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hamidiye med j

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Volume 5
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hamidiye med j

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hamidiye med j

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hamidiye med j

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2024
Volume 5
Issue 3

Contents

ORIGINAL ARTICLES

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Are the Clinical and Radiological Characteristics of Pulmonary Embolism Differential in Patients with Cancer?

Kanser Hastalarında Pulmoner Embolinin Klinik-Radyolojik Özellikleri Farklı mıdır?

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ABSTRACT

Background: In general, it is known that many cancers and chemotherapy regimens administered to prevent cancer increase the tendency for thrombosis by disrupting hemostasis physiology. In this study, the prognostic differences between pulmonary embolism (PE) in patients diagnosed with cancer and those without a cancer diagnosis were investigated.

Materials and Methods: The records of patients diagnosed with PE in our clinic between December 2021 and January 2023 were retrospectively examined. Patients were divided into 2 groups: those with and without a history of cancer. Clinical, demographic, radiological, and laboratory characteristics of the patients in both groups were compared. Pulmonary Embolism Severity Index (PESI) score was used for the prognostic evaluation of PE. For the classification of the severity of PE and early mortality assessment (EMD) patients were stratified into low, moderate-low, moderate-high, and high-risk categories. The data of these two groups were compared.

Results: A total of 108 patients, with a mean age of 65.5±18 years, were included in the study. Of these patients, 30 (27.7%) (Group 1) had a history of cancer, and 78 (72.3%) (Group 2) had no history of cancer. The mean duration of hospitalization was 7.3±5.4 days in Group 1 and 9.7±5.2 days in Group 2 ($p<0.05$). No significant difference was observed in D-dimer, brain natriuretic peptide, and troponin values ($p>0.05$). Thoracic computed tomography-angiography findings of both groups were also similar ($p>0.05$). In Group 1; mean PESI score and rate of the number of patients PESI-III and above were significantly higher ($p<0.05$). In terms of EMD, the rate of high-risk patients and incidence of hemodynamic instability were significantly higher in Group 1 ($p<0.05$). Concerning the 30 day mortality, the rate of number of patients in Group 1 was significantly higher ($p<0.05$).

Conclusion: The presence of an additional cancer diagnosis did not have a notable impact on the radiological and laboratory parameters of PE; however, it did significantly change the early mortality associated with PE.

Keywords: Pulmonary embolism, cancer, mortality

ÖZ

Amaç: Genel olarak birçok kanserin ve kanseri önlemek için verilen kemoterapilerin hemostaz fizyolojisini bozarak tromboza eğilimi artırdığı bilinmektedir. Bu çalışmada kanser tanısı olan olgularda gelişen pulmoner emboli (PE) ile kanser tanısı olmayan PE olguları arasındaki prognostik farklılıklar araştırılmıştır.

Gereç ve Yöntemler: Aralık 2021-Ocak 2023 tarihleri arasında kliniğimize PE tanısı ile yatan olguların dosyaları retrospektif olarak incelendi. Olgular öncesinde kanser tanısı olanlar ve olmayanlar olmak üzere 2 gruba ayrıldı. Her iki gruptaki olguların klinik, demografik, radyolojik ve laboratuvar özellikleri karşılaştırıldı. PE'nin prognostik değerlendirmesi için Pulmoner Emboli Şiddet İndeksi (PESİ) skoru kullanıldı, PE'nin şiddetinin sınıflandırılması ve erken mortalite değerlendirmesi (EMD) için olgular düşük, orta-düşük, orta-yüksek ve yüksek riskli olarak sınıflandırıldı. İki grubun verileri birbirleri ile karşılaştırıldı.

Bulgular: Çalışmaya yaş ortalaması 65,5±18 olan toplam 108 olgu dahil edildi. Olguların 30'unda (%27,7) (Grup 1) öz geçmişinde kanser öyküsü var iken, 78'inin (%72,3) (Grup 2) öz geçmişinde kanser öyküsü yoktu. Grup 1'de olguların ortalama hastanede yatış gün

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ÖZ

sayısı 7,3±5,4 gün iken Grup 2'de 9,7±5,2 gündü (p<0,05). İki grup arasında D-dimer, beyin natriüretik peptidi, Troponin değerlerinde fark izlenmedi (p>0,05). Her iki grubun toraks bilgisayarlı tomografi-anjiyo bulguları benzerdi (p>0,05). Grup 1'de ortalama PESİ skoru, PESİ-III ve üstü olgu sayısı oranı anlamlı olarak yüksekti (p<0,05). EMD açısından Grup 1'de yüksek riskli olgu sayısı oranı ve hemodinamik instabilite varlığı saptanan olgu sayısı oranı anlamlı olarak yüksekti (p<0,05). Otuz günlük mortalite açısından Grup 1'de olgu sayısı oranı istatistiksel anlamlı olarak fazlaydı (p<0,05).

Sonuç: Kanser tanısı varlığı PE'nin radyolojik ve laboratuvar değerlerini belirgin şekilde etkilememektedir, fakat kanser tanısı PE'nin erken mortalitesini anlamlı oranda değiştirmektedir.

Anahtar Kelimeler: Pulmoner emboli, kanser, mortalite

Introduction

Pulmonary embolism (PE) is characterized by occlusion of pulmonary arteries by thrombus, and it has an incidence of 23%-269 per 100,000 population annually. Although treatment outcomes are favorable with rapid and early diagnosis, mortality may exceed 50% in patients who cannot be treated for various reasons (1). There are more than 30 identified risk factors categorized as major, moderate and weak for PE (2).

PE is a common complication in individuals with cancer, attributable both to its presence as a risk factor and the heightened risk associated with chemotherapy regimens administered for cancer treatment. Although conclusive evidence is lacking, the exponential increase in PE risk among patients with cancer is linked to the intrinsic prothrombotic activity of cancer cells, a tendency toward hypercoagulation mediated by cytokine release, and the prothrombotic effects of chemotherapy treatment (3).

With advances in diagnostic/imaging methods, particularly thoracic computed tomography-angiography (CT-angiography), along with improvements in treatment options, the mortality of PE has been decreasing over the years (4,5). On the other hand, it is undeniable that the incidence of PE is likely to rise in patients with cancer due ongoing developments in diagnostic and therapeutic methods, advances in both the diagnosis and treatment of oncological diseases, and the extended life expectancy of patients with cancer (6).

Mortality from PE is directly correlated with comorbidities, notably cancer, and age (4). The hypothesis of this study was that the clinical, radiologic and laboratory aspects of PE in patients diagnosed with cancer may differ from those without a cancer diagnosis; and to explore this, cases of PE diagnosed with cancer were compared to those without a cancer diagnosis.

Materials and Methods

We conducted a retrospective study in accordance with the Declaration of Helsinki and obtained approval from the İstanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee (decision no: 2023/0587, date: 20.09.2023). Since our study was a retrospective file-scanning study, an informed consent form was not obtained. We retrospectively examined the records of patients diagnosed with PE by thoracic CT angiography at our clinic between December 2021 and January 2023 and documented their clinical and demographic characteristics. A detailed analysis of the thoracic CT angiograms was performed. The analysis included documenting the bilateral distribution of detected thrombi within the pulmonary arterial system, presence of thrombi in the main pulmonary root, right and left main pulmonary arteries, and bilateral lobar, segmental, and subsegmental branches. We also noted the presence of PE-related pleural effusion and parenchymal infiltration. We also recorded the routine laboratory values obtained during the diagnosis and treatment of PE, such as hemogram, white blood count (WBC), platelets (PLT), neutrophils, neutrophil percentage, lymphocytes, lymphocyte percentage, mean platelet volume (MPV), C-reactive protein (CRP), procalcitonin (PRC), alanine aminotransferase (ALT), aspartate aminotransferase, lactate dehydrogenase, urea-creatinine, electrolytes, troponin, brain natriuretic peptide (BNP), and D-dimer levels. Additionally, the highest values of CRP, PRC, BNP, and troponin observed during hospitalization were documented, as were the oxygen saturation and partial arterial pressure of oxygen values from arterial blood gas examinations during hospitalization. Echocardiography and Doppler ultrasonography (USG), if available, were recorded, noting the presence of right ventricular overload and pulmonary artery systolic pressure (PABs) on echocardiography as well as the presence of thrombus on Doppler USG.

Evaluation of Prognostic Status and Early Mortality

We used the Pulmonary Embolism Severity Index (PESI) scoring system developed by Aujesky et al. (7) for the prognostic evaluation of PE (Supplement 1). In accordance with the European Society of Cardiology (ESC) guidelines, Class I and II in PESI scoring were considered low-risk groups, whereas Class III and above (Class III-

IV-V) in PESI scoring were considered high-risk in terms of early mortality. For the classification of the severity of PE and early mortality assessment (30 day mortality) (EMD), patients were stratified into low, moderate-low, moderate-high, and high risk categories (8) (Supplement 2). Furthermore, hemodynamic instability in PE was defined as the presence of cardiac arrest, obstructive shock, and persistent hypotension according to the ESC guidelines (8) (Supplement 3). The points from these scoring systems were recorded for each patient.

Patients were divided into 2 groups: those with a history of cancer (Group 1) and those without a history of cancer (Group 2). The duration of cancer diagnosis and patients who underwent chemotherapy were also documented for further analysis and comparison between the two groups.

Patients with an uncertain diagnosis of PE, those diagnosed with cancer by methods other than thoracic CT angiography [ventilation perfusion (V/Q) scintigraphy and/or clinical diagnosis], pregnant women, and those under 18 years of age were excluded. Artificial intelligence-supported technologies were not used in this paper.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 (IBM IncRelased 2008. SPSS Statistics for Windows (Chicago, USA). In descriptive statistics, continuous variables were expressed as mean ± standard deviation for normally distributed values and as median (minimum-maximum) for values not fitting the normal distribution. Categorical variables are expressed as percentages. Normal distribution was assessed using the Kolmogorov-Smirnov test. The chi-square, independent sample t-test and Mann-Whitney U tests were employed to evaluate data from groups, when necessary. For all tests, $p<0.05$ was considered significant.

Results

We reviewed the medical records of a total of 110 patients admitted to the Chest Diseases clinic with a diagnosis of PE between December 2021 and January 2023. Two patients were excluded because their diagnosis was made by V/Q scintigraphy. Thus, the final analysis included a total of 108 patients, 62 (52.4%) females and 46 (42.6%) males, with a mean age of 65.5±18 years. Of these patients, 30 (27.7%) (Group 1) had a history of cancer and 78 (72.3%) (Group 2) had no history of cancer. While 86 (79.6%) of the patients had comorbidities, whereas 22 (20.4%) did not. The most common comorbidity was hypertension in 38 (35.1%) patients. The mean duration of hospitalization was 8.8±5.2 days for all patients, specifically 7.3±5.4 days in Group 1 and 9.7±5.2 days in Group 2 (p=0.049).

The cancer group included 8 (26.6%) patients with lung cancer, 6 (20%) with breast cancer, 6 (20%) with colon cancer, 3 (10%) with ovarian cancer, 2 (6. 6%) with bladder cancer, 2 (6.6%) with pancreatic cancer, 2 (6.6%) with glioblastoma and 1 (3.3%) with prostate cancer (Figure 1).

An analysis of the laboratory values showed that the mean PLT, MPV, and D-dimer values for Group 1 during admission were 208566±95241x103/µL, 10. 1±1fl, and 11.47±11.2 µg/L, respectively, while these parameters were 254794±98108x103/µL, 10.8±1.4fl, and 10.5±9.6 µg/L for Group 2, respectively (p=0.029, p=0.013, p=0.596). The highest laboratory values at admission and during hospitalization are presented in Table 1.

An analysis of the radiologic findings showed that the number of patients with embolism of the right main pulmonary artery was 4 (13.3%) in Group 1 vs. 13 (16.6%) in Group 2 (p=0.844) while those with embolism of the left main pulmonary artery was 0 in Group 1 and 5 (6.4%) in Group 2 (p=0.355). The detailed radiologic examination results (Thoracic CT angiography, echocardiography, bilateral lower extremity venous doppler USG) of the patients are presented in Table 2.

In Group 1, 28 patients (93.3%) patients received low-molecular-weight heparin (LMWH) and 2 patients (6.7%) received oral anticoagulant therapy. In Group 2, 30 cases (38.5%) received LMWH, while 11 patients (14.1%) received oral anticoagulants and 37 patients (47.4%) received new oral anticoagulants.

The mean PESI score was 121.6±23.3 in Group 1 vs. 95.5±35.4 in Group 2 (p<0.001). The mean number of patients with PESI-III and above was 28 (93.3%) in Group 1 vs. 46 (58.9%) in Group 2 (p=0.024) (Table 3). In terms of EMD, there were 8 high-risk patients (26.9%) in Group 1 and 9 (5.8%) were in Group 2 (p=0.05). The number of patients with hemodynamic instability was 8 (26.6%) in Group 1 and 8 (10.2%) in Group 2 (p=0.032) (Figure 2). The number of

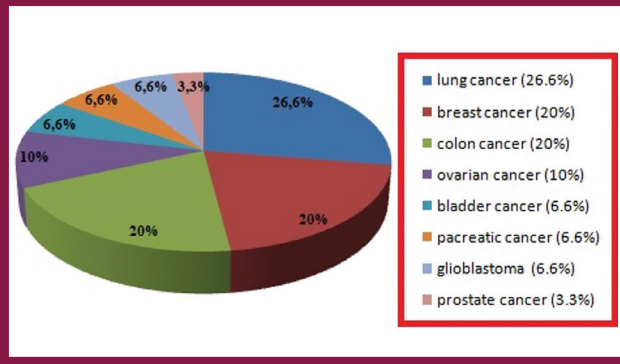

Figure 1. Distribution of cancer diagnoses of patients in Group 1

Table 1. Clinical, demographics, laboratory, and treatment characteristics of patients

	History of cancer (Group 1; n=30)	No history of cancer (Group 2; n=78)	p-value
Age (mean ± SD)	62.9±15.1	66.4±19	0.363
Gender (F/M) (n%)	14-46.7%/16-53.3%	48-61.5%/30-38.5%	0.104
Comorbidities			
Comorbidities (+/-) (n%)	30-100%	56-71.8%/22-28.2%	**0.003**
Hypertension (n%)	8-26.6%	30-38.4%	0.355
Diabetes (n%)	9-30%	16-20.5%	0.428
CAD (n%)	2-6.6%	8-10.2%	0.837
Heart failure (n%)	0-0%	8-10.2%	0.158
COPD (n%)	3-10%	7-8.9%	0.870
Asthma (n%)	1-3.3%	5-6.4%	0.876
CRD (n%)	0-0%	2-2.5%	0.929
Temparature (°C) (mean ± SD)	36.5±0.2	36.4±0.2	0.120
Pulse (min.) (mean ± SD)	97±27	95±19	0.678
SBP (mmHg) (mean ± SD)	114±28	127±27	**0.019**
DBP (mmHg) (mean ± SD)	70±14	73±13	0.239
Duration of hospitalization (days) (mean ± SD)	7.3±5.4	9.7±5.2	**0.049**
Laboratory values			
WBC (10^3/µL) (mean ± SD)	9086±3849	10828±4042	**0.045**
Hgb (g/dL) (mean ± SD)	10.9±2	12±2	**0.004**
PLT (10^3/µL) (mean ± SD)	208566±95241	254794±98108	**0.029**
MPV (fl) (mean ± SD)	10.1±1	10.8±1.4	**0.013**
Sodium (mEq/L) (mean ± SD)	134±4.2	137±3.8	**0.001**
Potassium (mEq/L) (mean ± SD)	4.1±0.6	4.3±0.8	0.119
CRP (mg/dL) (mean ± SD)	90±67	73±77	0.279
PRC (qg/L) (mean ± SD)	0.30±0.5	0.43±1.6	0.754
LDH (U/L) (mean ± SD)	356±249	274±151	0.052
D-dimer (µg/L) (mean ± SD)	11.47±11.2	10.5±9.6	0.753
Urea (mg/dL) (mean ± SD)	53±83	43±27	0.358
Creatinine (mg/dL) (mean ± SD)	0.76±0.2	0.99±0.5	**0.039**
ALT (U/L) (mean ± SD)	26±25	30±37	0.580
AST (U/L) (mean ± SD)	32±31	32±36	0.987
Albumin (g/L) (mean ± SD)	32±6.2	37±5.9	**0.002**
BNP (ng/L) (mean ± SD)	3173±4876	3103±5607	0.966
Troponin (ng/L) (mean ± SD)	32±37	50±90	0.359
ABG PaO_2 (mmHg) (mean ± SD)	72±20	62±14	0.075
Hospitalization SaO_2 (%)	91±4	90±4	0.485
PESI score	121.6±23.3	95.5±35.4	**<0.001**

SD: Standard deviation, F/M: Female/Man, CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, CRD: Chronic renal disease, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, WBC: White blood count, Hgb: Hemoglobin, PLT: Platelets, MPV: Mean platelet volume, CRP: C-reactive protein, PRC: Procalcitonin, LDH: Laktate dehydrogenase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BNP: Brain natriuretic peptide, ABG: Arterial blood gas, PESI: Pulmonary Embolism Severity Index, SaO_2: Oxygen saturation, PaO_2: Partial arterial pressure of oxygen, Min.: Minimum

Table 2. Radiological and ECHO characteristics of patients

Thoracic CT angiography findings	History of cancer (Group 1; n=30)	No history of cancer (Group 2; n=78)	p-value
Presence of embolism in the main pulmonary artery (n%)	0-0%	1-1.2%	1.000
Presence of embolism in the left pulmonary artery (n%)	0-0%	5-6.4%	0.355
Embolization in the right pulmonary artery (n%)	4-13.3%	13-16.6%	0.844
Presence of embolism in both pulmonary arteries (n%)	6-20%	21-26.9%	0.583
Presence of embolism in unilateral segment branches (n%)	9-30%	25-32%	0.938
Presence of embolism in bilateral segment branches (n%)	18-60%	41-52.5%	0.747
Presence of embolism in unilateral subsegment branches (n%)	9-30%	21-26.9%	1.000
Presence of embolism in bilateral subsegment branches (n%)	16-53.3%	43-55.1%	0.903
Presence of pleural fluid due to embolism (n%)	8-26.6%	22-28.2%	0.989
Presence of parenchymal infarction area (n%)	14-46.6%	38-48.7%	0.873
Presence of thrombi on Doppler USG (n%)	10-33.3%	21-26.9%	0.515
ECHO presence of right ventricular involvement (n%)	9-30%	34-43.5%	0.380
ECHO PAP value (mmHg) (mean ± SD)	31.9±11.3	40.6±15.8	**0.026**

ECHO: Echocardiography, PAP: Pulmonary artery pressure, USG: Ultrasonography, CT: Computed tomography

Table 3. Distribution of patients according to PESI scores

PESI score	History of cancer (Group 1; n=30)	No history of cancer (Group 2; n=78)	p-value
PESI-I (n%)	0	13-16.6%	0.017
PESI-II (n%)	2-6.6%	19-24.3%	0.037
PESI-III (n%)	5-16.6%	19-24.3%	0.389
PESI-IV (n%)	9-30%	14-17.9%	0.171
PESI-V (n%)	14-46.6%	13-16.6%	0.171
PESI-III and above	28-93.3%	46 58.9%	0.001

PESI: Pulmonary Embolism Severity Index

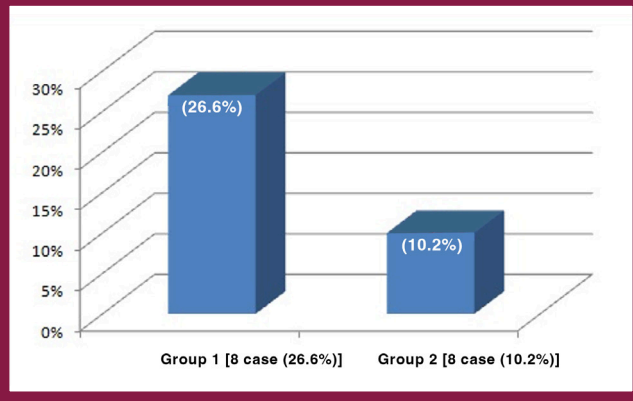

Figure 2. The number of patients with hemodynamic instability

patients who died within 30 days was 7 (23.3%) in Group 1 and 7 (8.97%) in Group 2 (p=0.047) (Figure 3).

Upon analyzing the treatment characteristics of patients in Group 1, it was found that 23 (76.7%) patients underwent chemotherapy, whereas 7 (23.3%) did not receive chemotherapy. Among patients who received chemotherapy, the mean time elapsed between chemotherapy and the onset of PE was 397±856.14 days. Among the included

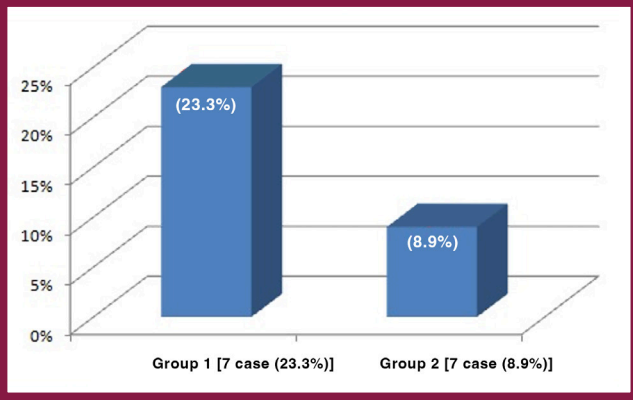

Figure 3. Death within 30 days

patients, 13 (56.5%) were diagnosed with PE within the first 30 days following chemotherapy.

Discussion

In this retrospective observational study, we examined the clinical, laboratory, and radiological characteristics of patients with PE with and without a diagnosis of cancer. PESI scores were higher in the PE group with cancer. Group 1 patients had statistically significantly higher PESI-III scores and higher scores, a higher proportion of high-risk patients in terms of PE, and a greater incidence of hemodynamic instability at the time of PE diagnosis. In terms of laboratory parameters, the WBC, HB, MPV, and PLT levels were lower in the cancer group than in the non-cancer group. No significant differences were observed in D-dimer, BNP, and troponin levels. Radiologically, there was no difference between the two groups. In the cancer group, most patients (56%) underwent chemotherapy within the first month. Interestingly, the duration of hospitalization was shorter in the cancer group.

Wang et al. (9) investigated the association between cancer and PE in two groups of patients with PE and cancer (n=52) and without cancer (n=44). They found that WBC counts were significantly higher in the cancer group than in the non-cancer group. In another study by Connolly et al. (10) involving 4,405 cancer outpatients receiving chemotherapy, the leukocyte levels of patients who developed PE were elevated. The authors suggested that leukocytes directly contribute to thrombus formation and disease progression by releasing tissue factors and vascular endothelial growth factor. In the present study, WBC and PLT counts were significantly lower in the cancer group. Considering that myelosuppression in bone marrow is a common side effect of chemotherapy drugs (11) we believe that this difference may be attributed to the fact that majority of our patients (56.5%) recently underwent chemotherapy.

An evaluation of the radiologic findings of the patients (thoracic CT angiography, echocardiography, Doppler USG) demonstrated a significant difference in Group 2 in terms of echocardiography-PABs; otherwise, the radiologic features of both groups were similar. The results are contradictory in the literature on this topic. Some publications suggest a higher incidence of central PE on thoracic CT angiography in patients with cancer and PE (12,13,14), whereas other studies (9,15), including ours, found no significant difference. Radiologically, there was no difference between the two groups in our study. One hypothesis on this topic is that the embolic material entering the pulmonary artery may fragment and evenly distribute to multiple segmental or subsegmental vessels, resulting in occlusion. This way, we can explain why we could not find the difference. Another radiological difference noted was the higher prevalence of echocardiography -derived PABs pressures in Group 2, but this finding may be coincidental or due to a significantly higher proportion of cardiac diseases in Group 2.

The risk of venous thromboembolism (VTE) is known to increase during cancer treatment, with chemotherapy contributing to thrombosis by showing toxic effects against the vascular endothelium and increasing cytokine release (16). A previous study reported a 5.3 fold higher risk of PE complications in patients with cancer treated with systemic chemotherapy compared with other treatments, highlighting the thrombogenic effects of systemic chemotherapy (17). Another study found a rate of 5.3% of VTE in 1921 patients receiving chemotherapy, with one-third experiencing PE complications (18). Otten et al. (19) detected VTE in 15 (7.3%) patients among a cancer group of 206 patients who underwent chemotherapy. When analyzing the duration of chemotherapy in these patients, it was observed that 86.6% received chemotherapy within the first month (9 patients were diagnosed with VTE during chemotherapy treatment, 2 patients within one week and 2 patients within the first month). Considering thatthe majority of our patients (56.5%) were diagnosed with PE within the first month of chemotherapy, chemotherapy-related PEs may be associated with the early phase of chemotherapy.

Most studies investigating the hospitalization duration of patients with PE, with or without a cancer diagnosis, have indicated longer hospital stays among patients with cancer (14,20). This could be attributed to older age, higher comorbidity, and potentially different treatment features for PE. Previous studies have shown a lower rate of thrombolytic administration in patients with cancer than in those without cancer, and the use of direct-acting oral anticoagulants has been associated with shorter hospitalization periods (20,21). In our

study, the majority of cancer patients (90%) were discharged with LMWH, which resulted in shorter hospitalizations.

PESI is commonly used to predict early mortality following PE. However, it hashas been developed for the general PE population, and its effectiveness in patients with cancer has not been extensively studied. In a literature review, Li et al. (22), who compared the sensitivity of PESI with other scoring methods in patients with cancer, reported that cancer-specific PE prognostic scores [Registro Informatizado de la Enfermedad Trombo Embólica (RIETE) and POMPE-C] outperformed PESI. Another study by Weeda et al. (23) compared the POMPE-C and RIETE criteria with PESI, revealing that the sensitivity of PESI was >96.0%, and the specificity was very low (<19%). In our study, the rates of patients with PESI-III and above (93.3%-58.9%) (p=0.024), high-risk (26.9%-5.8%) (p=0.05), and detection of hemodynamic instability (26.6%-10.2%) (p=0.032) were significantly higher in Group 1 compared with Group 2, and death within the first 30 days in group 1 (23.3%-8.9%) was also significantly higher than in Group 2. Given the lack of studies directly comparing PESI rates in patients with cancer in the literature, our study results suggest that PESI scoring can be valuable for predicting early mortality in this patient population.

However, it is important to acknowledge the limitations of this study, including itsits small sample size, retrospective design reflecting a single center experience, and the absence of cancer subgroups. Future research focusing specifically on PE in patients with lung cancer, which directly impacts cardiopulmonary reserve, may provide additional insights. These limitations should be considered when interpreting the results.

Conclusion

One significant finding from our investigation into the clinical, radiological, and laboratory aspects of PEs accompanied by cancer diagnosis was that the presence of an additional cancer diagnosis did not have a notable impact on the radiological and laboratory parameters of PE; however, it did significantly change the mortality associated with PE.

Ethics

Ethics Committee Approval: We conducted a retrospective study in accordance with the Declaration of Helsinki and obtained approval from the İstanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee (decision no: 2023/0587, date: 20.09.2023).

Informed Consent: Since our study was a retrospective file-scanning study, an informed consent form was not obtained.

Authorship Contributions

Surgical and Medical Practices: E.E.Y., Concept: S.D.T., Design: S.D.T., C.D., Data Collection or Processing: S.İ., S.S., Analysis or Interpretation: C.D., Literature Search: S.D.T., Z.N.T., Writing: S.D.T., C.D.

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REFERENCES

1. Turkish Thoracic Association ©2021. Pulmonary thromboembolism diagnosis and treatment consensus report. [Crossref]
2. Rosendaal FR. Risk factors for venous thrombotic disease. Thromb Haemost. 1999;82:610-619. [Crossref]
3. Gimbel IA, Mulder FI, Bosch FTM, Freund JE, Guman N, van Es N, et al. Pulmonary embolism at autopsy in cancer patients. J Thromb Haemost. 2021;19:1228-1235. [Crossref]
4. Lehnert P, Lange T, Møller CH, Olsen PS, Carlsen J. Acute pulmonary embolism in a national danish cohort: increasing incidence and decreasing mortality. Thromb Haemost. 2018;118:539-546. [Crossref]
5. West J, Goodacre S, Sampson F. The value of clinical features in the diagnosis of acute pulmonary embolism: systematic review and meta-analysis. QJM. 2007;100:763-769. [Crossref]
6. Doğan C, Fidan A, Kıral N, Parmaksız ET, Salepçi B, Çağlayan B, et al. Characteristics of metachronous second primary lung cancers. Eurasian J Pulmonol. 2019;21:127-131. [Crossref]
7. Aujesky D, Obrosky DS, Stone RA, Auble TE, Perrier A, Cornuz J, et al. Derivation and validation of a prognostic model for pulmonary embolism. Am J Respir Crit Care Med. 2005;172:1041-1046. [Crossref]
8. Konstantinides SV, Meyer G, Becattini C, Bueno H, Geersing GJ, Harjola VP, et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). Eur Heart J. 2020;41:543-603. [Crossref]
9. Wang H, Huang Y, Xu CW, Lin L. Clinical analysis of tumor and non-tumor patients complicated with pulmonary embolism. Int J Clin Exp Med. 2015;8:18729-18736. [Crossref]
10. Connolly GC, Khorana AA, Kuderer NM, Culakova E, Francis CW, Lyman GH. Leukocytosis, thrombosis and early mortality in cancer patients initiating chemotherapy. Thromb Res. 2010;126:113-118. [Crossref]
11. Çakan A, Erbaycu AE, Aksel N, Özsöz A. Hematologic complications seen after chemotherapy in cases with lung cancer. Eurasian J Pulmonol. 2004;6:53-61. [Crossref]
12. Jin YJ, Jin YF, Zhu XY, Zhang BL, Chen C. Intermediate risk pulmonary embolism concomitant with or without lung cancer: a wide spectrum of features. Clin Exp Hypertens. 2022;44:589-594. [Crossref]
13. Hasenberg U, Paul T, Feuersenger A, Goyen M, Kröger K. Cancer patients and characteristics of pulmonary embolism. Eur J Radiol. 2009;69:478-482. [Crossref]
14. Karippot A, Shaaban HS, Maroules M, Guron G. The clinical characteristics of pulmonary embolism in patients with malignancy: a single medical institutional experience. N Am J Med Sci. 2012;4:600-604. [Crossref]
15. Kim MS, Chang H, Lee SY, Shin SH, Park H, Chang SA, et al. Differential clinical manifestations and clinical outcome of cancer-related pulmonary embolism. Korean J Intern Med. 2020;35:360-368. [Crossref]
16. Haddad TC, Greeno EW. Chemotherapy-induced thrombosis. Thromb Res. 2006;118:555-568. [Crossref]

17. Li G, Li Y, Ma S. Lung cancer complicated with asymptomatic pulmonary embolism: clinical analysis of 84 patients. Technol Cancer Res Treat. 2017;16:1130-1135. [Crossref]
18. Di Nisio M, Ferrante N, De Tursi M, Iacobelli S, Cuccurullo F, Büller HR, et al. Incidental venous thromboembolism in ambulatory cancer patients receiving chemotherapy. Thromb Haemost. 2010;104:1049-1054. [Crossref]
19. Otten HM, Mathijssen J, ten Cate H, Soesan M, Inghels M, Richel DJ, et al. Symptomatic venous thromboembolism in cancer patients treated with chemotherapy: an underestimated phenomenon. Arch Intern Med. 2004;164:190-194. [Crossref]
20. Shalaby K, Kahn A, Silver ES, Kim MJ, Balakumaran K, Kim AS. Outcomes of acute pulmonary embolism in hospitalized patients with cancer. BMC Pulm Med. 2022;22:11. [Crossref]
21. Kohn CG, Fermann GJ, Peacock WF, Wells PS, Baugh CW, Ashton V, et al. Association between rivaroxaban use and length of hospital stay, treatment costs and early outcomes in patients with pulmonary embolism: a systematic review of real-world studies. Curr Med Res Opin. 2017;33:1697-1703. [Crossref]
22. Li X, Hu Y, Lin P, Zhang J, Tang Y, Yi Q, et al. Comparison of different clinical prognostic scores in patients with pulmonary embolism and active cancer. Thromb Haemost. 2021;121:834-844. [Crossref]
23. Weeda ER, Caranfa JT, Zeichner SB, Coleman CI, Nguyen E, Kohn CG. External validation of generic and cancer-specific risk stratification tools in patients with pulmonary embolism and active cancer. J Natl Compr Canc Netw. 2017;15:1476-1482. [Crossref]

Supplement 1. PESI score

Predictors	PESI	
Age>80 years	Age/years	
Male sex	+10	
History of cancer	+30	
History of heart failure	+10	
History of chronic lung disease	+10	
Heart rate≥110	+20	
SBP <100 mmHg	+30	
Respiratory rate ≥30	+20	
Temperature <36 °C	+20	
Altered mental health	+60	
O_2 saturation <90%	+20	
	Low risk Class I: ≤65 Class II: 66-85	**High risk** Class III: 86-105 Class IV: 106-125 Class V: >125

PESI: Pulmonary Embolism Severity Index, SBP: Systolic blood pressure

Supplement 2. Classification of PE severity and the risk of early death

		Risk indicators		
Early mortality risk	**Hemodynamic instability**	**PESI Class III-IV**	**Right ventricular dysfunction on TTE or CT**	**Increased cardiac troponin levels**
High	+	+	+	+
Moderate-high	-	+	+	+
Moderate-low	-	+	Either (+) or both (-)	
Low	-	-	-	-

CT: Computed tomography, PESI: Pulmonary embolism severity index, PE: Pulmonary embolism, TTE: Transthoracic echocardiography

Supplement 3. Definition of hemodynamic ınstability in pulmonary embolism		
Cardiac arrest	**Obstructive shock**	**Persistent hypotension**
Presence of ardiac arrest requiring cardiopulmonary resuscitation	-Systolic blood pressure <90 mmHg or -The need for vasopressors to maintain systolic blood pressure ⩾90 mmHg despite adequate fluid support and -Presence of end-organ hypoperfusion (altered consciousness, cold-clammy skin, oliguria/anuria, increased serum lactate level)	-Systolic blood pressure <90 mmHg or -Decrease in systolic blood pressure by >40 mmHg (Lasting longer than 15 minutes and unexplained by new onset arrhythmia, hypovolemia and sepsis)

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Scapulothoracic Arthroscopy for Snapping Scapula Syndrome: Clinical Outcomes and Complications

Snapping Skapula Sendromu Tedavisinde Skapulotorasik Artroskopi: Klinik Sonuç ve Komplikasyonlar

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ABSTRACT

Background: Operative treatment of a snapping scapula is reserved for refractory patients who do not respond to conservative treatment. Although open techniques have provided significant symptomatic relief, arthroscopic techniques have gained popularity in recent years, and promising outcomes have been reported. The aim of our study was to evaluate the surgical outcomes and complications of patients with symptomatic snapping scapula treated with scapulothoracic arthroscopy.

