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ORIGINAL ARTICLE

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Evaluation of the Relationship Between Delirium, Laboratory Parameters, and Falls in Patients Admitted to the Internal Medicine Ward

Dahiliye Servisine Yatan Hastalarda Deliryum ile Laboratuvar Parametreleri ve Düşme Arasındaki İlişkinin Değerlendirilmesi

Vahit Can ÇAVDAR, Yalçın GÖKMEN, Emre Cem GÖKÇE, Günışıl YALÇIN, Mert ARİÇ, Hasan ZERDALİ, Feray AKBAŞ

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ABSTRACT

Aim: This study aimed to evaluate the relationship between delirium, laboratory parameters, and falls in geriatric patients admitted to the internal medicine ward, focusing on differences between infectious and non-infectious delirium etiologies.

Materials and Methods: A prospective study was conducted between March 10 and May 10, 2025, involving 125 patients aged 65 years and older who were hospitalized in the Clinic of Internal Medicine of University of Health Sciences Türkiye, İstanbul Training and Research Hospital. Delirium was diagnosed with 4-AT scores ≥4. Patients were categorized into infectious and non-infectious groups. Demographics, laboratory values, delirium subtypes, fall history, fractures, and mobility status were compared. Statistical tests employed were Mann-Whitney U and chi-square.

Results: The mean age was 79.7±7.9 years, with balanced gender distribution. Infectious causes accounted for 40.8% of delirium cases; urinary tract infections were most common (19.2%). Falls averaged 1.9±1.8 in the prior year, with 20% having fall-related fractures. Hypoactive delirium predominated (70.4%), and 81.6% were mobile. No significant differences existed in age, sex, falls, fractures, or delirium subtype between groups. However, mobility was higher in the non-infectious group (p=0.030). C-reactive protein, erythrocyte sedimentation rate, leukocyte, platelet, neutrophil counts, and sodium were significantly lower in non-infectious delirium (p<0.05).

Conclusion: Delirium is common and serious in elderly inpatients, with infections as a major cause. Greater mobility in non-infectious cases may indicate lower systemic inflammation. Early diagnosis, etiology-focused management, and fall prevention are vital to improve outcomes.

Keywords: Delirium, geriatric, internal medicine

ÖZ

Amaç: Bu çalışma, dahiliye servisine yatan geriatrik hastalarda deliryum, laboratuvar parametreleri ve düşme arasındaki ilişkiyi, enfeksiyöz ve enfeksiyöz olmayan deliryum etiyolojileri açısından değerlendirmeyi amaçladı.

Gereç ve Yöntem: 10 Mart-10 Mayıs 2025 tarihleri arasında, Sağlık Bilimleri Üniversitesi, İstanbul Eğitim ve Araştırma Hastanesi Dahiliye Kliniği'ne yatırılan 65 yaş ve üzeri 125 hasta ile prospektif bir çalışma yürütüldü. Deliryum tanısı 4-AT skoru ≥4 olan hastalara konuldu. Hastalar enfeksiyöz ve enfeksiyöz olmayan gruplara ayrıldı. Demografik bilgiler, laboratuvar değerleri, deliryum alt tipleri, düşme öyküsü, kırıklar ve mobilite durumu karşılaştırıldı. İstatistiksel analizlerde Mann-Whitney U ve ki-kare testleri kullanıldı.

Bulgular: Ortalama yaş 79,7±7,9 yıl olup cinsiyet dağılımı dengeliydi. Deliryum olgularının %40,8'i enfeksiyöz nedenlere bağlıydı; en sık kabul tanısı üriner sistem enfeksiyonuydu (%19,2). Geçen yıl ortalama düşme sayısı 1,9±1,8 idi ve hastaların %20'sinde düşmeye bağlı kırık vardı. Hipoakitif deliryum yaygındı (%70,4) ve %81,6'sı hareketliydi. Gruplar arasında yaş, cinsiyet, düşme, kırık ve deliryum alt tipi açısından anlamlı fark yoktu.

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Ancak mobilite enfeksiyöz olmayan grupta anlamlı olarak yüksekti (p=0,030). C-reaktif protein, eritrosit sedimantasyon hızı, lökosit, trombosit, nötrofil sayıları ve sodyum seviyeleri enfeksiyöz olmayan grupta anlamlı olarak daha düşüktü (p<0,05).

Sonuç: Deliryum, hastanede yatan yaşlı hastalarda yaygın ve ciddi bir durum olup enfeksiyonlar önemli bir neden teşkil etmektedir. Enfeksiyöz olmayan olgularda daha yüksek mobilite, daha az sistemik enflamasyonu gösterebilir. Erken tanı, etiyolojiye yönelik tedavi ve düşme önleyici stratejiler, bu hassas hasta grubunda klinik sonuçların iyileştirilmesinde kritik öneme sahiptir.

Anahtar Kelimeler: Deliryum, geriatrik, iç hastalıkları

INTRODUCTION

Delirium is a condition characterized by alterations in attention, awareness, and cognitive function, and arises in the context of an underlying medical issue that is not more appropriately attributed to a previously diagnosed neurocognitive disorder. Numerous predisposing and precipitating factors for delirium have been identified. It is thought that, in most patients, both types of factors contribute to the onset of delirium[1]. Delirium is an extremely life-threatening and critically important clinical condition that is often overlooked by clinicians. Its etiology is multifactorial, with advanced age and neurocognitive disorders being among the most significant and commonly observed risk factors. Acute medical conditions such as infections, life-threatening organ failures, and sepsis, as well as adverse drug reactions and medical complications, often play central roles in the etiology of delirium[2].

In the United States, delirium is identified in more than 2.6 million geriatric patients aged 65 years and older each year, leading to an estimated annual healthcare expenditure exceeding $164 billion. Given its detrimental effects on patients' functional status and quality of life, as well as the substantial healthcare costs it imposes on governments, it is evident that clinicians should place greater emphasis on this critical clinical condition, approach it with increased vigilance, and prioritize early diagnosis[3].

Delirium is highly prevalent among hospitalized geriatric patients. In one study, delirium was identified in one third of patients aged 70 years and older admitted to general internal medicine wards. Among these patients, delirium was present at the time of hospital admission in half of the cases, while the other half developed delirium during the course of hospitalization[4].

In this study, we aimed to investigate the prevalence of delirium among geriatric patients hospitalized in the internal medicine ward, its distribution according to demographic characteristics, laboratory alterations based on different etiologies, and its association with falls.

MATERIALS AND METHODS

Patients

The clinical data for this study were obtained from of University of Health Sciences Türkiye, İstanbul Training and Research Hospital. A total of 356 geriatric patients who were hospitalized in the internal medicine ward for various medical reasons between March 10 and May 10, 2025, were included in the analysis. Inclusion criteria were defined as follows: individuals aged 65 years and older admitted to the internal medicine department who did not meet any exclusion criteria were included. Additionally, only patients who experienced their first episode of delirium were included. Patients with a prior history of delirium were excluded to minimize potential confounding effects.

Exclusion criteria included patients under the age of 65, those with severe cognitive impairment or a documented diagnosis of dementia, individuals with advanced hearing or visual impairment, patients with speech difficulties, foreign nationals with a potential language barrier that could impair communication, and those with incomplete medical records in the hospital information system. Additionally, patients with a history of alcohol or substance use, as well as those using central nervous system-active agents such as antidepressants, antipsychotics, anxiolytics, or mood stabilizers, were excluded to prevent potential confounding in the clinical assessment of delirium.

Based on these criteria, 231 patients were excluded from the study. A total of 125 patients met the eligibility requirements and were included in the final analysis. The Ethics Committee of University of Health Sciences Türkiye, İstanbul Training and Research Hospital granted approval for this study (decision no: 55, date: 07.03.2025). All study procedures complied with the ethical standards of the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all participants prior to their inclusion in the study.

Data Collection

The following variables were retrieved from the hospital's electronic health database: patients' age, sex, reason for hospitalization, hemogram and biochemical parameters,

number of falls within the past year, fall-related bone fractures, causes of delirium, delirium subtypes, and mobility status.

Delirium was diagnosed according to the 4-AT score assessed during daily bedside evaluations. The 4-AT test is a clinically practical and easily applicable screening tool composed of four components: alertness, abbreviated mental test 4, attention, and acute change or fluctuating course. Each subcomponent is scored individually. Accordingly, patients with a score of 0 were considered negative for delirium, with a low probability of cognitive impairment. Patients scoring between 1 and 3 were considered unlikely to have delirium but possibly had cognitive impairment. Those with a score of 4 or higher were considered to have delirium and were included in the study[5].

Subjects were divided into two groups based on the etiology of delirium: those with delirium due to infectious causes and those with delirium due to non-infectious causes. The relationship between these groups and clinical variables such as hematological and biochemical parameters, as well as fall history, was analyzed.

Statistical Analysis

Descriptive statistics including mean, standard deviation, median, minimum, maximum, frequency, and percentage values were used to summarize the data. Normality of the variables was evaluated by applying the Kolmogorov-Smirnov and Shapiro-Wilk tests. For the analysis of quantitative independent variables that were not normally distributed, the Mann-Whitney U test was used. For the analysis of qualitative independent variables, the chi-square test was applied, and when the assumptions of the chi-square test were not met, Fisher's exact test was used. All statistical analyses were implemented using SPSS version 27.0.

RESULTS

The mean age of the patients included in the study was 79.7±7.9 years. Among the participants, 64 patients (51.2%) were male and 61 patients (48.8%) were female. Regarding age distribution, 37 patients (29.6%) were between 65-74 years, another 37 (29.6%) were between 75-84 years, and 51 patients (40.8%) were aged 85 years or older. The most common reason for hospital admission among the patients was urinary tract infection (UTI), observed in 24 patients (19.2%). Other reasons for hospitalization are summarized in Table 1. The most frequent etiology of delirium was infection-related conditions, accounting for 51 cases (40.8%). Additional etiological factors contributing to delirium are also presented in Table 1. The mean number of falls within the past year was 1.9±1.8. Fall-related fractures were identified in 25 patients (20%). Hypoactive delirium was observed in 88 patients (70.4%), while hyperactive delirium was identified in 37 patients (29.6%). A total of 102 patients (81.6%) were classified as mobile, whereas 23 patients (18.4%) were immobile (Table 1). The primary causes of immobility among patients were sequelae of previous cerebrovascular events and immobilization secondary to fractures.

The complete blood count and biochemical parameters of all subjects included in the study are collectively presented in Table 2.

Table 1. Demographic, clinical, and delirium-related characteristics of subjects

		Min-max			Median	Mean ± SD/n-%		
Age (year)		65.0	-	95.0	80.0	79.7	±	7.9
Gender	Male					64		51.2%
	Female					61		48.8%
Geriatric age group	65-74					37		29.6%
	75-84					37		29.6%
	>84					51		40.8%
Reason for hospitalization								
Urinary tract infection						24		19.2%
Pneumonia						20		16.0%
Gastrointestinal system bleeding						13		10.4%
Others						11		8.8%
Acute kidney injury						9		7.2%
Decompensated heart failure						8		6.4%
Malignancy workup						8		6.4%
Chronic obstructive pulmonary disease exacerbation						7		5.6%

Table 1. Continued

		Min-max			Median	Mean ± SD/n-%		
Catheter infection						6		4.8%
Pancreatitis						6		4.8%
Decompensated cirrhosis						5		4.0%
Cholecystitis						4		3.2%
Diabetic ketoacidosis						4		3.2%
Number of falls in the last 1 year		0.0	-	7.0	2.0	1.9	±	1.8
Fracture due to a fall	(-)					100		80.0%
	(+)					25		20.0%
Etiology of delirium								
Infection						51		40.8%
Electrolyte imbalance						36		28.8%
Lung diseases accompanied by hypoxemia						13		10.4%
Renal failure						10		8.0%
Drug related						8		6.4%
Heart failure						6		4.8%
Liver failure						1		0.8%
Delirium type	Hypoactive					88		70.4%
	Hyperactive					37		29.6%
Mobility status	Immobile					23		18.4%
	Mobile					102		81.6%

Min: Minimum, Max: Maximum, SD: Standard deviation

Table 2. Descriptive statistics of laboratory parameters in geriatric inpatients

	Min-max			Median	Mean ± SD		
C-reactive protein (mg/L)	2.0	-	477.0	75.0	110.3	±	100.1
Erythrocyte sedimentation rate (mm/h)	2.0	-	102.0	44.0	41.3	±	29.9
White blood cell (10^9/L)	2.7	-	30.6	9.4	10.5	±	5.2
Hemoglobin (g/dL)	5.1	-	14.8	11.4	11.1	±	2.2
Platelet count (10^9/L)	39.0	-	604.0	256.0	283.9	±	142.4
Neutrophil (10^9/L)	0.1	-	28.3	7.3	8.6	±	5.0
Lymphocyte (10^9/L)	0.1	-	4.0	1.3	1.3	±	0.7
Monocyte (10^9/L)	0.1	-	1.3	0.3	0.4	±	0.3
Total protein (g/dL)	4.2	-	9.2	6.5	6.4	±	1.0
Albumin (g/dL)	1.8	-	4.7	3.3	3.3	±	0.6
Total cholesterol (mg/dL)	76.0	-	285.0	144.0	151.5	±	46.9
Glucose (mg/dL)	69.0	-	1066.0	112.0	149.8	±	121.1
Creatinine (mg/dL)	0.4	-	8.6	1.1	1.5	±	1.3
Urea (mg/dL)	17.0	-	248.0	49.5	66.0	±	43.5
Aspartate aminotransferase (U/L)	9.0	-	326.0	28.0	39.8	±	40.4
Alanine aminotransferase (U/L)	5.0	-	156.0	27.0	31.2	±	21.2
Sodium (mEq/L)	106.0	-	168.0	132.0	131.9	±	9.8
Potassium (mEq/L)	3.2	-	6.3	4.5	4.5	±	0.8
Corrected calcium (mg/dL)	7.6	-	11.3	8.9	8.9	±	0.7

Table 2. Continued

	Min-max			Median	Mean ± SD		
Phosphorus (mg/dL)	1.3	-	5.8	2.7	2.9	±	1.0
Parathyroid hormone (pg/mL)	17.2	-	234.5	57.3	89.5	±	59.2
Magnesium (mg/dL)	1.1	-	3.0	1.8	1.8	±	0.3
Vitamin B12 (pg/mL)	76.0	-	2100.0	302.0	385.2	±	273.7
Vitamin D (ng/mL)	4.5	-	86.7	13.8	18.5	±	15.7

Min: Minimum, Max: Maximum, SD: Standard deviation

A total of 125 patients were divided into two groups based on the etiology of delirium: 51 patients were identified to have infection-related delirium, while 74 patients had delirium due to non-infectious causes. Table 3 provides a comparative overview of these two groups in terms of mean age, distribution across geriatric age categories, sex, number of falls within the past year, presence of fall-related fractures, delirium subtype, and mobility status.

There were no notable differences between the infectious and non-infectious groups regarding age, geriatric age distribution, sex, number of falls in the past year, fall-related fracture rates, or delirium subtype (p>0.05). Specifically, no statistically significant differences were observed between the geriatric age subgroups (65-74 years, 75-84 years, and ≥85 years) in terms of delirium etiology distribution (p=0.475). However, the proportion of mobile patients was significantly higher in the non-infectious group compared to the infectious group (p=0.030) (Table 3).

There were no meaningful differences between the infectious and non-infectious groups in terms of hemoglobin, lymphocyte, monocyte, total protein, albumin, and total cholesterol levels (p>0.05). Similarly, no significant differences were observed in glucose, creatinine, urea, aspartat aminotransferaz, alanin aminotransferaz, potassium, or corrected calcium levels between the two groups (p>0.05). Additionally, phosphorus, parathyroid hormone, magnesium, vitamin B12, and vitamin D levels did not differ significantly between the groups (p>0.05) (Table 4).

However, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), leukocyte count, platelet count, neutrophil count, and sodium levels were significantly lower in the non-infectious group compared to the infectious group (p<0.05) (Table 4).

Table 3. Comparison of patients with infectious and non-infectious etiology of delirium

		Delirium due to infection group (n=51)				Non-infectious delirium group (n=74)				p	
		Mean ± SD/n-%			Median	Mean ± SD/n-%			Median		
Age (year)		79.5	±	7.9	80.0	79.8	±	7.9	80.0	0.823	m
Geriatric age group	65-74	18		35.3%		19		25.7%		0.475	X^2
	75-84	13		25.5%		24		32.4%			
	>84	20		39.2%		31		41.9%			
Gender	Male	24		47.1%		40		54.1%		0.442	X^2
	Female	27		52.9%		34		45.9%			
Number of falls in the last 1 year		1.9	±	1.8	2.0	1.9	±	1.7	2.0	0.839	m
Fracture due to a fall	(-)	42		82.4%		58		78.4%		0.585	X^2
	(+)	9		17.6%		16		21.6%			
Delirium type	Hypoactive	38		74.5%		50		67.6%		0.403	X^2
	Hyperactive	13		25.5%		24		32.4%			
Mobility status	Immobile	14		27.5%		9		12.2%		**0.030**	X^2
	Mobile	37		72.5%		65		87.8%			

m: Mann-Whitney U test, X^2: Chi-square test, SD: Standard deviation

Table 4. Comparison of laboratory parameters between patients with infectious and non-infectious etiology of delirium

	Delirium due to infection group (n=51)				Non-infectious delirium group (n=74)				p	
	Mean ± SD			Median	Mean ± SD			Median		
C-reactive protein (mg/L)	187.0	±	93.2	186.0	57.5	±	64.4	35.0	**0.000**	m
Erythrocyte sedimentation rate (mm/h)	57.9	±	23.0	56.0	29.9	±	28.9	15.0	**0.000**	m
White blood cell (10^9/L)	12.9	±	5.8	14.4	8.9	±	4.0	8.3	**0.000**	m
Hemoglobin (g/dL)	11.5	±	1.6	11.5	10.8	±	2.5	11.2	0.191	m
Platelet count (10^9/L)	325.7	±	158.9	318.0	255.1	±	122.9	241.5	**0.003**	m
Neutrophil (10^9/L)	11.0	±	5.5	12.2	7.0	±	3.9	6.3	**0.000**	m
Lymphocyte (10^9/L)	1.3	±	0.8	1.4	1.3	±	0.6	1.2	0.844	m
Monocyte (10^9/L)	0.4	±	0.3	0.3	0.4	±	0.3	0.3	0.707	m
Total protein (g/dL)	6.6	±	0.6	6.6	6.2	±	1.2	6.4	0.089	m
Albumin (g/dL)	3.4	±	0.4	3.4	3.2	±	0.7	3.2	0.065	m
Total cholesterol (mg/dL)	149.9	±	43.4	144.0	152.6	±	49.5	146.0	0.890	m
Glucose (mg/dL)	137.9	±	63.6	98.0	158.1	±	148.2	124.5	0.880	m
Creatinine (mg/dL)	1.6	±	1.3	1.3	1.5	±	1.3	1.0	0.217	m
Urea (mg/dL)	66.4	±	34.0	56.0	65.7	±	49.2	44.7	0.190	m
Aspartate aminotransferase (U/L)	38.6	±	36.8	25.0	40.6	±	43.0	29.0	0.293	m
Alanine aminotransferase (U/L)	29.7	±	20.1	26.0	32.3	±	22.1	30.0	0.349	m
Sodium (mEq/L)	134.0	±	6.4	133.0	130.5	±	11.4	130.0	**0.014**	m
Potassium (mEq/L)	4.6	±	0.8	5.0	4.4	±	0.8	4.5	0.134	m
Corrected calcium (mg/dL)	8.9	±	0.8	8.9	8.9	±	0.7	8.8	0.850	m
Phosphorus (mg/dL)	3.0	±	1.1	2.7	2.9	±	0.9	2.7	0.918	m
Parathyroid hormone (pg/mL)	81.7	±	58.8	57.3	95.0	±	59.3	81.8	0.207	m
Magnesium (mg/dL)	1.8	±	0.3	1.8	1.9	±	0.3	1.8	0.704	m
Vitamin B12 (pg/mL)	383.0	±	224.9	319.0	386.7	±	304.3	300.0	0.314	m
Vitamin D (ng/mL)	18.4	±	14.2	13.8	18.6	±	16.7	13.1	0.356	m

m: Mann-Whitney U test, Min: Minimum, Max: Maximum, SD: Standard deviation

DISCUSSION

Among the patients included in our study, 64 were male (51.2%). This finding is consistent with the results of a study conducted in Egypt by Ibrahim et al.[6], which included 588 patients and reported a male prevalence of 58.5%. These data suggest that delirium may be more common in geriatric males compared to females. Similarly, in a study conducted in 2018, Trzepacz et al.[7] reported that 63.5% of 406 patients with delirium were male, further supporting the notion that delirium may occur more frequently in men.

In our research, the proportion of patients was 29.6% in both the 65-74 and 75-84 age groups, whereas it increased to 40.8% in patients aged 85 years and older. This supports the notion that the occurrence of delirium increases with advancing age. In a study conducted on 708 patients aged 85 and above, the prevalence of delirium was reported as 17% at age 85, 21% at age 90, and 39% in individuals aged 95 years and older, with this increase being statistically significant[8]. Similarly, the medical research council cognitive function and ageing study also demonstrated that the prevalence of delirium rises with age, with the highest rates observed in individuals aged 85 and over, thereby supporting the findings of our study[9].

Infectious diseases are commonly observed among the causes of hospitalization in geriatric patients, with UTIs in particular accounting for a significant proportion of admissions. In a study conducted by Artero et al.[10], the median age of subjects admitted to the internal medicine ward due to UTIs was found to be 76 years, highlighting that UTIs are a major reason for hospitalization, especially in the geriatric population. Similarly, in our study, UTIs were identified as the most common cause of admission to the internal medicine ward in the geriatric age group, accounting for 19.2% of all hospitalizations.

With aging, one of the most significant factors affecting the quality of life in geriatric patients is falls and fall-related fractures. Each year, approximately 3 million older adults present to emergency departments in the United States due to falls[11]. About 20% of fall-related injuries are serious and result in conditions that require hospitalization and medical intervention[12]. According to the World Health Organization, around 684,000 fatal falls occur globally each year, with the majority involving adults aged 65 years and older[13]. Falls are considered a major public health issue due to their contribution to increased morbidity and mortality. The frequency of falls increases with age; it is estimated that approximately one-third of individuals over the age of 65 experience at least one fall annually, and this rate approaches 50% among those aged 80 years and older[14-16].

Falls not only result in physical injuries but also have significant psychological consequences. They can result in fear of falling, loss of self-confidence, avoidance of daily activities, decreased functionality, and social withdrawal. These outcomes not only reduce the quality of life but also increase the likelihood of recurrent falls[16]. When all these negative factors are considered together, it becomes evident that falls significantly impair the quality of life in the geriatric population, leading to reduced outdoor activity and increased susceptibility to depression[17]. In our study, patients experienced an average of 1.9 falls in the past year, and 20% sustained fractures as a result of these falls. These findings underscore the urgent need to raise public awareness about fall prevention in elderly individuals and to implement appropriate measures. In this way, healthcare systems may reduce expenditures while decreasing morbidity and mortality rates among the elderly population.

