 63.86±6.04 in the TY group. The preoperative QDASH score was statistically similar in both groups (p=0.229). The mean postoperative QDASH score was 4.79±7.65 in the TN group and 4.24±3.86 in the TY group. The postoperative QDASH score was statistically similar in both groups (p=0.799). The QDASH score delta (preoperative-postoperative) value was 57.79±8.97 in the TN patient group and 59.61±5.99 in the TY group. The QDASH delta score was statistically similar in both groups (p=0.240) (Table 2).

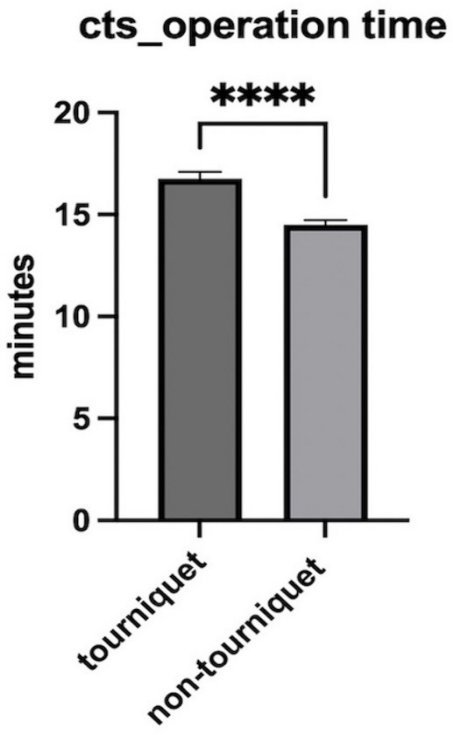


Figure 2. Comparison of the operative times in tourniquet groups (TY) and non-tourniquet groups (TN) groups
**** Statistically significant (p<0.001)

Table 2. Comparison of other variables by tourniquet groups

	Tourniquet		Test Statistics	
	No	**Yes**	**Test value**	***p* value**
Age				
x±sd	56.7±13.5	53.1±12.6	z=-1.640	0.101
M (min-max)	57 (26-88)	54 (32-88)		
Gender, n (%)				
Famale	37 (77.1)	57 (80.3)	χ^2=0.177	0.674
Male	11 (22.9)	14 (19.7)		
QDASH Preop				
x±sd	62.6±6.7	63.9±6.1	z=-1.204	0.229
M (min-max)	62.9 (62.5-80)	66.2 (47.8-72.7)		
QDASH Postop 3rd Monhts				
x±sd	4.8±7.7	4.3±3.9	z=-0.255	0.799
M (min-max)	4.5 (0-9)	4.5 (0-9)		
QDASH Delta				
x±sd	57.8±8.9	59.6±6	z=-1.176	0.240
M (min-max)	57.8 (14.7-73.2)	61.2 (43.7-70.4)		
Tourniquet				
x±sd	0±0	4.5±0.6	z=-9.787	<0.001
M (min-max)	0 (0-0)	4 (3-6)		
Operation Time				
x±sd	16.8±2.4	14.5±1.9	z=-5.399	<0.001
M (min-max)	16 (10-25)	14 (11-20)		
Bipolar Use, n(%)				
No	41 (85.4)	71 (100.0)		
Yes	7 (14.6)	0 (0.0)		
Complications n (%)				
Infection	0 (0)	0 (0)		
Vascular Nerve Injury	0 (0)	0 (0)		
Nerve Injury	0 (0)	1 (1.4)		

x: Mean, sd: Standard Deviation, M: Median, %: Row Percent, z: Mann-Whitney U test, χ^2: Chi-Square test statistics
QDASH: Quick Disabilities of the Arm, Shoulder and Hand scale

Discussion

Pain management and homeostasis control in patients are among the most important problems in CTS surgery. Sedation, regional block, or general anesthesia may be required to keep the patient from suffering. However, all of these interventions have the potential for risks and side effects [15, 16]. Therefore, most surgeons prefer less invasive techniques for this procedure that can be performed in less time [17]. In recent years, CTS surgeries are increasingly being performed in non-operating room settings, such as in procedure rooms, with field sterility [5, 6, 18, 19]. Surgery can be performed under LA with or without a tourniquet in less time and with very low infection rates. Another advantage of this technique is that it eliminates the need for overnight fasting, preoperative testing, and intravenous (IV) catheterization [14]. According to the literature, this technique is safe and well tolerated by patients [5, 18]. LeBlanc et al. [5] reported a superficial infection rate of 0.4% in a series of 1504 patients with CTS operated on in a minor intervention room with field sterility. No deep infection was observed in any patient [5]. In a systematic review by Jagodzinski et al. [20] articles on hand surgery performed in procedure rooms were reviewed. No infection was observed in three studies, and the infection

rate was 0.4% in two studies, which included 1962 CTS cases [20]. In addition, studies in the literature show that using the main operating room for CTS surgery is more costly and less efficient [6, 19]. To ensure field sterilization, all patients in our study underwent surgery in a procedure room separate from the main operating room. Consistent with the literature, none of the patients developed a superficial or deep infection.

There are many options in the surgical treatment of CTS such as open surgery, endoscopic methods, ultrasound-guided surgery. The most common method is to cut the carpal ligament with open surgery. In recent years, endoscopic surgical methods have also gained popularity. Eroglu et al. [21] compared 60 patients who underwent open and endoscopic CTS surgery. They reported that endoscopic surgery was feasible, well tolerated and performed with low morbidity compared to standard open methods. In their study, which included a 10-year outpatient analysis of 571.403 patients diagnosed with CTS, Williamson et al. [22] reported no significant difference between open surgery and endoscopic surgery in terms of perioperative complications, including infection, nerve damage and wound complications. The only significant difference reported was higher cost for endoscopic surgery. In our study, all patients underwent open mini-incision surgery. Endoscopic or ultrasound-guided surgery was not performed [22].

Working in a blood-free environment during surgery is important for reassuring both the surgeon and the patient. In CTS, the surgeon can achieve intraoperative hemostasis in several ways [1, 12, 23]. Tourniquets are used to restrict blood flow and control bleeding, resulting in a blood-free surgical field. Therefore, better visualization of anatomical structures is possible, and dissection becomes easier. The choice that surgeons make regarding tourniquet use varies culturally and depending on the specific health system. In a survey of more than 700 respondents conducted by the “American Society for Surgery of the Hand”, 95% of surgeons reported using tourniquets in CTS surgeries [24].

However, tourniquet use is not without risk. When inflated, they may cause discomfort and pain for patients with direct mechanical pressure and the resulting anoxia [12, 13]. In their prospective randomized controlled study, Iqbal et al. [13] reported that the rate of pain complaints in the group of patients undergoing CTS surgery with tourniquet use was significantly higher than in the group of patients operated on without tourniquet use and that tourniquets caused unnecessary pain without any additional benefit. Furthermore, prolonged tourniquet use may cause deep vein thrombosis, pulmonary embolism, local soft tissue, nerve damage, and a temporary/permanent neurologic deficit [12, 25, 26]. Lim et al. [26] reported that patients could tolerate tourniquets for approximately 20 min in their study. Maury and Roy [27] determined the mean tourniquet tolerance time to be 18 min (range 10-26 min).

In our study, a tourniquet was used for hemostasis in 71 patients. The mean duration of tourniquet use was 4.4±0.60 min and was well tolerated by the patients. Only one patient experienced postoperative transient ulnar nerve paralysis, which resolved after 24 h of follow-up without the need for additional treatment. In terms of hemostasis, the TY group showed less bleeding and did not require the use of bipolar for bleeding control and did not require bipolar use for bleeding control.

CTS surgery with LA without tourniquet use has become popular in the last decade. Many centers use epinephrine in combination with local anesthetic to secure a blood-free surgical field through vasoconstriction (Wide awake local anesthesia no tourniquet (WALANT) technique) [9, 14, 28]. However, because epinephrine administration creates a more acidic solution, it irritates the tissue and causes a burning sensation [27]. Therefore, it should be buffered with sodium bicarbonate [8, 29]. In addition, its optimal effect of providing maximal vasoconstriction and hemostasis in the surgical field has been proven to take effect/last >25 min after local injection [10]. This prolongs the surgical time and adversely affects efficacy and cost savings in outpatient surgical cases [8, 14, 28].

Bleeding control in surgical procedures with only LA is often related to the surgeon’s success in managing soft tissue bleeding. Bleeding occurs mostly at the edge of the incision and from subcutaneous tissues. These bleedings can be easily controlled by cauterization. In addition,

the tension supplied by the retractor and the injection's swelling effect, which enhances the local pressure in the subcutaneous tissue, may also even donate to hemostasis. In our study, surgery was performed only under LA without epinephrine administration in the TN group. Regarding hemostasis, the bleeding at the incision site was controlled with the help of bipolar use. In the TN group, bipolar use was employed for bleeding control in seven (14.6%) patients, which is statistically significant compared to the TY group.

One of the goals of our study was to compare operative time and complications in patients with and without tourniquet use. Olaiya et al. [11] showed that tourniquet application shortened the mean operative time by 1.82 min. In this study, no significant difference was reported between the intraoperative blood loss and the complication rates [11]. In our study, the TY group had a statistically significant mean operative time variance of 2.28 min. However, this period included only the operative time. The preoperative preparation time for the tourniquet was not included in this time frame. There are many complications that can occur during CTS surgeries. These include tendon ruptures, nerve injuries, infections, and incomplete decompression [8, 13, 20, 27, 30]. In our study, no intraoperative complications were observed in either group, and they were comparable in this respect.

In our study, all patients were evaluated preoperatively and at the 3rd month postoperatively using the QDASH questionnaire. Hudak et al. [31] described the Arm, Shoulder and Hand Disability Scale (DASH) in 1996 and reported that it was reliable in evaluating CTS results. The Arm, Shoulder and Hand Quick Disability Scale (QDASH) is a shortened and improved scale. The validity and reliability of the DASH version and the Turkish version have been demonstrated in CTS patients [32]. The QDASH questionnaire has been reported as one of the most frequently used and reliable questionnaires for evaluating individuals with upper extremity injuries [33]. In our study, we found a statistically significant difference between the QDASH values measured in the preoperative period and at the postoperative 3rd month in both groups (p<0.001). The mean preoperative QDASH score was 62.58±6.67 in the TY group and 63.86±6.04 in the TN group. The preoperative QDASH score was statistically similar in both groups (p=0.229). The mean postoperative QDASH score was 4.79±7.65 in the TY group and 4.24±3.86 in the TN group. The postoperative QDASH score was statistically similar in both groups (p=0.799). The QDASH score delta (preoperative-postoperative) value was 57.79±8.97 in the TN group and 59.61±5.99 in the TY group. The QDASH delta score was statistically similar in the TY and TN groups (p=0.240).

Some prognostic factors affecting the success of CTS operations have been reported in the literature [34, 35]. Gunes and Ozeren [34] emphasized the importance of age and body mass index (BMI) and reported that better results were obtained in younger patients. In this study, it was reported that age is an unchangeable factor, but since BMI can be changed, surgical success can be increased by developing weight loss strategies in patients [34]. Tonga and Bahadir [35] reported that high BMI and Vitamin B12 deficiency exacerbate CTS symptoms and weight control with appropriate diet can reduce the severity of these symptoms.

As a result, the use of tourniquet may slightly shorten the operative time and may be more effective in controlling bleeding. However, the overall complication rate was low in both groups, and there was no significant difference in postoperative outcomes. The findings indicate that operating with a local anesthetic alone is an effective alternative and safe option to tourniquet use.

The limitations of this study are presented in this section. First, the study was not double-blinded. Second, the follow-up period was relatively short, i.e., 3 months, but within this time, patients in both groups achieved satisfactory symptomatic and functional improvement. Third, this is a single-center study, and studies with better design and more participants are needed to confirm our findings.

Conflict of interest: No conflict of interest was declared by the authors

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Ethics committee approval: Permission was obtained from Bursa Medicana Hospital Clinical Research Ethics Committee for the study (date: 06.07.2023, number: 2023/03).

Authors' contributions to the article

B.A. and A.T. constructed the main idea and hypothesis of the study. B.A. developed the theory and arranged the material and method section. B.A. and A.T. have done the evaluation of the data in the Results section. Discussion section of the article was written by A.T.; A.T. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Seroprevalence of patients presenting with a pre-diagnosis of *Toxoplasma gondii*, Türkiye

Toxoplasma gondii ön tanısı ile başvuran hastalarda seroprevalans, Türkiye

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Abstract

Purpose: Toxoplasma gondii is a unicellular protozoan parasite. With this study, the aim was to evaluate the *T. gondii* test results that came to the General Directorate of Public Health, National Parasitology Reference Laboratory between January 2019 and December 2020.

Materials and methods: The seroprevalence were studied with the Sabin-Feldman Dye Test which is gold standard, Anti-*T. gondii* IgG and IgM and *T. gondii* IgG avidity with ELISA were evaluated in 1160 patient. The distribution of cases according to gender and age groups was also evaluated statistically.

Results: In this study, SFDT was performed on 589 patients from 1160 patients with suspected Toxoplasmosis samples and Anti-*T. gondii* IgG and IgM tests were studied by ELISA in 478 patients. IgG avidity test by ELISA was performed on 93 cases with positive Anti-*T. gondii* IgG. In addition to these, cases in which Anti-*T. gondii* IgM and IgG were studied together and were positive were also evaluated. The number of samples both positive together was 17 (3.6%). According to Anti-*T. gondii* IgG/IgM results, no significant relationship was found between toxoplasmosis and gender. Moreover, of 93 cases with positive Anti-*T. gondii* IgG values, 71% had high avidity, 16% had cutoff value and 13% had low avidity.

Conclusion: Further studies and surveillance studies should be performed to determine the epidemiology and current prevalence of toxoplasmosis.

Keywords: Avidity, ELISA, Sabin-Feldman Dye Test, *Toxoplasma gondii*.

Aslaner B, Nalbantoglu AS, Yucesan B. Seroprevalence of patients presenting with a pre-diagnosis of *Toxoplasma gondii*, Türkiye. Pam Med J 2024;17:53-60.

Öz

Amaç: *Toxoplasma gondii*, tek hücreli bir protozoon parazittir. Bu çalışma ile Halk Sağlığı Genel Müdürlüğü Ulusal Parazitoloji Referans Laboratuvarına Ocak 2019-Aralık 2020 tarihleri arasında gelen *T. gondii* test sonuçlarının değerlendirilmesi amaçlanmıştır.

Gereç ve yöntem: Altın standart olan Sabin-Feldman Boya Testi ile seroprevalans çalışıldı, Anti-*T. gondii* IgG ve IgM ve *T. gondii* IgG aviditesi ELISA ile 1160 hastada değerlendirildi. Vakaların cinsiyet ve yaş gruplarına göre dağılımı da istatistiksel olarak değerlendirildi.

Bulgular: Bu çalışmada Toksoplazmoz şüphesi olan 1160 hastadan 589'una SFDT yapıldı ve 478 hastada ELISA ile Anti-T. gondii IgG ve IgM testleri çalışıldı. Anti-*T. gondii* IgG'si pozitif olan 93 olguya ELISA ile IgG avidite testi uygulandı. Bunlara ek olarak Anti-*T. gondii* IgM ve IgG birlikte çalışıldı ve pozitif olanlar da değerlendirildi. İkisi birlikte pozitif olan örnek sayısı 17 (%3,6) idi. Anti-*T. gondii* IgG/IgM sonuçlarına göre toksoplazmoz ile cinsiyet arasında anlamlı bir ilişki bulunmadı. Ayrıca Anti-*T. gondii* IgG değerleri pozitif olan 93 olgunun %71'inde yüksek avidite, %16'sında cutoff değeri ve %13'ünde düşük avidite vardı.

Sonuç: Toksoplazmozun epidemiyolojisini ve güncel prevalansını belirlemek için ileri çalışmalar ve sürveyans çalışmaları yapılmalıdır.

Anahtar kelimeler: Avidite, ELISA, Sabin-Feldman Boya Testi, *Toxoplasma gondii*.

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Introduction

Toxoplasma gondii is a single-celled protozoan parasite that needs a host cell to reproduce and is frequently encountered throughout the world. It is an infective agent that can cause infection in all mammals. In its life cycle, the feline is definitive hosts and other warm-blooded vertebrates are intermediate hosts. *T. gondii* has three effective forms. These are tachyzoites [the form that can be found in all cells except rapidly proliferating erythrocytes], the tissue cyst form [the form in which slowly proliferating bradyzoites can be found in the latent and chronic periods], and oocysts [the form found in the intestinal epithelial cells of cats and felines and excreted with their feces]. Transmission of *T. gondii* to humans occurs by ingestion of tissue cysts [with raw/undercooked meat] or by ingestion of contaminated water and food through oocysts dispersed from the feces of infected cats [1, 2]. In cases where the immune system is intact, the infection usually develops in an asymptomatic course. In some infected patients, clinical findings may develop as cervical type lymphadenopathy, ocular findings, central nervous system diseases and abscess within the brain [3]. Asymptomatic infections, especially in pregnant women, constitute an important risk factor for the fetus. In the first trimester of pregnancy, when infected with this parasite, the agent can pass to the fetus. When it causes infection in the fetus, it can cause serious consequences, from miscarriage to stillbirth. Congenital toxoplasmosis can lead to severe symptoms in the newborn such as hydrocephalus, microcephaly, cerebral calcifications, retinal disorders in the eye, advanced hearing problems and mental retardation [2]. Other routes of transmission are blood transfusion, solid organ or stem cell transplantation, and laboratory accidents [4]. In diseases that suppress the immune system (Acquired Immune Deficiency Syndrome (AIDS) or the use of immunosuppressive drugs, etc.), the infection may flare up again in a latent and severe manner and cause case deaths. Therefore, it is known that T. *gondii* infection is an important cause of morbidity and mortality in patients with AIDS [5].

Diagnosis of *Toxoplasma gondii* infection, determination of its acute and chronic stages and genetic characteristics are very important for the treatment, control, prevention, and epidemiology of toxoplasmosis. Indirect diagnostic methods are frequently used to determine the population prevalence in terms of public health [6, 7]. Due to the disease caused by T. *gondii,* which is usually asymptomatic or clinical findings are not specific to the infection, anti-*T. gondii* antibodies should be supported by serological tests. If the infection is detected at an early stage, it can be treated [8]. The ELISA and the Sabin-Feldman Dye Test (SFDT) are the two most frequently used methods in laboratories today. Although SFDT has high sensitivity and specificity, ELISA is more reliable, economic and easier method [9]. While ELISA IgM positivity is interpreted in favor of acute toxoplasmosis, it is important that these antibodies remain positive for months or even years. In addition, the possibility of false positive IgM antibodies can lead to difficulties in diagnosis, and issues such as confirmation of positive values by other methods and understanding the stage of infection require the application of IgG avidity test. Understanding early or late toxoplasmosis is of great clinical importance in pregnant women and immunocompromised cases [10-13]. The aim of this study is to evaluate the frequency of the presence of *T. gondii* antibodies between different sexes and age groups by Sabin Feldman Dye test and ELISA IgG/IgM seroconventional methods in cases sent to the laboratory in 2019-2020.

Materials and methods

This study was carried out with 1160 serum samples sent to the General Directorate of Public Health, Department of Microbiology Reference Laboratories, National Parasitology Reference Laboratories between January 2019, and December 2020 to investigate *Toxoplasma gondii* antibodies. In the study, antibody determination was made with SFDT on 589 serum samples. Anti-*T. gondii* IgG and IgM positivity were investigated by ELISA in the serum of 478 patients, avidity values of 93 patients with Anti-*T. gondii* IgG positive were determined. Data obtained for all patient

and test groups, and gender and age group analyses were performed. ELISA test kits from different manufacturers were studied in accordance with the manufacturer's directives during the study periods. The *T. gondii* strain used in this study was reported by Ekmen et al. (1974) [14], and uploaded to NCBI with the name of *T. gondii* TR01 by defining whole genome studies in UPRL. Pearson Chi-Square was used for statistical analysis of the study data, and the Kappa test was used for univariate logistic regression analysis and fit tests. In the Pearson Chi-Square analysis, $p<0.05$ values were considered statistically significant. Risk assessment tests were also carried out, and all statistical tests were carried out with the SPSS Windows, Version 14.1 package program.

This publication, number 715087, dated 2022, was carried out by BA and approved as a master thesis, and its permission was obtained from the Ministry of Health, Public Health Institution of Türkiye on 05/03/2021.

Results

In this study, a total of 1160 toxoplasmosis suspected patients who applied to UPRL were studied. In the study, SFDT was studied in 589 serum samples to investigate Anti-*T. gondii* antibodies and the results were evaluated in terms of demographic findings in Table 1. Of the 589 cases, 348 (59.1%) of the total 589 cases were found to be positive for SFDT, of which 211 (60.6%) were female and 137 (39.4%) were male. According to gender groups, this difference was found to be statistically significant. When the SFDT seropositivity rates are analyzed by age groups, the lowest is in the 2-14 age group (3.7%), and the highest in the 0-1 age group (51.1%) (Table 1). A statistically significant correlation was found between patient age groups and the presence of *T. gondii* infection in SFDT ($p<0.05$). The high seropositive rates seen in the infant patient group in the SFDT study results indicate that the presence of mother-to-infant antibody titers was detected.

In this study, Anti-*T. gondii* IgG and IgM tests were studied together and Anti-*T. gondii* IgG avidity test was performed on 93 cases with positive Anti *T. gondii* IgG. Anti-*T. gondii* IgG values are presented in Table 2. Anti-*T. gondii* IgG positive result was detected in 45% of all samples, 44.7% of men and 55.3% of women. The lowest anti-*T. gondii* IgG results were found in the 2-14 (4.7%) age group and the highest in the 15-45 (58.1%) age group (Table 2).

In this study, Anti-*T. gondii* IgM values were evaluated with demographic findings (Table 3). Anti-*T. gondii* IgM result was found positive in 4.2% of the samples and in 30% of the men and 70% of the women. Anti-*T. gondii* IgM positivity was observed most frequently in the 15-45 age group with 75%, while the lowest frequency was detected at the same rate with 5% in the 2-14 and >45 age group (Table 3). In this respect, it has been observed that the 15-45 age group is riskier in terms of acute toxoplasmosis infection. According to the anti-Toxo- IgM/IgG results, no statistically significant difference was found between the sex and age groups in positive patients ($p>0.05$).

In this study, Anti-*T. gondii* IgG was positive in 215 samples and Anti-*T. gondii* IgM was positive in 20 samples of 478 patients aged 0-88 years. During this study, there were 17 patients in whom IgG and IgM were positive together (common positives). IgG and IgM were both positive in 17 patients (3.6%), IgG positive in 198 patients (41.4%), and IgM positive in 3 (0.6%). *T. gondii* seropositivity of the total patients in whom ELISA IgG and IgM were studied in 2019-2020 was 45.6% with the number of 218 patients. Of these 218 positive patients, 120 (55%) were female and 98 (45%) were male (Table 4).

In the study, the results of 93 patients with positive ELISA IgG results and *T. gondii* IgG avidity requests were evaluated. Of the 12 (13%) patients whose avidity test was low (Avidity index value <40%), 7 were female, 5 were male, and 15 (16%) patients had a cutoff value (Avidity index value of 40-50%). Of the 66 (71%) patients, 8 were female, 7 were male, with a high value (Avidity index value >50%), 44 were female and 22 were male (Table 5).

Table 1. Distribution of Anti-*T. gondii* antibody results detected by SFDT by gender and age group (n=589)

Anti-*T. gondii* IgG 2019-2020		**Positive**		**Negative**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
Gender	**Female**	211	60.6	120	49.8	331	56.2
	Male	137	39.4	121	50.2	258	43.8
	Total	348	59.1	241	40.9	589	100
Age	**0-1**	178	51.1	92	38.2	270	45.8
	2-14	13	3.7	26	10.8	39	6.6
	15-45	139	39.9	93	38.6	232	39.4
	>45	18	5.2	30	12.4	48	8.1
	Total	348	100.0	241	100.0	589	100.0

Table 2. Distribution of seropositivity by gender and age groups according to Anti- *T. gondii* IgG (n=478)

Anti-*T. gondii* IgG 2019-2020		**Positive**		**Negative**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
Gender	**Female**	119	55.3	124	47.1	243	50.8
	Male	96	44.7	139	52.9	235	49.2
	Total	215	45	263	55	478	100
Age	**0-1 Age**	50	23.3	13	4.9	63	13.2
	2-14 Age	10	4.7	29	11.0	39	8.2
	15-45 Age	125	58.1	201	76.4	326	68.2
	>45 Age	30	14.0	20	7.6	50	10.5
	Total	215	100.0	263	100.0	478	100.0

Table 3. Distribution of Anti-*T. gondii* IgM seropositivity by gender and age group (n=478)

Anti-*T. gondii* IgG 2019-2020		**Positive**		**Negative**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
Gender	**Female**	14	70	229	50	243	50.8
	Male	6	30	229	50	235	49.2
	Total						
Age	**0-1 Age**	3	15.0	60	13.1	63	13.2
	2-14 Age	1	5.0	38	8.3	39	8.2
	15-45 Age	15	75.0	311	67.9	326	68.2
	>45 Age	1	5.0	49	10.7	50	10.5
	Total	20	100.0	458	100.0	478	100

Table 4. Combination and distribution of Anti-*T. gondii* IgG and IgM between 2019-2020 (n=218)

IgM(+)/IgG(-)		IgM(+)/IgG(+)		IgM(-)/IgG(+)		Total	
n	%	n	%	n	%	n	%
3	0.6	17	3.6	198	41.4	218	45.6

Table 5. IgG Avidity test results (n=93)

			Avidity			
		Genes	Low Value	Breakdown	High Value	Total
IgG	Positive	Female	7	8	44	59
		Male	5	7	22	34
		Total	12	15	66	93

Discussion

Toxoplasmosis is an infection caused by *T. gondii* protozoa. According to the results of this study's investigation of the seroprevalence of *T. gondii,* it was stated that although the prevalence is high globally, it changes in conjunction with socio-economic conditions, increases with age, and is more common in hot climates [10]. Poor socio-economic conditions in countries, cause the prevalence of the disease to rise [5]. While the seropositivity of the disease was found to be below 3% in Australia and North America, rates exceeding 50% were detected in Europe and Africa [15]we describe the effects of global climate change for one specific pathogen: the parasite Toxoplasma gondii. It is postulated that an increase of T. gondii prevalence in humans can occur in some regions of North-Western Europe as a result of changing environmental conditions. Such a change can be predicted by using Global Climate Change models. We have elaborated such a prediction for one scenario (SRES A1. Due to the parasite's asymptomatic feature, clinical findings are rare [16].

Toxoplasma gondii-IgG antibodies appear within two weeks of contracting toxoplasmosis and these antibodies reach their highest levels within 3 months. IgM antibodies specific to *T. gondii*, which are accepted as an indicator of acute infection, can be detected by ELISA in the first weeks, since they are the first antibodies [17]. IgM-type antibodies disappear earlier than expected in some acute infections, making it difficult to distinguish between acute infection and chronic infection [18]. In such cases, the IgG avidity ELISA test helps us to obtain information about the time of detection by calculating the avidity of *T. gondii*-specific IgG in the acute and chronic phases of the infection [19]. In order to increase the reliability of the diagnosis, as Western Blot and PCR should be used [20].

Considering the recent studies in Türkiye, Anti-*T. gondii* IgG positivity is between 17.5% and 69.5% and Anti-*T. gondii* IgM has been reported between 0-5.4% [21]. In a retrospective study conducted in the same laboratory before, SFDT was found to be 52%, Anti-*T. gondii* IgG 47.1% and Anti-*T. gondii* IgM positive 10.2% [10].

In this study, SFDT 59.1%, Anti-*T. gondii* IgG 45% and Anti-*T. gondii* IgM 4.2% seropositivity was detected. These results are similar to *T. gondii* seropositivity studies conducted in Türkiye so far.

According to the ELISA results performed in this study, only IgG positivity was found in 198 (41.4%) of the patients, only IgM positivity was found in 3 patients (0.6%), and both were positive in 17 (3.6%) patients. 17 samples form a common group that were evaluated as positive according to both IgG and IgM. It has been determined that the results of the study show similarities with other studies conducted in Türkiye, including the studies conducted in the same laboratory before, and have consistent results.

In this study, all three tests (SFDT, IgG, IgM) were evaluated statistically and it was found that women had a higher rate of contracting *T. gondii*. It is thought that the reason for this is that women are more likely to feed cats at home and therefore have a higher number of interactions with feces in the cleaning of cat feces and the contact with both the parasite-contaminated food in the kitchen and the garden. Studies carried out in Malatya in Türkiye, and in the Netherlands are compatible with this study [22, 23]. In the large surveillance study in which they compared the data collected from the National Health and Research Survey (NHANES) system in the USA between 1988/1994 and 1999/2000, it was stated that no significant differences were observed between the sexes [24]. In the studies conducted in Kayseri and Manisa in Türkiye, as well as in Korea and Israel, gender differences were not found either [25-28]. In a study conducted in 2008 using the National Inpatient (NIS) data in the USA, seropositivity was found to be higher in HIV-positive patients and in another study in France, males [29, 30].

In many studies, it has been shown that a significant relationship between seroprevalence and age is frequently observed [31-33]. In this study, the difference between the high Anti-*T. gondii* IgG seroprevalence in the 15-45 age group (58.1%) and the 0-1 age group (23.3%) was found to be statistically significant by univariate regression analysis (p<0.05). However, when we evaluated Anti-*T. gondii* IgM seroprevalence, it was seen that the results were not statistically significant in age groups (p>0.05). Moreover, in the univariate logistic regression analysis performed between SFDT and age groups, the difference was found to be significant (p<0.05).

The avidity test is used to diagnose infections acutely or chronically. In the test evaluated according to antibody and antigen binding concentration, weak binding detects low avidity indicating infection within 3-4 months, strong binding detects high avidity indicating exposure to the agent 6 months or before and chronic infection [34]. In this study, *T. gondii* IgG avidity test was performed in 93 patients with *T. gondii* IgG positive, 12 (13%) patients with low avidity, 15 (16%) patients with cutoff avidity, and 66 (71%) patients with high avidity. In other studies, conducted in Türkiye, a high avidity of 30% was found in Akdeniz University hospital [35], and 70.8% in the study of Yazar et al. [36] (2005). In the study conducted in Iran, 92.7% high avidity values were determined. Values seen in this study are similar to other avidity values.

In conclusion, with this study, SFDT, ELISA Anti *T. gondii* IgG - Anti *T. gondii* IgM and Avidity tests were performed on patients who applied with the suspicion of toxoplasmosis and were evaluated prospectively with demographic data. The high rates detected in Türkiye and in our study show that the disease can be overlooked, as toxoplasmosis generally continues with an asymptomatic course. At the same time, this study emphasizes the importance of screening for *T. gondii* before and during pregnancy in order to protect the population from parasites and especially to prevent the risk of congenital toxoplasmosis. The fact that there are some difficulties in the detection of the disease from a serological point of view has shown that the detection of acute infections should not be satisfied with a single test. In these cases, ELISA IgG/IgM, as well as avidity tests are required. We believe that the application of molecular tests (PCR and qPCR) will be reliable in order to prevent interventional approaches and unnecessary drug treatments. Providing treatment for this disease, which is of great importance for public health all over the world in the future and cannot be eradicated yet, will prevent the emergence of dangerous dimensions such as congenital toxoplasmosis.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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Ethical committee approval: This publication, number 715087, dated 2022, was carried out by BA and approved as a master thesis, and its permission was obtained from the Ministry of Health, Public Health Institution of Türkiye on 05/03/2021.

Authors' contributions to the article

B.A. and A.S.N. constructed the main idea and hypothesis of the study. B.A., A.S.N. and B.Y. developed the theory and arranged/edited the material and method section. B.A., A.S.N. and B.Y. have done the evaluation of the data in the Results section. Discussion section of the article written by B.A., A.S.N. and B.Y.

A.S.N. and B.Y. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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A single-center study on the factors affecting the surgical approach in emergent inguinal hernias

Acil inguinal hernilerde cerrahi yaklaşıma etki eden faktörler: tek merkez deneyimi

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Abstract

Purpose: The aim is to establish the principles of surgery for patients who have undergone emergency groin hernia surgery by examining risk factors, diagnostic modalities, time to operation, and surgical techniques.

Materials and methods: A retrospective analysis was conducted on patients who underwent groin hernia repair surgery between the years 2017 and 2022. The study evaluated various parameters such as demographic characteristics, physical examination findings, co-morbidities, radiologic assessments, operation notes, time of arrival to the hospital, and the duration of the operation. Data was collected from the hospital's electronic medical records system. Based on the mode of pre-operative assessment, patients were classified into two groups: group 1, comprising patients whose decision for surgery was based on physical examination alone, and group 2, comprising patients who underwent radiologic assessment prior to the operation.

Result: The risk evaluation of patients who underwent emergent hernia surgery revealed a higher incidence of women gender (25%) and femoral hernia type (16.6%) as compared to the elective surgery group, where the incidence was 6.7% and 1.6%, respectively. The diagnosis of patients was primarily based on physical examination findings, although radiologic methods were used preoperatively in 75% of the cases. It was observed that radiologic assessments increased the duration of the operation and resulted in higher morbidity and intestinal resection rates.

Conclusion: Radiologic methods, apart from in cases of suspected conditions such as obesity and recurrent hernias, may lead to delayed treatment and increased morbidity and mortality rates in patients undergoing emergent hernia surgery. Prompt surgical intervention based on physical examination findings is crucial in cases of strangulation or incarceration. In instances of spontaneous reduction, incisional exploration, hernioscopy, laparoscopy, or laparotomy should be considered if there is any suspicion of intestinal viability.

Keywords: Inguinal, hernia, emergency, surgery.

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Öz

Amaç: Kasık fıtığı nedeniyle acil girişim gereken hastalarda risk faktörlerini, tanı yöntemlerini, operasyon zamanını ve tekniklerini inceleyerek cerrahi prensipleri belirlemek.

Gereç ve yöntem: 2017 ile 2022 yılları arasında kasık fıtığı onarımı ameliyatı geçiren hastalar retrospektif olarak incelendi. Çalışmada; demografik özellikler, fizik muayene bulguları, eşlik eden hastalıklar, radyolojik değerlendirmeler, operasyon notları, hastaneye varış zamanı ve operasyon süresi gibi çeşitli parametreler değerlendirildi. Veriler hastanenin elektronik tıbbi kayıt sisteminden toplandı. Hastalar, ameliyat öncesi değerlendirme şekline göre iki gruba ayrıldı: grup 1, ameliyat kararı sadece fizik muayene bulgularına dayanan hastaları içerirken; grup 2, ameliyattan önce radyolojik değerlendirmeye tabi tutulan hastaları içermekteydi.

Bulgular: Kadın cinsiyet (%25) ve femoral fıtığa (%16,6) sahip hastalarda inkarserasyon ve buna bağlı acil cerrahi gereksinimi daha yüksek oranda izlenmiştir. Hastaların tanısı genellikle fizik muayene bulgularına dayanıyordu ancak radyolojik yöntemler de vakaların %75'inde ameliyat öncesi değerlendirmede kullanıldı. Radyolojik değerlendirmelerin operasyon süresini artırdığı ve daha yüksek morbidite ve bağırsak rezeksiyonu oranlarına neden olduğu gözlemlendi.

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Sonuç: Obezite ve nüks fıtık gibi şüpheli durumlar dışında, acil fıtık cerrahisi geçiren hastalarda radyolojik yöntemler, tedaviyi geciktirebilir ve morbidite ve mortalite oranlarını artırabilir. İnkarserasyon veya strangülasyon durumlarında fizik muayene bulgularına dayalı hızlı cerrahi müdahale hayati önem taşır. Spontan redüksiyon durumlarında bağırsak canlılığına dair herhangi bir şüphe varsa eksplorasyon, hernioskopi, laparoskopi veya laparotomi düşünülmelidir.

Anahtar kelimeler: İnguinal, herni, acil, cerrahi.

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Introduction

Groin hernias (GH) are a common occurrence and typically require surgical intervention, with emergency surgery being necessary in 5-13% of cases [1, 2]. However, there is controversy regarding the identification of risk factors for incarceration (IC) or strangulation (SG), the use of diagnostic methods, and the optimal timing for surgery [3]. Factors such as femoral hernias and a history of hospitalization within the past year increase the likelihood of IC or SG and the need for emergent intervention, particularly among women [4, 5]. While GH surgery is generally considered low risk, emergency situations with SG or IC can lead to complications and even mortality, particularly in patients who are older than 65, experiencing intestinal obstruction, taking anticoagulants, classified as ASA III and IV, female, or have a body mass index over 30 [4-8]. Among the factors that contribute to increased mortality and morbidity rates are the presence of a long-term hernia, delayed diagnosis and treatment, IC hernias lasting exceeding 24 hours, symptoms lasting extending beyond 3 days, a prolonged duration between diagnosis to surgery, femoral hernia, a previous occurrence of exploration through midline incision post hernia reduction, and hernia recurrence [6, 8-11]. The present study seeks to examine the risk factors associated with the development of surgical emergencies such as strangulated (SG) or incarcerated (IC) hernias, as well as the timing of surgical intervention and the methods of treatment utilized in patients who have undergone emergent groin hernia (GH) surgery.

Materials and methods

The study titled “Surgical Treatment of Inguinal Hernia and Cord Lipomas” was conducted between 2017-2022 after obtaining approval from the Clinical Research Ethics Committee of Istanbul Medeniyet University Goztepe Training and Research Hospital.

Between 2017 and 2022, patients who were diagnosed with incarcerated groin hernia and underwent surgery in our general surgery emergency department were included in the cohort. All patients underwent a physical examination by the on-call general surgeon after initial assessment in the emergency department. Pre-operative imaging was requested in selected cases for differential diagnosis according to the preference of the on-call general surgeon. Initially, ultrasound (US) was the preferred imaging modality, and if access to US was not available after midnight, computed tomography (CT) was performed. All surgeries were performed by on-call general surgeons with a minimum of 3 years of experience under emergency conditions. Hernia repair was performed either mesh or primary repair based on the contamination status of the wound site. Hernioscopy was performed in patients with spontaneous hernia sac reduction, and laparotomy was performed in patients requiring bowel resection.

In the cohort group, various parameters such as demographic characteristics, physical examination findings, comorbidities, radiological evaluations, operation notes, time of arrival

at the hospital, and operation duration were retrospectively evaluated.

Two distinct patient groups were established: Group 1, whose surgeries were based solely on physical examination results, and Group 2, whose surgeries were preceded by preoperative imaging. The data collected was subjected to analysis utilizing SPSS Statistics Version 26, descriptive statistics were presented as counts and percentages for categorical variables, and as mean ± standard deviation, median, and minimum-maximum values for continuous variables. The presence of a normal distribution was assessed using histograms and the Shapiro-Wilk test. Depending on whether the data followed a normal distribution or not, Student's t-test or Mann-Whitney U test was used for comparing continuous variables between two independent groups. Categorical variables were compared using Pearson's chi-squared test or Fisher's exact test. A statistical significance level of $p<0.05$ was considered.

Result

Over a five-year period, 2321 patients received groin hernia (GH) surgery, of which 2098 were male and 151 were female among the 2249 patients who underwent elective surgery. Recurrent hernia was the cause of surgery in 139 patients, while 36 had femoral hernia ($p<0.01$). Among the 72 patients who required emergency surgery, 54 were male and 18 were female, and 12 had strangulated or incarcerated recurrent or femoral inguinal hernias, all of which were diagnosed through pre-operative physical examination. Furthermore, Ultrasonography (US) was conducted on 39 patients, while 15 patients underwent a Computerized Tomography (CT) scan. Notably, 18 patients received surgery based solely on physical examination, without the aid of any imaging modality.

The average age of our patients is 61.9±17.5 years, with Group 1 having an average age of 64.9±12.9 and Group 2 having an average age of 60.8±18.7 (p:0.462). In Group 1, 77.8% of the patients were male, while in Group 2, 74.1% were male (p:0.753). In both groups, inguinal hernia was the most common, but in Group 2, femoral and recurrent hernias were more common compared to Group 1 (femoral 20.4% and 5.6%, recurrent 18.5% and 11.1%, respectively) (p:0.157). Mesh repair was performed in 83% of patients in both groups (p:1.000). Strangulation was observed in 22.2% of Group 1 and 27.8% of Group 2 (p:0.643). Intestinal resection was performed in 11.1% of Group 1 and 14.8% of Group 2, with no significant difference (p:0.694). Hernioscopy was performed in 2 patients in each group due to spontaneous reduction (p:0.235). In Group 1, omentum was most commonly found in the hernia sac (58%), while in Group 2, small intestine was found in 50.9%, which was statistically significant (p:0.033). The time from emergency room admission to surgery was 6.2±4.5 hours in Group 1 and 9.8±11.1 hours in Group 2 (p:0.203); the length of hospital stay was 48.6±37.4 hours in Group 1 and 78.0±67.6 hours in Group 2 (p:0.074), with no statistically significant difference observed. There was no significant difference in pre-operative WBC and CRP values in both groups (Pre-op WBC, p:0.123; Pre-op CRP, p:0.396). The information provided above has been summarized in Table 1.