Materials and Methods: Retrospective database scanning for patients with painful snapping scapula treated with the scapulathoracic arthroscopic approach between 2013 and 2022 was performed. Demographic information, clinical outcomes, and complication rates of the patients were reviewed. The QuickDASH, Constant-Murely, and Visual Analogue Scale (VAS) scores were used to assess the pain and function levels preoperatively and at the final follow-up.

Results: Nineteen individuals who met the inclusion criteria were included; the average age of the patients was 31±10 years. The study included 13 females and 6 males. At the final follow-up, five of the 19 patients reported no improvement after their operations. Although the remaining 14 patients were satisfied with the operation results, 4 patients continued to have pain, but to a lesser extent. A significant improvement was observed in the VAS, with a mean preoperative score of 7±0.9 and postoperative score of 2±2.4 (p<0.001). In addition, there was a significant improvement in the mean Quick-DASH Score and Constant-Murley Score From 43±11.3 and 53.3±10.2 to 16.3±17.7 (p<0.001) and 75.9±17.2 (p<0.001), respectively. Four patients had depressed fractures in the infraspinatus fossa of the scapula that did not require additional treatment.

Conclusion: Arthroscopy for snapping of the scapula is an effective, reproducible, and safe procedure. Simple depression fractures of the infraspinatus fossa of the scapula can occur during arthroscopy, especially when performed by less experienced surgeons. These fractures require no additional treatment and do not adversely affect surgical outcomes.

Keywords: Snapping scapula syndrome, scapulothoracic, arthroscopy, bursectomy, scapuloplasty, complications

ÖZ

Amaç: Snapping skapulanın cerrahi tedavisi, konservatif tedaviye yanıt vermeyen dirençli hastalara yönelik yapılmaktadır. Açık teknikler önemli oranda semptomatik iyileşme sağlasa da artroskopik teknikler son yıllarda popülerlik kazanmış ve ümit verici sonuçlar bildirilmektedir. Çalışmamızın amacı, skapulotorasik artroskopi ile tedavi edilen semptomatik snapping skapula hastalarının cerrahi başarısını ve komplikasyonlarını değerlendirmektir.

Gereç ve Yöntemler: 2013-2022 yılları arasında skapulatorasik artroskopik yaklaşımla tedavi edilen ağrılı snapping skapula hastalarına ait retrospektif veri tabanı taraması yapıldı. Hastaların demografik bilgileri, cerrahi detayları, klinik sonuçları ve komplikasyon oranları incelendi. Ameliyat öncesi ve son takipte ağrı ve fonksiyon düzeylerini değerlendirmek için QuickDASH Skoru, Constant-Murely Skoru ve Görsel Analog skoru kullanıldı. Ek olarak hastalara ameliyat sonrası krepitasyonun meydana gelip gelmediği ve prosedürden genel memnuniyetleri soruldu.

Bulgular: Dahil edilme kriterlerini karşılayan 19 kişi çalışmaya dahil edildi. Hastaların yaş ortalaması 31±10 yıl, ortalama takip süresi 68,4±24,5 ay idi. Araştırmaya 13 kadın ve 6 erkek katıldı. Son takipte toplam 19 hastanın 5'i ameliyat sonrası herhangi bir iyileşme bildirmedi. Geriye kalan 14 hasta operasyon sonuçlarından memnun olmasına rağmen 4 hastada ağrı daha az da olsa devam etti. Görsel Anolog skorunda ameliyat öncesi ortalama skor 7±0,9 ve ameliyat sonrası skor 2±2,4 ile anlamlı iyileşme gözlendi (p<0,001). Ayrıca ortalama Quick-DASH Skoru ve Constant-Murley Skorunda sırasıyla 43±11,3 ve 53,3±10,2'den 16,3±17,7'ye (p<0,001) ve

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ÖZ

75,9±17,2'ye (p<0,001) anlamlı bir iyileşme saptandı. Dört hastada skapula infraspinatus fossada çökme kırığı ameliyat sırasında gelişti ve herhangi bir ek tedavi gerektirmedi.

Sonuç: Snapping skapula tedavisi için artroskopi etkili, tekrarlanabilir ve güvenli bir prosedür olmaktadır. Artroskopi sırasında özellikle daha az deneyimli cerrahlar tarafından yapıldığında skapula infrasupinatus fossada basit çökme kırıkları oluşabilmektedir. Bu kırıklar ek bir tedavi gerektirmemekte ve cerrahi sonucu olumsuz etkilememektedir. Bu potansiyel kırıklardan kaçınmak için dikkatli bir cerrahi teknik kullanılması ve anatomik olarak hassas bölgede kuvvetli manevralardan kaçınılması önerilir.

Anahtar Kelimeler: Snapping scapula sendromu, skapulotorasik, artroskopi, bursektomi, skapuloplasti, komplikasyonlar

Introduction

The scapulothoracic (ST) joint is an articulation between the volar concave surface of the scapula and the convex posterior thoracic cage. Congruent joint surfaces and smooth interposed soft tissue are necessary for the normal gliding motion of the scapula. Any conditions that interfere with smooth gliding may lead to disorders ranging from mild intermittent shoulder pain to persistent crepitus accompanied by considerable pain during attempts at overhead arm motion. Snapping scapula syndrome (SSS) is a disorder of the shoulder girdle. It is characterized by painful crepitation during ST movements (1). However, mechanical blocks, such as bony exostosis or muscular hypertrophy, may be the primary cause of SSS. Most patients present with no demonstrable anatomic lesions (2). Scapular dyskinesia resulting in an increased anterior tilt of the scapula, which compresses the superomedial border of the scapula against the ribs, is thought to be the cause of SSS with non-identifiable structural underlying lesions (3).

Regardless of underlying causes, available evidence supports initial non-surgical management (4). It is generally accepted that symptoms occurring without an anatomic lesion respond more favorably to non-operative management than do patients with an anatomic lesion. Surgical interventions are reserved for patients who do not respond to conservative treatment or those with structural abnormalities. Traditional open techniques have typically provided significant symptomatic relief (5), but the invasiveness of the approach by releasing trapezoid muscle insertions from the scapula, with consequent morbidity and slow rehabilitation, has shifted the preference of surgeons toward less invasive arthroscopic approaches.

Arthroscopic treatment of this disorder has gained popularity in recent years, and promising outcomes have been reported in the literature (3). However, many studies in this field have provided inconsistent recommendations for surgical treatment, and various studies have employed various arthroscopic procedures, such as bursectomy alone or superomedial angle scapuloplasty, with differing degrees of bone excision, which are customized to preoperative planning or arthroscopic findings. Apart from the reported high rates of residual symptoms (6), only a few neurovascular complications during portal placement of ST arthroscopy have been reported (2).

Herein, we aimed to present the surgical outcomes of patients with symptomatic SSS without underlying structural abnormalities, describe our preferred arthroscopic technique, and report our experience with complications.

Materials and Methods

Following the approval of the Memorial Bahçelievler Hospital Ethics Committee (approval number: 129, date: 11.06.2024), the database of our institution and that of the senoir author were scanned for patients with painful snapping of the scapula who underwent ST arthroscopic approach between 2013 and 2022. Patients with no history of trauma, absence of structural abnormalities (like osteochondroma, rib deformity, or tubercle of Luschka), and a minimum postoperative follow-up duration of 2 years were included in the study. However, the presence of inferior scapular pole symptoms, inaccessibility of medical records, interrupted follow-up, prior surgery on the ipsilateral shoulder, cervical spine pathology, and refusal to participate were the exclusion criteria. Informed consent was obtained from all participants. All patients were diagnosed by clinical examination and conventional X-ray followed by magnetic resonance imaging for better visualization, confirmation of bursal inflammation, and ruled out the presence of ST masses. Additionally, the diagnosis was supported by temporary symptom relief after the injection of a mixture of local anesthetic and corticosteroids. Each patient continued to complain of mechanical symptoms despite at least 6 months of conservative treatment, including completion of a physiotherapy-guided rehabilitation program. Demographic information, clinical outcomes, and complication rates of the patients were reviewed.

Currently, no specific score for evaluating SSS. To assess functional level and pain preoperatively and postoperatively, the QuickDASH and Constant-Murely Scores were utilized (7). The visual analog scale (VAS) was used to gage pre- and postoperative pain levels. Additionally, patients were questioned about the incidence of postoperative clicking and their overall satisfaction with the procedure.

Surgical Technique

All patients were operated under general anesthesia. Patients were laid down prone, with their hands in a "chicken-wing" position in the middle of the back. All surgical procedures were performed using a two-portal technique for soft tissue debridement and superomedial scapuloplasty. The initial inferior portal was localized halfway between the scapular spine and the inferior scapular angle, 2-3 cm medial to the medial border of the scapula. The skin and subcutaneous tissue were incised vertically, and forceps were passed to the ST space by blunt dissection. The angle of view was increased by gentle blunt dissection after confirmation of the location of the trochar under the scapula. The superior working portal was placed by triangulation and localized 2-3 cm medial to the medial border of the scapula at the level of or just inferior to the root of the scapular spine (Figure 1). After adequate visualization, diagnostic arthroscopy was performed. The inflamed bursa and interposed soft tissues were removed using a shaver and radiofrequency ablation probe. After localization of the superomedial scapular angle using a spinal needle, a radiofrequency ablation probe was used to elevate the underlying muscular attachments, debride the soft tissue, and skeletonize the anterior surface of the superomedial scapular angle. All encountered fibrotic tissue was considered abnormal and debrided. Then, a high-speed burr was used to excise the undersurface prominence of the superomedial scapular angle. The cutting-block technique was used for bone resection progressing from the proximal to distal and medial to lateral (Figure 2). Meticulous attention was paid to avoid penetrating the dorsal surface of the bone to maintain the overlying muscle insertions. At the end of the operation, the following hemostasis and irrigation, the portals were sutured.

Postoperative Care

Postoperatively, no immobilization except arm sling was applied. Rehabilitation of the shoulder and ST joints was started on the second postoperative day by initiating active and passive motion of both the scapular and glenohumeral joints. Patients were enrolled in a supervised periscapular rehabilitation program administered by a physiotherapist. The objective of this program was to facilitate the achievement of full active shoulder movement within a week. Typically, isometric strengthening exercises for the glenohumeral joint are initiated in the third postoperative week, while periscapular strengthening exercises are initiated around the fifth postoperative week. After six weeks post-surgery, patients were permitted to resume their regular and sports activities as tolerated.

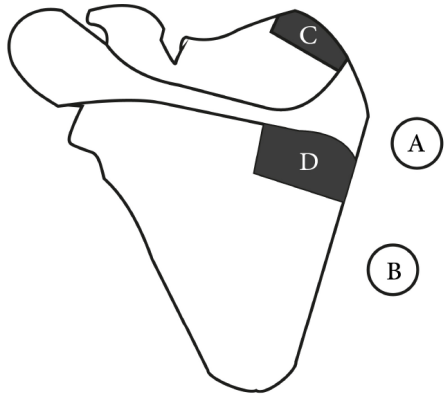

Figure 1. Schematic representation of the posterior surface of the scapula. A) Superior medial working portal, B) Inferior medial entry portal, C) Resection zone of anterior scapuloplasty, D) Area in the infraspinatus fossa that has the potential for depressed fracture during arthroscopy

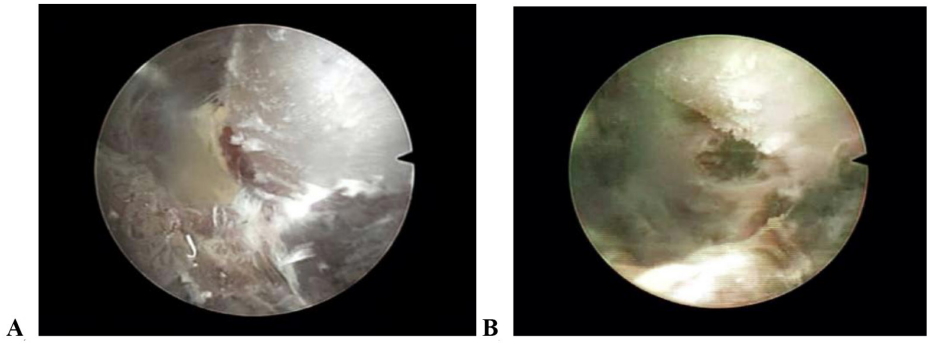

Figure 2. Arthroscopic images of scapula's superomedial angle (A) before and (B) after resection

All patients were seen at the clinic within 4 weeks after their respective surgeries. Further clinic reviews were scheduled as necessary. To ensure complete follow-up, patients were invited to attend a clinical review at the appropriate time.

Statistical Analysis

Statistical analysis was conducted using SPSS version 22.0 (IBM, Inc., Chicago, Illinois, USA). All values are expressed as means ± standard deviation and range. The preoperative and postoperative values of each pain and functional score were compared using the Wilcoxon signed-rank test. A p-value 0.05 was considered significant.

Results

During the study period, 28 patients diagnosed with SSS were treated with ST arthroscopy. Out of these patients, six were unreachable for follow-up, and three declined to participate, leaving 19 individuals for final evaluation. The average age of the patients at the time of surgery was 31±10 (range, 19-52) years, and the mean period of symptoms before surgical intervention was 16.8±8 (range, 8-34) months. The average time of following up was 68.4±24 (range, 26-108) months. The study included 13 females and 6 males. Symptoms were observed in the dominant limb of 13 and the non-dominant limb of 6 patients. The right side was affected in 12 patients and the left side in 7 patients. Baseline patient demographics and assessment scores are presented in Table 1.

At the final follow-up, five of the 19 patients reported no improvement after surgery. Although the remaining 14 patients were satisfied with the surgical results, 4 continued to experience pain to a lesser extent than their preoperative symptoms. Out of 19 patients, 11 reported persistent snapping at follow-up, and only 5 of them were painful and disturbing the patients. A significant improvement was observed in the VAS with a mean preoperative score of 7±0.9 (range, 6-9) and a postoperative score of 2±2.4 (range, 0-6) (p<0.001). Additionally, there was a significant improvement in the mean Quick-DASH and Constant-Murley Score From 43±11.3 (range, 36-66) and 53.3±10.2 (range, 34-68) to 16.3±17.7 (range, 2-46) (p<0.001) and to 75.9±17.2 (range, 53-95) (p<0.001), respectively.

Table 1. Patients baseline demographics, characteristics and clinical outcomes

Patient	Age (years)	Gender	Side	Dominant limb	Symptom duration (months)	Follow-up duration (months)	Visual Analog Score		Quick-DASH Score		Constant Score		Satisfaction	Postoperative criptation	Complication
							Pre-op.	Post-op.	Pre-op.	Post-op.	Pre-op.	Post-op.			
1	24	F	R	Y	14	108	6	2	42	11	58	74	S	Y	N
2	32	F	R	Y	10	102	7	5	45	42	34	53	D	Y	N
3	20	M	R	N	9	102	9	4	36	14	66	62	S	N	Y
4	21	F	L	N	12	96	7	3	38	8	60	83	S	Y	N
5	47	M	R	Y	26	90	6	0	39	5	53	86	V	N	Y
6	24	F	L	N	33	78	8	0	46	3	62	91	V	N	Y
7	23	F	R	Y	22	75	6	3	43	22	55	66	S	Y	N
8	52	M	R	Y	15	72	6	5	55	44	50	55	D	Y	N
9	22	F	L	N	18	70	8	0	47	2	68	95	V	Y	Y
10	26	F	R	Y	23	69	6	0	35	4	52	87	V	N	N
11	26	M	L	N	34	66	7	0	42	6	63	86	V	N	N
12	31	F	R	Y	23	64	7	5	56	42	48	54	D	Y	N
13	43	F	R	Y	9	60	8	6	63	46	44	49	D	Y	N
14	29	F	L	Y	15	60	7	0	21	4	57	92	V	N	N
15	19	M	L	N	11	59	8	0	39	5	46	88	V	Y	N
16	37	M	R	Y	19	43	6	0	24	2	43	90	V	Y	N
17	45	F	R	Y	10	31	8	6	66	46	32	48	D	Y	N
18	30	F	L	Y	8	29	7	0	38	2	59	90	V	N	N
19	38	F	R	Y	8	26	6	0	42	2	64	94	V	N	N

F: Female, M: Male, R: Right, L: Left, Y: Yes, N: No, V: Very satisfied, S: Satisfied, D: Dissatisfied, Pre-op.: Pre-operative, Post-op.: Post-operative

Complications

A depressed fracture at the infraspinatus fossa, located just below the scapula, was encountered in four patients during the surgery. In each of these cases, surgery was completed only after the necessary debridement and scapuloplasty had been performed, and no further action was taken. Postoperative X-rays did not show a fracture line, and no symptoms related to the fracture were observed. Despite the presence of these fractures, all patients followed the standard rehabilitation protocol without any issues. Other complications, such as peripheral nerve or vascular injuries, were not reported.

Discussion

In the present study, we investigated the clinical outcomes and complications of ST arthroscopy in patients with resistant SSS. Our results demonstrated that despite a number of patients who did not respond to the surgical intervention, arthroscopic debridement of the ST space and superomedial scapuloplasty provided significant improvements in functional outcomes and remarkable pain relief in patients with recalcitrant symptoms. Our second finding was that ST arthroscopy was safe and did not encounter serious complications. However, depressed fracture can occur in the infraspinatus fossa of the scapula in a non-negligible number of patients who do not need extra measures other than following an ordinary postoperative rehabilitation protocol.

Literature shows conflicting evidence regarding the outcomes of arthroscopic procedures for patients with SSS. Studies reported that average postoperative functional scores remained lower than expected (8), while others reported that 100% of patients experienced improvement in symptoms (9). Our findings indicate that 26% of the patients did not experience any postoperative improvement. The multifactorial nature of SSS makes it difficult to determine specific reasons for variations in outcomes among patients undergoing surgery. Surgery only addresses a portion of the underlying structural cause, whereas other functional causes, such as psychologic and neurologic disorders, cannot be addressed (10). Therefore, selecting the right patient for ST arthroscopy is crucial, and ST arthroscopy should only be considered after a specific rehabilitation program has been attempted and failed to alleviate symptoms. Patients who rely solely on surgery without physical therapy are unsuitable candidates for this procedure.

Owing to the lack of clarity regarding the etiology and impact of any bone anatomy variation on the initiation of snapping scapula, we preferred to combine superomedial angle scapuloplasty with bursectomy in our approach for all of these patients, regardless of preoperative images or intraoperative findings. We believe that a more extensive intervention can address potential undiagnosed structural abnormalities. However, a recent comparative study showed that both bursectomy alone and bursectomy with scapuloplasty had comparable pain levels, functional improvement, and additional shoulder operation demand (11).

Despite its potential for neurovascular complications, ST arthroscopy is a considerably safe procedure with no complications other than a few intraoperative neurovascular injuries reported in the literature (12). Among the 19 patients who underwent ST arthroscopy, four had depressed fractures in the infraspinatus fossa just inferior to the root of the scapular spine (Figure 1). Fortunately, these fractures did not affect the clinical outcome or alter the postoperative rehabilitation protocol. In the last follow-up, three of these cases were pain-free, whereas the other patient reported persistent pain, although it was reduced in severity. We propose that these fractures could occur during trochar insertion through the initial inferior portal. An anatomically weak region of the scapula located just anterior to the inferior entry portal when the trochar is forcefully propagated in an attempt to penetrate the bulk of subcutaneous tissue and muscles, resulting in depressed fractures. In addition, excessive forces applied to thrust the medial side of the scapula away from the thoracic wall might be a factor that facilitates this injury by allowing the anatomically weak part to be struck perpendicularly by the tip of the trochar.

To avoid such complications, we recommend using blunt-tip forceps to open the initial portal, ensuring that the portal is sufficiently wide for the trochar to be inserted without difficulty. The projection of the trochar should be parallel to the bony surface of the scapula upon entry, and debridement should be performed as gently as possible while avoiding aggressive maneuvers. All these fractures occurred in our early experience, but as our learning curve improved, we did not encounter any such complications. Therefore, beginners must be aware of this complication and know how to avoid it.

Our study has certain limitations that cannot be overlooked. First and foremost, the retrospective study design and the absence of a control group are significant limitations. The scarcity of information on the incidence rate of the syndrome hinders the planning of randomized studies that would generate more credible and consistent outcomes. However, despite these constraints, the current study provides valuable information about the safety, surgical outcomes, and complications of this approach.

Conclusion

In conclusion, despite the potential for complications, arthroscopy for snapping scapula appears to be an effective, reproducible, and safe procedure. Simple depression fractures at the infraspinatus fossa of the scapula can occur during arthroscopy, especially when performed by a less experienced surgeon. These fractures do not require additional treatment and do not adversely affect surgical outcomes. To avoid these potential fractures, a cautious surgical technique is recommended, and forceful maneuvers should be avoided in the anatomically vulnerable region.

Ethics

Ethics Committee Approval: This study approved by Memorial Bahçelievler Hospital Ethics Committee (approval number: 129, date: 11.06.2024).

Informed Consent: Informed consent was obtained from all participants.

Authorship Contributions

Surgical and Medical Practices: A.M.H., M.K., Concept: A.M.H., M.K., Design: A.M.H., M.K., Data Collection or Processing: A.M.H., M.K., Analysis or Interpretation: A.M.H., M.K., Literature Search: A.M.H., M.K., Writing: A.M.H., M.K.

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REFERENCES

1. Baldawi H, Gouveia K, Gohal C, Almana L, Paul R, Alolabi B, et al. Diagnosis and Treatment of snapping scapula syndrome: A scoping review. Sports Health. 2022;14:389-396. [Crossref]
2. Blønd L, Rechter S. Arthroscopic treatment for snapping scapula: a prospective case series. Eur J Orthop Surg Traumatol. 2014;24:159-164. [Crossref]
3. Gambhir N, Alben MG, Kim MT, Pines Y, Virk MS, Kwon YW. Outcomes after arthroscopic scapulothoracic bursectomy for the treatment of symptomatic snapping scapula syndrome. JSES Int. 2022;6:1042-1047. [Crossref]
4. Warth RJ, Spiegl UJ, Millett PJ. Scapulothoracic bursitis and snapping scapula syndrome: a critical review of current evidence. Am J Sports Med. 2015;43:236-245. [Crossref]
5. Nicholson GP, Duckworth MA. Scapulothoracic bursectomy for snapping scapula syndrome. J Shoulder Elbow Surg. 2002;11:80-85. [Crossref]
6. Memon M, Kay J, Simunovic N, Ayeni OR. Arthroscopic management of snapping scapula syndrome improves pain and functional outcomes, although a high rate of residual symptoms has been reported. Knee Surg Sports Traumatol Arthrosc. 2018;26:221-239. [Crossref]
7. Tytherleigh-Strong G, Gill J, Griffiths E, Al-Hadithy N. Prognosis after arthroscopic superior medial scapuloplasty for snapping scapula syndrome improves after a transient beneficial response with an ultrasound-guided subscapular cortisone injection. Arthroscopy. 2020;36:2965-2972. [Crossref]
8. Millett PJ, Gaskill TR, Horan MP, van der Meijden OA. Technique and outcomes of arthroscopic scapulothoracic bursectomy and partial scapulectomy. Arthroscopy. 2012;28:1776-1783. [Crossref]
9. Pavlik A, Ang K, Coghlan J, Bell S. Arthroscopic treatment of painful snapping of the scapula by using a new superior portal. Arthroscopy. 2003;19:608-612. [Crossref]
10. Menge TJ, Horan MP, Tahal DS, Mitchell JJ, Katthagen JC, Millett PJ. Arthroscopic treatment of snapping scapula syndrome: outcomes at minimum of 2 years. Arthroscopy. 2017;33:726-732. [Crossref]
11. Featherall J, Christensen GV, Mortensen AJ, Wheelwright JC, Chalmers PN, Tashjian RZ. Arthroscopic scapulothoracic bursectomy with and without superomedial angle scapuloplasty: a comparison of patient-reported outcomes. J Shoulder Elbow Surg. 2023;32:1945-1952. [Crossref]
12. Dey Hazra RO, Elrick BP, Ganokroj P, Nolte PC, Fossum BW, Brown JR, et al. Anatomic safe zones for arthroscopic snapping scapula surgery: quantitative anatomy of the superomedial scapula and associated neurovascular structures and the effects of arm positioning on safety. J Shoulder Elbow Surg. 2022;31:e465-72. [Crossref]

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Effects of Low-Molecular-Weight Heparins on Bacterial Translocation in an Experimental Mesenteric Ischemia Reperfusion Injury Model

Moleküler Ağırlıklı Heparinlerin Bakteriyel Translokasyon Üzerine Etkileri

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ABSTRACT

Background: Low molecular weight heparin (LMWH) has been reported to prevent intestinal bacterial translocation (BT), although this is not certain. The aim of this study was to determine the effect of LMWH used to prevent BT after mesentery ischaemia-reperfusion (I/R).

Materials and Methods: In this controlled experimental study, 21 female Wistar-Albino rats with an average weight of 250-350 grams were used. The rats were randomly divided equally (n=7) into 3 groups (control group, I/R group and I/R+LMWH group). Control group, no procedure other than explorative laparotomy (Ex-lab.) was performed. I/R group, superior mesenteric artery was clamped for 45 minutes and reperfusion was performed for 60 minutes after Ex-lab. I/R+LMWH group, 1 mg/kg enoxaparin sodium was given to the rats 4 hours before the I/R group. At the end of the experiment, samples taken from the ileum were evaluated histopathologically. The number of microorganisms per gram of tissue was analysed in blood, mesenteric lymph node (MLN), spleen and liver samples. Serum nitric oxide (NO) levels were also measured.

Results: When the groups were evaluated histopathologically in terms of ileal tissue damage, the difference between I/R and I/R+LMWH groups was found to be statistically insignificant (p=0.318). However, BT levels in tissue cultures of I/R+LMWH group were significantly decreased compared to I/R group (39% vs. 75%, p<0.001). There was no statistical difference between the bacterial counts per gram of total tissue of MLN, spleen and liver tissues of I/R and I/R+LMWH groups (4458.37 vs. 3157.14 colony-forming units/g, respectively, p=0.101). Although there was no statistical difference between them, NO levels in I/R+LMWH group tended to be higher than control and I/R groups [295 (149-437), 165 (89-298) and 192 (80-263) pg/mL; p=0.0626].

Conclusion: Since LMWH decreased BT and increased NO levels in patients with mesenteric ischaemia, it was determined that LMWH could be used as a therapeutic option to prevent sepsis.

Keywords: Low molecular weight heparin, ischemia-reperfusion, bacterial translocation, nitric oxide

ÖZ

Amaç: Düşük moleküler ağırlıklı heparinin (DMAH), kesin olmamakla birlikte intestinal bakteriyel translokasyonu (BT) önleyebileceği bildirilmektedir. Bu çalışmanın amacı, mezenter iskemi-reperfüzyon (İ/R) sonrası gerçekleşen BT engellemek için kullanılan DMAH'nin etkisini belirlemekti.

Gereç ve Yöntemler: Bu kontrollü deneysel araştırmada ortalama 250-350 gram ağırlığında 21 adet dişi Wistar-Albino sıçan kullanıldı. Sıçanlar eşit şekilde (n=7) randomize olarak 3 gruba ayrıldı (kontrol grubu, İ/R grubu ve İ/R+DMAH grubu). Kontrol grubuna eksploratif laparotomi (Ex-lab.) dışında, herhangi bir işlem yapılmadı. İ/R grubunda; Ex-lab. sonrasında, süperior mezenterik arter 45 dakika klemplendikten sonra 60 dakika reperfüzyon yapıldı. İ/R+DMAH grubunda ise İ/R grubuna uygulanan işlemler yanı sıra, 4 saat

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ÖZ

önce sıçanlara 1 mg/kg enoksaparin sodyum verildi. Deney sonunda ileumdan alınan örnekler histopatolojik olarak değerlendirildi. Kan, mezenterik lenf nodu (MLN), dalak ve karaciğer örneklerinde ise doku gramı başına düşen mikroorganizma sayısına bakıldı. Ayrıca serum nitrik oksit (NO) düzeyleri ölçüldü.

Bulgular: Gruplar histopatolojik olarak ileal doku hasarı açısından değerlendirildiğinde, İ/R ve İ/R+DMAH grupları arasındaki farkın istatistiksel olarak anlamsız olduğu saptandı (p=0,318). Ancak İ/R+DMAH grubunun doku kültürlerindeki BT düzeylerinin, İ/R grubuna kıyasla anlamlı düzeyde azaldığı görüldü (%39 vs. %75, p<0,001). İ/R ve İ/R+DMAH gruplarının MLN, dalak ve karaciğer dokularına ait total doku gramı başına düşen bakteri sayıları arasında istatistiksel olarak fark saptanmadı (sırasıyla, 4458,37'e karşı 3157,14 koloni oluşturan birimler/g, p=0,101). Aralarında istatistiksel fark saptanmamakla birlikte İ/R+DMAH grubunun serum NO düzeyleri, kontrol ve İ/R gruplarından daha yüksek çıkma eğilimindeydi [295 (149-437), 165 (89-298) and 192 (80-263) pg/mL; p=0,0626].

Sonuç: DMAH'nin mezenter iskemi geçiren hastalarda oluşan BT'yi azalttığından ve NO düzeylerini arttırdığından, terapötik bir seçenek olarak sepsisi engellemek amacıyla kullanılabileceği saptandı.

Anahtar Kelimeler: Düşük moleküler ağırlıklı heparin, iskemi-reperfüzyon, bakteriyel translokasyon, nitrik oksit

Introduction

Acute Mesenteric Ischemia (AMI), a vascular condition with an increasing incidence worldwide, has a mortality rate of 60-80% and requires urgent intervention. Reperfusion-induced damage may also occur after therapeutic procedures to restore mesenteric blood circulation without the development of intestinal necrosis. Therefore, an early recognition of pathology is important for effective treatment.

I/R injury in the small intestine after mesenteric ischemia is characterized by microvascular and mucosal changes. Due to decreased resistance of damaged tissues and mucosa to endogenous microorganisms, bacteria translocate to extraintestinal areas, such as the bloodstream, mesenteric lymph node (MLN), liver, and spleen. The physiopathological basis of microvascular changes due to ischaemia-reperfusion (I/R) is attributed to nitric oxide (NO) and oxidative events, which are vascular tone regulators, anti-aggregants, and regulators of leukocyte activation/migration. This can progress to Systemic Inflammatory Response Syndrome, in which many organs are affected. Therefore, correcting the pathology causing microcirculatory dysfunction in the intestine is one of the main goals of treatment. Research is increasingly focused on this issue (1-3). Recently, it has been suggested that heparin-based anticoagulant therapy may be effective in preventing I/R injury, although this is not certain. Low molecular weight heparin (LMWH), which is obtained by depolymerization of unfractionated heparin with an average of 5000 Da, can be effective even at low doses because it binds to plasma proteins. In addition, when administered subcutaneously, its bioavailability is 100%, reaching a peak level in an average of 4 hours and inactivating factor Xa just like unfractionated heparin (4). Many studies have shown that LMWH is also anti-inflammatory (5,6). Moreover, LMWH has been shown to have a protective effect against endothelial damage by preventing leukocyte adhesion in rats with endotoxemia (7). The possible reason for this effect has been attributed to the effect of LMWH to prevent microcirculatory dysfunction. In other words, this effect may be considered as the repair of pathophysiological mechanisms that occur with reperfusion after mesenteric ischemia. Bleeker et al.(8) reported that heparin prevented endothelial cell dysfunction and decreased leukocyte adhesion after ischemia. Another study supporting this finding is the report by Zapata-Sirvent et al. (9) that heparin decreases the incidence of computed tomography (CT) in burn injury.

In light of the above information, we investigated the effects of LMWH, which has been proven to have properties other than its anticoagulant effect, on reperfusion injury, CT, and NO in mesenteric ischemia, which is difficult to treat and manage, and has a high mortality rate.

Materials and Methods

Experimental Animals and Group Creation

In the study, 21 female Wistar-Albino rats, 6-8 weeks old, with an average weight of 250-350 g, and given standard rat chow and water ad libitum, were used. The rats were randomly divided into 3 groups, each group consisting of 7 rats.

Group 1. Sham group (n=7): Rats underwent laparotomy only, and mesenteric ischemia was not induced.