Delirium is often a complex process that arises from the interaction of multiple factors, particularly in the elderly. Predisposing and precipitating factors both play a role in the occurrence of this condition. Alcohol use, psychoactive medications, anticholinergic drugs, and polypharmacy can all trigger delirium. Other precipitating factors include surgical interventions, anesthesia, hypoxia, organ failure, untreated pain, infections, acute illnesses, and acute exacerbations of chronic diseases. In some sensitive patients, even constipation, dehydration, sleep deprivation, or urinary retention may be sufficient to trigger delirium[18].

In our research, infections were identified as the most common cause of delirium among individuals. The literature also supports this finding, with studies reporting delirium associated with UTIs and sepsis[19,20].

Delirium is also frequently observed after surgical procedures and constitutes an important cause of morbidity and mortality. While delirium occurs in approximately 10-20% of patients after major elective surgeries, its incidence can reach up to 50% following high-risk procedures. Postoperative delirium is associated with a 7-10% increase in 30-day mortality risk and prolongs the length of hospital stay by 2-3 days[21].

In our research, no significant differences were found between the infectious and non-infectious delirium groups in terms of age, sex, number of falls, presence of fall-related fractures, and delirium subtype. However, patients in the non-infectious delirium group were found to be significantly more mobile. This finding was interpreted as being related to the generalized inflammation and physical debilitation caused by infectious diseases. Given that infections were the most common cause of delirium in our study, it is crucial to promptly recognize and initiate treatment of infections in elderly patients to reduce the risk and severity of delirium episodes.

In the non-infectious delirium group, CRP, ESR, leukocyte count, platelet count, neutrophil count, and sodium levels were found to be significantly lower compared to the infectious group. This was attributed to the acute phase response commonly observed in infectious diseases, reactive thrombocytosis, and neutrophilia, especially associated with bacterial infections[21].

Sodium levels were found to be significantly lower in the non-infectious delirium group compared to the infectious group. This was thought to be due to the presence of diseases associated with hypervolemia in the non-infectious group. Conditions such as acute kidney injury, congestive heart failure, and liver failure frequently cause hypervolemic hyponatremia observed during hospital admissions[22].

Study Limitations

Among the limitations of this study is the sample size, which may affect the generalizability of the findings. The single-center design of our study was also considered an important limiting factor. Additionally, the exact hospital day on which delirium was diagnosed and the total length of hospital stay were not consistently recorded in our electronic health system during the study period. This limited our ability to analyze these parameters in detail. Furthermore, mixed-type (fluctuating) delirium was not systematically recorded during bedside evaluations, which restricted our ability to classify and analyze this specific subtype.

CONCLUSION

Delirium is a prevalent and serious clinical condition in the elderly, often presenting with acute confusion, attention deficits, and disorientation. It is frequently underdiagnosed, leading to increased morbidity and mortality. Early recognition and non-pharmacological management—such as environmental adjustments, orientation aids, and addressing underlying causes like infections or metabolic disturbances—are essential for improving outcomes. Pharmacologic treatments

should be used cautiously and only in exceptional cases due to potential cognitive side effects in older adults. Preventive strategies, including minimizing polypharmacy, ensuring mobility, and correcting sensory deficits, play a critical role in reducing the incidence and impact of delirium. In particular, early detection and timely management of infections in the elderly are essential steps to prevent delirium and improve patient outcomes.

Ethics

Ethics Committee Approval: The Ethics Committee of University of Health Sciences Türkiye, İstanbul Training and Research Hospital granted approval for this study (decision no: 55, date: 07.03.2025). All study procedures complied with the ethical standards of the 1964 Helsinki Declaration and its later amendments.

Informed Consent: Written informed consent was obtained from all participants prior to their inclusion in the study.

Footnotes

Authorship Contributions

Concept: V.C.Ç., Y.G., E.C.G., H.Z., F.A., Design: V.C.Ç., Y.G., G.Y., M.A., H.Z., Data Collection or Processing: V.C.Ç., Y.G., E.C.G., G.Y., M.A., H.Z., Analysis or Interpretation: V.C.Ç., G.Y., F.A., Literature Search: V.C.Ç., E.C.G., M.A., F.A., Writing: V.C.Ç.

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ORIGINAL ARTICLE

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Examination of the Corpus Callosum from Magnetic Resonance Images of Patients with Parkinson's Disease

Parkinson Hastalığı Olan Hastaların Manyetik Rezonans Görüntülerinden Korpus Kallozumun İncelenmesi

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ABSTRACT

Aim: In recent years, studies investigating white matter involvement, which is considered the cause of sensory symptoms that appear earlier than motor symptoms in Parkinson's disease, have increased. This study aims to investigate the involvement of the corpus callosum, the largest white matter structure connecting the two hemispheres, in Parkinson's disease, taking into account age and sex differences.

Materials and Methods: Our study were retrospectively compared the measurements of corpus callosum length, width, and angle on midsagittal magnetic resonance images from 120 controls without any diagnosis affecting the corpus callosum and from 120 patients diagnosed with Parkinson's disease.

Results: The height of the corpus callosum, the distance from the anterior tip to the top, and the distance from the anterointernal tip to the vertex increased, and the genu and rostrum width and the ratio of corpus callosum width to height decreased significantly in Parkinson's disease patients ($p<0.05$). Based on the angular measurements, it was determined that the mean value of Angle 2 decreased in patients with Parkinson's disease, while the values of Angle 4 and Angle 5 increased significantly ($p<0.05$).

Conclusion: It appears that there are few studies examining the involvement of the corpus callosum in Parkinson's disease, and to the best of our knowledge, no studies have evaluated the known angle parameters. Therefore, it is believed that research in this area may provide a novel perspective for clinicians and surgeons.

Keywords: Corpus callosum, magnetic resonance images, Parkinson's disease, white matter

ÖZ

Amaç: Son yıllarda, Parkinson hastalığında motor semptomlardan daha erken ortaya çıkan duyusal semptomların nedeni olarak kabul edilen beyaz cevher tutulumunu araştıran çalışmalar artış göstermiştir. Bu çalışma, iki beyin yarımküresini birbirine bağlayan en büyük beyaz cevher yapısı olan korpus kallozumun Parkinson hastalığındaki tutulumunu, yaş ve cinsiyet farklılıklarını da dikkate alarak incelemeyi amaçlamaktadır.

Gereç ve Yöntem: Çalışmamızda, korpus kallozumu etkileyen herhangi bir tanısı olmayan 120 kontrol grubuna ait ve Parkinson hastalığı tanısı konmuş 120 hastaya ait mid-sagittal manyetik rezonans görüntülerinde korpus kallozumun uzunluk, genişlik ve açı ölçümleri geriye dönük olarak karşılaştırıldı.

Bulgular: Parkinson hastalarında, korpus kallozumun yüksekliği, ön uçtan tepeye olan mesafe ve anterointernal uçtan tepeye olan mesafe arttı; genu ve rostrum genişliği ile korpus kallozum genişliğinin yüksekliğe oranı anlamlı düzeyde azaldı ($p<0,05$). Açısal değerlere bakıldığında, Parkinson hastalarında, Açı 2 ortalama değerinin azaldığı, Açı 4 ve Açı 5 değerlerinin ise anlamlı şekilde arttığı belirlendi ($p<0,05$).

Sonuç: Parkinson hastalığında korpus kallozum tutulumunu inceleyen az sayıda çalışma bulunmaktadır ve bildiğimiz kadarıyla açı değerlerin değerlendirildiği herhangi bir çalışma bulunmamaktadır. Bu nedenle, bu alanda yapılacak araştırmaların klinisyenler ve cerrahlar için yeni bir bakış açısı sağlayabileceği düşünülmektedir.

Anahtar Kelimeler: Korpus kallozum, manyetik rezonans görüntüleme, Parkinson hastalığı, beyaz cevher

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a decrease or complete loss of dopamine release[1]. While gray matter structures in the brain are emphasized in the pathogenesis of PD, recent studies have revealed their effects on white matter structures and increased the tendency to investigate this subject.

Many studies using various techniques have demonstrated that the corpus callosum, the largest white matter structure facilitating the transfer and integration of lateralized cognitive, motor, and sensory information between the cortices, is affected in PD[2-5]. Some studies indicate that these effects may manifest much earlier than changes in gray matter, underscoring their significance for early-stage diagnosis[6,7].

The aim of this study is to investigate the changes in width, length, and angle that occur in the corpus callosum, taking into account measurement parameters that have not been previously examined, through magnetic resonance images (MRI).

MATERIALS AND METHODS

Study Population

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University (decision no: 2021.74.03.14, date: 30.03.2021). In this study, images archived in the hospital's picture archiving and communication system were retrospectively reviewed. Ethical approval was obtained, and the ethics committee stated that informed consent was not required due to the retrospective nature of the study. The data obtained from the PD group and the control group were analyzed by stratified sampling method, grouped by age and gender. A total of 500 MRI images from patients with PD were available as the study population. Based on the calculations, considering the small effect size, a 95% confidence interval, a 5% margin of error, and the sample sizes in other studies, the sample size was determined to be 120 MRI images from the PD group, obtained between 2018 and 2021 from patients without any other diagnosis affecting the corpus callosum, and 120 MRI images from the control group.

MRI images, including T1 and T2 sequences, were obtained in the cranial sagittal plane using a 1.5 Tesla MRI machine (Canon Vantage Elan or Philips Intera; Philips Medical Systems) and an eight-channel head coil. The images had a slice thickness of 5 mm and were analyzed using the programs OrDICOM 1.0 and Weasis 3.7.1.

The demographic data and group distributions of the participants are presented in Table 1.

Corpus Callosum Measurement Parameters

The corpus callosum is divided into four parts: rostrum, genu, truncus, and splenium. Measurements of length, height, width, and angle were taken for both groups using MRI images from the midsagittal section. Length, height, and width measurements are given in mm, while angle measurements are in degrees. All measurement sites are shown in Figure 1.

Statistical Analysis

The data were analyzed using IBM SPSS 24.0 software (Armonk, New York, USA), with a significance value of $p<0.05$. To compare changes across different age groups, the data were divided into six groups: 30-40 years (Group I), 41-50 years (Group II), 51-60 years (Group III), 61-70 years (Group IV), 71-80 years (Group V), and 81 years and above (Group VI).

RESULTS

Corpus Callosum Metric Measurement Results

The mean values of all morphometric measurements for both groups are presented in Table 2.

The averages of corpus callosum length (CCL), corpus callosum height (CCH), cerebrum length (CL), distance between the corpus callosum front end to vertex distance (FV), and distance between the corpus callosum anterointernal tip to vertex distance (AIV) were found to be greater in men than in women ($p<0.05$).

Analysis of the group of individuals diagnosed with PD by sex revealed statistically significant differences in CCL ($p=0.007$), CCH ($p=0.008$), rostrum width (RW) ($p=0.012$), and CL ($p<0.001$). The averages of the other parameters did not differ significantly by sex. As a result of the data analysis based on age groups, it was observed that the average values of corpus callosum width (CCW), genu width (GW), splenium width (SW), RW, and trunk width maximum (TW_{max}) decreased as the age of patients with Parkinson's disease increased, while the average values of CCH, FV, and AIV increased.

Table 1. Demographic distributions and group data

	PD group	Control group	p-value
Female/male (%)	55/65 (45.8%/54.2%)	81/39 (67.5%/32.5%)	0.001
Age (mean ± SD)	68.82±10.0	57.91±12.4	0.000

PD: Parkinson's disease, SD: Standard deviation

in patients with PD, especially those aged 81 years and older, CCW, GW, SW, and TW_{max} showed a significantly greater decrease compared to controls ($p<0.05$). However, AIV was significantly higher in patients with PD, aged 81 years and older. In patients with PD, it has been observed that these significant changes begin to become noticeable between the ages of 51 and 60 and accelerate after the age of 81. The ratio of CCW to CCH was also significantly lower in the PD group ($p=0.001$).

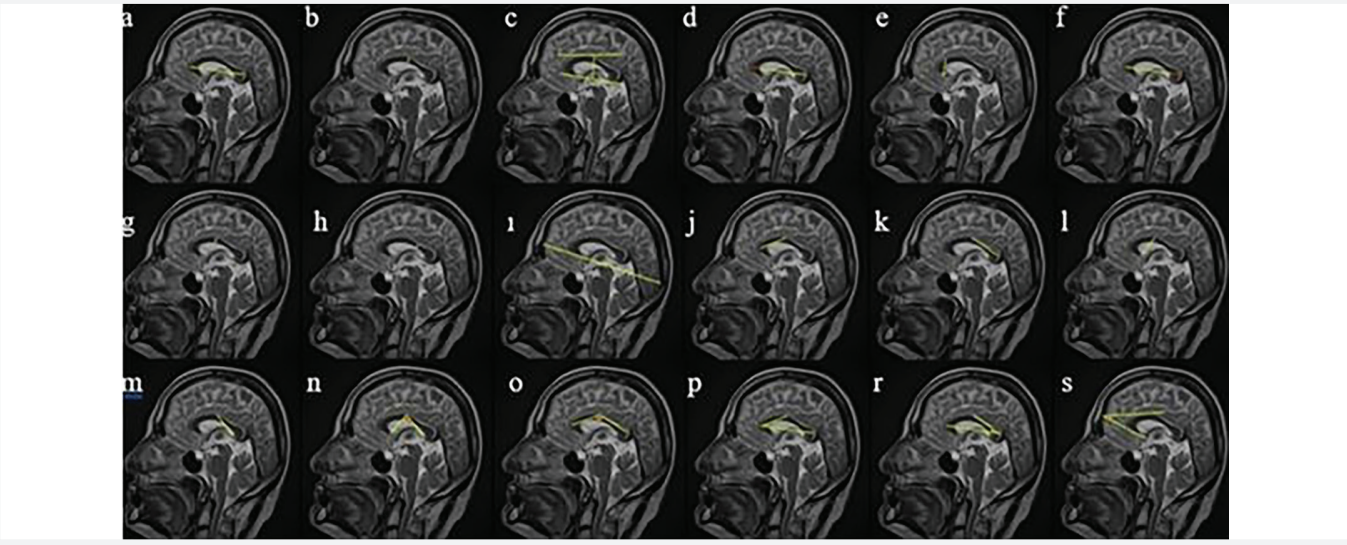

Figure 1. Corpus callosum measurements

a. Corpus callosum length (CCL), b. Corpus callosum width (CCW), c. Corpus callosum height (CCH), d. Genu width (GW), e. Rostrum width (RW), f. Splenium width (SW), g. Truncus maximum width (TW_{max}), h. Truncus minimum width (TW_{min}), i. Cerebrum length (CL), j. Front-end to vertex distance (FV), k. Posterior-end to vertex distance (PV), l. Anterointernal tip-to-vertex distance (AIV), m. Posterior inner tip-to-vertex distance (PIV), n. Angle between AIV and PIV (Angle 1), o. Angle between FV and PV (Angle 2), p. Angle between FV and CCU (Angle 3), r. Angle between AV and CCL (Angle 4), s. The angle between the line passing through the lower edge of the commissura anterior and genu and the tangent line passing over the anterior part of the corpus callosum (Angle 5)

Table 2. Comparison of average corpus callosum length across groups and by sex (mm)

	Control group			PD group			
	Female Mean ± SD	Male Mean ± SD	Total Mean ± SD	Female Mean ± SD	Male Mean ± SD	Total Mean ± SD	p*
CCL	68.3±4.2	69.8±4.5	68.8±4.3	67.8±4.2	70.1±4.7	69.1±4.6	0.654
CCW	4.6±0.9	4.3±0.7	4.5±0.9	4.3±0.9	4.6±1.0	4.5±1.0	0.534
CCH	24.0±2.9	24.9±2.9	24.3±2.9	25.3±3.3	27.1±3.7	26.3±3.6	0.000
GW	9.0±1.9	8.0±1.4	8.7±1.8	7.5±1.6	8.1±2.0	7.8±1.8	0.000
RW	4.9±1.1	4.7±1.4	4.8±1.2	3.8±1.2	4.4±1.0	4.1±1.1	0.000
SW	9.8±1.8	9.8±1.7	9.8±1.8	9.1±1.6	9.6±1.5	9.4±1.6	0.059
TW_{max}	5.1±1.0	4.7±0.7	4.9±1.0	4.7±0.9	5.0±0.9	4.9±0.9	0.825
TW_{min}	2.8±1.0	2.6±0.9	2.8±0.9	2.7±1.0	2.8±1.1	2.7±1.0	0.877
CL	153.8±7.3	160.3±7.2	155.9±7.9	152.2±6.4	159.4±6.7	156.1±7.4	0.886
FV	36.3±3.7	37.7±5.1	36.8±4.3	38.7±4.8	40.2±4.6	39.5±4.7	0.000
PV	39.8±4.4	40.0±4.6	40.6±5.1	38.2±3.8	39.6±4.4	39.0±4.2	0.060
AIV	29.3±3.9	31.0±4.5	29.9±4.1	32.5±4.2	33.3±4.2	32.9±4.2	0.000
PIV	34.2±3.6	34.7±4.4	34.4±3.9	33.1±3.6	34.6±4.5	33.9±4.1	0.429

*: Independent sample t-test $p<0.05$, SD: Standard deviation, CCL: Corpus callosum length, CCW: Corpus callosum width, CCH: Corpus callosum height, GW: Genu width, RW: Rostrum width, SW: Splenium width, TW_{max}: Trunk width maximum, TW_{min}: Trunk width minimum, CL: Cerebrum length, FV: Front end to vertex distance, PV: Posterior end to vertex distance, AIV: Anterointernal tip to vertex distance, PIV: Posterointernal tip to vertex distance

Corpus Callosum Angle Measurement Results

All the measurement results are presented in Table 3.

After comparing the average angles across different age groups, significant differences were found in Angle 1, Angle 2, and Angle 4 between the PD group and the control group, particularly in the 30-40 age group. Further analysis within the PD group showed statistically significant differences in Angle 1, angle 2, and Angle 5 values across different age groups ($p<0.05$). Specifically, Angle 2 decreases with age in patients with PD, while Angle 5 increases with age.

According to the analysis of the data set, there were weak negative correlations between age and SW (r=-0.263) and between age and TW_{min} (r=-0.216), and moderate negative correlations between age and CCW (r=-0.352), GW (r=0.568), RW (r=-0.347), and TW_{max} (r=-0.394). Additionally, weak positive relationships were detected between age and CCH (r=0.289), FV (r=0.205), Angle 4 (r=0.280), and Angle 5 (r=0.266). In the PD group, there was a weak negative correlation between age and Angle 2 (r=-0.197), but a weak positive correlation between age and Angle 4 (r=0.207) and Angle 5 (r=0.250). It is important to note that these correlations were not observed in the control group.

In the PD group, certain length measurements showed different correlation results compared to those in the control group. Specifically, there was a weak negative correlation between GW and CCH (r=-0.269, p=0.003). Moreover, moderate positive correlations were observed between CCH and RW (r=0.416, $p<0.001$) as well as between CCH and CL (r=0.310, p=0.001). Additionally, a weak positive relationship was found between CL and TW_{max} (r=0.187, p=0.041).

DISCUSSION

In PD, Lewy bodies affect gray matter structures and damage white matter connections, leading to non-motor symptoms such as cognitive impairment and depression[8]. Investigating changes in white matter fiber tracts may provide a better understanding of the underlying mechanism of PD. Our study is one of the most comprehensive studies to measure a wide range of parameters of the corpus callosum in MRI images of patients diagnosed with PD, including parameters that have not been previously evaluated.

Our research shows that women have smaller CCL and CL values than men, consistent with the findings of Mohammadi et al.[9] ($p<0.005$). When comparing patients with PD with healthy individuals, we found that genu and SW decreased in the PD group, while CCL and CL increased; however, these changes were not statistically significant except for GW. Mohammadi et al.[9] also found a positive relationship between CCL, CL, and CCW. Our findings support this result. We found a moderate positive correlation between CCL and CL in both the control group and the PD group.

Studies comparing patients with PD with healthy control groups have found that the corpus callosum thickness, particularly in the anterior half, is reduced in Parkinson's patients[10,11]. Significant decreases in volume in the anterior 2/5 of the corpus callosum and fractional anisotropy have also been observed in Parkinson's patients[2]. This is associated with impaired information processing in the prefrontal cortex, motor, and supplementary motor areas[12]. Gattellaro et al.[13], in their functional MRI study, suggested that the microstructure of the corpus callosum genu in patients with PD was impaired even at an early stage, while Guimarães et al.[3] suggested that changes in the corpus callosum, which they examined with diffusion tensor imaging, began to be observed at later stages. Furthermore, a clear connection between vascular parkinsonism and issues in the genu of the corpus callosum has been identified[14]. Reduced fiber density in the genu and body is related to gait asymmetry[2,15]. Our findings indicate that genu and RW are reduced in the PD group ($p<0.001$) and that the genu section is more affected in older patients with PD. The genu section of the corpus callosum is associated with cognitive functions, attention, and executive disorders; therefore, genu involvement may affect symptoms such as dementia, distractibility, and impaired control of planned movements[10,16].

Table 3. Comparing the angle values among different groups (°)

	Control group			PD group			
	Female Mean ± SD	Male Mean ± SD	Total Mean ± SD	Female Mean ± SD	Male Mean ± SD	Total Mean ± SD	p*
Angle 1	86.3±11.0	81.3±7.8	86.6±10.1	86.7±7.8	83.9±8.4	85.2±8.2	0.240
Angle 2	126.3±12.9	126.1±7.0	126.2±11.3	124.2±6.9	123.0±7.2	123.5±7.1	0.027
Angle 3	26.6±3.8	27.2±4.7	26.8±4.1	26.6±3.9	27.0±4.0	26.8±3.9	0.957
Angle 4	24.4±4.3	25.3±4.4	24.7±4.4	27.2±4.5	27.7±4.3	27.5±4.4	0.000
Angle 5	26.6±3.2	25.7±3.9	26.3±3.5	28.2±3.1	27.7±3.6	27.9±3.3	0.000

*: Independent sample t-test $p<0.05$, SD: Standard deviation, PD: Parkinson's disease

In the study by Bledsoe et al.[17], the corpus callosum in patients with PD was divided into five regions and examined using the diffusion tensor imaging method. The study showed an increase in axial diffusivity and a decrease in fractional anisotropy in the anterior 3/5 of the corpus callosum. These findings indicate damage to myelinated fibers and white matter atrophy. Our study results are also consistent with these findings. This supports the observation of a reduction in the number of fibers and microstructural atrophy in the affected regions identified through metric measurements.

In a study in which the distance from the vertex of the corpus callosum to the anterior commissure was evaluated as the CCH, it was reported that the CCH increased with age[18]. As in our study, in another study where the CCH was determined by measuring the distance between the tangent lines to the highest and lowest points of the corpus callosum, it was found that the height increased with age[19]. Our study revealed that height increased with age in both the control group and the group diagnosed with PD, with the group diagnosed with PD having a significantly greater mean CCH ($p<0.001$). This increase in height also resulted in a significant difference between the two groups in the average values of the FV and AIV parameters ($p<0.05$). Additionally, in patients with PD as CCH increased, RW and CL also increased, while GW decreased.