Table 1. The demographic, laboratory, and surgical data of the patients

	Total	Group I	Group II	*p*	N
Age, mean±SD	61.9±17.5	64.9±12.9	60.8±18.7	0.462	72
Gender, n (%)					
Female	18 (25)	4 (22.2)	14 (25.9)	0.753	72
Male	54 (75)	14 (77.8)	40 (74.1)		
Hernia side, n (%)					
Right	44 (61.1)	11 (61.1)	33 (61.1)	1.000	72
Left	28 (61.1)	7 (61.1)	21 (61.1)		
Hernia Type, n (%)					
Inguinal	48 (66.7)	15 (83.3)	33 (61.1)		
Femoral	12 (16.7)	1 (5.6)	11 (20.4)	0.157	72
Recurrent	12 (16.7)	2 (11.1)	10 (18.5)		
Repair Technique, n (%)					
Mesh	60 (83.3)	15 (83.3)	45 (83.3)	1.000	72
Suture	12 (16.7)	3 (16.9)	9 (16.9)		
Strangulation, n (%)					
Yes	19 (26.4)	4 (22.2)	15 (27.8)	0.643	72
No	53 (73.6)	14 (77.8)	39 (72.2)		
Bowel resection, n (%)					
Yes	10 (13.9)	2 (11.1)	8 (14.8)	0.694	72
No	62 (86.1)	16 (88.9)	46 (85.2)		
Hernioscopy, n (%)					
Yes	4 (5.6)	2 (11.1)	2 (3.7)	0.235	72
No	68 (94.4)	16 (88.9)	52 (96.3)		
Structure in hernia sac, n (%)					
Omentum	24 (34.3)	10 (58.8)	14 (26.4)		
Small intestine	32 (45.7)	5 (29.4)	27 (50.9)	0.033	70
Colon	14 (20.0)	2 (11.8)	12 (22.6)		
Length of hospitalization, h, mean±SD	70.7±62.5	48.6±37.4	78.0±67.6	0.074	72
Time to operation, h, mean±SD	8.8±9.9	6.2±4.5	9.8±11.1	0.203	72
Pre-op WBC, 10^3/uL, mean±SD	11.4±4.7	10.3±4.8	11.8±4.7	0.123	72
Pre-op CRP, mg/dl, mean±SD	2.7±6.1	1.8±3.0	3.0±6.7	0.396	65

Discussion

After analyzing patients who underwent surgery for IC or SG the study's observation was that 75% of the patients were male, while 25% were female. However, when elective cases were examined, it was found that 93.3% of male patients and 6.7% of female patients underwent surgery, indicating a higher incidence of SG and IC in women [7, 12, 13]. Moreover, our study showed that women were more commonly affected by SG and IC when compared to patients who underwent elective surgery.

Patients with femoral hernias had a significantly higher risk of developing SG [7, 8, 14]. Despite the fact that femoral hernias constituted only 1.6% of elective hernia surgeries, they constituted 16.6% of all emergency hernia surgeries, indicating a much higher risk of emergency SG and IC in these patients than in those who underwent elective inguinal hernia surgery.

According to the literature, physical examination is generally deemed adequate for diagnosing emergent inguinal hernias and should be performed on all patients [3]. Conducting a physical examination not only enables earlier surgical intervention but also minimizes the requirement for imaging techniques [15]. In our study, all patients were diagnosed with hernias based on physical examination, indicating the effectiveness of this method in diagnosing hernias. Additionally, 25% of the patients underwent surgery solely based on physical examination.

Research suggests that ultrasound (US) is an effective diagnostic tool for evaluating incarcerated organs, identifying recurrent hernias, and differentiating groin-related pathologies such as abscesses, hydrocele, and painful lymphadenopathy especially in obese patients [16-18]. The low cost, accessibility, non-invasive nature, and ease of application of US contribute to its increased usage in selected cases. In our study, 21% of patients received preoperative US examinations in addition to physical examinations.

CT scans are not commonly recommended for incarcerated and strangulated groin hernias, but may be used in some cases to diagnose patients with obstruction [3, 19]. In our study, CT scans were performed in only 21% of cases to investigate other intra-abdominal pathologies. Prolonged duration of IC or SG lasting more than 24 hours increases the risk of requiring intestinal resection [11]. Many studies have identified the 24-hour mark as the cut-off value for IC duration [2, 20, 21]. In our study, patients who underwent intestinal resection had a duration of IC or SG exceeding 24 hours. In patients with IC or SG duration longer than 24 hours, intestinal resection was deemed necessary in 10% of cases.

Postponing surgery following a hernia diagnosis may heighten the likelihood of morbidity and mortality [9]. A higher duration of IC and SG is linked to a greater probability of intestinal resection [22, 23]. Among patients who needed intestinal resection, we found that Group 2 had a longer mean duration from hospital admission to surgery than Group 1. Moreover, the use of imaging tests in Group 2 resulted in a longer time until surgery, increasing the likelihood of intestinal resection.

The decision to perform preoperative imaging does not create a significant difference between the two groups in terms of strangulation, bowel resection rates, and the surgical techniques applied as a result. Especially considering that the viability of the bowel is significantly related to the time elapsed until surgery, particularly in cases of incarcerated and/or strangulated hernias, there is no need for unnecessary time delay by performing preoperative imaging. Furthermore, our findings suggest that the delay in surgery caused by the use of imaging tests resulted in a higher length of hospital stay in Group 2.

Exploration is recommended in cases of groin hernias (GH) with incarcerated (IC) or strangulated (SG) hernias, even in cases of spontaneous reduction when intestinal viability is suspected [3]. The surgeon's experience and type of surgery determine the exploration technique. The available options comprise of open exploration via the groin, standard laparoscopy, hernia sac laparoscopy (hernioscopy) or laparotomy [24]. In our study 2 patients in each group underwent hernioscopy and laparotomy was performed only in patients requiring bowel resection. Routine laparoscopic exploration was not performed.

Performing emergent groin hernia surgery in a timely manner is crucial to prevent complications, irrespective of the hernia type (incarcerated or strangulated). For emergent hernia surgeries, mesh repair is generally considered the preferred technique for clean and clean-contaminated cases [25-27]. However, for infected and contaminated cases, suture repairs are typically preferred, although mesh repair may be appropriate for well-cleaned and protected surgical sites, while weighing the risks of infection. Therefore, it is important to carefully evaluate the benefits and potential risks of mesh repair in such cases [28, 29]. In our study, the majority of patients (73%) were classified as clean or clean-contaminated and underwent mesh repair. In our study 83.3% of the patients underwent mesh repair, while 16.7% underwent suture repair.

In conclusion; prompt surgical intervention is essential in cases of incarcerated or strangulated GH to prevent complications and minimize the risks of morbidity and mortality. Preoperative imaging should not be undertaken as it does not alter the type of surgical procedure and prolongs the time to surgery. Mesh repair is the optimal option for clean and clean-contaminated cases in emergent hernia surgery, while suture repair is preferred for contaminated and infected cases. In the latter cases, mesh repair should be cautiously considered after carefully evaluating the risk of infection. Exploration or hernioscopy ought to be conducted when doubts arise regarding intestinal viability subsequent to the spontaneous reduction of a hernia. Overall, early surgical intervention is necessary to avoid potential complications in cases of incarcerated and strangulated GH.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

F.B. constructed the main idea and hypothesis of the study. H.B. developed the theory and arranged/edited the material and method section. M.S.O. has done the evaluation of the data in the results section. Discussion section of the article was written by M.A.A. and S.T. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Evaluation of medicolegal death cases sent from abroad to Denizli province

Yurt dışından Denizli iline gönderilen medikolegal ölüm olgularının değerlendirilmesi

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Abstract

Purpose: In this study, we aimed to contribute to the literature by discussing the difficulties encountered in the approach to suspicious death cases reported abroad and sent to our country.

Materials and methods: The findings were obtained by retrospectively reviewing all medico-legal death cases sent from abroad to Denizli province between January 2012 and December 2022, in which postmortem examination and autopsy were performed.

Results: Of the 12 cases included in the study, 3 were female and 9 were male, the youngest case was 2 years old and the oldest case was 66 (median 49) years old. Germany was the country with the highest number of suspicious deaths with nine cases (75%), while one case each was sent from Turkmenistan, the Netherlands and Austria. When the cases were evaluated in terms of origin, it was observed that 5 (41%) cases were of natural origin followed by 4 (33%) cases of suicide origin.

Conclusion: In order to minimize the problems encountered in the re-evaluation of medico-legal death cases from abroad, we think that it would be appropriate for all countries to perform medico-legal autopsy in accordance with international standards for suspicious death cases sent abroad, and to share the report containing the autopsy findings and all documents that may help to clarify the cause of death of the person with the forensic medicine experts in the country of destination.

Key words: Abroad, autopsy, embalming.

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Öz

Amaç: Bu çalışmada, yurt dışında şüpheli ölüm olarak bildirilen ve ülkemize gönderilen şüpheli ölüm olgularına yaklaşımda karşılaşılan zorluklar bulgularımızla tartışılarak literatüre katkıda bulunulması amaçlanmıştır.

Gereç ve yöntem: Bulgular Ocak 2012-Aralık 2022 yılları arasında yurt dışından Denizli iline gönderilen, ölü muayene ve otopsisi yapılan tüm medikolegal ölüm olguları retrospektif olarak taranarak elde edilmiştir.

Bulgular: Çalışmaya alınan 12 olgunun 3'ü kadın, 9'u erkek, en genç olgu 2 yaşında, en yaşlı olgu ise 66 (medyan 49) yaşındadır. Dokuz (%75) olgu ile en fazla şüpheli ölüm olgusu gönderen ülke Almanya olurken, Türkmenistan, Hollanda ve Avusturya'dan birer olgu gönderilmiştir. Olgular orijin yönünden değerlendirildiğinde, doğal orjinli 5 (%41) olguyu, intihar orjinli 4 (%33) olgunun takip ettiği görülmektedir.

Sonuç: Yurt dışından gelen medikolegal ölüm olgularını yeniden değerlendirmede karşımıza çıkan sorunları en aza indirebilmek için tüm ülkeler tarafından yurt dışına gönderilen şüpheli ölüm olgularına uluslararası standartlar dahilinde medikolegal otopsi yapılması, yapılan otopsi bulgularını içeren raporun ve kişinin ölüm sebebini aydınlatmaya yardımcı olabilecek tüm belgelerin gittiği ülkedeki adli tıp uzmanları ile paylaşılmasının uygun olacağı düşüncesindeyiz.

Anahtar kelimeler: Yurt dışı, otopsi, tahnitleme.

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Introduction

The approach to suspicious death cases, the methods of investigating these cases and the scope of such investigations vary greatly from country to country [1, 2]. In order to minimise these differences, there are studies such as “Medical Autopsy Rules” established between the member countries of the European Union and the United Nations “Minnesota Protocol” [3, 4]. Brinkmann emphasised that although autopsies are carried out, they are extremely inadequate and attributed the main reasons for such shortcomings to: the lack of sufficiently specialised doctors, the lack of adequate provisions in the country concerned guaranteeing the investigation of suspicious cases, deficiencies in sampling and planning of further investigations, lack of quality control, problems of independence of the investigating doctor, etc. [5].

With the development of transportation and especially after the industrial revolution in Europe, there has been an increase in the number of people migrating from Türkiye to abroad. According to current official data, 6.5 million Türkiye citizens live abroad [6]. When citizens of the Republic of Türkiye who are reported as suspicious deaths or non-natural deaths abroad are brought to Türkiye for burial, the judicial authorities may request the determination of the exact cause of death and for this purpose, these cases are re-evaluated by forensic medicine specialists. Procedures such as the evaluation of medico-legal death cases, the need for autopsy and embalming procedures may vary from country to country. For this reason, the cases sent to us may have gone through different procedures.

It is a very difficult procedure to perform a re-autopsy in a case where an autopsy has been performed due to various reasons such as the integrity of the organs being disrupted due to previous dissection, tissue samples being taken for histopathological examination, whether or not embalming has been performed, the onset of putrefaction, and difficulty in accessing the autopsy report and medical documents.

Materials and methods

The population of this study consisted of all medico-legal death cases that were sent from abroad to Denizli province between January 2012 and December 2022 and were examined and autopsied in the autopsy room of Pamukkale University Faculty of Medicine, Department of Forensic Medicine. All 12 cases identified in the population were included in the sample group. The aim of this retrospective study was to discuss the autopsy techniques, embalming procedures, international differences in the approach to suspicious death cases and the problems encountered in autopsy and re-autopsy with the information obtained from 12 cases.

This study was conducted in accordance with the principles of the Declaration of Helsinki by obtaining ethics committee approval from Pamukkale University Non-Invasive Clinical Research Ethics Committee.

Results

In this study, 12 cases that were sent from abroad as suspicious deaths and were examined and autopsied in the autopsy room of Pamukkale University Forensic Medicine Department were discussed in terms of gender, age, country, origin, whether autopsy and embalming were performed in the country where they were sent, and whether they had medical documents (Table 1).

Of the 12 cases, 3 were female and 9 were male, the youngest case was 2 years old and the oldest case was 66 years old (median 49). Germany was the country with the highest number of suspicious deaths with nine cases (75%), while one case each was sent from Turkmenistan, the Netherlands and Austria. When the cases were analysed in terms of origin, it was observed that 5 (41%) cases of natural origin were followed by 4 (33%) cases of suicide origin.

Autopsy was performed in 2 (16%) cases and embalming was performed in only 1 (8%) case. Three (25%) cases had medical documents issued by health institutions abroad, while in the other 9 (75%) cases, no documents other than the burial permit and transfer protocols were found.

Table 1. Distribution of cases

	Gender	Age	Year	Country	Origin	Was there an autopsy in the country of origin?	Embalming status	Medical Document
Case 1	Male	57	2020	Turkmenistan	Natural	No	Yes	Supplied
Case 2	Male	53	2015	Germany	Suicide	No	No	None
Case 3	Male	50	2012	Germany	Homicide	Yes	No	None
Case 4	Male	47	2012	Germany	Natural	No	No	None
Case 5	Woman	61	2017	Germany	Suicide	No	No	None
Case 6	Woman	66	2015	Germany	Natural	No	No	Supplied
Case 7	Male	59	2021	Austria	Suicide	No	No	None
Case 8	Male	48	2021	Germany	Traffic Accident	No	No	None
Case 9	Woman	2	2014	Germany	Natural	Yes	No	None
Case 10	Male	32	2017	Germany	Natural	No	No	Supplied
Case 11	Male	22	2021	Netherlands	Suicide	No	No	None
Case 12	Male	2	2022	Germany	Accident	No	No	None

Case-1

As a result of the examination of the medical documents, medico-legal autopsy, histopathological and toxicological examinations of the 57-year-old male case, who was sent to us from Turkmenistan without an autopsy and died in the hospital where he was hospitalized due to bronchitis, it was understood that he died due to acute pneumonia and related intense pulmonary edema and respiratory failure, and that he was embalmed with 15% formaldehyde.

Case-2

It was reported that the male case sent from Germany jumped into the river from a bridge, autopsy and embalming were not performed, and as a result of the postmortem examination and autopsy performed in the autopsy room of Pamukkale University Forensic Medicine Department and histopathological examinations performed afterwards; the person was reported to have died due to asphyxia caused by drowning in water.

Case-3

In the postmortem examination and re-autopsy of a 50-year-old man who was stabbed to death in Germany and autopsied in the autopsy room of Pamukkale University, Department of Forensic Medicine, dissection cuts and sharps injuries to the internal organs could not be clearly distinguished.

Case-4

As a result of the postmortem examination and autopsy performed in the autopsy room of Pamukkale University Department of Forensic Medicine, it was determined that the person died due to acute myocardial infarction developing on the basis of atherosclerotic coronary artery disease.

Case-5

A 61-year-old woman who was sent to us from Germany without autopsy and embalming was learnt from her relatives that she had jumped from the fourth floor of her house. During the postmortem examination and autopsy performed in the autopsy room of Pamukkale University Department of Forensic Medicine, it was determined that there was laceration on the posterior surface of the left lung and haemorrhage/contusion in the parenchyma around this region and the person died due to lung laceration.

Case-6

As a result of the examination of the medical documents of the 66-year-old woman who was sent from Germany without autopsy and who died while being treated for subarachnoidal haemorrhage due to vertebral artery aneurysm, and as a result of the postmortem examination and autopsy performed in the autopsy room of Pamukkale University Forensic Medicine Department, it was understood that she died as a result of subarachnoidal haemorrhage due to right vertebral artery aneurysm.

Case-7

As a result of the postmortem examination and autopsy performed in the autopsy room of Pamukkale University Department of Forensic Medicine, it was determined that the 59-year-old male patient, who was sent to us from Austria without autopsy and embalming, died due to asphyxia caused by live hanging.

Case-8

A 48-year-old man who died at the scene as a result of a road traffic accident sent to us from Germany without autopsy and embalming was found to have died due to liver laceration and intra-abdominal haemorrhage during the postmortem examination and autopsy performed in the autopsy room of Pamukkale University Forensic Medicine Department.

Case-9

The case of a 2-year-old girl who was sent to us from Germany was thought to have died due to volvulus with the history taken from her relatives, and a more definite opinion could not be reported because almost all internal organs were removed in the first autopsy and medical documents could not be accessed.

Case-10

As a result of the postmortem examination performed in the autopsy room of Pamukkale University Department of Forensic Medicine and examination of the medical documents of the 32-year-old male case sent to us from Germany without autopsy and embalming, it was determined that the person died due to acute cardiac arrhythmia developing on the basis of cardiovascular disease.

Case-11

As a result of the postmortem examination and autopsy performed in the autopsy room of Pamukkale University Department of Forensic Medicine, it was determined that the 22-year-old male case who was sent to us from the Netherlands without autopsy and embalming died due to asphyxia caused by live hanging.

Case-12

As a result of the postmortem examination and autopsy performed by Pamukkale University Department of Forensic Medicine, it was determined that the 2-year-old boy, who was sent to us from Germany without autopsy and embalming, died due to diffuse SAH accompanied by comminuted skull bone fractures and contusion of the brain, cerebellum and brain stem.

Discussion

The first problem we encounter in the re-evaluation of suspicious death cases received from abroad is the inadequate access to the postmortem examination and autopsy reports and, if available, to the medical documents from the health institutions where the person was recently admitted. While medical documents could be accessed in 3 of the 12 cases in the study, the postmortem examination and autopsy report of 2 cases in which autopsy had been performed previously could not be accessed. In a study conducted in the UK, it was reported that in 15 of 44 cases (34%), documents related to the forensic investigation abroad could be accessed, but only the report of the first autopsy of 1 case could be obtained [7]. In our first case, the exact cause of death, which was reported as acute pneumonia after autopsy and histopathological examination, was found to be consistent with the medical documents. When the medical documents of our sixth case were translated into Turkish and analysed, it was understood that he had subarachnoid haemorrhage (SAH) due to right vertebral artery aneurysm, was operated and stenting was performed. In the autopsy, it was found that there was intense SAH on all surfaces of the brain and basis, cerebellum and medulla spinalis surfaces, metallic stent material in the right vertebral artery and the findings were found to be compatible with the medical documents. A review of the medical documentation of our tenth case revealed that the person died of acute cardiac arrhythmia secondary to cardiovascular disease.

It is controversial whether the second autopsy can provide sufficient information due to reasons such as dissection incisions left over

from the previous autopsy, tissue samples taken for histopathological examination, the onset of putrefaction, inability to access the autopsy report and medical documents. In a study conducted by Boukis, it is stated that many corpses were examined by performing second autopsies and that, with some exceptions, the second autopsy would not provide much information due to lack of information, incorrect data, dissatisfaction of the forensic pathologist and inability to access new information [8]. In a study by Holz et al. [9] it was reported that 62.6% of 91 previously autopsied cases were accompanied by a document stating the cause of death, in approximately 75% of these cases the cause of death stated in the first autopsy was compatible with the cause of death determined in the second autopsy, and in 5 (10.2%) cases the cause of death determined in the second autopsy was not the same as the cause of death stated in the accompanying document. In our third case who underwent re-autopsy, it could not be determined whether the sharps injuries were penetrating to the thorax due to the dissection of the intercostal muscles and the dissection incisions made in the lungs in the previous autopsy and no opinion could be expressed about which injuries were fatal. In addition, it was observed that the skull was filled with a paper-cardboard-like material, the sella turcica was opened and the pituitary gland was not present in it, all organs were dissected but not fixed and were in a bag in the thoracic cavity, internal organs could be evaluated suboptimally due to dissection incisions and sharps injuries could not be clearly distinguished and for these reasons, the lack of the previous autopsy report in this case constituted an important problem. In the autopsy of our ninth case who died probably due to volvulus, it was observed that the skull was filled with paper, the sphenoid sinuses were opened, the hyoid bone and thyroid cartilage were not dissected, and the brain, thymus, stomach, intestines, pancreas, bladder, internal genital organs and the majority of the right and left ventricles of the heart were absent. In this case, the medical documents from the health institution where the person was admitted or the documents related to the autopsy performed in Germany could not be accessed.

Embalming slows down the putrefaction of the corpse and prevents the loss of autopsy findings. However, it was observed that only one of our 12 cases was embalmed and decomposition started in 4 of the 11 non-embalmed cases. In a study by Williams et al. [7] it was reported that the organs were preserved in 9 of the 24 embalmed cases and partial or complete decomposition occurred in the others. In the toxicological examination of the first case, which was embalmed due to methanol in the formaldehyde used in embalming, high dose methanol was detected, but formic acid, which is its metabolite, was not detected. In addition, a 6 cm incision was found in the upper inner part of the right thigh in this case, and dissection of this area revealed that the embalming was performed from this area.

There may be differences in the approach to suspicious death cases between countries. In 4 of our twelve cases, we observe that the origin was suicide and autopsy was not performed in the countries of origin. In two cases in which autopsy was performed in the country of origin, homicidal and natural origin were observed. In our sixth case, although vertebral artery dissection and intense SAH were determined as the cause of death in the health institution where the patient was admitted before death, it was reported as suspicious death due to the argument of the patient's relatives with the doctors, but it was observed that the first autopsy was not performed in the country of origin.

Although there are some standardisation studies such as "Medico-legal Autopsy Rules" and the United Nations "Minnesota Protocol" established for the standardisation of autopsy procedures, differences in autopsy techniques and procedures can be seen from country to country and even within countries. In a study conducted by Grellner et al. [10] 4 out of 5 autopsies performed abroad and in a study conducted by Erbas et al. [11] it was reported that all 3 autopsies performed abroad were completely or partially inadequate. In our cases, no autopsy was performed in 6 cases of suicide and accident, and autopsy was performed only in the third and ninth cases of homicide and natural causes. In the first autopsy of the ninth

case, which was thought to have died due to volvulus, it was observed that the hyoid bone and thyroid cartilage were not dissected properly. In 2 other autopsy cases, it was determined that all three large body cavities were opened, all other organs were properly dissected as far as can be evaluated, and tissue samples were taken for histopathological examination.

In conclusion, it is a difficult and limited endeavour to obtain additional information about the death of the person through the re-autopsy procedure. According to the Convention on the Transfer of Corpses, signed by 23 countries, including Türkiye and many European countries, the international transfer of corpses requires the issuance of a passport containing information such as the name, age, date of death, cause of death and place of death [12]. In order to minimise the problems encountered in the re-evaluation of medico-legal death cases from abroad, we think that it would be appropriate for all countries to perform medico-legal autopsy in accordance with international standards for suspicious and non-natural death cases sent abroad, to send the report containing the autopsy findings and all documents that may help to clarify the cause of death of the person, such as crime scene investigation report, medical documents and hospital records, imaging information, and to share them with forensic medicine specialists in the country of destination.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

K.A. and A.K.D. constructed the main idea and hypothesis of the study. A.I. and K.A. They developed the theory and arranged/edited the material and method section. K.A., A.K.D. and A.I. have done the evaluation of the data in the Results section. Discussion section of the article was written by A.I. and K.A.

K.A., A.K.D. and K.A.A. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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HT22 cell differentiation reduces insulin receptor levels

HT22 hücre farklılaşması insülin reseptör seviyelerini azaltır

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Abstract

Purpose: The brain is an insulin-sensitive organ and has widespread insulin receptor (IR) expression. IR signaling in the brain is essential for neuronal development, feeding behavior, body weight, and cognitive processes such as attention, learning, and memory. HT22 cells, which are derived from parent HT4 cells that are immortalized from primary mouse hippocampal neuronal cells are used in research related to insulin signaling. However, the role of these cells in insulin signaling is not known. In this study, we aimed to examine IR levels in cells differentiated using neurobasal medium.

Material and methods: For the study, briefly, the cells were seeded in 6-well plates at 2x105 cells/well for 24 h. After the cells reached 80% confluence, the normal growth medium was replaced with a differentiation medium and the cells were incubated for 72 hours at 37^0C in 5% CO_2. Western blot procedure was used to determine the expression of the IR.

Result: Our results show that differentiation of HT22 cells stimulates neurite outgrowth. Furthermore, IR protein levels were significantly downregulated in differentiated HT22 cells.

Conclusion: This finding may require careful consideration of the use of neurobasal medium in conditions where IR signaling is important.

Keywords: HT22 cell, differentiation, insulin receptor.

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Öz

Amaç: Beyin, yaygın insülin reseptörü (IR) ekspresyonu olan insüline duyarlı bir organ olarak kabul edilmektedir. Beyindeki IR sinyali; nöronal gelişim, beslenme davranışı, vücut ağırlığı, dikkat, öğrenme ve hafıza gibi bilişsel süreçler için gereklidir. Primer fare hipokampal nöronal hücrelerinden ölümsüzleştirilen ana HT4 hücrelerinden türetilen HT22 hücreleri, insülin sinyali ile ilgili araştırmalarda kullanılmaktadır. Bununla birlikte, bu hücrelerin insülin sinyallemesindeki rolü bilinmemektedir. Bu çalışmada, nörobazal besiyeri kullanılarak farklılaştırılmış hücrelerde IR düzeylerini incelemeyi amaçladık.

Gereç ve yöntem: Çalışma için öncelikle, hücreler 24 saat boyunca 2x105 hücre/kuyu olacak şekilde 6 oyuklu plakalara ekildi. Hücreler %80 konfluansa ulaştıktan sonra normal büyüme ortamı farklılaşma ortamı ile değiştirildi ve hücreler 72 saat 37^0C'de %5 CO_2'de inkübe edildi. İnsülin reseptörünün (IR) ekspresyonunu belirlemek için western blot prosedürü kullanıldı.

Bulgular: Sonuçlarımız, HT22 hücrelerinin farklılaşmasının nörit büyümesini desteklediğini göstermektedir. Ayrıca, IR protein seviyeleri farklılaşmış HT22 hücrelerinde önemli ölçüde azalmıştır.

Sonuç: Bu bulgu, IR sinyalinin önemli olduğu durumlarda nörobazal ortamın kullanımının dikkatli bir şekilde değerlendirilmesini gerektirebilir.

Anahtar kelimeler: HT22 hücre, farklılaşma, insülin reseptörü.

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Introduction

Insulin is a significant growth factor that binds to the insulin receptor (IR) in the brain and then activates intracellular signaling pathways. The IR density is the highest in the olfactory bulb, hypothalamus, hippocampus, cerebral cortex, striatum, and cerebellum in the brain [1, 2] The widespread distribution of IR suggests that insulin signaling has important and diverse roles in the brain. Neuronal insulin signaling is known to regulate synaptic plasticity [3]. Evidence that insulin signaling is impaired in dementia indicates that this signaling is particularly important in maintaining or reversing symptoms in neurodegenerative diseases [4]. In addition, brain insulin resistance is the inability of brain cells to respond to insulin [5]. The reason for this could be due to the downregulation of IR, an inability of IR to bind insulin, or faulty activation of the insulin signaling cascade. This dysfunction may occur as the impairment of neuroplasticity, receptor regulation in neurons, or the impairment of processes more directly implicated in insulin metabolism, such as neuronal glucose uptake in neurons expressing GLUT4, or inflammatory responses to insulin [6]. In that respect, knowing the exact mechanism of brain insulin signaling and, its receptor is clinically important. Neuronal cell models are frequently used in studies on insulin signaling and IR.

HT22 cells are known as the mouse hippocampal neuroblastoma cell line and have similar properties to nerve cells and are used to understand neural development, synaptic functions, and neurodegeneration. In recent years, a medium called neurobasal medium has become a tool for HT22 cells to differentiate and acquire neural phenotypes. A study on the differentiation of HT22 cells showed that mature hippocampal neurons express very low cholinergic markers and glutamate receptors [7, 8]. However, the differentiation of HT22 cells caused increased levels of N-methyl-D-aspartate receptor (NMDAR) mRNA, making them more susceptible to glutamate-induced excitotoxicity [8]. In addition, another study showed that the differentiation of HT22 cells enhanced their functional serotonergic properties [9].

In line with these effects revealed by the differentiation of neuronal cells, we aimed to investigate the effect of HT22 cell differentiation with the neurobasal medium on IR expression level. Examining IR levels on differentiated HT22 cells may contribute to the understanding of insulin signaling and interactions in nerve cells.

Material and method

Antibodies and reagents

Neurobasal Medium (21103049), Dulbecco's modified Eagle's medium (DMEM) (2375262), and penicillin/streptomycin (2087433) were obtained from Gibco (Grand Island, NY, United States). N-2 supplement (100X) (CP18-2070) and fetal bovine serum (FBS) were purchased from Capricorn Scientific (Grand Island, NY, United States). L-glutamine (15323117) and Tripsin-EDTA (0.25%) (03-052-1B) were obtained from Biological Industries (Israel). Antibodies used in this study were as follows: IR (rabbit polyclonal, E-AB-60069, USA), β-actin (BTLab, BT-AP00213).

Cell culture

This study was carried out using HT22 mouse hippocampal cells. HT22 were cultured in DMEM supplemented with 10% FBS and antibiotics-penicillin 100 IU/mL and streptomycin 100 mg/mL, at 37^{0}C in 5% CO_2. Briefly, HT22 cells were seeded into 60 mm petri dishes, allowed to reach 80% confluence, and passaged. After obtaining a sufficient number of cells, they were then seeded in 6-well plates at a density of 2x10^5 cells/well. It was incubated at 37°C for 24 hours. HT22 differentiation was performed by previous literature [9], with a slight modification (Neurobasal medium containing 2 mmol/L-glutamine and 100xN2 supplement) for 72 h. HT22 cell proliferation, passages, and follow-up of cells were monitored with an inverted microscope. The number of undifferentiated samples used in the study was five and the number of differentiated samples was four. Differentiation medium: neurobasal medium, Pen-strep (1% v/v), L-glutamine (1% v/v), N2 supplement (1% v/v). Undifferentiation medium: DMEM, FBS (10% v/v), Pen-strep (1% v/v), L-glutamine (1% v/v)

Western blot procedure

HT22 cells were seeded at 2x10^5 cells/well in 6-well plates and incubated for 24h at 37^{0}C. Following the incubation, the cells were washed twice with phosphate-buffered saline

(PBS). The cells were scraped from the wells by using 500 µl of radioimmunoprecipitation assay buffer (RIPA) in the presence of a protein inhibitor cocktail (Santa Cruz Biotechnology, sc- 24948). Cell lysates were centrifuged for 12 min at 10000×g, 4˚C, and the supernatant was collected. Total protein extraction kit was used to determine the protein concentration. (DC protein assay-Bio-Rad). After denaturation with 2X Laemmli sample dilution buffer (1:1) for 5 minutes at 95^0C, 50 µg/µl of proteins were separated by 4-10% SDS-PAGE (Novex, Invitrogen) and transferred onto membranes (Invitrogen) using a wet transfer method (Bio-Rad Wet/Tank Blotting Systems). Membranes were blocked for 1 hour at room temperature by using 5% skim milk powder in PBS-Tween (PBST). Membranes were incubated at 4^0C overnight with the appropriate primary antibodies (1:1000) in PBST containing 5% skim milk powder. The membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:1000) (E-AB-1003) for 1 h at room temperature. After washing with PBST, SignalFire ECL Reagent (Cell Signaling) was placed on the membrane and specific binding was detected using a UVP Biospectrum chemiluminescence detection system (HR-410, USA). Specific bands were quantified using Image J studio software. Signals were normalized to the selected reference protein. Antibodies used in this study were as follows: IR (rabbit polyclonal, E-AB-60069, USA), β-actin (BTLab, BT-AP00213).

Statistical analysis

Data analysis was done with the GraphPad package program. Continuous variables were expressed as the mean±standard deviation (SD). The Shapiro-Wilk test was used to determine the suitability of the data for normal distribution. The Independent Samples T-test was used to compare the groups. In analyses, $p<0.05$ was considered statistically significant.

Results

Figure 1 shows 3-day images of HT22 cells in a normal growth medium and differentiation medium. In the differentiation medium, HT22 cells showed an increase in the number and length of cellular neurites, similar to that of mature neurons. Moreover, differentiated HT22 cells expressed significantly less IR levels than cells grown in a normal growth medium ($p=0.0028$, Figure 2).

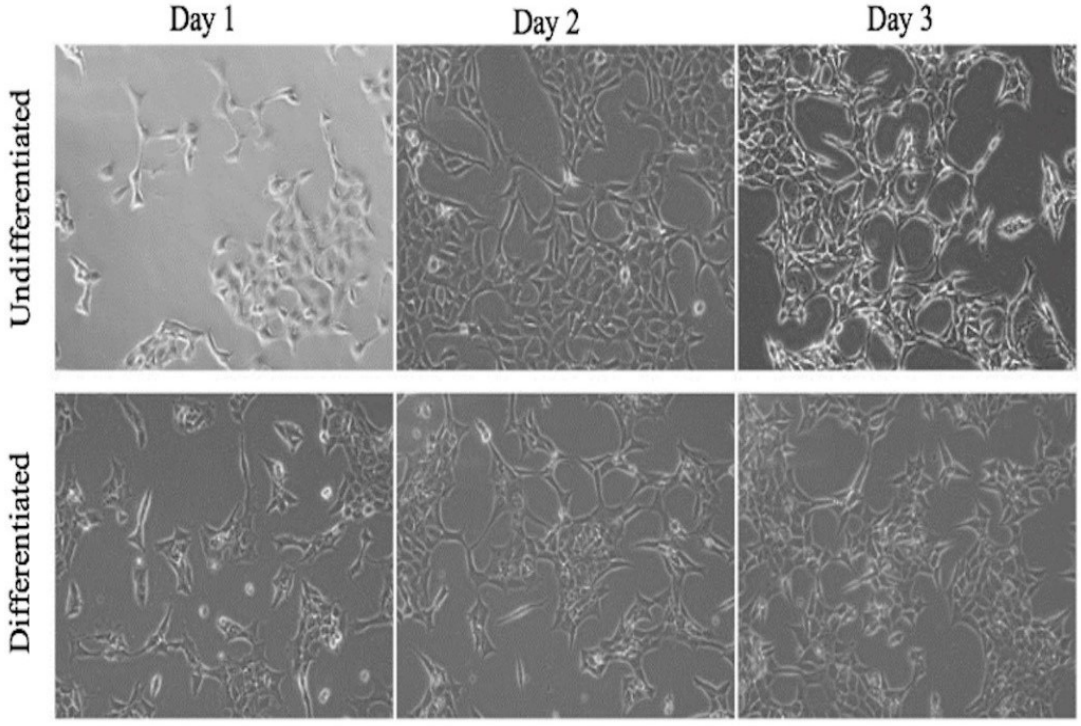

Figure 1. First three-day image of differentiated and undifferentiated mouse hippocampal HT22 cells

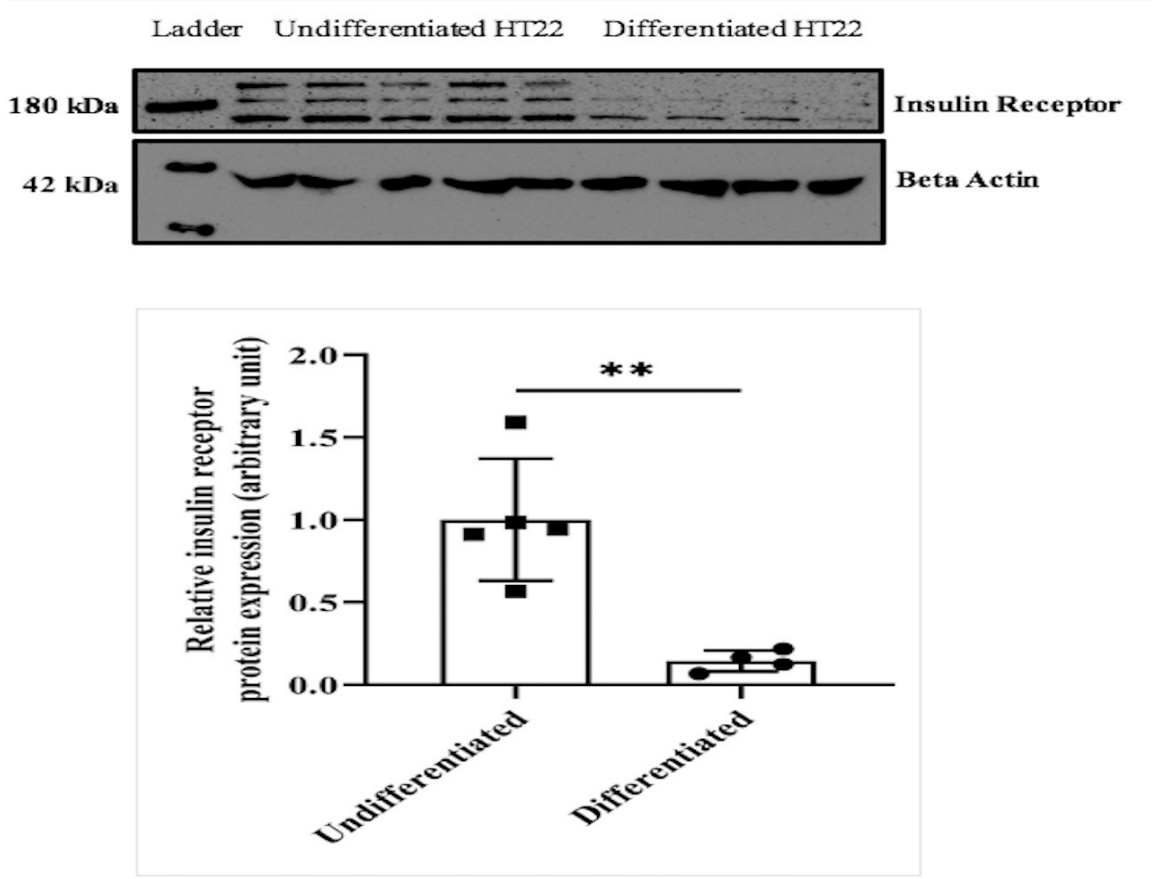

Figure 2. IR expression level in differentiated and undifferentiated HT22 cells after three days IR: insulin receptor

Discussion

In the brain, insulin facilitates differentiation, proliferation, and neuritis growth [10-12], while it also has a neuro-preservative role in preventing β-amyloid toxicity, oxidative stress, and damage caused by apoptosis. [13-16]. Conversely, these protective effects of insulin are diminished when brain insulin-receptor signaling and level are impaired [17], resulting in brain insulin resistance associated with cognitive dysfunction [18, 19].

When the literature is examined, numerous studies using different cell models and applications show that insulin and its receptors in the brain play key roles in biological functions [20-22]. One of the cell models used in these studies, HT22, is an immortalized mouse hippocampal cell line. It is an important research topic that the results of this cell line may change with their differentiation. In line with this information, we aimed to examine the IR level in differentiated HT22 cells using the neurobasal medium, which is a differentiation medium. Our results show that the differentiation of HT22 cells supports neurite growth and reduces IR levels. As far as we know, this is the first study to examine the level of IR in HT22 cells differentiated by the neurobasal medium.

Differentiation of neuronal cells in vivo has been reported to stimulate neurite outgrowth and promote up-regulation of NMDAR and choline acetyltransferase, thus displaying phenotypic features similar to those of mature hippocampal neurons [9]. Neural differentiation is achieved by complex cellular processes, including morphological changes. Maekawa et al. [23] in their study aiming to examine the pathogenesis of retinal ganglion cell diseases, observed enhanced neurite outgrowth after differentiation of mouse embryonic stem cells in neurobasal-A medium supplemented with B27 for 14 days. Yang et al. [24] aimed to

investigate the potential neurotrophic effects of a neurobasal medium containing glucagon like peptide-1 (GLP-1), retinoic acid, and B27 on neuronal differentiation. In this study, it was shown that retinoic acid and GLP-1 can trigger the morphological differentiation of SH-SY5Y cells. Consistent with the above results, we obtained an enhanced neurite morphology in HT22 cells differentiated using the neurobasal medium. N-2 supplement is provided as a 100X concentrate and is intended for use with neurobasal medium supplemented with growth factors such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). The differentiation of HT22 cells may be due to the growth factors present in the N-2 supplement added into the neurobasal medium. This result showed that our differentiation procedure was effectively accomplished.

Numerous studies examining the positive effect of insulin on neuronal cells emphasize the critical function of insulin stimulation and IR. When these studies are examined; Bassit et al. [25] showed that administration of insulin (10 nM, 24 hours) with human adipose tissue-derived stem cell exosomes after H_2O_2 treatment in undifferentiated HT22 cells increased proliferation in HT22 cells via protein kinase C delta (PKCδII). They also showed that it significantly enhances the relationship between metastatic-related pulmonary adenocarcinoma transcript 1 (MALAT1) and serine and arginine rich splicing factor 2 (SRSF2), which significantly supports cell vitality and proliferation of insulin in these cells. In another study, the positive effects of insulin (10 nM) on apoptosis induced by H_2O_2 (200 μM) in undifferentiated HT22 cells were investigated. In this study, it was shown that insulin administration inhibits apoptosis and upregulates pAKT, pGSK3β, and β-catenin protein expression levels. However, it was stated that these changes were abolished when IR in HT22 cells were silenced [26]. Another study examined the efficacy of the GLP-1 receptor agonist exendin-4 in activating insulin signaling and reducing tau phosphorylation in undifferentiated HT22 neuronal cells. In this study, insulin (100 nM) has been shown to play a fundamental role in exendin-4 eliciting these effects [20].

On the other hand, there are studies in the literature showing that real responses of the cell can be obtained by differentiation of these cells [27, 28]. Studies of differentiated neuronal cells have shown that these cells represent a better hippocampal neuron model than undifferentiated cells. For example, Lim et al. [9] showed that differentiation of HT22 cells (Neurobasal medium containing 2 mmol/ L-glutamine and. 5x N-2 supplement, 3 days), promotes neurite outgrowth and up-regulation of the NMDAR and choline acetyltransferase, as in primary cultured hippocampal neurons. In this study, proteins required for serotonergic neurotransmission were also upregulated in differentiated HT22 cells. With these findings, the authors showed that the differentiation of HT22 cells enhanced their functional serotonergic properties. Zhao et al. [29] investigated the responses of differentiated and undifferentiated HT22 cells to homocysteine toxicity. In this study, both cell models were exposed to homocysteine toxicity at different concentrations, and cell death was evaluated. It has been observed that undifferentiated and differentiated cells have different expression levels of the NMDA glutamate receptor. Our data showed a decrease in IR levels in differentiated HT22 cells.