Group 2. Mesenteric I/R group (n=7): 45 minutes mesenteric ischemia followed by 60 minutes reperfusion.

Group 3. Mesenteric I/R+LMWH group (n=7): A single subcutaneous dose of 1 mg/kg LMWH was administered 4 hours before mesenteric ischemia was induced and 45 minutes of mesenteric ischemia was followed by 60 minute of reperfusion.

Enoxaparin sodium (Clexan, Aventis Pharma, France) was used as the LMWH.

After these procedures, blood, MLN, intestinal (ileum), liver, and spleen tissue samples were obtained from all 3 groups.

Surgery Procedure

Group 1 (Sham group): A mixture of Xylazine (5 mg/kg) and Ketamine (40 mg/kg) was administered as a single dose via subcutaneous injection. The rats were placed on the experimental table in the supine position and were secured by their front and hind legs. The abdominal skin of all experimental animals was shaved on an operating table and cleaned with 10% povidone iodine. The abdomens of the rats were then opened (Figure 1A), and a 3-cm laparotomy was performed. The cecum and large intestines were removed from the incision site and preserved by superior mesenteric artery (SMA) observation (Figure 1B). Without any procedure, 2 cm of the terminal ileum was resected and placed in formalin solution for pathological examination. Then, approximately 1x1 cm samples were taken from the MLN, liver, and spleen and placed in microbiologic tubes. Finally, blood was collected from the v. port and placed in microbiological tubes, after which the subjects were sacrificed.

Group 2 (I/R group): The same procedures were performed in the I/R group until SMA was detected. The ligament of Treitz was found and cut, the SMA was occluded with an atraumatic microvascular clamp from the aorta, and the intestines were left to ischemia for 45 minutes. After 45 minutes of ischemia following the observation of pallor in the intestines and disappearance of pulse, reperfusion was performed for 60 minutes after the intestines turned pink and pulses returned when the clamps were opened. The following reperfusion, tissue and blood samples were collected as in the first group.

Group 3 (I/R+LMWH group): Enoxaparin sodium was administered subcutaneously at a single dose of 1 mg/kg 4 hours before the procedure (peak plasma level reached 3-4 hours). Subsequently, the same procedures were applied to the second group.

Histopathological Investigations

For histopathological examination, ileal tissue sections were fixed with 10% formaldehyde. After dehydration with alcohol and embedding in paraffin blocks, thin sections were obtained. These sections were stained with Hematoxylin and Eosin stain. Histologic changes were quantitatively evaluated by light microscopic examination. Tissue damage in the terminal ileum was graded using the Chiu classification (10).

Microbiological Analysis

Sterile tissue (MLN, spleen and liver) samples were weighed on a precision balance and placed in sterile tubes containing 1 mL of thioglycolate broth with known weights. The tissues were crushed under sterile conditions and homogenized by cortexin. Aerop cultures were inoculated on 5% sheep blood agar, chocolate agar, and eosin methylene blue (EMB) agar (RTA, Türkiye) and incubated at 37 °C for 24-48 hours. Colonies in cultured Petri dishes were stained with Gram-stained slides. Bacterial colony counts (Phoenix 100, BD Diagnostics, USA) were determined.

In anaerobic media prepared using gas kits (Anaero-Gen, Oxoid, UK), samples were inoculated with blood, EMB, and Schaedler medium (RTA, Türkiye) and incubated at 37 °C for 72 hours. The morphology of the colonies in growth media was compared with that of the colonies in aerobic media, and gram-stained slides were prepared and analyzed. For aerotolerance control, blood was passaged and incubated at 37 °C for 24 hours in aerobic medium.

The following formula was used to calculate the number of microorganisms per gram of tissue as the CT index in tissues with growth:

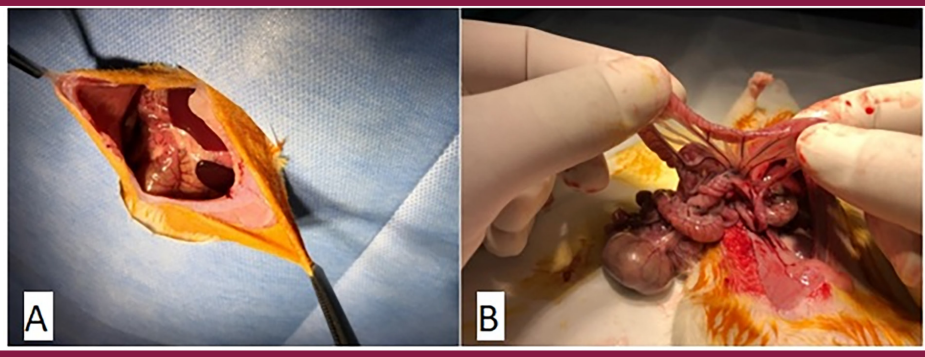

Figure 1. Explorative laparotomy procedure. A) Open the abdomen of rats after skin cleansing with povidone-iodine, B) Visualizing and protecting the Superior Mesenteric Artery

Number of colonies per tissue (CFU/g)=[Number of colonies (CFU)xReconstitution valuex10]/[Tissue weight]

Blood samples were added to automated blood culture bottles (BACTEC 9120, BD Diagnostics, USA) and monitored for 7 days. Samples that did not show any growth signals within this period were Gram-stained slides, passaged on 5% sheep blood agar media, and confirmed as negative. Signaling samples were passaged onto 5% sheep blood agar and EMB medium and incubated at 37 °C for 24 hours. At the end of this period, the colonies in the medium that grew in aerobic cultures were first placed on Gram-stained slides and then identified by microbiological methods.

NO measurements

NO levels in sera obtained from rats were measured in a spectrophotometer (Pharmacia Biotech, Novaspec II, Cambridge, England) using 550 nm wavelength with a colorimetric method kit (Elabscience, USA). Test principle; NO is oxidized by the formation of NO_2 in solution *in vivo*. With a color-developing agent, it gives a red (azo compound). The color intensity is proportional to the concentration.

Ethical Approval

This study was reviewed by the Bolu Abant İzzet Baysal University Faculty of Medicine Animal Experiments Ethics Committee and approved on 10/05/2017 with the decision number 2017/26. The surgeries were performed in Bolu Abant İzzet Baysal University Faculty of Medicine Experimental Animals and Research Laboratory.

Statistical Analysis

The chi-square test was used to compare the presence of categorical data in the control, I/R, and I/R+LMWH groups. In addition, the I/R and I/R+LMWH groups were compared according to the number of bacteria per gram of tissue and ileal damage scoring. Since the data for these comparisons did not show a normal distribution and the number of bacteria in the control group had a value of 0, the non-parametric Mann-Whitney U test was used. Kruskal-Wallis test was used to compare the parametric data of the three independent groups. SPSS 22.0 software was used for data analysis and α=0.05 was used for statistical significance.

Results

Histopathological Examination Results

As a result of histopathological examination of ileal tissue samples obtained from the subjects (Table 1), no difference was found between the I/R and I/R+LMWH groups in terms of damage scoring [3 (3-5) vs. 5 (2-5), p=0.318]. In addition, the histopathological differences between the two groups is as shown in Figure 2. According to these findings, although the I/R+LMWH group had higher damage scores than the I/R group, the difference was not statistically significant. This effect was not interpreted as tissue damage by LMWH.

Microbiological Analysis Results

Bacterial growth in MLN, spleen, liver, and blood tissue samples obtained from subjects was calculated in a sterile environment. When the percentages of bacterial growth in the tissue samples of the subjects in the control, I/R, and I/R+LMWH groups were statistically compared, no bacterial growth was observed in any of the tissue samples of the control group (Table 2). In the chi-square statistic in which the three groups were evaluated together, while there was a statistical difference in the number of MLN,

Table 1. The results of the damage score determined by Chiu Scoring of histopathological changes in the tissue samples of the subjects separately according to the groups

Group Rat number	Control	I/R	I/R+LMWH
1	0	3	5
2	0	3	5
3	0	3	3
4	0	4	5
5	0	5	5
6	0	3	4
7	0	4	2
Mean ± SD Median (Min.-Max.)	0	3.6±0.8 3 (3-5)	4.1±1.2 5 (2-5)
Mann-Whitney U test. P-value.		0.318	

The statistical significance level was taken as α=0.05. Control: The group that underwent only laparotomy without mesenteric ischemia. I/R: Ischemia/reperfusion, LMWH: Low molecular weight heparin, SD: Standard deviation, Min.: Minimum, Max.: Maximum

spleen, and liver tissue samples in which microbiological translocation (growth) was detected (p=0.001; p=0.005; p=0.001, respectively), when only the I/R and I/R+LMWH groups were compared, translocation was detected in 7 MLN tissues of the I/R group (n=7), while translocation was detected in 5 tissues in the I/R+LMWH group. The difference between the two groups was not statistically significant (p=0.127). There were translocations in 6 splenic tissues in the I/R group (n=7) and 3 splenic tissues in the I/R+LMWH group. The difference between the two groups was statistically significant (p=0.031). There were translocations in 7 liver tissue in the I/R group (n=7) and 3 liver tissue in the I/R+LMWH group. The difference between the two groups was statistically significant (p=0.018). However, there was no significant difference in terms of microbiological growth in blood samples obtained from the I/R and I/R+ LMWH groups (p=0.299). In total, bacterial growth was detected in 75% of the tissue samples of the I/R group and 39% of the tissue samples of the I/R+LMWH group. The difference was statistically significant (p<0.001) (Figure 3 and Table 3). According to these results, no growth was observed in any tissue sample from the control group. In addition, we found that bacterial translocation was significantly decreased in the spleen and liver tissue samples of LMWH-treated subjects.

Number of Bacteria per Gram of Tissue

No bacterial growth was observed in any tissue samples from the control group. Bacterial growth was observed in blood samples from one rat in the I/R group. The mean numbers of bacteria per gram of tissue in MLN, spleen, and liver samples of the groups are shown in Figure 4. Although the number of bacteria in the spleen and liver tissues of

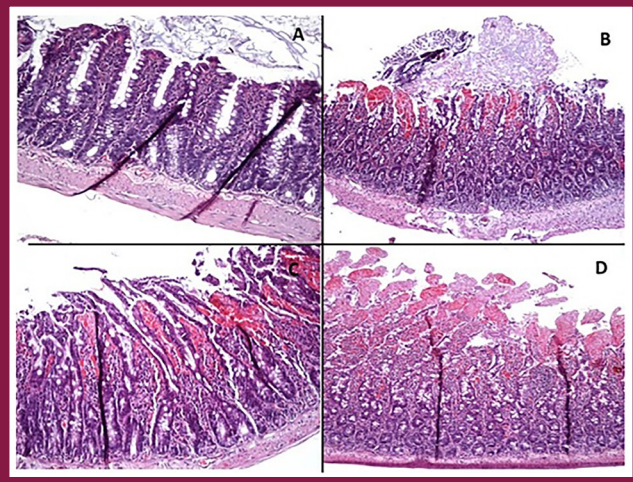

Figure 2. Chiu Scores for Hematoxylin and Eosin stain. A) 0; B) 3; C) 4; D) 5

Table 2. Bacterial growth in mesenteric lymph node, spleen, liver, and blood tissue samples according to groups

Tissue	Groups	NTS, n	NRTD, n (%)	p-value
MLN	A: Control	7	0 (0%)	0.001[a]
	B: I/R	7	7 (100%)	
	C: I/R+LMWH	7	5 (71%)	
Comparison p	0.127			
Spleen	A: Control	7	0 (0%)	0.005[a]
	B: I/R	7	6 (86%)	
	C: I/R + LMWH,	7	3 (43%)	
Comparison p	0.031			
Liver	A: Control	7	0 (0%)	0.001[a]
	B: I/R	7	7 (100%)	
	C: I/R + LMWH	7	3 (43%)	
Comparison p	0.018			
Blood	A: Control	7	0 (0%)	0.350[a]
	B: I/R	7	1 (14%)	
	C: I/R + LMWH	7	0 (0%)	
Comparison p	0.299			

[a]Chi-square test. The comparison of p with the chi square was made only between B and C. The statistical significance level was taken as α=0.05. MLN: Mesenteric lymph node, Control: Group that underwent only laparotomy without mesenteric ischemi, I/R: Ischemia/reperfusion, LMWH: Low molecular weight heparin, NTS: Number of tissue samples, NRTD: Number of reproductive tissues detected

the I/R+LMWH group was lower than that of the I/R group (spleen: 1400.84±2288.82 vs. 5534.18±6811.85 CFU/g p=0.165; liver: 961.42±1551.84 vs. 4099.74±5105.15 CFU/g p=0.165), this difference was not statistically significant. In MLN tissue, the number of bacteria observed in the I/R+LMWH group was higher than that in the I/R group, but the difference was not statistically significant (7109.17±8196.16 vs. 3741.20±3853.67 CFU/g p=0.535, respectively).

NO Levels

Although there was no statistical difference between the groups (Figure 5), the NO levels of the I/R+LMWH group tended to be higher than those of the control and I/R groups [295 (149-437), 165 (89-298) and 192 (80-263) pg/mL, respectively].

Discussion

Mesenteric ischemia remains an important health problem with a high mortality rate (11). Intestinal obstruction, incarcerated hernia, small-bowel volvulus, pneumoperitoneum, and necrotizing colitis are encountered in I/R injury due to AMI in various clinical situations. Mesenteric ischemia causes various morbidities, such as malabsorption, severe diarrhea, short-bowel syndrome, and high mortality rates. The most important reason for the high mortality rate in AMI is the lack of disease-specific investigations and physical examination methods that can be used for early disease diagnosis. Therefore, treatment is delayed. Early diagnosis can prevent the release of several vasoactive mediators (cytokines, endothelins and free oxygen radicals) that block mesenteric blood circulation and reduce leukocyte activation, endothelial dysfunction,

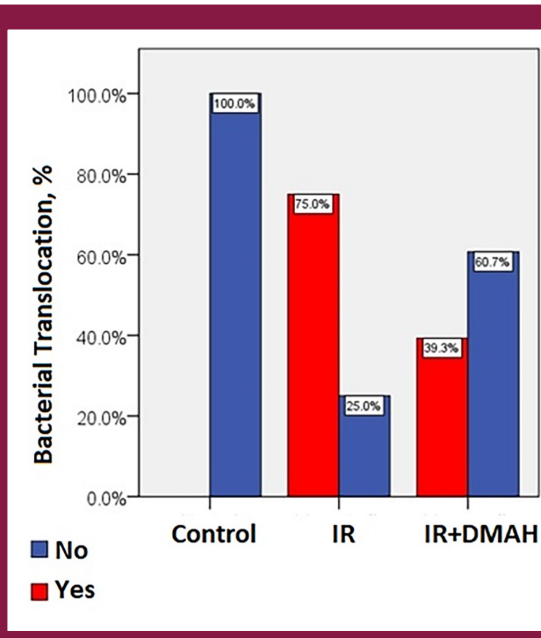

Figure 3. Bacterial growth rates by groups. It appears that bacterial translocation occurs at a higher rate in the I/R group
I/R: Ischaemia-reperfusion, DMAH: Low molecular weight heparin (LMWH)

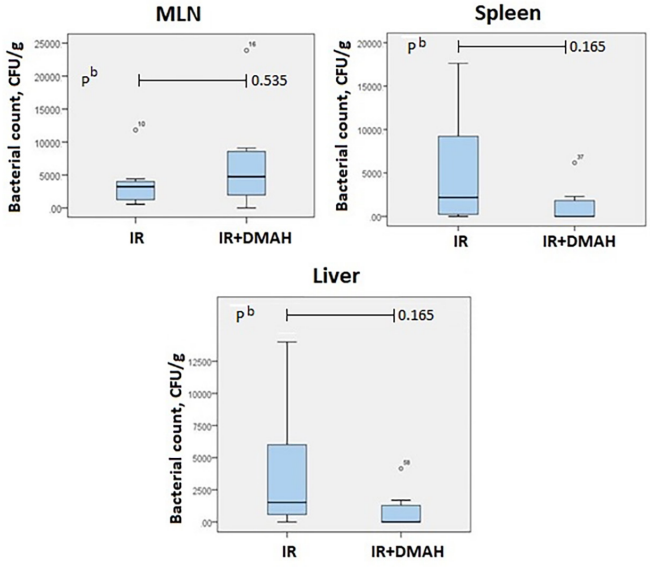

Figure 4. Average bacterial counts of the groups. It is observed that the average bacterial counts are higher in the spleen and liver tissue samples of the I/R group
[b]Mann-Whitney U test, I/R: Ischaemia-reperfusion, DMAH: Low molecular weight heparin (LMWH), MLN: Mesenteric lymph node

Table 3. Comparison of total bacterial reproduction rates among the groups

Groups	NTS	NRTD	p-value
Control	28	0 (0%)	<0.001[a]
I/R	28	21 (75%)	
I/R+LMWH	28	11 (39%)	
Total	84	32 (38%)	

[a]Chi-square test. The statistical significance level was taken as α=0.05. Control: Group that underwent only laparotomy without mesenteric ischemia, I/R: Ischemia/reperfusion, LMWH: Low molecular weight heparin, NTS: Number of tissue samples, NRTD: Number of reproductive tissues detected

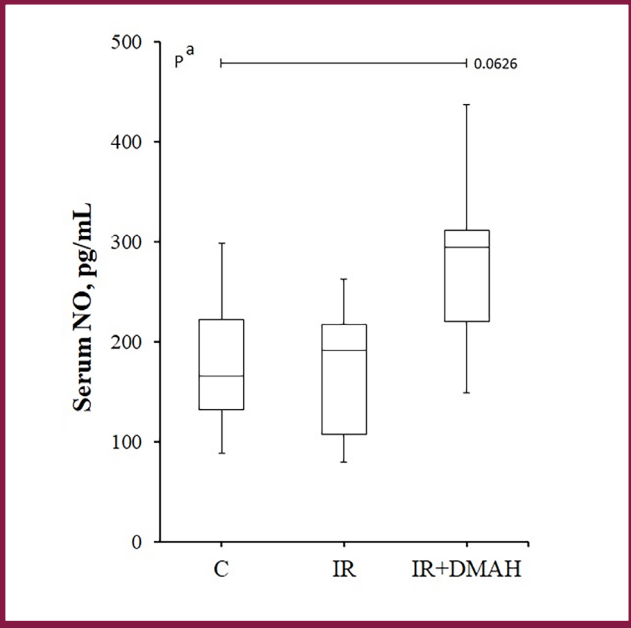

Figure 5. Box plot graph showing NO levels of groups. It was observed that the NO levels of the I/R group are higher than those of the control and I/R+LMWH groups, although there was no statistical difference
[a]Kruskal-Wallis test (non-parametric analysis of variance), I/R: Ischaemia-reperfusion, DMAH: Low molecular weight heparin (LMWH), NO: Nitric oxide

and tissue edema. Therefore, mucosal damage that may occur in the reperfused tissue will be mitigated.

In the studies conducted to date, single or combined pharmacological agents have been tried, but only partial benefits have been found in intestinal I/R injury (12). The main objective of most studies was to prevent or eliminate the formation of inflammatory and toxic mediators that trigger mechanisms that cause multiple damage during the I/R process. In this study, we aimed to investigate whether ischemia-induced tissue damage can be minimized by LMWH in premedication.

One of the consequences of I/R injury in the small intestine after mesenteric ischemia is the translocation of live bacteria and/or their products, termed bacterial translocation (BT), across the intestinal barrier to other tissues. This translocation mostly occurs at sterile body sites such as MLN, spleen, liver, and blood circulation. In a study by Ozban et al. (13) In which intestinal I/R injury was induced by SMA occlusion in rats, BT was demonstrated. Berg (14) described the three primary mechanisms leading to BT. These mechanisms were explained by intestinal bacterial overgrowth, host immune defense inadequacy, and increased or damaged intestinal mucosal permeability. It has also been reported that BT occurring in mesenteric I/R injury may be triggered by oral ricinoleic acid, endotoxemia, zymosan injection, thermal damage, and hemorrhagic shock (14,15). Morehouse et al. (16) showed that intragastric inoculation of ricinoleic acid damages the intestinal mucosa and causes translocation of many endogenous bacteria. In our study, tissue damage caused by mesenteric ischemia in the ileum was demonstrated by pathological examinations and was considered the cause of the increase in CT. Although LMWH was administered, no expected decrease in ileal tissue damage scores in group 3 was not observed. This result was attributed to the small number of subjects or biological differences. Therefore, this finding should be investigated in a larger population.

Prevention of I/R-induced vasoconstriction, oxidative stress, neutrophil migration, platelet aggregation, microcirculatory dysfunction, and BT are considered therapeutic targets. In this context, LMWH, which has a protective effect against endothelial damage and thus is thought to prevent microcirculatory dysfunction, was thought to be used in the treatment of AMI, and its effects on gastrointestinal anatomical changes and CT findings after mesenteric ischemia were investigated. In this context, the fact that NO levels tended to be higher in the I/R+LMWH group than in the control and I/R groups in the blood obtained from rats was interpreted as LMWH prevented tissue damage by inhibiting platelet aggregation and neutrophil migration via NO and regulating microvascular function. In a study by Waisman et al. (17) it was reported that NO was important in the protection of microvascular function and decreased vascular resistance and neutrophil-endothelial cell interaction, which explains the physiopathological

mechanism of our study finding. However, a larger sample size is required to confirm this conclusion.

Alexander et al. (18) have shown that enteric bacteria first settle in the MLN, which is the most vulnerable region, through damaged epithelial cells or cell components along the mucosa. Some bacteria may survive in the MLN or spread to the liver, kidney, and spleen via blood. In our study, although the number of bacteria spreading to the MLN was not statistically significant, a higher bacterial load was found in the MLN compared with the liver and spleen when the number of bacteria per gram of tissue was compared, which is consistent with the literature. This showed that the BT first spread from the MLN, and the bacteria primarily settled in this region.

In recent years, researchers have suggested that antithrombotic molecules such as enoxaparin may also have antioxidant effects. In traumatic brain injury, this molecule has been shown to reduce COX-2 overexpression, increase thiobarbituric acid reactive substances, and increase oxidized protein levels (19,20). In support of these findings, Okutan et al. (21) showed that LMWH (deltaparin, enoxaparin, nadroparin) reduced acute inflammation by reducing early neutrophil infiltration in the vein wall in rats with venous thrombosis. In more detail, Wang et al. (22) reported that the potent anti-inflammatory effects of heparins were mediated by the blockade of P- and L-selectins. Harada et al. (23) showed that a heparinoid derivative (danaparoid sodium) increased the release of the calcitonin gene-related peptide, which ameliorates neuronal damage in rats exposed to I/R injury. In the present study, we demonstrated that enoxaparin can reduce mesenteric ischemia-induced intestinal translocation. However, further studies are required to explain which molecular pathways are involved in this effect. The available data suggest that the antioxidant property of enoxaparin, a LMWH, contributes to this result.

The ability of enoxaparin to provide adequate perfusion in reducing CT has been attributed to its ability to reach sufficient density in small vessels and its anti-inflammatory properties (5,6,24). In a study conducted Iba and Miyasho (5) in rats, it was reported that enoxaparin decreased circulating pro-inflammatory cytokine levels, which may be related to mechanisms that prevent organ dysfunction. In a study in which mesenteric artery ischemia was induced in rats and CT was examined in MLN, it was observed that the decrease in inflammatory molecules was associated with a decrease in the amount of bacteria (25). Another study by Zhang et al. (26) LMWH reduced cerebral I/R injury by regulating energy metabolism and inhibiting apoptosis, in addition to its anti-inflammatory properties.

The anticoagulant properties of heparins are essential to prevent venous thrombosis and improve microcirculation during reperfusion therapy after ischemia. On the other hand, heparins are also known to cause bleeding complications after severe mesenteric I/R (27). In addition, many studies on intestinal I/R injury have reported that heparin sodium administered at therapeutic doses aggravates intestinal injury instead of benefiting (28-30). To investigate this problem, Walensi et al. (31) examined the effect of subtherapeutic doses of enoxaparin (heparin sodium) without anticoagulant effects on intestinal I/R injury in a model of SMA ischemia. This study showed that enoxaparin administered as a premedication provided intestinal protection independent of changes in the intestinal microcirculation and may reduce the risk of ischemia-reperfusion-induced gastrointestinal clinical complications. Again, in a study by Yeh et al. (3) it was reported that enoxaparin prevented intestinal microcirculatory dysfunction by preventing microvascular thrombosis and maintaining arterial pressure in rats with endotoxemia. In our study, when ileal damage classification according to the Chiu score was compared, we found that there was no significant increase or decrease in ileal damage in LMWH-treated rats, and the damage was predominantly observed in grades 4 and 5. This result supported that 1 mg/kg LMWH did not have a positive or negative effect on ulceration and hemorrhage in rats.

The groups included in our study were compared in terms of MLN, liver, spleen, and blood; CT, bacterial load; and ileal damage. Although no significant difference was found between the groups in terms of the number of bacteria per gram of tissue and ileal damage, BT was induced by mesenteric ischemia in all extraintestinal tissues, and this translocation was alleviated by LMWH treatment.

Study Limitations

Since this was an experimental animal study, we were able to work on rats with fewer subjects. Therefore, to clearly assess the effects of LMWH in mesenteric ischemia, studies in humans and more data are needed. The effects on tissues should have also been observed by administering LMWH alone without inducing mesenteric ischemia. This study could be confirmed by a study including other markers of mesenteric ischemia, such as D-dimer and intestinal free fatty acid-binding protein.

Conclusion

It was found that LMWH used in rats with mesenteric I/R was not effective in preventing tissue damage in the terminal ileum, but its effect on NO and microbiological CT was positive. This finding indicated that LMWH could be a therapeutic option for premedication.

Acknowledgments

This publication is based on a thesis (Bolu-2018). NO measurements could not be written in the thesis due to the late arrival of the kit.

Ethics

Ethics Committee Approval: This study was reviewed by the Bolu Abant İzzet Baysal University Faculty of Medicine Animal Experiments Ethics Committee and approved on 10/05/2017 with the decision number 2017/26.

Informed Consent: Not required.

Authorship Contributions

Surgical and Medical Practices: S.K., N.A.K., E.B.K., Concept: S.K., N.A.K., E.B.K., Design: S.K., N.A.K., E.B.K., Data Collection or Processing: S.K., N.A.K., E.B.K., Analysis or Interpretation: S.K., N.A.K., E.B.K., M.Ş., O.H.Ö., Z.M.Y.K., Literature Search: S.K., N.A.K., E.B.K., Writing: S.K., N.A.K., E.B.K., M.Ş., O.H.Ö., Z.M.Y.K.

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REFERENCES

1. Oldenburg WA, Lau LL, Rodenberg TJ, Edmonds HJ, Burger CD. Acute mesenteric ischemia: a clinical review. Arch Intern Med. 2004;164:1054-1062. [Crossref]
2. Kozuch PL, Brandt LJ. Review article: diagnosis and management of mesenteric ischaemia with an emphasis on pharmacotherapy. Aliment Pharmacol Ther. 2005;21:201-215. [Crossref]
3. Yeh YC, Wang MJ, Lin CP, Fan SZ, Tsai JC, Sun WZ, et al. Enoxaparin sodium prevents intestinal microcirculatory dysfunction in endotoxemic rats. Crit Care. 2012;16:R59. [Crossref]
4. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, et al. Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. Intensive Care Med. 2008;34:2210-2217. [Crossref]
5. Iba T, Miyasho T. Danaparoid sodium attenuates the increase in inflammatory cytokines and preserves organ function in endotoxemic rats. Crit Care. 2008;12:R86. [Crossref]
6. Li LF, Huang CC, Lin HC, Tsai YH, Quinn DA, Liao SK. Unfractionated heparin and enoxaparin reduce high-stretch ventilation augmented lung injury: a prospective, controlled animal experiment. Crit Care. 2009;13:R108. [Crossref]
7. Iba T, Takayama T. Enoxaparin attenuates endothelial damage with less bleeding compared with unfractionated heparin in endotoxemic rats. Shock. 2009;32:530-534. [Crossref]
8. Bleeker WK, Agterberg J, Rigter G, Hack CE, Gool JV. Protective effect of antithrombin III in acute experimental pancreatitis in rats. Dig Dis Sci. 1992;37:280-285. [Crossref]
9. Zapata-Sirvent RL, Hansbrough JF, Greenleaf GE, Grayson LS, Wolf P. Reduction of bacterial translocation and intestinal structural alterations by heparin in a murine burn injury model. J Trauma. 1994;36:1-6. [Crossref]
10. Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg. 1970;101:478-483. [Crossref]
11. Tendler DA. Acute intestinal ischemia and infarction. Semin Gastrointest Dis. 2003;14:66-76. [Crossref]
12. Yasuhara H. Acute mesenteric ischemia: the challenge of gastroenterology. Surg Today. 2005;35:185-195. [Crossref]
13. Ozban M, Aydin C, Cevahir N, Yenisey C, Birsen O, Gumrukcu G, et al. The effect of melatonin on bacterial translocation following ischemia/reperfusion injury in a rat model of superior mesenteric artery occlusion. BMC Surg. 2015;15:18. [Crossref]
14. Berg RD. Bacterial translocation from the gastrointestinal tract. Adv Exp Med Biol. 1999;473:11-30. [Crossref]
15. Berg RD. Translocation of enteric bacteria in health and disease. Curr Stud Hematol Blood Transfus. 1992;44-65. [Crossref]
16. Morehouse JL, Specian RD, Stewart JJ, Berg RD. Translocation of indigenous bacteria from the gastrointestinal tract of mice after oral ricinoleic acid treatment. Gastroenterology. 1986;91:673-682. [Crossref]
17. Waisman D, Brod V, Dickstein R, Abramovich A, Rotschild A, Bitterman H. Effects of inhaled nitric oxide on lung injury after intestinal ischemia-reperfusion in rats. Shock. 2005;23:150-155. [Crossref]
18. Alexander JW, Boyce ST, Babcock GF, Gianotti L, Peck MD, Dunn DL, et al. The process of microbial translocation. Ann Surg. 1990;212:496-510. [Crossref]
19. Demirtas S, Karahan O, Yazıcı S, Guclu O, Calıskan A, Tezcan O, et al. Investigation of possible prophylactic,renoprotective,and cardioprotective effects of thromboprophylactic drugs against ischemia-reperfusion injury. Kaohsiung J Med Sci. 2015;31:115-122. [Crossref]
20. Župan Ž, Pilipović K, Dangubić B, Frković V, Šustić A, Župan G. Effects of enoxaparin in the rat hippocampus following traumatic brain injury. Prog Neuropsychopharmacol Biol Psychiatry. 2011;35:1846-1856. [Crossref]
21. Okutan H, Eroğlu E, Karahan N, Aydin A, Tunç B, Çandir Ö, et al. Comparison of different low-molecular-weight heparins (dalteparin, enoxaparin, nadroparin) and standard heparin in rat venous thrombosis model. Turk J Vasc Surg. 2004;13:017-022. [Crossref]
22. Wang L, Brown JR, Varki A, Esko JD. Heparin's anti-inflammatory effects require glucosamine 6-O-sulfation and are mediated by blockade of L- and P-selectins. J Clin Invest. 2002;110:127-136. [Crossref]
23. Harada N, Okajima K, Kohmura H, Uchiba M, Tomita T. Danaparoid sodium reduces ischemia/reperfusion-induced liver injury in rats by attenuating inflammatory responses. Thromb Haemost. 2007;97:81-87. [Crossref]
24. Hagiwara S, Iwasaka H, Hidaka S, Hishiyama S, Noguchi T. Danaparoid sodium inhibits systemic inflammation and prevents endotoxin-induced acute lung injury in rats. Crit Care. 2008;12:R43. [Crossref]
25. Idrovo JP, Yang WL, Jacob A, Aziz M, Nicastro J, Coppa GF, et al. AICAR attenuates organ injury and inflammatory response after intestinal ischemia and reperfusion. Mol Med. 2015;20:676-683. [Crossref]
26. Zhang ZG, Sun X, Zhang QZ, Yang H. Neuroprotective effects of ultra-low-molecular-weight heparin on cerebral ischemia/reperfusion injury in rats: involvement of apoptosis, inflammatory reaction and energy metabolism. Int J Mol Sci. 2013;14:1932-1939. [Crossref]
27. Petrat F, Swoboda S, de Groot H, Schmitz KJ. Quantification of ischemia-reperfusion injury to the small intestine using a macroscopic score. J Invest Surg. 2010;23:208-217. [Crossref]
28. Balogh Z, Wolfárd A, Szalay L, Orosz E, Simonka JA, Boros M. Dalteparin sodium treatment during resuscitation inhibits hemorrhagic shock-induced leukocyte rolling and adhesion in the mesenteric microcirculation. J Trauma. 2002;52:1062-1069. [Crossref]
29. Huwer H, Winning J, Straub U, Isringhaus H, Kalweit G. Clinically diagnosed nonocclusive mesenteric ischemia after cardiopulmonary bypass: retrospective study. Vascular. 2004;12:114-120. [Crossref]

30. Kazmers A, Zwolak R, Appelman HD, Whitehouse WM Jr, Wu SC, Zelenock GB, et al. Pharmacologic interventions in acute mesenteric ischemia: improved survival with intravenous glucagon, methylprednisolone, and prostacyclin. J Vasc Surg. 1984;1:472-481. [Crossref]

31. Walensi M, de Groot H, Schulz R, Hartmann M, Petrat F. Mesenteric ischemia-reperfusion injury: clearly improved hemodynamics but only minor protection of the rat small intestine by (sub)therapeutic heparin sodium and enoxaparin doses. J Surg Res. 2013;179:e57-e69. [Crossref]

ORIGINAL ARTICLE

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Antifungal Susceptibilities of Candida Species Isolated to Clinical Samples

Klinik Örneklerden İzole Edilen Candida Türlerinin Antifungal Duyarlılıkları

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ABSTRACT

Background: Candidiasis is a skin, mucosal, and organ infection caused by Candida fungi, with Candida albicans being the most common. These infections pose significant morbidity and mortality risks, necessitating rapid identification and characterization methods for diagnosis and personalized antifungal therapy based on Candida's pathogenic properties.