In some studies, the values measured as Angle 1 and Angle 2 have been expressed as the corpus callosum bending angle. In studies involving patients with schizophrenia spectrum disorders and niemann-pick type C, no significant differences were observed in Angle 1 with respect to age, sex, or between groups[20,21]. Similarly, in a study comparing patients with Williams syndrome to healthy individuals, no significant difference was found in terms of Angle 2[22]. In our study, while there was no difference in Angle 1 between sexes or groups ($p>0.05$), the value of Angle 2 was significantly lower in the PD group ($p=0.027$). Both angles were observed to be higher in the 41-50 age group of patients with PD and decreased with advancing age. This study found that the decrease in Angle 2 was more significant in the Parkinson's group compared to the control group, especially after the age of 41-50. This finding may indicate a potential increase in the bending of the corpus callosum due to the progression of PD.

Angle 5 indicates the position of the anterior part of the corpus callosum relative to the floor of the 4th ventricle[23]. In one study, frontal dysplasia was found to increase Angle 5, and no difference was observed between autistic and non-autistic individuals with macrocephaly[24]. In our study, the increase in the average value of Angle 5 in the PD group was found to be statistically significant ($p<0.001$), which may indicate an increased tendency for frontal localization of the corpus callosum in PD patients.

Study Limitations

This study has some limitations. First, the retrospective design limits the ability to control for potential confounding variables. Second, the evaluation was based solely on midsagittal MRI images, which may not fully reflect all microstructural changes in the corpus callosum. We believe that including clinical data such as disease duration, medication use, or cognitive status in future studies would allow for a more comprehensive analysis, and this study serves as a guiding basis for such research.

CONCLUSION

In conclusion, PD is a progressive neurodegenerative condition that affects daily activities and reduces quality of life, making early diagnosis crucial for successful treatment. Therefore, there is a necessity to conduct research on white matter, as developing new perspectives and focusing on new methods in this area is essential. The consistency of results across studies using different methodologies enhances the reliability of these findings. We believe our study is significant for supporting findings from other techniques, being one of the most comprehensive studies focusing specifically on the corpus callosum, and adding new measurement parameters to the literature.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University (decision no: 2021.74.03.14, date: 30.03.2021).

Informed Consent: The study is a retrospective study.

Footnotes

Authorship Contributions

Concept: D.T., A.Z., Design: D.T., A.Z., Data Collection or Processing: D.T., A.Z., S.A., Analysis or Interpretation: D.T., A.Z., S.A., Literature Search: D.T., A.Z., S.A., Writing: D.T., A.Z.

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Retrospective Experience of Hyperferritinemia in a Single-center

Tek Merkezde Retrospektif Hiperferritinemi Deneyimi

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ABSTRACT

Aim: Malignancy, infection, inflammation, liver and renal diseases, hematological disorders, iron overload, metabolic syndrome, and alcohol consumption can cause hyperferritinemia. This study aimed to identify the underlying causes of hyperferritinemia in patients at a tertiary care medical center.

Materials and Methods: We retrospectively evaluated the patients with serum ferritin (SF) levels higher than 1000 µg/L between 2014 and 2016. Among these patients (n=94), 89 patients with hematological disorders (n=69) or oncological diseases (n=20) were included in the study. For patients with multiple SF measurements, the highest level was considered. The association between SF levels and patients' demographics, clinical characteristics, laboratory parameters, and the total number of red blood cell transfusions received were evaluated using the median SF value as a comparison point.

Results: The patients' median (min-max) age was 61 (20-94) years, and 49 (55.1%) patients were female. The median (min-max) SF level of the patients' was 1739 µg/L. Serum aspartate aminotransferase and gamma-glutamyl transferase levels were higher in patients with SF levels above the median value than those with SF below the median (p=0.001, p=0.003, respectively). No significant difference was found in erythrocyte sedimentation rate and C-reactive protein levels between patients with SF levels above the median and those below the median (p=0.689, 0.230, respectively). Patients with SF levels above the median received a higher total number of red blood cell transfusions compared to those with levels below the median (p<0.001).

Conclusion: In this study, hematological disorders were the predominant underlying cause of hyperferritinemia potentially due to chronic red blood cell transfusions and inflammation.

Keywords: Hematological disorder, hyperferritinemia, inflammation, iron overload

ÖZ

Amaç: Malignite, enfeksiyon, enflamasyon, karaciğer ve böbrek hastalıkları, hematolojik hastalıklar, aşırı demir yüklenmesi, metabolik sendrom ve alkol tüketimi hiperferritinemiye neden olabilir. Bu çalışmanın amacı, üçüncü basamak bir tıp merkezindeki hastalarda hiperferritineminin altında yatan nedenleri belirlemektir.

Gereç ve Yöntem: 2014-2016 yılları arasında serum ferritin (SF) düzeyi 1000 µg/L'den yüksek olan hastalar retrospektif olarak değerlendirildi. Bu hastalar arasından (n=94), hematolojik (n=69) veya onkolojik hastalık (n=20) tanıları olan 89 hasta çalışmaya dahil edildi. Birden fazla SF ölçümü olan hastalarda en yüksek düzey dikkate alındı. SF düzeyleri ile hastaların demografik özellikleri, klinik özellikleri, laboratuvar parametreleri ve aldıkları toplam eritrosit transfüzyonu sayıları arasındaki ilişki, karşılaştırma noktası olarak medyan SF değeri kullanılarak değerlendirildi.

Bulgular: Hastaların medyan (min-maks) yaşı 61 (20-94) yıldı ve 49 (%55,1) kadındı. Hastaların medyan SF düzeyi 1739 µg/L idi. Serum aspartat aminotransferaz ve gama-glutamil transferaz düzeyleri, SF düzeyi medyan değerin üzerinde olan hastalarda, SF düzeyi medyan değerin altında olanlara kıyasla daha yüksekti (sırasıyla p=0,001, p=0,003). SF düzeyleri medyanın üzerinde olan hastalar ile medyanın altında olan hastalar arasında eritrosit sedimantasyon hızı ve C-reaktif protein değerleri açısından anlamlı bir fark bulunmadı (sırasıyla p=0,689, 0,230). SF düzeyleri medyan değerin üzerinde olan hastalar, medyan değerin altında olanlara kıyasla daha fazla sayıda eritrosit transfüzyonu almıştır (p<0.001).

Sonuç: Bu çalışmada hematolojik bozukluklar, muhtemelen kronik eritrosit transfüzyonlarına ve enflamasyona bağlı olarak, hiperferritineminin temel nedeniydi.

Anahtar Kelimeler: Hematolojik bozukluklar, hiperferritinemi, enflamasyon, aşırı demir yükü

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INTRODUCTION

Ferritin, mainly a cytosolic protein, regulates iron homeostasis by storing and buffering intracellular iron to prevent free iron toxicity and releasing iron as needed for essential processes. Serum ferritin (SF) measurement is the primary non-invasive method for assessing body iron storage, though levels generally represent iron-poor extracellular ferritin in clinical practice[1]. Hyperferritinemia is generally defined as SF levels above 200 µg/L in adult females and 300 µg/L in adult males[2]. Hyperferritinemia can result from various conditions, including malignancy, infection, inflammation, liver disease, hematological disorders, renal disease, metabolic syndrome, chronic alcohol intake, and iron overload[2-8]. The pattern of SF elevation varies among these conditions; however, the degree of this elevation is infrequently quantified, except when applied as a diagnostic criterion for hemophagocytic lymphohistiocytosis or as an indication for iron chelation therapy in patients with iron overload[9,10]. It was demonstrated that individuals with moderately elevated SF levels ≥200 µg/L have an increased risk of total, cancer-related, endocrinological, and cardiovascular mortality compared to those with SF levels <200 µg/L[11]. SF levels exceeding 1000 µg/L warrant a thorough evaluation, regardless of transferrin saturation, as they reliably indicate an underlying pathology[1]. Considering the clinical importance of such elevated SF levels, we aimed to investigate the underlying causes in patients managed at a tertiary care medical center.

MATERIALS AND METHODS

Selection and Description of the Cases

We conducted a retrospective, descriptive study at a single center. We retrieved the medical records of patients who were 18 years and older with SF levels higher than 1000 µg/L at Dokuz Eylül University Hospital, between January 2014 and February 2016. Among these patients (n=94), 5 patients (chronic kidney disease, n=2; hereditary hemochromatosis, n=1; still's disease, n=1; and pectus excavatum, n=1) were excluded from the study to simplify the grouping of the patient population. The remaining 89 patients all had hematological (n=69) disorders or oncological (n=20) disease diagnoses and were included in the study.

Our study was based on the 2013 amendment of the Helsinki Declaration, and ethical approval was obtained from the Dokuz Eylül University Institutional Review Board (protocol number: 2584-GOA, decision no: 271, date: 24.03.2016).

Study Design

Demographic and clinical data of the patients were obtained from medical and/or electronic hospital records. The highest value was considered for patients with multiple ferritin measurements exceeding 1000 µg/L. The patients' ages, genders, laboratory parameters (transferrin saturation, alanine aminotransferase (normal range: 0-35 U/L), aspartate aminotransferase (AST) (normal range: 0-35 U/L), gamma-glutamyl transferase (GGT) (normal range: 0-38 U/L), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) (normal range: 0-5 mg/L) at the time of the highest ferritin levels and total number of erythrocyte transfusions they received in 25 months at Dokuz Eylül University Hospital were noted. Underlying disorders were classified as either hematological disorders or oncological diseases. Hematological disorders encompass benign and malignant conditions affecting the blood, blood cells, and organs involved in hematopoiesis. Oncological diseases, on the other hand, refer to malignant conditions characterized by the uncontrolled proliferation of cells, resulting in the formation of solid tumors. The association between SF levels and patients' demographics, clinical characteristics, laboratory parameters, and the total number of erythrocyte transfusions received was evaluated using the median SF value as a comparison point. Additionally, patients who received iron chelation therapy were documented, and ferritin levels of these patients were evaluated in the 3rd-6th and 10th months of iron chelation therapy.

Technical Information

SF levels were measured by a chemiluminescent method in the Beckman Coulter DxI-800 autoanalyzer and measurements were recorded as µg/L.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 15.0. Descriptive findings were presented as percentage distributions for categorical variables and mean ± standard deviation for continuous variables. The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. As the distributions were not normal, the non-parametric Mann-Whitney U test was used to compare the two independent groups. The chi-square test was used to analyze categorical variables when comparing groups based on the median ferritin value.

RESULTS

Demographic and General Clinical Features

The patients' median (min-max) age was 61 (20-94) years, and 49 (55.1%) patients were female. Sixty-nine (77.5%) patients had a hematological disorder diagnosis, and 20 (22.5%) patients had an oncological disease diagnosis. The association between SF level and gender and clinical diagnoses is shown in Table 1.

Laboratory Parameters of the Patients

The median (min-max) SF level of the patients was 1739 µg/L (1005-10,475). The patients' (n=66) mean transferrin saturation was 51.2±26.6%. When patients were divided into two groups according to median SF level (SF level <1739 µg/L and SF level ≥1739 µg/L), a statistically significant difference was found between these two groups in terms of AST and GGT levels (p=0.001, p=0.003 respectively) (Table 2). However, no significant difference was found in ESR and CRP values between patients with SF levels above the median value and patients with SF levels below the median value (p=0.689, p=0.230 respectively) (Table 2).

Red Blood Cell Transfusions

The mean total number of red blood cell transfusions received by the patients was 21.8±26.5. The total number of red blood cell transfusions received was higher in patients with SF levels above the median value compared to those with SF levels below the median (p<0.001) (Table 3).

Iron Chelation Therapy

Twenty (22.5%) of the patients received iron chelation therapy. Among the 20 patients, 18 had hematological disorders,

while 2 were diagnosed with oncological diseases. During the first assessment, 3 to 6 months after initiation of iron chelation therapy, two patients were lost to follow-up. Among the remaining 18 patients, 15 (83.3%) exhibited a ≥10% reduction in SF levels compared with baseline. During the second assessment at the 10th month of iron chelation therapy, six patients were lost to follow-up. Among the remaining 14 pateints, 10 (71.4%) showed a≥10% reduction in SF levels compared with baseline.

DISCUSSION

In this study, hematological disorders (77.5%) were identified as the most common underlying cause of hyperferritinemia, followed by oncological diseases (22.5%). Hyperferritinemia observed in these patients may be attributed to iron overload resulting from chronic red blood cell transfusions and chronic inflammation associated with malignancy[2]. On the other hand, red blood cell disorders characterized by ineffective

Table 1. Comparison of demographic and clinical features of the patients according to median serum ferritin levels

Patients (n=89)	Ferritin level <1739.0 µg/L		Ferritin level ≥1739.0 µg/L		p-value*
	n	%	n	%	
Gender					
Male	19	47.5	21	52.5	0.741
Female	25	51.0	24	49.0	
Diagnoses					
Hematological disorder	33	47.8	36	52.2	0.572
Oncological disease	11	55.0	9	45.0	

*: Chi-square test

Table 2. Comparison of laboratory parameters of the patients according to median serum ferritin levels

		Ferritin level<1739.0 µg/L	Ferritin level≥1739.0 µg/L	
Laboratory parameters	**n**	**Median (IQR)**	**Mean ± SD**	**p-value***
AST (U/L)	78	21 (18)	34 (30)	**0.001**
ALT (U/L)	85	18 (33)	24 (63)	0.07
GGT (U/L)	78	38 (53)	64 (132)	**0.003**
ESR (mm/h)	78	65.8±8.2	57.1±9.7	0.689
CRP (mg/L)	85	25.8±6.5	75.6±20.5	0.230

*: Mann-Whitney U test, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, SD: Standard deviation, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, IQR: Interquartile range

Table 3. Comparison of red blood cell transfusions received by patients based on median serum ferritin levels

	Ferritin level <1739.0 µg/L		Ferritin level ≥1739.0 µg/L		
Total number	**n**	**Mean ± SD**	**n**	**Mean ± SD**	**p-value***
Red blood cell transfusions	44	14.7±20.0	45	28.7±30.3	<0.001

*: Mann-Whitney U test, SD: Standard deviation

erythropoiesis or hemolysis can result in increased iron absorption and elevated SF levels even in the absence of red blood cell transfusions[12], representing an additional mechanism in patients with hematological disorders.

In another study of 95 patients with SF levels above 1000 µg/L, the following conditions were identified: 20.0% had liver disease; 17.9% had renal disease; 17.9% had malignant conditions; 16.8% were infected with human immunodeficiency virus (HIV); 15.8% had non-HIV-related systemic infections; 10.5% required chronic blood transfusions; and 10.5% had sickle cell syndromes[5]. Variations in reported prevalence may be attributed to differences in demographic characteristics, geographic distribution, genetic predisposition, environmental exposures, healthcare infrastructure, and study design.

Inflammation stimulates ferritin synthesis through the action of pro-inflammatory cytokines and promotes its release via apoptosis and cellular damage[13]. Although mean CRP and ESR levels were elevated in this study, no significant association was found between acute-phase reactants and patients stratified by SF levels above or below the median. This lack of association may be attributed to several factors, including the presence of outliers, the timing of biomarker measurement, variability in individual inflammatory responses, and the multifactorial role of ferritin in inflammation. Interestingly, the mean ESR value was higher among patients with SF levels below the median, suggesting a greater inflammatory burden in this subgroup. However, it is essential to acknowledge that ESR is a non-specific marker and may be influenced by non-inflammatory factors, such as age, sex, and changes in plasma protein composition.

McKinnon et al.[14] showed a correlation between GGT and SF levels in Australian adult males and females ($p<0.0001$). This correlation was consistently evident across all age groups and was unaffected by body mass index adjustment. In the current study, patients with SF levels above the median level demonstrated significantly higher serum GGT levels than those with SF levels below the median ($p=0.003$). Our findings align with previous research, suggesting a link between metabolic dysfunction and an iron overload syndrome characterized by hyperferritinemia[15]. Non-alcoholic steatohepatitis may have contributed to these elevated GGT levels[2].

Chronic red blood cell transfusions can result in toxic iron accumulation in organs, leading to tissue damage and dysfunction. Excess iron presents ongoing toxicity risks; however, damage is often reversible with the prompt elimination of iron[16]. Iron chelators have been shown to effectively reduce tissue iron levels, mitigate complications associated with iron overload, and enhance event-free survival outcomes[17]. In the current study, only 22.5% of the patients received iron chelation therapy. This low percentage is likely due to varying opinions on the optimal timing for initiating treatment, challenges with patient adherence, and issues related to cost and accessibility[17]. In developed countries, the monitoring of iron load and the assessment of chelation therapy progress in regularly transfused patients are typically conducted through SF measurements every three months[16].

Study Limitations

Our study was retrospective, which constrained the availability of detailed information, including specific subgroups of the hematological disorders. The number of patients was small. Our patient population was derived from a tertiary care medical center; it may not represent the full spectrum of diseases or conditions commonly encountered in general practice. The number of red blood cell transfusions received by patients outside our medical center was not recorded, which may result in an underestimate of the iron burden. The study's strengths include the high ferritin cut-off value (>1000 µg/L), which allowed for the exclusion of numerous factors that could contribute to elevated SF levels.

CONCLUSION

This study examined the underlying causes of hyperferritinemia in patients at a tertiary care medical center. Our results suggest that hematological disorders were the primary underlying cause of hyperferritinemia, potentially as a result of ineffective erythropoiesis, hemolysis, chronic inflammation, and chronic red blood cell transfusions.

Ethics

Ethics Committee Approval: Our study was based on the 2013 amendment of the Helsinki Declaration, and ethical approval was obtained from the Dokuz Eylül University Institutional Review Board (protocol number: 2584-GOA, decision no: 271, date: 24.03.2016).

Informed Consent: A retrospective, descriptive study was conducted at a single center.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ş.A.G.D., A.O., M.A.Ö., Concept: Ş.A.G.D., A.O., M.A.Ö., Design: Ş.A.G.D., A.O., M.A.Ö., Data Collection or Processing: Ş.A.G.D., Analysis or Interpretation: Ş.A.G.D., A.O., M.A.Ö., Literature Search: Ş.A.G.D., A.O., M.A.Ö., Writing: Ş.A.G.D.

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Disability and Related Factors in Patients with Psoriatic Arthritis; A Single Center Study

Psoriatik Artritli Hastalarda Sakatlık ve İlgili Faktörler; Tek Merkezli Bir Çalışma

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ABSTRACT

Aim: Psoriatic arthritis (PsA) is a complex chronic inflammatory condition that may limit daily activities, with detrimental effects on patients' physical function. For patients with PsA, physical ability may be influenced by various factors. The aim of the study is to gain a more comprehensive understanding of the effects of PsA on disability and the factors that contribute to impaired physical function.

Materials and Methods: The study was a cross-sectional survey conducted with patients diagnosed with PsA according to the classification criteria for PsA at a single tertiary center. Demographic, social, and disease-related characteristics were collected. Both univariate and multivariable analyses were used to evaluate characteristics that might be associated with disability.

Results: A total of 214 patients with PsA (67.3% of whom were female; mean age ± standard deviation, 52.2±12 years) were included in the study. The median (interquartile range) health assessment questionnaire-disability index (HAQ-DI) for the patient group was HAQ median (interquartile range 25-75) 0.32 (0.00-1.10), and 31.8% of patients had moderate-to-high disability. HAQ-DI scores were correlated with disease activity, function, and quality of life measurements. Patients with disabilities were predominantly women, of advanced age, and had higher body mass index and lower education levels. In addition, enthesitis, axial involvement, tender joints, nail involvement, and serum C-reactive protein level were found to be associated with disability in univariate analysis. In regression analysis, tender joint count [odds ratio (OR): 1.07, 95% confidence interval (CI): 0.02-1.12], nail involvement (OR: 2.09, 95% CI: 1.05-4.13; p=0.035), and enthesitis (OR: 2.25, 95% CI: 1.13-4.48; p=0.021) were the main determinants of disability in patients with PsA.

Conclusion: Approximately one-third (31.8 %) of patients with PsA had disability according to HAQ-DI. PsA was intimately associated with disease involvements irrespevtive of duration of psoriazsis.

Keywords: Psoriatic arthritis, function, disability

ÖZ

Amaç: Psoriatik artrit (PsA), hastaların günlük aktivitelerini kısıtlayabilen ve fiziksel işlevleri üzerinde olumsuz etkilere yol açabilen karmaşık bir kronik enflamatuvar durumdur. PsA hastalarının fiziksel yetenekleri çeşitli faktörlerden etkilenebilir. Çalışmanın amacı, PsA'nın engellilik üzerindeki etkileri ve fiziksel işlev bozukluğuna katkıda bulunan faktörler hakkında daha kapsamlı bir anlayış kazanmaktır.

Gereç ve Yöntem: Çalışma, kesitsel bir araştırma olup, tek bir üçüncü basamak merkezde PsA sınıflandırma kriterleri temelinde PsA tanısı almış hastalarla yürütülmüştür. Demografik, sosyal ve hastalıkla ilgili özellikler toplanmıştır. Engellilikle ilişkili olabilecek özellikleri değerlendirmek için hem tek değişkenli hem de çok değişkenli analiz kullanılmıştır.

Bulgular: Çalışmaya 214 PsA hastası (%67,3'ü kadın, ortalama yaş ± standart sapma 52,2±12 yıl) dahil edildi. Hasta grubunun ortanca (çeyrekler arası aralık) sağlık değerlendirme anketi-engellilik indeksi (HAQ-DI) değeri HAQ medyan (çeyrekler arası aralık 25-75): 0,32 (0,00-1,10) idi ve

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hastaların %31,8'inde orta-yüksek düzeyde engellilik vardı. HAQ-DI skorları hastalık aktivitesi, fonksiyon ve yaşam kalitesi ölçümleriyle ilişkiliydi. Engellilik yaşayan hastalar ağırlıklı olarak kadındı ve ileri yaştaydı, ayrıca daha yüksek vücut kitle indeksi ve daha düşük eğitim seviyesine sahiptiler. Ayrıca entezit, aksiyel tutulum, hassas eklem, tırnak tutulumu ve serum C-reaktif protein düzeyinin engellilikle ilişkili olduğu bulundu. Regresyon analizinde hassas eklem [olasılık oranı (OO): 1,07, %95 güven aralığı (GA): 0,02-1,12)], tırnak tutulumu (OO: 2,09, %95 GA: 1,05-4,13; p=0,035), entezit (OO: 2,25, %95 GA: 1,13-4,48; p=0,021) ve PsA'lı hastalarda sakatlığın başlıca belirleyicileriydi.

Sonuç: HAQ-DI'ya göre PsA'lı hastaların üçte biri engelliydi. PsA sedef hastalığı dsüresinden bağımsız olarak yaşam kalitesi, fonksiyon ve aktivitelerle yakından ilişkiliydi.