In conclusion, although the data we obtained from this study indicate that the differentiation of HT22 cells negatively affects the level of the IR these cells will not be a suitable in vitro model for assessing the signaling mechanism of insulin. The lack of B27 supplementation in the neurobasal medium used in our study may be a limitation of this study.

This study has limitation. The results of the study are based on western blot results. These results need to be supported by other experiments (rPCR analysis).

Conflict of interest: The authors declare that they have no conflict of interest.

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Author contributions

Conceptualization, M.T.A. and F.A. Data curation M.T.A and F.A. Formal analysis F.A. Investigation M.T.A. and F.A. Methodology M.T.A. Supervision M.T.A. Validation, F.A. Visualization, F.A. Writing – original draft, M.T.A. Reviewing, M.T.A. and F.A. All authors have read and agree to the published version of the manuscript.

Research Article

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Effects of various conditions related to circadian rhythm disturbances on plasma and erythrocyte lipids in rats: a peroxisomal perspective

Sıçanlarda sirkadiyen ritim bozuklukları ile ilgili çeşitli koşulların plazma ve eritrosit lipidleri üzerindeki etkileri: peroksizomal bir bakış açısı

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Abstract

Purpose: Lipidomics studies of sleep restriction, which is known to be associated with circadian perturbations, revealed alterations in some plasma phospholipid levels including plasmalogens which are partly synthesized in liver peroxisomes. To this end it was aimed to investigate effects of various conditions known to cause circadian rhythm disturbances on various peroxisomal parameters and to compare those effects with that of fenofibrate, a peroxisome proliferator-activated receptor alpha agonist.

Materials and methods: Plasmalogens and some fatty acids in erythrocyte lysates were analyzed by GC. Peroxisomal metabolites including very long chain fatty acids as well as phytanic and pristanic acids in plasma were measured by GC-MS. Immunohistological analyses by catalase antibodies were conducted on liver sections.

Results: All the conditions tested exhibited increased catalase immunoreactivity in liver sections compared to that of controls. Both calorie restriction, time-restricted feeding, as well as fenofibrate treatment exhibited lower C18:0 plasmalogen contents of erythrocyte lysates. As plasmalogens are known to be synthesized by peroxisomes, the present results suggest that the peroxisomal lipid content in membranes might be affected by conditions co-occuring with circadian perturbations.

Conclusion: Shared effects of conditions associated with circadian rhythm disturbances and peroxisomal induction by fenofibrate on erythrocyte membrane lipids might indicate a link between them.

Keywords: Calorie restriction, circadian rhythm, fenofibrate, peroxisome, plasmalogen.

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Öz

Amaç: Lipidomik çalışmalar; sirkadiyen ritim bozukluklarıyla ilişkili olduğu bilinen uyku kısıtlamasının karaciğer peroksizomlarında sentezlenen plazmalojenler dahil olmak üzere bazı plazma fosfolipid düzeylerinde değişikliklere neden olduğunu ortaya koymuştur. Bu nedenle bu çalışmada, sirkadiyen ritim bozukluklarına neden olduğu bilinen çeşitli koşulların bazı peroksizomal parametreler üzerindeki etkilerinin araştırılması ve bu etkilerin, bir peroksizom proliferatör reseptör agonisti olan fenofibrat ile karşılaştırılması amaçlandı.

Gereç ve yöntem: Eritrosit lizatlarındaki plazmalojenler ve bazı yağ asitleri GC ile analiz edildi. Plazmadaki çok uzun zincirli yağ asitlerinin yanı sıra fitanik ve pristanik asitleri içeren peroksizomal metabolitler GC-MS ile ölçüldü. Karaciğer kesitlerinde katalaz antikorları ile immünohistolojik analizler gerçekleştirildi.

Bulgular: Test edilen tüm koşullar, kontrollere kıyasla karaciğer kesitlerinde artmış katalaz immünoreaktivitesi gösterdi. Hem kalori kısıtlaması, hem de zaman kısıtlamalı beslenme, ayrıca fenofibrat tedavisi, eritrosit lizatlarında daha düşük C18:0 plazmalojen içeriğini sergiledi. Plazmalogenlerin peroksizomlar tarafından sentezlendiği bilindiğinden, mevcut sonuçlar, eritrosit membranındaki peroksizomal lipit içeriğinin, sirkadiyen ritim bozukluklarından etkilenebileceğini göstermektedir.

Sonuç: Sirkadiyen ritim bozuklukları ve fenofibratın peroksizomal indüksiyonunun eritrosit membran lipidleri üzerindeki ortak etkileri, bunlar arasında bir bağlantı olduğunu göstermektedir.

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Anahtar kelimeler: Kalori kısıtlaması, sirkadiyen ritim, fenofibrat, peroksizom, plazmalojen.

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Introduction

Several studies highlighted the interrelatedness of sleep deprivation, metabolism and circadian disruptions. Seven plasmalogen species were reported to be raised in the rat under acute or chronic sleep restriction conditions. As the plasmalogens are partly synthesized in peroxisomes, the authors pointed out the induction of peroxisome proliferator-activated receptors and disruptions of the circadian clock [1]. However, effects of circadian rhythm disturbances caused by conditions other than sleep restriction have not yet been considered. Restricted feding [2, 3] calorie restriction [4], continuous light exposure [5, 6] metabolic parameters, circadian rhythm activity patterns, and behavior were observed in rats subjected to a 12-h/12-h light/dark cycle (LDand agonists of PPARs, such as fenofibrate [7] have all been reported to cause circadian arythmicity. Currently no reliable biomarker is available to be used in large scale to diagnose circadian disturbances and to monitor the effectiveness of the intervention strategies. To this end, restricted feeding, calorie restriction and continuous light exposure conditions known to interfere with clock rhythm mechanisms in rats were tested for their effect on some peroxisome-related parameters (e.g., plasmalogens, long-chain fatty acids, pristanic acid, and phytanic acid) in blood and liver tissue samples obtained at the termination of 2 weeks of exposure. In order to evaluate the effects of these conditions linked to circadian rhythm disturbances on peroxisomes as well as peroxisomal lipids and to consider their usage as biomarkers. An additional approach was implemented in which rats were fed with fenofibrate (a PPARα agonist) supplemented feeds in order to provoke peroxisomes. Then the results were compared with those of circadian rhythm disturbances tested.

Materials and methods

Experimental design

Seventy five Sprague Drawley male rats weighing 360-380 g were obtained from Inonu University Laboratory Animals Research Center. Inonu University Scientific Ethical Committee on Animal Experimentation approved the study protocol. The animals' care, experimental procedures were carried out by the National Institutes of Health Animal Research Guidelines and ARRIVE guidelines [8]. During the adjustment period for one week, the rats were assigned to five groups, 15 of each, and kept ar 21-22^{o}C under 12:12 hours light-dark cycle and allowed free access to standard rat chow pellet before starting the experiment. Following this period, in the case of time restricted feeding group (TRF), the rats were allowed access to food (rat chow pellet) only between 8:00 a.m. and 11:00 a.m. for two weeks. The rats were allowed daily access to 60% of their normal daily calorie consumption starting at 08:00 am every day in the case of calorie restricted feeding (CRF) experimental condition. Rats in continuous light exposure (CLE) group were kept under continuous light. Additionally, another fifteen of rats were fed with chow pellet contaning 0.1% fenofibrate (Lipanthyl, Reciparm Fontaine or Alembic Pharmaceuticals) (FSD: Fenofibrate Supplemented Diet) for two weeks. Finally, control groups (CTR) were kept under the conditions same as described for the adjustment period. Body weight of rats exposed to various experimental conditions for two weeks were recorded at the commencement and cessation of the experimental procedures.

Collection of the samples

Blood samples obtained from the bifurcation of the femoral artery were collected in tubes containing EDTA. Then the samples were centrifuged at 3.000 rpm for 10 min. at 15^{0}C. The resultant plasma was kept at -80^{0}C until analysed. For plasmalogen analysis, the erythrocyte pellet was washed with an equal volume of saline (0.9% NaCl). The pellets were placed in an Eppendorf tube containing 100µL of 1% butylated hydroxytoluene (BHT) in ethanol dried under the nitrogen stream and kept at -80^{o}C until analysed.

Histopathological analyses

2-3 mm thick liver specimens were taken from the same lobe of the rat liver were fixed in 10% formalin and was embedded in paraffin. Tissue sections were cut at 4μm, mounted on slides, stained with hematoxylin-eosin (H-E) for general liver structure. Hydropic changes in the liver was assessed in 10 randomly selected fields on each section. Alterations in structure were evaluated using a histopathological score as follows: 0, normal; 1, mild; 2, moderate; 3, severe [9].

Immunohistological analyses

Briefly, sections were blocked with 0.3% hydrogen peroxide and incubated with primary catalase antibodies. The sections were then incubated with a biotinylated secondary antibody followed by streptavidin peroxidase and chromogenic substrate AEC. Tissue sections were counterstained with hematoxylin. According to the diffuseness of the staining, the sections were graded as 1=0-25% staining; 2=25-50% staining; 3=staining 51-75%; 4=staining 76-100%. According to the staining intensity, the sections were graded as follows: 0=no staining; 1=weak but detectable staining; 2=distinct; 3=intense staining. Total staining score was obtained as (diffuseness)X(intensity) [10].

Analyses of C16:0 and C18:0 plasmalogens, arachidonic acid (AA) and docosahexaenoic acid (DHA) content in erythrocyte lysates

By adding 3N methanolic HCl to erythrocyte lysates and heating the mixture at 90°C for 4 h, fatty acid glycerol esters are transmethylated resulting in the formation of fatty acid methyl esters whereas the alkyl-1-enyl ether linkage in plasmalogens is cleaved with acidified methanol, leading to the quantitative formation of the fatty aldehyde dimethyl acetals. After cooling the sample, the fatty acid methyl esters and the dimethylacetals are extracted with hexane. Then 1 μl of the resulting hexane solution was injected to GC. 18-methyl-C19:0 was used as the internal standart. The instrumental configuration and analytical conditions were summarized in the following; Shimadzu 2010 GC-FID instrument equipped with a RT-2560 capillary column (100 m x 0.25 mm x 0.20 μm, RESTEK Scientific) and FID Detector. AA and DHA content were expressed as percentages of total fatty acid methyl esters in erythrocyte lysates. The plasmalogen values were not expressed in absolute values, but as a percentage of the level of the corresponding fatty acid. Hence, the C16:0 dimethylacetal is compared with the C16:0 fatty acid methylester, the C18:0 dimethylacetal with the C18:0 fatty acid methyl ester [11].

Very long chain fatty acids, pristanic acid and phytanic acid analyses in plasma

Following alkaline and acid hydrolysis, plasma very long chain free fatty acids, phytanic and pristanic acids extracted with hexane were derivatized with N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide and 1% tert-butyldimethylchlorosilane to tertiarybutyl-dimethylsilyl derivatives. The resultant sample dissolved in hexane were injected into GC-MS (Agilent 6850 GC/Agilent 5977E MS) equipped with a column (Agilent HP5ms, 30 m x 0.25 mm x 0.25 μ). These analyses were conducted in Synlab Türkiye-Laboratory (Ankara/Türkiye).

Quantitative analysis of plasma triacylglycerol

The analysis was carried out spectrophotometrically by Abbott Triacylglyceride Kit via Abbott Architect c16000 automatic analyzer according to the manufacturer's instruction.

Statistical analyses

The sample size of this study was determined by power analysis, G*power 3.1 program. By the priori sample size calculation, the required minimum sample size was calculated as 13 per group for the effect size=0.45 (large) at 95% confidence level (α=0.05) and 80% power (β=0.20). R version 3.5.0 and IBM SPSS Statistics 22.0 software were used for the statistical analyses of triacylglycerol, VLCFA, plasmalogen, immunohistochemical analysis and the rats' body weight measurements. The data were summarized using median, minimum value, maximum value and interquartile range (IQR) statistics. Shapiro-Wilk test was used to determine whether or not the data fit the normal distribution. Kruskal-Wallis H test was used for comparisons between independent groups. Multiple comparison tests were performed with Conover test. Wilcoxon test was used for comparisons between dependent groups. $P<0.05$ was considered to be statistically significant level.

Results

Body weight changes in the rats

Body weight changes among experimental groups were compared. The calorie restricted group lost average 9.6% of their body weight at the end of the experimental period. The decrease was found to be significant. Body weight changes recorded in other groups were found to be insignificant.

Effects of experimental conditions influencing circadian rhythm on some blood lipid parameters

Plasma triacylglycerol levels

In plasma of rats fed on diet supplemented with 0.1% fenofibrate, which is known for its lipid lowering effect, average triacylglycerol concentration was found to be decreased by 66% compared to that of average control values. Whereas calorie restriction in rats resulted in a 45% decrease in average plasma triacylglycerol level compared to the control values. Plasma triacylglycerol levels of TRF and CLE were also lower compared to controls albeit insignificant.

Erythrocyte lysate C16:0 and C18:0 plasmalogen levels and long chain PUFA compositions

The chromatogram of fatty acids and C16:0 and C18:0 plasmalogen is depicted in Figure 1. The percentage C16:0 plasmalogen level in erythrocyte haemolysates of FSD rats was found to be slightly lower compared to that of control values. Whereas plasmalogen levels in other groups were similar to that of control values. In the case of percentage C18 plasmalogen levels in erythrocyte lysates, all but TRF group exhibited significantly lower level in comparison to that of control group. The decrease in all the groups varied from 6% to 10%. FSD and CRF groups showed highest level of decrease in erythrocyte plasmalogen level (Figure 2). AA (C20:4 (n-6)) and DHA (22:6 (n-3) percentages in erythrocyte haemolysate were found to be significantly decreased by 11% and 35% in FSD group respectively compared to that of control group. Both fatty acids were also lower in CRF group however in the case of DHA, the difference was not significant (Figure 2).

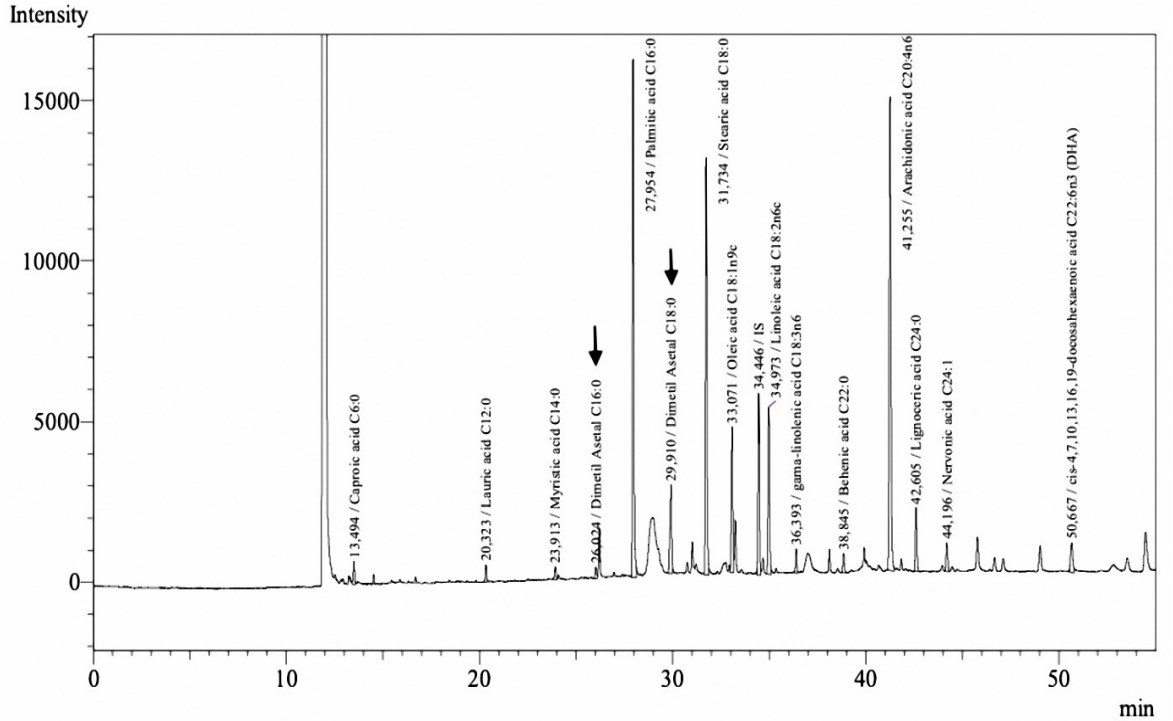

Figure 1. GC chromatogram overlay of fatty acid methyl esters and plasmalogen dimethylacetals in erythrocyte lysates

The plasmalogens can be distinguished next to their corresponding fatty acid methyl esters. Arrows indicate C16:0 or C18:0 plasmalogen dimethylacetals

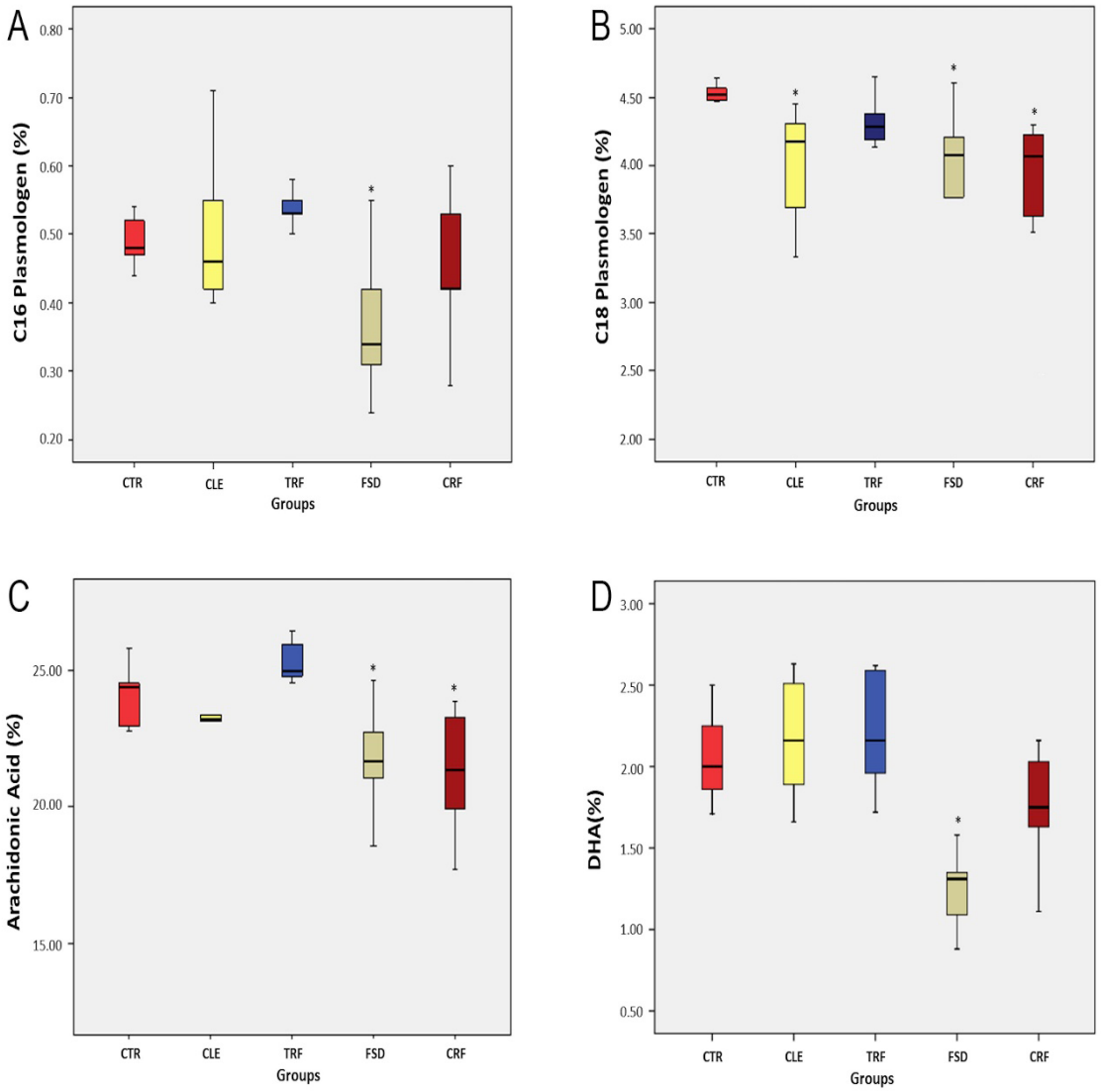

Figure 2. Effects of various experimental conditions associated with circadian disturbances on erythrocyte plasmalogens, arachidonic acid and docosahexaenoic contents

Effects of circadian disturbances on erythrocyte C16:0 plasmalogen content (A), on C18:0 plasmalogen content (B), on arachidonic acid content (C) and on docosahexaenoic acid (DHA) (D). The plasmalogen levels are expressed as a percentage of the level of the corresponding fatty acid. The results of arachidonic acid and docosahexaenoic acid compositions are expressed as percentages of total fatty acid methyl esters. Error bars represent median (n=10), *p<0.05 compared with CTR group

Plasma concentrations of very long chain and branched chain fatty acids

Comparison of C22:0, C24:0 and C26:0 very long chain fatty acids concentrations made among groups or between control group and either of the experimental groups indicated no significant difference (Table 1). However slightly higher C22:0 and C24:0 levels (2-3 nmol/L) was discernable in CRF group compared to the others. In the case of branched chain fatty acids, calorie restriction produced higher level of plasma phytanic acid concentrations whereas fenofibrate treatment yielded lower concentrations compared to the average control value. However, plasma pristanic acid concentrations were found to be similar among all the groups (Table 1).

Table 1. Levels of plasma very long chain fatty acids, pristanic acid and phytanic acid in various circadian disorder conditions of rats

		nmol/mL				
	Group	**Median**	**Minimum**	**Maximum**	**IQR**	***p***
C22:0	**CTR**	6.73	5.34	10.01	1.95	**0.22**
	CLE	7.37	3.12	15.30	6.27	
	TRF	5.92	3.87	10.22	4.28	
	FSD	6.17	3.12	10.60	4.60	
	CRF	8.51	5.72	12.56	4.21	
C24:0	**CTR**	15.28	9.32	21.37	3.50	**0.275**
	CLE	17.3	4.84	29.30	10.9	
	TRF	14.74	9.59	22.46	8.61	
	FSD	13.14	6.76	26.30	9.26	
	CRF	18.13	13.36	27.05	9.17	
C26:0	**CTR**	0.07	0.05	0.10	0.02	**0.49**
	CLE	0.06	0.05	0.10	0.03	
	TRF	0.07	0.05	0.60	0.02	
	FSD	0.06	0.05	0.61	0.02	
	CRF	0.06	0.04	0.09	0.02	
Phytanic Acid	**CTR**	0.44	0.32	0.59	0.12	**0.0001**
	CLE	0.48	0.26	0.96	0.30	
	TRF	0.43	0.29	0.71	0.16	
	FSD*	0.22	0.12	0.36	0.13	
	CRF*	0.58	0.42	0.72	0.16	
Phytanic Acid	**CTR**	6.46	4.76	11.50	3.41	**0.571**
	CLE	6.45	3.05	11.38	3.23	
	TRF	5.59	4.5	9.42	2.88	
	FSD	5.49	3.16	11.10	3.50	
	CRF	5.97	5.15	9.54	1.64	

Data are presented as median, minimum, maximum and IQR. Significance of differences compared with CTR group was indicated as $^{*}p<0.05$
n=12 for each group

Morphology of the liver

Hydropic changes were assessed in 10 randomly selected fields in each liver section and scored (Table 2). The liver sections of CTR group were normal in their histological appearance. Hepatocyte cordons were radially organized around the central vein in an orderly way. Sinusoids associated with hepatocyte cordons were open. In portal areas around liver lobules, arterial, venal and bile canalicular structures were prominently observed. Hepatocytes displayed an eosinophilic cytoplasm with round euchromatic nucleus (Figure 3). Hepatocytes of CRF and TRF groups however, showed marked hydropic changes (p=0.001) (Figure 3.B and C), whereas hydropic changes in that of CLE group was less noticeable. These changes were found to be significant in comparison to histological findings of control liver sections. Fenofibrate supplementation produced no noticable changes in hepatocyte histology. Histopathologic scoring of hydropic changes can be seen in Figure 3.

Table 2. Hydropic changes in hepatocytes and catalase immunoreactivity score results

Groups	Hydropic Changes			Catalase Immunreactivity		
	Median	Minimum	Maximum	Median	Minumum	Maximum
CTR	0.0	0.0	0.0	8.0	4.0	12.0
CLE [a,b]	1.0	0.0	3.0	10.0	4.0	12.0
TRF [a,b]	3.0	0.0	3.0	12.0	4.0	16.0
FSD [b]	0.0	0.0	0.0	3.0	1.0	6.0
CRF [a,b]	2.5	1.0	3.0	12.0	12.0	16.0

Results are expressed as minimum, maximum and median. Total staining score were used as an indication for catalase concentration
The scores are displayed in the table below the pictures, [b]$p \leq 0.001$, compared with CTR group (n:10 for each group)
Scores of hydropic changes can be seen in the [a]$p \leq 0.001$, compared to CTR group

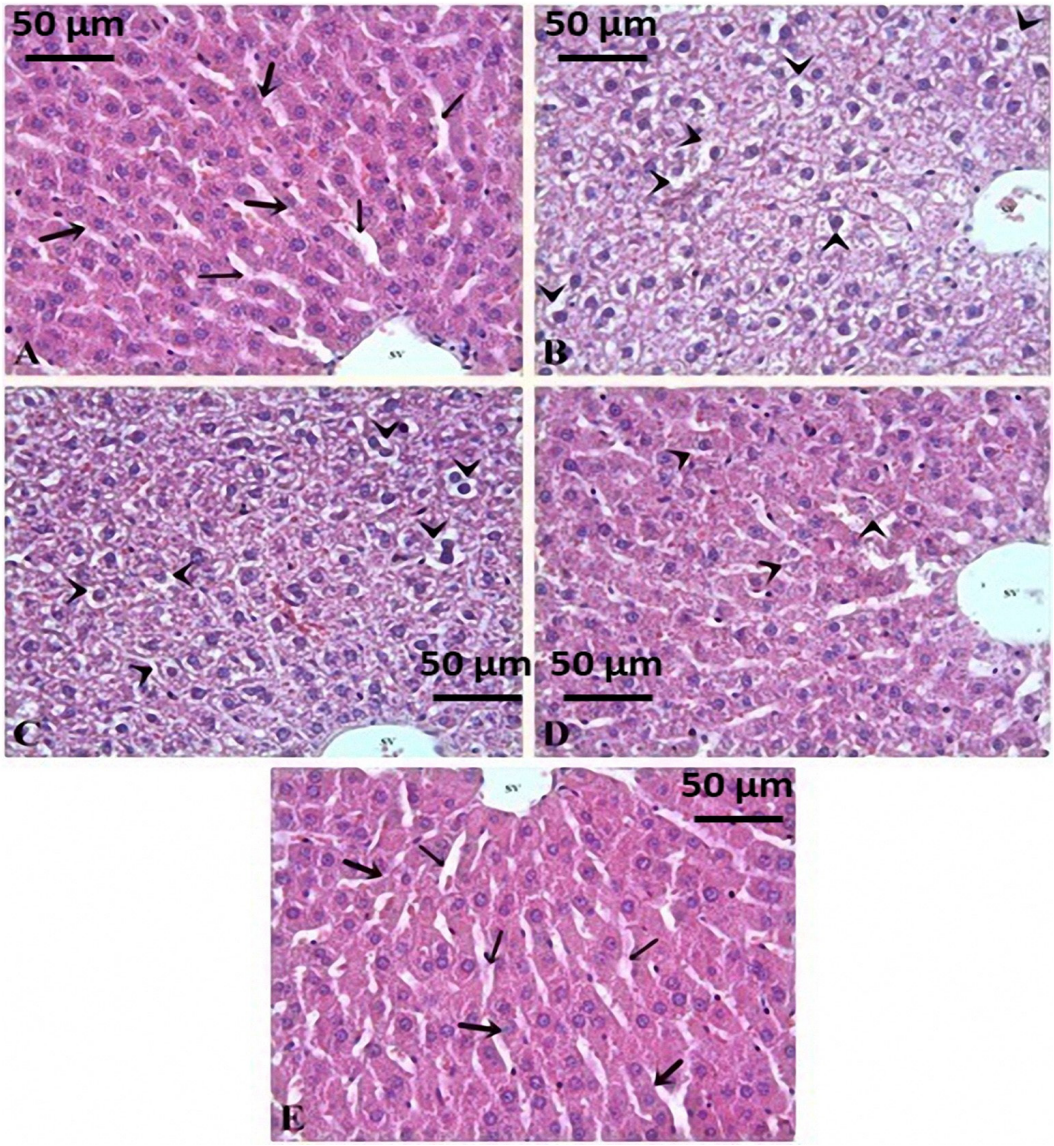

Figure 3. Histopathological picture of H&E-stained sections and scores of hydropic changes in rat livers exposed to various conditions of circadian disruptions

Hydropic changes was assessed in randomly selected 10 fields and scored. (A), A nearly normal liver architecture with CTR group. Various degrees of hydropic changes can be discerned in CRF (B), TRF (C) and CLE (D) groups. Histological features of the liver from FSD group (E) was similar to that of CTR group. SV: Central vein, thick arrows indicate hepatocyte cordons, thin arrows point sinusoids, arrowheads indicate hydropic changes ($p \leq 0.001$, compared with CTR group, n=10 for each group; magnification: x40)

Immunoreactivity of catalase in liver tissue sections of rats

The highest catalase immunoreactivity was observed in liver sections of CRF group whereas the lowest in that of fenofibrate supplemented group. Catalase immunoreactivity of control group had homogeneous distrubition throughout the liver section and was scored as 8 (4.0-12.0) (Table 2). Average catalase scores of CRF, TRF and CLE groups were found to be 12.0, 12.0 and 10.0 respectively being significantly higher as compared to that of control group. As in the case of control group, catalase immunoreactivity in liver sections of these groups exhibited a uniform distribution (Figure 4). On the other hand, heterogenous distrubition of catalase immunoreactivity in liver sections from fenofibrate supplemented group was observed (scored 3.0). The treatment with fibrates induced stronger proliferation of peroxisomes in zone 3 (pericentral hepatocytes) than in zone 1 hepatocytes. Since 10 randomly selected fields on each section was assessed according to the staining intensity, average catalase score of the selected fields became lower than it actually was in fenofibrate treated liver sections.

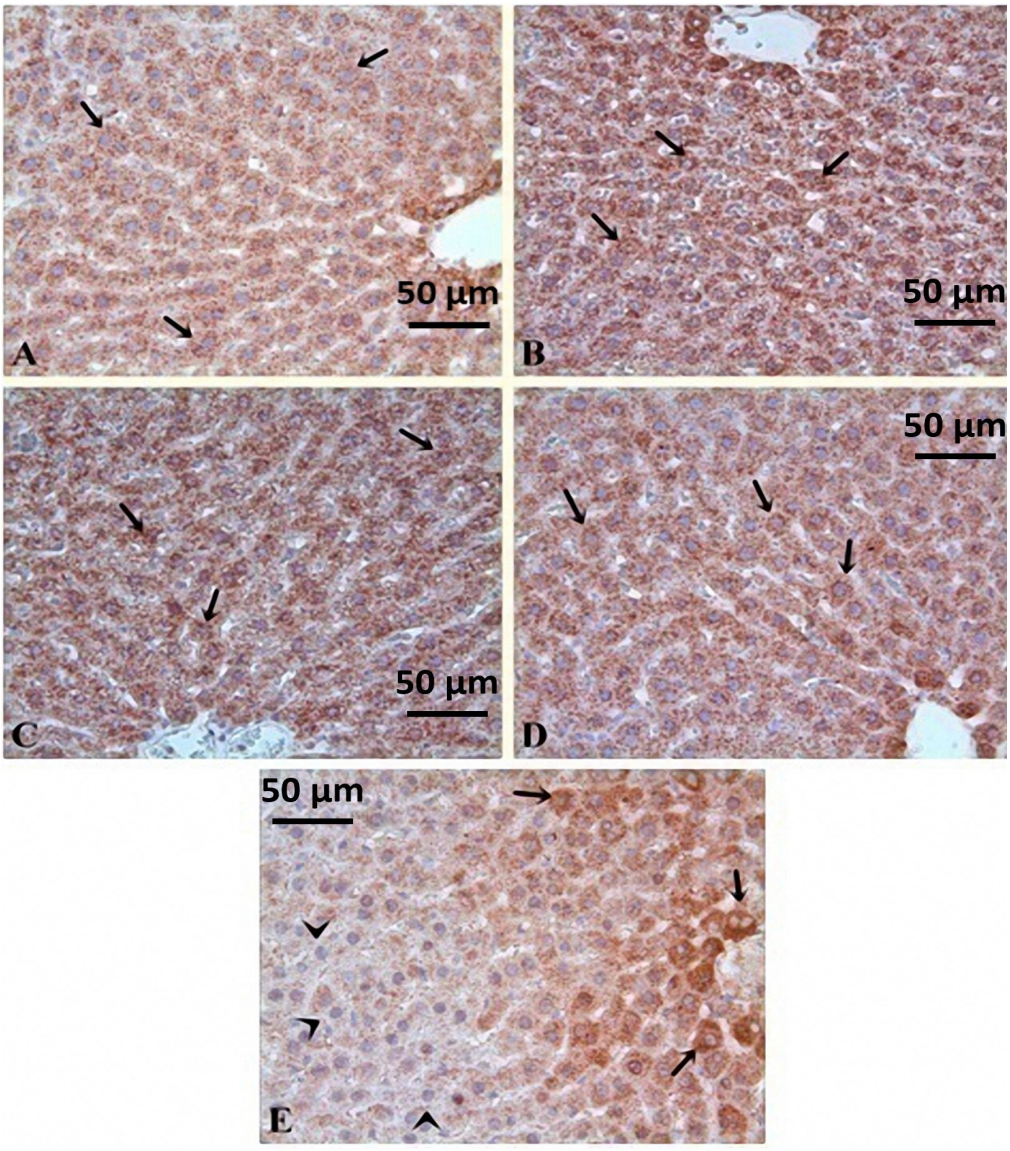

Figure 4. Immunohistochemical reactivity for catalase in livers of rats subjected to various circadian perturbations

Catalase immunoreactivity of CRF (B), TRF (C) and CLE (D) were found to be higher compared to that of the CTR group. These groups and (A) also showed uniform distribution of catalase immunoreactivity. Heterogenous distribution of catalase immunureactivity in liver sections from FSD group are discernable (E) which were more prominent in zone 3. Arrows indicate catalase immunoreactivity. Arrowheads point hepatocytes without catalase immunoreactivity. Total staining score were used as an indication for catalase concentration ($p \leq 0.001$ compared with CTR, n=10 for each group; magnification: x40)

Discussion

Lipids with ether linkage are plasmenyl (plasmalogens) glycerophospholipids that compose approximately 20% of the mammalian total phospholipid pool. Plasmalogens are enriched of AA and DHA in sn-2 position and generally either C16:0 or C18:0 fatty acid is linked to sn-1 position [12, 13]. In the current study, percentage of C18:0 plasmalogen level in erythrocyte lysates were found to be lower in all but TRF group. Lower level of C16:0 plasmalogen level was also detected in FSD group. However, in humans, circadian rhythm disturbances induced by sleep restriction, higher plasma plasmalogen levels were reported [1]. We measured C16:0 and C18:0 plasmalogen levels in erythrocyte lysates representing erythrocyte membranes. Whereas Weljie et al. [1] (2015) measured phospholipids including plasmalogens in human serum. Authors put forward possibility that a source for the elevated phospholipids could be membrane breakdown or release from circulating lipoprotein particles. Accordingly, the discrepancy between our results and that of Weljie [1] can be accounted for by the fact that the release of membrane phospholipids induced by circadian disturbances into serum can cause an increase in serum plasmalogen level and a decrease in membrane plasmalogen level. This possibility requires further research. On the other hand, several plasma plasmalogens were reported to decrease with acute calorie restriction in humans [14]. This discrepancy could be attributed to numerous situations that can affect plasmalogen levels one of which reported to decrease plasmalogen level is lipid oxidation. Plasmalogens are highly susceptible to oxidation [15] and are consumed in this reaction [16]. It is also possible that erythrocyte membrane plasmalogen levels might have been decreased by increased myeloperoxidase which was reported to react with vinyl ether bond of cellular plasmalogens by its ROS [16, 17].

Biochemically, various peroxisome dependent parameters are abnormal in peroxisome deficient mice which include accumulation of VLCFA (because of impaired VLCFA oxidation), lack of plasmalogen, accumulation of phytanic acid, lower DHA in erythrocytes [18]. In peroxisomal biogenesis disorders, VLCFAs accumulate, demonstrating the indispensable nature of peroxisomes in oxidizing these substrates [19]. In our study, none of the conditions of circadian rhythm disturbances tested led to significant variations in plasma levels of very long chain fatty acids (C22:0, C24:0 and C26:0). It is well established that catabolism of phytanic acid, a branched-chain fatty acid, by alpha-oxidation in peroxisomes yields pristanic acid [18]. In the current work, calorie restriction produced higher levels of plasma phytanic acid. This might suggest a slowing in α-oxidation of peroxisomes due to calorie restriction. On the other hand, fenofibrate supplementation resulted in lower plasma levels of phytanic acid and triacylglycerol levels indicating occurence of peroxisomal induction by fenofibrates as expected. Overall our data points out that calorie restriction might lower peroxisomal oxidation whereas fenofibrates enhance it. Calorie restriction and fenofibrates appear to have diverse effects on α-oxidation in peroxisomes.

Hepatocytes of CRF and TRF groups showed marked hydropic changes, whereas hydropic changes in that of CLE group was less pronounced. It is possible that excess lipolysis resulting from calorie restriction might have caused lipotoxicity [20-22] and subsequent hydropic changes in liver sections. On the contrary, fenofibrate supplementation produced no noticable hydropic changes in liver tissue sections. PPAR alpha agonists are known to suppress inflammatory events via the NF-κB pathway and inhibit ROS production [23, 24], Therefore, it is possible that damaging effect of hydrogen peroxide by-product of peroxisomal oxidation activity might have been served as an offset through antioxidant and antiinflammatory action of fibrates.

The most highly expressed and best characterized peroxisomal antioxidant enzyme is catalase [25]. Catalase activity is largely or completely located in peroxisomes [26]. Calorie restriction or body weight loss have been reported to be associated with enhanced catalase activity [23, 27, 28]. Increased peroxisomal β-oxidation and as a result, increased production of hydrogen peroxide at peroxisomal acyl CoA oxidase stage might have been the cause of high uniform catalase activity seen in the experimental groups other than FSD in our work. It has been reported that treatment with fibrates induced stronger proliferation

of peroxisomes in zone 3 rather than in zone 1 hepatocytes [29] which is in line with our findings in FSD group indicating heterogenously increased catalase immunoreactivity in liver sections. It is known that CRF increased the expression of the genes which involve in fatty acid metabolism and PPAR signalling pathway [30]. It is possible that excess lipolysis resulting from calorie restriction might have caused lipotoxicity [20-22] and subsequent hydropic changes in liver sections. On the contrary it is reported that TRF may attenuate the lipid homoeostasis in liver by regulating the clock components [31]. Moreover, fenofibrate supplementation produced no noticable hydropic changes in liver tissue sections.

The present results might imply that altered feeding regiments and fenofibrates might influence different subpopulation of peroxisomes. Both calorie restriction and fenofibrate treatments were reported to have induced changes in fatty acid compositions in tissues. Some workers reported a decrease in relative amount of arachidonic acid in membrane phospholipids [32, 33]. On the other hand, treatment with fibrates was reported to induce shifts in fatty acid composition [34] including EPA and DHA contents in tissues [35]. Our findings also indicate lower arachidonic acid and DHA contents in erythrocytes of FSD and CRD group. Arachidonic acid is the major essential fatty acid component of membrane phospholipids. Enzymatic breakdown of arachidonic acid yields a variety of bioactive lipid molecules that have diverse physiological roles in many cellular processes [36] which might be mediators in metabolic disorders with concurrent circadian disruptions.

Consequently, some common effects of the various conditions co-occuring with circadian rhythm disturbances and fenofibrate inducement on the C-18 plasmalogen contents in erythrocyte membranes and on catalase activity in liver sections might point out a link between peroxisomal function and circadian rhythm disturbances. Evaluation of peroxisomal lipids in erythrocyte membranes could be a new tool for the diagnosis of circadian rhythm disturbances.

In the present study, the effect of acute circadian rhythm disturbances on membrane lipid composition was examined and the short duration of the application period was a limitation in the evaluation of the results. The short-term rhythm disturbance model did not produce significant changes on the erythrocyte membrane, and the chronic rhythm disturbance model may give more meaningful results in determining the effect of long-term rhythm disturbances. In addition, since circadian rhythm shows individual differences, we believe that comparisons within individuals will give more accurate results rather than evaluating on a group basis.

Conflict of interest: No conflict of interest was declared by the authors.

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Author contributions

T.G. wrote manuscript and performed data acquisition. H.G.O. performed the experiments and acquisition of the data. S.E., Y.U., A.Y. and N.V. performed part of the experiments and methodology. S.Y. carried out statistical analyses. All authors read and approved the final manuscript.

Research Article

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Is high body mass index a factor that increases postoperative hemorrhage and bile complication rates in right lobe living donor liver transplant recipients?

Yüksek vücut kitle indeksi, sağ lob canlı donörden karaciğer nakli alıcılarında postoperatif kanama ve safra komplikasyon oranlarını artıran bir faktör mü?

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Abstract

Purpose: High Body Mass Index emerges as an important perioperative and postoperative risk factor for liver transplantation. We aimed to examine the effect of increased BMI on perioperative follow-up parameters and per-postoperative complications in liver transplant recipients.