Materials and Methods: Candida species isolated from diverse clinical samples from patients were included in this study. A total of 86 isolates were analyzed at the genus and species levels using mass spectrometry, while susceptibility profiles to amphotericin B (AmB), anidulafungin (AND), fluconazole (FLC), and voriconazole were regulated using the gradient test method.

Results: Candida spp. were detected in 1254 clinical samples obtained from 736 patients. Antifungal susceptibility was tested on 86 isolated samples, including C. albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida kefyr, Candida guilliermondii, and Candida krusei. The corresponding rates of resistance to AmB and FLC in C. albicans isolates were 2.2% and 13.3%. No resistance to AND was determined.

Conclusion: Candida species C. albicans is the most regularly isolated, but infections from other Candida species have increased. No resistance to AND was observed, but species-specific resistance to other antifungal agents was identified, emphasizing the need for continuous monitoring.

Keywords: Candida spp, antifungal susceptibility, fluconazole, voriconazole, anidulafungin

ÖZ

Amaç: Kandidiyaz, Candida mantarlarının neden olduğu bir tür cilt, mukoza ve organ enfeksiyonudur; en yaygın olanı Candida albicans'tır. Bu enfeksiyonlar önemli morbidite ve mortalite riskleri oluşturmakta, tanı için hızlı tanımlama ve karakterizasyon yöntemleri ve Candida'nın patojenik özelliklerine dayalı olarak kişiselleştirilmiş antifungal tedaviyi gerektirmektedir.

Gereç ve Yöntemler: Hastalardan alınan çeşitli klinik örneklerden izole edilen Candida türleri bu çalışmaya dahil edildi. Kütle spektrometresi kullanılarak cins ve tür düzeyinde toplam 86 izolat tanımlanırken, gradyan test yöntemi kullanılarak amfoterisin B (AmB), anidulafungin (AND), flukonazol (FLC) ve vorikonazole duyarlılık profilleri belirlendi.

Bulgular: Candida spp. 736 hastadan alınan 1254 klinik örnekte üreme tespit edildi. C. albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida kefyr, Candida guilliermondii ve Candida krusei'nin de aralarında bulunduğu 86 izolat üzerinde antifungal duyarlılık testi yapıldı. C. albicans izolatlarında AmB ve FLC'ye direnç oranları sırasıyla %2,2 ve %13,3'tür. AND'ye karşı direnç tespit edilmedi.

Sonuç: Candida türü C. albicans en sık izole edilen türdür ancak diğer Candida türlerinden kaynaklanan enfeksiyonlar da artış göstermiştir. AND'ye karşı herhangi bir direnç gözlenmedi ancak diğer antifungal ajanlara karşı türe özgü direnç belirlendi ve bu da sürekli izleme ihtiyacını vurguladı.

Anahtar Kelimeler: Candida türleri, antifungal duyarlılık, flukonazol, vorikonazol, anidulafungin

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Introduction

Candidiasis is a prevalent infection caused by Candida fungi, and it affects the skin, mucosal membranes, and internal organs (1). These infections can occur at any age and are frequently linked to particular risk factors. Candida albicans is the predominant pathogen causing candidiasis globally, followed by Candida parapsilosis, Candida tropicalis, and Candida glabrata (1-3). Invasive candidiasis is a highly prevalent condition and is associated with high morbidity and mortality. Hence, it is imperative to develop effective methods for immediately detecting and classifying the condition to ensure precise diagnosis and appropriate antifungal therapy (4,5).

Several studies have examined the clinical significance and therapeutic consequences of different Candida species, highlighting the need to understand their separation, identification, and resistance to antifungal drugs (1,3). Candida species, particularly C. albicans, are frequently found in clinical samples. However, there has been a rise in infections caused by non-albicans species, such as C. parapsilosis, C. tropicalis, and C. glabrata (2,3,6,7). These non-albicans species possess unique characteristics that contribute to their ability to cause disease and respond to antifungal treatment. This highlights the importance of accurately identifying species and developing customized therapeutic strategies (1,2,8-10).

Multiple methodologies, including phenotypic, genotypic, and proteomic techniques, have been devised to precisely determine Candida species (11). Conventional phenotypic approaches have drawbacks in terms of precision and speed, but molecular techniques including polymerase chain reaction, DNA sequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have significantly transformed the area of Candida species identification (11-13). MALDI-TOF MS is a method that involves comparing databases of reference spectra to identify the protein fingerprints of different microorganisms. MALDI-TOF MS exhibits a sensitivity and specificity of up to 100%, enabling rapid and accurate detection and identification of Candida species (11,14,15). The utilization of modern techniques has improved our understanding of the dispersion and frequency of distinct Candida species in diverse clinical environments (16).

The management of candidiasis is increasingly influenced by the development of antifungal resistance. Candida species exhibit different levels of resistance to commonly prescribed antifungal drugs, such as azoles [e.g., itraconazole, voriconazole, fluconazole (FLC)], echinocandin [e.g., anidulafungin (AND), caspofungin], and polyenes [e.g., amphotericin B (AmB)] (9,13,17-19). Comprehending the resistance patterns exhibited by various Candida species is crucial for selecting the most suitable antifungal treatment and avoiding treatment ineffectiveness (1,6,10). Resistance mechanisms, including changes in drug targets, increased production of efflux pumps, and genetic mutations, have been discovered and are associated with decreased susceptibility to antifungal drugs (17,20,21). Candida species play a key role in human illness because of their capacity to create drug-resistant biofilms, which are highly resistant to antifungal treatments (22-24). Treatment options are complicated by Candida isolates that are resistant to many drugs, requiring the improvement of new antifungal techniques. The global prevalence of intrinsic resistance in Candida species, except for C. albicans, is increasing (18,25). Non-albicans have been rising regularly in the last decade. The major cause of therapy failure is the presence of Candida species that exhibit limited sensitivity, collected resistance, or inherent resistance to existing antifungal drugs (16). Overall, candidiasis poses a substantial therapeutic obstacle because of its varied symptoms and the development of resistance to antifungal treatments (19,26,27). To address these challenges, this study aimed to enhance our understanding of the isolation, identification, and antifungal resistance profiles of Candida species.

Materials and Methods

In this study, yeast isolates classified as members of Candida genus, which were obtained from various clinical samples submitted to the Central Laboratory Bacteriology Unit of Dicle University Hospital between 22.12.2019 and 22.10.2020, were included. Only the first isolate of duplicate yeast fungi isolated from the same patient was included in the study, and the isolated samples were stored at -80 °C until the time of analysis in a nutrient medium containing 16% glycerol.

This study was approved by the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 157, date: 20.06.2019) and informed consent was obtained from the participants.

Sample Cultivation and Selection Criteria of Subcultures

Cerebrospinal fluid, pleural and joint fluid, and peripheral and catheter blood samples were collected from adult and pediatric patients in Bactec Plus aerobic/F or Becton Dickinson Bactec Peds Plus/F tubes and incubated in the BACTEC FX system (Becton Dickinson, USA). Subcultures were performed on solid culture media including 5% Sheep Blood Agar (RTA, Türkiye), Eosin Methylene Blue Agar (RTA, Türkiye), or Sabouraud Dextrose Agar (SDA) (Oxford, UK) from bottles displaying growth. The study used quantitative methods for cultivating tracheal aspirates, bronchoalveolar

lavage, urine, and nephrostomy samples, with dilution for the other samples. Colonization was determined by the presence of yeast in respiratory samples, whereas pathogenic growth was detected by Gram staining of wound culture samples. Vaginal samples were stained using the Gram method and were assessed using the Nugent scoring system. For catheter-related bloodstream infections, simultaneous cultures were performed from the catheter tip, blood, and peripheral vein blood. If growth was observed in both cultures, the isolate was considered the causative agent. Colonies suspected of being infectious agents were identified via Gram staining and subcultures on SDA culture media.

Gram Staining

Candida species are identified as gram-positive budding yeast cells or pseudo-hyphae with regular constrictions when stained using the Gram method. Clinical samples or colony suspensions are fixed, stained with crystal violet, iodine solution, or fuchsin, and examined under a light microscope. The yeasts are then stained with a dark or light purple. The process involves spreading, drying, and fixing the samples before examination.

Identification of Fungal Species using MALDI-TOF MS

After 24-48 hours of incubation, the identification of the growing fungal species on subculture plates was performed using mass spectrometry. MALDI-TOF MS was used for the identification of yeast. Samples were prepared in accordance with the MALDI Biotyper standard protocol. Initially, a clean wooden stick was used to transfer the sample collected from the colony onto the circular area of the target plate. The MALDI-TOF MS target plate is a recyclable stainless steel plate with 96 circular areas for testing different colonies, each of which can be used. The sample cells were treated with 70% formic acid on the target plate for application as yeast and then dried. Subsequently, 1-2 µL of matrix solution was applied to the spot and dried. The plate was then placed in the ionization chamber of the mass spectrometer.

The MALDI-TOF MS system, using positive linear mode and 337 nm nitrogen laser ionization, generated data spectra in the mass range of 2-20 kDa to identify Candida species. Using Bruker Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany), these data spectra were identified.

Antifungal Susceptibility Testing Using Gradient Tests

The antifungal susceptibility of the identified yeast species was determined using the gradient test (e-test). The agar-based gradient test is a quantitative method used to identify the minimum inhibitory concentration (MIC) of antifungal agents that inhibit the growth of Candida species, expressed in µg/mL (Table 1).

The e-tests consisted of a narrow and non-porous plastic strip. On one side of the strip (A), there is a MIC reading scale in µg/mL to indicate the identity of the antifungal agent, along with a two- or three-letter code. Four antifungal agents included in our study (FLC, voriconazole, AmB, AND) and their e-test codes are presented in Table 1. The other side of the strip (B) was affixed with a dried and stabilized antifungal agent, which was previously defined with an increasing gradient ranging from the maximum concentration to the minimum concentration (Figure 1).

Four antifungal agents were used in this study, and the MICs were determined for identified Candida spp. Prior to the study, each isolate was subcultured on an SDA plate. Purified isolates were suspended in physiological saline, homogenized using a vortex mixer, and adjusted to a McFarland of 0.5 using a nephelometer.

The prepared suspensions were poured onto RPMI 1640 agar plates (RPMI 1640 Agar w/MOPS and 2% Glucose) with a diameter of 90 mm, ensuring even distribution across the entire surface of the agar. The agar plates were dried, and the gradient test strips (FLC, voriconazole, AmB, AND) were maintained at room temperature until equilibrium was reached. The antifungal gradient was then placed on agar plates at increasing concentrations in a consistent pattern. The plates were then incubated at 37 °C after the process.

Statistical Analysis

The test was conducted following manufacturer's instructions, with areas of inhibition around the gradient test strips assessed after incubation. The MIC of the isolate for a specific antifungal agent was determined by comparing the strip with areas where growth was 80% inhibited. If the culture appeared mixed or faint, the test was repeated. The MICs were analyzed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. Culture and quality control for the test of antifungal resistance were performed using the C. albicans American Type Culture Collection 10231 standard strain. Table 1 presents the MIC values of the antifungal drugs used in the study according to EUCAST standards (28).

The study recorded patient information, clinical specimens, yeast species, and antifungal susceptibility results. The isolates were compared with four tested antifungal agents based on specimen type, patient settings, and fungal species. The data were evaluated to determine the effectiveness of the antifungal agents. One-Way analysis of variance was applied to data that is showing a normal distribution among the groups, whereas the Kruskal-Wallis is analysis of variance test was applied to data not showing a normal distribution. Additionally, descriptive statistical calculations were employed in the data analysis.

Table 1. The antifungal drugs used in this study and their characteristics. The EUCAST MIC breakpoint values for susceptibility (S) and resistance (R) of the specified Candida species are expressed

Candida spp.	Antifungal agents and EUCAST MIC breakpoints (mg/L)				
		AmB	AND	FLC	VRC
Candida albicans	S≤	1	0.03	2	0.06
	I			4	0.125-0.25
	R>	1	0.03	4	0.25
Candida glabrata	S≤	1	0.06	0.001	-
	I			≤16	-
	R>	1	0.06	16	-
Candida kefyr	S≤	-	-	-	-
	I	-	-	-	-
	R>	-	-	-	-
Candida krusei	S≤	1	0.06	-	-
	I			-	-
	R>	1	0.06	-	-
Candida parapsilosis	S≤	1	4	2	0.125
	I			4	0.25
	R>	1	4	4	0.25
Candida tropicalis	S≤	1	0.06	2	0.125
	I			4	0.25
	R>	1	0.06	4	0.25
Candida gulliermondii	S≤	1	0.03	2	0.06
	I				
	R>	1	4	4	0.25

AmB: Amphotericin B, EUCAST: European Committee on Antimicrobial Susceptibility Testing, AND: Anidulafungin, FLC: Flukonazol, VRC: Vorikonazol, MIC: Minimal inhibitory concentration

Results

Between December 2019 and November 2020, a total of 1254 Candida spp. isolates were obtained from various clinical samples from 736 patients (477 females, 269 males) from the clinics and outpatient departments of Dicle University Hospital. The clinic-specific distribution of samples collected from patients with positive Candida spp. cultures is presented in Table 2.

A total of 1254 samples were found to exhibit Candida spp. growth, with 756 (60.29%) obtained from patients in intensive care units, 269 (21.45%) from patients in regular hospital wards, and 229 (18.26%) from outpatient clinic patients. The superiority of Candida spp. isolates was determined from urine, vaginal, and blood samples.

In total, 270 clinical samples exhibiting Candida spp. growth were isolated from blood cultures. The number of blood culture samples was 13,213, with 2,094 cultures suspected of being causative agents. Among these suspected cultures, 12.9% were identified as Candida species. C. albicans was the most regularly isolated Candida species from blood cultures, accounting for 37.41%, followed by C. parapsilosis at 32.22%. Other Candida species isolated from blood samples included C. tropicalis (14.07%), C. glabrata (8.15%), Candida kefyr (5.18%), Candida lusitaniae (1.48%), Candida krusei (1.11%), and Candida guilliermondii (0.37%).

The number of urine samples was 11,391, with a total of 3,037 (26.7%) urine cultures yielding positive results, regardless of pathogenicity or colonization. Among the 669 isolates obtained, C. albicans was found in 324 samples (48.43%), making it the most identified Candida species in urine samples. C. tropicalis was the second most frequently isolated species, found in 192 samples (28.7%). C. parapsilosis was detected in 48 samples (7.17%), C. kefyr in 38 samples (5.68%), C. glabrata in 32 samples (3.78%), Cyberlindhera fabiani in 10 samples (1.5%), C. krusei in 8 samples (1.2%), C. lusitaniae in 4 samples (0.6%), and C. dubliniensis in 4 samples (0.6%). The yeast species in 9 samples could not be classified.

Out of 352 vaginal swab cultures performed, Candida species were isolated in 227 (64.5%) samples. C. albicans and C. glabrata accounted for more than 90% of the Candida species isolated from vaginal cultures. C. albicans was isolated in over half of the samples (144 samples, 63.44%), followed by C. glabrata (68 samples, 29.96%). Rarely isolated species in vaginal samples included C. krusei (4 samples, 1.76%), C. tropicalis (3 samples, 1.32%), C. kefyr (3 samples, 1.32%), and C. parapsilosis (1 sample, 0.44%), while 4 samples could not be classified.

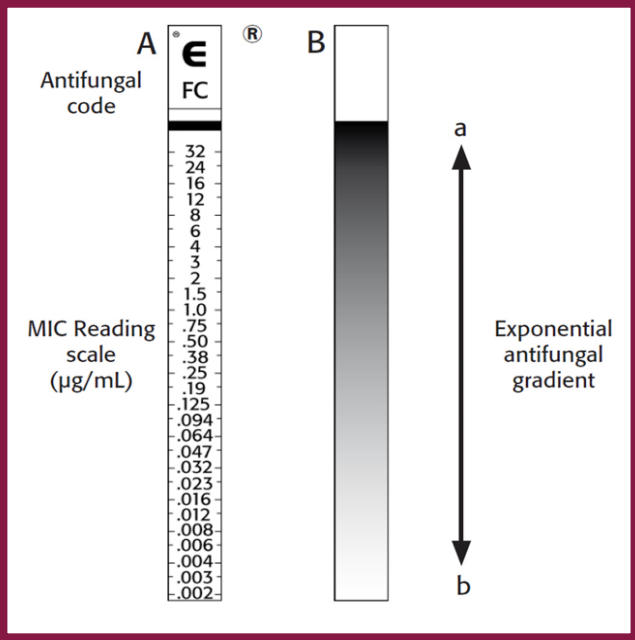

Figure 1. E-test strip for the antifungal agent gradient test used in this study. A) Front side of the strip containing the MIC reading scale. B) Backside of the strip containing an exponential antifungal gradient. Where a represents the maximum and b represents the minimum concentration of the antifungal agent MIC: Minimum inhibitory concentration

Antifungal Susceptibility Test

Out of the 86 Candida spp., the distribution of clinical samples in which isolated Candida species were tested for antifungal susceptibility is shown in Table 3.

From female patients, a total of 59 clinical samples were isolated, whereas a total of 27 samples were obtained from male patients with a mean age of 44.82. Significant differences were observed in terms of patient age and gender distribution among Candida species ($p<0.05$).

The Candida spp. isolates included in this study were obtained from different units, including clinical, intensive care, and outpatient departments. It was statistically significant that C. glabrata was more frequently isolated from outpatients, and C. tropicalis and C. parapsilosis were more frequently isolated from intensive care and clinical patients ($p<0.05$). Among the 45 C. albicans isolates in our study, 7 were derived from clinical samples, 13 from intensive care samples, and 25 were from outpatient sources. Of the C. glabrata isolates, 5 were from clinical samples, 2

Table 2. Distribution of samples obtained from patients with positive Candida spp. culture according to clinics. The number of isolates was determined according to the clinical sample type

Clinical sample type	Number of isolates	%
Urine	669	59.77
Blood	270	18.57
Vagina	227	15.61
Respiratory tract	35	2.41
Catheter	19	1.31
Wound	11	0.76
Abscess	8	0.55
Ear	5	0.34
Peritoneum	4	0.28
Pleura	3	0.21
Cerebrospinal fluid	3	0.24
Total	1254	100

Table 3. Clinical sample distribution for candida antifungal susceptibility testing. Distribution of clinical samples for antifungal susceptibility testing of isolated Candida species

Agent	Vagina	Blood	Urine	Abscess	Wound	Cerebrospinal fluid	Total
Candida albicans	25	14	3	1	1	1	45
Candida glabrata	15	3	1	1	-	-	20
Candida tropicalis	-	7	1	-	1	-	9
Candida parapsilosis	-	6	-	-	-	-	6
Candida kefyr	-	1	3	-	-	-	4
Candida guilliermondi	-	1	-	-	-	-	1
Candida krusei	-	1	-	-	-	-	1
Total	40	33	8	2	2	1	86

Table 4. Candida spp. MIC values and resistance profiles for antifungal agents. MIC values and resistance profiles of Candida spp. isolates based on gradient test results for antifungal agents

Candida spp. (n)	Antifungal drugs	MIC range (mg/L)	Susceptible (S) n (%)	Moderately susceptible (I) n (%)	Resistant (R) n (%)
Candida albicans	AmB	<0.002-1.5	44 (%97.8)	-	1 (2.2%)
	AND	<0.002-0.125	45 (100%)	-	-
	FLC	<0.016-> 56	39 (86.7%)	-	6 (13.3%)
	VRC	<0.002-0.25	39 (86.7%)	6 (13.3%)	-
Candida glabrata (20)	AmB	0.002-1	20 (100%)	-	-
	AND	<0.002-0.064	20 (100%)	-	-
	FLC	1-32	-	12 (60%)	8 (40%)
	VRC	0.5-8	-	-	-
Candida tropicalis (9)	AmB	0.25-3	8 (88.9%)	-	1 (11.1%)
	AND	<0.002	9 (100%)	-	-
	FLC	<0.016-8	8 (88.9%)	-	1 (11.1%)
	VRC	0.032-0.38	8 (88.9%)	-	1 (11.1%)
Candida parapsilosis (6)	AmB	<0.002-1	6 (100%)	-	-
	AND	<0.002-0.5	6 (100%)	-	-
	FLC	0.016-256	5 (83.3%)	-	1 (16.7%)
	VRC	<0.002-0.75	5 (83.3%)	1 (16.7%)	-
Candida kefyr (4)	AmB	0.12-1.5	-	-	-
	AND	<0.002-1	-	-	-
	FLC	0.125-1	4 (100%)	-	-
	VRC	0.032-0.125	-	-	-
Candida guilliermondi (1)	AmB	1	-	-	-
	AND	0.5	-	-	-
	FLC	2	1 (100%)	-	-
	VRC	0.125	-	-	-
Candida krusei (1)	AmB	0.5	1 (100%)	-	-
	AND	0.064	1 (100%)	-	-
	FLC	>256	-	-	1 (100%)
	VRC	0.094	-	-	-

AmB: Amphotericin B, AND: Anidulafungin, FLC: Flukonazol, VRC: Vorikonazol, MIC: Minimal inhibitory concentration

from intensive care samples, and 13 from outpatients. Four C. tropicalis isolates were obtained, 4 from clinical and 5 from intensive care patients. One C. parapsilosis isolate was derived from clinical samples, while 5 were from intensive care sources. Four isolates were examined for C. kefyr, including 2 clinical and 2 intensive care samples. One isolate was clinically confirmed as C. guilliermondii, while one isolate of C. krusei was obtained from the intensive care unit.

EUCAST reported susceptibility breakpoints for seven antifungals against C. albicans isolates, including AmB, AND, FLC, vorikonazol (VRC), itraconazole, posaconazole, and micafungin. The MICs of the isolates and the distribution of resistance profiles among Candida spp. based on gradient test results are shown in Table 4.

In this study, a total of 45 isolates were obtained, including one isolate from a burn intensive care unit patient. Among the 6 isolates showing resistance to AmB from the C. albicans species, it was determined that 4 were obtained from patients in the intensive care units and 2 were obtained from outpatient clinic patients. The test of antifungal susceptibility was shown that 8 out of 8 C. glabrata isolates tested had MIC values above 16 for FLC. Among these isolates, 1 was obtained from an intensive care unit patient and 4 were obtained from outpatient clinic patients, with 3 isolates identified as clinical isolates. One

C. tropicalis isolate showing resistance to AmP was isolated from an 81-year-old male patient in the internal medicine intensive care unit. This isolate also showed resistance to FLC and VRC agents. It was observed that the resistant C. krusei isolates identified in the antifungal susceptibility testing also showed resistance to VRC and were obtained from patients infected with C. parapsilosis in intensive care units. Intensive care units are noteworthy for being where both fungal infections and resistant isolates are detected.

Discussion

Candidiasis refers to various infections caused by C. fungi that affect the skin, mucous membranes, and deep-seated organs. Commonly caused by C. albicans, these invasive infections pose significant morbidity and mortality risks (6,19). The symptoms of candidiasis include oral lesions, redness, burning, bleeding, cracking, loss of taste, and spread into the esophagus in patients with weakened immune systems (28,29). Treatment for candidiasis depends on the type of infection. Antifungal medications such as FLC (oral antifungals), clotrimazole (antifungal lozenges), and nystatin (antifungal mouth wash) are used to treat fungal infections (28). Candida species can develop resistance to antifungal medications through efflux pump and biofilm formation, which protect cells against azoles. Candida biofilms, which are resistant to azoles, can also protect cells against antifungal medications by altering ergosterol (17,18,22).

Analysis of the clinical samples revealed the presence of Candida species in a substantial number of cases, with 1254 clinical samples yielding positive growth for Candida spp. Among the Candida isolates, C. albicans was the most frequently identified species, accounting for 46.8% of the isolates. However, infections caused by other Candida species demonstrated an increasing trend. Studies conducted worldwide have demonstrated variations in Candida species occurrence across regions. While C. albicans is frequently isolated as a pathogen in Northern and Central Europe as well as the United States, non-albicans Candida species are predominant in Asia, Southern Europe, and South America. The highest rate of C. glabrata isolates has been reported in Northern and Central Europe, whereas C. parapsilosis is most commonly found in Slovakia, Southern Europe, South America, and Asia. C. tropicalis, on the other hand, exhibits dominance in Eastern Asia and Argentina. In contrast, the prevalence of C. krusei is relatively low across all geographic regions. The underlying medical conditions of patients can also have an impact on the frequency of Candida species, in addition to regional variance, the administration of antifungal agents, and local factors related to the hospital environment (30).

When comparing the conducted studies, it was observed that the decreasing order of Candida species in the American population generally consisted of C. albicans, C. glabrata, C. parapsilosis, and C. tropicalis. In our study, the most commonly encountered Candida species was C. albicans (46.8%). On the other hand, when our study was compared with other studies, C. glabrata was not identified as the most frequently encountered non-albicans Candida species. The frequency of C. tropicalis and C. parapsilosis was higher than that of C. glabrata. The discrepancy in the ranking between C. glabrata, C. tropicalis, and C. parapsilosis in our study can be attributed to population differences. The epidemiology of candidemia varies according to region. As observed in the study conducted by Horn et al. (31) in 2009, our study also demonstrated a higher isolation rate of non-albicans Candida species from blood cultures (62.59%) (32,33).

As observed, different populations influence the frequency of Candida species occurrence. In a study conducted in Singapore, it was found that the most commonly encountered Candida species differed from our study, as C. albicans was not predominant (33). The frequency of non-C. albicans Candida species, particularly C. tropicalis and C. parapsilosis, appeared to be consistent with our findings.

In accordance with nearly all studies, C. albicans, C. tropicalis, C. parapsilosis, and C. glabrata, which exhibit variable rankings, were the most commonly encountered Candida species in our investigation, occupying the top four positions (34). Consistent with the prevalent Candida species observed in our study population, C. albicans emerged as the predominant species. Some studies conducted in Türkiye have demonstrated the occasional inclusion of C. krusei among the four most frequently isolated Candida species (35). However, contrary to these findings, C. krusei (1.29%) was the least regularly isolated species in our study.

In our study, consistent with previous studies, C. parapsilosis was found to be the third most frequently isolated species. C. parapsilosis has been reported as the third most common Candida species obtained from blood cultures in North America. However, in our study, C. parapsilosis was the second most common species isolated from blood cultures, despite ranking third overall. Growth of C. parapsilosis was observed in isolates obtained from blood cultures of intensive care unit patients, accounting for 90% of cases, and was suggested to be associated with catheter usage (33). Antifungal drug resistance can be categorized microbiologically or clinically, with microbiological resistance referring to a fungal pathogen's *in vitro* susceptibility to an antifungal agent (30). According to the Infectious Diseases Society of America guidelines,

FLC is recommended for less severe infections, whereas echinocandin are the first-line treatment for systemic candidiasis in patients with moderate-to-severe infections and those who have previously been exposed to azoles. The European Society of Clinical Microbiology and Infectious Diseases in Europe recommends echinocandin for all cases (30). Different varieties of Candida exhibit different levels of susceptibility to widely used antifungal medications. For instance, C. krusei is naturally resistant to FLC with a global resistance rate of 78.3%, whereas C. glabrata exhibits dose-dependent low susceptibility with a global resistance rate of 15.7% compared to other Candida species (30).

Fundamental resistance to FLC is known to be extraordinary in C. albicans (1.4%), C. parapsilosis (3.6%), and C. tropicalis (4.1%). In our study, the FLC resistance rates of C. albicans, C. parapsilosis, and C. tropicalis isolates were 13.3%, 16.7%, and 11.1%, respectively. This resistance profile may be attributed to the frequent use of FLC as a prophylactic treatment, particularly in hospitalized patients. Echinocandins have strong antifungal properties against most Candida species, except for C. parapsilosis, which has been reported to have higher MICs (30). None of the tested isolates in our study showed resistance to echinocandin. This finding is consistent with the existing literature. As observed in other studies (35), we did not observe resistance to echinocandin in our study. The most common resistance was found against FLC, both in C. albicans and non-albicans Candida species. The isolates from clinical samples showed an increasing prevalence of non-albicans Candida species, including C. parapsilosis, C. tropicalis, and C. glabrata. The observed increase is attributed to invasive procedures, prolonged stays, and long-term use of antibiotics. Prophylactic antifungal therapy may also contribute to antifungal resistance. Monitoring antifungal resistance profiles will help guide empirical fungal infections treatment, highlighting the need for more effective treatment strategies.

Conclusion

Candida species C. albicans is the most frequently isolated, but infections from other Candida species have increased. No resistance to AND was observed, but species-specific resistance to other antifungal agents was identified, emphasizing the need for continuous monitoring.

Ethics

Ethics Committee Approval: This study was approved by the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 157, date: 20.06.2019)

Informed Consent: Informed consent was obtained from all participants.

Authorship Contributions

Surgical and Medical Practices: N.K., S.A., N.Ö., Concept: N.K., S.A., N.Ö., Design: N.K., S.A., N.Ö., Data Collection or Processing: N.K., N.Ö., Analysis or Interpretation: N.K., S.A., Literature Search: N.K., N.Ö., Writing: N.K., S.A., N.Ö.

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

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REFERENCES

1. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nat Rev Dis Primers. 2018;4:18026. [Crossref]
2. Gómez-Gaviria M, Ramírez-Sotelo U, Mora-Montes HM. Non-albicans Candida species: immune response, evasion mechanisms, and new plant-derived alternative therapies. J Fungi (Basel). 2023;9:11. [Crossref]
3. Kadosh D, Mundodi V. A re-evaluation of the relationship between Morphology and Pathogenicity in Candida species. J Fungi (Basel). 2020;6:13. [Crossref]
4. Quindós G, Marcos-Arias C, San-Millán R, Mateo E, Eraso E. The continuous changes in the aetiology and epidemiology of invasive candidiasis: from familiar Candida albicans to multiresistant Candida auris. Int Microbiol. 2018;21:107-119. [Crossref]
5. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20:133-163. [Crossref]
6. Gonzalez-Lara MF, Ostrosky-Zeichner L. Invasive Candidiasis. Semin Respir Crit Care Med. 2020;41:3-12. [Crossref]
7. Singh DK, Tóth R, Gácser A. Mechanisms of pathogenic Candida species to evade the host complement attack. Front Cell Infect Microbiol. 2020;10:94. [Crossref]
8. Arendrup MC, Patterson TF. Multidrug-resistant Candida: epidemiology, molecular mechanisms, and treatment. J Infect Dis. 2017;216:S445-S451. [Crossref]
9. Fioriti S, Brescini L, Pallotta F, Canovari B, Morroni G, Barchiesi F. Antifungal combinations against Candida species: from bench to bedside. J Fungi (Basel). 2022;8:1077. [Crossref]
10. Kumar S, Kumar A, Roudbary M, Mohammadi R, Černáková L, Rodrigues CF. Overview on the infections related to rare Candida species. Pathogens. 2022;11:963. [Crossref]
11. Musinguzi B, J. Sande O, Mboowa G, Baguma A, Itabangi H, Achan B. Laboratory diagnosis of Candidiasis. In: Candida and Candidiasis. IntechOpen; 2023. [Crossref]
12. Clancy CJ, Nguyen MH. Diagnosing invasive Candidiasis. J Clin Microbiol. 2018;56:e01909-e01917. [Crossref]
13. Fang W, Wu J, Cheng M, Zhu X, Du M, Chen C, et al. Diagnosis of invasive fungal infections: challenges and recent developments. J Biomed Sci. 2023;30:42. [Crossref]
14. Paul S, Singh P, Shamanth AS, Rudramurthy SM, Chakrabarti A, Ghosh AK. Rapid detection of fluconazole resistance in Candida tropicalis by MALDI-TOF MS. Med Mycol. 2018;56:234-241. [Crossref]

15. Mehramouz B, Khodadadi E, Khodadadi E, Taghizadeh S, Ghanbarov K, Köse Ş, et al. The use of MALDI-TOF mass spectrometry technology in molecular analysis of microbial pathogenesis. Vol. 13, Biointerface Research in Applied Chemistry. AMG Transcend Association; 2023. [Crossref]
16. Aslani N, Kokabi R, Moradi F, Abbasi K, Vaseghi N, Afsarian MH. Characterization of Candida species isolated from vulvovaginal candidiasis by MALDI-TOF with *in vitro* antifungal susceptibility profiles. Curr Med Mycol. 2021;7:6-11. [Crossref]
17. Cowen LE, Sanglard D, Howard SJ, Rogers PD, Perlin DS. Mechanisms of antifungal drug resistance. Cold Spring Harb Perspect Med. 2014;5:a019752. [Crossref]
18. Kaur J, Nobile CJ. Antifungal drug-resistance mechanisms in Candida biofilms. Curr Opin Microbiol. 2023;71:102237. [Crossref]
19. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole antifungal resistance in Candida albicans and emerging non-albicans Candida species. Front Microbiol. 2017;7:2173. [Crossref]
20. Krishnasamy L, Krishnakumar S, Kumaramanickavel G, Saikumar C. Molecular mechanisms of antifungal drug resistance in Candida species. Vol. 12, Journal of Clinical and Diagnostic Research. 2018. [Crossref]
21. Lee Y, Puumala E, Robbins N, Cowen LE. Antifungal drug resistance: molecular mechanisms in Candida albicans and beyond. Chem Rev. 2021;121:3390-3411. [Crossref]
22. Silva S, Rodrigues CF, Araújo D, Rodrigues ME, Henriques M. Candida species biofilms' antifungal resistance. J Fungi (Basel). 2017;3:8. [Crossref]
23. Rodrigues CF, Rodrigues ME, Silva S, Henriques M. Candida glabrata biofilms: how far have we come? J Fungi (Basel). 2017;3:11. [Crossref]
24. Mohammadi F, Hemmat N, Bajalan Z, Javadi A. Analysis of biofilm-related genes and antifungal susceptibility pattern of vaginal Candida albicans and non-Candida albicans species. Biomed Res Int. 2021;2021:5598907. [Crossref]
25. Sobel JD. Role of antifungal susceptibility tests in the treatment of vulvovaginal Candidiasis. Vol. 25, Current Infectious Disease Reports. Springer. 2023;25:29-32. [Crossref]
26. Pristov KE, Ghannoum MA. Resistance of Candida to azoles and echinocandins worldwide. Clin Microbiol Infect. 2019;25:792-798. [Crossref]
27. Arendrup MC, Friberg N, Mares M, Kahlmeter G, Meletiadis J, Guinea J, et al. How to interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European committee on antimicrobial susceptibility testing (EUCAST). Clin Microbiol Infect. 2020;26:1464-1472. [Crossref]
28. Taylor M, Brizuela M, Raja A. Oral Candidiasis. StatPearls. 2023 Mar 19. [Crossref]
29. Hu L, He C, Zhao C, Chen X, Hua H, Yan Z. Characterization of oral candidiasis and the Candida species profile in patients with oral mucosal diseases. Microb Pathog. 2019;134:103575. [Crossref]
30. Sanguinetti M, Posteraro B, Lass-Flörl C. Antifungal drug resistance among Candida species: mechanisms and clinical impact. Mycoses. 2015;58 Suppl 2:2-13. [Crossref]
31. Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis. 2009;48:1695-1703. [Crossref]
32. Bhattacharya S, Sae-Tia S, Fries BC. Candidiasis and mechanisms of antifungal resistance. Antibiotics (Basel). 2020;9:312. [Crossref]
33. Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, et al. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance®) registry, 2004-2008. Diagn Microbiol Infect Dis. 2012;74:323-331. [Crossref]
34. Teo JQM, Candra SR, Lee SJY, Chia SYH, Leck H, Tan AL, et al. Candidemia in a major regional tertiary referral hospital-epidemiology, practice patterns and outcomes. Antimicrob Resist Infect Control. 2017;6:27. [Crossref]
35. Badiee P, Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study. Iran J Microbiol. 2011;3:183-188. [Crossref]

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Values of Plasma Vascular Endothelial Growth Factors in Patients with Obstructive Sleep Apnea-Hypopnea Syndrome

Plazma Vasküler Endotelyal Büyüme Faktörünün Obstrüktif Uyku Apne Hipopne Sendromlu Olgulardaki Değerleri

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ABSTRACT

Background: Obstructive sleep apnea-hypopnea syndrome (OSAHS) is an important public health problem. Plasma levels of vascular endothelial growth factor (pVEGF), which is believed to play an active role in the pathogenesis of cardiac pathologies developing in OSAHS. In this study, the correlation between pVEGF levels in OSAHS patients and polysomnographic values was examined.