Anahtar Kelimeler: Psöriatik artrit, fonksiyon, sakatlık

INTRODUCTION

Psoriatic arthritis (PsA) is a complex chronic inflammatory disease characterized by several symptoms, including axial involvement, peripheral arthritis, enthesitis, dactylitis, and skin psoriasis[1]. PsA typically manifests between the ages of 30 and 55, affecting approximately 0.3-1% of the general population and 5-30% of psoriasis patients[2]. Its influence on quality of life is extensive, presenting with symptoms of pain, exhaustion, sadness, anxiety, diminished physical function, reduced social involvement, disability, and loss of employment[3,4]. Assessment of the outcomes is important in both clinical practice and in a trial setting to enable evaluation of disease activity and treatment effects. On the other hand, physical function and disability are fundamental metrics of patient-reported disease impact, as shown in randomized controlled trials, longitudinal observational studies, and clinical practice[5]. A well-validated patient self-report questionnaire for the evaluation of physical function in rheumatic diseases is the health assessment questionnaire-disability index (HAQ-DI)[6]. Nevertheless, the process is time-consuming, and the scoring can be intricate. The scoring method may result in the comparison of various activities from visit to visit, and scores can also be artifactually elevated when aids are used, despite the improvement in patient function[7]. Therefore, it is crucial to illustrate the relationship between the results of the HAQ-DI questionnaire and patient and disease-related features. In the current study, our objective was to investigate the prevalence of disability in patients with PsA, the correlation between disability and various patient-reported outcomes, and the relationship between disability, demographic variables, and other disease-related features.

MATERIALS AND METHODS

Patients and Data Collection

All patients with PsA were aged 18 years and older, in accordance with the classification criteria for PsA (CASPAR). Patients from the PsA cohort of İzmir Katip Çelebi University, Rheumatology Clinic, were included in the study from 15 August 2023 to 16 October 2024[8]. Demographic features, smoking history, educational level, and disease-related characteristics were collected. The subsequent components of the disease were assessed: disease activity with the disease activity index for psoriatic arthritis (DAPSA)[9], PsA disease activity score (PASDAS)[10], the tender joint count (TJC), swollen joint count, and leeds enthesitis index[11]; functional status with the bath ankylosing spondylitis functional index (BASFI)[12]; disease-related quality of life with the short-form 36 (SF-36) questionnaire[13] and dermatology life quality index (DLQI)[14]; and psoriasis severity with body surface area. HAQ-DI was used to evaluate disability[15]. The HAQ-DI contains 20 questions that are divided into eight categories: dressing and grooming, hygiene, arising, reach, eating, grip, walking, and outside activities. Each item has four response possibilities, ranging from "no difficulty" to "unable to do", corresponding to scores from 0 to 3. Better function is indicated by lower HAQ-DI scores. In our investigation, we defined moderate-to-high disability as a score of 1 or greater. This study was approved by the Ethics Committee of İzmir Katip Çelebi University (decision no: 0411, date:11.09.2023). The research was performed in compliance with the principles of the Declaration of Helsinki.

Statistical Analysis

Both analytical (Kolmogorov-Smirnov or Shapiro-Wilk) and visual (histograms, probability plots) techniques were used to examine the distribution of continuous variables. Values were displayed as percentages for categorical variables and as the mean and standard deviation (SD) or median and interquartile range for continuous variables. Normally distributed variables were compared between the groups using the Student's t-test, while non-normally distributed variables were compared using the Mann-Whitney U test. To compare categorical data, the chi-square test and Fisher's exact test were used. The factors linked to disability were evaluated using binary logistic regression analysis. Demographic and/or disease-related variables were initially selected based on a univariable analysis with a significance level of $p<0.05$, as well as clinical relevance supported by the existing literature. These variables were then entered into a multivariable model using the backward elimination method to identify the final set of covariates. We have clearly described the selection criteria and provided a complete list of variables included in the final model. The correlations between HAQ-DI with BASFI scores, DAPSA, PASDAS, DLQI, SF-36 physical component summary

score (PCS), and SF-36 mental component summary score (MCS) were analyzed using correlation analysis. Due to the majority of variables failing to adhere to a normal distribution, the Spearman's rank correlation analysis was conducted. All statistical tests were two-tailed, and p-values of less than 0.05 were considered statistically significant. Version 18.0 of the Statistical Package for the Social Sciences (SPSS) software package (IBM®, Armonk, NY, USA) was used for all statistical analyses.

RESULTS

Physical and Disease-related Characteristics

A total of 214 patients with PsA were included. The mean age (SD) was 52.2 years, and 144 (67.3%) patients were female. Of the patients, 37.3% were never smokers, and the mean body mass index (BMI) (SD) of the patients was 28.6 (5.2). The mean psoriasis disease duration (SD) was 16.1 (12.3) years, and PsA disease duration (SD) was 7.4 (6.9) years. Axial involvement was observed in 52% of patients, while polyarticular, oligoarticular, and monoarticular phenotypes were 48%, 20%, and 10%, respectively. Enthesitis was detected in 39% of the patients, while 41% and 26% of the patients exhibited nail involvement and dactylitis, respectively. The mean (SD) HAQ-DI was 0.32 (1.1).

Disability and Related Factors

Sixty-eight (31.8%) of patients [the mean age (SD) was 54.4 (11.7) years, and 77.9% of patients were female] had moderate-to-high disability. The baseline demographic and disease-related characteristics of the disabled and non-disabled patients were summarized in Table 1. Of the patients, 30% were never smokers, and the mean BMI (SD) of the patients was 29.7 (5.5). The mean psoriasis disease duration (SD) was 16.5 (13.1) years, and PsA disease duration (SD) was 7.6 (7.0) years. Axial involvement was observed in 84% of patients, while polyarticular, oligoarticular, and monoarticular phenotypes

Table 1. Demographic and clinical characteristics of patients with and without disability

	HAQ <1 (n=146)	HAQ >1 (n=68)	p
Age, mean (SD)	50.9 (11.9)	54.4 (11.7)	**0.020**
Gender, female, n (%)	91 (62.3)	53 (77.9)	**0.023**
PsA disease duration, mean (SD)	7.2 (6.9)	7.6 (7.0)	>0.05
Pso disease duration, mean (SD)	15.8 (11.8)	16.5 (13.1)	>0.05
Smoking history, yes, n (%)	86 (59.3)	47 (70.1)	>0.05
Education duration, mean (SD)	9.5 (4.1)	7.5 (3.8)	**0.001**
BMI >30, yes, n (%)	102 (69.9)	58 (85.3)	**0.016**
Peripheral arthritis, n (%)	114 (78.1)	57 (83.8)	>0.05
Enthesitis, n (%)	51 (34.9)	46 (67.6)	**<0.001**
Dactylitis, n (%)	40 (27.4)	20 (29.4)	>0.05
Axial involvement, n (%)	90 (61.6)	57 (83.8)	**0.001**
Nail involvement, n (%)	57 (39.6)	38 (55.9)	**0.026**
Methotrexate use, n (%)	89 (61.4)	41 (60.3)	>0.05
Biologic therapy use, n (%)	30 (20.5)	22 (32.4)	>0.05
DAPSA, mean (SD)	14.3 (12.3)	27.0 (17.9)	**<0.001**
PASDAS, mean (SD)	2.7 (1.2)	4.5 (0.9)	**<0.001**
TJC, mean (SD)	3.3 (6.6)	10 (12.8)	**<0.001**
SJC, mean (SD)	0.4 (1.2)	0.5 (1.2)	>0.05
LEI, mean (SD)	0.6 (1.3)	1.8 (2.1)	**<0.001**
BASFI, mean (SD)	1.2 (1.3)	4.8 (2.3)	**<0.001**
BSA, mean (SD)	1.1 (1.9)	1.3 (1.9)	>0.05
CRP, mg/dL, mean (SD)	6.3 (10.5)	8.2 (9.4)	**0.028**
DLQI, mean (SD)	1.6 (2.6)	3.5 (4.6)	**0.012**
SF-36 MCS, mean (SD)	63.3 (10.5)	57.5 (11.1)	**<0.001**
SF-36 PCS, mean (SD)	57.6 (12.3)	40.9 (9.1)	**<0.001**

PsA: Psoriatic arthritis, Pso: Psoriazis, BMI: Body mass index, DAPSA: Disease activity index for psoriatic arthritis, PASDAS: Psoriatic arthritis disease activity score, TJC: Tender joint count, SJC: Swollen joint count, LEI: Leeds enthesitis index, BASFI: Bath ankylosing spondylitis functional index, BSA: Body surface ares, CRP: C-reactive protein, DLQI: Dermatology life quality index, SF-36: Short form-36 health survey, MCS: Mental component summary score, PCS: Physical component summary score, SD: Standard deviation, HAQ: Health assessment questionnaire

were 68%, 28%, and 4%, respectively. Enthesitis was detected in 68% of the patients, while 56% and 29% of the patients exhibited nail involvement and dactylitis, respectively. Patients with disabilities were predominantly women, older, had higher BMI, and had lower education levels. Moreover, enthesitis, axial involvement, tender joints, nail involvement, serum CRP level, BASFI score, DLQI score, DAPSA score, and PASDAS were found to be associated with disability (Table 1). Disease activity (PASDAS and DAPSA), function (BASFI and SF-36 PCS), and quality of life (DLQI and SF-36 MCS) measurements were found to be correlated with HAQ-DI (Table 2). We established a multivariable model to assess the independent factors and covariates with disability and showed that TJC, [odds ratio (OR):1.07, 95% confidence interval (CI): 1.02-1.12; p=0.003], nail involvement (OR: 2.09, 95% CI: 1.05-4.13; p=0.035), and enthesitis (OR: 2.25, 95% CI: 1.13-4.48; p=0.021) were the main determinants of disability in patients with PsA (Table 3).

DISCUSSION

This study provides an informative overview of the burden of disability associated with PsA in patients, emphasizing both demographic and disease-related factors that contribute to impaired physical function. As assessed by the HAQ-DI, our results indicate that nearly one-third of patients with PsA experience moderate-to-high levels of disability. In accordance with previous research, our findings indicate that patients exhibiting elevated HAQ-DI scores were predominantly female, older, had higher BMI, and had lower levels of education. These demographic features have previously been recognized as independent risk factors for disability in multiple chronic rheumatologic conditions. A Turkish multicenter study found a substantial correlation between obesity and higher disease activity and poorer functional results in patients with PsA[16]. Likewise, advanced age and poorer educational levels have been linked to diminished self-efficacy, reduced health literacy, and restricted access to timely care, potentially exacerbating disability. Several clinical manifestations, such as enthesitis, axial involvement, tender joints, nail involvement, and elevated CRP levels, were substantially associated with worse physical function. Enthesitis and axial disease, in particular, are frequently more difficult to manage and are associated with a higher degree of disease severity. A recent study indicated that patients with axial disease and enthesitis exhibited higher disease activity and HAQ scores[17]. Although often disregarded, nail involvement may function as a visible indicator of a more extensive disease and has been associated with distal interphalangeal joint arthritis[18]. We found that functional status was more strongly correlated with current disease activity than disease duration. This aligns with previous research indicating that the influence of disease activity on functional scores diminishes with the progression of the disease duration. Moreover, there is a scarcity of substantial evidence indicating that the impact of clinical harm escalates as the illness progresses[19]. This result emphasizes the importance of early and aggressive intervention to control inflammation and prevent irreversible damage. Furthermore, the study demonstrated a close link between quality of life and functional capacity. Patients who reported higher HAQ-DI scores also reported a lower overall quality of life, which is consistent with previous research that has shown the extensive impact of PsA beyond joint-related symptoms. PsA has the potential to exacerbate the perceived disability and disease burden by disrupting sleep, mood, work productivity, and social participation[20,21]. These findings demonstrate the importance of a comprehensive and individualized treatment approach.

Table 2. Correlation of HAQ-DI and other patient reported outcomes of PsA patients

	r	p
DAPSA	0.53	**<0.001**
PASDAS	0.66	**<0.001**
BASFI	0.74	**<0.001**
DLQI	0.16	**0.02**
SF-36 MCS	-0.61	**<0.001**
SF-36 PCS	-0.29	<0.001

DAPSA: Disease activity index for psoriatic arthritis, PASDAS: Psoriatic arthritis disease activity score, BASFI: Bath ankylosing spondylitis functional index, DLQI: Dermatology life quality index, SF-36: Short form-36 health survey, MCS: Mental component summary score, PCS: Physical component summary score, HAQ-DI: Health assessment questionnaire-disability index, PsA: Psoriatic arthritis

Table 3. Multivariate analysis of disability

	CI (95%)	p
Age	1.03 (0.99-1.06)	0.13
Gender	0.73 (0.34-1.59)	0.43
Education duration	0.93 (0.85-1.02)	0.10
BMI >30	2.33 (1.06-5.41)	0.50
Enthesitis	2.25 (1.13-4.48)	**0.02**
Axial involvement	1.67 (0.84-3.31)	0.14
Nail involvement	2.09 (1.05-4.13)	**0.035**
TJC	1.07 (0.02-1.12)	**0.003**
CRP, mg/dL	1.01 (0.98-1.04)	0.37

BMI: Body mass index, TJC: Tender joint count, CRP: C-reactive protein, CI: Confidence interval

Study Limitations

Our study has some limitations. First, the single-center and cross-sectional design significantly limits the generalizability of the findings to broader populations. Additionally, due to the cross-sectional nature of the data, it is not possible to establish causal relationships between the variables studied. It is imperative to carefully consider these design limitations when interpreting the results, and future studies with multi-

center and longitudinal designs are required to confirm and expand upon these findings. Another important limitation of this study is the absence of a healthy control group or a disease control group. In the absence of comparison groups, it is challenging to ascertain if the observed connections are exclusive to patients with PsA. This limits the ability to draw disease-specific conclusions and may affect the interpretability and clinical relevance of the findings. Future research incorporating appropriate control groups is essential to clarify the specificity and significance of these associations. Lastly, the HAQ-DI is a well-known and generally accepted tool, but it might not measure all areas of disability that are important in PsA, such as fatigue, mental health, and work disability. The main strengths of the study were in its patient groups and sample size, as well as those treated with conventional and biologic DMARDs. Furthermore, we assessed the relationship between several outcome measures and the patient's disability. Conversely, there are also favorable features of the study. This study offers new perspectives on the functional impairment associated with PsA in a Turkish cohort, a population that has been underrepresented in the current literature. Although other countries have conducted similar studies, differences in culture, genetics, lifestyle, and healthcare access may impact disease expression and disability outcomes. Consequently, this investigation addresses a critical deficiency by conducting an assessment of not only clinical characteristics but also sociodemographic variables, including BMI and educational attainment, within a Turkish context.

CONCLUSION

In conclusion, our study highlights the importance of PsA on physical function and identifies critical demographic and clinical determinants of disability. Clinicians should be cognizant of early identification of high-risk patients and a multidisciplinary treatment strategy that encompasses both physical and psychosocial dimensions of the condition. Future research should prioritize longitudinal evaluations of impairment and the creation of comprehensive patient-reported outcome measures.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of İzmir Katip Çelebi University (decision no: 0411, date:11.09.2023). The research was performed in compliance with the principles of the Declaration of Helsinki.

Informed Consent: This study the single-center and cross-sectional design.

Footnotes

Authorship Contributions

Concept: E.D.E., Design: E.D.E., Data Collection or Processing: S.G., K.Y., E.Ç., H.K., E.E., Analysis or Interpretation: S.A., D.S., Literature Search: A.Ö., K.G., Writing: E.D.E.

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

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The Role of miR-330-3p in UV-induced Photokeratitis: A Pilot Experimental Study

UV ile Indüklenen Fotokeratitis Üzerine miR-330-3p Uygulamasının Rolü: Pilot Çalışma

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ABSTRACT

Aim: Ultraviolet (UV) radiation-induced ocular diseases pose a growing challenge to public health. In recent years, miRNA-based therapeutics have gained attention in the treatment of ocular diseases. miR-330-3p has anticarcinogenic, anti-inflammatory, and anti-apoptotic effects. This study aimed to investigate the effects of miR-330-3p against photokeratitis following UV radiation.

Materials and Methods: Eighteen Wistar albino male rats were randomly divided into three groups (n=6): control, UV, and UV+eye drop. Chronic exposure to UV radiation was conducted for 30 days, 2 hours a day. One µL of miR-330-3p-based eye drops was applied to the UV+eye drop group once daily for 7 days. Following the treatments, eye tissues were harvested and evaluated microscopically.

Results: There was no statistically significant difference between groups in inflammation, neovascularization, epithelial proliferation, and collagen density parameters. However, the edema levels in the UV group increased compared to the control and UV+eye drop groups (all $p<0.001$). The collagen density, however, increased in the UV group and decreased in the UV+eye drop group, but the results did not indicate a significant difference ($p>0.05$).

Conclusion: miR-330-3p presents a promising treatment option for corneal damage arising from photokeratitis. Our study is the first to explore the alleviating effects of miR-330-3p in photokeratitis, yielding encouraging results.

Keywords: Ultraviolet rays, eye diseases, miRNA, ophthalmic solutions, eye drops

ÖZ

Amaç: Ultraviyole (UV) radyasyon ile indüklenen oküler hastalıklar büyüyen bir halk sağlığı sorunudur. Son yıllarda yapılan çalışmalar miRNA-tabanlı tedavilerin önemini artırmaktadır. miRNA'lardan biri olan miR-330-3p anti-karsinojenik, anti-enflamatuvar ve anti-apoptotik etkileri mevcuttur. Bu bilgiler ışığında çalışmamızın amacı UV radyasyonu sonucundaki fotokeratitise karşı miR-330-3p'nin etkilerini incelemektir.

Gereç ve Yöntem: Çalışmada 18 adet erkek Wistar Albino rat kontrol, UV ve UV+göz damlası olmak üzere üç farklı gruba ayrılmıştır. Otuz gün süresince günde 2 saat kronik UV radyasyonuna maruz bırakılmıştır. Bunu takiben 7 gün süresince UV+göz damlası grubuna 1 µL miR-330-3p içeren göz damlası uygulaması yapılmıştır. Son uygulamadan 24 saat sonra hayvanlar sakrifiye edilerek histopatolojik incelemeler için uygun koşullarda saklanmıştır.

Bulgular: Yapılan mikroskobik incelemeler sonucunda enflamasyon, neovaskülarizasyon, epitel proliferasyonu ve kolajen densitesi parametrelerinde gruplar arasında istatistiksel olarak bir farklılık belirlenmedi. Bununla birlikte istatistiksel olarak farklılık olmamasında rağmen kolajen densitesinin UV grubunda Kontrol grubuna göre yükseldiği ve göz damlası uygulamasının bunu düzenlediği görüldü. Ödem parametresi UV grubunda hem kontrol hem de UV+göz damlası grubuna göre istatistiksel olarak anlamlı şekilde yüksek olduğu ortaya koyuldu (her iki p-değeri $p<0,001$).

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Sonuç: Çalışmamızda miR-330-3p'nin fotokeratit üzerine etkileri ortaya koyulmuştur. miR-330-3p, fotokeratitten kaynaklanan umut verici bir tedavi seçeneği olarak görülmektedir.

Anahtar Kelimeler: Ultraviyole ışınlar, göz hastalıkları, miRNA, oftalmik solüsyon, göz damlası

INTRODUCTION

Ultraviolet (UV) radiation has many effects on the human body, including both beneficial and hazardous impacts. Eyes, especially the anterior sites, are the most affected by UV radiation and the most UV-sensitive part of the body[1]. Due to multiple protection methods, the cornea, retina, and epithelial compartments are exposed to macro and micro damage. There are various pharmacological aspects for alleviating corneal or retinal damage that are lacking, and further studies are still essential[2]. Growing evidence demonstrates the risk of UV exposure to corneal damage and visual impairment, which has been an emerging health care problem. Ocular exposure to UV may cause several detrimental effects, as well as loss of vision. It has been reported that corneal tissues absorb almost 80% of the UV light directly. Depending on age-related differences, during late phases of life, the absorption levels increase and have detrimental effects on the cornea and ocular layers. People should be aware of the long-term UV exposure and use protective tools. The UV radiation-induced pathophysiological cascade includes photokeratitis, oxidative stress, edema, and apoptosis of epithelial cells[3]. The ocular fluid and layers include multiple antioxidant substances that may protect the cornea and surrounding ocular tissues. High exposure to UV radiation has negative impacts on ocular tissues and fluid through the increase of corneal fluid level and photo absorption. Photokeratitis is a painful condition and is mostly attributed to corneal damage following long-term exposure to UV. The loss of epithelial cells in the superficial layers of the cornea triggers edema and vision impairment[4]. Edema is initially identified as an adaptation and prevention from UV exposure; however, the exposure level is the main determinant of the corneal damage[5]. Non-pharmacological treatment options mostly include ice application or resting in a dark place. However, in serious cases, pharmacological therapies might be essential to reduce edema and inflammatory response to the UV exposure-induced corneal damage. Over the past three decades, gene-based therapies have gained attention in most pathological conditions. MicroRNAs are small non-coding RNAs and regulate tissue-specific RNA transcription, which mostly depends on the degradation of expressed proteins[6,7]. miRNAs were involved in several cellular responses, like the cellular cycle, inflammation, and apoptosis. It was reported that over two thousand miRNAs have been expressed in the human body, and yet most of them have not been identified about their functionality. The clarification of miRNA functions has the potential to provide promising adjunctive therapies for multiple disorders. Recent studies declared that there are crucially important miRNAs for the prevention of corneal damage[8-10]. These suggestions mostly originated from experimental diabetes studies and have become apparent. miR-330-3p is a miRNA cluster that is mostly associated with anti-aging, anti-apoptotic, and anticarcinogenic effects. miR-330-3p was used in several types in a variety of studies, including rheumatoid arthritis, osteosarcoma, and melanoma, with promising alleviating effects[11-13]. Therefore, in this study, we aimed to investigate the effects of miRNA-based eye drops (miR-330-3p) on UV exposure-induced corneal damage.

MATERIALS AND METHODS

Preparation of miR-330-3p-based Eye Drop

For the preparation of the eye drop, miR-330-3p mimic was purchased from (Med Chem Express Cat No: HY-R03031, Lot no: 326920) and encapsulated with lipofectamine 2000TM. For the encapsulation, lipofectamine and miR-330-3p were incubated for fifteen minutes according to the manufacturer's protocol. The eye drops were applied 1 µL to the UV+miR-330-3p group bilaterally after 30 days of UV radiation for 7 days, once a day[14,15].

Animals

The present study included eighteen Wistar albino male rats (7-8 months; G*Power: n ≥18) purchased from Çanakkale University Experimental Research Application and Research Center, with ethical approval from Çanakkale University Animal Experiments Local Ethics Committee (decision no: 2024/04-02, date: 18.04.2024). Rats were housed individually at standard humidity and temperature (45%-50% humidity and 22±2 °C), with a 12-h dark/light cycle and fed standard pellets and water ad libitum.

Experimental Groups and Procedure

The purchased rats were randomly divided into three groups (n=6) as control, UV, and UV+eye drop. The Control group was not exposed to UV radiation and did not receive any treatment. UV and UV+eye drop groups were exposed to 2 hours of daily UV radiation for 30 days at a dose of UV-A 12.5 J/cm^2 and UV-B 0.22 J/cm^2 [16,17]. At the end of the 30 days, the UV+eye drop group was administered with the miR-330-3p-based eye drop. The UV group did not receive treatment.