Materials and methods: One hundred and seventy-two patients who underwent living-donor liver transplantation for end stage liver cirrhosis were included in the study. Whether there was a difference between cold ischemia time, operation time, blood product transfusion rates, hospital, biliary complications, hepatic vein thrombosis, portal vein thrombosis, NASH etiology, postoperative hemorrhage, sepsis, and primary graft dysfunction were analyzed statistically in terms of those with a BMI of 25 and above and those below 25.

Results: Anhepatic phase duration (p=0.047) and cold ischemia duration (p=0.009) were statistically longer in patients with BMI >25.

Conclusion: Prolonged anhepatic phase and cold ischemia times may be important in terms of the effect of increased BMI on postoperative graft survival.

Keywords: Obesity, body mass index, liver, transplantation, complication.

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Öz

Amaç: Yüksek Vücut Kitle İndeksi, karaciğer transplantasyonu için önemli bir perioperatif ve postoperatif risk faktörü olarak ortaya çıkmaktadır. Karaciğer transplantasyonu alıcılarında artmış BMI'nin perioperatif takip parametreleri ve postoperatif komplikasyonlar üzerine etkisini incelemeyi amaçladık.

Gereç ve yöntem: Son dönem karaciğer sirozu nedeniyle canlı vericiden karaciğer nakli yapılan 172 hasta çalışmaya dahil edildi. Bunlara göre soğuk iskemi süresi, operasyon süresi, kan ürünü transfüzyon oranları, hastane yatış süresi, safra komplikasyonları, hepatik ven trombozu, portal ven trombozu, NASH etiyolojisi, postoperatif kanama, sepsis ve primer greft disfonksiyonu arasında fark olup olmadığı BMI'sı 25 ve üzeri olan ve 25'in altında olan kişiler açısından istatistiksel olarak analiz edildi.

Bulgular: BMI >25 olan hastalarda anhepatik faz süresi (p=0,047) ve soğuk iskemi süresi (p=0,009) istatistiksel olarak daha uzundu.

Sonuç: Uzamış anhepatik faz ve soğuk iskemi süreleri, artan vücut kitle indeksinin postoperatif greft sağ kalımına etkisi açısından önemli olabilir.

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Anahtar kelimeler: Obezite, vücut kitle indeksi, karaciğer, transplantasyon, komplikasyon.

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Introduction

High Body Mass Index (BMI) and obesity are among the most important risk factors of our age in terms of chronic diseases, but they are also important causes that increase morbidity and mortality during or after surgery. Again, it emerges as an important perioperative and postoperative risk factor for liver transplantation (LT), which is one of the major surgical operations [1]. Factors such as increased operative time and implantation difficulties of the graft, prolongation of organ ischemia times, and risk of embolism in large vessels may adversely affect the surgery. In addition, complications such as thrombosis in large organ vessels, small for size syndrome (SFSS) and graft deficiencies that may occur in the postoperative period are also observed as conditions that obesity might cause [2, 3]. In our study, we examined the effect of increased BMI on perioperative follow-up parameters and per-postoperative complications in liver transplant recipients.

Materials and methods

Collected LT database was retrospectively reviewed. One hundred and seventy-two patients who underwent living-donor liver transplantation (LDLT) for end stage liver cirrhosis between July 2021 and July 2023 were included in the study. Demographic data, gender, age, MELD, Child score, (non-alcoholic steatohepatitis) NASH etiology rates were analyzed. Anhepatic phase, cold ischemia time, operation time, blood products transfusion rates were analyzed as perioperative anesthesia findings. The patients were separated and examined in terms of BMI. At first, those with a BMI below 18.5 were evaluated as underweight, those with 18.5-24.9 as normal weight, those with 25-29.9 as overweight, those with 30-34.9 as obese, and those 35 and over as severely obese. In order to compare the under/normal weight and overweight/obese patients in terms of perioperative anesthesia findings and postoperative complications, the recipients were subsequently divided into those with a BMI of 24.9 and below and those of 25 and above. Whether there was a difference between cold ischemia time, operation time, and blood product transfusion rates was statistically analyzed in terms of these BMI groups. Also, hospital stay was statistically analyzed in terms of BMI groups. Biliary complications, hepatic vein thrombosis, portal vein thrombosis, NASH etiology, postoperative hemorrhage, sepsis, and primary graft dysfunction were analyzed statistically in terms of those with a BMI of 25 and above and those below 25. The patients were informed about the study and their consent forms were obtained. All procedures were conducted in accordance with the ethical standards of the committees concerned with human experimentation (institutional and national) and the 1964 Declaration of Helsinki and its later editions. This study was approved by the Istanbul Aydın University Human Experiments Ethics Committee with an Ethics Committee with decision number on date.

Statistical analysis

Nominal and ordinal parameters were described with frequency analysis, whereas scale parameters were described with means and standard deviations. Chi-Square Test and Chi-Square Likelihood tests were used for differences between categorical parameters. Kolmogorov-Smirnov test was used for normality of scale parameters. Mann-Whitney U test was used for difference analysis, since distributions were non-normal. Spearman's rho correlation and Cox Regression tests were used for relational analysis. SPSS 17.0 for Windows was used at 95% Confidence Interval. When referring to SPSS versions prior to the IBM acquisition, authors should cite 'SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, 3., USA).

Results

The mean age was 53.7 (range: 18-78 years). 44% of the patients were female and 56% were male. The mean weight was 76, the mean height was 166 cm. The rate of A blood group was 45%, the rate of group B was 20%, the rate of 0 group was 28%, and the rate of AB group was 7%. 30% of the patients were Child A, 38% Child B,

and 32% were Child C. The mean MELD score of adult patients was 16.2. NASH etiology rate was 19%. The mean anhepatic phase duration was 89 minutes, and the mean cold ischemia time was 68 minutes. The mean operative time was 468 minutes, and the mean hospital stay was 14.9 days. In patients with BMI <25, mean anhepatic phase duration was 82.3 minutes, cold ischemia time was 60.1 minutes, operation time was 459.1 minutes, and hospitalization time was 14.2 days; In patients with BMI >25, the mean anhepatic phase duration was 93.2 minutes, cold ischemia time was 73.7 minutes, operation time was 472.2 minutes, and hospitalization time was 15.3 days. Anhepatic phase duration (p=0.047) and cold ischemia duration (p=0.009) were statistically longer in patients with BMI >25 (Table 1). Liver cirrhosis due to NASH was statistically more common in those with BMI >25 (p=0.001). There was no statistically significant difference between those with BMI >25 and BMI <25 in terms of perioperative blood product transfusion (p=0.646), biliary complications (p=0.556), portal vein (p=0.422) and hepatic vein (p=0.169) thrombosis/occlusions, postoperative hemorrhage (p=0553), sepsis (p=0.590), and primary graft failure (p=0.577) (Table 2). Postoperative hepatic artery thrombosis or insufficiency was not detected in any recipient. In addition, in the statistical evaluation of underweight, healthy weight, overweight, obese and severely obese recipients, no significant difference was observed in terms of perioperative findings, hospital stay and complications (p>0.05).

Table 1. Ratio and statistical results of age, length of hospital stay and peroperative phase duration in patients with BMI below 25 and 25 and above

	BMI <25	**BMI ≥25**	***P* value**
Anhepatic phase (minute)	82.3	93.2	**0.047***
Cold ischemia time (minute)	60.1	73.7	**0.009***
Hospital stay (day)	14.2	15.3	0.125*
Operation time (minute)	459.1	472.2	0.324*

BMI: body mass index, *Chi-Square Test and Chi-Square Likelihood tests

Table 2. Statistical results of etiology, perioperative blood product and postoperative complications in patients with BMI Below 25 and 25 and above

	BMI <25	**BMI ≥25**	***P* value**
Etiology			
• NASH	3.8%	96.2%	**0.001***
• Others	26.1%	73.9%	
Peroperative Blood Product	16.5%	20.8%	0.646*
Bile Complication	17%	20.9%	0.556*
Portal Vein Thrombosis	3.8%	1.7%	0.422*
Hepatic Vein Occlusion	1.9%	2.6%	0.169*
Postoperative Hemorrhage	7.5%	5.2%	0.553*
Sepsis	11.3%	8.7%	0.590*
Primary Graft Failure	1.9%	0.9%	0.577*

BMI: body mass index, NASH: nonalcoholicsteatohepatitis, *Chi-Square Test and Chi-Square Likelihood tests

Discussion

Obesity and high BMI increase the risk factors and complication rates of surgical operations as an increasingly common health problem all over the world. This increased risk is also valid for liver transplant recipients, and it can cause morbidity and mortality by creating both perioperative risk factors and postoperative vascular and bile complications [1, 4, 5]. BMI elevation may affect all perioperative and postoperative stages of transplant surgery. Considering that obesity shortens the graft survival, it can be said that the complication rates that increase as the BMI increases may be one of the reasons for this [6, 7]. In addition to the results in which there is no significant difference in terms of operative time, hospital stay or perioperative complications in patients with high BMI [3, 8, 9], there are also studies showing that perioperative hot and cold ischemia times, operative time, and hospital stay increase as BMI increases [1, 2, 10, 11]. In addition, when the relationship between the need for perioperative blood product transfusion, bile complications, postoperative bleeding [1], portal vein and hepatic vein thrombosis/occlusion and increased BMI was examined, in some studies, these rates increase with increasing BMI [2, 12-14], while in others it does not change [3, 7, 8, 15-19]. However, it can be said that the risk of sepsis increases as BMI increases [2, 20-22] but there is no conclusive data that shows that it can increase primary graft dysfunction [15]. When we examine the findings of our study in the light of these heterogeneous results, although the rates of operation time, hospital stay, blood product transfusion requirement, bile and vascular complications, postoperative bleeding, sepsis and primary graft dysfunction rates do not change with increasing BMI, we see that the anhepatic phase and cold ischemia times are prolonged. This may be important in terms of the effect of increased BMI on postoperative graft survival.

Obesity and BMI above normal may be the causes of problems that develop both in the perioperative and postoperative period for liver transplant recipients. It will serve to reduce complications if liver transplant recipient candidates with high BMI undergo an effective preparation process before surgery. High-volume patient studies in terms of BMI elevation will be important to show complications and graft survival after liver transplantation.

A higher number of patients, the ability to conduct detailed BMI group analyzes as the increasing number of patients BMI >30, and taking into clinical follow up weight loss after effective ascites treatment will eliminate the limitations of the current study.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

E.A. constructed the main idea and hypothesis of the study. A.O., F.S.T. and H.C. developed the theory and arranged/edited the material and method section. M.B. and E.S. have done the evaluation of the data in the results section. Discussion section of the article was written by E.A. B.U and A.D. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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The relationship between salivary flow rate, oral health and malnutrition in elderly; a cross-sectional study

Yaşlılarda tükürük akış hızı, ağız sağlığı ve malnütrisyon arasındaki ilişki; kesitsel bir çalışma

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Abstract

Purpose: This study aimed to evaluate the association between salivary flow rate (SFR), oral health and nutritional status in the elderly.

Materials and methods: The study included 63 elderly people (20 men, 43 women, age: 70.83±6.42 years) who applied to a private dental clinic. Data were collected by face-to-face interview, and participants' sociodemographic characteristics, employment status information and lifestyle habits were recorded in the questionnaire. To determine nutritional status, 3-day food consumption records were taken, the Mini Nutritional Assessment Test (MNA) was performed and anthropometric parameters were measured. To assess oral health, the decayed teeth (DMFT) index and oral health impact scale (OHIP-14) were used, and unstimulated SFR was measured.

Results: According to MNA, 23.8% of the elderly were malnourished or at risk of malnutrition. The mean SFR of the elderly was 0.40±0.31 mL/min and 15.9% of them had low SFR. The mean SFR of the elderly with normal body weight was higher than that of the obese (p<0.05). There was a negative correlation between SFR and Body Mass Indexs (r=-0.291, p=0.021), calf circumference (r=-0.260, p=0.014), Mid-Upper Arm Circumference (r=-0.254, p=0.044) and body fat percentage (r=-0.308, p=0.014), and a negative correlation between energy (r=0.345, p=0.006), carbohydrate (r=0.251, p=0.047), protein (r=0.326, p=0.009), fat (r=0.354, p=0.006) and phosphorus (r=0.287, p=0.023) intake.

Conclusion: No significant evidence was found regarding to a direct association between SFR, DMFT index, and OHIP-14 scores with malnutrition. However, an assaciation was found between low SFR and obesity. This suggests that there might be a potential link between SFR and nutritional status, which requires further investigation. To gain a better understanding of the relationship between SFR and malnutrition in the elderly, it is recommended to conduct multicenter clinical trials.

Keywords: Elderly, salivary flow rate, oral health, malnutrition.

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Öz

Amaç: Bu çalışmanın amacı; yaşlılarda tükürük akış hızı, ağız sağlığı ve beslenme durumu arasındaki ilişkinin değerlendirilmesidir.

Gereç ve yöntem: Çalışmaya, özel diş hekimi kliniğine başvuran 63 (20 erkek, 43 kadın, yaş: 70,83±6,42 yıl) yaşlı dahil edildi. Veriler yüz yüze görüşme yöntemiyle toplanmış olup katılımcıların sosyodemografik özellikleri, çalışma durumuna ilişkin bilgileri ve yaşam tarzı alışkanlıkları ankete kaydedildi. Beslenme durumu saptanması için 3 günlük besin tüketim kaydı alındı, Mini Nütrisyonel Değerlendirme Testi (MNA) uygulandı; antropometrik ölçümler yapıldı. Ağız sağlığını değerlendirmek için çürük dişler indeksi (DMFT) ve ağız sağlığı etki ölçeği (OHİP-14); tükürük akış hızını saptamak için uyarımsız tükürük akış hızı hesaplaması kullanıldı.

Bulgular: MNA'ya göre yaşlıların %23,8'inin malnutrisyonlu veya malnütrisyon riski altında olduğu belirlenmiştir. Yaşlıların tükürük akış hızı ortalamaları 0,40±0,31 mL/dk olarak bulunmuş olup %15,9'unun düşük tükürük akış hızına sahip olduğu saptanmıştır. Normal vücut ağırlığına sahip yaşlıların tükürük akış hız ortalaması obezlerden daha yüksektir (p<0,05). Tükürük akış hızı ile beden kütle indeksi (r=-0,291, p=0,021), baldır çevresi (r=-0,260, p=0,014), üst orta kol çevresi (r=-0,254, p=0,044) ve vücut yağ oranı (r=-0,308, p=0,014) arasında negatif yönlü, diyetle enerji (r=0,345, p=0,006), karbonhidrat (r=0,251, p=0,047), protein (r=0,326, p=0,009), yağ (r=0,354, p=0,006) ve fosfor (r=0,287, p=0,023) alımı arasında pozitif yönlü anlamlı ilişki mevcuttur.

Sonuç: Tükürük akış hızı, DMFT indeksi ve OHİP-14 skorları ile malnütrisyon arasında doğrudan bir ilişki olduğuna dair anlamlı bir kanıt bulunmamıştır. Bununla birlikte, düşük SFR ile obezite arasında bir ilişki tespit edilmiştir. Bu durum, SFR ile beslenme durumu arasında daha fazla araştırma gerektiren potansiyel bir bağlantı olabileceğini düşündürmektedir. Yaşlılarda tükürük akış hızı ve malnütrisyon arasındaki ilişkinin daha iyi anlaşılması için çok merkezli klinik çalışmaların yapılması önerilmektedir.

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Anahtar kelimeler: Yaşlı, tükürük akış hızı, ağız sağlığı, malnütrisyon.

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Introduction

Malnutrition is a condition in which a deficiency or excess (or imbalance) of energy, protein, and other nutrients has adverse effects on body/tissue structure and function and clinical course [1, 2]. Malnutrition is a common problem in the elderly, and the prevalence of malnutrition has been reported to vary from 13% to 54% [3, 4]. Malnutrition is associated with several adverse health outcomes, including deterioration of functional and cognitive abilities and increased morbidity and mortality [5]. The causes of malnutrition in the elderly are multifactorial. It has been reported that many factors such as age-related physiological changes, inadequate nutrient intake, mental and physical illness, substance use, economic problems, and social isolation can lead to malnutrition in the elderly [6].

Good oral health refers to healthy oral tissues without sores, inflammation, or other painful conditions, well-functioning teeth, adequate saliva, and no oral cavity or masticatory disorders [7]. Oral health problems such as tooth loss, dry mouth, periodontal disease, dental caries, painful mucosal disease, and decreased masticatory function are common in the elderly [7]. It has also been found that salivary glands degenerate with age and therefore salivary flow rate (SFR) decreases in the elderly [8].

Saliva is a physiological fluid that plays an important role in normal oral functions such as swallowing, chewing, and food taste perception [9]. Saliva plays an important role in the acceptance of food and beverages by altering the perception of mouthfeel and the release of flavors. It also converts food into a form that can be safely swallowed. In addition, it dilutes and removes substances from the oral cavity after swallowing [9]. Decreased SFR (hyposalivation), mouth sores, and poorly functioning dentition can affect appetite as well as chewing and swallowing function. It has been reported that this condition can alter food choices and reduce the consumption of balanced foods, increasing the risk of malnutrition [10]. Therefore, it is suggested that impaired oral health may be a factor in the pathogenesis of malnutrition [10]. In a study of the elderly, it was found that the prevalence of malnutrition increased steadily with the increase in oral health problems (oral pain, chewing problems, dryness, and difficulty swallowing) [10]. Another study reported that there was no association between decreased SFR and malnutrition in community-dwelling elderly [11]. Considering the high prevalence of malnutrition in the elderly and the negative consequences of malnutrition on the health of the elderly, studies investigating the relationship between malnutrition and oral health are needed.

In Türkiye, there is no study investigating the association between SFR, oral health status, and malnutrition in the elderly. Therefore, the main objective of this study was to investigate the relationship between SFR, Oral health impact scele-14 (OHIP-14), Decayed, Missing, and filled teeth (DMFT) index and nutritional status. In addition, the relationship between SFR, DMFT, and OHIP scores by Body Mass Index (BMI) groups was investigated. The first null hypothesis states that there are no significant differences in SFR, the DMFT, and (OHIP-14) between older people with normal nutritional status and those with malnutrition or at risk of malnutrition. The second null hypothesis is that there is no association between SFR, DMFT, OHIP-14, and BMI.

Materials and methods

Study design and participants

This cross-sectional study was conducted in independently living individuals over 60 years of age who applied to a private dental clinic in Balıkesir between October 2021 and June 2022. To determine the relationship between SFR and MNA for d=0.4, effect size, 90% power, and 5% type 1 error, the sample size was calculated to be 67 using G power software. Before data collection, 5 volunteers aged 60 years and older underwent a pretest (the results of the pretest were not analyzed) to check the comprehensibility of the questionnaire, and

after the pretest, a total of 63 elderly people were included in the study. The inclusion criteria were as follows: Applying to the dental clinic, being 60 years of age or older, agreeing to participate in the study and signing the informed consent form, answering the research questions completely, and using a telephone. Individuals younger than 60 years of age, individuals who could not answer all research questions, and individuals who had a disease that could affect SFR (sjögren's syndrome, rheumatoid arthritis, systemic lupus erythematosus, parkinson's disease, diabetes mellitus, and other endocrine diseases, crohn's disease, inflammatory bowel disease, periodontitis, mucositis), as well as subjects who received radiotherapy to the head and neck region and subjects who were fasting were not included in the study [12]. The Non-Interventional Clinical Research Ethics Committee of Izmir Katip Celebi University approved the study.

Data collection

Between October 2021 and June 2022, participants who applied to a private dental clinic in Balıkesir, and met the inclusion criteria received general information about the content and purpose of the study, and each participant who agreed to participate in the study was read and signed the informed consent form.

Data for the study were collected by face-to-face interview using a questionnaire. Each interview lasted approximately 30 minutes. In the questionnaire, participants were asked questions about sociodemographic characteristics and information about diseases; a three-day food consumption record was taken to determine nutritional status, and the Mini Nutritional Assessment (MNA) was used. Anthropometric parameters [(body weight, height, mid-upper arm circumference (MUAC), calf circumference, triceps skinfold thickness (TST) were measured. All anthropometric measurements, MNA, and food consumption record were evaluated by a standardized investigational dietitian who had been trained on this topic.

The OHIP-14 was used to assess the oral health problems of the eldery and their impact on nutritional status [13]. The World Health Organization (WHO) criteria for decayed (D), missing (M), and filled (F) teeth (DMFT) were used to assess caries in the permanent dentition [14]. A dentist performed the clinical examinations of the oral cavity using dental mirrors, artificial light, and WHO dental probes, and the participants' teeth were cleaned before the examination. The "unstimulated SFR calculation" was used to determine the SFR.

Anthropometric measurements and bioelectrical impedance analysis

The body weights and body fat percentage of the subjects participating in the study were measured using the bioelectrical impedance analyzer "TANITA BC532 InnerScan" (Tanita, Amsterdam, The Netherlands). The height of the participants was measured with a stadiometer, with the feet side by side and the head in the Frankfort plane. BMI was calculated by dividing body weight (kg) by height squared (m^2). Participants were classified as "obese (≥30 kg/m^2)", "overweight (25-29.9 kg/m^2)", or "normal weight (<25 kg/m^2)" according to BMI [15].

For the TST measurement, the right arm was bent at the elbow 90° and the midpoint between the acromion (shoulder) and olecranon (elbow) was sought and marked in the upright position. The arm was released, and the layer was held with the index finger and thumb of the left hand. The marked area was measured with the right hand using a Holtain caliper [15]. Calf circumference was determined by measuring the widest part of the lower leg between the patella and Achilles tendon with a tape measure [15].

Dietary intake

Each participant was informed about the recording of food consumption in the first interview, in which they were asked the questions of the questionnaire, and participants were asked to take notes so as not to forget the foods they consumed. Another interview was scheduled for the fourth day after the first interview with the participants. For three days, participants were called by telephone at the end of each day after the first interview, and notes were taken on when and in what quantities they had consumed which foods during the day. At the second interview, participants were given the foods and quantities noted in the records as measurements in the book "Food and Nutrition Photo Catalogue: Measurements and

Quantities" and calculated by asking participants to choose the portion they consumed [16]. After determining the amount of food consumed daily by the participants, daily energy, macro- and micronutrient intakes were determined using the "Nutrition Information Systems Package Programme" 9 (Ebispro for Windows, Stuttgart, Germany; Turkish version, BEBIS 9) [17].

"Mini nutritional assessment" (MNA)

In the study, the Turkish version of the MNA was used as a screening test for the nutritional status of the elderly. In this context, the participants were asked questions under the titles "anthropometric assessment," "general assessment," "food intake assessment," and "subjective assessment" in the MNA and the necessary measurements were taken. After completion of the assessment test, participants' total scores were calculated. Participants' nutritional status was categorised as "no nutritional problem" with a score above 23.5, "at risk of malnutrition" with a score between 23.5-17.0, and "malnutrition" with a score below 17.0 [18].

"Oral health impact scale-14" (OHIP-14)

Oral health-related quality of life is an individual's personal perception of how oral health affects their quality of life and overall health. The OHIP-14 is a scale that assesses this perception with two questions about functional limitations, physical pain, psychological discomfort, physical, psychological, and social disability and handicap. Because the scale is concerned with patient problems, high scores indicate patients with oral health-related problems and low scores indicate healthy individuals. An increase in the total score means that the severity of the problem increases and the quality of life decreases [13]. The severity of oral problems and quality of life related to the condition of the elderly who participated in the study were assessed with this scale. The validity and reliability of the scale was determined by Basol et al. [13] in 2014.

SFR

To eliminate the risk that interventional procedures might not be accepted and might reduce the sample size, especially in frail elderly, a nonstimulated SFR measurement was performed in this study.

SFR measurements were performed between 09:00 and 11:00 am, and participants were asked not to eat breakfast on the day of measurement and not to drink water for 2 hours before measurement. Participants rested for a few minutes before the measurement. Participants were asked to swallow 1 time before starting the measurement. Unstimulated saliva samples were collected in cylindrical tubes calibrated from 0 mL to 10 mL by placing subjects with their heads in front of them for 5 minutes and allowing only saliva to flow into the tube in front of the lips without making a spitting motion. The obtained mL value was divided by 5 and the saliva flow rate per minute was calculated. Of the calculated values, the range of 0.3-0.4 mL was considered normal and values below 0.1 mL were considered low salivary flow rate (hyposalivation) [19-21].

DMFT index

Clinical examinations of the elderly were performed by a dentist to determine the number of teeth affected by caries and their results. The sum of the 28 permanent teeth (excluding teeth 18, 28, 38, 48) of the participants and the number of decayed (Decayed-D), missing (Missing-M) and filled (Filled-F) teeth resulted in the DMFT values of the participants [14].

Statistical analysis

Data were analysed with the programme "IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA)". Descriptive statistics are reported as number (n), percentage (%), mean ± standard deviation, median, and IQR. Normal distribution of quantitative data was analysed using the Kolmogorov-Simirnov test. Parametric tests were used for numerical variables that had a normal distribution, and nonparametric methods were used for variables that did not have a normal distribution. Intergroup comparisons for numeric variables were evaluated with the independent t test for two groups when data were normally distributed and with the Mann-Whitney U test when data were not normally distributed. The ANOVA test was used to determine whether there was a difference in SFR by BMI groups, and the difference in DMFT and OHIP-14 scores was determined with the Kruskal Wallis test. Pearson's or Spearman's correlation analysis was used to evaluate the relationships between continuous variables. In

all analyses, a *p* value of less than 0.05 was considered statistically significant.

Results

Table 1 shows the general characteristics of the elderly. A total of 63 elderly people, 20 men (32%) and 43 women (68%), participated in the study. The mean age was 70.83±6.42 years, 87.3% of the elderly did not consume alcohol, and 61.9% did not smoke. Of all the elderly, 76.2% had normal nutritional status and 23.8% were malnourished or at risk of malnutrition. Most of the elderly were overweight and 38.1% of them did 150 minutes of moderate exercise per week. The mean SFR of the elderly was 0.40±0.31 mL/min and 15.9% of them had low SFR.

Table 1. General characteristics of the elderly

	n	%
Age (year) (x̄±SD)	70.8±6.42	
Alcohol consumption status		
Yes	8	12.7
No	55	87.3
Smoking status		
Yes	9	14.3
No	54	85.7
Doing exercise regularly (150 minutes/week, moderate intensity)		
Yes	24	38.1
No	39	61.9
Nutritional status of the elderly according to MNA		
Normal nutritional status	48	76.2
At risk of malnutrition	12	19.0
Malnourished	3	4.8
BMI group		
Normal body weight	12	19.0
Overweight	29	46.0
Obese	22	34.9
Anthropometric measurements	**x̄±SD**	
Calf circumference (cm)	33.9±4.16	
TST (mm)	20.6±11.36	
MUAC (cm)	29.8±4.88	
Body Fat (%)	35.6±9.20	
Dietary intake		
Energy (kcal)	1501.3±556.49	
Carbohydrate (%)	41.1±10.63	
Protein (%)	17.4±4.06	
Fat (%)	41.1±9.29	
Oral health variables		
OHIP-14 (median (IQR)	6.0 (8.0)	
DMFT (median (IQR)	24.0 (12.0)	
Salivary flow rate (mL/min)	0.40±0.31	
Low salivary flow rate (<0.1 mL/min) n (%)	10 (15.9)	

OHIP 14: Oral Health Impact Profile, DMFT: Decayed, Missing, and Filled Teeth, TST: triceps skinfold thickness
MUAC: Mid-upper arm circumference, SD: standart deviation

The relationship between SFRs, DMFT indices, and OHIP scores of the elderly according to MNA is shown in Table 2. It was found that there was no statistically significant difference in SFRs, DMFT indices, and OHIP-14 scores between the elderly with normal nutritional status and those at risk of malnutrition/ malnourished according to MNA (p>0.05).

Salivary flow rates, DMFT, and OHIP scores of the elderly by BMI group are shown in Table 3. Although there was no statistically significant difference between the DMFT and OHIP-14 scores of elderly people in the different BMI groups, a statistically significant difference was found in the SFR averages (p<0.001). The SFRs of the elderly with normal body weight were higher than those of the elderly with overweight (p<0.001).

The relationship between SFR, OHIP, DMFT, and MNA, anthropometric measurements, and food intake is shown in Table 4. There was a negative correlation between SFR and BMI (r=-0.291, p=0.021), calf circumference (r=-0.260, p=0.014), MUAC (r=-0.254, p=0.044) and body fat percentage (r=-0.308, p=0.014) and a positive correlation between energy (r=0.345, p=0.006), carbohydrate (r=0.251, p=0.047), protein (r=0.326, p=0.009), fat (r=0.354, p=0.006) and phosphorus (r=0.287, p=0.023) intake. There was a significant positive correlation between OHIP and energy intake (r=0.346, p=0.006), carbohydrates (r=0.272, p=0.031), protein (r=0.261, p=0.038), fat (r=0.340, p=0.006), calcium (r=0.343, p=0.006) and phosphorus (r=0.270, p=0.032). There was no statistically significant relationship between DMFT and MNA, anthropometric measurements, and food intake values (p>0.05).

Table 2. Salivary flow rates, DMFT indices and OHIP scores of the elderly according to MNA groups

	Healthy (n=48)	**Risk of malnutrition/ malnourished (n=15)**	***p***
	x̄±SD or median (IQR)	**x̄±SD or median (IQR)**	
Salivary flow rate	0.39±0.27	0.43±0.41	0.617[a]
DMFT	25.0 (13.0)	19.0 (12.0)	0.291[b]
OHIP-14	7.0 (7.5)	7.0 (8.0)	0.999[b]

OHIP-14: Oral Health Impact Profile, DMFT: Decayed, Missing, and Filled Teeth · [a]Independent t test, [b]Mann Whitney U test

Table 3. Salivary flow rates, DMFT and OHIP scores of the elderly according to BMI groups

	Normal (n=12)	**Overweight (n=29)**	**Obese (n=22)**	***p***
	x̄±SD or median (IQR)	**x̄±SD or median (IQR)**	**x̄±SD or median (IQR)**	
Salivary flow rate	0.72±0.48[1]	0.34±0.23[2]	0.30±0.14[3]	<0.001[a], 1>3
DMFT	20.0 (8.0)	25.0 (14.0)	24.0 (11.0)	0.683[b]
OHIP-14	5.5 (4.0)	6.0 (7.0)	6.5 (7.5)	0.677[b]

OHIP-14: Oral Health Impact Profile, DMFT: Decayed, Missing, and Filled Teeth, [a]One Way Anova, [b]Kruskal Wallis Test

Table 4. Correlations between the variables

	Salivary flow rate		OHIP-14		DMFT	
	r	*p*	r	*p*	r	*p*
Salivary flow rate	-	-	0.225	0.077	0.018	0.886
MNA	-0.007	0.955	0.027	0.836	0.040	0.753
OHIP-14	0.225	0.077	-	-	0.098	0.447
DMFT	0.018	0.886	0.098	0.447	-	-
Body weight (kg)	-0.160	0.211	0.032	0.805	0.147	0.252
BMI (kg/m²)	-0.291	0.021	0.005	0.971	0.066	0.609
Calf circumference (cm)	-0.260	0.014	-0.150	0.240	0.134	0.295
MUAC (cm)	-0.254	0.044	-0.139	0.279	0.123	0.337
TST (mm)	-0.131	0.307	0.034	0.791	0.025	0.848
Body fat (%)	-0.308	0.014	0.082	0.522	0.50	0.700
Energy (kkal)	0.345	0.006	0.346	0.006	0.177	0.165
Carbohydrate (g)	0.251	0.047	0.272	0.031	0.196	0.124
Carbohydrate (%)	-0.158	0.216	-0.166	0.194	-0.012	0.928
Protein (g)	0.326	0.009	0.261	0.038	0.108	0.400
Protein (%)	0.024	0.851	-0.068	0.596	-0.047	0.716
Fat (g)	0.345	0.006	0.340	0.006	0.131	0.306
Fat (%)	0.117	0.360	0.204	0.209	0.009	0.945
Calcium (mg)	0.021	0.873	0.343	0.006	0.140	0.274
Phosphorus (mg)	0.287	0.023	0.270	0.032	0.141	0.269

TST: triceps skinfold thickness, MUAC: Mid-upper arm circumference, MNA: Mini nutritional assessment, OHIP-14: Oral Health Impact Profile DMFT: Decayed, Missing, and Filled Teeth, BMI: body mass index

Discussion

Oral health is an important component of "healthy ageing" as it affects the overall health and quality of life of individuals [22]. Nutritional status is also a key component of overall health in the elderly [23]. Oral problems are likely to contribute to poorer nutritional status, as certain foods are avoided due to chewing or pain problems. However, the relationship between oral health and nutritional status in the elderly is still neglected. The main objective of this study was to investigate the relationship between SFR, OHIP-14, DMFT index, and nutritional status. The first null hypothesis of this work, namely that SFR, DMFT index and OHIP-14 score are not predictors of malnutrition, was accepted. An inverse statistically significant relationship was found between BMI and SFR, and obese elderly were found to have lower SFR in this study. Therefore, the second null hypothesis was rejected.

Insufficient SFR, also known as hyposalivation, has been associated with a number of chronic diseases such as diabetes [24], asthma [25], and obesity [26]. Therefore, screening and early treatment of SFR play an important role in maintaining oral health, especially in people living in a rapidly ageing society [27, 21]. In the literature, older people with reduced SFR have been shown to consume less of certain foods, including vegetables and seafood. Low SFR has been associated with taste perception, food pleasure, food enjoyment, quality of life, and malnutrition problems [28-30]. A meta-analysis by Pina et al. [8] found that the prevalence of unstimulated low SFR in the elderly was 33.3% (n=2425 individuals). In a 6-year follow-up study in Japan that examined the association between anorexia nervosa and low SFR, it was found that approximately 20% of healthy elderly developed decreased SFR [21]. Similarly, in this cross-sectional study,

15.9% of the elderly were found to have a low SFR. Because saliva not only maintains oral cavity health but also plays a role in maintaining overall health, these findings underscore the importance of early screening and management in the elderly [31, 27-29].

SFR varies according to population, age group, or climatic conditions. The mean value of unstimulated SFR for the general population is 0.3-0.5 mL/min [32]. In this study, in accordance with the literature, the mean SFR of the elderly was found to be 0.40±0.31 mL/min. However, in this study, no statistically significant difference was found between the SFRs of elderly at risk of malnutrition or malnutrition and elderly with normal body weight. In addition, no statistically significant difference was found in DMFT and OHIP-14 scores by BMI and MNA. Similarly, in a study conducted in Finland with 157 community dwelling elderly aged 75 years and older, it was shown that there was no assaciation between low SFR and malnutrition [11]. In contrast to these results, low SFR was associated with malnutrition in a study conducted in elderly hospital patients [33]. The absence of an association between malnutrition and SFR in this study may be attributed to the fact that the participants lived in the community and were probably healthier than the hospitalised elderly. At the same time, these results may be related to the small number of malnourished elderly who participated in the study.

There are many factors that influence SFR. Many drugs such as anxiolytics, diuretics, antidepressants, antihypertensives, analgesics, and neuroleptics can cause a decrease in SFR. In addition, age, women, and obesity are other known risk factors for low SFR in young patients [26, 34]. In a study conducted in Brazil, a negative correlation was found between BMI and perception of oral health in the elderly [35]. Accordingly, in this study, it was found that the SFR of elderly people with normal body weight was higher than that of obese people, and an inverse statistically significant relationship was found between SFR and BMI. In contrast to the results of the present study, Dormenval et al. [33] showed that the SFR of elderly people with BMI <21 kg/m² was significantly lower than that of people with BMI >21 kg/m². It is well known that the term malnutrition does not only refer to inadequate food intake and/or underweight. Malnutrition is the imbalance between the nutrients consumed (energy, protein and other nutrients) and the coverage of the changing metabolic needs. From this perspective, malnutrition is a concept that includes obesity. In previous studies, SFR has been associated with malnutrition, which is characterised by inadequate nutrition. Based on the results of this study in relation to BMI and considering that obesity is also a form of malnutrition, it can be said that SFR is higher in elderly with normal BMI values than in malnourished elderly. These results suggest that low SFR may influence nutritional status.

Previous studies have reported that nutritional status may also influence SFR. [28, 30]. A study based on frequency of food intake showed that dry mouth affects quantity and quality of food intake and ultimately quality of life, and that dry mouth was associated with low carbohydrate and protein intake [36]. In addition, it was reported that people with dry mouth tend to avoid crunchy, dry, and sticky foods and have lower intakes of cellulose, potassium, vitamin B6, iron, calcium, and zinc [37, 38]. Similarly, in a study in the elderly, dry mouth was associated with lower intake of omega-3 fatty acids, micronutrients (vitamin E, folate, fluorine), and water [39]. Similarly, this study found a statistically significant positive association between SFR and carbohydrate, protein, and fat intake. In addition, a significant positive association was found between OHIP and energy, carbohydrate, protein, fat, calcium, and phosphorus intake. The ESPEN guideline states that these changes in dietary intake in the elderly may put people with dry mouth at risk of malnutrition [40]. In this context, routine consideration of SFR in determining and monitoring the nutritional status of the elderly is expected to play a critical role in preventing malnutrition.

This study has limitations. First, due to the fact that this study was designed as a cross-sectional study, a cause-effect relationship could not be established as a result of the analyzes conducted. Second, the sample consisted of older people living in the community; therefore, they likely represent a healthier proportion of older adults overall. Despite these limitations, the study also has its strengths. This study is the first to examine malnutrition and SFR in community-dwelling older people in Türkiye. Another strength is that

the clinical examinations of the participants are conducted by a professional team that includes a dentist and a dietitian. At the same time, the evaluation of food intake with a three-day consumption protocol instead of consumption frequency in this study allowed a more accurate determination of nutritional status.

In conclusion, no significant evidence was found regarding to a direct association between SFR, dental health as measured by the DMFT index, and oral health-related quality of life as measured by the OHIP-14 scores with malnutrition. However, an association was found between low SFR and obesity. This suggests that there might be a potential link between SFR and nutritional status, which requires further investigation. To gain a better understanding of the relationship between SFR and malnutrition in the elderly, it is recommended to conduct multicenter clinical trials.

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Authors' contributions to the article

G.A. and G.K constructed the main idea and hypothesis of the study. G.A, and G.K developed the theory and arranged/edited the material and method section. G.Y.D. wrote the introduction section. G.A and G.Y.D have done the evaluation of the data in the Results section. Discussion section of the article was written by G.K., G.Y.D and G.K reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Fractal analysis and radiomorphometric indices: comparison of mandibular bone structure changes on digital panoramic radiographs of smokers

Fraktal analiz ve radyomorfometrik indeksler: sigara içenlerin dijital panoramik radyografilerinde mandibular kemik yapısındaki değişikliklerin karşılaştırılması

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Abstract

Purpose: The purpose of the study was to assess how smoking affected the mandibular bone structure using a variety of radiomorphometric indices and fractal dimension (FD) analysis.

Material and methods: 56 patients—28 smokers and 28 non-smokers—were included in this retrospective study. In the trabecular bone of the mandible, eight areas of interest of 45x45 pixels were chosen, and fractal dimension analysis was carried out. All digital panoramic radiographs were used to measure the mandibular index and mandibular cortical width. All values were compared between groups. Utilizing the Shapiro-Wilk test, independent samples t-test and Mann-Whitney U test, collected data were evaluated.

Results: A total of 56 patients between the ages of 18 and 52 were evaluated, 28 in the study group (12 female and 16 male) and 28 in the control group (12 female and 16 male). There was a statistically significant difference between the groups in ROI4 and ROI8, but not in the mean FD values of ROI1, ROI2, ROI3, ROI5, ROI6 and ROI7 in the mandibular trabecular bone. No statistically significant differences between groups were seen for PMI and MCW measures (p>0.05). There was a statistically significant difference between genders only in FD values of ROI5 and PMI measurements (p<0.05). The intraclass correlation coefficient (ICC) values for the FD, PMI and MCW measurements had excellent reliability.

Conclusions: The trabecular bone structures of the anterior of the mental foramen and the condyle were different in smokers. There was no difference in cortical bone structures.

Keywords: Fractal dimension, radiomorphometric indices, smoker, panoramic radiography, osteoporosis.

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Öz

Amaç: Çalışmanın amacı, çeşitli radyomorfometrik indeksler ve fraktal boyut (FB) analizi kullanarak sigaranın mandibular kemik yapısını nasıl etkilediğini değerlendirmektir.

Gereç ve yöntem: Bu retrospektif çalışmaya 56 hasta (28 sigara içen ve 28 sigara içmeyen) dahil edildi. Mandibulada, trabeküler kemikte 45x45 piksellik sekiz ilgi alanı seçildi ve fraktal boyut analizi yapıldı. Mandibular indeks ve mandibular kortikal genişliği ölçmek için dijital panoramik radyografiler kullanıldı. Tüm değerler gruplar arasında karşılaştırıldı. Shapiro-Wilk, bağımsız örneklem t-testi ve Mann-Whitney U testleri kullanılarak toplanan veriler değerlendirildi.

Bulgular: Çalışma grubunda 28 (12 kadın, 16 erkek), kontrol grubunda 28 (12 kadın, 16 erkek) olmak üzere yaşları 18 ile 52 arasında değişen toplam 56 hasta değerlendirildi. Gruplar arasında mandibular trabeküler kemikte ROI4 ve ROI8'de istatistiksel olarak anlamlı bir fark vardı, ancak ROI1, ROI2, ROI3, ROI5, ROI6 ve ROI7'nin ortalama FB değerlerinde fark yoktu. PMI ve MCW ölçümleri için gruplar arasında istatistiksel olarak anlamlı bir fark görülmedi (p>0,05). Cinsiyetler arasında yalnızca ROI5'in FB değerleri ve PMI ölçümlerinde istatistiksel olarak anlamlı farklılık vardı (p<0,05). FB, PMI ve MCW ölçümleri için sınıf içi korelasyon katsayısı (ICC) değerleri mükemmel güvenilirliğe sahipti.

Sonuç: Mental foramenin anterioru ve kondil bölgesindeki trabeküler kemik yapıları sigara içenlerde farklıydı. Kortikal kemik yapılarında fark yoktu.

Anahtar kelimeler: Fraktal boyut, radyomorfometrik indeksler, sigara, panoramik radyografi, osteoporoz.