Materials and Methods: Patients who applied to our center for the first time and consecutively who were "informed or referred from another unit" were admitted to this study. First, a standard questionnaire for the diagnosis of OSAHS and the Epworth Sleep Test were administered to the selected cases. Polysomnography (PSG) was performed in the Sleep Disorders Laboratory of our hospital. On the morning of the PSG test, blood was drawn to measure pVEGF levels.

Results: The study was evaluated on 34 cases and 20 healthy individuals who were accepted as OSAHS with apnea-hypopnea index (AHI) value greater than 5. OSAHS patients who were admitted to the study, 5 were female and 29 were male. The pVEGF levels of the patients included in the study were higher than those of the control group. The AHI value and pVEGF levels were correlated. pVEGF levels were higher in patients with severe OSAHS were higher than mild OSAHS. There was a moderate correlation between desaturation and pVEGF levels. There was a moderate correlation between pVEGF levels and mean duration of apnea, number of total hypopneas, mean duration of hypopnea, apnea index, total apnea, and obstructive apnea.

Conclusion: We found that pVEGF levels were elevated in patients with OSAHS, which is considered to account for 1-5% of the population. pVEGF levels were directly correlated with the AHI value, which is linked to disease severity. In severe OSAHS cases, changes in pVEGF levels can be effective in the development of cardiovascular pathologies.

Keywords: OSAHS, apnea, VEGF

ÖZ

Amaç: Obstrüktif uyku apne hipopne sendromu (OSAHS) önemli bir halk sağlığı problemidir. Vascular endothelial growth factor (pVEGF), OSAHS'da gelişen kardiyak patolojilerin patogenezinde etkin olduğu düşünülen anjiogenetik bir sitokindir. Biz çalışmamızda, plazma VEGF düzeylerinin OSAHS'lı olgularda polisomnografik değerlerle korele olup olmadığını göstermeyi amaçladık.

Gereç ve Yöntemler: Bu çalışmaya, merkezimize "kendisi bilgilenerek gelen ya da başka bir birimden refere edilen" ilk kez ve ardarda başvuran hastalar kabul edildi. Seçilen olgulara öncelikle OSAHS tanısına yönelik standart bir anket formu ve Epworth Uyku Testi uygulanmıştır. Hastanemiz Uyku Bozuklukları Laboratuvarında Polisomnografik inceleme (PSG) uygulandı. Çalışmaya katılan olgulardan ve kontrol grubundan PSG incelemenin ertesi sabahı pVEGF incelemesi için kan alındı.

Bulgular: Çalışma, apne-hipopne indeksi (AHİ) değeri 5'den büyük bulunarak OSAHS olarak kabul edilen 34 olgu ve 20 sağlıklı birey üzerinden değerlendirildi. Çalışmaya kabul edilen OSAHS'lı olguların 5'i kadın ve 29'u erkekti. Çalışmaya dahil edilen hastaların pVEGF düzeyleri kontrol grubuna göre daha yüksekti. AHİ değeri ile pVEGF düzeyleri korele idi. Şiddetli OSAHS'li hastaların plazma VEGF düzeyleri hafif OSAHS'li hastalara göre anlamlı olarak yüksekti. Desatürasyon sayıları ile pVEGF düzeyleri arasında orta derecede korelasyon vardı. pVEGF düzeyleri ile ortalama apne süresi, total hipopne sayısı, ortalama hipopne süresi, apne indeksi, total apne ve obstrüktif apne arasında orta derecede korelasyon vardı.

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This study was produced from "Plasma Vascular Endothelial Growth Factor Levels in Patients With Obstructive Sleep Apnea Hypopnea Syndrome" titled theses.

ÖZ

Sonuç: Sonuç olarak; biz, toplumun %1-5'ini oluşturduğu kabul edilen OSAHS'lı olgularda plazma VEGF düzeylerini yükselmiş bulduk. Plazma VEGF düzeyleri direkt hastalığın ciddiyetiyle bağlantılı olan AHİ değeri ile koreleydi. Ağır OSAHS'lı olgularda, kan VEGF düzeyindeki değişiklikler, bu olgulardaki kardiyovasküler patolojilerin gelişmesine etkili olabilmektedir.

Anahtar Kelimeler: OSAHS, apne, VEGF

Introduction

About one-third of human life is spent asleep. For this reason, scientists have focused on the physioanatomy of sleep for centuries. In 8th century BC, a researcher named Hesiod described sleep as "the brother of death". In Shakespeare's Hamlet and Cervantes' Don Quixote, sleep is interpreted as "a temporary suspension of life and a chance to dream" (1). It is known today that these views are not true.

In 1929, Hans Berger recorded the electrical activity of the human brain and revealed the existence of different rhythms between sleep and wakefulness (2). This development; he accelerated sleep research and, for the first time in 1966, Gastaut added a new dimension to sleep research using polysomnography (3). Obstructive sleep apnea-hypopnea syndrome (OSAHS) is a condition that manifests as recurrent episodes of upper airway closure during sleep and a decrease in blood oxygen saturation (4).

OSAHS is observed in 5% of the population. Given its high prevalence, OSAHS is an important public health problem because it is not less common than diseases such as diabetes mellitus and allergic bronchial asthma (5,6). Although some negative problems are observed in sleep even in healthy individuals, the results of OSAHS, which we consider the most important picture of respiratory disorders during sleep, lead to various morbidities and an increase in mortality rates in these patients. The most severe effects of OSAHS are on the cardiovascular system, which may even result in myocardial infarction and sudden death during sleep (3,6).

Vascular Endothelial Growth Factor (VEGF) is an angiogenetic, heparin-dependent-soluble glycoprotein of 34-36 kilodalton. This cytokine regulates many endothelial cell responses, including apoptosis, mitogenesis, vascular permeability, and tone (7,8). Hypoxia is the main stimulant that controls VEGF synthesis, gene transcription, and mRNA stability. In addition to hypoxemia, synthesis is stimulated when cells are deprived of glucose and inflammation (7,9).

VEGF plays a major role in physiological and pathophysiological angiogenesis. The measurement of circulating VEGF levels is believed to have diagnostic and prognostic value in cardiovascilar diseases, inflammatory diseases, and malignancies (10,11).

VEGF is an angiogenetic cytokine that is believed to be effective in the pathogenesis of cardiac pathologies in OSAHS. The present study aimed to determine whether plasma VEGF (pVEGF) levels correlate with polysomnographic values in patients with OSAHS.

Materials and Methods

This study was conducted in the Sleep Disorders Laboratory of the Chest Diseases and Tuberculosis Clinic of University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital between January 2003 and May 2003.

Study Population

Patients who applied to our center for the first time and consecutively who were "informed or referred from another unit" were admitted to this study.

Patients aged between 25 and 70 (without distinction between men and women), common complaints of habitual snoring, witnessed apnea and/or daytime sleepiness, an apnea-hypopnea index (AHI) of 5> as a result of overnight polysomnographic examination (PSG), and sleep efficiency of at least 60% or more were included.

Those who do not meet these criteria; patients younger than 25 or older than 70 years, those with chronic respiratory diseases, those with hypothyroidism, those with class II (progeny) and class III (retrogenic) jaw occlusion abnormalities, patients who had previously undergone surgery for snoring, those with sleep efficiency below 60%, those with cancer or who were screened for suspected cancer, and individuals with a chronic inflammatory disease or with leukocytes-sedimentation elevation were excluded from the study.

First, a standard questionnaire for the diagnosis of OSAHS and the Epworth Sleep Test were administered to the selected cases. Then, whole system physical examinations, pulmonary function tests, laboratory tests (complete blood count, sedimentation level, total biochemistry, thyroid function tests, Antistreptolizin O-rheumatoid factor-C3-C4 tests), electrocardiography, arterial blood gases, and posteroanterior chest radiographs of the patients were completed before PSG. Neck circumference (NC) was measured at the cricothyroid membrane level. Body mass index (BMI) of the patients was calculated. In addition, Doppler Echocardiography was performed in all patients

included in the study, and pulmonary artery pressure (PAP) and left ventricular function were evaluated. All patients underwent endoscopic examination at the Ear-Nose-Throat clinic. Patients were informed that they should not consume alcohol on the day of PSG, should not sleep until the time of the test in the afternoon, and should not use any sedative drugs from 1 week before the day of PSG.

Cases with non-OSAHS sleep-disordered breathing disorders, such as obesity hypoventilation syndrome, Upper Respiratory Tract Resistance Syndrome, leukocytosis, thrombocytosis, hypothyroidism after PSG, laboratory tests, and patients with malignancies and inflammatory diseases were excluded from the study.

In the case group, a total of 49 patients (41 men and 8 women) were included in the study. A total of 20 healthy individuals were included in the control group; 12 women and 8 men without any complaints. Common complaints were habitual snoring, witnessed apnea, and/or daytime sleepiness, and patients with an AHI>5 as a result of PSG examination were accepted as OSAHS and were included in the study program. All patients and the control group were first informed about the study and its purpose, and their consent was obtained. A 5 cc volume of venous blood was taken from the patients participating in the study and the control group into the EDTA tube at 07:00 on the morning after the PSG examination. The blood was centrifuged at 3000 rpm for 10 minute in 1 hour. Two 1 cc plasma samples were separated from each patient and stored at 20 °C until the study was conducted.

Materials Used

Questionnaire Forms

A routine questionnaire prepared by the Sleep Disorders Laboratory of the Chest Diseases and Tuberculosis Clinic at SSK Dışkapı Ankara Training and Research Hospital was applied to all cases. Identity information, personal and family history information, symptoms and signs related to the diagnosis of OSAHS, history information, physical examination results, and laboratory findings of the cases were recorded on this form. Second, the "Epworth Sleepiness Scale", which is a subjective test that is currently the most commonly used method to determine sleepiness throughout the day, was applied.

Polysomnography

Sleep efficiency was assessed using the Oxford Medilog SAC-SRI device in the Sleep Disorders Laboratory of our Polysomnography Hospital (Sleep efficiency: the ratio of the time spent in sleep to the entire recording time). This rate was at least 60% or more than 60%. Electroencephalography, electrooculography, electromyography, electrocardiography, thoraco-abdominal movements, body position, oro-nasal airflow, tracheal microphone, pulse oximetry, and fingertip oxygen saturation were measured as standard measurement parameters.

Apnea in polysomnographic study; It was considered as the cessation of airflow for 10 second or more. The condition was interpreted as "obstructive" if there was apnea despite the presence of thoraco-abdominal movements, "central" if there was no respiratory effort with apnea, and "mixed apnea" if the apnea was central at the beginning and continued despite the onset of respiratory effort. Since it was decided at the ATS congress held in Boston in 1998 that mixed apneas should be evaluated as obstructive apnea; We evaluated mixed apneas as obstructive apnea. Hypopnea; It was interpreted as a 3% decrease in oxygen saturation or arousal development with at least a 50% decrease in airflow for 10 second or more (12).

After the sleep test recording, manual scoring was performed. Sleep, respiratory, and cardiac evaluations were performed by scoring. In this way, sleep staging, changes in breathing patterns (apnea, hypopnea, arousal, etc.), changes in heart rate, presence of arrhythmias, if any, and periodic limb movement scoring were recorded. As a result of the PSG study, patients with an AHI>5 and/or 5 and the total number of obstructive/mixed apneas was found to be >80% of the total number of apneas were diagnosed with OSAHS.

Measurement of pVEGF Levels

Human VEGF (hVEGF) levels; worked with hVEGF ELISA test (BioSource, Nivelles, Belgium) kit. In this method, microplaques coated with specific polyclonal antibodies for hVEGF are used. In the first incubation, hVEGF-specific monoclonal antibodies were added after the hVEGF antigens found in the patient and control samples bind to the antibodies. The amount of color formed by the added enzyme and substrate is then evaluated. The darkness of the resulting color is directly proportional to the hVEGF concentration in the sample. The lowest detected value by this method was 5 pg/mL. In measurements taken from 15 healthy individuals, pVEGF values ranged from 0 to 120 pg/mL (mean: 20 pg/mL).

Ethics Committee Approval

This study was conducted before the ULAKBİM-TR Dizin decision dated 25.02.2020; therefore, an ethics committee decision was not reached. This study was produced from "Values of pVEGF in patients with obstructive sleep apnea hypopnea syndrome" titled theses. A clinical suitability certificate was obtained for the study (Sleep Disorders Laboratory of the Chest Diseases and Tuberculosis Clinic

of University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital, approval number: 6, date: 07.01.2003).

Statistical Analysis

The data obtained from the cases were encoded and recorded on a computer in SPSS for Windows 10.0. The Kruskal-Wallis test and Mann-Whitney U analysis were used for statistical evaluation, $p<0.05$ was accepted as significant.

Results

As a result of PSG, a total of 15 cases (12 men and 3 women) whose sleep duration and quality were not sufficient and whose AHI value was less than 5 were excluded from the study. The study included 34 cases and 20 healthy individuals who were accepted as OSAHS with an AHI>5.

Of the patients with OSAHS who were admitted to the study, 5 were female (14.7%) and 29 were male (85.3%). The mean age was 48.7±10 years. BMI was 32.7±5.7. The NC was 43.8±3.7 cm. The mean age of the control group was 43.4±5.8 years, BMI was 23.2±3.6 and NC was 34.4±2.7 cm. The demographic characteristics of patients with OSAHS and controls are presented in Table 1. As can be seen in the table, there was a difference in the BMI and NC measurement values between the patients and the control groups. As expected, the Epworth Sleepiness Scale scores were high in patients with OSAHS who had excessive daytime sleepiness.

The average recording time of the PSG test performed on the patients was 7 hours, and sleep adequacy did not fall below 60% in any patient. The changes in sleep stage rates observed in patients with OSAHS were also observed in our study cohort. An increase in superficial sleep level and a decrease in deep sleep and rapid eye movement sleep were observed. Table 2 presents the PSG results of patients with OSAHS.

The first aim of this study was to investigate whether there was a difference between the pVEGF levels of the control group and patients with OSAHS. There was a significant difference between our patients and the control group included in the study ($p<0.001$) (Table 3).

Table 1. Demographic parameters of patients with OSAHS

	OSAHS	Control	
Number of cases (M/F)	34 (29/5)	20 (12/8)	
Age, year, mean ± SD (min.-max.)	48.7±10 (31-67)	43.4+5.8 (26-56)	p<0.05
BMI, mean ± SD (min.-max.)	32.7±5.7 (24.8-49.9)	23.2±3.6 (18.6-33.2)	p<0.05
NC (cm) , mean ± SD (min.-max.)	43.8±3.7(36-51)	34.4±2.7 (30-39)	p<0.05

Control: Healthy individual, OSAHS: Obstructive sleep apnea hypopnea syndrome, M: Male, F: Female, SD: Standard deviation, BMI: Body mass index, min.-max.: Minimum-maximum, NC: Neck circumference

Table 2. PSG results in patients with OSAHS

	OSAHS, mean ± SD	Control, mean ± SD
Total recording time (minute)	432.2±55.3	442.4±45.6
Sleep efficiency (%)	85.4±11.8	92.2±12.3
Phase 0 (%)	14.4±11.8	10.4±6.2
Phase 1 (%)	12.0±9.0	8.1±4.2
Phase 2 (%)	49.2±14.6	50.3±14.6
Phase 3 (%)	9.6±9.0	6.6±2.2
Phase 4 (%)	3.2±5.2	14.2±4.7
REM (%)	11.4±8.8	19.1±7.3
Non-REM (%)	74.2±12.1	79.5±11.1
Number of desaturation steps	198.4±140.0	10.8±3.1
During sleep, mean sO_2	89.26±7.19	93.9±2.6
During sleep, minimum sO_2	65.88±13.30	82.1±5.7
AHI	33.1±27.2	1.0±0.7

Control: Healthy individual, OSAHS: Obstructive sleep apnea hypopnea syndrome, SD: Standard deviation, REM: Rapid eye movement, AHI: Apnea-hypopnea index, sO_2: Oxygen saturation, PSG: Polysomnography

pVEGF levels of patients with OSAHS were compared with AHI values, which are considered to indicate disease severity. A moderate correlation was found between these two values (Table 4). Patients with OSAHS were divided into 3 groups according to AHI values (AHI: 5.0-14.9 mild OSAHS, AHI: 15-29.9 moderate OSAHS, ⩾30 severe OSAHS). There were 12 patients in the mild OSAHS group, 8 in the moderate OSAHS group, and 14 in the severe OSAHS group. pVEGF levels of these 3 groups were compared with each other. pVEGF levels were significantly higher in patients with severe OSAHS than in patients with mild OSAHS. ($p<0.05$); However, no difference was found between the other groups.

Again, the number of total apneas, obstructive apneas, and central apneas was compared with pVEGF. Mean apnea duration, longest apnea duration, apnea index (AI), total hypopnea count, mean hypopnea duration, hypopnea index, and pVEGF levels were compared. There was a moderate correlation between pVEGF levels and the mean duration of apnea, total number of hypopneas, mean duration of hypopnea, AI, total apnea, and number of obstructive apnea (Table 4).

The desaturation numbers, mean saturation levels, minimum saturation levels, and pVEGF levels of patients with OSAHS were compared. Although there was a moderate correlation between desaturation numbers and pVEGF levels, there was no correlation between mean saturation levels and minimum saturation levels and pVEGF values (Table 5).

We found elevated PAP in 14 (41.1%) of 34 patients diagnosed with OSAHS. Because PAP is directly related to quality of life and possible complications, we compared pVEGF levels and PAP values to determine whether elevated pVEGF levels in patients with OSAHS are significant in terms

Table 3. Group pVEGF levels

	Number of cases	pVEGF (pg/mL)	
Control (mean ± SD)	20	86.0±43.9	p<0.001
OSAHS (mean ± SD)	34	152.4±58.2	

OSAHS: Obstructive sleep apnea hypopnea syndrome, SD: Standard deviation,cpVEGF: Plasma vascular endothelial growth factor

Table 4. Correlation matrix analysis between AHI and pVEGF levels

	pVEGF (pg/mL)	
	r-value	p-value
AHI	0.454	0.007*
Total apnea count	0.474	0.005*
Obstructive apnea count	0.493	0.003*
Central apne count	0.259	0.139
Mean apnea duration (sec)	0.343	0.047*
Longest apnea duration (sec)	0.293	0.093
Apnea index	0.441	0.009*
Total hypopne count	0.354	0.040*
Mean hypopne duration (sec)	0.391	0.022*
Hypopne index	0.294	0.092

AHI: Apnea-hypopnea index, pVEGF: Plasma vascular endothelial growth factor

Table 5. Correlation matrix analysis of saturated VEGF and pVEGF levels

	pVEGF (pg/mL)	
	r-value	p-value
Number of desaturation steps	0.409	0.046*
During sleep, mean sO_2	-0.261	0.137
During sleep, minimum sO_2	-0.202	0.252

pVEGF: Plasma vascular endothelial growth factor, sO_2: Oxygen saturation

of prognosis. We did not find any difference in pVEGF levels between patients with and without high PAP. In addition, we did not find any correlation between PAP and pVEGF levels in patients with high PAP.

Discussion

OSAHS is an important public health problem because its prevalence is approaching 5%, similar to that of diabetes mellitus and allergic bronchial asthma (5,6). Even healthy individuals exhibit anomalies during sleep. OSAHS, the most important sleep disorder, leads to different morbidities and an increase in mortality rates in these patients (3,6).

OSAHS is a syndrome that manifests itself with recurrent episodes of upper airway closure during sleep and is often accompanied by a decrease in blood oxygen saturation (4). AHI is the most commonly used PSG test parameter in determining the diagnosis and severity of OSAHS. The AHI parameter's limit value in OSAHS patients is not clear and precise. In various studies, AHI ranges from 5 to 20. However, clinically important patients with AHI>20 have increased mortality risks (13). More than half of our patients had AHI values >20. It is known that there is a relationship between apnea and obesity, and weight loss may lead to improvement in OSAHS. Obesity, especially central obesity, increases the tendency to develop OSAHS at a remarkable rate by affecting the patency of the upper respiratory tract and the complicity of the region with fat storage and by affecting the respiratory pattern with abdominal fat accumulation. In individuals over the age of 40 years, the risk of OSAHS increased 8-12 times in individuals with a BMI>29 kg/m^2 compared with individuals who were not overweight. This risk is much higher, particularly in those with more pronounced fat accumulation in the upper body region and in morbidly obese patients with a BMI>40 kg/m2 (14,15). In recent studies, NC was shown to be a determining factor for OSAHS. More than 43 cm in male individuals and 38 cm in female individuals was considered significant (15). BMI and NC measurements were found to be significantly higher in our cases.

VEGF regulates multiple endothelial cell responses, including apoptosis, mitogenesis, vascular permeability, and tone (7,8). pVEGF levels increase mainly in hypoxia, inflammations and cancers. In our study, we showed that the pVEGF levels of patients with OSAHS were higher than those of healthy individuals ($p<0.001$).

Previous studies have shown that pVEGF levels are elevated due to hypoxia. Hypoxia. It is the main stimulant that controls VEGF synthesis, gene transcription, and mRNA stabilization (9). The relationship between VEGF concentration and nocturnal hypoxia in patients with OSAHS leads to increased hypoxia-sensitive gene expression and consequently increased protein production in recurrent intermittent nocturnal hypoxemic episodes (16). Apart from hypoxia, the most common conditions associated with an increase in VEGF level are; for example, disseminated cancer, chronic inflammatory, and autoimmune diseases were not present in our patients and, consequently, could not be responsible for changes in the VEGF concentration. Our findings were the same as those of Lavie et al. (16), Schulz et al. (17), Gozal et al. (18), Imagawa et al. (19), and Teramoto et al. (20).

Schulz et al. (17) observed a significant increase in serum VEGF levels in obstructive sleep apnea (OSA) patients with severe nocturnal hypoxemia compared with OSA patients with mild hypoxemia and the control group (17). Gozal et al. (18) found VEGF levels to be significantly higher in both children and adults with OSAHS compared with very few sick and non-sick individuals.

Imagawa et al. (19) measured hemoglobin, serum erythropoietin, and VEGF levels in 106 patients with severe OSAHS. Their results showed that transient hypoxemia increased hemoglobin. In addition, they found a small increase in erythropoietin levels and a large increase in VEGF levels (19). Teramoto et al. (20) showed that serum VEGF levels are elevated due to nocturnal hypoxia in patients with OSAHS. They observed that serum VEGF levels decreased in patients with OSAHS whose nocturnal hypoxemia was corrected by applying oxygen (O_2) at 2 L/min during the night. However, the administration of compressed air did not affect pVEGF levels and O_2 desaturation in patients with OSAHS (20).

Lavie et al. (16) found that patients with OSAHS had high circulating VEGF. They also found that VEGF levels were significantly higher in patients with OSAHS than in snoring and healthy young adult participants of the same age. Researchers have reported that VEGF levels also decreased in patients whose hypoxemia improved after non-invasive continuous positive airway pressure treatment. This result supports the idea that elevated VEGF levels in patients with OSAHS result from nocturnal hypoxemia.

In our study, although we established a relationship between pVEGF levels and apnea-related hypoxia; We did not find a linear relationship between the depth of nocturnal dephased and pVEGF levels. For this purpose, we investigated the number of desaturations, average saturation times, and minimum saturation values. Although there was a correlation between the number of desaturation and pVEGF levels ($p<0.05$), there was no correlation between the mean saturation levels and minimum saturation levels and pVEGF values. This result led us to suggest that although recurrent hypoxemic attacks are direct stimulants of VEGF release, deepening hypoxia does not act as an extra stimulator of

VEGF release. In contrast, Schulz et al. (17) reported that serum VEGF concentration was significantly correlated with the degree of oxygen desaturation in OSAHS (17). Gozal et al. (18) also found a correlation between serum VEGF and oxygen levels in their studies (18).

The second issue is whether there is a difference between plasma and serum samples in VEGF levels due to hypoxia in OSAHS. Schulz et al. (17), Gozal et al. (18), and Imagawa et al. (19) detected VEGF in serum and not plasma, and their values were higher than those of ours and Lavie et al. (16-19). Serum VEGF was released from platelets and other blood cells during blood clotting. And that is why they reported a 2-7 fold increase in VEGF. In addition, their values reflect blood platelet counts rather than VEGF synthesis by peripheral tissues; It may not directly reflect hypoxia. In Jelkmann's review study and two separate studies, the necessity of using plasma to measure VEGF levels was emphasized (11,21,22).

In their study, Lavie et al. (16) reported that some patients responded to hypoxia with high VEGF mRNA synthesis *in vitro*, whereas others had little or no response (16). In our study, we did not detect elevated pVEGF levels secondary to deep hypoxia in 3 patients. This personal answer difference; In the study conducted by Schultz et al. (17), it was reported that this was correlated with the height of the coronary collateral tree (23).

We compared pVEGF with AHI, apnea, and hypopnea parameters to evaluate its efficacy in patients with OSAHS. We found a correlation between pVEGF levels and AHI, which is considered to indicate the weight of OSAHS ($p<0.05$). When we divided our cases into 3 groups according to the AHI value recommended by the American Sleep Disorders Association and then compared them with the pVEGF level again; We found that there was a significant difference between the light and severe groups in terms of pVEGF levels. This may explain the high pVEGF levels caused by hypoxemia due to apnea and hypopnea, which are more common in patients with severe OSAHS. In addition, we found a correlation between pVEGF levels and obstructive apnea count, total apnea count, AI, mean apnea duration, total hypopnea number, and mean hypopnea duration. These correlations strengthen the link between recurrent apnea-related hypoxia and pVEGF. Lavie et al. (16) and Gozal et al. (18) found a significant correlation between pVEGF concentrations and respiratory distribution index (16,18).

While planning the study, we assessed the difference in pVEGF levels among 14 (41.2%) patients with high PAP, considering that elevated PAP changes may affect pVEGF levels. We did not find any correlation between these two values ($p>0.05$). Although the location of echocardiography for PAP measurement is discussed; We preferred echocardiographic imaging for PAP measurement because it is non-invasive (24). We could not detect daytime hypoxemia and concomitant lung disease, which are believed to cause high PAP in patients with OSAHS. Considering that VEGF levels are elevated due to nocturnal hypoxemic attacks in all our cases; PAP pressure elevation was observed in only 14 (41.2%) patients, indicating that PAP may have increased due to another physiopathological mechanism and not due to nocturnal hypoxemic attacks.

There are no accepted opinions on the physiological significance of elevated VEGF levels in the blood of patients with OSAHS. The tissues in which VEGF secretion due to hypoxia was observed were the beginning of theories for the researchers. VEGF has been shown to be upregulated in cardiac myocyte, vascular smooth muscle cells, and endothelial cells under hypoxic conditions, as well as in cardiac tissues following microvascular flow index (16). When we examine our own study, we cannot determine the main source of VEGF secretion observed in patients with OSAHS. Unlike endothelium, the site responsible for increased VEGF production is likely activated platelets in untreated patients with OSAHS. However, in vitro experiments have shown that platelets do not release significant amounts of VEGF against hypoxia (17).

As mentioned above, the fact that most cardiac responses are due to hypoxic conditions has led to two hypotheses in patients with OSAHS. The first of these; This is the view put forward by Lavie et al. (16) in their work. According to these findings, current studies suggest that VEGF may contribute to the atherogenic process on its own, not to mention its role in angiogenesis. VEGF-induced monocyte activation and migration regulate the growth of smooth muscle cells and are closely linked to the development of coronary atherosclerosis in humans (25). The second hypothesis was proposed by Schulz et al. (17). They are considered to be of the pathophysiological significance of their results; They hypothesized that increased VEGF production in OSAHS is an adaptive mechanism that offsets the urgency of OSAHS-related cardiovascular diseases. Theoretically, an increase in VEGF production in patients with OSAHS may facilitate the formation of new vessels in ischemic and atherosclerotic vascular areas. This prediction is supported by a study in which collateral vessel formation was correlated with hypoxic VEGF induction in coronary artery patients (23,26). Again, they are partial to the results of the study. They said it may be part of their review of the Sleep Heart Health Study, which explains that not all cardiovascular risk factors in OSAHS are directly linked to apnea severity (17).

In our opinion, VEGF, the most prominent function in the body of angiogenesis, is more likely to contribute to the development of atherosclerosis. As mentioned above and as

determined, pVEGF levels are higher in patients with severe OSAHS. It may not be a coincidence that cardiac and other complications are more common in these cases. Increased *in vivo* platelet activation was noted in patients with OSAHS, as shown in a study. In some studies, this event; It has been stated that OSAHS may increase cardiovascular outcomes (27,28). In addition, in one study, a decrease in plasma fibrinolytic activity was observed in patients with OSAHS, although the mechanism was unknown (29). However, Zakrzewski et al. (30) explained that patients with OSA had a severe risk of cardiovascular disorders due to increased pro-thrombotic activity. Risk factors such as elevated blood pressure, advanced age, obesity, and hyperlipidemia may contribute to the development of atherosclerosis along with increased VEGF levels.

Study Limitations

Because this was a prospective study, the number of participants was limited.

Conclusion

As a result; We found that pVEGF levels were elevated in patients with OSAHS, which is considered to constitute 1-5% of the population. pVEGF levels were correlated with AHI levels, which are directly related to disease severity. In patients with severe OSAHS, changes in blood VEGF levels may affect the development of cardiovascular pathologies.

Ethics

Ethics Committee Approval: This study was conducted before the ULAKBİM-TR Dizin decision dated 25.02.2020; therefore, an ethics committee decision was not reached.

A clinical suitability certificate was obtained for the study (Sleep Disorders Laboratory of the Chest Diseases and Tuberculosis Clinic of SSK Dışkapı Ankara Training and Research Hospital, decision no: 6/07.01.2003).

Informed Consent: All patients and the control group were first informed about the study and its purpose, and their consent was obtained.

Authorship Contributions

Surgical and Medical Practices: M.İ.B., S.K., Concept: M.İ.B., S.A., Design: M.İ.B., S.A., Data Collection or Processing: M.İ.B., S.K., Analysis or Interpretation: M.İ.B., S.A., S.K., Literature Search: M.İ.B., S.A., Writing: M.İ.B., S.K.