Tissue Harvesting

At the end of 7 days of treatment, all rats underwent general anesthesia (ketamine-xylazine) for sacrifice. Following euthanasia with cervical dislocation, the whole eye tissue and optic nerves were harvested immediately and stored in 10% formaldehyde until histopathological analysis. Histopathological analysis the eye specimens were used for hematoxylin-eosin (H-E) and masson's trichrome (M-T) staining analysis for histopathological assessment. First, all specimens were fixed and processed for staining assessments. After all tissues were embedded in paraffin, 4-µm sections were then cut and stained with H-E and M-T[18-20]. H-E-stained sections were used to evaluate the edema, inflammation, neovascularization, and epithelial proliferation; and M-T-stained sections were used to evaluate the collagen density and structure.

Statistical Analysis

The data were analyzed by the SPSS program version 27.0 (SPSS, version 27, IBM of Armonk, New York, U.S.). One-way ANOVA test was used for determining differences. Post-hoc determinations were performed by Tukey HSD test for comparing the groups. Data were presented as means and standard errors. P<0.05 was considered statistically significant.

RESULTS

The histopathological analysis indicated that there were no significant changes in inflammation, neovascularization, and epithelial proliferation according to the H-E results, and in collagen density according to the M-T results. However, the edema levels in the UV group (3.00±0.00) were significantly increased compared to the Control group as shown in Figure 1 (0.00±0.00; p<0.001). Additionally, there was no statistically significant difference between the Control group and UV+eye drop group as shown in Figure 2 (0.83±0.54; p=0.178). Moreover, in the UV+eye drop group, edema levels were significantly decreased compared to the UV group (p<0.001). The collagen density, however, increased in the UV group (0.50±0.22) and decreased in the UV+eye drop group (0.16±0.16), but the results did not indicate a significant difference (p>0.05; Figure 2).

DISCUSSION

The present study mainly aimed to evaluate the effects of a miRNA-based eye drop in chronic exposure to UV radiation. Our results indicated that, in accordance with the literature, the UV exposure increased the edema levels in the UV exposure group significantly compared to the Control group. miR-330-3p-based eye drop significantly decreased the edema levels compared to the UV group. However, inflammation, neovascularization, and epithelial proliferation did not indicate any significant change. Additionally, collagen density decreased in the UV+eye drop group, but the results were not significant. The UV light penetrates the eye tissue layers, increasing the risk of eye cancer, inflammation, and visual disorders[21]. The overall eye tissues represent an adaptation to low exposure to UV radiation in daily life. However, prolonged

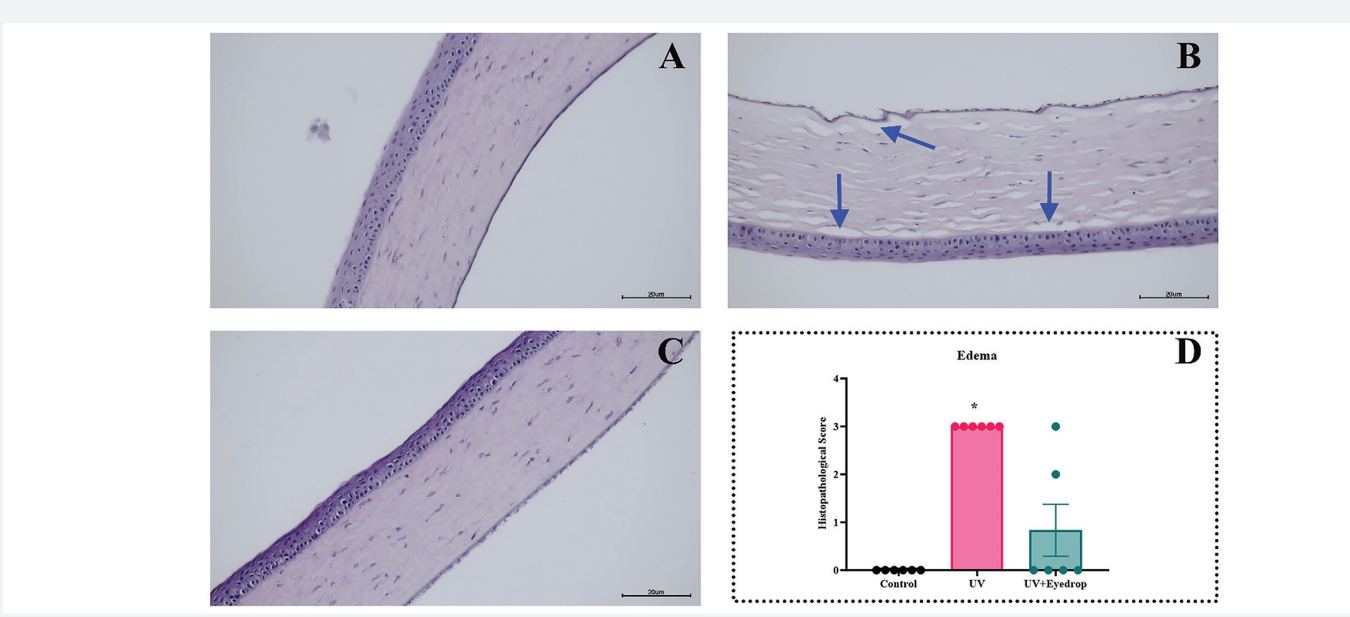

Figure 1. (A) Control, (B) UV, (C) UV+eye drop group of H-E-stained sections and (D) Histopathological scores for edema in all groups (*p<0.05 compared to the control and UV+eye drop groups)

H-E: Hematoxylin-eosin, UV: Ultraviolet

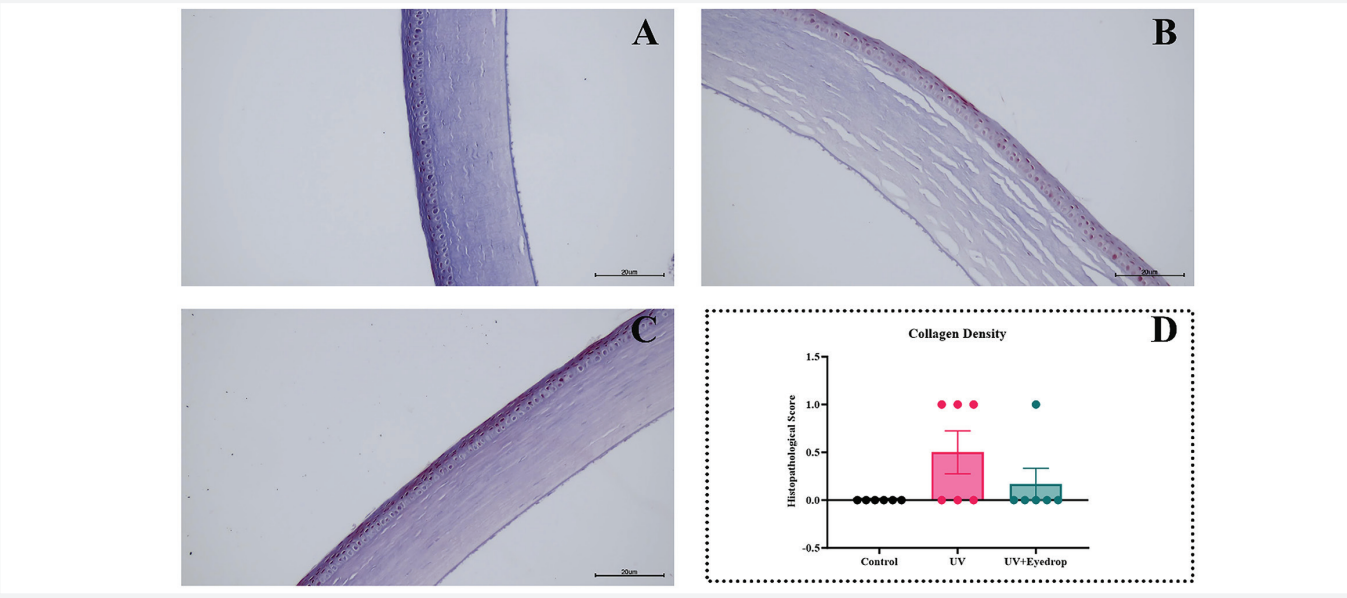

Figure 2. (A) Control, (B) UV, (C) UV+eye drop group of M-T stained sections and (D) Histopathological scores for collagen density in all groups

UV: Ultraviolet, M-T: Masson's trichrome

exposure without protection may provoke visual disorders, pain, or itchy eyes[22]. Photokeratitis is one of the major detrimental outcomes of prolonged UV exposure to the eyes. Corneal epithelial damage implicates an edema, which may also alter vision and result in clouds and haze[23]. In a study, the rats' eyes were locally exposed to the UV dose of 6.5 J/cm² daily for 5 consecutive days. Histopathological results indicated that there was a significant increase in interstitial edema and disruption of collagen structure[24]. A comparative study was established to evaluate the acute effects of 0.08 and 0.225 J/cm² UV radiation on eye tissue. It was claimed that 0.08 J/cm² UV exposure exhibited a mild edema and nerve injury, whereas 0.225 J/cm² destroyed epithelial nerves[25]. In our study, 30 days of daily 2-h exposure to UV-A 12.5 J/cm² and UV-B 0.22 J/cm² significantly increased epithelial edema levels. Considering local or acute exposure, it is suggested that daily exposure to UV radiation may also trigger epithelial disorders. Nucleic acid-based therapeutics provide promising outcomes when used for a variety of disorders, including cancer, neurodegenerative disorders, inflammatory conditions, and apoptosis-related diseases[19]. Currently, there is a wide range of disorders for which nucleic acid-based therapeutics have completed phase II studies[20]. Since the discovery of the first miRNA in 1993, miRNA-based applications have gained attention over the past decade due to their regulatory effects on transcription factors[20]. In addition to most ocular diseases, photokeratitis has become a target for miRNA-based therapeutics. In a previous study, the effects and tolerability of anti-miR-328 were assessed, and the outcomes were promising, indicating its appropriateness for phase II and phase III trials[21]. The effects of miR-127-5p in UV-exposed photokeratitis demonstrated anti-apoptotic and antioxidant effects in an *in vitro* study[22]. In an animal study on acute UV exposure eye damage, miR-129-5p eye drops reduced corneal epithelial damage and photokeratitis-induced visual loss[23]. miR-330-3p has primarily been defined as an anti-carcinogenic miRNA, exhibiting anti-inflammatory, anti-apoptotic, and antioxidant effects[24]. To date, no study exists that has investigated the effects of miR-330-3p on ocular diseases. In the present study, following chronic exposure to UV radiation, a miR-330-3p-based eye drop was applied to the rats' eyes for 7 days. Depending on chronic application, there were no statistically significant changes in inflammation, neovascularization, and epithelial proliferation. However, edema, which is the most important signal for the development of photokeratitis, was increased significantly in the non-treated UV exposure group. miR-330-3p application significantly decreased edematous levels and alleviated photokeratitis-based vision loss and haze. It is suggested that miR-330-3p might be a good candidate to reduce corneal damage based on photokeratitis following chronic UV exposure. However, further studies with acute exposure to UV light have to be established to elucidate the main molecular and effect mechanisms of miR-330-3p eye drops.

Study Limitations

There are some limitations of the study given below:

• Our study is a pilot study so lower numbers of animals were used. Further studies have already been established to elucidate the effects of chronic and acute exposure to UV radiation on photokeratitis.

• Additionally, our model was different from previous studies as the exposure of UV light has been established to the whole body representing the real exposure in daily life, not locally to the eyes.

CONCLUSION

Future Directions

Ocular diseases present challenges due to limited treatment options and the sensitivity of corneal tissues to chemicals. Nevertheless, vision loss can have serious implications for public health. While UV exposure has its benefits, it can also pose significant risks to eye tissues. Acute and chronic exposure has different detrimental effects on eye health. Although protective measures are essential, chronic exposure to UV radiation for outdoor workers may lead to visual disorders like photokeratitis. Therefore, there is an urgent need for emerging therapies to address UV exposure-related ocular diseases. miRNA-based therapeutics have been instilling hope, yielding successful outcomes in nearly all diseases. Depending on their transcriptional pathway effects, these therapies may pave the way for future pharmacotherapy. miR-330-3p, known for its anti-inflammatory and anti-apoptotic properties, presents a promising treatment option for corneal damage arising from photokeratitis. Our study is the first to explore the alleviating effects of miR-330-3p in photokeratitis, yielding encouraging results. Future studies are suggested to fully understand the underlying mechanisms in acute and chronic high exposure to UV radiation in ocular degenerative disorders.

Ethics

Ethics Committee Approval: Çanakkale University Experimental Research Application and Research Center, with ethical approval from Çanakkale University Animal Experiments Local Ethics Committee (decision no: 2024/04-02, date: 18.04.2024).

Informed Consent: It is an animal experiment study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.A.E., C.A., D.A., Concept: H.A.E., H.E., Design: H.A.E., H.E., Data Collection or Processing: C.A., D.A., Analysis or Interpretation: B.B., Writing: H.A.E., H.E., C.A.

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

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ORIGINAL ARTICLE

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Preoperative Prediction of Stone Composition Using Hounsfield Units in Non-Contrast CT Imaging: A Single-Center Study from Turkey

Kontrastsız BT Görüntülemede Hounsfield Üniteleri Kullanılarak Taş Kompozisyonunun Preoperatif Tahmini: Türkiye'den Tek Merkezli Bir Çalışma

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ABSTRACT

Aim: Numerous studies have shown that urinary stone composition is directly associated with the effectiveness of extracorporeal shock wave lithotripsy and is significantly related to various factors, including stone-free rates and fragmentation time during endoscopic procedures. Therefore, this study aimed to predict stone composition using Hounsfield unit (HU) measurements obtained from non-contrast computed tomography (NCCT).

Materials and Methods: Urinary stones were classified according to their predominant composition. HU measurements were compared with spectrophotometric analysis results to assess the accuracy of predicting stone composition. Additionally, patient demographic data, clinical characteristics, and stone-related parameters—including HU values, HU density, stone size, volume, and composition were recorded and analyzed.

Results: A total of 571 patients' stone analysis data were retrospectively analyzed: mean core HU, peripheral HU, and average HU values. The calcium oxalate group had significantly higher values than those in the cystine, uric acid, and calcium oxalate + uric acid groups (all $p<0.001$). Furthermore, a statistically significant correlation was found between stone size and core HU values ($r=0.291$, $p<0.001$).

Conclusion: NCCT may aid in selecting the most appropriate treatment by identifying stone composition. HU measurements (core, peripheral, and density) can be utilized to differentiate calcium-based stones from cystine- and uric acid-based stones.

Keywords: Computed tomography, Hounsfield unit, stone, stone composition, stone type

ÖZ

Amaç: Üriner sistem taşlarının bileşiminin, ekstrakorporeal şok dalgası litotripsi etkinliği ile doğrudan ilişkili olduğu ve endoskopik prosedürlerde taşsızlık oranları ve taş fragmantasyon süresi gibi çeşitli faktörlerle önemli derecede ilişkili olduğu birçok çalışmada gösterilmiştir. Bu çalışmada kontrastsız bilgisayarlı tomografi (NCCT) ile elde edilen Hounsfield ünitesi (HU) ölçümlerinin taş bileşimini tahmin etmedeki rolünün araştırılması amaçlanmıştır.

Gereç ve Yöntem: Taşlar baskın kompozisyonlarına göre sınıflandırıldı. HU ölçümleri, taş bileşimini tahmin etmedeki doğruluğu değerlendirmek için spektrofotometrik analiz sonuçları ile karşılaştırıldı. Ayrıca, hasta demografik verileri, klinik özellikler ve taş ile ilişkili parametreler (HU değerleri, HU yoğunluğu, taş boyutu, hacmi ve kompozisyonu) kaydedilerek analiz edildi.

Bulgular: Toplam 571 hastanın taş analiz verileri retrospektif olarak incelendi. Kalsiyum oksalat grubunda ortalama kor HU, periferik HU ve ortalama HU değerleri; sistin, ürik asit ve kalsiyum oksalat + ürik asit gruplarına kıyasla anlamlı derecede daha yüksek bulundu (tüm $p<0,001$). Ayrıca, taş boyutu ile kor HU değerleri arasında istatistiksel olarak anlamlı bir korelasyon saptandı ($r=0,291$, $p<0,001$).

Sonuç: NCCT, taş kompozisyonunu belirleyerek en uygun tedavi seçiminin yapılmasına yardımcı olabilir. HU ölçümleri (kor, periferik ve yoğunluk) kullanılarak kalsiyum bazlı taşlar, sistin ve ürik asit bazlı taşlardan ayırt edilebilir.

Anahtar Kelimeler: Bilgisayarlı tomografi, Hounsfield ünitesi, taş, taş kompozisyonu, taş tipi

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INTRODUCTION

Urinary stone disease has seen significant advancements in diagnosis and management with the widespread use of non-contrast computed tomography (NCCT). NCCT enables the measurement of the stone's radiodensity through Hounsfield units (HU), which indirectly provides information about the stone composition. Preoperative prediction of stone composition is crucial, as it helps determine the most appropriate treatment approach, especially in predicting the success rates of extracorporeal shock wave lithotripsy (ESWL) and endoscopic procedures. Computed tomography (CT) additionally facilitates visualization of the stone within the surrounding tissues and allows HU measurements. Therefore, NCCT has replaced other conventional methods, including urinary system ultrasonography, plain radiography, and intravenous pyelography, due to these advantages[1,2].

The literature indicates that the composition of urinary stones is directly correlated with the efficacy of ESWL and is also significantly associated with several factors, including stone-free rates and the duration of stone fragmentation during endoscopic procedures[3]. Several studies in the literature demonstrate a clear correlation between urinary stone composition and HU values[4-6]. Furthermore, HU is recognized as a crucial marker for predicting stone composition. This study aims to investigate the relationship between HU values obtained from NCCT and urinary stone composition in a Turkish patient population. Specifically, it seeks to evaluate whether HU values can distinguish different stone types, establish possible cut-off points to enhance preoperative prediction of stone composition, and assess the applicability of these findings to the Turkish population.

MATERIALS AND METHODS

The retrospective study received approval from the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethical Committee (protocol number: 2023.73.04.09, date: 25.04.2023) and was carried out in accordance with the Declaration of Helsinki. Data were collected from patients who underwent surgical interventions for urolithiasis, including ureteroscopy, retrograde intrarenal surgery, percutaneous nephrolithotomy, and laparoscopic or open stone surgery, at our clinic, between August 2016 and January 2024. Patients who underwent surgical stone removal, had complete preoperative imaging with NCCT, and provided stone samples for infrared spectrophotometric analysis were included in the study.

The patient group was examined using a sixteen-channel, multi-slice BrightSpeed Series CT scanner (GE Healthcare, Milwaukee, WI, USA). The NCCT scans were obtained with a 16 × 1.25 mm collimation, an average slice width of 5 mm, and an instrument rotation speed of 27.50 rotations per 0.80 seconds (pitch 1.375), utilizing 120 kVp and 250 effective mAs. The field of view was calibrated to each individual's dimensions, extending from the upper abdomen to the pubis, and no intravenous contrast agent was used. The NCCT images were transmitted digitally to a computer (Sectra PACS Linköping, Sweden). The HU values of stones were measured at both the core and the periphery using region of interest techniques, with measurements performed at 25× magnification for enhanced accuracy (Figure 1). Two experienced endourologists (Ç.D. and M.F.Ş.) performed HU measurements with a specific focus on stone disease. The results demonstrated high agreement, with a correlation coefficient of 0.89. Stone size and volume were calculated according to the Sorokin formula (volume = π × length × width × height × 0.167)[7]. After obtaining HU values, the stone composition was analyzed for correlation with these radiodensity measurements.

Stone samples collected during surgical procedures were analyzed spectrophotometrically in the institutional laboratory. The stones were categorized by dominant composition as calcium oxalate (CaOx) (monohydrate or

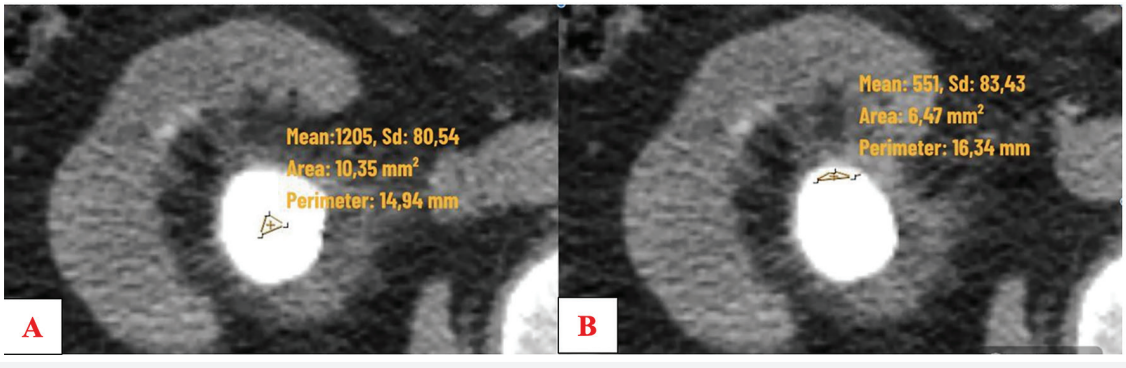

Figure 1. Demonstration of measuring HU value A) From the core and B) Periphery of the stone

HU: Hounsfield unit, SD: Standard deviation

dihydrate), calcium phosphate, uric acid (UA), struvite, cystine, or mixed. HU measurements were subsequently compared with spectrophotometric results to evaluate predictive accuracy. Patient demographics, clinical characteristics, and stone parameters [(HU values, HU density (HUD) size, volume, and composition)] were recorded.

Statistical Procedure

Statistical analyses were performed using SPSS 23.0 for Macintosh (IBM, New York, U.S.A.). Numerical data were presented as means and standard deviations, whereas categorical data were reported as counts and percentages. Chi-square and Mann-Whitney U tests were employed to compare patient groups. The Spearman correlation test was used for non-parametric data.

RESULTS

A total of 571 patients' stone analysis data were retrospectively included in the study. The average age of the patients was 50±13.9 years (range, 13 to 85 years), and the mean stone volume was 1662.85 mm^3. A total of 388 patients had a single stone, while 183 patients had multiple stones. Based on stone analysis, the urinary stones were classified into seven groups[8]. There was no significant difference between the groups in terms of gender distribution. The demographic data and stone-related properties are presented in Tables 1 and 2. The average values of core HU, peripheral HU, and average HU in the CaOx group were significantly elevated compared to those in the cystine, UA, and CaOx + uric acid (UA) groups ($p<$**0.001**, $p<$**0.001**, and $p<$**0.001**, respectively). However, there was no statistical difference among the CaOx and carbonate apatite, brushite, or carbonate apatite + CaOx + struvite groups in terms of peripheral, mean, and core HU values ($p>0.05$). In addition, HUD values were consistent with the results of peripheral HU, mean HU, and core HU measurements. A statistically significant relationship existed between stone size and core HU values ($r=+0.291$, $p<$**0.001**); however, no correlation was observed between HUD and stone volume ($p>0.05$). The classification of stone types and their HU values is detailed in Table 3. We were unable to identify a statistically significant HU-related cut-off value to predict stone type in our ROC analyses.