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Introduction

Universally, tobacco use is one of the most serious public health risks, killing about 8 million people worldwide each year [1]. The main addictive substance in tobacco is nicotine [2]. Apart from being addictive, nicotine has adverse effects on various body systems such as cardiovascular, gastrointestinal and respiratory systems. In addition to its systemic side effects, nicotine use is among the modifiable risk factors for certain types of cancer and bone diseases such as osteoporosis [2, 3]. In the world, smoking is the most popular way to consume tobacco [1].

Smoke exposure has been associated with the bone regeneration process, as it affects the reduction of bone formation and the increase of the mechanism of bone resorption [4]. It has been reported that smoking is a significant risk factor for both bone loss and fractures [5].

Osteoporosis (OP), described as asymptomatic bone disease, is a significant health problem that is increasingly common worldwide. It is characterized by low bone mass and degeneration of the bone's microarchitecture, which increases bone fragility and fracture risk [3, 6]. It is known that osteoporosis causes relatively more trabecular bone loss than cortical bone [3].

By using the box-counting algorithm, the mathematical technique of "fractal analysis" (FA) assesses changes in trabecular bone tissue [3, 7]. FA quantitatively describes the image complexity of the bone structure and correlates with the bone strength of the trabecular bone [3, 7, 8]. A more complex bone structure is characterized by a higher fractal dimension (FD) [7-9]. According to certain reports, fractal analysis is enough to detect osteoporotic disorders in the jaw bones [7, 8].

The fractal analysis method was used to assess complex structures in biology and medicine [3]. In the literature, there are studies showing that FA is useful in detecting changes in bone in the early period, especially in the medical field [10]. FA is an advantageous method that can quantitatively evaluate the trabecular bone structure and is cost-effective, non-invasive, accessible, and unaffected by variable parameters such as projection geometry and density. Due to these advantages, it has been widely used in many fields, including dentistry, in recent years [11]. With fractal analysis in dentistry, studies have been carried out on different subjects such as the analysis of bone structure of patients with osteoporosis [6], using various systemic drugs [7-9, 12, 13] and systemic diseases [14-16], temporomandibular joint (TMJ) dysfunction [17], bruxism [11, 18], and evaluation of the bone around the implant [19, 20].

Apart from fractal analysis, radiomorphometric measurements such as panoramic mandibular index (PMI), mandibular cortical width (MCW) are essential markers for detection osteoporosis. These indices are simple and effective methods that can be used to detect osteoporotic conditions [7, 8, 10, 21, 22]. Both fractal analysis and radiomorphometric index measurements can be performed on panoramic radiographs used for routine dentistry examinations [9].

The objective of this study is to assess and compare both fractal analysis and radiomorphometric measurements of the mandibular bone on digital panoramic radiography in smokers and non-smokers.

Materials and methods

Ethical issues

The research was carried out in accordance with the guidelines of the Helsinki Declaration. The University's Ethical Committee approved the presented study. The panoramic radiographs of patients who requested hospital examinations in 2021 for a variety of reasons were examined for this retrospective investigation.

Patient selection and study design

Information about the medical conditions of all patients and the drugs they used, as well as demographic information such as age and gender of the patients, were gleaned from anamnesis records and the hospital's automation system.

The study was selected from panoramic radiographs containing explicit images of the mandibular anterior and posterior areas, TMJ,

mental foramen, and mandibular lower cortices. The individuals were between the ages of 18 and 52, smokers (smoking a pack of cigarettes for at least 1 year), and healthy (without a systemic illness that affects bone metabolism specifically). Exclusion criteria for individuals; panoramic radiography of the mandibular or maxillary arch with more than one missing tooth (excluding third molars), low diagnostic value, ghost image or artifact that would prevent analysis and measurements of the mandibular bone, complete or ongoing orthodontic radiographs with jaw fracture or pathological lesions in the evaluated area treatment history, parafunctional habits such as bruxism and TMJ disease, early or late menopause, alcohol and drug addiction, using anti-resorptive drugs, neurological and psychiatric diseases, taking long-term corticosteroid treatment and using drugs that affect bone metabolism patients were excluded.

A total of 56 patients were selected. Systemically healthy individuals who have smoked for at least 1 year were selected for the smoker group, and non-smokers were selected as the control group as non-smokers. The smoker and non-smoker groups were matched based on gender, with the groups consisting of 28 patients (16 males and 12 females).

Digital panoramic radiography

The same radiology technician utilized the dental panoramic device (Planmeca, Helsinki, Finland) to take all digital panoramic radiographs used for measurements with an exposure time of 16 seconds at 70 kVp, 12.5 mA. To ensure standardization in patients, the positioning recommendations recommended by the manufacturer were followed (Frankfurt plane horizontal to the ground, light beam markers were placed in appropriate areas). All patients were subjected to the same standard protocol.

Image preprocessing

The high resolution Tagged Image File Format (TIFF) was used to store the panoramic radiographs that matched the requirements and 8-bit grayscale depth, and a data set was created. For image standardization, all panoramic radiographs were set to 2976x1536 pixels using adobe photoshop CS6 Extended (Adobe Systems Inc., San Jose, CA, USA). Image manipulation (magnification, contrast, and brightness) was strictly prohibited. The radiographs were examined using the 1920x1080 pixel resolution Windows XPTM Professional operating system.

Fractal dimension analysis

For FD analysis, the free ImageJ 1.53k (National Institutes of Health, USA) software was downloaded (https://imagej.nih.gov/ij). Each DPR performed a fractal dimension analysis using the box-counting technique suggested by White and Rudolph [23]. On the digital panoramic image of each patient, a total of eight region of interest (ROI) were selected in four different regions, bilaterally in the mandible trabecular bone: ROI1-8: the condyler subcortical region; ROI2-7: the gonial supracortical region; ROI3-6: above mandibular canal, the interdental area between the second premolar and first molar; ROI4-5: the area adjacent to the mental foramen (Figure 1). Due to anatomical difficulties and artifacts in the maxilla, a relevant area was not selected. The lamina dura, cortical structure, periodontal space and mandibular canal were not included in ROIs. Each ROI 45×45 pixel area was selected. The fractal dimension for each ROI was performed according to the steps defined by White and Rudolph (Figure 2) [23].

Radiomorphometric indices

Panoramic mandibular index

The thickness of the mandibular cortex (a) and the distance (b) between the lower edges of the mental foramen and the mandible were divided to create the PMI values (a/b) (Figure 3) [22].

Mandibular cortical width

For MCW measurement in accordance with Ledgerton et al. [24] definitions, a tangent line parallel to the lower nerve of the mandibular cortex was drawn at the level of the mental foramen in the premolar area. A line was stretched vertically from the mental foramen to the second parallel line, which was drawn along the superior nerve of the mandibular cortex. On this vertical line, the mandibular cortical width was calculated as the distance between two parallel lines (Figure 3).

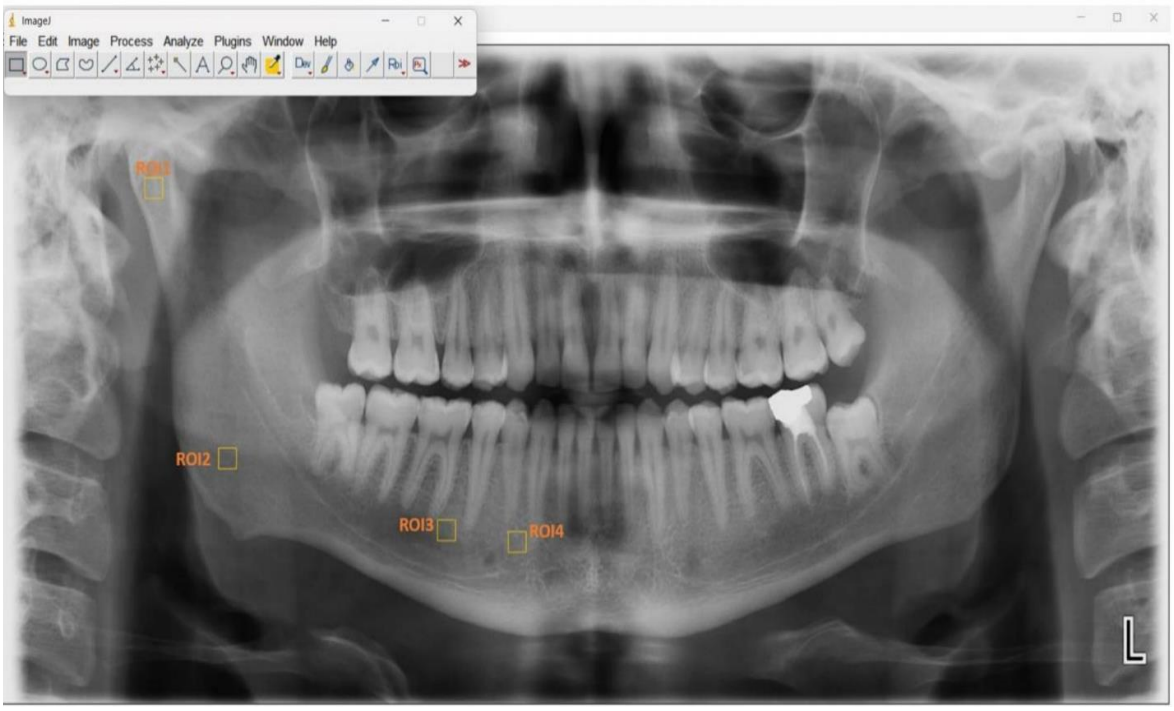

Figure 1. Selection of ROI in digital panoramic radiography with imagej

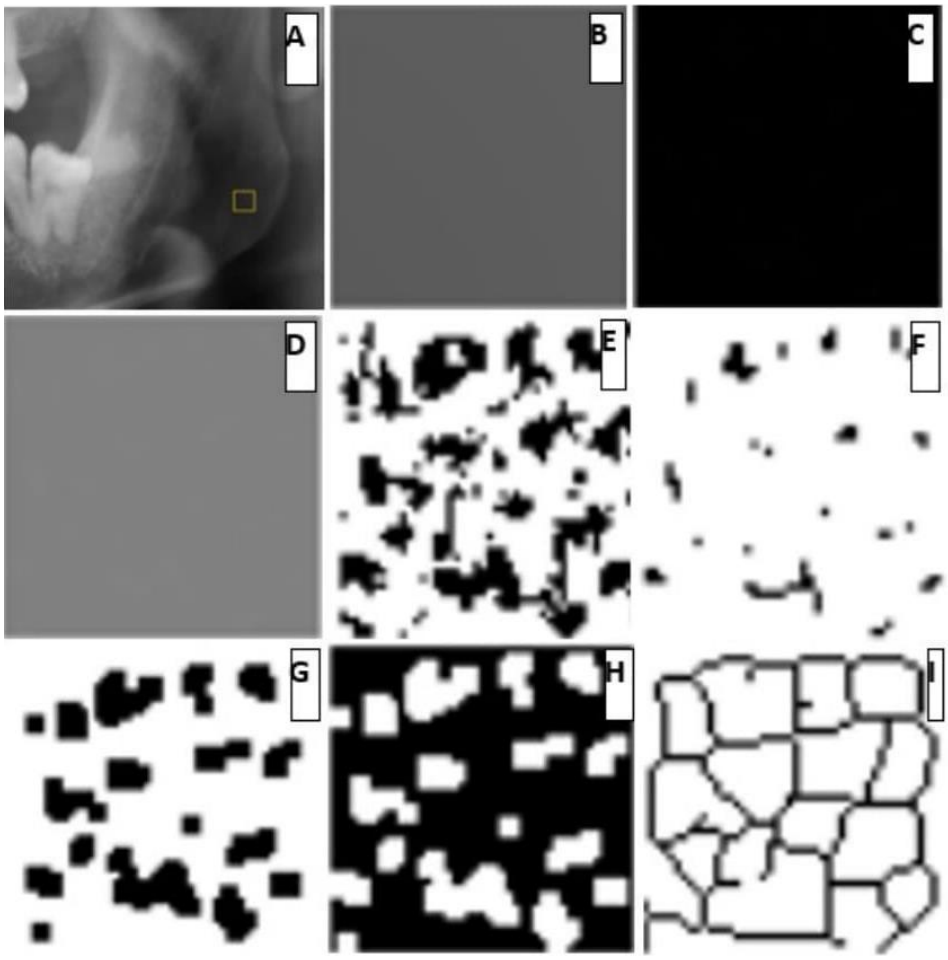

Figure 2. Steps of fractal dimension analysis of ROI7. a:ROI selection, b:Gaussian blur, c:subtraction d:Addition of 128 gray value, e:Binarization, f:Erosion, g:Dilatation, h:Inversion, ı:Skleletonization

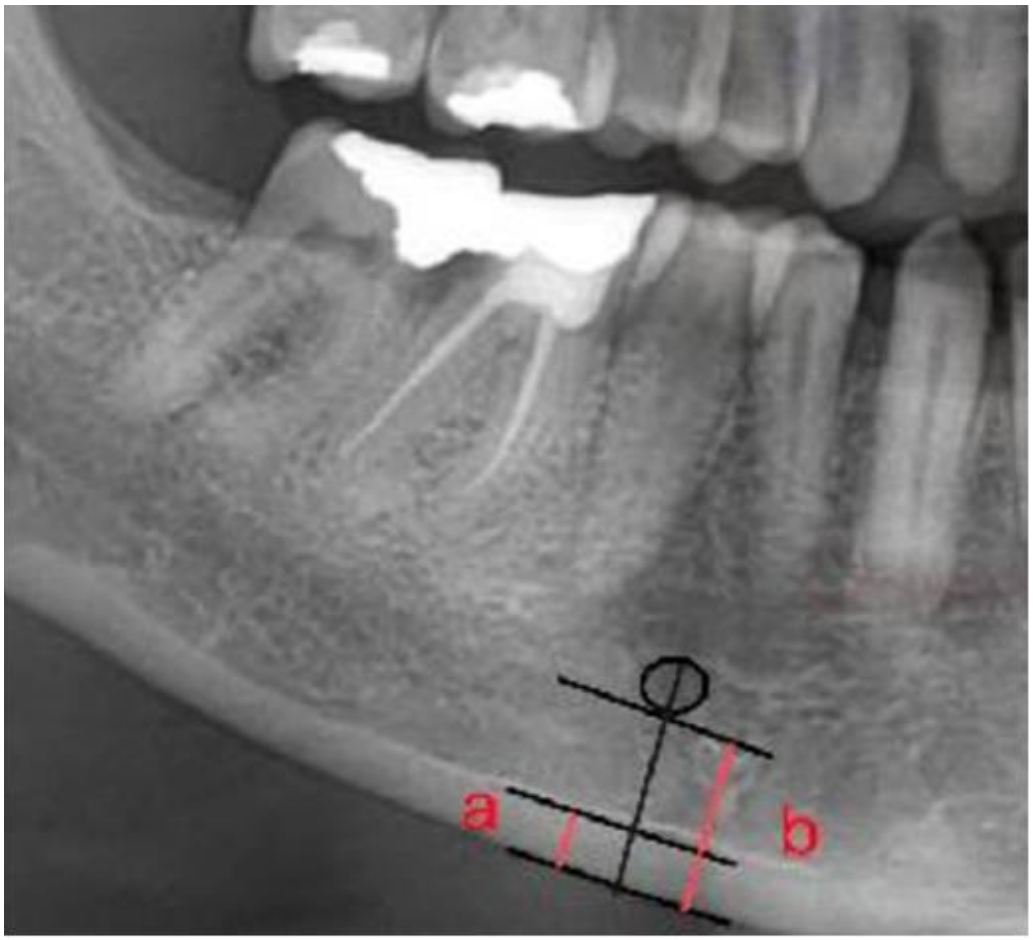

Figure 3. Measurements of mandibular cortical width (a) and panoramic mandibular index (a/b)

In the mandible, the mean values of the PMI and MCW measures were computed bilaterally. An expert in Oral and Maxillofacial Radiology (B.Ç) with six years of clinical experience conducted the FD analysis. Radiomorphometric measurements were performed in a dark room by an expert in Oral and Maxillofacial Radiology (Z.B.A.) with six years of clinical experience. Experts were blind to all information about individuals. For the intraobserver reliability of the obtained values, two weeks after the first measurements were made, 14 randomly selected panoramic images (25%) were re-measured and evaluated.

Statistical analysis

The IBM-SPSS (International Business Machines-Software Package for Social Sciences) statistical package, version 26 (SPSS, Chicago, IL, USA), was used to analyze the data from this study. At .05, the statistical significance level was determined. Intra-observer agreement was evaluated with the intraclass correlation coefficient (ICC). Descriptive statistics were made for all parameters. To evaluate whether the data had a normal distribution, the Shapiro-Wilk test was performed. To compare normally distributed FD, PMI, and MCW values between groups and gender, an independent samples t-test was utilized. The Mann-Whitney U test was used to compare variables that did not exhibit normal distribution.

Results

A total of 56 patients between the ages of 18-52 were evaluated in all groups. 28 smokers (12 females and 16 males) made up the group of smokers, while 28 non-smokers made up the group of non-smokers (12 females and 16 males). The proportion of females in all group was 42.86%. Smokers and non-smokers had respective mean ages of 29.6±9.40 years and 28.1±7.63 years (Table 1).

Table 1. Distribution of smokers and non-smokers groups by age and gender

Group	Age (Mean±SD)	Sex (N, %)		
		Female	Male	Total
Smoker group	29.6±9.40	12 (21.43%)	16 (28.57%)	28(50%)
Non-smoker group	28.1±7.63	12 (21.43%)	16 (28.57%)	28(50%)
All groups	28.86± 8.52	24 (42.86%)	32 (57.14%)	56 (100%)

SD: standard deviation N: number of cases

For all metrics, including ROI1 (ICC=0.978), ROI2 (ICC=0.981), ROI3 (ICC=0.978), ROI4 (ICC=0.995), ROI5 (ICC=0.996), ROI6 (ICC=0.996), ROI7 (ICC=0.999), ROI8 (ICC=0.975), MCW (ICC=0.981), PMI (ICC=0.969), the intraclass correlation coefficient values showed excellent reliability.

FD values according to regions in smoker and non-smoker groups are shown in Table 2. The FD values assessed in ROI1, ROI2, ROI3, ROI5, ROI6, and ROI7 locations did not differ statistically significantly between the groups. However, ROI4 and ROI8 were found statistically significantly higher in the non-smoker group (p<0.05) (Table 2).

No group differences in MCW or PMI were found to be statistically significant (p>0.05) (Table 3). PMI values were obtained as 0.31 in both groups. Smokers group had lower MCW values than non-smokers group (4.40 mm vs. 4.46 mm).

Between the genders, only FD in ROI 5 and PMI values were revealed to be significantly higher in female patients than in male patients (p≤0.05) (Table 4).

Table 2. Comparison of FD values according to regions in the smoker and non-smoker groups

ROI	Group	Mean+SD	*p* value	Test value
ROI1	**S**	1.51± 0.04	0.870	z=-0.164
	NS	1.51± 0.05		
ROI2	**S**	1.49±0.06	0.207	z=-1.262
	NS	1.48±0.05		
ROI3	**S**	1.50±0.05	0.057	z=-1.901
	NS	1.48±0.04		
ROI4	**S**	1.52±0.04	0.013*	z=-2.483
	NS	1.50±0.03		
ROI5	**S**	1.50±0.04	0.403	z=-0.836
	NS	1.49±0.04		
ROI6	**S**	1.49±0.04	0.533	z=-0.623
	NS	1.48±0.05		
ROI7	**S**	1.49±0.07	0.298	z=-1.041
	NS	1.47±0.07		
ROI8	**S**	1.52±0.03	0.008*	t=2.743
	NS	1.50±0.04		

ROI, region of interest: ROI1,8 condylar region, ROI2,7 gonial region
ROI3,6 interdental area between the first molar and the second premolar, ROI4,5 adjacent to the mental foramen
S: smoker, NS: non-smoker, **: significant at the 0.05 level, t: independent samples t-test, z: Mann-Whitney U test*

Table 3. Comparison of radiomorphometric indices in the smoker and non-smoker groups

	Group	Mean+SD	*p* value	Test value
MCW	Smoker	4.40±0.67	0.718	z= -0.361
	Non-smoker	4.46±0.69		
PMI	Smoker	0.31±0.07	0.890	t=0.139
	Non-smoker	0.31±0.06		

MCW: Mandibular cortical width, PMI: Panoramic mandibular index, t: independent samples t-test, z: Mann-Whitney U test

Table 4. Comparison of FD values and radiomorphometric indices according to gender

	Female (n:24) Mean+SD	Male (n:36) Mean+SD	*P* value	Test value
ROI1	1.51± 0.03	1.50±0.05	0.351	t=0.940
ROI2	1.49±0.05	1.49±0.06	0.513	z=-0.654
ROI3	1.49±0.04	1.49±0.05	0.797	t=0.258
ROI4	1.51±0.04	1.50±0.04	0.790	t=0.267
ROI5	1.50±0.03	1.49±0.05	0.044*	t=2.067
ROI6	1.49±0.05	1.48±0.05	0.524	z=-0.638
ROI7	1.48±0.06	1.48±0.07	0.855	t=0.183
ROI8	1.51±0.04	1.51±0.03	0.585	z=-0.547
MCW	45.83±7.09	43.12±6.35	0.197	z=-1.291
PMI	0.35±0.06	0.29±0.05	0.000*	t=4.116

*: significant at the 0.05 level, n: number of cases, t: independent samples t-test, z: Mann-Whitney U test

Discussion

Osteoporosis is an age-related bone disease with a higher risk of fracture and is characterized by decreasing bone microstructure and density [25]. Risk factors for osteoporosis include a low body mass index, genetics, smoking, a deficiency in vitamin D, hormonal state, aging inadequate calcium intake, excessive caffeine and alcohol and consumption, a decline in physical activity, female gender [26, 27]. One of the most significant osteoporosis risk factors that can be changed is smoking [28]. The compounds in cigarette smoke cause changes in BMD due to their negative effects on osteoclastogenesis, bone angiogenesis, calcium-phosphate balance, sex and adrenal hormones. Due to their detrimental effects on calcium-phosphate balance osteoclastogenesis, adrenal and sex hormones, bone angiogenesis, the chemicals in cigarette smoke alter BMD [29].

Although dual-energy x-ray absorptiometry (DEXA), which measures bone mineral density (BMD), is a costly method, it is regarded as the gold standard for osteoporosis screening [30]. Additionally, as the population ages, the costs related to this condition likely to rise [31]. The application of panoramic radiography in the early detection of low bone mass will provide those suffering from osteoporosis with the significant benefit of early treatment because it is a more frequent and affordable examination than DEXA and shows the complete maxilla and mandible on a single film [32].

By analyzing structural components such as bone tissue and complex shapes, FA [6], reveals the microarchitectural structure of trabecular bone, allowing the detection of osteoporotic changes in alveolar bone [33]. FA allows the computation of existing fractals with digitized images, but these images need to be preprocessed. After the image preprocessing process is completed, various algorithms are used to calculate the FD [34]. Since the box-counting algorithm is the most used technique in the literature for determining the fractal dimension [6, 35], it was also preferred

in our study. In the present work, we used radiomorphometric indices and FD analysis to assess the changes in the mandibular bone structure in smokers. In the field of dentistry, FA is used to assess the jaw's bone structure [3]. Fractal analysis is thought to be a metric that assesses the distinction between healthy and osteoporotic bone tissue [36]. According to a comprehensive review, investigations have been conducted using a variety of imaging modalities, with panoramic radiographs being the most common [3]. They argued that the use of panoramic radiography in FA is more advantageous because cone beam computed tomography (CBCT) shows lower image resolution and the radiation dose is higher in the examination of the trabecular bone [37]. In addition, the processing capacity of DPR allows qualitative and quantitative analysis of bone density and structure [2]. In the light of all this information, in this study, without using additional radiation, DPRs collected from patients who sought treatment at our facility for a variety of reasons were evaluated.

Existing studies in the medical literature on smoking and osteoporosis risk have reported that smoking increases the incidence of fractures by decreasing bone density [38]. Male smokers were shown to have lower forearm BMD than non-smokers [39]. In the study of Hijazi et al. [40], in which they evaluated the incidence of osteoporosis, it was shown that smoking and non-smokers had different incidences. In addition, smoking causes an increase in chronic oxidative stress in the body, which affects bone metabolism, causing the bone mineral density to decrease. By inhibiting vitamin D and calcium absorption, tobacco disrupts the calcium-phosphate balance necessary for bone matrix mineralization and affects bone mineral density [41].

The results of the investigations in the literature show that there is no unambiguous agreement on how alterations in bone microarchitecture and FD values are related. While some of the studies found an inverse relationship between osteoporosis and FD, others found that reduction in complexity in trabecular bone correlated with FD [42].

According to the findings of the present study, the FD of the trabecular bone of smokers was high. Similar to our study, in comparison to the osteoporotic group, Yasar et al. [43], Mostafa et al. [44], Tosoni et al. [33] obtained lower FD values in the control groups. The premolar and condyle region's results for the fractal dimension were statistically significant. Alman et al. [45], and Cosgunarslan et al. [7], who evaluated the mineral density of the mandible, discovered a substantial variation in FD values in the premolar area, consistent with our study.

Only a few recent studies that examine the relation between bone mineral density and smoking can be found in the dental literature. Basavarajappa et al. [3] found that FD measurements taken only from the anterior of the mental foramen in digital panoramic radiography in male smokers were found to be lower in smoking tobacco (ST) and smokeless tobacco (SLT) users than in the control group. In addition, lower FD values were obtained in SLT users were compared to ST users. In Santolia et al. [46] studies where the FD values in three different areas changed between groups when they assessed patients with oral lesions linked to areca nut and tobacco use. Mean values were lowest in SLT users [46]. In the present research, the front of the mental foramen (ROI4) and condyle region (ROI8) showed a statistically significant difference, but the values were lower in the non-smoker group. This difference between the results of the studies is due to the methodological difference such as ROI selection and sizes, the superposition of the surrounding anatomical structures to the examined areas, the anatomical difference between individuals [47], and different demographic characteristics such as patient gender, number and age range, smoking frequency, and the type of cigarette used may be due to the difference. Compared to those who use smoking tobacco, since the dose of nicotine in smokeless tobacco users is relatively high, nicotine can cause more severe side effects on the bone [29]. We think that the use of smoke tobacco by the people included in the study in the smokers group may have caused the difference between the results.

Most studies published in the literature successfully assess osteoporotic abnormalities on the jawbones in dentistry using both FA and radiomorphometric indices [7, 10, 13-15]. The ability to assess osteoporotic abnormalities using a variety of radiomorphometric indices is one of the most important benefits of dental radiography [48]. The measurement that is

least affected by image distortions in panoramic radiographs is considered to be PMI [22]. However, the size and location of the mental foramen, its radiographic appearance, and its distance to the mandibular cortex differ between individuals [16]. Zihni Korkmaz et al. [8] reported that patients with lack of vitamin D had lower PMI values. Aytekin et al. [15] indicated that it is advantageous to employ in the earlier detection of osteoporotic abnormalities and reported lower PMI values on radiographic images of individuals with hyperthyroidism. Limeira et al. [49] found that patients with Type 1 Diabetes Mellitus (T1DM) that is not well controlled have lower PMI values than healthy people. On the other hand, some studies did not find a relationship between PMI measurements and BMD changes [21, 24].

According to the data, there wasn't any statistically significant variation in PMI among smokers [46]. There was no significant statistical difference in PMI values among non-smokers and smokers, which is in line with the findings of this study.

Some researchers did not discover a statistically significant distinction between the PMI values in the patient group and the healthy group; they found lower MCW values. They argued that MCW measurements would be helpful in the objective evaluation of osteoporotic changes, unlike PMI [7, 10]. They discovered that MCW values were lower in patients with T1 DM when compared to the control groups, even though there was no statistically significant difference in MCW index between patient groups with T1 and T2 DM [14]. In the study performed on patients using antidepressant, no MCW difference was observed [12]. The MCW values across smokers varied significantly, with ST users having the lowest values, but values were not less than 3 mm between groups [46]. No statistically significant difference existed between the groups in our investigation. However, while lower values (4.40 mm) were obtained in smokers, the results were not found to be less than 3 mm, which is consistent with the other study.

Some of the studies investigating FD values with various diseases did not detect a gender-related correlation [8]. However, Demiralp et al. [50], in their study of panoramic radiographs of cancer patients taking bisphosphonates, observed a significant difference between genders in the FD values around the premolars and mental foramen. Cosgunarslan et al. [7] showed that in individuals using proton pump inhibitors, males had a higher number of affected parameters compared to females. In the current study, PMI and FD values of the region around the left mental foramen were found to be significantly higher in female patients. Due to the risk of sex-related characteristics and hormonal factors that may influence the results, studies with larger samples including different ages and sexes are needed to confirm these results.

Due to the retrospective nature of our current study, the inaccessibility of information such as smoking frequencies, vitamin D levels and supplementation, calcium use, and body mass index are the limitations of the study. Individual variances in this information could have influenced our current findings. Detailed studies should be focused on by considering other risk factors with larger samples.

In conclusion; it is important to determine its effect on the jaw bones due to tobaccos direct and indirect effects on bone mineral density. Our current study found changes in trabecular bone structure anterior to the mental foramen and in the condyle region. No difference was observed in the cortical bone structure. Differences were observed in some measurements between genders. Our study showed that fractal dimension in mandible trabecular bone and PMI are a parameter to be used in the evaluation of osteoporosis in smokers. Since dental radiographs are frequently used during dental control, it is of great importance to obtain the opportunity to screen for possible osteoporosis in dental clinics.

Conflict of interest: There was no conflict of interest among the authors in this study.

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Authors' contributions to the article

B.C: Concept, Design, Definition of content, Literature search, Radiological studies (Fractal Analysis), Data acquisition, Data analysis, Preparation of Manuscript and Proofreading. Z.B.A: Design, Definition of content, Literature search, Radiological studies (Radiomorphometric indices), Data acquisition, Data analysis, Statistical Analysis, Preparation of Manuscript.

Research Article

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Assessing latent tuberculosis infection prior to biologic therapy in psoriasis: a new diagnostic approach with an online interpreter

Psoriasis'te biyolojik tedavi öncesi latent tüberküloz enfeksiyonunun değerlendirilmesi: web tabanlı yorumlayıcı ile yeni bir tanı yaklaşımı

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Abstract

Purpose: The use of biological agents, particularly anti-TNF-alpha treatments, is associated with an elevated risk of tuberculosis (TB). Hence, a comprehensive assessment of latent tuberculosis infection (LTBI) before biologic therapies is imperative. The objective of this study was to evaluate the utility of an online tuberculin skin test (TST)/ interferon-γ release assay (IGRA) interpreter (OI-TST/IGRA) in assessing the risk of LTBI prior to initiating biological therapies in psoriasis patients.

Materials and methods: 116 psoriasis patients who were previously evaluated for TB by a pulmonologist before being treated with a biologic agent were re-evaluated retrospectively with OI-TST/IGRA (tstin3d.com). Mean positive predictive value (PPV), mean annual risk of development of active tuberculosis (ARDATB), and mean cumulative risk of active tuberculosis (CRATB) values were calculated with OI-TST/IGRA and compared with previous results. Group comparisons were performed using Kruskal-Wallis and Mann-Whitney U tests.

Results: The PPV of the LTBI-positive group was significantly higher than the LTBI-negative group. The PPV and ARDATB values of the TST size of >15 mm group were significantly higher than the TST size of 5-9 mm and TST size of 10-15 mm groups. The PPV, ARDATB, and CRATB values of the QuantiFERON-TB Gold In-tube test (QFT-GIT)-positive group were significantly higher than the QFT-GIT-negative group. And the same values of the chest X-ray (CXR)-positive group were significantly higher than the CXR-negative group. The PPV, ARDATB, and CRATB values were positively correlated with TST, QFT-GIT and CXR results. In addition, the PPV was positively correlated with previous LTBI decisions.

Conclusion: OI-TST/IGRA in which many factors are questioned and PPV, ARDATB, and CRATB values are evaluated together, may be a valuable tool for assessing the risk of active TB in psoriasis patients and preventing overdiagnosis and unnecessary prophylaxis.

Keywords: Latent tuberculosis infection, psoriasis, biologic therapy.

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Öz

Amaç: Biyolojik ajanların, özellikle de anti-TNF-alfa tedavilerinin kullanımı artmış tüberküloz (TB) riski ile ilişkilidir. Bu nedenle, biyolojik tedavilerden önce latent tüberküloz enfeksiyonunun (LTBI) kapsamlı bir şekilde değerlendirilmesi gereklidir. Bu çalışmanın amacı, psöriazis hastalarında biyolojik tedavilere başlamadan önce LTBI riskini değerlendirmek için web tabanlı bir uygulama olan tüberkülin deri testi (TST) /interferon-γ salınım testi (IGRA) (OI-TST/IGRA) yorumlayıcısının faydasını değerlendirmektir.

Gereç ve yöntem: Biyolojik bir ajanla tedavi edilmeden önce göğüs hastalıkları uzmanı tarafından TB açısından değerlendirilen 116 psoriasis hastası retrospektif olarak OI-TST/IGRA (tstin3d.com) ile yeniden değerlendirildi. OI-TST/IGRA ile ortalama pozitif prediktif değer (PPV), ortalama yıllık aktif tüberküloz gelişme riski (ARDATB) ve ortalama kümülatif aktif tüberküloz riski (CRATB) değerleri hesaplandı ve önceki sonuçlarla karşılaştırıldı. Grup karşılaştırmaları Kruskal-Wallis ve Mann- Whitney U testleri kullanılarak yapılmıştır.

Bulgular: LTBI-pozitif grubun PPV'si, LTBI-negatif gruptan anlamlı olarak daha yüksekti. TST boyutu >15 mm olan grubun PPV ve ARDATB değerleri, TST boyutu 5-9 mm ve TST boyutu 10-15 mm olan gruplardan anlamlı olarak daha yüksekti. QuantiFERON-TB Gold In-tube test (QFT-GIT) pozitif grubun PPV, ARDATB ve CRATB değerleri QFT-GIT negatif gruptan anlamlı derecede yüksekti. Akciğer grafisi (CXR) pozitif grubun aynı değerleri CXR negatif gruptan anlamlı derecede yüksekti.

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PPV, ARDATB ve CRATB değerleri TST, QFT-GIT ve CXR sonuçları ile pozitif korelasyon göstermiştir. Ayrıca, PPV önceki LTBI kararları ile pozitif korelasyon göstermiştir.
Sonuç: Birçok faktörün sorgulandığı ve PPV, ARDATB ve CRATB değerlerinin birlikte değerlendirildiği OI-TST/IGRA, psoriasis hastalarında TB riskinin değerlendirilmesinde, yanlış tanı ve gereksiz profilaksinin önlenmesinde değerli bir araç olabilir.

Anahtar kelimeler: Latent tüberküloz enfeksiyonu, psöriazis, biyolojik tedavi.

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Introduction

Psoriasis is an inflammatory and chronic disease with about 3% of prevalence worldwide. It may be associated with significant comorbidities, such as psoriatic arthritis, uveitis, metabolic syndrome, psychiatric and cardiovascular diseases that poorly affect patients' quality of life [1-3].

The use of biologics targeting tumor necrosis factor-α (TNF-α), the primary cytokine in the development of psoriasis, has proven to be highly effective in the treatment of the disease. With the increasing use of anti-TNF-α biologics, a new group of high-risk patients for tuberculosis (TB) has developed. This has led to the need for increased awareness and effort to screen latent tuberculosis infection (LTBI) with clinical, radiographic, and laboratory measures [4, 5].

Before the treatment with anti-TNF-α biologics, detailed personal and familiar anamnesis about TB, including BCG vaccination, household contact with a TB case, immigration from or journey to a nation with a high incidence of TB, occupation (healthcare workers, etc.), smoking, alcohol consumption, and diabetes mellitus should be evaluated with great attention. On the other hand, chest X-ray (CXR), computed tomography (CT) of thorax, tuberculin skin test (TST), and interferon-γ release assays (IGRAs) like QuantiFERON-TB Gold In-tube test (QFT-GIT) are widely used to determine the TB risk more objectively [6]. For more than a century, TST has been employed as a standard method for screening TB. However, some TST limitations, such as koebnerization phenomena and false positivity associated with previous BCG or contacting with non-tuberculous mycobacteria, maybe the reasons that resulted in the growing acceptance and popularity of the IGRAs in clinical practice in the last decade [7-9]. A gold standard test is still not available for establishing LTBI. The difficulty in inventing a novel test is related neither to sensitivity nor specificity but to the predictive value [10].

The 'Online TST/IGRA Interpreter' (OI-TST/IGRA) (tstin3d.com) is suggested to be a useful clinical tool for screening LTBI in adult patients. This calculator aims to approximate the risk of active TB in a patient with a TST size of ≥ 5 mm, based on his/her related medical outcomes and specific conditions [8, 11, 12]. The calculation needs the information about current and immigration age (for individuals who migrated to a country with a low incidence of TB), size of TST, IGRA, BCG status, place of birth, and recent contact with an active TB patient. Besides, a total of 15 parameters are asked, such as anti-TNF-α therapy, diabetes mellitus, abnormal CXR findings, chronic renal failure, etc. (Table 1).

OI-TST/IGRA calculates the positive predictive value (PPV) of the test, the annual risk of development of active TB (ARDATB), the cumulative risk of active TB (CRATB) up to age 80, and the probability of drug-induced hepatitis and related hospitalization if the patient treated with isoniazid. Table 2 presents the OI-TST/IGRA results of a sample patient.

In this study, it was aimed to use the OI-TST/IGRA to re-assess the previously established diagnosis of LTBI before the biologic therapies in psoriasis by conventional procedures that are thus far mainly based on TST and/or IGRA and clinical profile. Based on literature reviews, this study is the first, investigating the usability of OI-TST/IGRA to determine active TB risk in patients with psoriasis. Considering the challenges of LTBI investigation and overestimation risk of LTBI diagnosis, OI-TST/IGRA may provide a new and hopeful perspective on this issue.

Table 1. Parameters of the online TST/IGRA interpreter

Age				
Age at immigration (If person immigrated to a low TB incidence country)				
Country of birth				
TST size (TST<5 mm is not included in the calculator)	Not done	5-9 mm	10-15 mm	15> mm
IGRA result	Not done	Negative	Positive	
BCG vaccination	Never vaccinated or unknown	Vaccinated age<2 years	Vaccinated age≥2 years	
Recent contact with active tuberculosis	No contact	Close contact	Casual contact	
Other conditions				
· AIDS				
· Abnormal chest x-ray: granuloma				
· Abnormal chest x-ray: fibronodular disease				
· Carcinoma of head and neck				
· Chronic renal failure requiring hemodialysis				
· Cigarette smoker (>1 pack/day)				
· Diabetes mellitus (all types)				
· HIV infection				
· Recent tuberculosis infection (tuberculin skin test conversion≤ 2 years ago)				
· Transplantation (requiring immune-suppressant therapy)				
· Silicosis				
· Treatment with glucocorticoids				
· TNF-α inhibitors (e.g., infliximab/etanercept)				
· Underweight (<90% of ideal body weight or a body mass index≤ 20)				
· Young age when infected (0-4 years)				

TB tuberculosis, *TST* tuberculin skin test, *IGRA* interferon-γ release assay

Table 2. Online TST/IGRA Interpreter results of a sample patient

Patient Data
1. TST size of>15 mm
2. Positive IGRA test
3. 68 years old, born in Türkiye
4. BCG status; vaccinated age<2 years
5. No contact with active tuberculosis
6. Abnormal chest X-ray: fibronodular disease
7. TNF-α inhibitors (e.g., infliximab/etanercept)
Results
1. The likelihood that this is a true positive test (PPV) is: 99.71%
2. The ARDATB is estimated to be 1.78%
3. The CRATB, up to the age of 80, is: 21.36%
4. If treated with INH the probability of drug-induced hepatitis is 5% and the probability of hospitalization for drug-induced hepatitis is 2.4%

TST tuberculin skin test, *IGRA* interferon-γ release assay, *PPV* positive predictive value, *ARDATB* annual risk of development of active TB *CRATB* cumulative risk of active TB, *INH* isoniazid

Materials and methods

Subjects

One hundred-sixteen psoriasis patients treated with a biologic agent between October 2016 and March 2019 in Kayseri City Education and Research Hospital (Department of Dermatology and Venereology) were reviewed using a software called PSORTAKSIS. The software has been in use for psoriatic patients since 2016 [13]. It includes patients' data such as demographics, overall personal and familial medical history, accompanying diseases, laboratory results, previous and current treatments, etc. Records of the local tuberculosis dispensary (Melikgazi Tuberculosis Dispensary, Kayseri, Turkey) were also evaluated. The study approved by Ethics Committee of Kayseri City Education and Research Hospital.

Conventional searching procedures for LTBI

Screening LTBI was performed routinely before all biologic therapies for psoriasis according to the Turkish Psoriasis Biologic Agent Usage Guideline (2010) [14]. Diagnosis of LTBI, the decision for prophylactic anti-tuberculosis treatment, the initiation time of biologics (simultaneously or one month later, etc.), and follow-up were all performed by a pulmonologist.

All TSTs were performed at a local tuberculosis dispensary, and the QFT-GIT tests were performed at a private laboratory. TSTs were conducted conventionally by injecting five tuberculin units. The measurement of skin induration was documented 48-72 hours after injection. The cutoff for considering a result positive was determined as ≥ 10 mm for the patients; all were BCG-vaccinated. QFT-GIT tests were administered following the guidelines provided by the manufacturer. Interferon-γ value of 0.35 IU/mL or greater was defined as a positive result. In all patients, TST was repeated every three months and QFT-GIT test annually [14]. Furthermore, after the initial assessment, CXR was repeated every six months. A CT scan was obtained in patients with suspicious CXR findings.

Re-assessment of predetermined LTBI with OI-TST/IGRA

Patients previously diagnosed with LTBI by a pulmonologist underwent a re-assessment using OI-TST/IGRA, targeting those with a TST size of ≥5 mm. Forty-three patients had a <5 mm TST size and were eliminated to re-assess. Therefore, calculations were made individually for seventy-three patients. PPV, ARDATB, and CRATB values were calculated with OI-TST/IGRA version 3.0 [11]. Test results were analyzed according to the groups based on previous LTBI decisions, TST sizes, QFT-GIT results, and CXR findings.