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REFERENCES

1. Dement WC. History of sleep medicine. Neurol Clin. 2005;23:945-965. [Crossref]
2. Kuhl W. History of clinical research on the sleep apnea syndrome. The early days of polysomnography. Respiration. 1997;64(Suppl 1):5-10. [Crossref]
3. Bresnitz EA, Goldberg R, Kosinski RM. Epidemiology of obstructive sleep apnea. Epidemiol Rev. 1994;16:210-227. [Crossref]
4. America Sleep Disorders Association. (2001). The international classification of sleep disorders 1997, revised. Rochester MN: ASDA. [Crossref]
5. Stradling JR. Sleep-related breathing disorders. 1. Obstructive sleep apnoea: definitions, epidemiology, and natural history. Thorax. 1995;50:683-689. [Crossref]
6. Strohl KP, Redline S. Recognition of obstructive sleep apnea. Am J Respir Crit Care Med. 1996;154:279-289. [Crossref]
7. Frelin C, Ladoux A, D'angelo G. Vascular endothelial growth factors and angiogenesis. Ann Endocrinol (Paris). 2000;61:70-74. [Crossref]
8. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989;246:1306-1309. [Crossref]
9. Harmey JH, Dimitriadis E, Kay E, Redmond HP, Bouchier-Hayes D. Regulation of macrophage production of vascular endothelial growth factor (VEGF) by hypoxia and transforming growth factor beta-1. Ann Surg Oncol. 1998;5:271-278. [Crossref]
10. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev. 1997;18:4-25. [Crossref]
11. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. Clin Chem. 2001;47:617-623. [Crossref]
12. Demirci AY. CPAP tedavisi verilen obstrüktif uyku apne sendromlu hastalarda pulmoner arter basıncı ve pro-BNP düzeylerinin değerlendirilmesi. Uzmanlık Tezi. Bursa Uludağ Üniversitesi. 2009. [Crossref]
13. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. Sleep. 1999;22:667-689. [Crossref]
14. Redline S, Strohl KP. Recognition and consequences of obstructive sleep apnea hypopnea syndrome. Clin Chest Med. 1998;19:1-19. [Crossref]
15. Schwab RJ, Goldberg AN, Pack AL. Sleep apnea syndromes. In: Fishman AP (ed). Fishman's Pulmonary Diseases and Disorders. New York: McGraw-Hill BookCompany, 1998; 1617-37. [Crossref]
16. Lavie L, Kraiczi H, Hefetz A, Ghandour H, Perelman A, Hedner J, et al. Plasma vascular endothelial growth factor in sleep apnea syndrome: effects of nasal continuous positive air pressure treatment. Am J Respir Crit Care Med. 2002;165:1624-1628. [Crossref]
17. Schulz R, Hummel C, Heinemann S, Seeger W, Grimminger F. Serum levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea and severe nighttime hypoxia. Am J Respir Crit Care Med. 2002;165:67-70. [Crossref]
18. Gozal D, Lipton AJ, Jones KL. Circulating vascular endothelial growth factor levels in patients with obstructive sleep apnea. Sleep. 2002;25:59-65. [Crossref]
19. Imagawa S, Yamaguchi Y, Higuchi M, Neichi T, Hasegawa Y, Mukai HY, et al. Levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea-hypopnea syndrome. Blood. 2001;98:1255-1257. [Crossref]
20. Teramoto S, Kume H, Yamamoto H, Ishii T, Miyashita A, Matsuse T, et al. Effects of oxygen administration on the circulating vascular endothelial growth factor (VEGF) levels in patients with obstructive sleep apnea syndrome. Intern Med. 2003;42:681-685. [Crossref]
21. Gunsilius E, Petzer AL, Gastl GA. Blood levels of vascular endothelial growth factor in obstructive sleep apnea-hypopnea syndrome. Blood.

2002;99:393-394. [Crossref]

22. Webb NJ, Bottomley MJ, Watson CJ, Brenchley PE. Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: implications for measurement of circulating VEGF levels in clinical disease. Clin Sci (Lond). 1998;94:395-404. [Crossref]
23. Schultz A, Lavie L. Hochberg I, Beyar R, Stone T, Skorecki K, et al. Interindividual heterogeneity in the hypoxic regulation of VEGF: significance for the development of the coronary artery collateral circulation. Circulation. 1999;100:547-552. [Crossref]
24. Erol Ç. Pulmoner hipertansiyonda ekokardiyografi. Tüberküloz ve Toraks, 1993; 41(Özel Sayı):53-55. [Crossref]
25. Dabravolski SA, Khotina VA, Omelchenko AV, Kalmykov VA, Orekhov AN. The role of the VEGF family in atherosclerosis development and its potential as treatment targets. Int J Mol Sci. 2022;23:931. [Crossref]
26. Zhu D, Kang W, Zhang S, Qiao X, Liu J, Liu C, et al. Effect of mandibular advancement device treatment on HIF-1α, EPO and VEGF in the myocardium of obstructive sleep apnea-hypopnea syndrome rabbits. Sci Rep. 2020;10:13261. [Crossref]
27. Geiser T. Buck F, Meyer BJ, Bassetti C, Haeberli A, Gugger M. *In vivo* platelet activation ıs ıncreased during sleep in patients with obstructive sleep apnea syndrome. Respiration. 2002;69:229-234. [Crossref]
28. Wang X, Fan J, Guo R, Hao W, Gong W, Yan Y, et al. Association of obstructive sleep apnoea with cardiovascular events in women and men with acute coronary syndrome. Eur Respir J. 2023;61:2201110. [Crossref]
29. Rangemark C, Hedner JA, Carlson JT. Gleerıp G, Winther K. Platelet function and fibrinolytic activity in hypertensive and normotensive sleep apnea patients. Sleep. 1995;18:188-194. [Crossref]
30. Zakrzewski M, Zakrzewska E, Kiciński P, Przybylska-Kuć S, Dybała A, Myśliński W, et al. Evaluation of fibrinolytic inhibitors: alpha-2-antiplasmin and plasminogen activator inhibitor 1 in patients with obstructive sleep apnoea. PLoS One. 2016;11:e0166725. [Crossref]

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Depression and Anxiety Disorders in Patients with Reported Prolactinoma Using Cabergoline Therapy: A Comparative Analysis with Controls

Remisyondaki Prolaktinomalı Hastalarda Kabergolin Tedavisi Altında Depresyon ve Anksiyete Bozuklukları: Kontrol Grubu ile Karşılaştırmalı Bir Analiz

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ABSTRACT

Background: Prolactinoma is the most common functional pituitary tumor. Although effective in reducing prolactin and tumor size, cabergoline can have psychiatric side effects. This study aimed to investigate the prevalence of depression and anxiety disorders in patients with recurrent prolactinoma receiving cabergoline treatment.

Materials and Methods: Patients aged 18 years, diagnosed with prolactinoma and achieving biochemical remission on cabergoline therapy, and the control group were included in the study. The participants completed the Hospital Anxiety and Depression Scale questionnaire. Data collected included the number of years of diagnosis, prolactin levels, cumulative cabergoline dosage, and duration of cabergoline use.

Results: The study included 56 patients with recurrent prolactinoma and 56 controls. The mean age of both groups was 39.4 years [standard deviation (SD)=10.5], with 62.5% female and 37.5% male. The average duration of cabergoline treatment was 40.39 months (SD=30.2). Patients with prolactinoma had a median depression score of 5 [interquartile range (IQR) 2.25-8], whereas the control group had 6 (IQR 4-9). For anxiety, the prolactinoma patients had a median score of 8 (IQR 5-11.75), compared to 7 (IQR 5-9) in the control group. The results revealed no significant difference in depression (p=0.32) and anxiety scores (p=0.19) between the groups. Among the prolactinoma patients, 37.5% (n=21) were found to have symptoms of depression, and 41.1% (n=23) of the control group exhibited symptoms of depression. Anxiety disorders were present in 37.5% (n=21) of the prolactinoma patients and 23.2% (n=13) in the control group. The prevalence of depression and anxiety disorders was not significantly different between the groups (p=0.69 and p=0.1 respectively).

Conclusion: The study found no significant differences in the prevalence of depression and anxiety disorders between patients with recurrent prolactinoma who received cabergoline treatment and the control group. These results suggest that cabergoline dosage and duration do not strongly influence the aforementioned psychiatric comorbidities.

Keywords: Prolactinoma, anxiety disorders, depression, cabergoline

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ÖZ

Amaç: Prolaktinoma, en yaygın fonksiyonel hipofiz tümörüdür. Prolaktin seviyelerini ve tümör boyutunu azaltmada etkili olan kabergolin, psikiyatrik yan etkilere neden olabilir. Bu çalışmanın amacı, remisyondaki prolaktinomalı hastalarda kabergolin tedavisi altındaki depresyon ve anksiyete bozukluklarının yaygınlığını araştırmaktır.

Gereç ve Yöntemler: Çalışmaya, 18 yaş ve üstü, prolaktinoma tanısı almış ve kabergolin tedavisi ile biyokimyasal remisyona ulaşmış hastalar dahil edildi. Katılımcılar, Hastane Anksiyete ve Depresyon Ölçeği anketini doldurdu. Hastaların tanı yılı, prolaktin seviyeleri, kümülatif kabergolin dozu, kabergolin kullanım süresi ve manyetik rezonans bulguları kaydedildi.

Bulgular: Çalışmaya remisyondaki 56 prolaktinoma hastası ve 56 kontrol dahi edildi. Her iki grubun ortalama yaşı 39,4 yıl [standart sapma (SS)=10,5] olup, %62,5'i kadın ve %37,5'i erkekti. Kabergolin tedavisinin ortalama süresi 40,39 ay (SS=30,2) saptandı. Prolaktinoma hastalarının medyan depresyon skoru 5 [çeyrekler açıklığı (IQR) 2,25-8], kontrol grubunun 6 (IQR 4-9) bulundu. Anksiyete için prolaktinoma hastalarının medyan skoru 8 (IQR 5-11,75), kontrol grubunun 7 (IQR 5-9) saptandı. Gruplar arasında depresyon (p=0,32) ve anksiyete skorlarında (p=0,19) anlamlı bir fark bulunmadı. Prolaktinoma hastalarının %37,5'i (n=21) depresyon belirtileri gösterirken, kontrol grubunun %41,1'i (n=23) depresyon belirtileri sergiledi. Anksiyete bozuklukları ise prolaktinoma hastalarının %37,5'inde (n=21) ve kontrol grubunun %23,2'sinde (n=13) mevcuttu. Depresyon ve anksiyete bozukluklarının yaygınlığı gruplar arasında anlamlı farklılık göstermedi (sırasıyla p=0,69 ve p=0,1).

Sonuç: Bu çalışma, remisyondaki prolaktinoma hastaları ile kontrol grubu arasında depresyon ve anksiyete bozukluklarının yaygınlığında anlamlı bir fark olmadığını bulmuştur. Sonuçlar, kabergolin dozajı ve süresinin bu psikiyatrik komorbiditeleri güçlü bir şekilde etkilemediğini göstermektedir.

Anahtar Kelimeler: Prolaktinoma, anksiyete bozuklukları, depresyon, kabergolin

Introduction

Prolactinoma is the most prevalent type of functional pituitary tumor, encompassing 30-60% of all pituitary tumors (1). In contemporary practice, medical therapy, particularly with cabergoline, a dopamine agonist, is the gold standard for treating prolactinoma (2). Cabergoline binds to dopamine receptors, thereby reducing prolactin synthesis and exerting its effects by shrinking the size of the adenoma (3). Although generally well tolerated, cabergoline has certain side effects, including nausea, postural hypotension, heart valve dysfunction, and psychiatric effects (4). Psychiatric side effects of cabergoline include cognitive fog, impulse control disorder, and depression.

Among the psychiatric side effects of cabergoline, impulse control disorder is frequently reported in the medical literature (5,6). However, research on the impact of depression and anxiety disorders remains limited. Depression, a neuropsychiatric disorder characterized by persistent feelings of low mood, anhedonia, and suicidal tendencies, has a lifetime prevalence of 10% (7). Its pathophysiology is believed to involve dopamine and its receptors, with dopamine playing a significant role in emotional regulation, reward, and neurosecretion (8,9). Consequently, alterations in the dopaminergic system, such as changes in dopamine or receptor levels, are associated with depression.

Anxiety, which is typically considered an adaptive response to acute stress, is deemed pathological when functionality is impaired (10). Animal studies have indicated a significant role for dopamine D1 and D2 receptors in anxiety-like behavior models, and dopamine metabolites are also potentially influential in these conditions (11,12).

In this study, we aimed to investigate the prevalence of depression and anxiety disorders among patients with remitted prolactinoma treated with cabergoline and to compare these findings with those of a control group to better understand the impact of cabergoline treatment on mental health outcomes.

Materials and Methods

The study was conducted with the approval of the University of Health Sciences Türkiye, Hamidiye Scientific Research Ethics Committee (approval number: 23/526, date: 22.09.2023) and was conducted in accordance with the Declaration of Helsinki. All participants provided informed consent.

Participants were recruited from the Endocrinology and Metabolism Outpatient Clinic of the University of Health Sciences Türkiye, Sultan Abdülhamid Han Training and Research Hospital between October 2023 and May 2024. Eligible participants were voluntary patients aged 18 years and over who had been diagnosed with prolactinoma and were currently receiving treatment with cabergoline, having achieved biochemical remission.

Patients diagnosed with prolactinoma were included if their prolactin levels were above the normal range, they exhibited an adenoma appearance in the pituitary on magnetic resonance imaging (MRI), and they had no

other conditions causing hyperprolactinemia (such as medication use, polycystic ovary syndrome, kidney or liver insufficiency, etc.). The participants completed the Hospital Anxiety and Depression Scale (HADS) questionnaire. Data collected included years of diagnosis, baseline and current prolactin levels, cumulative cabergoline dosage, duration of cabergoline use, MRI findings, demographic characteristics, and comorbidities.

The exclusion criteria were as follows: patients under 18 years old, those with macroprolactinemia, pregnant or breastfeeding women, individuals with hypothyroidism, polycystic ovary syndrome, advanced liver or kidney insufficiency, patients diagnosed with depression, anxiety disorders, schizophrenia, or parkinsonism, and those taking medications that could affect prolactin levels or alter questionnaire scores (e.g., glucocorticoids, oral contraceptives, antidepressants, antipsychotics, L-dopa). Patients for whom data were inaccessible through the system were also excluded. Control group participants were individuals without known diseases of similar age and gender who presented to the outpatient clinic for routine examinations.

The Hospital Anxiety and Depression Scale

The HADS was developed by Zigmond and Snaith (13) in 1983, and its Turkish validity study was conducted by Aydemir and Köroğlu (14). A total of 14 questions were included, seven of which measured anxiety and the other seven measured depression. Each question contained four items, and patients were asked to mark the item that best fit their condition. The cutoff score for the seven-item depression subscale was 8, while that for the anxiety subscale was 10 (14,15).

Statistical Analyses

Statistical analyses were performed using SPSS version 25 (IBM Corporation, Armonk, NY, USA). Descriptive statistics [mean ± standard deviation (SD) for continuous variables, frequencies and percentages for categorical variables] were used to summarize the baseline characteristics of the participants. Comparisons between the control and disease groups were conducted using (Mann-Whitney U for continuous variables, chi-squared tests for categorical variables). $p<0.05$ was considered statistically significant.

Results

The study comprised 56 patients with remitted prolactinoma who were on cabergoline treatment and 56 control participants without known diseases who were matched for age and gender and presented for routine examinations. The mean age of both groups was 39.4 years (SD=10.5), with 62.5% female and 37.5% male in each group. The mean baseline prolactin level of the patients was 30.31 ng/mL (SD=63.12). The cumulative cabergoline dose used by the patients was 105.25 mg (SD=108.58). The average duration of cabergoline treatment among patients with prolactinoma was 40.39 months (SD=30.2). Table 1 shows the demographic and clinical profiles of the two groups.

Among the prolactinoma patients, 37.5% (n=21) were diagnosed with depression according to the HADS. Moreover, 41.1% (n=23) of the control group exhibited symptoms of depression. Anxiety disorders, as measured by The HADS, were present in 37.5% (n=21) of the prolactinoma patients and 23.2% (n=13) in the control group (Table 2).

The chi-square test of independence was used to investigate the association between prolactinoma status and the presence of depression. The results indicated no significant association between prolactinoma status and depression (p=0.69) suggesting that the prevalence of depression was not significantly different between patients with recurrent prolactinoma and the control group. Additionally, the chi-squared test of independence indicated

Table 1. Demographics and clinical profiles of the study participants

Characteristic	Prolactinoma patients (n=56)	Control group (n=56)
Mean age (years)	39.4±10.5	39.4±10.5
Female, n (%)	35 (62.5%)	35 (62.5%)
Males, n (%)	21 (37.5%)	21 (37.5%)

Mean ± standard deviation for continuous variables and frequencies and percentages for categorical variables were used to summarize the baseline characteristics of the participants

Table 2. Prevalence of depression and anxiety in participants

Condition	Prolactinoma patients (n=56)	Control group (n=56)	p-value
Depression, n (%)	21 (37.5%)	23 (41.1%)	0.69
Anxiety, n (%)	21 (37.5%)	13 (23.2%)	0.1

Comparisons between the control and disease groups were conducted using Mann-Whitney U for continuous variables

no significant association between prolactinoma status and anxiety (p=0.1), suggesting similar anxiety prevalence in both groups (Table 2). Prolactinoma patients had a median depression score of 5 [interquartile range (IQR) 2.25-8], whereas the control group had a median score of 6 (IQR 4-9). For anxiety, the prolactinoma patients had a median score of 8 (IQR 5-11.75), whereas the control group had a median score of 7 (IQR 5-9) in the control group. A Mann-Whitney U test was used to compare depression and anxiety scores between the two groups. The results showed no significant difference in the depression (p=0.32) and anxiety scores (p=0.19) between the prolactinoma and control groups, indicating similar levels of depression and anxiety.

Discussion

The prevalence of psychiatric side effects, particularly impulse control disorder, associated with cabergoline therapy for prolactinoma has been documented in the medical literature (5,6). However, our study aimed to delve deeper into less explored areas, specifically depression and anxiety disorders, in patients with recurrent prolactinoma undergoing cabergoline treatment. Understanding the prevalence and potential contributing factors of these psychiatric conditions is crucial for comprehensive patient care and treatment management. We examined the frequency of these disorders with that of a healthy population to explore potential cabergoline-related side effects. Our findings revealed no significant differences in anxiety disorders and depression between patients with recurrent prolactinoma who received cabergoline therapy and the control group. This study is the first to investigate the relationship between cabergoline dosage/duration and depression or anxiety disorders in patients with prolactinoma.

Although studies have reported conflicting results, depression and anxiety disorders are frequently observed in patients with prolactinoma (16-19). This occurrence can be linked to several factors, including the chronic nature of prolactinoma, long-term medication use, and hypogonadism. In our study, the similarity in anxiety and depression scores between patients with prolactinoma who achieved biochemical remission and the general population indicated that the neuropsychiatric effects of the disease could improve with treatment. In a study by Buckman and Kelner (20), it was reported that patients successfully treated with bromocriptine experienced a decrease in anxiety and depression scores. Similarly, some studies have reported an increase in quality of life in prolactinoma patients receiving medical treatment (18). However, the findings of our study may have been influenced by the high prevalence of psychiatric disorders in the control group. In Türkiye, it is reported that 18% of the population experiences a mental disorder at some point in their lives, with the prevalence of depression ranging from 4% to 9%. Globally, it is reported to be around 15%. However, in our study, the prevalence of depression was reported to be remarkably high at 41%, which may obscure the depressive condition of patients with prolactinoma treated with cabergoline (21).

Cabergoline acts on dopamine D2 receptors, and understanding the complex realm of dopamine D2 receptors is necessary for comprehending the neuropsychiatric side effects of cabergoline. D2 receptors serve as the primary modulators of dopaminergic activity, operating through various mechanisms to fulfill this role. This pathway is particularly closely associated with reward systems, mood disorders, and motivation (22-24). D2 autoreceptor suppress dopaminergic neuron activation and inhibit dopamine secretion. Consequently, it has been hypothesized that alterations in dopamine levels or receptor sensitivity associated with D2 receptors could potentially be linked to depression and anxiety disorders (22,23). Cabergoline may induce these alterations and disrupt the delicate balance of neurotransmitter signaling, predisposing individuals to mood dysregulation and anxiety.

On the other hand, some studies have indicated the potential anxiolytic and antidepressant effects of cabergoline. In a study by Anokhin et al. (25), rats administered cabergoline showed an increase in brain-derived neurotrophic factor (BDNF) mRNA expression in the midbrain, suggesting possible anxiolytic effects. Furthermore, evidence from a study by Chiba et al. (26) confirmed the antidepressant effects of cabergoline in rats. It has been noted that the underlying mechanism involves increased levels of BDNF in the hippocampus, which is typically low in depression. In summary, results from studies examining the relationship between cabergoline and depression/anxiety disorders are conflicting, emphasizing the need for further evidence to elucidate this relationship. In parallel with these outcomes, our study did not find a relationship between the duration of cabergoline use, its cumulative dose, and elevated anxiety and depression scores.

This study addresses a significant gap in the literature by focusing on the prevalence of depression and anxiety disorders in patients with recurrent prolactinoma undergoing cabergoline treatment. By delving into these often-understudied psychiatric effects, this research provides valuable insights into the holistic management of prolactinoma patients. One of the strengths of our study lies in its inclusion of a control group, allowing for a comparative analysis of psychiatric symptomatology between patients with prolactinoma and individuals without known diseases

along with the long-term follow-up of patients and the inclusion of both genders in the study.

Study Limitations

It is important to acknowledge the limitations inherent to this study. First, the limited sample size may have limited the generalizability of the findings. Furthermore, the study did not comprehensively investigate other comorbidities or socioeconomic factors that could potentially contribute to depression and anxiety disorders. Additionally, the recruitment of participants from a single outpatient clinic may introduce selection bias, further limiting the generalizability of the results to other populations.

Conclusion

The present study investigated the prevalence of depression and anxiety disorders in patients with recurrent prolactinoma undergoing cabergoline treatment. Despite known psychiatric side effects of cabergoline, we found no significant differences in these disorders compared with the control group. This suggests that cabergoline dosage/duration does not strongly influence these psychiatric comorbidities in this population. However, the complex modulation of dopamine D2 receptors and conflicting evidence from preclinical studies warrant further investigation.

Ethics

Ethics Committee Approval: The study was conducted with the approval of the University of Health Sciences Türkiye, Hamidiye Scientific Research Ethics Committee (approval number: 23/526, date: 22.09.2023).

Informed Consent: All participants provided informed consent.

Authorship Contributions

Concept: M.C.Ş., N.H.E., F.D., A.Y., Design: M.C.Ş., N.H.E., F.D., A.Y., Data Collection or Processing: M.C.Ş., N.B., G.Y., Analysis or Interpretation: N.H.E., N.B., G.Y., F.D., A.Y., Literature Search: M.C.Ş., N.B., G.Y., F.D., A.Y., Writing: M.C.Ş., N.H.E., N.B., G.Y.,

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REFERENCES

1. Melmed S. Pituitary-tumor endocrinopathies. N Engl J Med. 2020;382:937-950. [Crossref]
2. Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, et al. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:273-288. [Crossref]
3. Wang AT, Mullan RJ, Lane MA, Hazem A, Prasad C, Gathaiya NW, et al. Treatment of hyperprolactinemia: a systematic review and meta-analysis. Syst Rev. 2012;1:33. [Crossref]
4. Colao A, di Sarno A, Pivonello R, di Somma C, Lombardi G. Dopamine receptor agonists for treating prolactinomas. Expert Opin Investig Drugs. 2002;11:787-800. [Crossref]
5. Barake M, Evins AE, Stoeckel L, Pachas GN, Nachtigall LB, Miller KK, et al. Investigation of impulsivity in patients on dopamine agonist therapy for hyperprolactinemia: a pilot study. Pituitary. 2014;17:150-156. [Crossref]
6. Dogansen SC, Cikrikcili U, Oruk G, Kutbay NO, Tanrikulu S, Hekimsoy Z, et al. Dopamine agonist-induced impulse control disorders in patients with prolactinoma: a cross-sectional multicenter study. J Clin Endocrinol Metab. 2019;104:2527-2534. [Crossref]
7. Tolentino JC, Schmidt SL. DSM-5 Criteria and depression severity: implications for clinical practice. front psychiatry. 2018;9:450. [Crossref]
8. Admon R, Kaiser RH, Dillon DG, Beltzer M, Goer F, Olson DP, et al. Dopaminergic enhancement of striatal response to reward in major depression. Am J Psychiatry. 2017;174:378-386. [Crossref]
9. Wang HQ, Wang ZZ, Chen NH. The receptor hypothesis and the pathogenesis of depression: Genetic bases and biological correlates. Pharmacol Res. 2021;167:105542. [Crossref]
10. Penninx BW, Pine DS, Holmes EA, Reif A. Anxiety disorders. Lancet. 2021;397:914-927. [Crossref]
11. Zarrindast MR, Khakpai F. The modulatory role of dopamine in anxiety-like behavior. Arch Iran Med. 2015;18:591-603. [Crossref]
12. Bananej M, Karimi-Sori A, Zarrindast MR, Ahmadi S. D1 and D2 dopaminergic systems in the rat basolateral amygdala are involved in anxiogenic-like effects induced by histamine. J Psychopharmacol. 2012;26:564-574. [Crossref]
13. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67:361-370. [Crossref]
14. Aydemir Ö, Köroğlu E. Psikiyatride Kullanılan Ölçekler. Ankara: HYB Basım Yayım; 2009. [Crossref]
15. Aydemir Ö, Güvenir T, Küey L, Kültür S. Hastane anksiyete ve depresyon ölçeği Türkçe formunun geçerlilik ve güvenilirliği. Turk Psikiyatr Derg. 1997;8:280-287. [Crossref]
16. Calva-González M, Villanueva-Solórzano PL, Crail-Meléndez ED, Loya-Murguia KM, Dehesa Hernandez IA, Robles-Ramirez F, et al. Neuropsychiatric effects in patients with invasive prolactinomas treated with cabergoline. Cureus. 2023;15:e39869. [Crossref]
17. Reavley A, Fisher AD, Owen D, Creed FH, Davis JR. Psychological distress in patients with hyperprolactinaemia. Clin Endocrinol (Oxf). 1997;47:343-348. [Crossref]
18. Castle-Kirszbaum M, Biermasz N, Kam J, Goldschlager T. Quality of life in prolactinoma: a systematic review. Pituitary. 2024;27:239-247. [Crossref]
19. Miao X, Fu Z, Luo X, Wang J, Yuan L, Zhao S, et al. A study on the correlations of PRL levels with anxiety, depression, sleep, and self-efficacy in patients with prolactinoma. Front Endocrinol (Lausanne). 2024;15:1369729. [Crossref]
20. Buckman MT, Kellner R. Reduction of distress in hyperprolactinemia with bromocriptine. Am J Psychiatry. 1985;142:242-244. [Crossref]
21. Erol N, Kılıç C, Ulusoy M, Keçeci M, Şimşek Z. Türkiye Ruh Sağlığı Profili Raporu. Birinci baskı, Ankara. 1998. [Crossref]
22. Ford CP. The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience. 2014;282:13-22. [Crossref]
23. Simpson EH, Gallo EF, Balsam PD, Javitch JA, Kellendonk C. How changes in dopamine D2 receptor levels alter striatal circuit function and motivation. Mol Psychiatry. 2022;27:436-444. [Crossref]

24. Grace AA. Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. Nat Rev Neurosci. 2016;17:524-532. [Crossref]
25. Anokhin PK, Veretinskaya AG, Pavshintsev VV, Shamakina IYu. Experimental studies of the effects of the dopamine D2 receptor agonist cabergoline on catecholamine content and BDNF mRNA expression in the midbrain and hypothalamus. Neurosci Behav Physi. 2020;50:830-834. [Crossref]
26. Chiba S, Numakawa T, Ninomiya M, Yoon HS, Kunugi H. Cabergoline, a dopamine receptor agonist, has an antidepressant-like property and enhances brain-derived neurotrophic factor signaling. Psychopharmacology (Berl). 2010;211:291-301. [Crossref]

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Long-Term Pulmonary Evaluation of Intensive Care Unit Patients with Severe COVID-19

Ağır COVID-19 Nedeniyle Yoğun Bakımda Takip Edilen Hastaların Uzun Dönem Akciğer Değerlendirmesi

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ABSTRACT

Background: The long-term pulmonary consequences and impact on respiratory function among patients discharged from intensive care unit (ICU) after severe COVID-19 are not well known. The purpose of this study was to investigate the pulmonary sequelae and long-term respiratory function of patients with severe COVID-19 who were discharged from the ICU.

Materials and Methods: This prospective cohort study. First, 3rd, and 6th month symptoms, laboratory data, 6-minute walk test, sit-to-stand test, BORG scale, pulmonary function test, chest X-ray, and thorax computed tomographys (CTs) of patients diagnosed with COVID-19 who were treated and discharged from the ICU.

Results: Sixty (74%) of the 81 patients included in the study were male, and the median age was 49 (43-61). The most common symptoms upon admission were dyspnea (69%), cough (42%), fever (47%), and fatigue (37%). At the 6-month follow-up, 30 (45%) of the 66 patients had at least one complaint, with dyspnea, cough, and muscle pain being the most common. When the symptoms of patients upon admission were compared with those during the 1st, 3rd, and 6th months of follow-up, it was found that the symptoms of dyspnea, cough, and fatigue regressed. Furthermore, laboratory parameters such as lymphocyte and eosinophil counts, neutrophil lymphocyte ratio, lactate dehydrogenase, C-reactive protein, ferritin, and D-dimer levels were improved ($p<0.01$). At the 6-month follow-up, thorax CT scans showed ground glass infiltration in 7 (28%) patients, fibrosis in 4 (16%), band atelectasis in 2 (8%), and fibrotic bands in 8 (32%). 12% of patients had normal thorax CT scans.

Conclusion: There has been a growing body of knowledge regarding the long-term impacts of COVID-19. Prolonged symptoms are linked to illness convalescence and the potential development of fibrosis, emphasizing the need for regular post-discharge monitoring of patients. It is recommended that a minimum of 6 months after the onset of COVID-19 is an appropriate duration to identify the long-term effects of COVID-19.

Keywords: COVID-19, intensive care unit, convalescence, severe COVID-19, long term

ÖZ

Amaç: Ağır COVID-19 nedeniyle yoğun bakım ünitesinde (YBÜ) takip edilen olguların taburculuk sonrası akciğerlerinde nasıl sekel kaldığı, pulmoner fonksiyon ve kapasitelerinin nasıl etkilendiği net bilinmemektedir. Çalışmamızda YBÜ'de COVID-19 nedeniyle takip edildikten sonra taburcu olan olguların uzun dönem pulmoner fonksiyon ve sekel durumunu araştırmayı amaçladık.

Gereç ve Yöntemler: Prospektif çalışmada, COVID-19 tanısıyla YBÜ'de tedavi edilip taburcu olan hastaların, taburculuk sonrası 1. 3. ve 6. ay semptomları, laboratuvar verileri, 6 dakika yürüme testi, otur kalk testi, BORG skalası, solunum fonksiyon testi, akciğer grafisi ve toraks bilgisayarlı tomografileri (BT) değerlendirildi.

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ÖZ

Bulgular: Çalışmaya alınan 81 olgunun 60'ı (%74) erkek olup, ortanca yaş 49 (43-61) idi. Olguların yatışta en sık görülen semptomları nefes darlığı (%69), öksürük (%42), ateş (%47) ve halsizlik (%37) idi. Altıncı ay kontrolüne gelen 66 olgunun 30'unun (%45) en az bir şikâyeti vardı, en sık görülen şikâyet nefes darlığı, öksürük ve kas ağrısı idi. Hastaların yatış, 1. 3. ve 6. ay takiplerindeki semptomları karşılaştırıldığında nefes darlığı, öksürük ve halsizlik semptomlarında gerileme olduğu, laboratuvar parametrelerinden ortalama lenfosit sayısı, eozinofil sayısı, nötrofil lenfosit oranı, laktat dehidrogenaz, C-reaktif protein, ferritin ve D-dimer değerlerinde düzelme olduğu görüldü (p<0,01). Altıncı ay kontrolünde çekilen toraks BT'lerin 7'sinde (%28) buzlu cam infiltrasyonu, 4'ünde (%16) fibrozis, 2'sinde (%8) band atelektazi ve 8'inde (%32) fibrotik bant görüldü. %12 olgunun toraks BT'leri normal idi.

Sonuç: COVID-19'un uzun dönem etkileri ile ilgili bilgiler zaman içerisinde artmaktadır. Uzamış semptomlar, hastalıktan iyileşme süreci ve gelişebilecek fibrozis ile ilişkili olup hastalar taburculuk sonrası düzenli takip edilmelidir. COVID-19'a bağlı sekelleri tanımlamak için hastalık sonrası en az 6 ayın geçmesinin uygun olacağı düşünülmektedir.

Anahtar Kelimeler: COVID-19, yoğun bakım ünitesi, iyileşme dönemi, ağır COVID-19, uzun dönem

Introduction

Although the epidemiological and clinical characteristics of COVID-19 were well established since December 2019, data regarding its long-term consequences is limited. As the number of patients who recovered from the disease increased, ongoing multisystemic organ involvement, including the lungs, was observed. COVID-19-related pulmonary fibrosis, vascular diseases, and mental disorders have also been reported (1-4). Post-severe COVID-19 pulmonary diffusion disorders, muscle weakness, and radiological sequelae were identified recently (5).

Although symptoms lasting longer than a month were 10-20% of patients who recovered from COVID-19, symptoms lasting 12 weeks were reported in 2.3% of patients (6). In cases of prolonged COVID-19, symptoms like dyspnea, cough, fatigue, chest pain, joint pain, muscle pain, and anxiety were persisted, while there were limited studies regarding the sequelae left in different organ systems (6-9). Furthermore, data on the duration of COVID-19-related ongoing symptoms, radiological abnormalities, and pulmonary function alterations are limited.