DISCUSSION

Urinary system stones may consist of a pure single component or multiple components. The most observed subtype is the CaOx group, which is resistant to ESWL. Similarly, cystine stones,

Table 1. Demographic properties of patients grouped by stone compositions

	Ca oxalate (n=379)	Uric acid (n=45)	Carbonate apatite (n=38)	Cystine (n=7)	Ca oxalate + Struvite + Carbonate apatite (n=70)	Ca oxalate + Uric acid (n=26)	Brushite (n=6)	p-value
Age (mean ± SD) W	49.3±14.6	57.6±15.2	47.7±15.5	26.7±11.3	50.5±15.0	57.7±15.8	45.8±16.3	**0.0011**
BMI (kg/m^2)$^{\Omega}$	27.4±3.9	29.4±3.7	25.2±5.8	23.1±2.2	27.4±3.7	28.6±3.8	23.5±4.4	**0.0042**
Positive preoperative urine culture$^{\Omega}$ (%)	9 (2.4%)	0 (2.2%)	3 (7.9%)	0 (0%)	6 (8.6%)	2 (7.7%)	1 (16.7%)	**0.0013**
Male$^{\Psi}$	247 (65.2%)	26 (57.8%)	12 (31.6%)	4 (57.1%)	38 (54.3%)	14 (53.8%)	3 (50%)	**0.235**
Female	132 (34.8%)	19 (42.2%)	26 (68.4%)	3 (42.9%)	32 (45.7%)	12 (46.2%)	3 (50%)	

$^{\Psi}$: The Chi-square test, $^{\Omega}$: The Mann-Whitney U test, SD: Standard deviation, BMI: Body mass index

Table 2. Stone related properties

	Ca oxalate (n=379)	Uric acid (n=45)	Carbonate apatite (n=38)	Cystine (n=7)	Ca oxalate + Struvite + Carbonate apatite (n=70)	Ca oxalate + Uric acid (n=26)	Brushite (n=6)	p-value
Stone volume$^{\Omega}$ mm^3	1324.8±315.7	3715.4±521.4	1320.7±979.8	4689.7±746.3	933.7±480.5	4300.3±898.8	3336.5±498.3	**0.0274**
Stone side$^{\Psi}$								**0.0016**
right	146 (38.5%)	20 (44.4%)	19 (50%)	3 (42.8%)	32 (45.7%)	15 (57.7%)	3 (50%)	
left	186 (49.1%)	23 (51.1%)	16 (42.1%)	2 (28.6%)	30 (42.9%)	9 (34.6%)	3 (50%)	
bilateral	47 (12.4%)	2 (4.5%)	3 (7.9%)	2 (28.6%)	8 (11.4%)	2 (7.7%)	0	

$^{\Psi}$: The Chi-square, $^{\Omega}$: The Mann-Whitney U test was used

Table 3. The presentation of measured HU and HU density based on stone compositions

HU variable	Ca oxalate (n=379)	Uric acid (n=45)	Carbonate apatite (n=38)	Cystine (n=7)	Ca oxalate + Struvite + Carbonate apatite (n=70)	Ca oxalate + Uric acid (n=26)	Brushite (n=6)	p-value
Core HU[Ω]	1073.9±342.3	789.6±318.8	1047.4±335.2	901.3±338.0	1090.3±371.7	846.5±213.3	1103.1±311.2	**0.001**
Periphery HUW	852.4±219.1	615.3±183.1	758.9±218.9	735.3±282.0	978.7±165.5	698.4±193.6	892.7±254.4	**0.001**
Mean HU[Ω]	1006.8±308.6	722.9±279.5	996.5±312.8	812.3±321.0	1023.1±337.2	767.9±252.9	1036.7±259.6	**0.001**
HU density[Ω]	96 (47-379)	51 (29-187)	81 (36-332)	69 (31-277)	99 (41-401)	63 (34-366)	87 (51-356)	**0.001**

[Ω]: The Mann-Whitney U test, HU: Hounsfield unit

frequently observed in the pediatric population, are naturally resistant to ESWL due to their intrinsic characteristics. On the other hand, UA stones can be chemolysed. Furthermore, in cases of infection stones, antibiotic treatment plays a vital role in preventing recurrences. Predicting kidney stone types is crucial for personalizing treatment strategies and implementing preventive measures, especially for specific compositions such as UA, cystine, and infection-related stones.

Recent papers on predicting stone composition from HU values report conflicting results. Numerous studies have demonstrated that stone composition can be predicted from NCCT measurements[9-12]. However, some studies report contradictory outcomes, including one that found NCCT is ineffective for accurately determining stone composition[13]. Two recent studies have focused on the use of HU values in distinguishing UA stones from CaOx stones[14,15]. These studies reported that the density of UA stones is lower than that of CaOx stones. In line with these two studies, our findings also demonstrated consistent results. However, in our research, HU measurements (peripheral, core, and density) failed to distinguish CaOx stones from carbonate apatite or cystine stones from UA stones, with no statistically significant difference observed.

Advancements in NCCT measurements have led to the definition of additional HU values, including the stone core HU, peripheral HU, and HUD were used to predict stone composition. A recent study reported that CaOx stones are typically associated with an HUD >80[15]. Similarly, Torricelli et al.[16] observed significant variations in core HU, HUD, and peripheral HU values among CaOx, UA, and cystine stones. Furthermore, the study demonstrated that CaOx stones can be effectively distinguished from UA and cystine stones; however, no significant distinction was observed between cystine and UA stones[4]. In our study, the outcomes were consistent with those reported by Torricelli et al.[16]. Significant differences were observed in peripheral HU, core HU, and HUD values among different calcium-based stone compositions; however, these parameters were insufficient to differentiate between UA and cystine stones. In contrast to the literature, we were unable to identify a statistically significant HU-related cut-off value in our ROC analyses. This may be due to the inclusion of mixed-type stones alongside pure stones in our study.

While our study primarily focuses on the role of HU values in predicting urinary stone composition, it is important to consider the clinical implications of these findings, particularly in the context of fluoroscopy usage and surgical complications. The HU value not only provides insights into stone composition but also has potential implications for intraoperative management. Higher HU values are often associated with harder stones, which may necessitate longer laser lithotripsy times and higher energy settings, potentially leading to longer operative durations and a higher risk of thermal injury to the urothelium. Additionally, stone density can influence the need for fluoroscopy during endourological procedures, as denser stones may require more frequent imaging for localization and assessment of fragmentation. Future studies should investigate the relationships between HU values, radiation exposure, and perioperative complications to optimize surgical planning and patient safety[17].

Study Limitations

Our study has some limitations, primarily its retrospective design. While the number of stone analyses may appear limited when only pure stone compositions are considered, we observed that including mixed-type stones significantly increases the sample size. Nevertheless, our study includes a sufficient number of patients to provide valuable insights and guide national data and future publications.

CONCLUSION

NCCT may help select the most effective treatment by identifying stone composition. Calcium-based stones can be differentiated from cystine and struvite stones using HU measurements (peripheral, core, and density). However, additional technical assessments and predictive markers should be explored to more accurately distinguish CaOx from carbonate apatite stones and cystine from UA stones.

Ethics

Ethics Committee Approval: The retrospective study received approval from the Tekirdağ Namık Kemal University Non-Interventional Clinical Research (protocol number: 2023.73.04.09, date: 25.04.2023) and was carried out in accordance with the Declaration of Helsinki.

Informed Consent: This is a retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ç.D., C.M.Y., Concept: Ç.D., M.F.Ş., M.S.K., Design: Ç.D., M.F.Ş., Data Collection or Processing: Ç.D., M.S.K., Analysis or Interpretation: Ç.D., M.F.Ş., C.M.Y., Literature Search: Ç.D., O.O., Writing: Ç.D., M.F.Ş., M.S.K., O.O., C.M.Y.

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ORIGINAL ARTICLE

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Simultaneous Correction of Pectus Excavatum During Median Sternotomy for Cardiac Surgery: A Case Series of Four Patients

Pektus Ekskavatumun Median Sternotomi ile Kardiyak Cerrahi Sırasında Eş Zamanlı Onarımı: Dört Olguluk Seri

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ABSTRACT

Aim: Simultaneous surgical correction of pectus excavatum (PE) and cardiac pathology has been increasingly considered in recent years. This approach may reduce the technical challenges and complication risks compared to performing chest wall correction as a separate procedure in patients with a history of median sternotomy. Although the literature on its feasibility, safety, and long-term outcomes is limited, current evidence suggests potential benefits when applied in appropriately selected patients.

Materials and Methods: This single-center retrospective study included four patients who underwent simultaneous correction of PE and open-heart surgery via median sternotomy between 2015 and 2023. Demographic, operative, and postoperative data were collected. All patients underwent the Nuss procedure after intracardiac intervention.

Results: The cohort comprised four males, mean age 37.0±26.2 years (range: 20-78). Concomitant procedures were atrial septal defect repair, aortic root replacement, mitral valve replacement, and multi-vessel coronary artery bypass grafting. One or two pectus bars were placed in each case, with no intraoperative complications. One patient (25%) died postoperatively from cardiac arrhythmia unrelated to chest wall repair. Among survivors, complications were limited to one pleural effusion. Two patients underwent elective bar removal. Median follow-up was 58 months (range: 14-108).

Conclusion: Simultaneous PE repair during median sternotomy for cardiac surgery is technically feasible and safe. It eliminates the need for staged procedures, minimizes retrosternal dissection risks, and optimizes surgical exposure. Careful planning and intraoperative coordination are critical for successful outcomes. These findings support the broader adoption of this strategy in selected patients with coexisting thoracic and cardiac anomalies.

Keywords: Pectus excavatum, sternotomy, cardiac surgery, Nuss procedure

ÖZ

Amaç: Pektus ekskavatum (PE) ve kardiyak patolojilerin eş zamanlı cerrahi olarak düzeltilmesi son yıllarda giderek daha fazla gündeme gelmektedir. Bu yaklaşım, daha önce median sternotomi uygulanmış hastalarda ikinci seansta gerçekleştirilecek göğüs duvarı düzeltme ameliyatlarına kıyasla teknik zorlukları ve komplikasyon risklerini azaltabilir. Literatürde bu yöntemin uygulanabilirliği, güvenliği ve uzun dönem sonuçlarına dair veriler sınırlı olmakla birlikte, mevcut bulgular uygun hasta seçiminde potansiyel faydalar sağlayabileceğini düşündürmektedir.

Gereç ve Yöntem: Bu tek merkezli retrospektif çalışmaya, 2015-2023 yılları arasında median sternotomi yoluyla PE onarımı ile açık kalp cerrahisi eş zamanlı olarak yapılan dört hasta dahil edildi. Demografik, operatif ve postoperatif veriler incelendi. Tüm hastalara intrakardiyak girişim sonrası Nuss prosedürü uygulandı.

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Bulgular: Dört hastanın tamamı erkekti; ortalama yaş 37,0±26,2 yıl (dağılım: 20-78). Eş zamanlı girişimler atrial septal defekt onarımı, aort kökü replasmanı, mitral kapak replasmanı ve çoklu damar koroner arter bypass idi. Her hastada bir veya iki pektus barı yerleştirildi ve intraoperatif komplikasyon olmadı. Bir hasta (%25) postoperatif dönemde, onarımla ilişkili olmayan kardiyak aritmi nedeniyle kaybedildi. Sağ kalanlarda komplikasyon yalnızca bir plevral efüzyon ile sınırlıydı. İki hastada elektif bar çıkarıldı. Medyan takip süresi 58 ay (dağılım: 14-108) idi.

Sonuç: Kardiyak cerrahi sırasında median sternotomi ile eş zamanlı PE onarımı teknik olarak uygulanabilir ve güvenlidir. Bu yöntem, aşamalı cerrahi gereksinimini ortadan kaldırır, retrosternal diseksiyonla ilişkili riskleri en aza indirir ve cerrahi görüş alanını optimize eder. Başarılı sonuçlar için dikkatli planlama ve ameliyat sırasında ekip koordinasyonu kritik önemdedir. Bulgularımız, torasik ve kardiyak anomalileri birlikte bulunan seçilmiş hastalarda bu stratejinin daha geniş ölçekte uygulanmasını desteklemektedir.

Anahtar Kelimeler: Pektus ekskavatum, sternotomi, kardiyak cerrahi, Nuss prosedürü

INTRODUCTION

Pectus excavatum (PE) is the most common congenital anterior chest wall deformity, characterized by a depression of the sternum and adjacent costal cartilages. The minimally invasive repair of PE (MIRPE) was first introduced by Donald Nuss in the late 1990s and has since become the standard technique for surgical correction[1]. PE constitutes over 90% of congenital chest wall anomalies and is estimated to occur in approximately one in 400 live births, with a male predominance of up to 5:1[2]. Although PE is usually an isolated deformity, it may coexist with congenital or acquired cardiac conditions[3]. When PE coexists with cardiac pathology, mechanical compression of the right heart chambers may exacerbate hemodynamic compromise, leading to worsened clinical status and supporting the necessity of chest wall correction[4]. Performing MIRPE after previous median sternotomy poses significant technical challenges and carries an increased risk of life-threatening complications, particularly iatrogenic cardiac injury[5]. Dense retrosternal adhesions, distortion of anatomical planes, and limited visualization may contribute to intraoperative cardiac injury, which has been reported in up to 7% of patients with previous sternotomy undergoing MIRPE[6]. To overcome the challenges and risks associated with delayed repair, several authors have proposed performing PE correction concurrently with cardiac surgery. This combined approach offers direct access to the retrosternal space, eliminates the need for re-entry through a previously operated field, and may reduce the risk of complications such as adhesions and cardiac injury. Furthermore, simultaneous correction can shorten the overall treatment course, reduce hospitalization time, and avoid repeated anesthesia exposure. Case reports and small series have demonstrated that this strategy is feasible, even in pediatric patients, with acceptable safety and satisfactory functional and cosmetic outcomes[7-10]. In this study, we present our experience with four patients who underwent simultaneous repair of PE and cardiac pathology via median sternotomy. The primary aim was to evaluate the feasibility and safety of the combined approach, while also highlighting key aspects of the surgical technique and procedural sequence. By sharing our results and operative details, we aim to contribute to the growing body of evidence supporting single-stage repair in selected cases with coexisting thoracic and cardiac anomalies.

MATERIALS AND METHODS

Selection and Description of the Cases

This retrospective study included patients who underwent simultaneous repair of PE and cardiac pathology between August 2015 and May 2023 at a single tertiary care institution. Inclusion criteria comprised patients diagnosed with PE who also required surgical correction of congenital or acquired cardiac disease via median sternotomy. Patients with previous chest wall surgery, incomplete medical records, or those undergoing isolated pectus or cardiac procedures were excluded. The indication for simultaneous repair in all four patients was hemodynamically significant cardiac compression due to PE, confirmed by imaging and intraoperative findings. The multidisciplinary heart team concluded that correcting the chest wall deformity during the index cardiac operation would optimize postoperative cardiac function and prevent persistent right heart compression. This study was approved by the Institutional Ethics Committee of Memorial Ataşehir Hospital (approval number: 2025/19, date: 13.08.025).

Data Collection and Outcome Measures

Demographic and clinical variables including age, sex, PE symmetry, associated anomalies, family history, and coexisting cardiac diagnoses were reviewed. Operative data such as the type of cardiac procedure performed, number of pectus bars implanted, and whether bars were subsequently removed were recorded. Postoperative outcomes were assessed in terms of hospital stay duration, complication rates, and any observed morbidity or mortality.

Technical Information

Prior to median sternotomy, standard marking for the pectus repair was performed with the patient in the supine position. The point of maximal sternal depression was identified as the

central axis for bar passage. In addition, the bilateral entry and exit points for the pectus bar along the lateral chest wall were marked. After determining the appropriate length and curvature on the aluminum model, the pectus bars were bent accordingly to achieve their final shape. Two 2 cm vertical skin incisions were made at the mid-axillary lines on both sides, and subpectoral tunnels were developed to facilitate bar passage. Cardiac surgery was then performed via median sternotomy. Upon completion of the intracardiac procedure, the sternal wires were placed but left untied, allowing sufficient space for the subsequent insertion of the pectus bars. The prepared pectus bars were then advanced along the pre-positioned guide under thoracoscopic guidance (Figure 1a). Bar fixation was performed using stabilizers, which were secured in synchronization with sternal wire closure to prevent tension-related failure or loosening of either fixation system (Figure 1b). Special care was taken to avoid mechanical interference between the bar construct and the sternal wires.

Statistical Analysis

Given the small sample size, only descriptive statistics were used. Continuous variables were expressed as mean ± standard deviation or median (range), and categorical variables as counts and percentages.

RESULTS

Patient Characteristics

Four male patients underwent simultaneous repair of PE and cardiac pathology during the study period. The mean age at the time of surgery was 37.0±26.2 years (range: 20-78 years). Two patients presented with symmetric PE, while the remaining two had asymmetric deformities. One patient had a history of surgically treated severe scoliosis as an associated musculoskeletal anomaly, whereas the other three had no additional congenital or acquired anomalies. A positive family history of pectus deformity was documented in one patient.

Operative Details

The surgical indications varied among the patients and included atrial septal defect (ASD), aortic root dilatation with valve insufficiency, coronary artery disease, and mitral valve prolapse. Accordingly, the concomitant cardiac procedures performed were: ASD repair (n=1), aortic root replacement using the David procedure (n=1), coronary artery bypass grafting (5-vessel, n=1), and mitral valve replacement (n=1). All patients underwent simultaneous Nuss repair in the same operative session. The mean operative time was 385±192 minutes (range: 250-690 minutes). Two patients required two

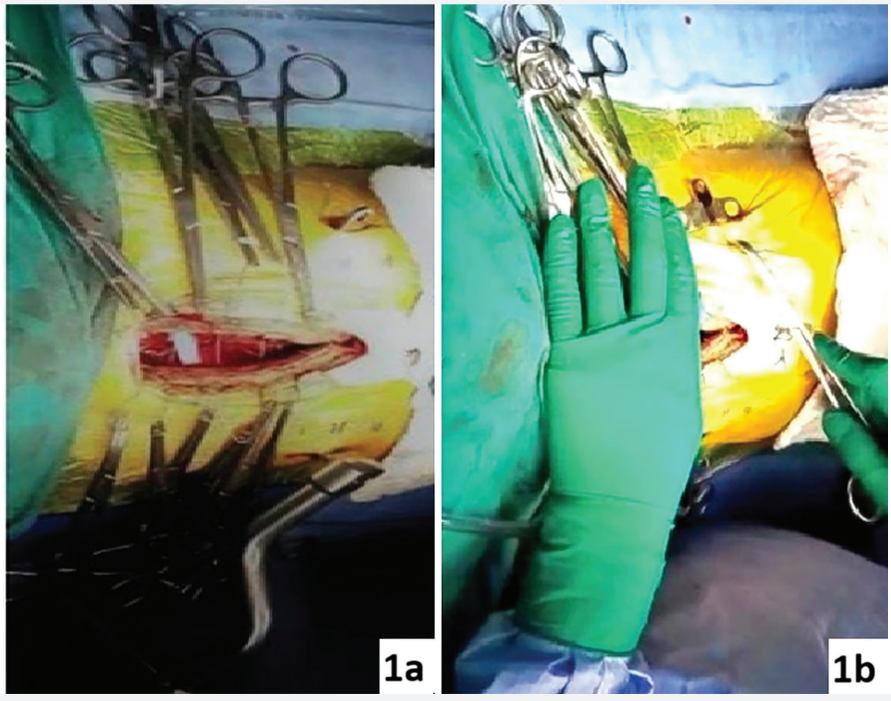

Figure 1. (a) Placement of the pectus bar after positioning sternal wires but before tightening, illustrating the sequence to avoid interference between sternal closure and bar insertion. (b) Final position of the pectus bar after 180° rotation, with stabilizers secured in place prior to sternal wire tightening

pectus bars, while the other two were treated with a single bar. In all cases, one stabilizer per bar was used, and no additional fixation such as wires or secondary stabilizers was needed. No intraoperative complications were encountered in any of the patients.

Postoperative Outcomes

One patient (25%) died on postoperative day 2 due to malignant arrhythmias and cardiac fibrillation, despite resuscitation. Among the remaining three patients, one developed a left-sided pleural effusion during the first postoperative month, which was successfully managed with tube thoracostomy. No postoperative complications were observed in the other two patients. Postoperative hospital stay durations for the surviving patients were 15, 19, and 6 days, respectively. During follow-up, pectus bars were electively removed in two patients, while one patient still retains the implanted bar. A summary of individual patient characteristics, surgical procedures, and postoperative outcomes is presented in Table 1. Representative preoperative and postoperative clinical and radiological images from two patients are presented in Figure 2. The median follow-up period for surviving patients was 58 months (range: 14-108 months).