Statistical analysis

Utilizing the "Statistical Package for the Social Sciences (SPSS ver. 21.0 for Windows, IBM Corp., Armonk, NY, USA)", we conducted analysis on the data. A level of significance was assigned to p-values, with $p<0.05$ deemed significant. Using the Shapiro-Wilk and Kolmogorov-Smirnov tests, the normality assumption for quantitative variables was assessed. The group comparisons of continuous variables in the study were carried out using the Kruskal-Wallis test, considering the fulfillment of assumptions. Following the Kruskal-Wallis test, pairwise comparisons of groups showing significant differences were executed using the Mann-Whitney U test, and the results were evaluated with Bonferroni correction (0.05/group number). The agreement between TST/IGRA (tstin3d.com) PPV value classes and LTBI, TST, QFTGT and CXR test decisions were examined using Kappa values.

Relationships between categorical variables were examined by taking into account the variable type and the number of categories of categorical variables. Relationships between continuous variables were examined with Spearman correlation analysis. Relationships between categorical variables were examined by taking into account Phi (φ) coefficient, Cramer's V correlation coefficients and Fisher Exact test results. The relationships between dichotomous nominal variables and continuous variables were examined with Point Biserial Correlation coefficients.

Results

Of the 116 patients, there was no medical history of HIV infection or immunosuppressive therapy. The country of birth was the Republic of Türkiye in all patients with no history of immigration. They were all vaccinated with BCG before two years of age and did not give a history of recent contact with an active TB patient.

Among the individuals excluded from the study due to a TST size of <5 mm (n=43), 13 received anti-TNF-α biologic treatment, while 30 underwent treatment with an interleukin (IL) antagonist. LTBI diagnoses were made in 10 of these patients; however, TST and QFT-GIT were negative for all. CXR had suspicious findings in two of them. Three patients with LTBI received anti-TNF-α biologic, and seven received IL antagonist.

Among the participants included in the study (TST size of ≥5 mm, n=73), the average age (±SD) was 45.50±11.46 years. 26 of them received anti-TNF-α biologic, which is accepted as one of the risk conditions in OI-TST/IGRA, and 47 were treated with an IL antagonist, which was not included as a risk factor. Additionally, 4 patients had diabetes mellitus which is also accepted as a risk factor in OI-TST/IGRA. The patients did not have any other conditions that could potentially increase the risk of tuberculosis.

Table 3 summarizes the statistical analysis of OI-TST/IGRA results according to the groups based on previous LTBI decisions, TST sizes, QFT-GIT results, and CXR findings. Of the patients, 89% (n=65) were grouped as LTBI-positive and 11% as LTBI-negative (n=8). The mean PPV value of the LTBI-positive group was significantly higher than the LTBI-negative group (p<0.05). Both ARDATB and CRATB values of the LTBI-positive group were higher than LTBI negative group, but this difference was not statistically significant (p>0.05).

When OI-TST/IGRA results were evaluated in terms of TST size (TST-a group: 5-9 mm, TST-b group: 10-15 mm, and TST-c group: >15 mm), the differences between TST size groups in terms of PPV and ARDATB values were found to be statistically significant (p<0.01, p<0.05, respectively). The difference between TST size groups in terms of CRATB values was not statistically significant (p>0.05). The difference between TST-a and TST-b groups in terms of mean PPV and ARDATB values was not statistically significant (p>0.05). However, mean PPV and ARDATB values of the TST-c group were significantly higher than the other groups (p<0.01, p<0.05, respectively). The mean PPV, ARDATB, and CRATB values of the QFT-GIT-positive group were significantly higher than the QFT-GIT-negative group (p<0.01). Furthermore, mean PPV, ARDATB and CRATB values of CXR-positive group were significantly higher than CXR-negative group (p<0.05, p<0.01, and p<0.01, respectively).

Correlations between LTBI decision, TST, QFT-GIT, CXR, mean PPV, ARDATB, and CRATB values are presented in Table 4. According to these coefficients, positive correlations were found between LTBI decision and mean PPV value (r=0.269, p<0.05), TST and CXR (r=0.293, p<0.05), TST and mean PPV value (r=0.310, p<0.01), TST and mean ARDATB value (r=0.323, p<0.01), TST and mean CRATB value (r=0.308, p<0.01), QFT-GIT result and mean PPV value (r=0.896, p<0.01), QFT-GIT result and mean ARDATB value (r=0.400, p<0.01), and QFT-GIT result and mean CRATB value (r=0.314, p<0.01). A positive correlation was also found between CXR and mean PPV value (r=0.272, p<0.05), CXR and mean ARDATB value (r=0.736, p<0.01), and CXR and mean CRATB value (r=0.720, p<0.01).

Patients were categorized into three groups based on their PPV as low (<10%), intermediate (10-50%), and high (50-100%) [12]. None of the patients participating in the study were in the low PPV group. There were three patients (4.1%) in the intermediate and 70 patients (95.9%) in the high group. Inter-rater reliability is determined by assessing the agreement between scores provided by two or more raters, representing the degree of consistency among evaluators [15]. The Kappa statistic, introduced by Cohen, is commonly utilized for evaluating inter-rater reliability [16]. This statistical measure gauges the extent to how observer agreement deviates from chance agreement, especially in contexts involving categorical or nominal scales. The Kappa value typically falls within the range of -1 to 1. Positive values indicate that the agreement between observers is greater than would be expected by chance, while negative values suggest that chance agreement is more prevalent. A value of 0 indicates that the

agreement between observers is at the level expected by chance. The close-to-0 Kappa coefficients in Table 5 indicate either a lack of genuine agreement among evaluators or agreement occurring at a random level. The relationship between PPV groups and LTBI decision, TST result, QFT-GIT result, and CXR finding was not statistically significant (p>0.05). When the Kappa values were examined, it was found that there was no significant agreement (p>0.05) (Table 5).

Table 3. Analysis of Online TST/IGRA Interpreter results according to the groups based on LTBI decision, TST size, QFT-GIT result, and CXR finding

		n (%)	PPV, median (minimum-maximum)	ARDATB, median (minimum-maximum)	CRATB, median (minimum-maximum)
LTBI decision	Negative	8 (11.0)	60.06 (44.75-80.10)	0.075 (0.05-0.32)	2.83 (1.79-14.12)
	Positive	65 (89.0)	69.07 (45.87-99.70)	0.30 (0.05-1.78)	8.96 (0.65-62.01)
	$p^{\&}$		0.018	0.095	0.497
TST size	TST-a group (5-9 mm)	38 (52.1)	63.18 (44.75-99.39)	0.17 (0.05-1.77)	6.16 (0.65-62.01)
	TST-b group (10-15 mm)	22 (30.1)	65.86 (49.40-99.45)	0.07 (0.05-1.24)	3.07 (1.43-42.89)
	TST-c group (> 15 mm)	13 (17.8)	84.62 (71.20-99.70)	0.89 (0.08-1.78)	22.76 (1.44-54.74)
	p^{*}		0.000	0.024	0.095
	p^{a-b}		0.311	0.975	
	p^{a-c}		0.000	0.014	
	p^{b-c}		0.000	0.010	
QFT-GIT result	Negative	53 (72.6)	64.56 (44.75-84.99)	0.24 (0.05-1.52)	7.65 (0.65-47.17)
	Positive	20 (27.4)	99.34 (99.25-99.70)	0.53 (0.10-1.78)	19.27 (2.48-62.01)
	$p^{\&}$		0.000	0.000	0.007
CXR finding	No suspect finding for tuberculosis	54 (74.0)	64.99 (44.75-99.70)	0.08 (0.05-0.81)	2.87 (0.65-17.51)
	Suspect findings for tuberculosis	19 (26.0)	70.30 (50.81-99.66)	0.92 (0.06-1.78)	32.20 (0.92-62.01)
	$p^{\&}$		0.018	0.000	0.000

p^{*}: Kruskal- Wallis, p^{a-b}, p^{a-c}, p^{b-c}, $p^{\&}$: Mann-Whitney U, PPV positive predictive value, *ARDATB* annual risk of development of active tuberculosis *CRATB* cumulative risk of active tuberculosis disease, *LTBI* latent tuberculosis infection, *TST* tuberculin skin test, *QFT-GIT* QuantiFERON-TB Gold In-tube test, *CXR* chest X-ray

Table 4. Correlation coefficients between the variables

Parameters	LTBI	TST	QFT-GIT	CXR	PPV	ARDATB	CRATB
LTBI decision	1	0.074	0.216	0.108	0.269*[a]	0.191	0.146
TST		1	0.121	0.293*[a]	0.310**[b]	0.323**[b]	0.308**[b]
QFT-GIT			1	0.196	0.896**[b]	0.400**[b]	0.314**[b]
CXR				1	0.272*[a]	0.736**[b]	0.720**[b]
PPV					1	0.531**[b]	0.130
ARDATB						1	0.801**[b]
CRATB							1

LTBI latent tuberculosis infection, *TST* tuberculin skin test, *QFT-GIT* QuantiFERON-TB Gold In-tube test, *CXR* chest X-ray
PPV positive predictive value, *ARDATB* annual risk of development of active tuberculosis, *CRATB* cumulative risk of active tuberculosis
[a] Weak positive correlation, [b] Strong positive correlation, *:<.05, **:<01

Table 5. The relationship between PPV groups and LTBI decision, TST result, QFT-GIT result, and CXR finding

		PPV				
		Intermediate (10-50%) (n=3) n (%)	**High (50-100%) (n=70) n (%)**	***P* (Fisher exact)**	**Kappa value**	***P* (Kappa)**
LTBI decision	**Negative**	1 (12.5)	7 (87.5)	0.298	0.130	0.205
	Positive	2 (3.1)	63 (96.9)			
TST result	**Negative**	3 (5.0)	57 (95.0)	0.550	0.018	0.410
	Positive	0 (0.0)	13 (100)			
QFT-GIT result	**Negative**	3 (5.7)	50 (94.3)	0.557	0.032	0.277
	Positive	0 (0.0)	20 (100.0)			
CXR finding	**Negative**	3 (5.6)	51 (94.4)	0.563	0.030	0.294
	Positive	0 (0.0)	19 (100.0)			

PPV positive predictive value, *LTBI* latent tuberculosis infection, *TST* tuberculin skin test, *QFT-GIT* QuantiFERON-TB Gold In-tube test
CXR chest X-ray

Discussion

In the diagnosis of LTBI and the recommendation of TB prophylaxis, accurately determining an individual's risk ratio for developing active TB is a crucial step. Proper identification of cases with LTBI before the anti-TNF-α treatment is a prerequisite to avoid possible over-treatment with anti-tuberculosis agents and to identify patients who would benefit from anti-tuberculosis treatment accurately. If proper identification is not performed, unnecessary cost, time-consuming, increased risk of anti-tuberculosis drug resistance, and adverse side effects may be inevitable [17]. Unfortunately, a widely accepted guideline or consensus is not existing in this issue [12]. Lee et al. [18] stated that evaluating patients on biologics for LTBI is critical to reducing the risk of active TB. They proposed clinicians consider the insufficiency of actual methods and use all available facilities, including risk factor assessment, to estimate risk extensively.

Although many current guidelines encourage LTBI screening before all biologics, OI-TST/IGRA does not regard IL antagonists as risk factors for progressing to active TB [14, 19, 20]. In a cohort study including 12.319 patients with psoriasis, psoriatic arthritis, or ankylosing spondylitis, it was found that LTBI was reported as an uncommon adverse event after secukinumab therapy. This finding contradicts earlier research that indicated secukinumab was not linked to a heightened risk of tuberculosis [21]. Cho et al. [22] reported that ustekinumab was not with an elevated risk of TB compared with the general population in South Korea. They suggested that

TB screening is not required with ustekinumab treatment, even in areas with high disease burden. As a result, conducting comprehensive studies on biological agents beyond anti-TNF-α would prevent LTBI overdiagnosis and provide a better caliber of predictors such as OI-TST/IGRA.

There are several studies in the literature investigating the reliability and confirmation of TST and IGRAs. In a meta-analysis conducted by Rangaka et al. [23], in which 15 studies with a combined sample size of 26.680 were analyzed, a rate of 4-48 TB cases per 1.000 person-years was found for IGRA positive patients, and a rate of 2-24 TB cases per 1.000 person-years was found for TST-positive patients. The authors emphasized that both IGRAs and the TST lack high accuracy in predicting active TB infection, and IGRAs could potentially decrease the number of patients recommended prophylactic treatment in certain populations. In another meta-analysis, a rate of 3.7-84.5 TB cases per 1.000 person-years was found for IGRA-positive patients, and a rate of 2-32 TB cases per 1.000 person-years was found for IGRA-negative patients [24]. The authors mentioned that IGRAs could be a more suitable choice than the TST in nations where BCG is administered after infancy or administered repeatedly. Laffitte et al. [25] observed the LTBI in 20% of patients with psoriasis and maintained to prefer IGRA instead of the TST for screening. The authors remarked that even with accurate LTBI diagnosis and prophylaxis, the risk of active TB is continued during anti-TNF-α therapy. Several guidelines were published for screening LTBI before biologics, some of which propose TST or IGRA alone, some others recommend using both of them [26].

In a study, positivity of TST was investigated in elevated levels in patients with psoriasis than in the control group [9]. This difference was suggested to have a connection with an enhanced skin response to mycobacterial antigens as opposed to Koebner's phenomenon. They adjusted a 10 mm threshold value as a rational approach in patients before biologics to avoid overdiagnosis and unnecessary TB prophylaxis. Finally, they concluded that the in vitro QFT-plus test may be preferred over TST in psoriasis patients due to higher specificity and being unaffected by disease severity.

In our study, it was aimed to use the OI-TST/IGRA before the biologic therapies in psoriasis patients. As relying solely on either TST or IGRA is insufficient, in determining the risk of LTBI, it is also important to consider the risk factors such as contact with an active TB patient, having transplant or dialysis, and receiving an anti-TNF-α therapy [27]. As indicated in Table 1, these factors and more are taken into account in the OI-TST/IGRA. Although it is recommended to make certain calibrations in the risk calculation for these multifactorial agents, OI-TST/IGRA exhibits satisfactory proficiency in distinguishing individuals with a high or low risk of being diagnosed with active TB [28].

In the present study, PPV in patients with a previous diagnosis of positive LTBI was higher than LTBI-negative patients. However, OI-TST/IGRA did not show a significant difference between LTBI positive and negative patients for ARDATB and CRATB values (Table 3). TST size is a stronger determinant in calculating the PPV. On the other hand, the algorithm calculation utilized in OI-TST/IGRA primarily considers the presence of medical and radiographic risk factors as the key indicators of active disease risk when determining ARDATB and CRATB values. Therefore, the size of the TST reaction is with modest significance for them [8].

PPV and ARDATB values were significantly higher in patients with a TST size of> 15 mm than 5-9 mm and 10-15 mm (Table 3). Surucuoglu et al. [9] suggested that adopting a 10 mm cutoff value, irrespective of BCG vaccination, would be a suitable approach in patients before biological treatment to reduce the number of patients who may receive unwarranted prophylactic treatment for TB. However, analyzing TST reactions requires thinking in three dimensions; not only the size of the skin reaction but also PPV and risk of disease related to the medical and radiographic results of individuals [8].

This study showed that PPV, ARDATB, and CRATB values of QFT-GIT-positive and CXR-positive patients were significantly higher than QFT-GIT-negative and CXR-negative patients. QFT-GIT was emphasized in many studies to be used in psoriasis patients since it is not affected by the severity of disease and its in vitro application and higher specificity [29-32]. We also found that there was no significant correlation between QFT-GIT and TST. This

finding may also be significant to underline inadequate decision-making for screening LTBI based on TST.

The analysis of Tables 3 and 4 reveals that QFT-GIT results were more effective than TST results in previous LTBI decisions. Although LTBI decisions correlated with PPV, they did not correlate with ARDATB and CRATB values. This observation may serve as an indicator of the extent to which factors other than TST and QFT-GIT were considered in the previous decision-making process for LTBI. Considering both our study results and the calculation methods of OI-TST/IGRA, it becomes apparent that radiographic and medical factors have a stronger association with ARDATB and CRATB values. It is worth noting that TST and QFT-GIT exhibit a strong correlation with PPV, ARDATB, and CRATB values. In addition, QFT-GIT exhibit a closer correlation with PPV. Further studies on OI-TST/IGRA, in which multiple factors are taken into consideration, may establish cutoff values and deliver objective results for making more precise decisions in LTBI.

Based on the findings presented in Table 5, it can be concluded that being classified in the intermediate or high PPV group does not demonstrate a substantial impact on the positivity or negativity of the LTBI, TST, QFT-GIT, and CXR test results. The Kappa coefficients being close to zero and statistically insignificant indicate that the agreement is at the expected level of random agreement. In other words, there is no significant agreement between the PPV categories and the decisions of LTB, TST, QFTGT, and CXR tests.

In conclusion, retrospectively analyzing the LTBI screening results and LTBI diagnosis decisions with online OI-TST/IGRA, important inferences can be made. Firstly, TST size alone cannot be sufficient for the diagnosis of LTBI and active TB risk. In patients >5 mm size of TST, clinical and radiographic evaluation should be considered to maintain TB risk. TST size of >15 mm may be more relevant to increased risk. Secondly, QFT-GIT and CXR are strong parameters to determine the TB risk. Thirdly, OI-TST/IGRA showed overdiagnosis of LTBI, which was diagnosed by the guideline used for the present patients. Therefore, it is thought that ARDATB and CRATB values appear to be significant parameters in the estimation of TB risk. It seems feasible to prevent the occurrence of false positive outcomes and mitigate the overdiagnosis of LTBI by comprehensively assessing the combined results of OI-TST/IGRA.

It is worthwhile to focus on the diagnosis process of LTBI in a deductive manner, reviewing current valid tools, guidelines, and perceptions to check the state of affairs. According to the literature information, this study is the first, assessing the risk of active TB in patients with psoriasis before the biologics with online OI-TST/IGRA. OI-TST/IGRA may be a valuable tool in this issue and prevent overdiagnosis and unnecessary TB prophylaxis. The limitations of our study include the small number and diversity of patients, as well as the inability to determine cutoff values for OI-TST/IGRA values in diagnosing LTBI. Prospective studies with larger and more diverse groups will yield more precise and detailed results.

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Author contributions

Designing the study and writing the first draft manuscript were performed by ZM, K.O., M.A., E.G.Y., R.E. and O.G. Data collection was performed by K.O., M.A., E.G.Y. and R.E. Material preparation was performed by Z.M., K.O., M.A., E.G.Y. and R.E. Analysis was performed by O.G. All authors provided critical feedback throughout the study, reviewed and edited subsequent drafts and approved the final draft.

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The effect of demographic characteristics of patients applying to the urology outpatient clinic on urinary incontinence awareness and attitudes

Üroloji polikliniğine başvuran hastaların demografik özelliklerinin üriner inkontinans farkındalığı ve tutumlarına etkisi

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Abstract

Purpose: This study was conducted to determine the effect of demographic characteristics of patients applying to the urology outpatient clinic on urinary incontinence awareness and attitudes.

Materials and methods: The data of this descriptive and cross-sectional study were collected between 07/09/2023-08/10/2023 with Personal Information Form, Urinary Incontinence Awareness Attitude Scale data collection tools from 180 people who applied to urology outpatient clinic by face-to-face interview method. Data were analyzed with the SPSS 25.0 package program. In all analyses, $p<0.05$ was considered statistically significant.

Results: In the factors that prevent the scale from being accepted as a health problem sub-dimension, educational status increases the scores; Educational status and frequency of caffeine&tea consumption had a decreasing effect on scores in the health motivation sub-dimension; In the coping with incontinence sub-dimension, being male lowers the scores, while education level, presence of a chronic disease and having undergone surgery increase the scores; In the restriction sub-dimension, it was found that age, marital status, place of residence, presence of chronic disease and frequency of changing underwear had a decreasing effect on scores. In the sub-dimension of fear of urinary incontinence, it was found that gender, marital status, and frequency of changing underwear had a decreasing effect.

Conclusion: Male are more likely to accept urinary incontinence as a health problem. The factors that prevent adults from accepting it as a health problem and the sub-dimensions of coping with urinary incontinence are good. It was determined that the health motivation sub-dimension and the fear of urination sub-dimension of the patients who applied to the urology outpatient clinic were at a weak level, and the restriction sub-dimension was at a medium level.

Keywords: Adult, attitudes, awareness, urinary incontinence.

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Öz

Amaç: Bu çalışma üroloji polikliniğine başvuran hastaların demografik özelliklerinin üriner inkontinans farkındalığına ve tutumlarına etkisini belirlemek amacıyla yapılmıştır.

Gereç ve yöntem: Tanımlayıcı ve kesitsel türde olan bu çalışmanın verileri 07/09/2023-08/10/2023 tarihleri arasında Kişisel Bilgi Formu ve Üriner İnkontinansla Farkındalık Tutum Ölçeği veri toplama araçları ile Üroloji polikliniğe başvuran 180 kişiden yüz yüze görüşme metodu ile toplanmıştır. Veriler SPSS 25.0 paket programıyla analiz edilmiştir. Tüm analizlerde $p<0,05$ istatistiksel olarak anlamlı kabul edilmiştir.

Bulgular: Ölçeğin sağlık sorunu olarak kabulünü engelleyen faktörler alt boyutunda eğitim durumunun puanları yükseltici olduğu; sağlık motivasyonu alt boyutunda eğitim durumu ve kafein-çay tüketimi sıklığının puanları düşürücü etki ettiği; inkontinansla başetme alt boyutunda erkek olmanın puanları düşürücü, eğitim durumu, kronik hastalık varlığı ve ameliyat geçirmiş olma durumunun puanları yükseltici olduğu; kısıtlanma alt boyutunda yaş, medeni durum, yaşanılan yer, kronik hastalık varlığı ve iç çamaşır değiştirme sıklığının puanları düşürücü etki gösterdiği bulunmuştur. İdrar kaçırma korkusu alt boyutunda ise cinsiyetin, medeni durumun ve iç çamaşır değiştirme sıklığının düşürücü etki ettiği bulunmuştur.

Sonuç: Erkeklerin üriner inkontinansı sağlık sorunu olarak kabullenmeleri daha yüksektir. Araştırma kapsamındaki yetişkin bireylerin, sağlık sorunu olarak kabulünü engelleyen faktörler, üriner inkontinans ile başetme alt boyutları iyi düzeydedir. Üroloji polikliniğine başvuran hastaların, sağlık motivasyonu alt boyutu ve ve idrar korkusu yaşama alt boyutu kötü düzeyde, kısıtlama alt boyutu orta düzeyde olduğu bulunmuştur.

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Anahtar kelimeler: Yetişkin, farkındalık, tutumlar, üriner inkontinans.

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Introduction

Urinary Incontinence (UI) is defined as the complaint of involuntary urine leakage [1]. It significantly impacts individuals' physical and mental health, disrupts their work lives, and leads to social isolation [2]. While UI affects both genders, it is more common in women. In men, it often results from damage to incontinence mechanisms during prostate cancer surgery or radiotherapy, or due to an enlarged prostate. In women, it is associated with dysfunction of the bladder or pelvic floor muscles during pregnancy, childbirth, or menopause [3].

The prevalence of UI tends to increase until middle age, stabilizes or decreases between 50 and 70 and then rises again in advanced age. The uptick in UI among the elderly may be linked to increased rates of idiopathic detrusor activity and risk factors such as diabetes, limited mobility, and medication use [4].

Due to stigma or the perception that UI is 'normal' in some societies, there are low rates of reporting, potentially leading to an underestimation of its actual prevalence [2]. Those affected by UI often deny or conceal the condition [5]. Reasons for not seeking medical attention include insufficient information about treatment options, embarrassment, considering UI symptoms as a natural age-related condition, fear of surgery, busy doctor schedules, economic constraints, and neglect by healthcare providers [6, 7]. Neglectful attitudes result in negative consequences such as psychosocial and physical restraint, social isolation, loss of self-confidence, anxiety, depression, impaired sexual life, and decreased physical activity [5].

Furthermore, individuals' denial, concealment, and unwillingness to acknowledge their incontinence problem reduce the likelihood of successful intervention, leading to either no treatment or delayed treatment [8]. The effectiveness of treatment depends on identifying the underlying causes of UI as early as possible, emphasizing the need for careful evaluation of these causes [2]. Understanding community attitudes and thoughts about UI can reshape approaches to the condition. Early diagnosis and treatment can alleviate the financial burden on the country by destigmatizing UI [9].

In conclusion, this study aims to assess the impact of urinary incontinence awareness and attitudes among patients attending the urology outpatient clinic. It is anticipated that this study will contribute valuable insights to the literature on this subject in our country.

Research question

What are adults' awareness and attitudes about urinary incontinence?

Materials and methods

Purpose and type of study

This study is both descriptive and cross-sectional, and its main purpose is to evaluate the effect of demographic characteristics of patients applying to the urology outpatient clinic on Urinary Incontinence Awareness and Attitudes.

Population and sample of the study

The study encompassed all patients admitted to the urology outpatient clinic at a training and research hospital in the western Black Sea region. The sample size was determined through a power analysis program. Referring to a previous study [10], the correlation between scales of self-esteem and factors that prevented the acceptance of the urinary incontinence scale as a health problem was found to have a low effect size (r=-0.187). Assuming a similar effect size for the relationship under investigation, it was calculated that a sample size of at least 172 individuals would provide 80% power at a 95% confidence level. Data was collected between 07/09/2023-08/10/2023. During the data collection period, 370 patients applied to the outpatient clinic, 142 patients were not

included in the study because they did not meet the inclusion criteria and 48 patients did not agree to participate in the study. Criteria for inclusion in the research are: Applying to the urology outpatient clinic as a patient, 2) Being 18 years of age or older, 4) Having no problem communicating, and 5) Accepting to participate in the research. Exclusion criteria: 1) The current situation of the patient who applied to the outpatient clinic is not suitable for communication (aggressive, unconsciousness, orientation problem). The study was ultimately conducted with 180 patients who met the inclusion criteria.

Data collection tools

Data were collected from the Personal Information Form and Urinary Incontinence Awareness Attitude Scale data collection tools by face-to-face interview method from people who applied to the urology outpatient clinic. The data were collected by the researcher using the Personal Information Form and Urinary Incontinence Awareness Attitude Scale data collection tools from people who applied to the Urology outpatient clinic by face-to-face interview method. The questions in the data collection tools were asked of the participants and filled in by the researcher. Data collection took an average of 15 minutes.

Personal information form: This form, prepared by the researcher in line with the relevant literature, includes 11 questions regarding the patients' descriptive characteristics, age, marital status, educational status, place of residence, fluid intake, and micturition characteristics. The form was filled out face to-face by the researchers [7, 9-12]. The form was filled in face-to-face by the researchers

Urinary incontinence awareness attitude scale (URINAS): The scale under consideration was developed and adapted by Aydın Avci et al. [11] in 2022. Comprising 5 sub-dimensions and 26 items, this 5-point Likert-type scale was utilized in the study. The instrument does not generate a cumulative score; it is scored based on subdimension scores as follows: (1) factors that prevent acceptance of UI as a health problem (minimum score 8, maximum score 40); (2) health motivation (minimum score 5, maximum score 25); (3) coping with UI (minimum score 6, maximum score 30); (4) restriction related to UI (minimum score 3, maximum score 15); and (5) fear related to UI (minimum score 4, maximum score 20). The internal consistency of the URINAS was evaluated using the Cronbach α for each subdimension. The Cronbach α values for the scale's 5 subdimensions vary between 0.60 and 0.92. In this study, the factors that prevent it from being accepted as a health problem sub-dimension are Cronbach's alpha value: 0.84, health motivation: 0.80, coping with urinary incontinence: 0.83, restriction: 0.79, fear of urinary incontinence: 0.72. Factors that prevent it from being accepted as a health problem, the sub-dimensions of restriction and fear of urinary incontinence include negative perceptions, while the sub-dimensions of health motivation and coping with urinary incontinence include positive perceptions. Factors that prevent it from being accepted as a health problem, restriction, and fear of urinary incontinence subscales indicate that high scores indicate that the effect is greater. Interpretation of the incontinence awareness and attitude scale is made as poor, moderate or good according to the median score. It is crucial to emphasize that the required permissions were secured for utilizing the scale.

Permission for the research was received from the Kastamonu University Clinical Research Ethics Committee Additionally, written permission was obtained from Kastamonu Training and Research Hospital, the institution where the study was conducted. The purpose and method of the study and the voluntary nature of participation were explained verbally, and in writing by the researcher, and written informed consent was obtained from the patients who agreed to participate in the study. All articles of the Declaration of Helsinki Principles were complied with throughout the study.

Data analysis

The data were analyzed using the SPSS 25.0 software package (IBM SPSS Statistics 25, Armonk, NY: IBM Corp.). Continuous variables were presented as mean, standard deviation, median, interquartile range (IQR), and minimum-maximum values, while categorical variables

were expressed as numbers and percentages. Univariate and multivariate Linear Regression analyses were employed to identify the risk factors influencing the dependent variable. Throughout all analyses, a significance level of $p<0.05$ was considered statistically significant.

Results

Of the individuals who participated in the study, 38.9% (n=70) were female and 61.1% (n=110) were male. 37.8% of the participants were between 60-69 years of age, 82.2% (n=148) were married, 47.2% were primary school graduates, and 37.2% lived in the city center. 96.7% of the participants consumed caffeine&tea, and 63.9% had chronic diseases. Of those with chronic diseases, 36.5% had diabetes and hypertension. The proportion of participants who have had surgery before is 17.2%. 61.3% of the previous surgeries were prostate surgeries (Table 1).

Among the sub-dimensions of the urinary incontinence awareness scale of the patients participating in the study, the factors that prevent it from being accepted as a health problem sub-dimension total score average is 32.23±6.16, health motivation sub-dimension total score average is 10.33±3.45, coping with urinary incontinence sub-dimension total score average is 19.8±5.63, restriction sub-dimension total score average is 9.01±3.59. Their mean total score was 10.36±3.06, fear of urinary incontinence subscale total score mean was 10.46±3.96 (Table 2).

When the factors that prevent the patients included in the study from accepting urinary incontinence awareness scale as a health problem are examined; First of all, as a result of the univariate examinations, as a result of the multivariate model established using factors that have a significant effect, it was seen that only being female had a decreasing effect on the scores preventing the acceptance of gender as a health problem (Table 3).

When the health motivation sub-dimension of the urinary incontinence awareness scale of the patients included in the research is examined, first of all, as a result of the univariate examinations and as a result of the multivariate model established using factors that have a significant effect, it was seen that only the increase in educational status had a decreasing effect on health motivation scores (Table 4).

When the incontinence coping sub-dimension of the urinary incontinence awareness scale of the patients included in the study is examined, as a result of the multivariate model established using the factors that have a significant effect, it was seen that the increase in education level, the presence of a chronic disease and having undergone surgery had an increasing effect on the coping with incontinence scores (Table 5).

When the restriction sub-dimension of the urinary incontinence awareness scale of the patients included in the study is examined, first of all, as a result of the univariate examinations and as a result of the multivariate model established using factors that have a significant effect, it was seen that only the increase in educational status had an increasing effect on the restriction scores (Table 6).

When the fear of urinary incontinence sub-dimension of the urinary incontinence awareness scale of the patients included in the study is examined, as a result of the multivariate model established using factors that have a significant effect as a result of the univariate examinations, it was seen that the increase in educational status had an increasing effect on the fear of urinary incontinence scores and the decrease in the frequency of changing underwear had a decreasing effect on the fear of urinary incontinence scores (Table 7).

When the total score of the urinary incontinence awareness scale of the patients included in the research is examined, as a result of the multivariate model established using factors that have a significant effect as a result of the univariate examinations, it was seen that being male had a decreasing effect on the total score, and increasing the education level had an increasing effect on the total score (Table 8).

Table 1. Descriptive characteristics of the participants

		n	%
Gender	Female	70	38.9
	Male	110	61.1
Age	40-49	19	10.6
	50-59	39	21.7
	60-69	68	37.8
	70-79	48	26.7
	80-89	6	3.3
Marital status	Married	148	82.2
	Single	2	1.1
	Separated from his wife	1	.6
	His wife passed away	29	16.1
Educational Status	Literate	24	13.3
	Primary school	85	47.2
	Middle school	19	10.6
	High school	43	23.9
	University	9	5.0
Living place	Bay	51	28.3
	District	62	34.4
	Town center	67	37.2
Caffeine&Tea	Yes	174	96.7
	No	6	3.3
If yes, tea and coffee	1 glass	8	4.6
	3 glasses	51	29.3
	6 glasses	46	26.4
	9 glasses	44	25.3
	15 glasses	25	14.4
Alcohol Use	Yes	16	8.9
	No	164	91.1
If Yes, Alcohol	1 double per day	14	87.5
	2 doubles a day	2	12.5
Cigarette	Yes	52	28.9
	No	128	71.1

Table 1. Descriptive characteristics of the participants (Continue)

		n	%
Frequency of admission to urology outpatient clinic	Every week	2	1.1
	Once in a month	10	5.6
	Once in 3 months	35	19.4
	Once in a year	84	46.7
	First time	49	27.2
Presence of Chronic Disease	Yes	115	63.9
	No	65	36.1
Chronic Disease	Yes	115	63.9
	No	65	36.1
Operation	Yes	31	17.2
	No	149	82.8
Surgery If yes	Prostate	19	61.3
	Kidney stone	10	32.3
	Bladder	2	6.5
Underwear	Every day	66	36.7
	Once every 3 days	70	38.9
	Once a week	43	23.9
	Every 15 days	1	0.6

n: Number of individuals, %: Percentage

Table 2. Women's awareness scale for coping with urinary incontinence scores and cronbach alpha values

	Mean±SD	Med (IQR)	Min-max	Cronbach Alpha
***Factors that prevent acceptance as a health problem**	32.23±6.16	32 (29-37.75)	14-40	0.842
***Health motivation**	10.33±3.45	11 (8-12)	5-21	0.802
***Coping with urinary incontinence**	19.8±5.63	19 (15-24)	6-30	0.834
***Restriction**	9.01±3.59	9 (6-12)	3-15	0.794
***Fear of urination**	10.46±3.96	10 (8-13.75)	4-20	0.721
Total Score	81.82±12.69	82 (74-90)	46-112	0.8

SD: Standart Deviation, Min-max: Minimum-maximum

Table 3. Comparison of some descriptive characteristics of women and factors preventing their acceptance as a health problem sub-dimension average scores

		Univariate					Multivariate				
		Std. Beta	**t**	***p***	**95% CI Lower**	**95% CI Upper**	**Std. Beta**	**t**	***p***	**95% CI Lower**	**95% CI Upper**
Factors that prevent acceptance as a health problem	**Gender**	-0.246	-3.393	0.001*	-4.915	-1.3	-0.217	-2.639	0.009*	-4.783	-0.69
	Age	-0.184	-2.504	0.013*	-1.998	-0.237	-0.16	-1.918	0.057	-1.97	0.028
	Marital status	-0.211	-2.881	0.004*	-5.718	-1.069	-0.078	-0.995	0.321	-3.741	1.233
	Educational status	0.317	4.458	0.0001*	0.958	2.48	0.162	1.918	0.057	-0.025	1.785
	Living place	-0.13	-1.755	0.081	-3.523	0.207	-	-	-	-	-
	Frequency of visiting the urology clinic	0.074	0.993	0.322	-0.509	1.539	-	-	-	-	-
	Caffeine & Tea consumption	0.123	1.649	0.101	-0.826	9.228	-	-	-	-	-
	Caffeine & Tea consumption frequency	0.101	1.327	0.186	-0.262	1.34	-	-	-	-	-
	Alcohol use	0.004	0.057	0.954	-3.102	3.288	-	-	-	-	-
	Frequency of alcohol use	0.021	0.08	0.937	-9.168	9.882	-	-	-	-	-
	Smoking	0.088	1.179	0.24	-0.804	3.192	-	-	-	-	-
	Presence of chronic disease	-0.108	-1.444	0.15	-3.259	0.505	-	-	-	-	-
	Operation	0.053	0.702	0.484	-1.55	3.26	-	-	-	-	-
	Underwear	-0.078	-1.037	0.301	-1.765	0.549	-	-	-	-	-

Linear Regression Analysis; *p<0.05 statistically significant effect; std.beta: Standardized beta coefficient; C.I: Confidence Interval

Table 4. Comparison of some descriptive characteristics of women and health motivation sub-dimension average scores

		Univariate					Multivariate				
		Std. Beta	t	*p*	95% CI Lower	95% CI Upper	Std. Beta	t	*p*	95% CI Lower	95% CI Upper
Health Motivation	Gender	0.029	0.383	0.702	-0.842	1.247	-	-	-	-	-
	Age	0.259	3.572	0.0001*	0.393	1.362	0.148	1.9	0.059	-0.02	1.032
	Marital status	0.267	3.701	0.0001*	1.123	3.691	0.127	1.601	0.111	-0.269	2.582
	Educational status	-0.298	-4.158	0.0001*	-1.333	-0.475	-0.191	-2.538	0.012*	-1.02	-0.127
	Living place	0.115	1.539	0.126	-0.23	1.863	-	-	-	-	-
	Frequency of visiting the urology clinic	-0.098	-1.319	0.189	-0.955	0.19	-	-	-	-	-
	Caffeine & Tea consumption	-0.108	-1.448	0.15	-4.89	0.752	-	-	-	-	-
	Caffeine & Tea consumption frequency	-0.245	-3.315	0.001*	-1.175	-0.298	-0.142	-1.928	0.056	-0.865	0.01
	Alcohol use	-0.093	-1.241	0.216	-2.902	0.661	-	-	-	-	-
	Frequency of alcohol use	-0.389	-1.578	0.137	-11.291	1.719	-	-	-	-	-
	Smoking	-0.044	-0.586	0.558	-1.456	0.789	-	-	-	-	-
	Presence of chronic disease	0.11	1.473	0.143	-0.267	1.841	-	-	-	-	-
	Operation	0.033	0.437	0.662	-1.049	1.647	-	-	-	-	-
	Underwear	0.117	1.577	0.117	-0.13	1.161	-	-	-	-	-

Linear Regression Analysis; *p<0.05 statistically significant effect; std.beta: Standardized beta coefficient; C.I: Confidence Interval

Table 5. Comparison of some descriptive characteristics of women and mean scores of the sub-dimension of coping with *incontinence*

		Univariate					Multivariate				
		Std. Beta	**t**	***p***	**95% CI Lower**	**95% CI Upper**	**Std. Beta**	**t**	***p***	**95% CI Lower**	**95% CI Upper**
Coping With Urinary İncontinence	**Gender**	-0.23	-3.147	0.002*	-4.298	-0.985	-0.094	-1.19	0.236	-2.888	0.715
	Age	-0.003	-0.044	0.965	-0.836	0.799	-	-	-	-	-
	Marital status	-0.038	-0.505	0.614	-2.724	1.614	-	-	-	-	-
	Educational status	0.209	2.857	0.005*	0.32	1.752	0.202	2.578	0.011*	0.235	1.768
	Living place	-0.023	-0.312	0.756	-1.987	1.445	-	-	-	-	-
	Frequency of visiting the urology clinic	-0.019	-0.26	0.795	-1.06	0.813	-	-	-	-	-
	Caffeine & Tea consumption	0.049	0.648	0.518	-3.1	6.134	-	-	-	-	-
	Caffeine & Tea consumption frequency	-0.015	-0.2	0.841	-0.826	0.674	-	-	-	-	-
	Alcohol use	0.126	1.694	0.092	-0.409	5.376	-	-	-	-	-
	Frequency of alcohol use	-0.177	-0.672	0.513	-9.286	4.858	-	-	-	-	-
	Smoking	0.018	0.245	0.807	-1.603	2.058	-	-	-	-	-
	Presence of chronic disease	0.167	2.26	0.025*	0.247	3.654	0.168	2.304	0.022*	0.282	3.654
	Operation	0.223	3.059	0.003*	1.178	5.462	0.191	2.661	0.009*	0.734	4.95
	Underwear	-0.036	-0.476	0.635	-1.313	0.803	-	-	-	-	-

Linear Regression Analysis; *p<0.05 statistically significant effect; std.beta: Standardized beta coefficient; C.I: Confidence Interval

Table 6. Comparison of some descriptive characteristics of women and constraint sub-dimension mean scores

		Univariate					Multivariate				
		Std. Beta	**t**	***p***	**95% CI Lower**	**95% CI Upper**	**Std. Beta**	**t**	***p***	**95% CI Lower**	**95% CI Upper**
Restriction	**Gender**	-0.132	-1.771	0.078	-2.046	0.111	-	-	-	-	-
	Age	-0.229	-3.137	0.002*	-1.317	-0.3	-0.037	-0.436	0.663	-0.728	0.464
	Marital status	-0.26	-3.595	0.0001*	-3.778	-1.1	-0.135	-1.716	0.088	-2.715	0.19
	Educational status	0.305	4.28	0.0001*	0.521	1.412	0.208	2.622	0.01*	0.163	1.155
	Living place	-0.185	-2.508	0.013*	-2.448	-0.292	-0.066	-0.877	0.382	-1.587	0.611
	Frequency of visiting the urology clinic	0.026	0.352	0.725	-0.492	0.705	-	-	-	-	-
	Caffeine & Tea consumption	0.018	0.234	0.815	-2.603	3.304	-	-	-	-	-
	Caffeine & Tea consumption frequency	0.111	1.463	0.145	-0.123	0.828	-	-	-	-	-
	Alcohol use	0.049	0.648	0.518	-1.25	2.472	-	-	-	-	-
	Frequency of alcohol use	-0.495	-2.13	0.051	-10.179	0.036	-	-	-	-	-
	Smoking	-0.066	-0.882	0.379	-1.689	0.646	-	-	-	-	-
	Presence of chronic disease	-0.157	-2.12	0.035*	-2.261	-0.081	-0.049	-0.647	0.518	-1.49	0.754
	Operation	-0.066	-0.888	0.376	-2.031	0.771	-	-	-	-	-
	Underwear	-0.211	-2.885	0.004*	-1.628	-0.306	-0.139	-1.835	0.068	-1.317	0.048

Linear Regression Analysis; *p<0.05 statistically significant effect; std.beta: Standardized beta coefficient; C.I: Confidence Interval

Table 7. Comparison of some descriptive characteristics of women and fear of urinary incontinence subscale score means

		Univariate					Multivariate				
		Std. Beta	**t**	***p***	**95% CI Lower**	**95% CI Upper**	**Std. Beta**	**t**	***p***	**95% CI Lower**	**95% CI Upper**
Fear of Urination	**Gender**	-0.17	-2.303	0.022*	-2.556	-0.197	-0.159	-1.872	0.063	-2.631	0.07
	Age	-0.124	-1.665	0.098	-1.052	0.089	-	-	-	-	-
	Marital status	-0.172	-2.323	0.021*	-3.274	-0.266	-0.062	-0.796	0.427	-2.258	0.96
	Educational status	0.305	4.278	0.0001*	0.573	1.554	0.217	2.617	0.01*	0.185	1.324
	Living place	-0.08	-1.071	0.286	-1.857	0.55	-	-	-	-	-
	Frequency of visiting the urology clinic	0.083	1.117	0.266	-0.285	1.028	-	-	-	-	-
	Caffeine & Tea consumption	-0.041	-0.552	0.582	-4.157	2.341	-	-	-	-	-
	Caffeine & Tea consumption frequency	0.163	2.173	0.031*	0.052	1.084	0.098	1.323	0.188	-0.167	0.847
	Alcohol use	0.053	0.708	0.48	-1.313	2.783	-	-	-	-	-
	Frequency of alcohol use	-0.051	-0.189	0.852	-5.281	4.424	-	-	-	-	-
	Smoking	0.072	0.969	0.334	-0.654	1.915	-	-	-	-	-
	Presence of chronic disease	-0.089	-1.193	0.234	-1.942	0.479	-	-	-	-	-
	Operation	-0.027	-0.354	0.723	-1.823	1.268	-	-	-	-	-
	Underwear	-0.175	-2.372	0.019*	-1.615	-0.148	-0.185	-2.331	0.021*	-1.725	-0.143

Linear Regression Analysis; *p<0.05 statistically significant effect; std.beta: Standardized beta coefficient; C.I: Confidence Interval

Table 8. Comparison of some descriptive characteristics of women and total score means of the urinary ıncontinence awareness scale

		Univariate					Multivariate				
		Std. Beta	**t**	***p***	**95% CI Lower**	**95% CI Upper**	**Std. Beta**	**t**	***p***	**95% CI Lower**	**95% CI Upper**
Total Point	**Gender**	-0.304	-4.258	0.0001*	-11.548	-4.234	-0.205	-2.74	0.007*	-9.16	-1.489
	Age	-0.124	-1.67	0.097	-3.378	0.282	-	-	-	-	-
	Marital status	-0.174	-2.354	0.02*	-10.572	-0.93	-0.076	-1.039	0.3	-7.324	2.273
	Educational status	0.348	4.946	0.0001*	2.332	5.429	0.244	3.073	0.002*	0.973	4.466
	Living place	-0.12	-1.61	0.109	-6.98	0.708	-	-	-	-	-
	Frequency of visiting the urology clinic	0.034	0.456	0.649	-1.624	2.6	-	-	-	-	-
	Caffeine & Tea consumption	0.044	0.586	0.559	-7.324	13.508	-	-	-	-	-
	Caffeine & Tea consumption frequency	0.058	0.766	0.445	-1.019	2.312	-	-	-	-	-
	Alcohol use	0.063	0.842	0.401	-3.762	9.365	-	-	-	-	-
	Frequency of alcohol use	-0.405	-1.66	0.119	-27.836	3.551	-	-	-	-	-
	Smoking	0.043	0.572	0.568	-2.929	5.322	-	-	-	-	-
	Presence of chronic disease	-0.021	-0.275	0.784	-4.439	3.352	-	-	-	-	-
	Operation	0.106	1.428	0.155	-1.362	8.495	-	-	-	-	-
	Underwear	-0.136	-1.831	0.069	-4.562	0.17	-	-	-	-	-

Linear Regression Analysis; *p<0.05 statistically significant effect; std.beta: Standardized beta coefficient; C.I: Confidence Interval

Discussion

Urinary incontinence is a physical health problem, but it also causes emotional and social problems. As its prevalence increases with age, incontinence is seen as a natural ageing process. In the literature, it has been reported that men are more likely to seek treatment for urinary incontinence than women [12-14]. In this study, being a female caused a low score in the factors preventing the acceptance of urinary incontinence as a health problem sub-dimension of the urinary incontinence awareness scale. Low scores in this dimension indicate that individuals do not accept urinary incontinence as a health problem. Similar to our study [15], in a study conducted with women, it was determined that although urinary incontinence reduces the quality of life, very few women accepted this condition as a health problem and applied to health services. Due to the reasons such as being perceived as a natural consequence of ageing/childbirth or embarrassment, women usually apply to health institutions late or not at all. In addition, only a tiny proportion of diagnosed women receive effective treatment [3, 16, 17]. This result of the study is thought to be due to the high prevalence of urinary incontinence in women and the fact that it is seen as a natural consequence of ageing and childbirth.