This study aimed to investigate the long-term effects of severe COVID-19 on pulmonary function and sequelae of the patients who were discharged from intensive care unit (ICU).

Materials and Methods

Patients who were followed-up in the ICU between August 1, 2020, and August 31, 2021 and discharged from the hospital due to severe COVID-19 were included in the prospective cohort study. Approval was obtained from the University of Health Sciences Türkiye, Süreyyapaşa Chest Diseases and Thoracic Surgery Clinical Research Ethics Committee (approval number: 116.217.089, date: 18.06.2020), in accordance with the Helsinki Declaration.

Patients

COVID-19 patients who were followed-up in the ICU and subsequently discharged were scheduled for control appointments at the 1st, 3rd, and 6th months after the discharge date. Demographic characteristics, symptoms at diagnosis, smoking history, comorbidities, polymerase chain reaction (PCR) test results, radiological images [chest X-ray, thorax computed tomography (CT)], length of stay, blood tests (hemogram, biochemistry, coagulation, cardiac markers), APACHE II score, Charlson comorbidity index, treatments applied during hospitalization and respiratory support treatments, treatments given after discharge, and radiological images from the hospital data system and intensive care records were recorded. The study flow chart is shown in Figure 1.

Inclusion Criteria

- Severe COVID-19 patients followed up in the ICU
- Patients discharged from the ICU
- Patients undergoing 1st, 3rd, and 6th month follow-up after discharge

Exclusion Criteria

- Dieting in a palliative care unit
- Living outside the province
- Immobile
- Lost to follow-up
- Refused to participate

Evaluation/Patient Follow-Ups

In the first month of follow-up of the patients participating in the study, questions regarding symptoms and oxygen support, saturation measurement, BORG scale, chest X-ray, biochemistry tests [glucose, urea, creatinine, aspartat transferaz, alanin aminotransferaz, alkaline phosphatase, gamma-glutamyl transferase, ferritin, C-reactive protein (CRP), lactate dehydrogenase (LDH)], coagulation tests (D-dimer, prothrombin time, international normalized ratio), and hemogram tests were performed. In the 3rd month

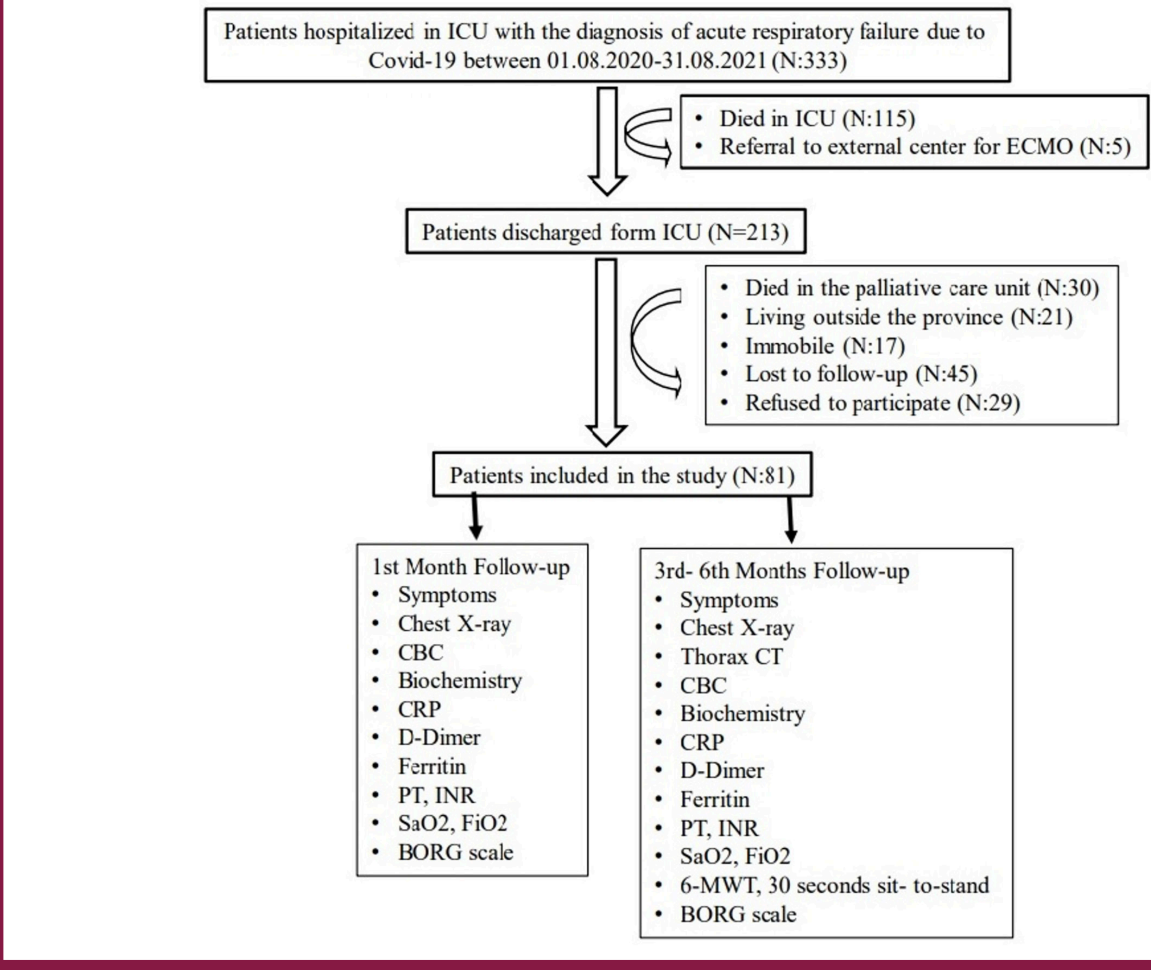

Figure 1. Flow chart of study
ICU: Intensive care unit, CRP: C-reactive protein, INR: International normalized ratio, ECMO: Extracorporeal membrane oxygenation, CBC: Complete blood count, PT: Prothrombin time, BORG: Dyspnea scale, 6-MWT: 6-minute walk test

follow-up, in addition to the 1st month outpatient clinic control, 6-minute walk test (6-MWT) and sit-stand tests, pulmonary function tests (PFTs) were performed, and thorax CT was requested. In the 6th month follow-up, in addition to the 3rd month outpatient clinic follow-up, thorax CT was performed if there was a sequel on X-ray. Lung function was evaluated using the PFT, BORG dyspnea scale, 6-min walk test, and sit-to- stand test.

Statistical Analysis

The minimum sample size required to achieve 80% study power was calculated to be 62. Statistical analysis was performed using SPSS 20 (IBM Corporation, Armonk, NY, USA) software to evaluate the study findings. Numerical values, such as age and hospital stay, were presented as mean and standard deviation (SD) if normally distributed and as median and 25%-75% if not normally distributed. Dichotomous values, such as sex and comorbidities, were presented as numbers and percentages. The patients' data from their intensive care hospitalization, 1st, 3rd, and 6th month follow-ups were evaluated. The data were compared by mean (SD) deviation and the Student t-test was performed if the numerical values were normally distributed. In addition to the numerical values that were not normally distributed, they were compared by the median (25%-75%) and the Mann-Whitney U test was performed. Cochran's analysis was used to evaluate the follow-up symptom data at the 1st, 3rd, and 6th months after admission to the ICU, and descriptive analysis was performed for radiological data.

Results

A total of 81 volunteers were included in the study out of the 333 patients who were hospitalized in the ICU

due to severe COVID-19. Among the included patients, 60 (74%) were male, with a median age of 49 (43-61). At least one comorbid disease was present in 52 (64%) cases, most frequently accompanied by hypertension, diabetes mellitus, and asthma. The most common symptoms during hospitalization were dyspnea (69%), cough (42%), fever (47%), and fatigue (37%). Table 1 summarizes the demographic characteristics.

Upon evaluation of the intensive care hospitalization data, the mean APACHE II score was 13, and the median Charlson comorbidity index was 2. PCR tests were positive in 89% of the 81 patients admitted to the ICU. Acute Respiratory Distress Syndrome was observed in 32% of the patients, whereas 33% had sepsis. Most cases had bilateral infiltration on chest X-ray and typical findings on thorax CT upon admission to the ICU. While 74 patients were followed up with nasal cannula oxygen therapy, 6 were followed up with invasive mechanical ventilation. The median length of stay in the ICU was 9 days. A summary of intensive care data and treatments is presented in Table 1.

On the day of discharge from the ICU, the mean SaO_2 was 95±2, and the median FiO_2 was 28% (21-40). Long-term oxygen therapy was administered on the day of discharge for 40% (n=32) patients. Methylprednisolone was administered to 36 patients, and dexamethasone was administered to 28 patients after discharge.

Table 2 summarizes the complaints, laboratory data, thorax CT scan, and pulmonary status evaluation of the patients at the 1st, 3rd, and 6th months after discharge. Among the 81 patients who attended the 1-month follow-up, 67% (n=54) reported complaints. Among the 80 patients who attended the 3rd-month follow-up, 56% (n=45) had complaints. At the 6-month follow-up, 45% (n=30) of the 66 patients had complaints, with dyspnea, cough, and myalgia being the most common. During the 6-month follow-up, 7 (28%) patients had ground glass infiltration, 4 (16%) had fibrosis, 2 (8%) had band atelectasis, and 8 (32%) had fibrotic bands. Normal thorax CTs were observed in 12% of the patients.

Comparison of admission symptoms with the 1st, 3rd, and 6th-month follow-ups showed a reduction in dyspnea, cough, and fatigue, and laboratory parameters such as lymphocyte and eosinophil counts, neutrophil lymphocyte ratio, laktat dehidrogenaz, CRP, ferritin, and D-dimer levels showed improvement ($p<0.01$) (Table 3).

Discussion

In the present prospective study, the 6-month follow-up of patients hospitalized in the ICU due to acute respiratory failure caused by COVID-19 was investigated. It has been shown that more than half of the patients still had at least

Table 1. Demographic characteristics, intensive care and laboratory data, radiological characteristics, and treatments of patients hospitalized in the ICU due to COVID-19 between 01.08-2020-31.08.2021 (n=81)

Age (years), median (Q1-Q3)	49 (43-61)
Sex, male, n (%)	60 (74)
BMI, (kg/m²), mean ± SD	27±4
Cigarettes, n (%) • Non-smoker • Smoker • Ex-smoker	 47 (58) 12 (15) 22 (27)
Co-morbidity, *n (%) • Hypertension • Diabetes mellitus • Asthma • Coronary artery disease • COPD • Cancer	52 (64) 30 (37) 24 (30) 11 (14) 6 (7) 3 (4) 2 (3)
Hospitalization symptoms, n (%) • Dyspnea • Cough • Fever • Fatigue • Myalgia • Nausea • Chest pain	 56 (69) 42 (52) 38 (47) 30 (37) 8 (10) 5 (6) 4 (5)
APACHE II, mean ± SD	13±4
Charlson comorbidity index, median (Q1-Q3)	2 (1-3)
Admission RT-PCR-positive, n (%)	72 (89)
ARDS, n (%)	26 (32)
Sepsis, n (%)	27 (33)
Laboratory data	
• Lymphocyte count, 10^9/L, median (Q1-Q3)	0.77 (0.43-1.04)
• NLR, median (Q1-Q3)	10.5 (6.6-16.6)
• Eosinophil count, 10^9/L, median (Q1-Q3)	0 (0-0)
• LDH, U/L, median (Q1-Q3)	399 (312-547)
• CRP level, mg/dL, median (Q1-Q3)	85 (45-181)
• Ferritin level, ng/mL, median (Q1-Q3)	742 (386-1402)
• D-dimer, mg/L; median (Q1-Q3)	0.81 (0.52-1.47)
Chest X-ray infiltration rate, n (%) • Unilateral • Bilateral	 6 (7) 75 (93)
Thorax CT classification, **n (%) • Normal • Typical • Likely • Atypical	 3 (4) 54 (68) 7 (9) 4 (5)
ICU treatment, n (%) • IMV • NIV • HFNC • Reservoir mask oxygen • Mask oxygen • Nasal cannula	 6 (7) 10 (12) 6 (7) 37 (45) 24 (30) 74 (91)

Table 1. Continued

Medical treatment, n (%)	
• Antibiotic	80 (99)
• Anticoagulant	77 (95)
• Favipiravir	76 (94)
• Methylprednisolone	73 (90)
• Tocilizumab	17 (21)
• Apharesis immune plasma	16 (20)
• Anakinra	7 (9)
Length of ICU stay, days, median (Q1-Q3)	9 (5-14)

*There was a case with more than one comorbid disease, **According to the American College of Radiology and The Radiological Society of North America.
Q1-Q3: 1st and 3rd quartile values, BMI: Body mass index, SD: Standard deviation, COPD: Chronic obstructive pulmonary disease, APACHE: Acute physiology and chronic health evaluation, RT-PCR: Reverse transcription-polymerase chain reaction, ARDS: Acute Respiratory Distress Syndrome, ICU: Intensive care unit, IMV: Invasive mechanical ventilation, NIV: Non-invasive mechanical ventilation, HFNC: High-flow nasal cannula, NLR: Neutrophil lymphocyte ratio, LDH: Lactate dehydrogenase, CRP: C-reactive protein, CT: Computed tomography

one complaint in the first month after discharge, but the symptoms significantly regressed after 6 months. Dyspnea, cough, and muscle pain were the most common symptoms at 6-month follow-up. Although most cases initially had bilateral infiltration, approximately 80% of the cases had normal radiological findings during follow-up. Although approximately half of the patients required oxygen therapy upon discharge from the ICU, the need for oxygen decreased during follow-up. PFTs were within normal limits, and no dyspnea was detected on the BORG dyspnea scale, which was used to assess the perception of dyspnea from the first month. There were no significant sequelae in terms of radiological and respiratory function after discharge in patients who were followed-up in the ICU due to severe COVID-19.

At the beginning of the pandemic, it was not known how the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease would progress and what kind of sequelae would occur in patients who recovered. However, post-viral syndromes have been well defined in previous outbreaks of COVID-19, such as SARS and Middle East respiratory syndrome (MERS). Patients who recovered from SARS were reported to have sequelae, such as pulmonary function deterioration, chronic muscle pain, and mental status deterioration. Similarly, pulmonary fibrosis-related changes have been described radiologically after MERS (10-12). As more people recovered over time, data emerged that symptoms associated with COVID-19 persisted after the acute stage of the disease (7,8). Symptoms lasting longer than 12 weeks that cannot be explained by an alternative diagnosis are defined as "long COVID" (7-9). It has been reported that 10-20% of patients who recover from COVID-19 have symptoms lasting longer than one month, whereas symptoms last for more than 12 weeks in 2.3% of patients (6). Common symptoms associated with prolonged COVID; dyspnea, cough, fatigue, chest pain, joint pain, muscle pain, and mood changes (6-8,13). In approximately half of our cases, there were symptoms that persisted at the 12th week after discharge. In the multicenter study of Baris et al. (4), which included a 1-year follow-up period after COVID-19 and evaluated 504 cases; presence of comorbidity (especially COPD), initial pneumonia, persistence of symptoms after treatment, and post-treatment emergency admission were defined as independent risk factors for prolonged COVID symptoms.

Hemoglobin breakdown and iron accumulation boost ferritin levels in patients with severe COVID-19, as indicated by a meta-analysis. Ferritin, CRP, and erythrocyte sedimentation rate are markers of inflammatory burden and are associated with disease severity (14). Sirayder et al. (3) conducted a study on 26 post-COVID cases and 26 healthy controls who were followed up for 6 months after being discharged from the ICU. No changes in leukocyte, neutrophil, lymphocyte, and platelet values were detected during the follow-up period, whereas creatinine, lactate dehydrogenase, D-dimer, ferritin, and CRP levels were decreased. Inflammatory markers returned to normal levels by the 3rd month (3). Similarly, Darcis et al. (5) evaluated 199 severe COVID-19 cases, 80% of whom received oxygen therapy after discharge; hemoglobin, lymphocyte values increased in the 1st and 3rd months after discharge, D-dimer, and CRP values decreased compared with discharge values. In our study, inflammatory marker levels were decreased in the 1st, 3rd, and 6th month controls, which is consistent with the literature. This may be due to the reduction of the inflammatory process activated by SARS-CoV-2 over time.

Chest graphy in the early phases of COVID-19 plays an important role in disease detection. Consolidation in bilateral subareas and peripheral and diffuse opacities are radiological manifestations of COVID-19 (15). In our cases, bilateral involvement was present in the chest X-ray in approximately all cases at the beginning of the study period and in ¾ of them at 1 month after discharge. At the 6-month follow-up, chest X-ray was found to be normal in approximately 75% of the patients. Among the studies evaluating the post-COVID period, chest radiography was mostly normal during follow-up (4,16). The typical tomographic features of COVID-19 were bilateral, peripheral ground glass appearance, consolidation, multifocal ground glass, focal edema, and organizing pneumonia (17).

During the recovery period from COVID-19 pneumonia, time is required for tomographic findings to improve. When

Table 2. Complaints, laboratory data, thorax CT, and pulmonary status evaluation of patients followed-up in the ICU due to COVID-19 at the 1st, 3rd, and 6th months after discharge

	1st month (n=81)	3rd month (n=80)	6th month (n=66)
Complaint, n (%)	54 (67)	45 (56)	30 (45)
Dyspnea	17 (21)	19 (23)	10 (15)
Cough	9 (11)	5 (6)	3 (5)
Chest pain	8 (10)	3 (4)	4 (6)
Fatigue/weakness	16 (20)	12 (15)	6 (9)
Myalgia	5 (6)	3 (4)	9 (13)
Hair loss	2 (2)	4 (5)	2 (3)
Drowsiness	5 (6)	6 (7)	2 (3)
Palpitation	3 (4)	1 (1)	1 (1)
Dizziness	2 (3)	1 (1)	0 (0)
Sweating	3 (4)	3 (4)	1 (1)
Laboratory data			
Lymphocyte count, 10^9/L, median (Q1-Q3)	2.10 (1.70-2.89)	2.33 (1.95-2.86)	2.41 (1.88-2.89)
NLR, median (Q1-Q3)	1.80 (1.38-2.78)	1.59 (1.17-2.07)	1.64 (1.16-2.21)
Eosinophil count, 10^9/L, median (Q1-Q3)	1.10 (0.60-1.80)	1.30 (0.8-19.5)	1.50 (0.9-2.40)
LDH, U/L, median (Q1-Q3)	232 (208-286)	191 (174-212)	187 (168-209)
CRP level, mg/dL, median (Q1-Q3)	3.8 (3.0-8.5)	3.0 (1.7-4.9)	3.1 (1.8-5.7)
Ferritin level, ng/mL, median (Q1-Q3)	188 (83-382)	59 (27-123)	69 (38-119)
D-Dimer, mg/L; median (Q1-Q3)	0.42 (0.25-0.70)	0.28 (0.19-0.48)	0.25 (0.19-0.42)
Chest X-ray infiltration rate, n (%)			
Normal	21 (26)	44 (55)	51 (76)
Unilateral	16 (19)	15 (19)	11 (16)
Bilateral	44 (55)	21 (36)	4 (8)
Thorax CT findings, n (%)	**n=6**	**n=5**	**n=26**
Ground glass	4 (68)	2 (40)	7 (28)
Fibrosis	1 (17)	0	4 (16)
Band atelectasis	1 (17)	3 (60)	2 (8)
Mosaic pattern	0	0	1 (4)
Fibrotic band	0	0	8 (32)
Normal	0	0	3 (12)
Pulmonary status evaluation results	**1st month (n=81)***	**3rd month (n=80)**	**6th month (n=66)**
SaO_2 %, mean ± SD	97±2	98±1	98±1
FiO_2, mean ± SD	22±4	21±2	21±2
LTOT, n (%)	28 (35)	10 (12)	5 (8)
PFT, n (%)	-	47 (59)	32 (50)
FEV_1 mL, median (Q1-Q3)	-	2850 (2250-3420)	2780 (2180-3440)
FEV_1 %, median (Q1-Q3)	-	93 (75-102)	93 (81-103)
FVC mL, median (Q1-Q3)	-	3180 (2480-3740)	3100 (2535-3680)
FVC %, median (Q1-Q3)	-	84 (72-94)	88 (71-98)
FEV_1/FVC, mean ± SD	-	92±11	88±9
6-MWT, n (%)	-	n=44 (55)	n=26 (39)

Table 2. Continued

	1st month (n=81)	3rd month (n=80)	6th month (n=66)
6- MWT, m, mean ± SD	-	475±95	496±71
6- MWT, expected walking %, mean ± SD	-	82±13	87±11
Thirty seconds sit-stand test, n (%)	-	n=45 (56)	n=27 (40)
Sit-to-stand time of 30 s/unit, mean ± SD	-	11±2	11±3
BORG scale, median (Q1-Q3)	1 (0-2)	0 (0-1)	0 (0-1)

*PFT, 6-MWT, sit-stand test were not performed during the 1-month follow-up. SD: Standard deviation, Q1-Q3: 1st and 3rd quartile values, NLR: Neutrophil lymphocyte ratio, LDH: Lactate dehydrogenase, CRP: C-reactive protein, CT: Computed tomography, FEV_1: Forced expiratory volume in 1 s, FVC: Forced vital capacity, 6-MWT: 6-minute walk test, SaO_2: Oxygen saturation, FiO_2: Fractionated oxygen, LTOT: Long-term oxygen therapy, PFT: Pulmonary function test, BORG: Dyspnea scale

Table 3. Comparison of admission, 1st, 3rd and 6th months symptom and laboratory parameters of cases followed in the ICU due to COVID-19 disease (n=67)

	Admission to the ICU	1st month	3rd month	6st month	p-value
Symptoms					
• Dyspnea	46 (68)	14 (21)	13 (19)	10 (14)	**<0.001***
• Cough	36 (54)	8 (12)	4 (1)	3 (1)	**<0.001***
• Myalgia	6 (1)	4 (1)	3 (1)	9 (2)	0.198*
• Fatigue	23 (34)	13 (19)	8 (12)	6 (1)	**<0.001***
• Chest pain	4 (1)	5 (1)	3 (1)	4 (1)	0.909*
Laboratory					
• Lymphocyte, 10^9/L	0.77 (0.43-1.04)	2.18 (1.77-3.12)	2.33 (1.88-2.87)	2.43 (1.89-2.90)	**<0.001ᵝ**
• NLR	10.5 (6.6-16.6)	1.63 (1.32-2.78)	1.58 (1.15-2.07)	1.64 (1.17-2.24)	**<0.001ᵝ**
• Eosinophil, 10^9/L	0 (0-0)	1.10 (0.55-1.75)	1.20 (0.80-1.90)	1.50 (0.85-2.50)	**<0.001ᵝ**
• LDH, U/L	399 (312-547)	232 (210-294)	192 (177-214)	187 (168-210)	**<0.001ᵝ**
• CRP, mg/dL	85 (45-181)	4.3 (3.0-9.5)	2.7 (1.6-4.5)	3.1 (1.8-5.6)	**<0.001ᵝ**
• Ferritin, ng/mL	742 (386-1402)	200 (90-399)	59 (27-118)	69 (35-119)	**<0.001ᵝ**
• D-dimer, mg/L	0.81 (0.52-1.47)	0.40 (0.26-0.70)	0.28 (0.20-0.49)	0.25 (0.19-0.42)	**<0.001ᵝ**

*Theochran Q test was used, ᵝFriedman analysis test was used. Data were expressed as number (%) or median interquartile range (25th-75th percentile). Q1-Q3: 1st and 3rd quartile values, NLR: Neutrophil lymphocyte ratio, LDH: Lactate dehydrogenase, CRP: C-reactive protein, ICU: Intensive care unit

CT scans at the time of hospitalization due to COVID-19 and at the 3rd month after the disease were compared, it was observed that the persistent main pattern was ground glass (5). In the study of Wu et al. (1), 83 COVID-19 cases discharged from the hospital were evaluated, and 78% of the cases showed regression in CT findings at the 3rd month after discharge; however, ground glass (78%), interlobular septal thickening (34%), reticular opacities (33%) were most common, and subpleural diffuse opacities (11%) were persisted In the present study, although the number of patients who underwent CT at 1st and 3rd months was low, ground glass and band atelectasis were often present. In a meta-analysis evaluating 30 studies that included 6- to 12-month follow-up after COVID-19, it was determined that while ground glass opacity continued in 34% of cases at 6th month, this rate decreased to 24% at the 12th month. During the follow-up period, no abnormal CT scan was detected in 43% of the patients (65% at 6 months, 36% at 12 months) (18). Although ground glass infiltration was observed in approximately 1/3 of our patients who underwent CT at 6 months, controls showed fibrotic bands at a similar rate. Thorax CT was normal in 12% of the cases. In a meta-analysis including 2018 cases in which 13 studies were evaluated, it was determined that 45% of the patients recovered with pulmonary fibrosis after COVID-19, and the most important risk factor for the development of fibrosis was disease severity. It was reported that hospitalization in intensive care increased the development of fibrosis 5.6 times, noninvasive ventilator support 8 times, and corticosteroid treatment 3.3 times among factors associated with disease severity (19). According to various studies mentioned, the time required for radiological improvement would be 6-12 months.

During the pandemic, severe COVID-19 patients receiving oxygen therapy in the ICU were discharged from

the hospital with long-term oxygen therapy. In the present study, nearly 50% of patients required long-term oxygen therapy at discharge, but the requirement for oxygen therapy significantly decreased during follow-up. In a study, patients who required high levels of oxygen during hospitalization no longer required oxygen therapy during the 6-month follow-up, which was similar to our study (2).

Severe COVID-19 can lead to various complications, such as lung injury, polyneuropathy, myopathy, weakness, and depression, all of which can result in decreased pulmonary function. Sirayder et al. (3) discovered that patients who recovered from COVID-19 had significantly lower FEV1, forced vital capacity (FVC), peak expiratory flow, and FEF25-75 values than the control group. Accordingly, the severity of the disease and the length of ICU stay were associated with a decrease in respiratory function, and it was concluded that COVID-19 could cause permanent lung damage, reducing respiratory function, functional capacity, and quality of life (3). Among the patients who recovered from COVID-19, 8% had decreased FVC and 14% had decreased total lung capacity. Furthermore, impaired diffusion capacity was found to be more common than restrictive patterns (1,3,18). Decreased diffusion capacity has been associated with the influence of COVID-19 on the interstitium or vascular bed (1). However, in our study, pulmonary function values remained within normal limits during follow-up. Consequently, restrictive dysfunction, which may emerge because of lung parenchymal degradation, might heal with time.

While dyspnea persisted in our study, it showed a decreasing trend during follow-ups, and the 6-MWT, sit-stand test, and BORG scale were within normal limits in the 3rd and 6th month controls. In a study that compared COVID-19 cases discharged from the ICU and a healthy control group, the 6-MWT of the COVID-19 group was lower than that of the healthy group (561 m and 652 m, respectively). It was shown that the COVID-19 group also had lower SaO_2 levels during the test and more weakness and dyspnea after the test (3). Damanti et al. (2) reported a positive association between high BORG scores and low PaO_2/FiO_2 during follow-up after severe COVID-19 and stated that this condition was associated with a longer hospital stay. Muscle weakness caused by severe illness and prolonged hospital stay might have contributed to the shorter-than-expected 6-MWT results.

Study Limitation

Our study has several limitations, including its single-center design, which may limit its generalizability to other populations. However, following the same protocol may provide valuable insights for similar patient groups. Furthermore, the pulmonary function values and radiological findings of the patients were unknown before the study, but it is likely that these parameters were within the normal ranges given the low prevalence of chronic pulmonary diseases.

CONCLUSION

The knowledge on COVID-19's long-term impacts is growing over time. Prolonged symptoms are associated with disease recovery and potential fibrosis; therefore, patients should be followed up regularly after discharge. It is believed that defining permanent sequelae due to COVID-19 might require at least 6 months post-disease.

Ethics

Ethics Committee Approval: Approval was obtained from the University of Health Sciences Türkiye, Süreyyapaşa Chest Diseases and Thoracic Surgery Clinical Research Ethics Committee (approval number: 116.217.089, date: 18.06.2020),

Informed Consent: Written informed consent was obtained from all patients.

Authorship Contributions

Concept: H.G.Ş., S.G., E.T., Design: H.G.Ş., S.G., E.T., Data Collection or Processing: H.G.Ş., S.G., E.T., B.N.E., B.D., E.A., Ö.Y.M., G.G., N.A., Z.K., Analysis or Interpretation: H.G.Ş., S.G., E.T., Z.K., Literature Search: H.G.Ş., S.G., E.T., Writing: H.G.Ş., S.G., E.T., Z.K.

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REFERENCES

1. Wu X, Liu X, Zhou Y, Yu H, Li R, Zhan Q, et al. 3-month, 6-month, 9-month, and 12-month respiratory outcomes in patients following COVID-19-related hospitalisation: a prospective study. Lancet Respir Med. 2021;9:747-754. [Crossref]
2. Damanti S, Ramirez GA, Bozzolo EP, Rovere-Querini P, De Lorenzo R, Magnaghi C, et al. Six-month respiratory outcomes and exercise capacity of COVID-19 acute respiratory failure patients treated with continuous positive airway pressure. Intern Med J. 2021;51:1810-1815. [Crossref]
3. Sirayder U, Inal-Ince D, Kepenek-Varol B, Acik C. Long-term characteristics of severe COVID-19: respiratory function, functional capacity, and quality of life. Int J Environ Res Public Health. 2022;19:6304. [Crossref]
4. Baris SA, Toprak OB, Cetinkaya PD, Fakili F, Kokturk N, Kul S, et al. The predictors of long-COVID in the cohort of Turkish Thoracic Society-TURCOVID multi center registry: One year follow-up results. Asian Pac J Trop Med. 2022;15:400-409. [Crossref]
5. Darcis G, Bouquegneau A, Maes N, Thys M, Henket M, Labye F, et al. Long-term clinical follow-up of patients suffering from moderate-to-severe COVID-19 infection: a monocentric prospective observational cohort study. Int J Infect Dis. 2021;109:209-216. [Crossref]

6. Sudre CH, Murray B, Varsavsky T, Graham MS, Penfold RS, Bowyer RC, et al. Attributes and predictors of long COVID. Nat Med. 2021;27:626-631. [Crossref]
7. Fernández-de-Las-Peñas C, Palacios-Ceña D, Gómez-Mayordomo V, Cuadrado ML, Florencio LL. Defining post-COVID symptoms (post-acute COVID, long COVID, persistent post-COVID): An Integrative Classification. Int J Environ Res Public Health. 2021;18:2621. [Crossref]
8. COVID-19 rapid guideline: managing the long-term effects of COVID-19. London: National Institute for Health and Care Excellence (NICE); 2020 Dec 18. [Crossref]
9. Shah W, Hillman T, Playford ED, Hishmeh L. Managing the long term effects of covid-19: summary of NICE, SIGN, and RCGP rapid guideline. BMJ. 2021;372:n136. [Crossref]
10. Chan KS, Zheng JP, Mok YW, Li YM, Liu YN, Chu CM, et al. SARS: prognosis, outcome and sequelae. Respirology. 2003;8 Suppl:S36-S40. [Crossref]
11. Moldofsky H, Patcai J. Chronic widespread musculoskeletal pain, fatigue, depression and disordered sleep in chronic post-SARS syndrome; a case-controlled study. BMC Neurol. 2011;11:37. [Crossref]
12. Choi WJ, Lee KN, Kang EJ, Lee H. Middle East respiratory syndrome-coronavirus ınfection: a case report of serial computed tomographic findings in a young male patient. Korean J Radiol. 2016;17:166-170. [Crossref]
13. Lescure FX, Bouadma L, Nguyen D, Parisey M, Wicky PH, Behillil S, et al. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. Lancet Infect Dis. 2020;20:697-706. [Crossref]
14. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med. 2020;58:1021-1028. [Crossref]
15. Jacobi A, Chung M, Bernheim A, Eber C. Portable chest X-ray in coronavirus disease-19 (COVID-19): A pictorial review. Clin Imaging. 2020;64:35-42. [Crossref]
16. Mandal S, Barnett J, Brill SE, Brown JS, Denneny EK, Hare SS, et al. Long-COVID: a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. Thorax. 2021;76:396-398. [Crossref]
17. Fonseca EKUN, Loureiro BMC, Strabelli DG, Farias LPG, Garcia JVR, Gama VAA, et al. Evaluation of the RSNA and CORADS classifications for COVID-19 on chest computed tomography in the Brazilian population. Clinics (Sao Paulo). 2021;76:e2476. [Crossref]
18. Lee JH, Yim JJ, Park J. Pulmonary function and chest computed tomography abnormalities 6-12 months after recovery from COVID-19: a systematic review and meta-analysis. Respir Res. 2022;23:233. [Crossref]
19. Hama Amin BJ, Kakamad FH, Ahmed GS, Ahmed SF, Abdulla BA, Mohammed SH, et al. Post COVID-19 pulmonary fibrosis; a meta-analysis study. Ann Med Surg (Lond). 2022;77:103590. [Crossref]

ORIGINAL ARTICLE

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Does Extracorporeal Shock Wave Therapy Used in the Treatment of Chronic Prostatitis and Erectile Dysfunction Help Premature Ejaculation?

Kronik Prostatit ve Erektil Disfonksiyon Tedavisinde Uygulanan Ekstrakorporeal Şok Dalga Tedavisi Prematür Ejakülasyona Fayda Sağlar mı?

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ABSTRACT

Background: We investigated the effect of extracorporeal shock wave therapy (ESWT) on premature ejaculation (PE).