DISCUSSION

PE, while often regarded as a cosmetic concern, may have functional cardiopulmonary implications, especially in patients requiring cardiac surgery[4]. In this single-center retrospective study, we present our institutional experience with four patients who underwent simultaneous open-heart surgery and PE repair using the Nuss procedure. Our findings suggest that simultaneous repair using this approach is technically feasible and can be performed safely in selected patients, though further studies with larger cohorts are needed to confirm its generalizability. These results add to the growing body of literature supporting the combined correction of chest wall deformities and cardiac pathology in selected patients[7-10]. Surgical correction of PE after prior median sternotomy remains technically challenging due to retrosternal adhesions, distorted mediastinal anatomy, and potential cardiac injury during bar placement[5,6]. In a multi-institutional analysis of 77 patients with prior sternotomy undergoing the Nuss procedure, Jaroszewski et al.[6] reported intraoperative cardiac injury in 7% of cases, while also concluding that MIRPE can be performed safely in experienced centers—highlighting the importance of careful planning in reoperative settings[6]. In our cohort, MIRPE was performed concurrently with median sternotomy (i.e., without prior sternotomy), and no intraoperative complications related to bar passage were observed. Simultaneous correction of PE and cardiac pathologies during a single surgical session offers notable clinical and logistical advantages. Compared to staged procedures, this approach eliminates the need for a second general anesthesia, reduces the cumulative operative burden,

Table 1. Demographic, operative, and postoperative details of patients undergoing simultaneous pectus excavatum and cardiac surgery

Variable	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	20	21	78	29
Sex	Male	Male	Male	Male
PE symmetry	Symmetric	Symmetric	Asymmetric	Asymmetric
Associated anomaly	None	Scoliosis (op) + Marfan	None	None
Family history of PE	No	No	No	No
Haller index	6.8	16.6	3.8	4.5
Cardiac diagnosis	ASD	Aortic root dilatation	CAD	Mitral valve prolapse/ regurgitation
Cardiac procedure	ASD repair	David procedure (aortic root replacement)	CABG ×5	Mitral valve replacement
Number of pectus bars used	2 bars	2 bars	1 bar	1 bar
Operation time (min)	250	690	290	310
Intraoperative complications	None	None	None	None
Postoperative complications	None	Malignant arrhythmia (exitus POD2)	None	Pleural effusion (drained)
Hospital stay (days)	15	2 (exitus)	19	6
Follow-up (months)	108	-	25	14
Bar removal	Yes	-	Yes	No

PE: Pectus excavatum, ASD: Atrial septal defect, CAD: Coronary artery disease, CABG: Coronary artery bypass grafting, POD: Postoperative day

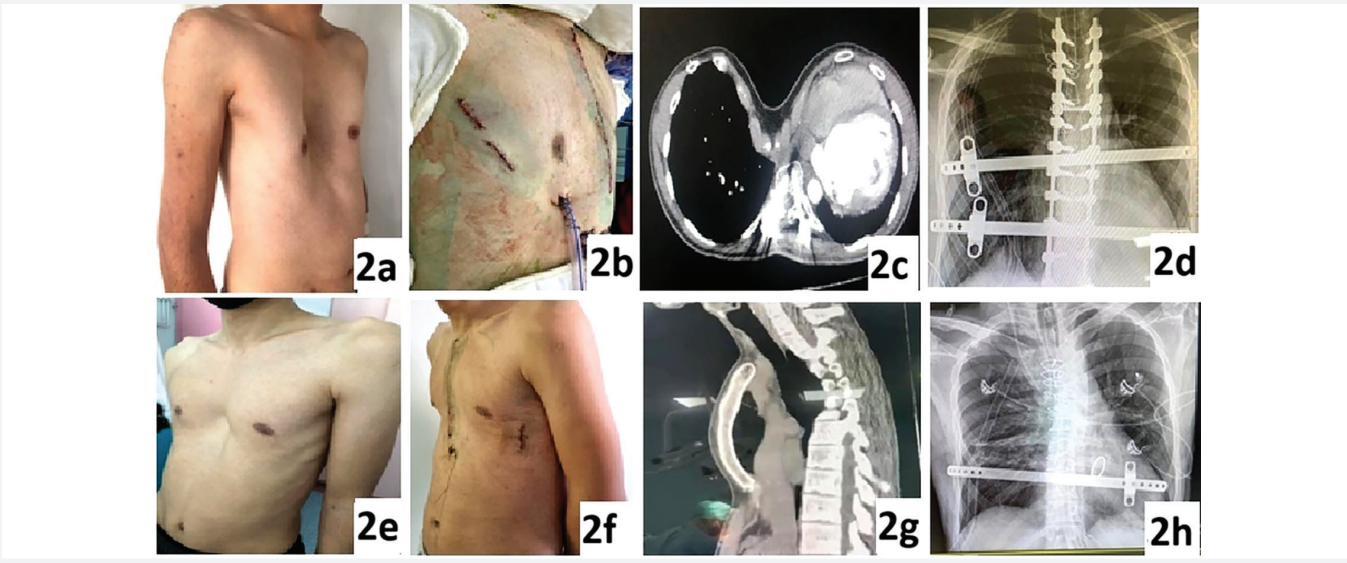

Figure 2. Representative images from two patients undergoing simultaneous cardiac surgery and MIRPE

2a, 2e: Preoperative clinical appearance, 2b, 2f: Postoperative clinical appearance, 2c, 2g: Preoperative chest CT, 2d, 2h: Postoperative chest X-rays showing bar position

MIRPE: Minimally invasive repair of pectus excavatum, CT: Computed tomography

and prevents the development of retrosternal adhesions that may complicate later pectus repair[7,8]. Additionally, performing the Nuss procedure in an untouched mediastinal plane provides optimal anatomical exposure and minimizes the risk of iatrogenic injury. These benefits are especially valuable in pediatric and young adult patients, in whom early repair may provide both cosmetic and cardiopulmonary improvements[7,8]. The feasibility and safety of such simultaneous interventions have been documented in various institutional series. Willekes et al.[11] reported a 26-year review including simultaneous intracardiac and chest wall deformity repairs, highlighting favorable outcomes in selected patients. Similarly, Javangula et al.[12] described successful correction of severe pectus deformity and aortic root pathology in a single-stage operation. In our series, simultaneous repair allowed optimal exposure for both procedures and eliminated the need for a second intervention, with no intraoperative complications observed. Bar preparation was performed while the cardiac procedure was ongoing, and the additional operative time attributable to bar placement and fixation was approximately 30 minutes. Thus, combining both procedures in a single session avoided reoperation and a second exposure to general anesthesia without increasing perioperative risk. One patient in our series died postoperatively due to complications related to the underlying cardiac condition, not the PE repair. During simultaneous cardiac surgery and pectus repair, care must be taken to avoid mechanical interference between the sternal wires and the pectus bar stabilizers. In our technique, bar fixation was synchronized with sternal closure, allowing for secure anchoring of both systems without complication. This approach eliminated the need for additional fixation methods such as supplemental sutures or bilateral stabilizers, thereby reducing hardware burden and operative complexity. Prior reports have similarly emphasized the importance of careful intraoperative planning in combined procedures to ensure structural integrity and minimize complications[8]. During a median follow-up of 58 months, no cases of sternal instability, bar displacement, or chronic pain were observed. Cosmetic outcomes were satisfactory in all surviving patients, with no residual chest wall deformity or functional limitation reported. Notably, the prolonged operative times observed in this cohort were primarily due to the complexity of the cardiac surgeries rather than the pectus repair itself, which typically requires approximately 60 minutes when performed in isolation. Based on our experience, simultaneous repair may be considered in carefully selected patients, particularly when the chest wall deformity is expected to cause persistent cardiopulmonary compromise despite successful cardiac surgery. This approach can optimize postoperative recovery by addressing both structural and functional issues in a single session.

Study Limitations

This study has several limitations. First, the small sample size and retrospective nature of the analysis limit the ability to draw definitive conclusions or perform statistical comparisons. Second, the absence of a control group precludes direct comparison with staged repair strategies. Additionally, this is a

single-center experience, and surgical expertise or institutional preferences may influence the reproducibility of the results in other settings.

CONCLUSION

Simultaneous correction of PE and cardiac pathology using the Nuss procedure during median sternotomy is technically feasible and safe, even in complex cases. This combined approach eliminates the need for a second surgery, reduces operative risk associated with retrosternal adhesions, and ensures optimal anatomical exposure. Our experience demonstrates that with appropriate planning and surgical coordination, favorable outcomes can be achieved without additional morbidity. These findings support the broader implementation of single-stage repair in selected patients undergoing open-heart surgery.

Ethics

Ethics Committee Approval: This study was approved by the Institutional Ethics Committee of Memorial Ataşehir Hospital (approval number: 2025/19, date: 13.08.025).

Informed Consent: This is retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ç,Ç, Concept: M.A., Design: L.Ç.O., Data Collection or Processing: T.B., Analysis or Interpretation: Ç.Ç., L.Ç.O., Literature Search: Ç.Ç., L.Ç.O., Writing: Ç.Ç.

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

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ORIGINAL ARTICLE

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Prevalence of Restless Legs Syndrome and Associated Factors in Nurses: A Multicenter Study in İstanbul (Türkiye)

Hemşirelerde Huzursuz Bacak Sendromu Prevalansı ve İlişkili Faktörler: İstanbul'da (Türkiye) Çok Merkezli Bir Çalışma

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ABSTRACT

Aim: Restless legs syndrome (RLS) primarily occurs in the evenings and is characterized by uncomfortable sensations such as numbness, tingling, burning, or pain that compel leg movement. This study aimed to determine the prevalence of RLS among hospital-based nurses and to explore its associations with health issues, symptoms, and quality of life.

Materials and Methods: This observational study included both retrospective and cross-sectional components and evaluated nurses aged 18-65 years working at two tertiary hospitals (University of Health Sciences Türkiye, Physical Medicine and Rehabilitation and University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital) between May 1, 2024, and February 1, 2025. A total of 488 participants were assessed, of whom 233 were diagnosed with RLS.

Results: RLS was diagnosed in 233 participants, with a prevalence of 47.7%. Patients in the severe group had a higher prevalence of doctor visits compared to other groups ($p<0.003$). Thyroid-stimulating hormone levels differed significantly according to disease severity (p-value). Older age at the onset was found to be a risk factor that decreases the severity of the disease. Lack of a family history of the disease was correlated with reduced disease severity. There was a difference between age at disease onset and having a chronic disease. Having a chronic disease increased the age at disease onset by 5.4 (confidence interval: 3.0-7.8) units. The age at the onset of the disease was older in patients who were non-alcohol users.

Conclusion: Certain factors, including age at disease onset, thyroid dysfunction, family history, and alcohol use, were found to influence the severity and onset of the disease. Patients in the severe group visit doctors more often than those in the mild or moderate groups. This may indicate that the severity of RLS leads to more frequent medical interventions or concerns.

Keywords: Chronic disease, nurses, prevalence, restless legs syndrome, thyroid-stimulating hormone, shift work

ÖZ

Amaç: Huzursuz bacak sendromu (RLS), genellikle akşam saatlerinde ortaya çıkan; uyuşma, karıncalanma, yanma veya ağrı gibi rahatsız edici hislerle karakterize, bireyi bacaklarını hareket ettirmeye zorlayan bir nörolojik hastalıktır. Bu çalışmanın amacı, hastanelerde görev yapan hemşirelerde RLS prevalansını belirlemek ve hastalığın sağlık sorunları, semptomlar ve yaşam kalitesiyle olan ilişkisini incelemektir.

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Gereç ve Yöntem: Bu gözlemsel çalışma hem retrospektif hem de kesitsel bileşenleri içermektedir. 1 Mayıs 2024-1 Şubat 2025 tarihleri arasında İstanbul'daki iki üçüncü basamak hastanede (Sağlık Bilimleri Üniversitesi, İstanbul Fizik Tedavi ve Rehabilitasyon Eğitim ve Araştırma Hastanesi ve Sağlık Bilimleri Üniversitesi, Bakırköy Dr. Sadi Konuk Eğitim ve Araştırma Hastanesi) çalışan 18-65 yaş arası hemşireler değerlendirilmiştir. Toplam 488 katılımcı incelenmiş olup, bunlardan 233'üne RLS tanısı konulmuştur.

Bulgular: Katılımcıların %47,7'sinde (n=233) RLS tespit edilmiştir. Şiddetli RLS grubundaki bireylerin diğer gruplara kıyasla daha sık doktora başvurduğu belirlenmiştir (p<0,003). Hastalık şiddeti ile tiroid uyarıcı hormon düzeyleri arasında istatistiksel olarak anlamlı bir fark bulunmuştur. Hastalık başlangıç yaşının artması, hastalık şiddetinde azalma ile ilişkili bulunmuştur. Aile öyküsünün olmaması, hastalık şiddetini azaltan bir diğer faktör olarak saptanmıştır. Kronik hastalığı olan bireylerde hastalık başlangıç yaşı ortalama 5,4 birim (güven aralığı: 3,0-7,8) daha ileri yaşta bulunmuştur. Ayrıca, alkol kullanmayan bireylerde hastalık başlangıç yaşının daha yüksek olduğu gözlenmiştir.

Sonuç: Hastalık şiddeti ve başlangıcı üzerinde etkili olan faktörler arasında hastalık başlangıç yaşı, tiroid fonksiyon bozukluğu, aile öyküsü ve alkol kullanımı yer almaktadır. Şiddetli RLS grubundaki bireylerin daha sık tıbbi yardım arayışında bulunmaları, hastalık şiddetinin artmasının sağlık hizmeti başvurularını artırabileceğini göstermektedir. Bulgular, hemşirelerde RLS'nin erken tanılanması ve risk faktörlerinin yönetimi açısından önemli bilgiler sunmaktadır.

Anahtar Kelimeler: Kronik hastalık, hemşire, prevalans, huzursuz bacak sendromu, tiroid stimülan hormon, vardiyalı çalışma düzeni

INTRODUCTION

Restless legs syndrome (RLS), also known as willis-ekbom disease, is a chronic, progressive movement disorder characterized by abnormal sensations that occur with the urge or need to move the legs[1-3]. Complaints are prominent in the evening hours and occur in resting or immobilized conditions, with partial or complete relief provided by walking and moving the legs[2-4]. The prevalence of RLS in nursing staff is around 25%, with the syndrome also linked to shift work disorder[5]. It has been reported to be twice as common in women as in men, and sleep disturbances are present in 60-90% of patients[6]. There is no specific test for the diagnosis of RLS, and the diagnosis is made according to the diagnostic criteria first established by the International RLS Study Group (IRLSSG) and last revised in 2014[7]. RLS is a very common and underdiagnosed disorder in the community. RLS is divided into two groups as primary and secondary. Primary RLS is an idiopathic condition without any symptomatic cause. Secondary RLS develops due to a cause such as chronic renal failure, diabetes mellitus, pregnancy, hypertension, anemia, polyneuropathy, Sjögren's syndrome, Parkinson's disease (PD), congestive heart failure, sleep apnea syndrome, rheumatoid arthritis, or multiple sclerosis[8]. Although the pathophysiology of RLS has been focused on dopaminergic system disorder and iron deficiency, the pathophysiology of the disease is not clearly clarified[9].

Healthcare workers work under severe stress factors (psychological and physiological violence) with long shift hours and are frequently on call. Studies have reported that shiftwork may be associated with various serious health problems[10]. RLS has been shown to decrease sleep quality and cause daytime sleepiness, insomnia, and daytime dysfunction[11]. Sleep apnea syndrome is frequently associated with RLS[12]. Lack of restorative sleep can lead to problems with concentration, mood, and overall cognitive functioning during the day.

RLS can be a significant health concern for health professionals who stand or walk for long periods of time, such as nurses. Prolonged standing, fast walking, and the need to be physically active can make the symptoms of RLS more pronounced. To understand whether RLS is an important health problem for nurses, to increase awareness among nurses about the symptoms and effects of this disorder, thus helping nurses to provide better support to patients and guide them correctly. It is aimed to contribute to improving the professional performance of nurses with RLS by providing effective treatment.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of University of Health Sciences Türkiye, İstanbul Physical Medicine and Rehabilitation Training and Research Hospital (approval number: 2024/2024-14, date: 30.04.2024) and conducted according to the Declaration of Helsinki principles. Since the study was retrospective, informed consent was not obtained from the patients.

Study Design and Population

This observational study included both retrospective and cross-sectional components. All nurses aged 18-65 who worked at University of Health Sciences Türkiye, Physical Medicine and Rehabilitation or University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Researc Hospital between 1 May 2024 and 1 February 2025 were evaluated. A total of 488 participants were assessed, of whom 233 were diagnosed with RLS.

Diagnosis of RLS was made according to the criteria of the International IRLSSG updated in 2014, and the RLS positive group was examined in this study[7]. Additionally, diagnosed patients were assessed with the severity scale questionnaires developed by the IRLSSG. Nurses who did not volunteer to participate in the study and who had diseases that increase the incidence of RLS (diabetes mellitus, chronic renal failure,

chronic kidney and liver diseases), used sedative drugs, and pregnant women were not included in the study.

The classification of RLS severity in the current study was indeed based on the IRLSSG rating scale, which is a validated and widely used tool for assessing RLS severity. According to this scale, scores are categorized as follows: mild (1-10), moderate (11-20), severe (21-30), and very severe (31-40)[13].

All volunteers who participated in the study were interviewed face-to-face, explained about the questionnaire, and informed about the purpose and method of the study. The questionnaire was administered by the same person (neurological specialist) without taking the name of the participant. Demographic characteristics, clinical features, and laboratory findings obtained during outpatient clinic follow-up of all cases were evaluated.

Laboratory Parameters

All blood samples were collected from the antecubital vein between 08:00-11:30 in the morning after 8-12 hours of fasting. Samples for thyroid-stimulating hormone (TSH), T4, vitamin B12, and 25-OH vitamin D tests were taken in a biochemistry tube with gel separator from Vacusera (İzmir, Türkiye). Following clotting, serum was obtained by centrifugation at 4000xg for 10 minutes. Tests were routinely measured on Roche Modular Analytics cobas 8000 Immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany) within 4 hours after centrifugation. Haemograms were performed on whole blood samples with EDTA from Vacusera (İzmir, Türkiye) using the Focusing Flow-DC method on a Mindray BC-6200 (Shenzhen/China) automated haematology analyzer.

Statistical Analysis

IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data. The normality of the variables was evaluated using the Kolmogorov-Smirnov test, Q-Q plots, and histograms. Continuous variables are presented as mean ± standard deviation and median (25^{th}-75^{th} percentile). Categorical data were presented as frequency (percentage). The relationship between categorical variables was evaluated by the chi-squared test or Fisher's exact test. The Mann-Whitney U test was used for two independent group comparisons, and the Kruskal-Wallis test was used for more than two independent group comparisons. The relationship between continuous data was evaluated with Spearman's correlation test. Variables with a p-value below 0.25 in univariate analyses were evaluated by multivariate logistic regression analysis to determine the factors affecting the development and severity of the RLS and the age of disease onset. Variance inflation factor was used to examine the interaction of independent variables in the logistic regression model. The model excludes variables with high correlation. Outliers in the data were examined by showing cook's values. Hosmer-Lemeshow fit statistics were used to assess model fit. For all analyses, $p<0.05$ was considered significant.

RESULTS

In this study, 488 participants with a mean age of 31.1±8.0 years and 79.1% women were evaluated. RLS was diagnosed

Table 1. Comparison of demographic and clinical characteristics between study groups

Characteristics	All participants	RLS (-) (n=255)	RLS (+) (n=233)	p-value
Age (years)	31.1±8.0	30.8±7.6	31.4±8.4	0.517
Sex				
Female	386 (79.1)	193 (75.7)	193 (82.8)	0.052
Male	102 (20.9)	62 (24.3)	40 (17.2)	
Smoking				
Yes	164 (33.6)	84 (32.9)	80 (34.3)	0.745
No	324 (66.4)	171 (67.1)	153 (65.7)	
Alcohol use				
Yes	86 (17.6)	48 (18.8)	38 (16.3)	0.466
No	402 (82.4)	207 (81.2)	195 (83.7)	
Chronic disease				
Yes	93 (19.1)	43 (16.9)	50 (21.5)	0.197
No	395 (80.9)	212 (83.1)	183 (78.5)	

Mann-Whitney U test, Pearson chi-square test, n (%), mean ± SD

RLS: Restless legs syndrome, SD: Standard deviation

in 233 participants, with a prevalence of 47.7% in the study. While the mean age of RLS positive cases was 31.4±8.4 years, the mean age of RLS negative cases was 30.8±7.6 years. There was no statistically significant difference in the demographic characteristics of the cases (p>0.05). The comparison of the demographic characteristics of the evaluated cases is presented in Table 1.

Risk factors for the development of RLS were evaluated by multivariate regression analysis. Participants' demographic variables of sex and chronic disease were not found to be independent risk factors for RLS (p>0.05). There were 233 RLS cases evaluated in the study, with an age range of 20-64 years, with a mean age of 31.4±8.4 years. 193 (82.8%) of the cases were female. Age at onset of the disease median value was 25.0 (23-29). The comparison of the demographic characteristics of the evaluated cases is presented in Table 2.

RLS disease severity [median (n: 233; 25-75) percentil] was classified as mild in 31(13.3), moderate in 121(51.9), severe in 77 (33.0), and very severe in 4 (1.7).

The association between demographic and clinical characteristics of RLS patients and disease severity was analyzed (Table 3). Patients in the severe group had a higher frequency of doctor visits than the other groups. Post-hoc analyses showed that doctor control was different in moderate and severe groups (p<0.003). There was a statistical difference between disease severity and TSH level (p=0.04). Post-hoc analysis showed that the mild group was different from the moderate and severe groups. Patients in the mild group had lower TSH levels compared to the other groups. There was no significant correlation between disease severity and other demographic and clinical parameters (p>0.05).

The factors related to the severity of the syndrome were evaluated. Older age at onset was found to be a risk factor that decreased the severity of the disease [odds ratio (OR): 1.047; 95% confidence interval (CI): 1.007-1.089, p=0.02]. The presence of a family history was a risk factor that increased the severity of the disease (OR: 1.808; 95% CI: 1.006-3.251, p=0.04). The severity of the disease was not influenced by either sex or the presence of a chronic disease (Table 4).

There was a significant difference between age at disease onset and having a chronic disease. Age at disease onset was older in patients with chronic disease compared to patients without chronic disease (respectively, 32.0±11.3; 26.5±6.4, p=0.005). Having a chronic disease increased the age at disease onset by 5.4 (95% CI: 2.986-7.819 p<0.001) units.

There was a significant difference between alcohol use and age at the onset of disease. The age at the onset of the disease was higher in patients who were non-alcohol users (respectively, 28.2±8.3; 25.3±6.2, p=0.046) (Table 5).