Health motivation is the general intention and desire to create the necessary behaviours to maintain and improve health [18]. In this study, the scores obtained from the health motivation sub-dimension of the urinary incontinence awareness scale were lower in those with higher education levels. A lower score in the health motivation sub-dimension indicates better health motivation. In a study [19] conducted with elderly patients, it was found that patients with primary school graduates had higher health motivation than illiterate patients. Previous studies have indicated that health awareness, health-seeking behaviour, and motivation increase as the level of education increases [20-22]. The results of this study are similar to the literature in this respect.

In this study, the scores obtained from the sub-dimensions of the urinary incontinence awareness scale, restriction, and fear of urinary incontinence sub-dimensions were higher in those with higher education levels. High scores from the restriction and urinary incontinence sub-dimensions indicate no fear of restriction and urinary incontinence. In the study conducted by Bulgak and Aydın Avcı (2022), it was found that the fear of urinary incontinence and restriction scores of those who graduated from primary school were higher than those who were illiterate. This result of the study is likely because the increase in health literacy as the educational level increases awareness and decreases the fear of restriction and urinary incontinence [19].

In this study, the score obtained from the coping with urinary incontinence sub-dimension of the urinary incontinence awareness scale was higher in participants who had surgery and in participants with chronic disease. High scores on the sub-dimensions of coping with urinary incontinence indicate that dealing with urinary incontinence is not good. When the literature was examined, it was stated that urinary incontinence was higher in those with chronic disease [23, 24]. This result is thought to be because treatment and costs of chronic diseases are prioritized, and urinary incontinence is seen as a natural consequence of ageing.

In this study, the score obtained from the fear of urinary incontinence sub-dimension of the urinary incontinence awareness scale was lower in participants whose frequency of changing underwear decreased. Low scores from the urinary incontinence sub-dimensions indicate that fear of urinary incontinence is experienced. The frequency of changing underwear increases in patients with urinary incontinence. In the literature, carrying and changing underwear are among the methods of coping with urinary incontinence [25-28]. This study result suggests that the fear of urinary incontinence decreases in patients with urinary incontinence by being more cautious against the possibility of urinary incontinence.

In conclusion, males are more likely to accept urinary incontinence as a health problem. The factors that prevent adults from accepting it as a health problem and the sub-dimensions of coping with urinary incontinence are at a good level. It was determined that the health motivation sub-dimension and the fear of urination sub-dimension of the patients who applied to the urology outpatient clinic were at a weak level, and the restriction sub-

dimension was at a medium level. Awareness should be raised that urinary incontinence is not a natural process and early admission to the clinic can accelerate the recovery process. Patients applying to health institutions should be questioned whether they have urinary incontinence complaints, and patients with complaints should be informed about non-pharmacological and pharmacological methods.

The limitation of the research is that it was conducted in a single center in the surrounding area. Another limitation is that the data was collected by face-to-face interview method. Additionally, the high average age of the patients can be considered a limitation. The reliability of the data is limited to the answers given by the patients. Another limitation is the lack of observation-based findings in the study. Despite all these limitations, it is thought that the study can contribute to future research by clarifying the effect of urinary incontinence awareness on attitudes with an updated scale.

Conflict of interest: The authors declare that they have no conflict of interest.

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Author contributions

M.D.I. is responsible for creating the idea and design of the study, managing the study, and analyzing the data. S.G. is responsible for preparing ethics committee documents, collecting data and entering them into SPSS. M.D.I. and S.G. are responsible for article writing, most of which is M.D.I. Authors are responsible for working together to create a format suitable for the journal.

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Examining the relationship between touch and visual perception: pareidolia perception in the social brain

Dokunma ve görsel algı arasındaki ilişkinin incelenmesi: sosyal beyinde pareidolia algısı

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Abstract

Purpose: Human perception is a complex system based on the interaction of different modalities. However, it is unclear how adults' perception of social touch influences their social dimension of visual perception. The aim of this study is to investigate the relationship between social touch perception and visual perception in the social brain.

Materials and methods: The survey study recruited 802 healthy participants. Thus, a self-report survey that included the Social Touch Questionnaire consisting of three factors and the pareidolia test were used. Pearson's Correlation and one- way ANOVA was performed for analysis.

Result: We display a statistically significant negative correlation between face reaction time, personal social touch factor, and liking personal social touch factor. An adverse important relationship emerged between pareidolia reaction time, liking of personal social touch, and social touch behavior factors.

Conclusion: According to our results, multimodal perception necessitates the simultaneous activation of multiple heteromodal associations in the social brain. Our findings can be interpreted as an interaction between the Dorsal and Ventral Attention Networks and the Social Brain Network.

Keywords: Social touch perception, visual perception, pareidolia, multimodal perception, social brain.

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Öz

Amaç: İnsan algısı, farklı modalitelerin etkileşimine dayalı karmaşık bir sistemdir. Ancak yetişkinlerin sosyal dokunma algısının görsel algının sosyal boyutunu nasıl etkilediği belirsizdir. Bu çalışmanın amacı, sosyal beyinde sosyal dokunma algısı ile görsel algı arasındaki ilişkiyi incelemektir.

Gereç ve yöntem: Çalışma 802 sağlıklı katılımcı ile gerçekleştirildi. Üç faktörden oluşan Sosyal Dokunma testini ve Pareidolia testini içeren öz bildirim anketi kullanıldı. Pearson Korelasyon ve tek yönlü ANOVA analizi gerçekleştirildi.

Bulgular: Yüzlere verilen tepki süresi ile kişisel beğenilen sosyal dokunma faktörü ve kişisel sosyal dokunma faktörü arasında istatistiksel olarak anlamlı bir negatif ilişki bulundu. Pareidoliaya verilen tepki süresi ile kişisel sosyal dokunma ve sosyal dokunma davranışı faktörleri arasında anlamlı negatif ilişki bulundu.

Sonuç: Sonuçlarımıza göre, multimodel algı, sosyal beyinde çoklu heteromodal ilişkilendirme alanları ile eş zamanlı olarak aktive olmasını gerektirmektedir. Bulgularımız, Dorsal ve Ventral Dikkat Ağları ile Sosyal Beyin Ağı arasındaki etkileşimi yansıtabilir.

Anahtar kelimeler: Sosyal dokunma algısı, görsel algı, pareidolia, multimodel algı, sosyal beyin.

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Introduction

The perception of touch has a prominent role in effective communication with both physical and social aspects of our environment. Due to social touch perception affecting empathy, bonding, communication, and social perception [1] social touch is regarded as a complex social cue. Complex social cues are defined as nonverbal or verbal indicators that are denoted by face and body to guide social interactions with others [2]. The face as another significant complex social cue is naturally perceived by vision modality for adaption to the social environment whereas social touch, another significant social cue, is dominated by tactile modality. In terms of cognitive limitations, humans tend to choose the most suitable social cues and integrate them during perception with sensory modalities working together in interaction [3]. Evidence of this interaction has been released in Dionne et al. [3] fMRI study in which a change in the Bold signal between performed simultaneous visual and tactile stimulus and single trials in the sensory-related motor task was shown. Additionally, Della Longa et al. [4] has found that social touch has a role in face perception in infants regarding complex social cues.

The pareidolia test has been used specifically in face perception studies. Pareidolia is interpreting information in a stimulus or image that does not exist as a familiar pattern [5]. Palmer and Clifford have stated that pareidolia is a phenomenon that shows how our visual system is sensitive to complex social cues and how fast we perceive them [6]. In a study conducted by Akdeniz with electroencephalography (EEG), face, and pareidolia conditions were compared and it was found that the N170 response was earlier and greater in response to faces [7]. In Liu et al. [8], study investigating face-specific neural and behavioral reactions throughout illusory visual processing, researchers compared neural and behavioral reactions of face pareidolia using letter pareidolia with fMRI and they have found specific right fusiform face activation in letter pareidolia conditions. Wardle et al. [9] also used fMRI to analyze how pareidolias are represented in category-selective areas. In addition, Wardle et al. [10] used MEG to evaluate individuals' brain activation patterns in reaction to illusory faces, non-facial items, and face images to comprehend the perception of illusory faces temporally.

Although there are many neuroimaging studies on this subject, the digital pareidolia test has not been performed until now. The purpose of this study is to investigate the relationship between touch and visual perception by using the digital pareidolia test. We have developed a new digital face pareidolia test to measure how touch and visual perception affects each other.

Materials and methods

Respondents

Data from 802 respondents have been included in the study. The sample consists of Turkish people currently residing in Türkiye. To find the relationship between visual sense perception and sense of touch perception and to find out how much the decrease in sense of touch several questions were asked to the respondents. Respondents were asked to fill out a self-report survey that took approximately 15 minutes. All surveys were formed and performed on the Qualtrics XLM platform. The survey consists of a social touch questionnaire to measure the effect and attitude to touch-related experiences (Adapted from STQ) [11] and a pareidolia test to measure visual perception. After approval by Ethics Committee of Training and Research Hospital in Türkiye, informed consent forms from the respondent were obtained.

Social Touch Questionnaire (STQ)

To examine the overall effect and attitude toward touch-related experiences, respondents were asked for 19 items rated on 5 points (1/ Not at all to 5/Extremely) from the Social Touch Questionnaire (STQ;20) [11]. The items were selected to examine various reactions to the social touch. The questionnaire contains 3 main factors: dislike of social touch, liking of personal social touch, and social touch behavior [12].

Face Pareidolia Test

To examine face pareidolia, three different types of images were shown to the respondents: face, pareidolia, and scramble. Visuals taken by the image-processing laboratory of Centro

Universitário da FEI, Sao Paolo, Brazil provided from Google Images. The luminosity of all the visuals used in the study was equalized. Every face image that was used in the study had a neutral facial expression. Scramble images were designed in MATLAB, Shine toolbox. (MathWorks, Inc., Natick, MA, USA). Pareidolia, a visual perception test with proven reliability and validity in Turkish [5] was performed. 10 images were used for each stimulus type, and a total of 30 images were shown to the respondents (Figure 1). As soon as the respondents see the images, they were asked whether they see a face in the given images. 3000 milliseconds were given to the respondents to answer this question.

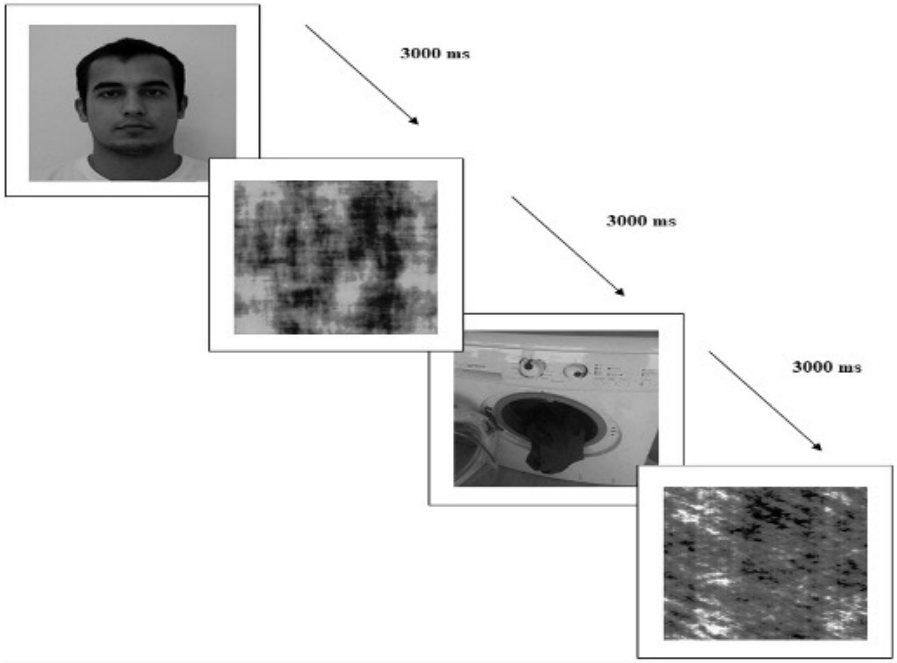

Figure 1. The example of stimuli and experimental design of the pareidolia test

Statistical analysis

Statistical Package for the Social Sciences (SPSS 22.0, SPSS Inc., Chicago, IL) was used to perform all the statistical analysis. Descriptive statistics, mean, and standard deviation were used to summarize the data. Pearson's Correlation was used for correlation analysis. One-way ANOVA analysis was used to compare the means. Eta squared was used to calculate the effect size.

Results

A total of 802 Turkish citizens (68.3% female, 31.7% male), 18-56 and above filled out the online survey named "Self-Report Touch and Vision Measurement". The socio-demographic characteristics of the participant was displayed in Table 1 and Table 2 shows the questions of the STQ. Figure 2 shows the frequency distribution of dislike of the social touch factor. 66.3% of respondents feel uncomfortable when they have to contact physically to a stranger on public transportation. Figure 3 includes the frequency distribution of liking personal social touch factor, 43% of respondents like touching animals. As shown in Figure 4, the frequency distribution of the Liking Social Touch Behavior Factor, 37.9% of respondents stated that they never feel comfortable when they are physically contacting a stranger.

The mean reaction time measurements of 802 participants are shown in Figure 5. The mean reaction time given to the face is 1250 milliseconds (1.25 seconds), the pareidolia is 1610 ms (1.61 seconds), and the scrambles is 2080 ms (2.08 seconds).

Table 1. Items of STQ

	ITEMS
I.	I feel uncomfortable if a stranger keeps holding my hand after shaking it.
II.	I feel annoyed if someone unexpectedly touches me.
III.	I feel uncomfortable if a stranger hugs me.
IV.	I feel disturbed if I have to have physical contact with a stranger person in public transportation.
V.	I get anxious if someone I have just met touches on my wrists.
VI.	I feel disturbed if a professor touches my shoulder in front of people.
VII.	I feel uncomfortable if I have to touch strangers to get their attention.
VIII.	I'd rather skip shaking hands with strangers.
IX.	I despise being tickled.
X.	I don't like when people physically contact each other in public. (e.g. hugging, kissing)
XI.	I generally received hugs from family members when I was a kid. (e.g. parents, relatives)
XII.	I kiss the cheeks of my close friends when I want to greet them.
XIII.	I like touching animals.
XIV.	I feel delighted if I give shoulder/neck massages to my friends when they are distressed.
XV.	I would like to get a professional massage if I have an opportunity.
XVI.	I describe myself as someone who loves touching while communicating.
XVII.	I like when people express their love for me physically. (e.g. hugging, kissing)
XVIII.	I generally like contacting people physically. (e.g. hugging, kissing, shaking hands)
XIX.	I am at ease making physical touch with strangers.

Table 2. Demographic table

Variables	Percentage	Respondents
Total		802
Gender		
Woman	(68.3%)	548
Man	(31.7%)	254
Age		
18-30	(63%)	505
31-40	(9.5%)	76
41-55	(22.7%)	182
56 and above	(4.9%)	39
Education status		
High School	(21.8%)	175
Graduate	(62.2%)	499
Postgraduate	(13.5%)	108
PhD	(2.5%)	20
Chronic disease		
Yes	(19%)	152
No	(81%)	650
Smoking status		
Smoker	(27.9%)	224
Nonsmoker	(72.1%)	578
Dominant hand		
Right-handed	(92.4%)	741
Left-handed	(7.6%)	61

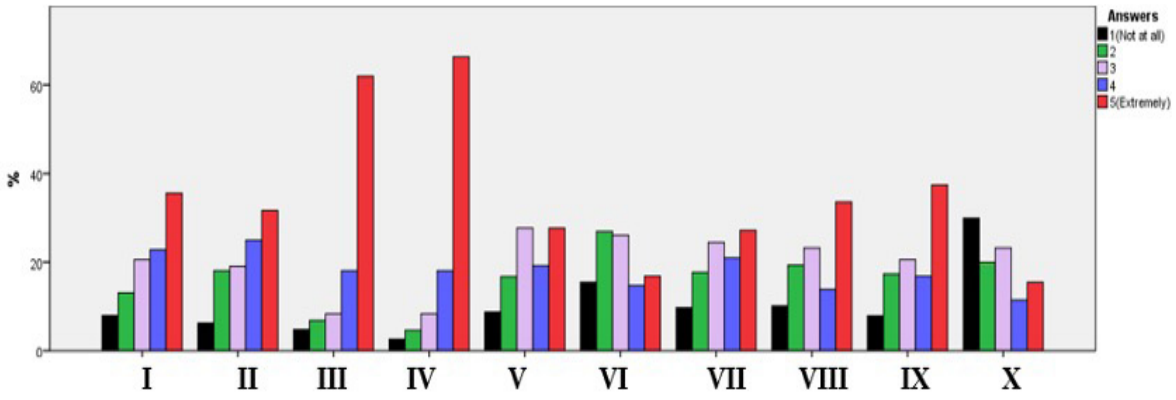

Figure 2. Frequency distribution of responses to Dislike of Social Touch Factor

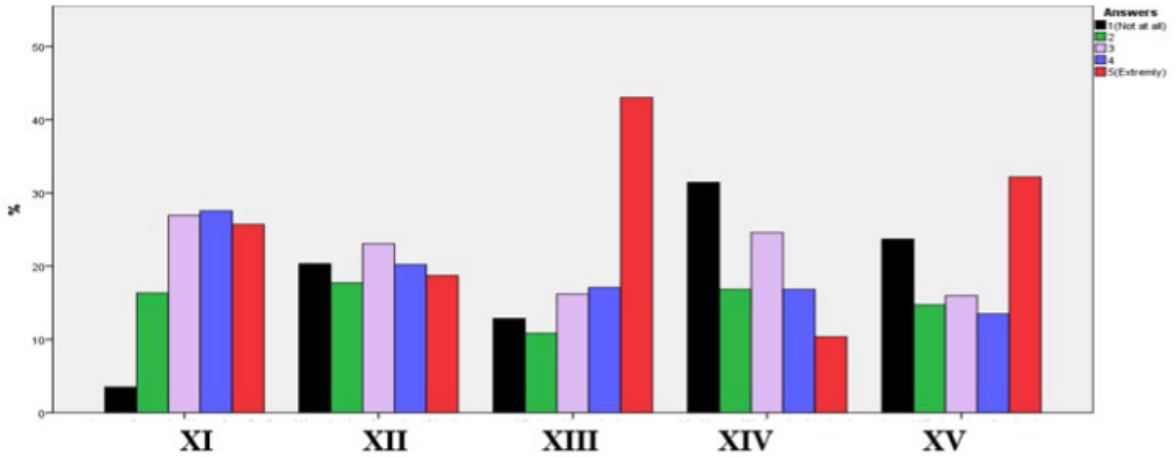

Figure 3. Frequency distribution of responses to liking of personal social touch factor

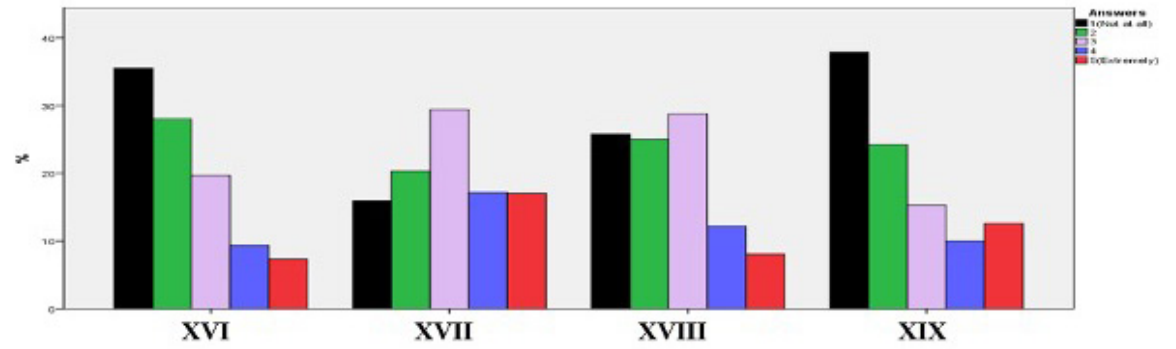

Figure 4. Frequency distribution of responses to liking of social touch behavior factor

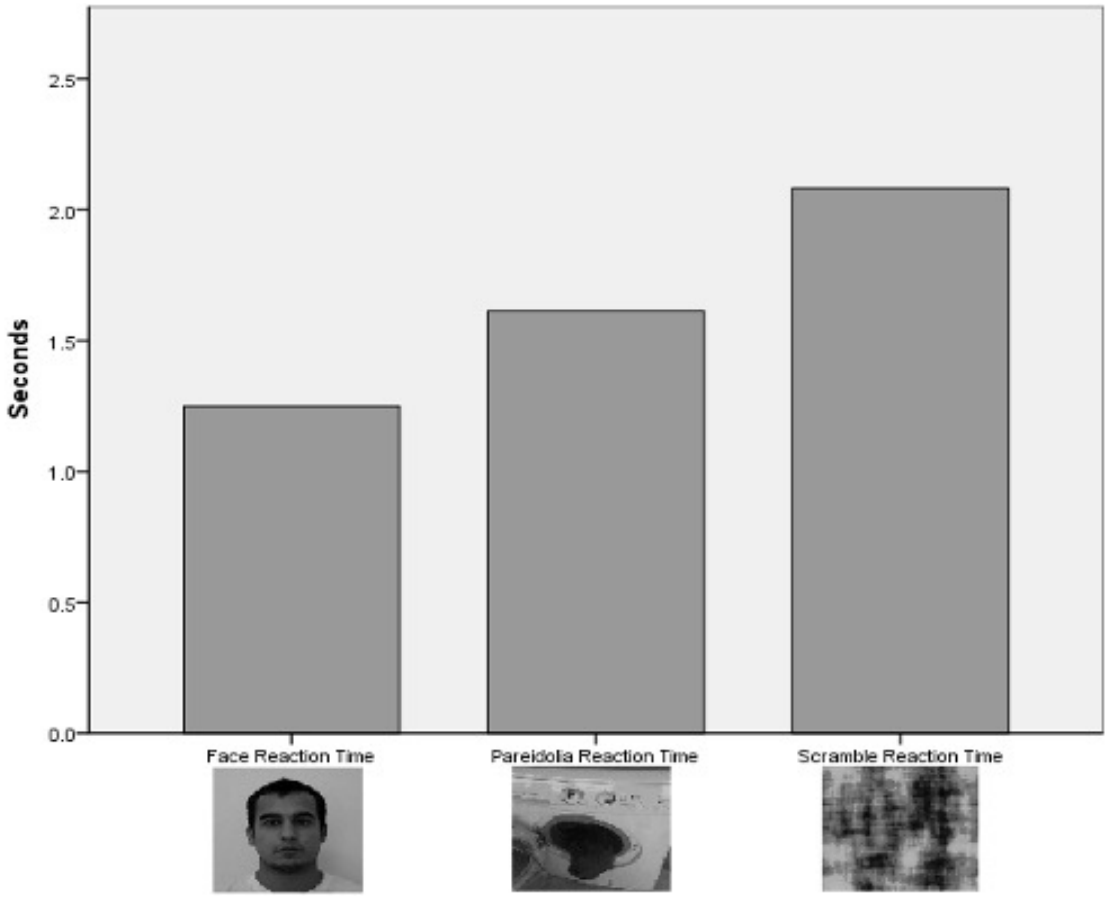

Figure 5. The mean of reaction time given to visual types in the pareidolia test

Table 3 shows Pearson Correlations related to reaction time and STQ factors. The output has revealed a positive correlation between face reaction time and dislike of touch factor. Also, there was a strong negative statistically significant correlation between face reaction time, liking of personal social touch factor, and social touch behavior. We have found a negative significant correlation between pareidolia reaction time, liking of personal social touch factor, and social touch behavior factor. There was no correlation between pareidolia reaction time and dislike of touch factor. We found a positive correlation between scramble reaction time and dislike of touch factor. Also, we have found a strong negative correlation between scramble reaction time, liking of personal social touch, and social touch behavior factors.

Reaction time for every condition (face, pareidolia, and scramble) were measured and compared with factors of social touch by means. Multiple comparisons between reaction times and STQ factors are displayed one-way ANOVA comparison in Table 4. One-way ANOVA revealed a significant difference in the main effect between pareidolia reaction time and dislike of social touch factor ($f(38.763)=1.502$, $p<0.05$). Furthermore, there is an important difference in the main effect between face reaction time and liking personal social touch factor ($f(20.781)=3.754$, $p<0.01$). Also, as a result of one-way ANOVA performed between scramble reaction time and dislike of social touch factor, a significant difference emerged between them($f(38,763)=1.726$, $p<0.05$). A significant difference emerged in the main effect between pareidolia reaction time and liking of personal social touch factor ($f(20.781)=1.513$, $p<0.01$). Also, there was a significant difference in the main effect between face reaction time and liking of social touch behavior factor ($f(16.785)=2.184$, $p<0.01$).

Table 3. Correlations related to reaction time and STQ

	M	SD	1	2	3	4	5	6
Dislike of Social Touch (1)	3.52	.802	1					
Personal Social Touch (2)	3.19	.829	**-.274****	1				
Social Touch Behavior (3)	2.53	.911	**-.394****	**.513****	1			
Face RT (4)	2.14	.547	**.070***	**-.160****	**-.093****	1		
Pareidolia RT (5)	1.62	.732	.049	**-.137****	**-.151***	-.**689****	1	
Scramble RT (6)	2.08	1.154	**.077***	**-.241****	**-.80****	**.659****	**.620**	1

M: mean, SD: standart deviation, RT: reaction time, *p<0.05, **p<0.01

Table 4. Multiple comparisons between reaction times and STQ factors

	SS	df	MS	f	*p*	η^2
Comparison of Reaction Time Means and Dislike of Social Touch Mean						
Face RT	15.449	38	.407	1.382	.065	.70
Pareidolia RT	29.881	38	.768	1.502	.028	.064
Scramble RT	84.518	38	2.224	.079	.005	.079
Comparison of Reaction Time Means and Liking of Personal Social Touch Mean						
Face RT	37.661	20	1.883	3.754	.000	.088
Pareidolia RT	12.638	20	.632	2.172	.002	.053
Scramble RT	40.262	20	2.013	1.513	.064	.038
Comparison of Reaction Time Means and Liking of Social Touch Behavior Mean						
Face RT	18.296	16	1.143	2.184	.005	.043
Pareidolia RT	7.068	16	.442	1.489	.097	.029
Scramble RT	25.504	16	1.594	1.201	.261	.024

RT: reaction time, SS: sum of the square, MS: mean of square, *p<0.05

Discussion

This study is the first to research a self-report test for measuring touch and vision perception and the relationship between visual perception and the touch experience of participants. The study demonstrates a negative correlation between face reaction time, liking of personal social touch factor, and social touch behavior. The more people dislike social touch, the longer they react to faces. To the best of our knowledge, the present study is the first study on the neuropsychological evaluation of touch and vision perception in the brain performed on a digital platform by now in Türkiye.

In the current study, we used STQ to examine attitudes towards the social touch. According to the dislike of social touch factor in STQ, the highest 'extremely' response given by participants was 'I feel disturbed if I have to have physical contact with a stranger person in public transportation' with 66%. In this context, Ceccato, Näsman, and Langefors conducted a study to assess patterns of sexual victimization in public transportations of a total

of 1122 college students in Sweden. The results revealed that 22.2% of participants experienced physical sexual harassment while using public transportation [13]. This kind of physical violation may be one of the reasons why our participants do not want to have physical contact with a stranger on public transportation. In addition, 61.6% of women stated that they have been exposed to this kind of violation at least once in the previous three years, while the ratings for men have affected aspects faces by only 26.6%. As a result, women, the majority of participants in the study, were found to be at higher risk with regard to the violation. Based on these findings, the reason why our participants avoid physical contact in public transportation can be explained in terms of gender differences [13]. Furthermore, strangers in public places like transportation can be a risk factor in terms of health. Harvey et al. [14] conducted a longitudinal study where 1815 people were observed in 12 different public areas, including a metro station, and RNA samples were taken from 348 surfaces to detect whether the COVID-19 virus was on those surfaces. 52% of these samples tested positive for COVID-19 at least once. These surfaces were touched 781 times with bare hands during observation. The study showed that touching surfaces with bare hands might be the secondary route for COVID-19 transmission. This may be a second reason for participants feeling uncomfortable being touched in public places like transportation during the COVID-19 pandemic. Moreover, 62% of our participants stated that they feel uncomfortable if a stranger hug them. Consistently, Suviletho et al. [15] conducted a cross-cultural study with a total of 1368 participants, in which 13 body regions were asked to be colored in order to determine which area can be touched by family, friends, acquaintances, and strangers. The touchability index was calculated based on the coloring measurement. Results have shown that emotional closeness explained the width of the touchable areas by 92%. Based on this finding, strangers were permitted to touch limited areas due to a lack of emotional closeness, which explains a reason why participants may be uncomfortable with the touch of strangers. Additionally, 33% of our participants stated that they avoid shaking hands with strangers. According to a cross-cultural study conducted on 2736 people in Türkiye, Iran, and Afghanistan in 2021, 80.1% of Turkish participants (1080) has stated that there was a tendency to avoid shaking hands during COVID-19 [16]. Health protection/ disease prevention might be an explanation for the lower percentage of handshaking avoidance in our study.

Furthermore, 35% of participants feel uncomfortable when a stranger keeps holding their hands after shaking them. This result is also consistent with Nagy et al. [17], cross-cultural study that examined 188 handshakes and found that a normal handshake lasted 3 seconds. According to Wundt, about 2.5 seconds is a duration that is needed for grouping complex stimuli. However, complex stimuli lasting longer than 2.5 seconds were perceived separately [18]. This duration is important for all modalities to perceive a group of stimuli. In visual perception, 3 seconds is duration to change the point of view when perceiving ambiguous visuals [19]. The participants have stated that they were uncomfortable because the longer-than-expected handshake violates temporal communication.

According to findings of the Liking Personal Social Touch Factor in STQ, the highest ‘extremely’ response given by participants was ‘I like touching animals’ with an average of 46%. Odendaal et al. [20] conducted a study to measure changes in blood levels before and after petting dogs and significant changes in dopamine, prolactin, oxytocin, and plasma b-endorphin were revealed [20]. This might be a reason that our participants liked petting animals. Another finding shows that 43% of our participants stated that they would like to get a professional massage if they had an opportunity. This finding is consistently supported by an fMRI study conducted by Lindgren et al. [21] which emphasized that the pregenual anterior cingulate cortex (pgACC) is activated after a massage. pgACC is the brain region including high amounts of opioid receptors serving as targets of opioids which are the substances used for pain relief with risk of addiction [22]. Our participants may have stated that they would like to get a professional massage for this reason. Surprisingly, the highest ‘not at all’ response given by participants was ‘I feel delighted if I give shoulder/neck massages to my friends when they are distressed’ with 31.4% in this factor.

According to findings of the Liking Social Touch Behavior Factor in STQ, the highest 'not at all' response was 'I am at ease making physical touch with strangers.' with an average of 37.9%. A study by Heslin et al. [23] investigated the meaning of touch from strangers or close friends of the opposite or same-sex in which 208 participants evaluated the touch of different body parts from strangers or close friends as pleasing or unpleasant. A touch from strangers of the same sex was found to be unpleasant for all participants. On the other hand, while women found touching strangers of the opposite sex uncomfortable, men found it pleasant. In our study, the majority of the participants were women (68.3%). However, a detailed investigation of this gender difference will contribute to the literature in future studies. The second highest 'not at all' response given by participants was 'I describe myself as someone who loves touching while communicating' with an average of 35.5%. In 2015, the Comfort with Interpersonal Touch scale was developed to measure individual differences that affect tactile communication. During the reliability and validity study of this scale, it was found that age, gender, and personality had an effect on touching while communicating. As a result, women were more comfortable touching while communicating compared to men, and older participants were more comfortable touching while communicating compared to young participants [24]. Additionally, culture plays a significant role in terms of attitudes towards touch. Sidney Jourard conducted an observational pilot study to reveal how culture influences the frequency of touching. In the study, couples were observed in coffee shops in four different locations in London (England), Paris (France), San Juan (Puerto Rico), and Gainesville (USA). Results showed that couples in London touched each other 0 times, couples in Gainesville touched each other 2 times, couples in Paris touched each other 120, and those in San Juan touched each other 180 times in an hour [25]. For the repetition of this study, the difference between rural and urban areas in the USA was examined where 52 people were observed to measure the frequency of touching during communication. The frequency of touching in the countryside was 43 while it was 19 in the city. Hence, touching while communicating may even vary according to the place of residency [26]. It is beyond the scope of this study to analyze demographic variables such as age, gender, personality traits, and culture.

To examine visual perception, we used the pareidolia test. According to reaction times given to stimuli, we found that the fastest response was given to the face among other stimuli. Consistent with these findings, Akdeniz has found that the face stimulus elicited an earlier N170 response than face pareidolias in EEG [7]. Our findings show that the participants gave a reaction to pareidolia stimulus on average of 1610 ms. However, Guillon et al. [27] found that the average reaction time given to upright pareidolia stimuli in typically developing children was 1303 ms. This delay may be due to the digital presentation of the stimuli in our study. Our results revealed that as the liking of social touch increased, the reaction time to face and pareidolia decreased. In a study by Della Longa et al. [4], two experimental conditions were applied to 40 infants. In both experimental conditions, infants were shown videos of two different unfamiliar female faces. The first video was given with tactile stimulation whereas the second one was given without tactile stimulation, and cardiac responses of the infants were measured during these experimental conditions. The two experimental conditions were separated according to the type of touch given in the first video. The tactile stimulus in the first experimental condition involved social touch by gently stroking the infants' heads by the researcher, while the tactile stimulus in the second experimental condition involved tapping the infants' heads with a brush. Afterward, infants were shown women's faces that were used in experimental conditions to assess their visual choice. As a result, infants looked longer at the faces shown with social touch and they elicit lower cardiac responses. Thus, it has been shown how social touch perception can affect visual perception in infants [4]. The present study used a different paradigm to investigate how adults' perceptions of social touch affect visual perception, and it demonstrates comparable findings. Similarly, Nava et al. [28], conducted a study that includes both adults and infants and investigates whether the social aspect of visual and tactile cues modulates the physiological response. A video clip of an unfamiliar woman as a social cue and a house that resembles a human face (pareidolia) as a non-social cue was shown to all participants. Tactile stimuli

were presented as social and non-social touch by researchers during the presentation of visual stimuli. Electrodermological responses of participants were also measured via using electrodes to measure responses given to tactile stimuli. The results revealed that electrodermal responses of infants decreased in terms of a socially meaningful visual stimulus combined with a social touch that can generate calming responses. On the other hand, adults showed a greater electrodermal response to social touch for every visual stimulus compared to the non-social touch condition. These results indicate a difference between infants and adults in terms of the effect of social touch on visual perception. Moreover, infants' behavioral responses were always strongly directed towards the face, regardless of the type of touch received. This study suggests that visual and social touch perception affects each other on a physiological level, not on a behavioral level. However, the reaction time measured in our study shows that this effect is also reflected in the behavior of adults. We have established that the more people dislike social touch, the longer they react to faces in the social brain.

We draw the conclusion that our new neuropsychological paradigm is a beneficial test to enlighten the relationship between social touch and visual perception. We hypothesize that our findings obtained from participants rely on the relation between the brain networks, which are activated with Social Brain Network and Dorsal (DAN) and Ventral Attention Networks (VAN) [29]. Social Brain Network includes temporoparietal junction, superior temporal sulcus, medial prefrontal cortex (mPFC), and anterior cingulate cortex (ACC). While the VAN includes temporoparietal junction (TPJ) and the ventral frontal cortex, the DAN has intraparietal sulcus (IPS) and the frontal eye fields (FEF) [30, 31]. It is possible that these brain networks can be integrated with the interaction of social touch and visual perception.

Here we have developed a self-report test for measuring touch and vision perception and the relationship between visual perception and the touch experience of participants was evaluated with this newly developed test. Specifically, our results are consistent with a causal role of touch perception in the emergence of visual perception and suggest that the amelioration of touch perception will result in the relief of fundamentally misunderstanding visual perception. Because there remains a lack of evidence for the use of medical imaging modalities, such studies are still needed. Further research on multiple perceptions will almost certainly improve our understanding of complex social cues and perceptions.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

G.A constructed the main idea and hypothesis of the study. O.D.D and A.C.F developed the theory and arranged/edited the material and method section. G.Y has done the evaluation of the data in the results section. Discussion section of the article was written by T.K.Y.