Materials and Methods: This patient-based prospective survey study. A total of 60 male patients aged 18 years and older who underwent perineal ESWT for chronic prostatitis (CP) and penile ESWT for erectile dysfunction (ED) were included in the study. The ages of the patients were recorded. The patients underwent ESWT for a total of 12 sessions (2 sessions per week for 6 weeks) without any anesthesia method. Intravaginal ejaculatory latency time (IELT), International Erectile Function Form, and PE Diagnostic Tool (PEDT) were evaluated before and 3 months after ESWT. PE symptoms and sexual function values of the patients were analyzed separately for the CP and ED groups before and 3 months after ESWT, and the groups were compared.

Results: The average age of the CP group was 33.17±8.32 years and the ED group was 33.30±7.03 years. In the CP group, there was a significant improvement in erectile function, sexual and general satisfaction, and IELT and PEDT scores after ESWT treatment; In the ED group, there was a significant improvement in erectile function, orgasmic function, sexual desire, and sexual and general satisfaction scores after treatment. When the groups were compared, the treatment was found to be more effective in terms of sexual desire and orgasmic function in the penile ESWT group, whereas it was more effective in terms of IELT and PEDT scores in the perineal ESWT group.

Conclusion: Our study showed that; ESWT may be effective for the treatment of PE. It has been determined that this effect is more evident in perineal applications. Prospective, randomized, multicenter and high-participation studies are therefore needed.

Keywords: Chronic prostatitis, erectile dysfunction, extracorporeal shock wave therapy, premature ejaculation

ÖZ

Amaç: Preamatür ejakülasyon (PE) tedavisinde ekstrakorporeal şok dalga tedavisinin (ESWT) etkisini araştırdık.

Gereç ve Yöntemler: Hasta bazlı prospektif bir anket çalışmasıdır. Kronik prostatit (KP), için perineal ve ereksiyon bozukluğu (ED) için penil ESWT uygulanan 18 yaş ve üzeri 30'ardan total 60 erkek hasta çalışmaya dahil edilmiştir. Hastaların yaşları kaydedilmiştir. Hastalara herhangi bir anestezi yöntemi uygulanmadan haftada 2 seans olmak üzere 6 hafta, toplam 12 seans ESWT uygulandı. ESWT öncesi ve 3 ay sonrasında olmak üzere intravajinal ejakülatuvar latens süresi (IELT), Uluslararası Erektil İşlev Formu ve Prematür Ejakülasyon Değerlendirme Anketi (PEDT) ile değerlendirilmiştir. Hastaların ESWT öncesinde ve 3 ay sonrasında KP ve ED grupları için ayrı ayrı PE semptomları ve cinsel fonksiyon değerleri analiz edilmiş ve gruplar karşılaştırılmıştır.

Bulgular: KP grubunun yaş ortalaması 33,17±8,32 yıl ve ED grubunun ise 33,30±7,03 yıl olduğu tespit edilmiştir. KP grubunda ESWT tedavisi sonrası erektil fonksiyon, cinsel ve genel memnuniyet, IELT ve PEDT skorlarında anlamlı düzelme olurken; ED grubunda ise tedavi sonrası erektil fonksiyon, orgazmik işlev, cinsel istek, cinsel ve genel memnuniyet skorlarında anlamlı düzelme olmuştur. Gruplar karşılaştırıldığında penil ESWT grubunda cinsel istek ve orgazmik işlevde tedavi daha etkili iken, perineal ESWT grubunda ise IELT ve PEDT skorları açısından tedavi daha etkili saptanmıştır.

Sonuç: Çalışmamız gösterdi ki; ESWT PE tedavisinde etkili olabilir. Bu etki perineal uygulamalarda daha belirgindir. Prospektif, randomize, çok merkezli ve yüksek katılımlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Kronik prostatit, erektil disfonksiyon, ekstrakorporeal şok dalgası tedavisi, erken boşalma

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Introduction

Premature ejaculation (PE) is a prevalent sexual dysfunction observed in approximately 30% of men (1). It was formally defined by the International Society for Sexual Medicine (ISSM) in 2014, and it is currently classified into two subtypes. Life-long PE (L-PE): This definition has three main elements: (a) ejaculation always or almost always occurs before or within a minute after vaginal penetration, (b) the inability to delay ejaculation, and (c) this condition causes frustration, sadness, mental distress, and sexual avoidance. Acquired PE (A-PE): A-PE is differentiated from lifelong PE by the onset of PE in individuals with previously normal ejaculatory performance and ejaculation occurring within approximately three minutes (2). However, although the definition accepted by ISSM is valid for penile-vaginal sexual activities, we have limited information on how to define PE in homosexual activities (3). Neurotransmitter pathologies in the central nervous system, genetic pathologies, erectile dysfunction (ED), prostate diseases, thyroid diseases, and psychological factors have been emphasized as factors generally associated with the etiology of PE (4). Antidepressants, topical anesthetics, and cognitive behavioral therapies are used in these therapies, but their effectiveness is limited (5).

Extracorporeal shock wave therapy (ESWT) was previously used as a treatment method for chronic wounds and musculoskeletal diseases, in which the common pathology was tissue hypoxia (6). ESWT contributed to neovascularization by creating mechanical tension in the tissue and exerted a therapeutic effect (7). In recent years, it has also been used for the treatment of ED, Peyronie's disease (PD), and chronic prostatitis (CP). In the pathophysiology of treatment efficacy, neovascularization, progenitor cell activation, penile tissue proliferation and differentiation, and cavernous nerve regeneration due to vascular endothelial growth factor and receptor upregulation have been shown for ED; inflammation due to neovascularization and increased blood flow and plaque lysis with macrophage activity have been shown for PD; and hyperstimulation of nociceptor, pain reduction, and perineal spasticity have been shown for CP (8-10). ESWT treatment has been shown to strengthen pelvic floor muscles and increase control over the muscles, and it has been reported that it may also benefit PE through this mechanism (11). Recent studies have shown significant improvements in PE symptoms, especially in those with perineal ESWT (12). In particular, the combination of dapoxetine and ESWT has been shown to increase treatment efficacy (13).

In our study, we examined patients who were also diagnosed with L-PE and received ESWT treatment for CP or ED to compare the improvement in PE symptoms. Perineal ESWT for CP may facilitate ejaculation control by strengthening the pelvic floor muscles. However, penile ESWT for ED may also contribute to the control of ejaculation via neovascularization and nerve regeneration. The present study aimed to determine the potential efficacy of ESWT for the treatment of PE using two different methods.

Materials and Methods

The study population consisted of 60 male patients, 30 with EDs and 30 with CPs aged 18 years and older. The patients were admitted to the Medicana International Ankara Hospital, Clinic of Urology in 2024 due to ED or CP and had a history of L-PE. As medical treatment, ED patients were treated with tadalafil for 8 weeks, whereas CP patients were treated with ciprofloxacin for 4 weeks and tamsulosin for 12 weeks. Patients underwent ESWT when they did not respond to medical treatment. Two cup tests were conducted on patients exhibiting CP symptoms. The exclusion criteria were as follows: (i) having a psychiatric illness (ii) the symptoms had been present for less than three months, (iii) a proven urinary tract infection, (iv) abnormal testosterone levels.

The patients were treated with the Medispect Bold ESWT device for 12 sessions for six weeks, (2 sessions per week) without any anesthesia. In each session, 500 shock waves (3000 shock waves in total) were applied to six points in the perineum for CP and three on both lateral sides of the cavernosal tissue proximal-distal line for ED. The energy setting was a 3-Hz frequency, and the maximum total energy flow density was 0.25 mJ/mm^2. The intravaginal ejaculatory latency time (IELT), International Index of Erectile Function (IIEF), and PE diagnostic tool (PEDT) were completed before and 3 months after treatment.

The IIEF is a standardized instrument designed to assess male sexual dysfunction in accordance with the guidelines established by the European Urological Association. The form comprises 15 questions that evaluate sexual function. The sexual function is scored according to the answers given (14). The validity of this form in Türkiye has been evaluated and approved by the researchers (14,15).

The PEDT is a 5-item instrument designed to facilitate the systematic application of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria for the diagnosis of PE (16). In the PEDT score evaluation, a score of ⩽8 is indicative of the absence of PE, a score of 9 or 10 suggests the possibility of PE, and a score of ⩾11 is indicative of the presence of PE. The definition of probable PE directs physicians to conduct further examinations to confirm the presence of PE. The PEDT is employed as a screening criterion for PE rather than a means of evaluating treatment

efficacy. The validated Turkish version of the PEDT, which comprises five questions, is a reliable instrument for use with patients, particularly given its positive correlation with IELT (17).

Statistical Analysis

In the analysis of the data, descriptive statistical measures (median, mean, and standard deviation) and skewness and kurtosis coefficients for the normal distribution of the measurements obtained from the measurement tool were employed. The independent samples t-test and dependent samples t-test were used to determine differences between groups. The data analysis was conducted using the SPSS Statistic (IBM Corp, 25 Version, Chicago, USA) package. The alpha level was set at 0.05 to assess statistical significance.

Ethical Approval

Ethics committee approval for the study was given by Niğde Ömer Halisdemir University Non-Interventional Clinical Research Ethics Committee (approval number: 2024/42, date: 01.07.2024). The study was conducted in accordance with the Declaration of Helsinki, and each participant was informed about the research at the beginning of the study, provided an informed consent form, and was then included in the study.

Results

The study group of the research consisted of 60 L-PE patients (CP 30 and ED 30) selected using purposive sampling. To measure the effect of ESWT, measurements were taken twice, before and 3 months after the treatment. The mean age of the CP group was 33.17±8.32 years, and the mean age of the ED group was 33.30±7.03 years, and they were statistically similar (t=0.07; p=0.947). No significant difference was observed between the groups in terms of average age.

Skewness and kurtosis coefficients were calculated to determine the normality of the measurements obtained from the scales used in this research. The findings are presented in Table 1. The skewness and kurtosis values of the measurements obtained from the scales examined within the scope of this research were within the range of ±3. Accordingly, it was determined that the measurements were close to a normal distribution, and parametric tests were used in the statistical analysis (Table 1).

In the context of this study, the initial objective was to present the findings related to the comparison of sexual function and PE parameters among patients classified according to their baseline scores. Upon examination

Table 1. Normality analysis of the measurements obtained from the scales

Chronic prostatitis				
	1st measurement		**2nd measurement**	
Variables	**Skewness**	**Kurtosis**	**Skewness**	**Kurtosis**
Erectyl function	-0.54	0.70	-0.33	0.21
Orgasmic function	-0.45	1.09	0.35	-0.17
Sexual desire	-0.09	1.35	-0.10	0.89
Sexual satisfaction	-0.45	-0.97	-1.08	2.36
General satisfaction	0.38	-0.91	-1.26	2.78
PEDT	0.26	-0.15	0.16	-0.24
IELT	0.22	1.02	0.11	-0.35
Erectyl dysfunction				
	1st measurement		**2nd measurement**	
Variables	**Skewness**	**Kurtosis**	**Skewness**	**Kurtosis**
Erectyl function	-0.14	-0.39	0.03	-1.19
Orgasmic function	-0.40	0.98	0.24	-0.25
Sexual desire	-0.76	0.63	0.50	0.03
Sexual satisfaction	-0.27	1.07	0.17	0.66
General satisfaction	-0.53	0.63	0.00	-0.12
PEDT	1.04	0.76	0.90	0.79
IELT	0.15	0.78	0.71	3.00

IELT: Intravaginal ejaculation latency time, PEDT: Premature ejaculation diagnostic tool

of Table 2, it becomes evident that there is a statistically significant difference in sexual function between the two groups prior to ESWT (p=0.000). Upon examination of the averages, the mean sexual function was higher in the CP group than in the ED group (p=0.000). The significant difference had a notable impact on practice. The findings revealed that the PE and IELT parameters of both groups were comparable (Table 2).

After comparing the sexual functions and PE parameters of both groups prior to ESWT, measurements were performed again after ESWT. The second measurements were then compared and are presented in Table 3. Upon examination of Table 3, upon examination of the averages, it was determined that the mean sexual function was higher in the CP group than in the ED group (p=0.000). Significant differences were found to have medium and large effects in practice. The data indicated that the PE and IELT parameters of both groups were comparable (Table 3).

Table 4 presents the comparison of pre-and post-ESWT score differences according to the groups. The analysis revealed that the scores for orgasmic function, sexual desire, and PE were statistically significant, whereas the other variables were not. When the averages were examined, it was found that the difference between the post-ESWT and pre-ESWT measurements of the ED group for orgasmic function (p=0.000) and sexual desire (p=0.001) was higher than that of the CP group. When the PE scores were analyzed, it was found that the difference between the CP and ED groups before and after ESWT was higher than that of the ED group (p=0.000). In other words, ESWT treatment was more effective against the PE status of the CP group (Table 4).

Following the comparison of the two groups, an analysis was conducted to identify the changes within each group. First, the two measurements of the CP group were compared, and the findings are presented in Table 5.

Upon examination of Table 5, it was determined that variables other than orgasmic function (p=0.662) and sexual desire (p=0.662) exhibited statistically significant differences between the pre-and post-ESWT puns of the CP group (p=0.000). Upon examination of the averages, the scores of the CP patients following ESWT treatment were higher than those before ESWT. A comparison of the PE scores revealed that the mean scores after ESWT were lower than those before ESWT (p=0.000), whereas the IELT was higher (p=0.000). Perineal ESWT contributed significantly to PE symptoms (Table 5).

Upon examination of Table 6, it was determined that the variables other than PE (p=0.104) exhibited statistically significant discrepancies between the pre-and post-ESWT scores of the ED group (p=0.000). Upon examining the averages, we determined that the sexual function scores of the ED patients following ESWT treatment were higher than their scores prior to ESWT. Upon examination of the PE scores, it was determined that the post-ESWT and pre-ESWT scores were similar and exhibited no statistically significant difference. In light of these findings, it can be concluded that ESWT treatment is not an effective intervention for PE,

Table 2. Comparison of sexual functions and premature ejaculation parameters between patients with CP and those with ED compared with initial values

Variables	Group	N	$\overline{X}$	SD	sd	t-value	p-value	Cohen-d
Erectyl function	CP	30	22.30	2.68	58	6.67	0.000	1.72
	ED	30	15.77	4.64				
Orgasmic function	CP	30	7.37	0.85	58	6.54	0.000	1.69
	ED	30	5.53	1.28				
Sexual desire	CP	30	7.37	0.85	58	5.82	0.000	1.51
	ED	30	5.70	1.32				
Sexual satisfaction	CP	30	11.10	1.21	58	6.99	0.000	1.81
	ED	30	8.20	1.92				
General satisfaction	CP	30	6.97	0.89	58	4.28	0.000	1.10
	ED	30	5.83	1.15				
PEDT	CP	30	13.13	3.84	58	0.21	0.838	-
	ED	30	12.93	3.71				
IELT	CP	30	35.27	7.32	58	0.09	0.931	-
	ED	30	35.10	7.54				

CP: Chronic prostatitis, ED: Erectyl dysfunction, PEDT: Premature ejaculation diagnostic tool, IELT: Intravaginal ejaculation latency time, N: Number of cases, $\overline{X}$: Mean, SD: Standard deviation, sd: Degree of freedom

although it does result in a partial increase in IELT in the ED group (Table 6).

Discussion

ESWT is an effective treatment for ED and can even partially improve PE symptoms (13). Additionally, it has been shown to positively affect both pain and sexual function during the treatment of CP (12). As far as we have scanned the literature, no study has attempted to measure and compare the effectiveness of perineal and penile ESWT for PE. The objective of this study was to investigate the effect of ESWT treatment applied to different regions that have been demonstrated to contribute to sexual functions in PE. Although there was no significant change in sexual

Table 3. Comparison of sexual function and premature ejaculation parameters between patients with CP and ED after ESWT

Variables	Group	N	$\overline{X}$	SD	sd	t-value	p-value	Cohen-d
Erectyl function	CP	30	24.37	3.48	58	6.25	0.000	1.61
	ED	30	18.77	3.46				
Orgasmic function	CP	30	7.40	0.81	58	3.35	0.001	0.87
	ED	30	6.50	1.22				
Sexual desire	CP	30	7.33	0.88	58	2.36	0.022	0.61
	ED	30	6.57	1.55				
Sexual satisfaction	CP	30	12.43	1.98	58	5.32	0.000	1.37
	ED	30	9.70	2.00				
General satisfaction	CP	30	8.23	1.30	58	3.61	0.001	0.93
	ED	30	7.00	1.34				
PEDT	CP	30	10.37	4.06	58	1.92	0.060	-
	ED	30	12.37	4.02				
IELT	CP	30	42.67	10.70	58	1.63	0.108	-
	ED	30	38.63	8.26				

CP: Chronic prostatitis, ED: Erectyl dysfunction, IELT: Intravaginal ejaculation latency time, PEDT: Premature ejaculation diagnostic tool, N: Number of cases, $\overline{X}$: Mean, SD: Standard deviation, sd: Degree of freedom

Table 4. Comparison of difference scores for sexual function and premature ejaculation parameters between patients with CP and ED after and before ESWT

Variables	Group	N	$\overline{X}$	SD	sd	t-value	p-value	Cohen-d
Erectyl function	CP	30	2.07	1.82	58	1.45	0.153	-
	ED	30	3.00	3.03				
Orgasmic function	CP	30	0.03	0.41	58	3.90	0.000	1.01
	ED	30	0.97	1.25				
Sexual desire	CP	30	-0.03	0.41	58	3.67	0.001	0.95
	ED	30	0.87	1.28				
Sexual satisfaction	CP	30	1.33	1.37	58	0.46	0.650	-
	ED	30	1.50	1.46				
General satisfaction	CP	30	1.27	0.94	58	0.34	0.733	-
	ED	30	1.17	1.29				
PEDT	CP	30	-2.77	2.46	58	3.92	0.000	1.01
	ED	30	-0.57	1.85				
IELT	CP	30	7.40	10.09	58	1.80	0.077	-
	ED	30	3.53	6.07				

CP: Chronic prostatitis, ED: Erectyl dysfunction, IELT: Intravaginal ejaculation latency time, PEDT: Premature ejaculation diagnostic tool, N: Number of cases, $\overline{X}$: Mean, SD: Standard deviation, sd: Degree of freedom

Table 5. Comparison of pre- and post-ESWT scores in the CP group

Variables	Measurement	N	$\bar{X}$	SD	sd	t-value	p-value	Cohen-d
Erectyl function	2nd measurement	30	24.37	3.48	29	6.23	0.000	1.14
	1st measurement	30	22.30	2.68				
Orgasmic function	2nd measurement	30	7.40	0.81	29	0.44	0.662	-
	1st measurement	30	7.37	0.85				
Sexual desire	2nd measurement	30	7.33	0.88	29	0.44	0.662	-
	1st measurement	30	7.37	0.85				
Sexual satisfaction	2nd measurement	30	12.43	1.98	29	5.32	0.000	0.97
	1st measurement	30	11.10	1.21				
General satisfaction	2nd measurement	30	8.23	1.30	29	7.35	0.000	1.34
	1st measurement	30	6.97	0.89				
PEDT	2nd measurement	30	10.37	4.06	29	6.16	0.000	1.13
	1st measurement	30	13.13	3.84				
IELT	2nd measurement	30	42.67	10.70	29	4.02	0.000	0.73
	1st measurement	30	35.27	7.32				

IELT: Intravaginal ejaculation latency time, PEDT: Premature ejaculation diagnostic tool, N: Number of cases, $\bar{X}$: Mean, SD: Standart deviation, sd: Degree of freedom

Table 6. Comparison of pre- and post-ESWT scores in the ED group

Variables	Measurement	N	$\bar{X}$	SD	sd	t-value	p-value	Cohen-d
Erectyl function	2nd measurement	30	18.77	3.46	29	5.43	0.000	0.99
	1st measurement	30	15.77	4.64				
Orgasmic function	2nd measurement	30	6.50	1.22	29	4.25	0.000	0.78
	1st measurement	30	5.53	1.28				
Sexual desire	2nd measurement	30	6.57	1.55	29	3.71	0.001	0.68
	1st measurement	30	5.70	1.32				
Sexual satisfaction	2nd measurement	30	9.70	2.00	29	5.64	0.000	1.03
	1st measurement	30	8.20	1.92				
General satisfaction	2nd measurement	30	7.00	1.34	29	4.96	0.000	0.91
	1st measurement	30	5.83	1.15				
PEDT	2nd measurement	30	12.37	4.02	29	1.68	0.104	--
	1st measurement	30	12.93	3.71				
IELT	2nd measurement	30	38.63	8.26	29	3.19	0.003	0.58
	1st measurement	30	35.10	7.54				

IELT: Intravaginal ejaculation latency time, PEDT: Premature ejaculation diagnostic tool, N: Number of cases, $\bar{X}$: Mean, SD: Standard deviation, sd: Degree of freedom

desire and orgasmic function after ESWT in the CP group, significant improvements were observed in erectile function, sexual and general satisfaction, PE, and IELT parameters. In the ED group, although there was no significant change in PE after ESWT, there was a significant improvement in erectile function, orgasmic function, sexual desire, sexual and general satisfaction, and IELT parameters. A comparison of the ED and CP groups after treatment revealed that penile ESWT had a significantly greater impact on orgasmic function and sexual desire, whereas perineal ESWT demonstrated greater efficacy in addressing PE.

The etiology of PE remains unclear (18). The etiology of L-PE, which occurs symptomatically from the first sexual experience, is suggested to be the disruption in the structure of neurotransmitters (19). Specifically, it is considered a neurobiological issue linked to neurotransmission irregularities in serotonin and 5-hydroxytryptamine receptors (20). Furthermore, selective serotonin reuptake

inhibitors (SSRIs) have been demonstrated to be effective in the treatment of PE through this mechanism (21). Furthermore, ED (22), prostate diseases such as CP (23), hormonal pathologies (24), and genetic diseases (25) also contribute to the pathophysiology of PE. The treatment of PE remains a significant challenge, and ongoing research is aimed at identifying the most effective therapeutic approach for this condition (3). Topical anesthetics and SSRIs are currently used to treat this condition (26). Adherence to topical anesthetics is relatively low (27), whereas the efficacy of SSRIs is rapidly lost when treatment is discontinued (1). Furthermore, cognitive behavioral therapies are employed to enhance self-assurance and mitigate anxiety and depression by meticulously instructing men in the acquisition of sexual abilities that can extend ejaculation duration. However, the success rate of this approach is approximately 50% (28). In the pathophysiology of PE, pelvic floor muscles, particularly the ischiocavernous and bulbar spongiosus muscles, play a pivotal role in the expulsion phase of ejaculation, as evidenced by an increase in electromyographic activity during ejaculation (29). The objective of physiotherapy and electrostimulation is to reinforce pelvic floor muscles and to facilitate a more comfortable control over ejaculation. Significant improvements in IELT were observed in the treatment groups that received physiokinesitherapy and electrostimulation (30). ESWT has also been demonstrated to enhance the strength of pelvic floor muscles, thereby facilitating greater control over these muscles and potentially improving the management of PE (31). A recent study demonstrated that the combination of dapoxetine and ESWT was more effective than dapoxetine alone for the treatment of L-PE (13). In our study, a statistically significant improvement was observed in IELT and PE symptoms following perineal ESWT through similar mechanisms. Although a slight increase in IELT was observed following the application of ESWT to the penis, no therapeutic effect on PE was identified.

ESWT applied to the penis enhances blood flow and optimizes endothelial function by stimulating angiogenesis in the corpus cavernosum (7). The precise mechanism of action of shock waves remains unclear; however, the mechanical stress and microtrauma produced by shock waves appear to initiate a biological cascade that promotes the release of angiogenic factors, leading to neovascularization and increased blood flow (32). ESWT applied perineally for CP has been demonstrated to induce the synthesis of nitric oxide (NO), which is essential for inflammatory reactions (33). Furthermore, NO has been shown to mediate neuromuscular junction formation, including synaptic plasticity and neurotransmission in the peripheral nervous system. Moreover, interruption of the flow of nerve impulses via stimulation of nociceptive receptors and reduction of muscle tone represent additional potential mechanisms of action (34). ESWT is employed in the management of ED, CP, and chronic pelvic pain (CPP) syndrome via different pathophysiologic mechanisms (8). A review of the literature revealed that perineal ESWT improves sexual function in patients with CPP (18). The present study demonstrated that both perineal and penile ESWT resulted in significant improvements in sexual function. Of particular note is the observation that ESWT applied to the penis had a more pronounced impact on both orgasmic function and sexual desire.

Study Limitations

Our study also has some limitations. This was a single-center survey study. The sample size was relatively small. There is only a 3-month control period. Chronic diseases and medications were not evaluated. Multicenter studies with longer follow-up periods are required for ESWT treatment of PE.

Conclusion

Our study showed that; ESWT may be effective in treating PE when applied perineally. Although it is more evident in penile application, it also contributes to sexual functions in perineal application. However, prospective, randomized, multicenter and high-participation studies are needed to obtain clearer results.

Ethics

Ethics Committee Approval: Ethics committee approval for the study was given by Niğde Ömer Halisdemir University Non-Interventional Clinical Research Ethics Committee (approval number: 2024/42, date: 01.07.2024).

Informed Consent: Informed consent was obtained.

Authorship Contributions

Surgical and Medical Practices: E.Y., Concept: E.Y., K.Ö., Design: E.Y., Data Collection or Processing: E.Y., K.Ö., Analysis or Interpretation: E.Y., K.Ö., Literature Search: E.Y., Writing: E.Y.

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REFERENCES

1. Raveendran AV, Agarwal A. Premature ejaculation-current concepts in the management: A narrative review. Int J Reprod Biomed. 2021;19:5-22. [Crossref]

2. Serefoglu EC, McMahon CG, Waldinger MD, Althof SE, Shindel A, Adaikan G, et al. An evidence-based unified definition of lifelong and acquired premature ejaculation: report of the second international society for sexual medicine ad hoc committee for the definition of premature ejaculation. Sex Med. 2014;2:41-59. [Crossref]
3. Shechter A, Gruenwald I. New technologies developed for treatment of premature ejaculation. Int J Impot Res. 2024. [Crossref]
4. Salonia A, Bettocchi C, Boeri L, Capogrosso P, Carvalho J, Cilesiz NC, et al. European Association of Urology Guidelines on Sexual and Reproductive Health-2021 Update: Male Sexual Dysfunction. Eur Urol. 2021;80:333-357. [Crossref]
5. Zhong C, Li C, Geng Q, Han Q, Gao Q, Zhang J, et al. Reasons and treatment strategy for discontinuation of dapoxetine treatment in premature ejaculation patients in China: A retrospective observational study. Andrologia. 2022;54:1598-1604. [Crossref]
6. Hayashi D, Kawakami K, Ito K, Ishii K, Tanno H, Imai Y, et al. Low-energy extracorporeal shock wave therapy enhances skin wound healing in diabetic mice: a critical role of endothelial nitric oxide synthase. Wound Repair Regen. 2012;20:887-895. [Crossref]
7. Gruenwald I, Kitrey ND, Appel B, Vardi Y. Low-intensity extracorporeal shock wave therapy in vascular disease and erectile dysfunction: theory and outcomes. Sex Med Rev. 2013;1:83-90. [Crossref]
8. Wu WL, Bamodu OA, Wang YH, Hu SW, Tzou KY, Yeh CT, et al. Extracorporeal shockwave therapy (ESWT) alleviates pain, enhances erectile function and ımproves quality of life in patients with chronic prostatitis/chronic pelvic pain syndrome. J Clin Med. 2021;10:3602. [Crossref]
9. Li G, Man L. Low-intensity extracorporeal shock wave therapy for male chronic pelvic pain syndrome: a systematic review and meta-analysis. Transl Androl Urol. 2021;10:1202-1211. [Crossref]
10. Hatzimouratidis K, Eardley I, Giuliano F, Hatzichristou D, Moncada I, Salonia A, et al. EAU guidelines on penile curvature. Eur Urol. 2012;62:543-552. [Crossref]
11. Kalyvianakis D, Hatzichristou D. Low-Intensity shockwave therapy ımproves hemodynamic parameters in patients with vasculogenic erectile dysfunction: a triplex ultrasonography-based sham-controlled trial. J Sex Med. 2017;14:891-897. [Crossref]
12. Sokmen D, Comez YI. Efficacy and safety of extracorporeal shock wave therapy in the treatment of chronic prostatitis/chronic pelvic pain syndrome and acquired premature ejaculation patients. Urol Int. 2023;107:872-876. [Crossref]
13. Dogan K, M Taskiran. A randomized controlled trial of combined low-intensity extracorporeal shockwave therapy and Dapoxetine use in the management of lifelong premature ejaculation. Journal of Men's Health. 2023;19:92-98. [Crossref]
14. Akkus E, Kadioglu A, Esen A, Doran S, Ergen A, Anafarta K, et al. Prevalence and correlates of erectile dysfunction in Turkey: a population-based study. Eur Urol. 2002;41:298-304. [Crossref]
15. Serefoglu EC, Atmaca AF, Dogan B, Altinova S, Akbulut Z, Balbay MD. Problems in understanding the Turkish translation of the international index of erectile function. J Androl. 2008;29:369-373. [Crossref]
16. Symonds T, Perelman MA, Althof S, Giuliano F, Martin M, May K, et al. Development and validation of a premature ejaculation diagnostic tool. Eur Urol. 2007;52:565-573. [Crossref]
17. Serefoglu EC, Cimen HI, Ozdemir AT, Symonds T, Berktas M, Balbay MD. Turkish validation of the premature ejaculation diagnostic tool and its association with intravaginal ejaculatory latency time. Int J Impot Res. 2009;21:139-144. [Crossref]
18. Culha MG, Tuken M, Gonultas S, Cakir OO, Serefoglu EC. Frequency of etiological factors among patients with acquired premature ejaculation: prospective, observational, single-center study. Int J Impot Res. 2020;32:352-357. [Crossref]
19. Waldinger MD. The pathophysiology of lifelong premature ejaculation. Transl Androl Urol. 2016;5:424-433. [Crossref]
20. Coskuner ER, Ozkan B. Premature ejaculation and endocrine disorders: a literature review. World J Mens Health. 2022;40:38-51. [Crossref]
21. Althof SE, McMahon CG, Waldinger MD, Serefoglu EC, Shindel AW, Adaikan PG, et al. An Update of the International Society of Sexual Medicine's Guidelines for the Diagnosis and Treatment of Premature Ejaculation (PE). Sex Med. 2014;2:60-90. [Crossref]
22. Guo L, Liu Y, Wang X, Yuan M, Yu Y, Zhang X, et al. Significance of penile hypersensitivity in premature ejaculation. Scientific reports. 2017;7:10441. [Crossref]
23. Sihotang RC, Alvonico T, Taher A, Birowo P, Rasyid N, Atmoko W. Premature ejaculation in patients with lower urinary tract symptoms: a systematic review. Int J Impot Res. 2021;33:516-524. [Crossref]
24. Abu El-Hamd M, Farah A. Possible role of serum testosterone, gonadotropins and prolactin in patients with premature ejaculation. Andrologia. 2018;50. [Crossref]
25. Eltonsi TK, Tawfik TM, Rashed LA, GamalEl Din SF, Mahmoud MA. Study of the link between dopamine transporter gene polymorphisms and response to paroxetin and escitalopram in patients with lifelong premature ejaculation. Int J Impot Res. 2017;29:235-239. [Crossref]
26. Mondaini N, Fusco F, Cai T, Benemei S, Mirone V, Bartoletti R .Dapoxetine treatment in patients with lifelong premature ejaculation: the reasons of a "Waterloo". Urology. 2013;82:620-624. [Crossref]
27. Abu El-Hamd M. Effectiveness and tolerability of lidocaine 5% spray in the treatment of lifelong premature ejaculation patients: a randomized single-blind placebo-controlled clinical trial. Int J Impot Res. 2021;33:96-101. [Crossref]
28. Bao B, Shang J, Wang J, Dai H, Li X, Zhang K, et al. Efficacy and safety of behavioral therapy for premature ejaculation: Protocol for a systematic review. Medicine (Baltimore). 2019;98:e14056. [Crossref]
29. Pischedda A, Fusco F, Curreli A, Grimaldi G, Pirozzi Farina F. Pelvic floor and sexual male dysfunction. Arch Ital Urol Androl. 2013;85:1-7. [Crossref]
30. Pastore AL, Palleschi G, Fuschi A, Al Salhi Y, Zucchi A, Bozzini G, et al. Pelvic muscle floor rehabilitation as a therapeutic option in lifelong premature ejaculation: long-term outcomes. Asian J Androl. 2018;20:572-575. [Crossref]
31. Fode M, Lowenstein L, Reisman Y. Low-intensity extracorporeal shockwave therapy in sexual medicine: a questionnaire-based assessment of knowledge, clinical practice patterns, and attitudes in sexual medicine practitioners. Sex Med. 2017;5:e94-e98. [Crossref]
32. Vardi Y, Appel B, Kilchevsky A, Gruenwald I. Does low intensity extracorporeal shock wave therapy have a physiological effect on erectile function? Short-term results of a randomized, double-blind, sham controlled study. J Urol. 2012;187:1769-1775. [Crossref]
33. Mariotto S, Cavalieri E, Amelio E, Ciampa AR, de Prati AC, Marlinghaus E, et al. Extracorporeal shock waves: from lithotripsy to anti-inflammatory action by NO production. Nitric Oxide. 2005;12:89-96. [Crossref]
34. Marszalek M, Berger I, Madersbacher S. Low-energy extracorporeal shock wave therapy for chronic pelvic pain syndrome: finally, the magic bullet? Eur Urol. 2009;56:425-426. [Crossref]