P.O reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Case Report

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Traumatic knee dislocation

Travmatik diz çıkıkları

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Abstract

Traumatic knee dislocations are serious injuries that threaten the entire extremity, requiring urgent evaluation and a multidisciplinary approach. The high frequency of limb-threatening vascular injury, misdiagnosis of knee injury, or failure to assess the limb's vascular status will result in a large number of potentially preventable amputations. We tried to demonstrate with case examples that this situation should be investigated carefully and aggressive treatment is mandatory in our study. 8 patients who applied to Pamukkale University Orthopedics and Traumatology Clinic and Alasehir State Hospital Orthopedics and Traumatology Clinic in the last 3 years due to traumatic knee dislocation were evaluated. It was revealed that the group with multiple ligament injuries and neurovascular injuries, which is in the subgroup according to the Schenck classification, was the worst injury group according to the postoperative evaluation tests. It has been shown that postoperative Lysholm and Cincinnati scores of patients in the KD II group with isolated cruciate ligament injury without collateral ligament injury were better than other injuries. Among the patients in the KD I group, it was shown that the postoperative Lysholm and Cincinnati scores were relatively better in the group with anterior cruciate ligament (ACL) injury compared to the group with posterior cruciate ligament (PCL) injury. Management of these serious injuries, the importance of post-operative rehabilitation comes to the forefront with the early and appropriate intervention of experienced orthopedic surgeons with a multidisciplinary approach, as well as the high awareness in terms of neurovascular injuries.

Keywords: Knee dislocation, knee joint, vascular injury, trauma.

Bektas M, Gungor HR, Gem K. Traumatic knee dislocation. Pam Med J 2024;17:173-184.

Öz

Travmatik diz çıkıkları, acil değerlendirme ve multidisipliner bir yaklaşım gerektiren ciddi ve ekstremitenin tamamını tehdit eden bir yaralanmadır. Ekstremiteyi tehdit eden vasküler yaralanmanın yüksek sıklığı, diz yaralanmasının yanlış teşhisi veya ekstremitenin vasküler durumunun değerlendirilmesinin yapılamaması, çok sayıda potansiyel olarak önlenebilir amputasyonla sonuçlanacaktır. Çalışmamızda bu durumun dikkatli bir şekilde araştırılması gerektiği ve agresif tedavisinin zorunlu olduğu vaka örnekleriyle ortaya konulmaya çalışılmıştır. Pamukkale Üniversitesi Ortopedi ve Travmatoloji Kliniği ile Alaşehir Devlet Hastanesi Ortopedi ve Travmatoloji Kliniğine son 3 yıl içerisinde travmatik diz çıkığı nedeniyle başvurmuş olan 8 hasta değerlendirilmiştir. Çoklu bağ yaralanmasının yanı sıra nörovasküler yaralanmaların eşlik ettiği grubun operasyon sonrası değerlendirme testlerine göre en kötü yaralanma grubu olduğu ortaya konmuştur. Yan bağ yaralanmasının eşlik etmediği izole çapraz bağ yaralanmasının olduğu Schenck sınıflamasına göre KD II grubu hastaların operasyon sonrası Lysholm ve Cincinnati skorlarının diğer yaralanmalara kıyasla daha iyi olduğu gösterilmiştir. KD I grubunda yer alan hastalardan ön çapraz bağ (ACL) yaralanması olan grubun arka çapraz bağ (PCL) yaralanması olan gruba kıyasla operasyon sonrası Lysholm ve Cincinnati skorlarının daha iyi olduğu gösterilmiştir. Bu zorlu ve ciddi yaralanmaların başarılı bir şekilde yönetiminde nörovasküler yaralanma açısından farkındalığın fazla olmasının yanı sıra multidisipliner yaklaşımla diz ligaman yaralanmalarına özel ilgi duyan tecrübeli ortopedi cerrahlarının erken ve uygun müdahaleleriyle birlikte operasyon sonrası rehabilitasyonun önemi ön plana çıkmaktadır.

Anahtar kelimeler: Diz çıkığı, diz eklemi, damar yaralanması, travma.

Bektaş M, Güngör HR, Gem K. Travmatik diz çıkıkları. Pam Tıp Derg 2024;17:173-184.

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Introduction

Knee dislocations are serious and threatening to the entire extremity and require an emergency assessment and a multidisciplinary approach. It is known that the incidence of traumatic knee dislocations, which is considered to be rare, is less than 0.02% among all musculoskeletal injuries [1, 2].

Knee dislocation is predominantly seen in the young population and the male/female ratio is 4:1 [3]. 75% of these injuries are caused by high-energy traffic accidents. 25% are caused by low-energy sports injuries and falls [4]. The risk of vascular damage can reach 65% in high-energy injuries [5].

When the knee dislocations are seen as isolated injuries, the diagnosis is relatively easy; diagnosis may be delayed, especially if spontaneous reduction occurs in multi-trauma patients. This may cause the accompanying popliteal artery injury to go undetected and even cause loss of the extremity.

Knee dislocation is the deterioration of the tibiofemoral joint integrity. Knee ligaments that provide joint integrity: anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), posterolateral corner (PLC) (lateral collateral, popliteus and popliteofibular ligament), medial collateral ligament (MCL). Disruption of joint integrity is considered a multiple ligament injury in which two or more of the knee ligaments are injured.

Anatomical classification was developed by Kennedy [6] in 1963 based on the relative orientation of the tibia to the femur (anterior, posterior, lateral, medial, rotatuvar / anteromedial, posteromedial, posterolateral). Although this classification is simple, it was not sufficient in terms of prognosis and treatment plan. The most widely accepted classification was made by Schenck [7] (Table 1).

Table 1. Schenck anatomic classification system for knee dislocations

Type	Description
KD I	Knee Dislocation with either cruciate intact
KD II	Bicruciate injury with collaterals intact
KD III	Bicruciate injury with one collaterals ligament injury
	KD IIIM Bicruciate injury with medial collateral ligament injury
	KD IIIL Bicruciate injury with lateral collateral ligament injury
KD IV	Bicruciate injury with both collaterals ligament injury
KD V	Periarticular fracture dislocation

Associated injuries: C: Arterial injury, N: Neural injury

Multiple ligaments injured knee dislocation treatment continues to be a challenging situation with many unanswered discussions. The management of this serious trauma and operational procedures can be challenging.

There are still discussions on the time of surgery, reconstruction technique and appropriate graft selection. The result, complications may be common and the morbidity rate of these injuries is high [8].

This study reviews the treatment processes of patients who applied to our clinic with multiple ligament injuries of the traumatized knee and evaluates their clinical outcomes.

Materials and methods

8 patients who applied to the Orthopedics and Traumatology Clinic of Pamukkale University and Alaşehir State Hospital in the last 3 years due to post-traumatic knee dislocation were evaluated in our study. First of all, informed consent forms were obtained from all patients. The patients were checked in the 1st, 3rd and 6th months after the operation.

Lysholm, Cincinnati, Visual Analogue Scale (VAS) and Tegner Activity Level scores were determined before and after the operation and at the 3rd and 6th months.

Results

The first patient with knee dislocation who was operated on in the last 3 years by us was a 27-year-old male patient who was brought to the emergency room after a motorcycle accident, and the dislocated knee was reduced by us in the first examination. The patient whose general condition is stabilized is in the KD I group according to the Schenck classification. The lateral collateral ligament (LCL), medial collateral ligament (MCL), anterior cruciate ligament (ACL), lateral meniscus and biceps femoris tendon of the patient who underwent surgical intervention were repaired. The operation was performed on the 7th day of admission to the hospital. The patient also has a subtrochanteric fracture of the left femur was repaired. Lysholm and Cincinnati scores of the patient before and after treatment are given in Table 2 and Table 3. The patient's VAS score was found to be 1 (0-no pain, 10-severe pain). Tegner Activity Level was found to be 4.

Table 2. Patients Cincinnati scores

CINCINNATI SCORE	Before Treatment	After Treatment	After 3 Month	After 6 Month
1st Patient	0	6	28	28
2nd Patient	0	0	6	9
3rd Patient	0	0	17	24
4th Patient	0	6	28	28
5th Patient	0	6	28	28
6th Patient	0	6	21	22
7th Patient	0	6	23	28
8th Patient	0	6	21	22

Table 3. Patients Lysholm scores

LYSHOLM SCORE	Before Treatment	After Treatment	After 3 Month	After 6 Month
1st Patient	25	37	100	100
2nd Patient	0	7	34	42
3rd Patient	0	12	34	55
4th Patient	25	37	100	100
5th Patient	25	37	100	100
6th Patient	0	17	50	73
7th Patient	9	26	64	83
8th Patient	0	17	50	66

The second patient was a 40-year-old male patient and his lower extremity was stuck in the agricultural machine. After the patient's emergency department admission, the popliteal artery was repaired urgently by the cardiovascular surgery unit and then an external fixator application was performed by us. The patient is in the subgroup C+N according to the Schenck classification. The patient underwent fasciotomy due to the development of compartment syndrome in the right lower extremity. Following the regression of the patient's circulatory problems, fasciotomy closure was performed. The peroneal nerve, posterolateral corner elements and medial collateral ligament (MCL) were repaired 10 days after the injury. Debridement procedures were performed many times due to the patient's wound problems. Therefore, anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL) repairs had to be left to the next session. Lysholm and Cincinnati scores of the patient before and after the treatment are given in Table 2 and Table 3. The patient's VAS score was 8 (0-no pain, 10-severe pain). Tegner Activity Level was found to be 0. Preoperative and postoperative knee radiographs and preoperative MRI of the patient are shown in Picture 1.

Picture 1. Preoperative and postoperative knee radiographs and Preoperative MRI of the 2nd patient

The third patient was a 46-year-old female patient who applied to the emergency department after motorcycle accident. The dislocated knee was reduced in the first examination of the patient. Further examinations of the patient included medial collateral ligament (MCL), lateral collateral ligament (LCL), anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), lateral meniscus were found to be ruptured and repaired. The operation was performed on the 5th day of admission to the hospital. The patient is in the KD IV group according to the Schenck classification. Lysholm and Cincinnati scores of the patient before and after the treatment are given in Table 2 and Table 3. The patient's VAS score was found to be 2 (0-no pain, 10-severe pain). Tegner Activity Level was found to be 4. Preoperative MRI, radiographs and postoperative radiographs of the patient are shown in Picture 2.

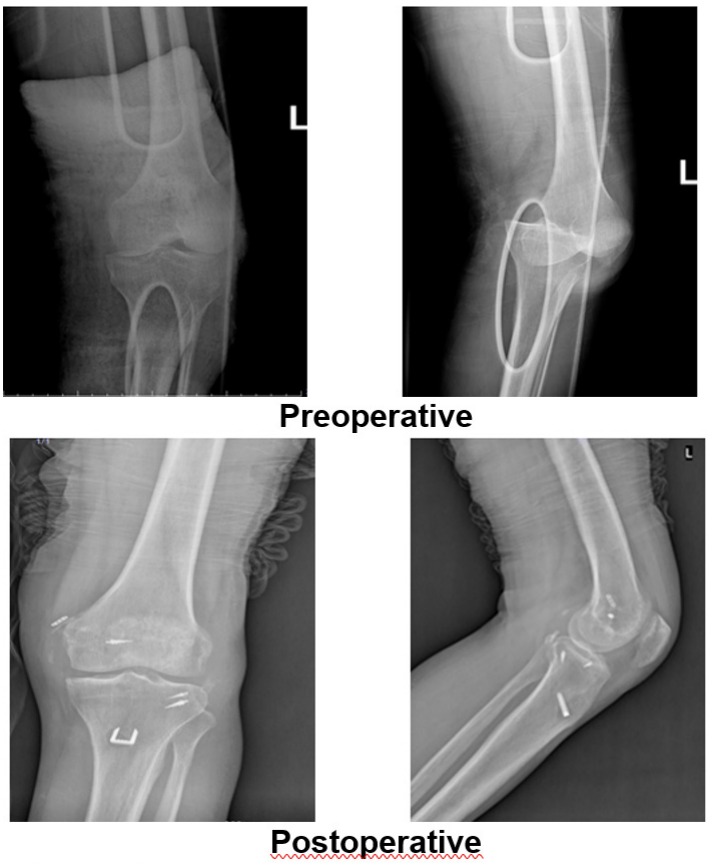
Picture 2. Preoperative MRI, radiographs and postoperative radiographs of the 3rd patient

The fourth patient was a 23-year-old female patient. She applied to the emergency service due to traffic accident. It was learned from the primary emergency physicians that the knee dislocation had spontaneous reduction in the first application of the patient. Anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL) tears were detected and repaired. The operation was performed on the 5th day of admission to the hospital. The patient is in the KD II group according to the Schenck classification. Lysholm and Cincinnati scores of the patient before and after the treatment are given in Table 2 and Table 3. The patient's VAS score was found to be 1 (0-no pain, 10-severe pain). Tegner Activity Level was found to be 5. Preoperative MRI, radiographs and postoperative radiographs of the patient are shown in Picture 3. Postoperative radiographs were taken after PCL surgery was performed before ACL surgery was performed.

Picture 3. Preoperative MRI, radiographs and postoperative radiographs of the 4th patient

The fifth patient was a similar patient to the previous patient and he was 30 years old. He was brought to the emergency room after traffic accident. Anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL) tear was detected and repaired. The operation was performed on the 3th day of admission to the hospital. The patient is in the KD II group according to the Schenck classification. Lysholm and Cincinnati scores of the patient before and after the treatment are given in Table 2 and Table 3. The patient's VAS score was found to be 1 (0-no pain, 10-severe pain). Tegner Activity Level was found to be 4.

The sixth patient was a 32-year-old male patient who applied to the emergency department after falling from the stairs. Knee dislocation was diagnosed in the patient with severe knee pain and a reduction was performed. The patient also has a distal radius fracture. The patient's posterior cruciate ligament (PCL), medial collateral ligament (MCL), lateral collateral ligament (LCL) tear was detected and repaired. The operation was performed on the 6th day of admission to the hospital. The patient is in the KD I group according to the Schenck classification. The patient's Lysholm and Cincinnati scores before and after treatment are shown in Table 2 and Table 3. The patient's VAS score was found to be 5 (0-no pain, 10-severe pain). Tegner Activity Level was found to be 3. The knee radiographs of the patient before and after the operation are shown in Picture 4.



Picture 4. Preoperative and postoperative knee radiographs of the 6th patient

The seventh patient was a 27-year-old male patient. He was brought to the emergency room after traffic accident. The knee dislocation of the patient who was unconscious was reduced. The patient had pneumothorax and emergency treatment was applied. Further examinations revealed damage to the anterior cruciate ligament (ACL), medial collateral ligament (MCL) and lateral collateral ligament (LCL). It was subsequently repaired. The operation was performed on the 12th day of admission to the hospital. The patient is in the KD I group according to the Schenck classification. Lysholm and Cincinnati scores of the patient before and after the treatment are given in Table 2 and Table 3. The patient's VAS score was found to be 2 (0-no pain, 10-severe pain). Tegner Activity Level was found to be 5. Preoperative and postoperative radiographs of the patient are shown in Picture 5.

Picture 5. Preoperative and postoperative knee radiographs of the 7th patient

The last patient was a 33-year-old female patient who applied to the emergency service after motorcycle accident. Anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), lateral collateral ligament (LCL), lateral meniscus tears were detected and repaired. The operation was performed on the 9th day of admission to the hospital. The patient is in the KD III L group according to the Schenck classification. Lysholm and Cincinnati scores of the patient before and after the treatment are given in Table 2 and Table 3. The patient's VAS score was found to be 3 (0-no pain, 10-severe pain). Tegner Activity Level was found to be 3. Preoperative radiographs, MRI and postoperative radiographs of the patient are shown in Picture 6.

Picture 6. Preoperative radiographs, MRI and postoperative radiographs of the 8th patient

ACL reconstruction with arthroscopic transtibial femoral tunnel method and PCL reconstruction with arthroscopic transtibial tunnel technique were applied as operation techniques. Tibial avulsions of the PCL were repaired with cannulated screws with arthroscopic support. Posterolateral and posteromedial corner repairs could be primarily repaired with the help of anchors without the use of grafts.

As a physical therapy protocol, immobilization in extension and weight restriction were applied to all patients in the first 6 weeks after the operation. Then, controlled weight loss, increase in ROM, gain of proprioceptive sense and regaining joint range of motion were focused on under the control of the physical therapy unit.

VAS scores and Tegner Activity Level of the patients are given in Table 4 and Table 5.

Table 4. Patients Visual Analogue Scale scores

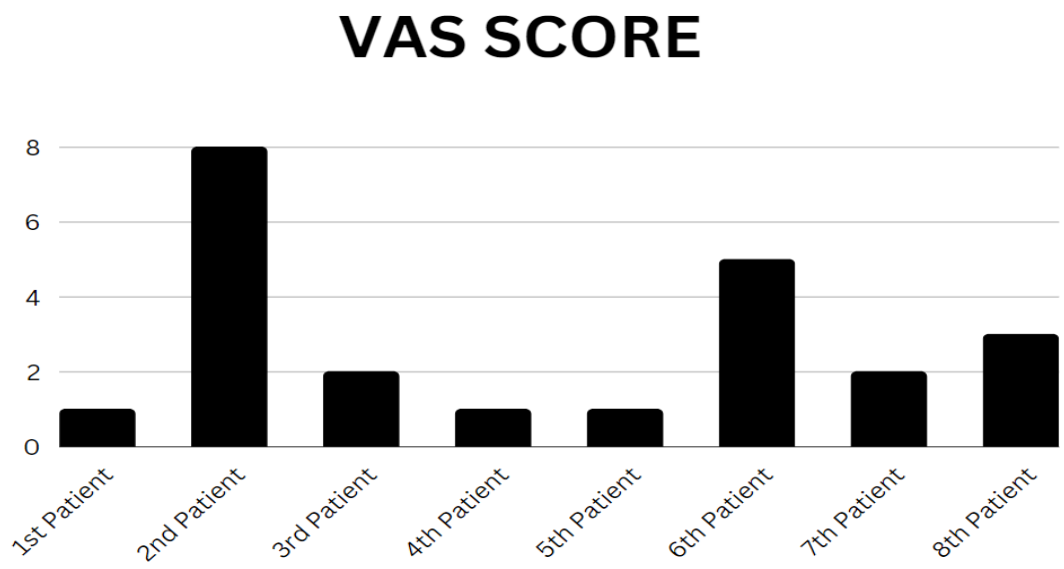
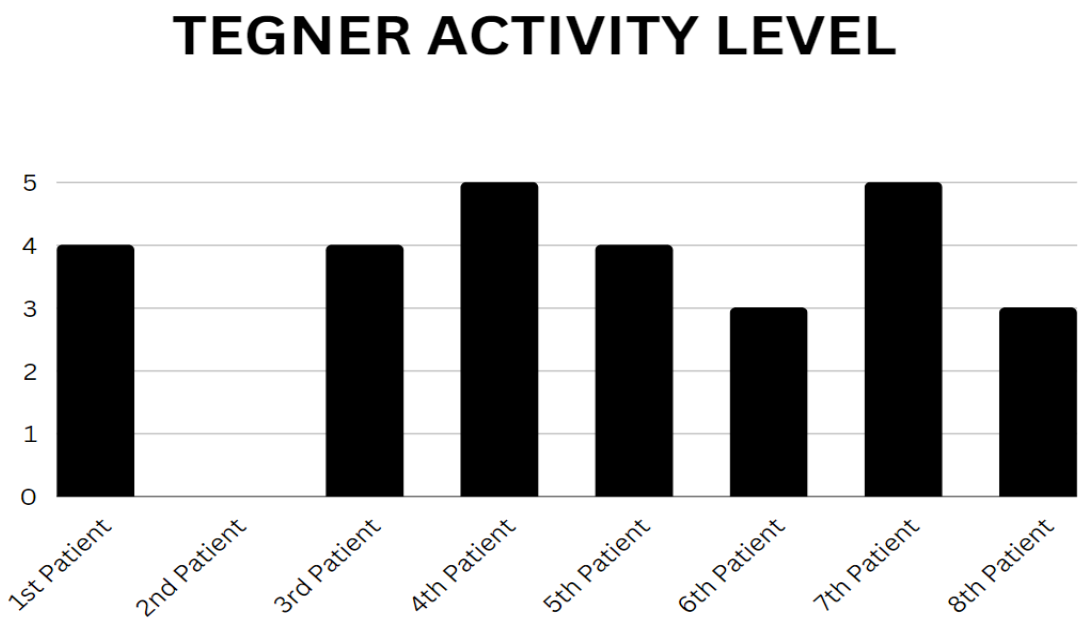


Table 5. Patients Tegner Activity Level



It has been shown that postoperative Lysholm and Cincinnati scores of patients in the KD II group with isolated cruciate ligament injury without collateral ligament injury were better than other injuries.

It was revealed that the group with multiple ligament injuries and neurovascular injuries, which is in the subgroup according to the Schenck classification, was the worst injury group according to the postoperative evaluation tests.

Among the patients in the KD I group, it was shown that the postoperative Lysholm and Cincinnati scores were relatively better in

the group with anterior cruciate ligament (ACL) injury compared to the group with posterior cruciate ligament (PCL) injury.

There was not correlation between the VAS scores and Tegner Activity Level scores of the patients and the group they belonged to according to the Schenck [7] classification.

Discussion

Traumatic knee dislocations are rare but serious injuries that may cause advanced functional problems and require an urgent and multidisciplinary approach. It has long-term negative effects that may disrupt the patient's return to routine life [9]. The term knee dislocation is known as the complete deterioration of tibiofemoral joint integrity. As a result of such injury, it should be noted that at least two of the main ligament structures responsible for the stability of the knee joint may be damaged and spontaneous reduction may be achieved.

Neurovascular injury is a common and serious limb threatening complication of traumatic knee dislocations. For this reason, neurovascular examination should be done primarily in every patient with suspicion of knee dislocation. If there is cyanosis, pallor and delayed capillary filling, major vascular injury should be considered [10]. If necessary, further examination should be performed by performing Doppler USG and CT angiography. Because of the severity of the management of vascular injuries accompanying knee dislocations, common protocols between vascular surgeons and orthopedic surgeons are required [11]. Nerve injuries occur most frequently in the peroneal nerve and have been reported in 16-50% of posterolateral corner injuries. It can occur in a range from only sensory loss to total motor loss and recovery can take up to 6-18 months. However, even if nerve continuity is preserved, recovery may not be complete in half of the cases [12].

The most important treatment aims are to define anatomical structures that contribute to instability and to shape or repair them in accordance with the original anatomy and isometry as much as possible [13]. There are few studies in the literature that provide long-term results for knee dislocation.

In the management of these serious injuries, the importance of post-operative rehabilitation comes to the forefront with the early and appropriate intervention of experienced orthopedic surgeons with a multidisciplinary approach, as well as the high awareness of neurovascular injuries.

Conflict of interest: The authors declare that there is no conflict of interest.

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Informed consent: Written informed consent was obtained from the patient.

Authors' contributions to the article

M.B. and K.G. have constructed/constructed the main idea and hypothesis of the study. M.B. and H.R.G. developed the theory and arranged/edited the material and method section. M.B., H.R.G. and K.G. have done the evaluation of the data in the results section. Discussion section of the article was written by M.B., H.R.G. and K.G. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Case Report

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Incidentally diagnosed appendix diverticulosis after appendectomy

Appendektomi sonrası insidental olarak saptanan appendiks divertikülü

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Abstract

Appenix diverticulitis was first described by Kelynack in 1893. Appendix diverticulosis is a rare clinical manifestation of the appendix with a changing rate of 0.004 to 2.1% of all appendectomies. Generally diagnosed in males, and the mean age is 38. Appendix diverticulosis may be inflamed and result in appendix diverticulitis or can be incidentally diagnosed by acute appendicitis. Clinical presentation of appendix diverticulitis may be similar to acute appendicitis but clinical signs may occur slowly and mildly.

The thirty-five-year-old male patient was admitted to the emergency department with right lower quadrant pain. The patient has abdominal tenderness, rebound, and defense reactions with other intraabdominal infections signs. Abdominal ultrasonography was performed and appendix diameter was measured enlarged, and inflammation signs were observed in the surrounding tissue in favor of acute appendicitis. A laparoscopic appendectomy was performed. During the operation, three small herniated tissue was observed on the anti-mesenteric surface of the appendix. Acute phlegmonous appendicitis and appendix diverticulosis diagnosis were confirmed with the histopathological examination.

Appendix diverticulosis may occur either acquired or congenital. The congenital diverticulosis is generally located on the anti-mesenteric edge and contains all layers of the appendix wall. Diagnosed appendix diverticulosis is acquired type predominantly. Appendix diverticulosis is diagnosed more commonly in patient who has cystic fibrosis or Hirschsprung disease. However, no relationship between colon diverticular disease and appendix diverticula has been found. There is a high risk of appendiceal neoplasms such as carcinoid tumors and mucinous adenomas. The gold standard to diagnose appendix diverticula is histopathological examination. The treatment of the appendix diverticula is appendectomy in case of diagnosis.

Keywords: Appendix diverticulosis, diverticular disease, laparoscopic appendectomy.

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Öz

Appendiks divertiküliti ilk olarak 1893'te Kelynack tarafından tanımlanmıştır. Sıklığı tüm appendektomilerin %0,004 ile 2,1 i arasında değişen nadir bir klinik tablodur. Genellikle erkeklerde teşhis edilir ve ortalama yaş 38'dir. Apendiks divertikülü inflamasyona sekonder apendiks divertiküliti ile sonuçlanabilir veya tesadüfen akut apandisit ile teşhis edilebilir. Apendiks divertikülitinin klinik prezentasyonu akut apandisite benzerdir, ancak klinik bulgular daha yavaş ve hafif bir şekilde ortaya çıkar.

Otuz beş yaşında erkek hasta, sağ alt kadran ağrısı şikayetleri ile acil servise başvurması sonucu değerlendirildi. Yapılan fizik muayenede diğer karın içi enfeksiyon bulguları ile birlikte batın sağ alt kadranda hassasiyet, defans ve rebound bulguları olduğu görüldü. Yapılan abdominal ultrasonografide apendiks lümen çapının arttığı, duvarı kalınlığı artmış ve çevre dokuda inflamasyon bulgularının olduğu görüldü ve akut apandisit lehine değerlendirildi. Laparoskopik apendektomi uygulandı. Ameliyat sırasında apendiksin anti-mezenterik yüzeyinde üç adet küçük divertikül yapısı olduğu görüldü. Histopatolojik değerlendirme sonucu akut flegmonöz apandisit ve appendiksin divertiküler hastalığı olarak raporlandı.

Apendiks divertikülü, konjenital veya edinsel olabilir. Konjenital divertiküller genellikle apendiks duvarının anti-mezenterik kenarında yerleşimlidir ve lümenin tüm katlarını içerir. Teşhis edilen apendiks divertikülleri ağırlıklı olarak edinsel tiptedir. Apendiks divertikülü, kistik fibroz veya Hirschsprung hastalığı olanlarda daha sık görülmektedir. Ancak apendiks divertikülü ile kolonun divertiküler hastalığı arasında bir ilişki bulunamamıştır. Appendiks divertikülü olgularınıda karsinoid tümörler ve müsinöz adenomlar gibi apendiks neoplazmaları riski yüksektir. Tanıda altın standart histopatolojik incelemedir. Apendiks divertiküllerinin tespit edilmesi halinde ise tedavisi apendektomidir.

Anahtar kelimeler: Appendiks divertikülü, divertiküler hastalık, laparoskopik appendektomi.

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Introduction

Acute appendicitis is the most common cause of acute abdomen. Gokce and Aren [1] stated that the diverticular disease of the appendix is a rare clinical condition. Chia et al. [2] reported that the appendix diverticulitis was first described by Kelynack in 1893. Sohn et al. [3] stated that that appendix diverticulosis is a rare manifestation, and it was only reported as case reports or case series in the literature. According to those clinical trials, the frequency of this disease changes between 0.004% and 2.1 among all appendectomies. Frade et al. [4] described the clinical signs of appendix diverticulitis generally mimic acute appendicitis but it has indolent progress than acute appendicitis. John et al. [5] found that the etiology of the disease is not clear but, it can occur as congenital or acquired. The appendicular diverticular disease remains asymptomatic unless inflammation presents, thus it can be only diagnosed if acute appendicitis clinic or appendicular diverticulitis occurs. Gokce and Aren [1], Chia et al. [2] stated that it is not possible to diagnose appendicular diverticulosis radiologically generally, histopathological examination is required most commonly. We aimed to present a diverticular disease of the appendix secondary to an acute appendicitis clinic.

Case report

The thirty-five-year-old male patient was admitted to the emergency department with right lower quadrant pain and vomiting and nausea. Abdominal tenderness, rebound, and defense reactions were observed on his abdominal examination. The patient's White Blood Cell (WBC): 15.000 10^3/uL, and C-Reactive Protein (CRP): 22 mg/L have resulted in his laboratory tests. Abdominal ultrasonography was performed and appendix diameter was measured at 8 mm, and inflammation signs were observed in the surrounding tissue in favor of acute appendicitis. Therefore, appendectomy operation was planned with a preliminary diagnosis of acute appendicitis, and the patient was informed, and hospitalized. A laparoscopic appendectomy was performed. During the operation, the appendix was observed as erected hypervascularized and inflamed, and surrounded with omental tissue adhesions. After the omental adhesions were dissected with surgical instruments, three small herniated tissue was observed on the anti-mesenteric surface of the appendix (Figure 1). Appendectomy was performed properly without any complications and the patient was discharged on postoperative day 1. In the histopathological examination, acute phlegmonous appendicitis and numerous diverticulum structures were observed on the mesenteric and anti – mesenteric surfaces of the appendix wall. The diverticula structures were defined as pseudodiverticula due to mucosa and submucosa layers extending beyond the muscularis propria, without containing muscularis propria layers (Figure 2).

Figure 1. Appendix diverticulosis

Figure 2. Histological image of pseudodiverticula located on the anti – mesenteric surface of the appendix wall, muscularis propria layer shown by marks. (HE x4)

Discussion

Appendix diverticulosis may occur either acquired or congenital. Frade et al. [4], and Lesi et al. [6] stated that diagnosed appendix diverticulosis cases are acquired type predominantly. Congenital diverticulosis of the appendix is rare. Albeeshi et al. [7] reported that the congenital diverticulosis is generally located on the anti-mesenteric edge and contains all four layers (mucosa, submucosa, muscular, serosa) of the bowel wall. Chia et al. [2] stated that acquired diverticulosis includes only the mucosa or submucosa layer, and both of them may be single or multiple. Sohn et al. [3], and Lesi et al. [6] reported that acquired type of diverticula occurs generally secondary to increased pressure of the appendix from the weakened point of the lumen wall caused by inflammation or atrophy of the muscular layer. Chia et al. [2], and Lesi et al. [6] described the risk factors of diverticular disease of the appendix are; male gender, age over 30, Hirschprung's Disease, and cystic fibrosis. Congenital diverticulosis may be related to Patau syndrome. However, no relationship between colon diverticular disease and appendix diverticula has been reported by Frade et al. [4]. Syed Muhammad Hammad [8] stated that the diverticular disease of the appendix may be associated with or without acute appendicitis, but diverticulitis is not an etiological factor for acute appendicitis. Chia et al. [2] stated that the diagnosis of appendix diverticulosis is still not exactly possible with preoperative imaging systems. Sohn et al.[3] reported that lower WBC count and higher CRP levels were found in appendix diverticulitis at laboratory test results compared to acute appendicitis. Lesi et al. [6] described the ultrasonographic findings of appendicular diverticulosis as hypoechoic inflamed diverticula surrounded by echogenic fatty tissue. In appendix diverticulosis, all the appendix wall layers are inflamed and visualized as thickened not only the mucosal and submucosal layers as different from acute appendicitis. Albeeshi et al. [7] stated that even though Ultrasonography

and Computer Tomography (CT) imaging might be helpful to diagnose preoperatively in some cases, histopathological examination is the gold standard for diagnosis. Lesi et al. [6] defined five types of appendix diverticulosis; Type 1 defines primary acute diverticulitis, with or without peridiverticulitis, type 2 defines acute diverticulitis secondary to acute appendicitis, type 3 defines diverticulum without inflammation, type 4 defines diverticulum with acute appendicitis, type 5 defines chronic peridiverticulitis with acute appendicitis. Our case is a type 4 appendix diverticulosis case according to this classification. Type I is the most frequently diagnosed type, with a prevalence of 40-50%. Lesi et al. [6] stated that due to the mildly and slowly progression of the appendix divertilitis may cause delayed diagnosis. Thus, it may cause several complications, such as perforation, intraabdominal abscess, pelvic pseudocyst, and vesicocaecal fistula. Chia et al. [2] reported that the perforation risk at the diverticular disease of appendix is four time higher than acute appendicitis. Therefore this is a reason of higher mortality and morbidity. John et al. [5] stated that there is a high risk of pseudomyxoma peritonei and appendiceal neoplasms such as carcinoid tumors and mucinous adenomas tubular adenomas, and primary appendiceal adenocarcinomas. Phillips and Perry [9] stated that the treatment of appendix diverticulosis is similar to acute appendicitis; appenedectomy. Gokce and Aren [1] recommended appendectomy as a treatment even if appendicular diverticulosis is diagnosed incidentally during the laparotomy for some other reasons except acute appendicitis.

In conclusion, appendicular diverticulosis is a rare clinical condition that is not foreseen at first sight, and hard to diagnose before the surgical intervention, is hard to manage the complications, and requires longer hospital stay and treatment duration. This clinical situation is also related to malignancy with a higher rate than acute appendicitis. The treatment modality is the same as acute appendicitis, but it is generally either delayed or diagnosed incidentally secondary to acute appendicitis. That's why it is important to suspect appendix diverticulosis if symptoms are similar to acute appendicitis but slowly and mildly.

Conflict of interest: No conflict of interest was declared by the authors.

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Informed consent: The patient consent that all information about this clinical condition may be published in an anonymous electronic journal or stored on the website

Authors' contributions to the article

M.B.K. constructed the main idea and hypothesis of the study. M.B.K. and N.B. developed the theory and arranged/edited the material and method section. N.B. has done the evaluation of the data in the Results section. Discussion section of the article was written by M.B.K.

M.B.K. and N.B. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Melkersson-rosenthal syndrome

Melkersson-rosenthal sendromu

Ufuk Çınkır

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Abstract
The Melkersson-Rosenthal syndrome (MRS) is a rare neuro mucocutaneous granulomatous disease presenting with recurrent peripheral facial paralysis, orofacial oedema, and fissured tongue. The coexistence of the classic clinical triad is rare. Clinical findings and examination make the diagnosis. It should be considered in the differential diagnosis of recurrent facial paralysis. In this case report, a 42-year-old female patient with the classical triad of MRS is presented.

Keywords: Recurrent peripheral facial palsy, orofacial oedema, fissured tongue, neuro mucocutaneous disease.

Cinkir U. Melkersson-rosenthal syndrome. Pam Med J 2024;17:191-194.

Öz
Melkersson-Rosenthal sendromu (MRS) yineleyici periferik fasiyal paralizi, orofasiyal ödem, fissürlü dil triadı ile kendini gösteren nadir bir nöromukokütan granülomatöz hastalıktır. MRS'nin klasik klinik triadının beraber görülmesi nadirdir. Tanısı klinik bulgular ve muayene ile konulur. Yineleyici fasiyal paralizilerin ayırıcı tanısında muhakkak düşünülmesi gerekir. Bu olgu sunumunda MRS'nin klasik triadının bir arada görüldüğü 42 yaşında, kadın hasta sunulmuştur.

Anahtar kelimeler: Tekrarlayıcı periferik fasiyal paralizi, orofasiyal ödem, fissürlü dil, nöromukokütan hastalık.

Çınkır U. Melkersson-rosenthal sendromu. Pam Tıp Derg 2024;17:191-194

Introduction

The Melkersson-Rosenthal syndrome is an uncommon neuro mucocutaneous granulomatous disease presenting with the triad of recurrent peripheral facial paralysis, orofacial oedema, and fissured tongue. It is more common in adults. All classical triad findings are seen in approximately 25% of patients with MRS [1, 2]. Its aetiology has not been fully elucidated. It is thought that genetic predisposition and factors that subsequently affect the individual play a role in the aetiology. Many diseases, such as intracranial space-occupying lesions, head trauma, intracranial haemorrhage, leukaemia, autoimmune diseases developing after infections, allergic disorders, thyroiditis, multiple sclerosis, diabetes mellitus are included in the aetiology [2-4]. There is no agreed treatment protocol to ensure complete remission and prevent recurrences in MRS. Medical or surgical methods are used in the treatment. Facial nerve decompression can be applied surgically in cases which do not respond to medical therapy [2, 3, 5].

Case report

A 42-year-old woman was admitted to the outpatient clinic of our hospital with complaints of recurrent inability to close her right eye, swelling in the lip and the tongue and fissured tongue. The complaints started a year ago. The frequency of the complaints was variable. There was no distinctive feature in her personal and family history. In the physical examination, her vital signs were stable; she could not close the right eyelid completely, consistent with right peripheral facial paralysis. The right nasolabial sulcus was erased. Mild, painless oedema was in the right upper lip (Figure 1) and fissured tongue (Figure 2).

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Figure 1. Right peripheral facial paralysis and oedema in the lip

Figure 2. Fissured tongue

In laboratory examinations, complete blood count (CBC), blood glucose, glycated haemoglobin (HbA1c), serum electrolyte levels, liver and renal function tests, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), vasculitis panel tests including antineutrophilic cytoplasmic antibody (ANCA), serum complement 3 (C3) and 4 (C4), folic acid, vitamin B12, homocysteine, free thyroxine (fT4), thyroid stimulating hormone (TSH), antithyroglobulin (anti-Tg) and anti thyroperoxidase antibodies (anti-TPO), serum angiotensin-converting enzyme (ACE), methylmalonic acid levels were normal. Serum and urine immunofixation electrophoresis tests showed no monoclonal gammopathies. Viral serology tests, including anti-human immunodeficiency virus (HIV), were negative.

The C1 esterase inhibitor test result did not suggest hereditary angioneurotic oedema.

Cranial and bilateral temporal bone magnetic resonance imaging (MRI) with gadolinium revealed no abnormalities. The bilateral facial nerve electrodiagnostic test with needle electromyography (EMG) demonstrated neither acute nor chronic pathologies.

The dermatology department examined the patient regarding possible Behcet's disease and angioedema. Serum immunoglobulin E (Ig-E) level was normal. Findings and examination did not suggest Behcet's disease or hereditary angioneurotic oedema. In terms of possible underlying rheumatological pathologies, a salivary gland biopsy was taken

from the lip. No pathology was found. The rheumatology department evaluated her with the biopsy results and vasculitis panel tests. No rheumatological pathology was detected. The electrocardiogram (ECG) was in normal sinus rhythm. Transthoracic echocardiography (TTE), cardiac stress test, and coronary computed tomography (CT) scan were within the normal range. The cardiology department assessed this patient with the previously mentioned cardiac tests, and cardiac pathologies were ruled out. Since the bilateral facial nerve electrodiagnostic test with needle EMG and the temporal bone MRI with contrast demonstrated no pathologies, she was consulted to the otorhinolaryngology department, and the examination was within the normal range. Otorhinolaryngological pathologies were excluded.

To evaluate sarcoidosis and tuberculosis, a thoracic CT scan was performed. There were no lesions in the thoracic CT scan.

She received oral methylprednisolone 64 mg/day treatment. In addition, to avoid any gastrointestinal haemorrhage, pantoprazole sodium 40 mg/day was added to the treatment. The dose of the corticosteroid was gradually tapered. After 26 days of oral methylprednisolone, her complaints and symptoms decreased; however, they did not disappear. A whole-body dual-energy x-ray absorptiometry (DEXA) scan was performed to examine the adverse effects of the corticosteroid treatment. Osteopenia in the left femur was detected. Due to this side effect, the methylprednisolone treatment was discontinued, and an anti-inflammatory non-steroidal agent, naproxen sodium 500 mg/day, was commenced. The frequency and severity of her complaints have decreased to almost non-existent.

Discussion

The Melkersson-Rosenthal syndrome is an infrequent neuro mucocutaneous granulomatous disease presenting with the triad of recurrent peripheral facial paralysis, orofacial oedema, and fissural tongue. It is more common in adults. All classical triad findings are seen in around 25% of MRS patients, making diagnosing the syndrome arduous [1, 2]. Its aetiology has not been fully elucidated. It is thought that genetic predisposition and factors that subsequently affect the individual play a role in the aetiology. Many diseases such as intracranial space-occupying lesions, head trauma, intracranial haemorrhage, leukaemia, autoimmune diseases developing after previous infections such as adenotonsillitis, allergic disorders, thyroiditis, multiple sclerosis, diabetes mellitus are included in the aetiology [2-4].

All the clinical findings might be seen simultaneously or separately. The presence of not less than one of the findings of idiopathic facial paralysis or fissured tongue at once with permanent or recurrent orofacial oedema is enough for MRS diagnosis [6-9].

The typical symptom of MRS is diffused, painless, acute orofacial oedema [6, 10-12]. Oedema usually recurs and lasts a few hours to weeks [13, 14]. MRS may mimic angioedema; however, it differs from angioedema by lasting longer and lacking response to antihistaminic treatments [15]. The second component of the triad of MRS is peripheral facial paralysis, which might be seen in almost 0.3 of the patients [16]. Paralysis might be both transient or permanent. Permanency is usually observed as the disease duration increases. Peripheral facial paralysis may be partial, bilateral and unilateral. Facial paralysis is found to be associated both with pressure due to oedema on the facial nerve within the temporal bone and its granulomatous infiltration [17, 18]. Our patient had a history of transient intermittent peripheral facial paralysis. The third symptom of MRS is the fissured tongue, a common finding in the population [19]. Therefore, it is slighter crucial in the diagnosis. Our patient also has a transient recurrent fissured tongue.

There is no agreed treatment protocol to ensure complete remission and prevent recurrences in MRS. Medical treatments such as corticosteroids, anti-inflammatory non-steroidal agents or surgical methods are used in the treatment. Facial nerve decompression can be applied surgically in cases that do not respond to medical therapy [2, 3, 5].

The patient showed all the elements of the characteristic triad. The corticosteroid and anti-inflammatory non-steroidal choices have worked in this patient. The frequency and severity of the symptoms have decreased to almost non-existent. Even though MRS might be under

control with the treatments, reoccurrence is always possible [20].

In conclusion, the diagnosis of MRS is arduous due to its infrequency. The typical symptom is orofacial oedema. Medical doctors should remember this syndrome, especially with patients with recurrent, transient or permanent swelling in the orofacial area and peripheral facial paralysis.

Conflict of interest: No conflict of interest was declared by the authors.

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Informed consent: An informed consent form was obtained from the patient to conduct this case report and use the photographs.