Table 2. Evaluation of clinical and demographic characteristics of Restless Legs Syndrome patients (n=233)

Characteristics	n (%) median (25-75 percentile)
Sex	
Female	193 (82.8)
Male	40 (17.2)
Age (years)	28.0 (26-33)
Chronic disease (yes)	50 (21.5)
Smoking (yes)	80 (34.3)
Alcohol use (yes)	38 (16.3)
Family history (yes)	75 (32.2)
Doctor check-up (yes)	27 (11.6)
Treatment (yes)	2 (0.9)
Upper extremity involvement (yes)	2 (0.9)
Age at onset of the disease (years)	25.0 (23-29)
Hemoglobin (g/dL)	12.9 (11.9-14.0)
Ferritin (ng/mL)	32.2 (15.6-57.1)
TSH (µIU/mL)	1.7 (1.2-2.5)
T4 (ng/dL)	1.2 (1.1-1.4)
BUN (mg/dL)	22.0 (18.1-27.0)
Creatine (mg/dL)	0.7 (0.6-0.8)
B12 vitamin (pg/mL)	324 (256-402.5)
D vitamin (ng/mL)	19.4 (12.3-26.0)

TSH: Thyroid-stimulating hormone, T4: Thyroxine, BUN: Blood urea nitrogen

Table 3. Comparison of demographic and clinical characteristics between RLS groups

Characteristics	Restless legs syndrome severity				
	Mild	Moderate	Severe	Very severe	p-value
Age (years)	27.0 (25-38)	28.0 (26-36)	28.0 (26-30)	37.5 (26.75-51.25)	0.459
Sex					
Female	27 (87.1)	98 (81.0)	65 (84.4)	3 (75.0)	0.704
Male	4 (12.9)	23 (19.0)	12 (15.6)	1 (25.0)	
Age at onset of the disease (years)	25.0 (24-35)	25.0 (23-30)	25 (23-28)	26.0 (20.75-38.0)	0.575
Family history					
Yes	10 (32.3)	32 (26.4)	31 (40.3)	2 (50.0)	0.156
No	21 (67.7)	89 (73.6)	46 (59.7)	2 (50.0)	
Doctor check-up					
Yes	1 (3.2)	8 (6.6)	17 (22.1)	1 (25.0)	0.003
No	30 (96.8)	113 (93.4)	60 (77.9)	3 (75.0)	
Treatment					
Yes	0 (0.0)	0 (0.0)	2 (2.6)	0 (0.0)	0.246
No	31 (100.0)	121 (100.0)	75 (97.4)	4 (100.0)	
Upper extremity involvement					
Yes	0 (0.0)	0 (0.0)	2 (2.6)	0 (0.0)	0.246
No	30 (100.0)	120 (100.0)	75 (97.4)	4 (100.0)	
Smoking					
Yes	14 (45.2)	38 (31.4)	28 (36.4)	0 (0.0)	0.255
No	17 (54.8)	83 (68.6)	49 (63.6)	4 (100.0)	
Alcohol use					
Yes	6 (19.4)	18 (14.9)	14 (18.2)	0 (0.0)	0.829
No	25 (80.6)	103 (85.1)	63 (81.8)	4 (100.0)	
Chronic disease					
Yes	9 (29.0)	21 (17.4)	18 (23.4)	2 (50.0)	0.167
No	22 (71.0)	100 (82.6)	59 (76.6)	2 (50.0)	
Hemoglobin (g/dL)	12.7 (11.5-14.2)	13.0 (12.2-13.9)	12.5 (11.7-14.0)	13.2 (12.9-15.1)	0.433
Ferritin (ng/mL)	31.1 (14.5-57.7)	38.4 (18.4-58.5)	25.6 (13.6-59.5)	26.6 (18.8-180.3)	0.252
TSH (µIU/mL)	1.3 (1.1-1.6)	1.9 (1.2-2.7)	1.8 (1.2-2.3)	1.5 (1.1-1.6)	0.04
T4 (ng/dL)	1.2 (1.1-1.3)	1.2 (1.1-1.3)	1.2 (1.1-1.4)	1.1 (0.5-1.3)	0.734
BUN (mg/dL)	24.1 (20-28)	21.7 (17.9-26.0)	22.2 (18.2-27.7)	27.3 (15.7-40.2)	0.340
Creatine (mg/dL)	0.7 (0.6-0.8)	0.7 (0.6-0.8)	0.7 (0.6-0.8)	0.7 (0.7-1.0)	0.803
B12 vitamin (pg/mL)	331 (256-417)	298 (234-386.5)	334 (279-418)	320 (223.2-482.0)	0.246
D vitamin (ng/mL)	18.4 (13.9-24.0)	19.8 (13.2-26.0)	19.7 (10.8-26.4)	23.5 (13.3-41.1)	0.721

Fisher's exact test, Kruskal-Wallis test, n (%), median (25.-75. percentil)

RLS: Restless legs syndrome, TSH: Thyroid-stimulating hormone, T4: Thyroxine, BUN: Blood urea nitrogen

DISCUSSION

The pathophysiology of RLS is not yet fully understood; however, a localized decrease in dopamine levels within the central nervous system is considered to play a major role by triggering a hyperadrenergic state. In the current study, RLS was diagnosed in 233 participants, with a prevalence of 47.7%. Older age at the time of disease onset was associated with a reduction in disease severity, while the presence of a family history of RLS appeared to reduce the severity as well. Furthermore, alcohol consumption and the presence of chronic diseases influenced the age of disease onset, with non-alcohol users and those with chronic conditions experiencing the disease onset at older ages. TSH levels were significantly different across the severity groups, suggesting that thyroid functions may play a role in the development or progression

Table 4. The factors related to the severity of the syndrome

Characteristics	Multivariate analysis-enter method		Multivariate analysis- forward: LR method	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age at onset of the disease	1.047 (1.007-1.089)	0.02	1.041 (1.002-1.081)	**0.03**
Family history (ref: yes)	1.808 (1.006-3.251)	0.04	1.909 (1.071-3.403)	**0.02**
Sex (ref: female)	1.103 (0.523-2.328)	0.796	-	-
Chronic disease (ref: yes)	0.668 (0.333-1.341)	0.256	-	-

Forward LR and enter methods were used for logistic regression analysis.

Enter model: Hosmer Lemeshow test p= 0.978, Cox & Snell R^2= 0.043, Nagelkerke R^2= 0.059, -2 Log Likelihood= 290.756

Forward LR model: Hosmer Lemeshow test p= 0.708, Cox & Snell R^2= 0.037 Nagelkerke R^2= 0.051 -2 Log Likelihood= 292.163

OR: Odds ratio, CI: Confidence interval, LR: Likelihood ratio

Table 5. Factors related to the age of disease onset

Characteristics	Unstandardized coefficients B	Std. error	Standardized coefficients beta	t	p-value	95% CI
Alcohol use	2.864	1.363	0.132	2.101	0.037	0.179-5.549
Chronic disease	5.402	1.226	0.276	4.405	0.000	2.986-7.819

CI: Confidence interval, Std: Standard

of RLS. The study emphasizes the significance of considering demographic, lifestyle, and clinical factors in understanding the progression and severity of the disease, which can inform future treatment and management strategies for RLS patients.

The prevalence of RLS in nursing staff is approximately 25%[5]. In the current study, RLS was diagnosed in 233 participants, with a prevalence of 47.7%. The results of our study showed that nearly half of the subjects had RLS (+), of which one-third had severe and higher-grade RLS. The results of a study targeting to investigate the effects of the circulating shift schedule, chronotype, and RLS on sleep quality of female nurses and midwives in Japan showed that the prevalence of RLS and restless foot movement was 2.5% and 15.5%, respectively[14]. In another study, it can be concluded that RLS plays a significant role in contributing to fatigue in critical care nurses. However, it's important to note that previous studies have suggested fatigue can also be a contributing factor to the development of RLS. The relationship between these two conditions may vary depending on the individual case, with different causes and effects in each situation[15]. In a study conducted by Waage et al.[16] in Norway for the same purpose, the prevalence of RLS in nurses was 12.4% and the general prevalence was 26.8%, which was higher than the values obtained in the other study. The fact that health policies exhibit differences in determining the nurse-to-patient ratio when countries are compared may be one of the reasons for negative outcomes, such as work stress, burnout syndrome, and RLS. This suggests that improving staffing conditions and addressing these policy differences could help reduce the prevalence of RLS and related issues in nursing professionals.

Patients in the severe group had a higher frequency of doctor visits than the other groups. The findings suggest that patients in the severe group tend to visit doctors more frequently compared to those in the mild or moderate groups. This may indicate that the severity of the disease leads to more frequent medical interventions or concerns. The post-hoc analysis revealed a significant difference in doctor visits between the moderate and severe groups, which reinforces the idea that the severity of symptoms directly impacts healthcare utilization. Allen et al.[17] investigated the medical treatment received by participants in their study of RLS patients 3 months before their inclusion in the study. During these 3 months, 57.6% of participants with primary RLS had at least 1 visit to a primary care/general practitioner, with 36.4% of the visits reporting RLS-related visits. In contrast, 64.1% of RLS patients had at least 1 primary care/general practitioner visit, with 44% of these visits related to RLS. Additionally, 29.8% of participants with primary RLS had specialist visits (31.2% related to RLS), compared to 36.6% of RLS patients (37.5% related to RLS). Both medication and healthcare resource use costs related to RLS were significantly associated with symptom severity[17].

In the secondary form of RLS, the most common of which are symptoms, various clinical conditions may accompany it, such as iron deficiency, pregnancy, end-stage renal disease (uremia), thyroid dysfunction, Parkinsonism, depression, rheumatoid arthritis, fibromyalgia, diabetes mellitus, and multiple sclerosis[6,18-25]. In the current study, the statistically significant difference between disease severity and TSH levels indicates a potential link between thyroid function and RLS severity. However, no patients with hypothyroidism were found in the two groups. Lower TSH levels may be associated with more severe symptoms, or thyroid

dysfunction could be a contributing factor to the intensity of RLS. The post-hoc analysis further indicated that the mild group differed from both the moderate and severe groups, which suggests that TSH levels may play a role in differentiating the severity of RLS. Ahmed et al.[26] reported a higher prevalence of hypothyroidism in RLS patients compared to healthy controls in a case-control study they conducted on RLS patients. In contrast, Tan et al.[27] found no significant difference in the prevalence of RLS between patients with thyroid disorders and normal individuals. In another study, when RLS (+) patients were compared with RLS (-), serum TSH levels and subclinical hypothyroidism prevalence were found to be significantly higher in RLS (+) patients[28]. The higher serum TSH levels and subclinical hypothyroidism prevalence in RLS patients suggest that the imbalance between thyroid hormones and the dopaminergic system may contribute to the development of primary RLS. Overall, these findings underscore the need to consider thyroid function as part of the broader clinical evaluation of RLS, as it may help better understand the factors influencing disease severity and inform treatment strategies. Parathormone (PTH), rather than calcium, phosphate, or even vitamin D itself, appears to be linked to the presence of RLS symptoms in PD, with this association not being significantly affected by the patient's motor symptoms. Vitamin D may potentially alleviate leg restlessness, suggesting possible future insights into the pathophysiology and treatment of RLS in PD patients. PTH is believed to play a potential role in modulating pain perception, and prior studies on hyperparathyroidism have indicated a possible link between elevated PTH levels and the development or exacerbation of RLS symptoms[29]. Since thyroid dysfunction is clinically easier to detect as well as treat, it should be taken seriously, given that it may be a potentially modifiable risk factor for RLS[28].

The factors related to the severity of the syndrome were also evaluated in the present study. Older age at the onset was found to be a risk factor that decreases the severity of the disease. The absence of a family history of the disease was a risk factor that decreased the severity of the disease. Sex and having a chronic disease were not independent risk factors for the severity of the disease. There was a difference between age at disease onset and having a chronic disease. Age at disease onset was older in patients with chronic disease compared to patients without chronic disease. Having a chronic disease increased the age at disease onset by 5.4 units. There was a significant difference between alcohol use and age at the onset of disease. The age at the onset of the disease was older in patients who were non-alcohol users. Geng et al.[28] found no significant differences in age, gender, body mass index, smoking, or alcohol consumption between the RLS group and the healthy group. The age of onset of the secondary form is late, and its progression is rapid[30-32]. RLS occurs in 22% of patients detoxifying from alcohol. RLS is more severe in opioid detoxification compared to alcohol detoxification[3,33].

Study Limitations

This study has several limitations. First, the retrospective and questionnaire-based design relied on existing records and participant recall, which may introduce information and recall bias, potentially affecting the accuracy of reported symptoms and exposures. Second, the study was conducted in only two tertiary hospitals in İstanbul, and participation was voluntary, which may introduce selection bias and limit the generalizability of the findings to other regions or healthcare settings. Third, despite using multivariable analyses, residual confounding from unmeasured factors such as iron levels, work stress, or lifestyle variables cannot be excluded. Finally, as an observational study, only associations rather than causal relationships can be inferred.

CONCLUSION

RLS is a neurological sensory-motor disorder that should not be overlooked in clinical evaluation. Certain factors, including age at disease onset, thyroid dysfunction, family history, and alcohol use, were found to influence the severity and onset of the disease. The findings suggest that patients in the severe group tend to visit doctors more frequently compared to those in the mild or moderate groups. This may indicate that the severity of the disease leads to more frequent medical interventions or concerns. In patients with a prediagnosis of RLS, thyroid function parameters should be evaluated to determine whether hypo-/or hyperthyroid. More attention is needed to RLS in those with impaired thyroid function to prevent or treat this syndrome. In thyroid gland diseases, dopamine levels will become imbalanced and may trigger RLS. These tests are low-cost, widely available and promising parameters that can be performed in all health institutions, including primary care.

Ethics

Ethic Committee Approval: The study was approved by the Ethics Committee of University of Health Sciences Türkiye, İstanbul Physical Medicine and Rehabilitation Training and Research Hospital (approval number: 2024/2024-14, date: 30.04.2024) and conducted according to the Declaration of Helsinki principles.

Informed Consent: Since the study was retrospective, informed consent was not obtained from the patients.

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Footnotes

Author Contributions

Surgical and Medical Practices: N.E., A.T., M.Ç., A.Ç.A., Concept: N.E., H.U., A.T., M.Ç., S.N.A., A.Ç.A., Design: N.E., H.U., A.T., M.Ç., G.G., S.N.A., A.Ç.A., Data Collection or Processing: N.E., A.T., M.Ç., A.Ç.A., Analysis or Interpretation: N.E., H.U., A.T., M.Ç., G.G., S.N.A., A.Ç.A., Literature Search: N.E., H.U., A.T., G.G., S.N.A., A.Ç.A., Writing: N.E., H.U., A.T., M.Ç., G.G., S.N.A., A.Ç.A.

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ORIGINAL ARTICLE

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The Effects of Ficus carica Latex on SH-SY5Y Neuroblastoma Cells and L929 Fibroblast Cells

Ficus carica Lateksinin SH-SY5Y Nöroblastoma Kanser Hücreleri ve L929 Fibroblast Hücreleri Üzerine Etkileri

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ABSTRACT

Aim: This study aims to evaluate the anti-cancer effect of latex obtained from Ficus carica on SH-SY5Y neuroblastoma cells and its wound healing potential on L929 fibroblast cells.

Materials and Methods: The effects of Ficus carica latex were investigated *in vitro*. A scratch wound model was created on L929 cells and treated with 1-1000 µg/mL of Ficus carica latex. Microscopic images were taken at 0 and 48 hours to measure the wound areas. In SH-SY5Y cells, cytotoxicity was assessed using the MTT assay, and the IC_{50} value was determined.

Results: Ficus carica latex promoted significant wound healing in L929 cells, particularly at concentrations of 5 µg/mL and above, associated with increased proliferation. In SH-SY5Y cells, no cytotoxic effects were observed at 1-50 µg/mL, whereas cytotoxicity was evident at 100 µg/mL and 1000 µg/mL.

Conclusion: Ficus carica latex significantly promoted wound healing even at low doses, mainly by enhancing cell proliferation. On the other hand, it exhibited marked cytotoxic effects on neuroblastoma cells at high doses. These findings suggest that Ficus carica latex could be a potential therapeutic agent for wound healing but should be used in a dose-controlled manner for cancer cells like neuroblastoma.

Keywords: Anti-cancer, Ficus carica latex, SH-SY5Y, L929, wound healing

ÖZ

Amaç: Bu çalışmada, Ficus carica bitkisinden elde edilen lateksin, SH-SY5Y nöroblastoma hücreleri üzerindeki antikanser etkisi ile L929 fibroblast hücreleri üzerindeki yara iyileştirici potansiyelinin değerlendirilmesi amaçlanmaktadır.

Gereç ve Yöntem: Ficus carica lateksinin etkileri *in vitro* olarak incelenmiştir. L929 hücrelerinde oluşturulan yapay yara modeli, 1-1000 µg/mL Ficus carica lateks ile işlenmiş, 0. ve 48. saatlerde mikroskobik olarak yara alanları ölçülmüştür. SH-SY5Y hücrelerinde MTT testi uygulanarak hücre canlılığı ve IC_{50} değeri belirlenmiştir.

Bulgular: Ficus carica lateks L929 hücrelerinde 5 µg/mL ve üzeri konsantrasyonlarda proliferasyona bağlı yüksek yara iyileşmesi sağlamıştır. Ayrıca Ficus carica lateks SH-SY5Y nöroblastoma hücrelerinde 1-50 µg/mL arası düşük dozlarda sitotoksik etki göstermemiş, ancak 100 µg/mL ve 1000 µg/mL gibi yüksek dozlarda sitotoksisite gözlemlenmiştir.

Sonuç: Ficus carica lateks, düşük dozlarda bile yara iyileşmesini anlamlı şekilde teşvik etmiş ve bu etkisi özellikle proliferasyon artışıyla desteklenmiştir. Öte yandan, nöroblastoma hücreleri üzerinde yapılan çalışmalarda, Ficus carica lateks yüksek dozlarda belirgin sitotoksik etkiler gösterdiği tespit edilmiştir. Bu bulgular, Ficus carica lateksinin yara iyileşmesi açısından potansiyel bir tedavi ajanı olabileceğini, ancak nöroblastoma gibi kanser hücreleri üzerinde doz-kontrollü kullanılması gerektiğini göstermektedir.

Anahtar Kelimeler: Antikanser, Ficus carica lateksi, SH-SY5Y, L929, yara iyileşmesi

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INTRODUCTION

Neuroblastomas are malignant solid tumors formed from the progenitor cells of the sympathetic nervous system[1]. Neuroblastoma is responsible for about 7-10% of all cancers occurring in children and primarily affects infants and young children. It represents approximately 8-10% of all pediatric malignancies, with an estimated incidence of one case per 7000 live births[2-4]. Neuroblastoma is a type of cancer that typically lacks disease-specific symptoms. Symptoms usually appear when the tumor grows and begins to compress surrounding organs or when metastasis occurs[1]. The goal of treatment is to fully restore the patient's health while minimizing side effects that may develop during the disease course or treatment. Care is adapted to reflect the stage of the disease, distinct clinical features, and the likelihood of the condition returning. In certain cases, surgical excision of the tumor may be adequate, whereas others may need further interventions such as drug therapy and radiation treatment. For patients with a significant risk of recurrence, autologous stem cell transplantation and antibody-based immunotherapy are also applied in addition to these treatments[1].

The genus *Ficus* (Moraceae) ranks among the most extensive groups of flowering plants and consists of approximately 800 species worldwide[5,6]. These species are critical genetic assets, appreciated for both their economic worth and nutritional contributions[7]. The healing properties of Ficus carica have been recognized across traditional medical practices such as Ayurveda, Unani, and Siddha. This species has been traditionally employed in managing a wide spectrum of disorders, including metabolic conditions like diabetes, respiratory ailments such as asthma, cough, and liver-related problems, gastrointestinal issues including ulcers and vomiting, reproductive disorders like menstrual pain, as well as infectious and dermatological diseases such as scabies, gonorrhea, and other skin conditions[8]. Ficus carica holds therapeutic importance in Unani medicine due to its mild purgative, diuretic, and expectorant actions, and is employed to alleviate obstructions and inflammation in hepatic and splenic conditions[8]. The latex found in *Ficus* species has long attracted attention due to its medicinal properties and has been used as a drug[9]. Owing to its antiseptic and anti-inflammatory properties, it is applied topically in the treatment of skin disorders such as warts, boils[10], and dermatitis[11]. Historically, latex has been used as a wound-healing agent for rabid dog bites, snake and scorpion stings, and has also been employed in the treatment of wasp stings and hard swellings[12]. Latex has been applied in various mixtures for the treatment of diverse dermatological conditions[12]. Phytochemical studies on Ficus carica have revealed that various plant tissues, particularly latex, as well as leaves, fruits, and roots, contain isolated bioactive compounds including phytosterols, anthocyanins, phenolic derivatives, amino and organic acids, fatty acids, aliphatic alcohols, volatile compounds, hydrocarbons, and various secondary metabolites[13]. According to the literature, numerous studies have investigated the anti-cancer properties of Ficus carica latex (FCL). These studies demonstrate the antiproliferative effects of FCL in cancers such as gastric[14], brain[15], cervix[16], breast, liver cancer, and acute myeloid leukemia[17]. This suggests that FCL is a promising agent in cancer treatment, in addition to its traditional uses. In this study, based on its traditional use in wound healing, it is aimed to investigate the wound healing-supportive effects of FCL on the L929 fibroblast cell line to provide new insights for the literature; additionally, since its antiproliferative effects have been shown in many cancer studies, it is aimed to evaluate, for the first time, the antiproliferative and cytotoxic effects of FCL on the SH-SY5Y neuroblastoma cell line.

MATERIALS AND METHODS

In this study, the cytotoxic and wound healing effects of FCL were investigated *in vitro* on SH-SY5Y human neuroblastoma and L929 fibroblast cell lines. FCL was obtained from (aydindansoframa.com, Aydın, Türkiye). According to the description of the supplier: the latex was collected in September from the stalks of unripe fig fruits in the Aydın region, stored in amber-colored vials at 3 mL per vial. All experiments were performed using material from the same batch to ensure consistency within the study. Until the work was done, FCL was kept at +2-4 °C. FCL was weighed as 100 mg into eppendorf tubes, and 10 mL of Roswell Park Memorial Institute (RPMI) medium (Thermo Fisher Scientific, Gibco) was added to prepare a stock solution at a concentration of 10,000 µg/mL. The solution was sterilized by filtration through a 0.22 µm pore-sized polytetrafluoroethylene filter. The resulting stock solution was used for subsequent dilutions, and the unused portion was stored at -80 °C. Serial dilutions of this stock solution were prepared to obtain concentrations of 1, 2, 5, 10, 50, 100, and 1000 µg/mL, which were used for all cell culture experiments. The concentration range selected in this study was based on previously reported concentrations used for the evaluation of FCL in different cell lines. Indeed, the literature has shown that FCL exerts cytotoxic effects at concentrations ranging from 25 to 100 µg/mL in various cell lines[18]. An additional high concentration (1000 µg/mL) was included to evaluate potential non-specific cytotoxicity and to define the upper limit of the response curve. A broad range encompassing both low and high concentrations was chosen to enable a detailed investigation of the potential dose-response relationship in the cells. The study was conducted using cells previously obtained commercially from the American Type Culture Collection (ATCC) and available in stocks of the Department of Pharmacology at Atatürk University Faculty of Pharmacy and cultured under the same laboratory conditions. Cells were used within passages 12-15 for all experiments.

Authentication of the cell line was performed by ATCC using short tandem repeat (STR) profiling prior to distribution. No additional authentication was performed in our laboratory. The cytotoxic effect of FCL on SH-SY5Y cells was evaluated, while its wound healing-supportive effects on L929 cells were analyzed. There are no cells obtained from any living being in the study. The cells were purchased commercially. Therefore, informed consent and ethics committee approval are not required. For cell culture, RPMI 1640 medium containing 10% FBS (Gibco) was used to maintain the L929 cells, and 1% penicillin, streptomycin, amphotericin B antibiotic-antifungal mixture containing penicillin, streptomycin, and amphotericin B (Gibco), whereas SH-SY5Y cells were cultured in Kaighn's modification of Ham's F-12 medium (Thermo Fisher Scientific, Gibco). Standard protocols were applied to support cell proliferation and viability; incubation of cells occurred at 37 °C in a humidified atmosphere of 5% CO_2, with culture media renewal every 48 hours. After reaching confluence, cells forming a monolayer were washed with phosphate-buffered saline (PBS) (Thermo Fisher Scientific, Gibco), detached using trypsin, centrifuged, resuspended in fresh medium, and transferred to new culture flasks. Viable and dead cells were distinguished by 0.4% trypan blue staining, and cell counts were performed using a Thoma counting chamber. Cells were seeded at a density of 5×10^3 cells per well in 96-well plates for SH-SY5Y cytotoxicity assays and 1×10^6 cells per well in 12-well plates for the wound healing assay using L929 cells. All procedures were carefully performed to ensure reliable analysis of FCL's biological effects on the cells. The number of cells per 1 mL was calculated using the formula: cell count (in 1 mL)=n (number of cells counted in 0.1 mm^3) × dilution factor × 10^4.

MTT Cytotoxicity Assay

The MTT assay relies on viable cells' function in transforming MTT to insoluble formazan crystals. In the MTT assay, SH-SY5Y cells were divided into experimental groups, including a control group without FCL exposure and groups treated with FCL at concentrations of 1, 2, 5, 10, 50, 100, and 1000 µg/mL. SH-SY5Y cells underwent exposure to a range of concentrations of FCL (1-1000 µg/mL) and incubated for 24 hours. Afterwards, 100 µL of medium and 10 µL of MTT reagent (Acros Organics, China) were applied to all wells, with incubation conducted at 37 °C for 4 hours. The medium was then aspirated, and the formazan deposits were dissolved in 100 µL of dimethyl sulfoxide (Interlab, Türkiye). Using an enzyme-linked immunosorbent assay reader (Multiscan Sky, Thermo, Singapore), optical density at 570 nm was used to determine cell viability. Viability percentages were calculated relative to the untreated control group (% cell viability=(sample absorbance)/(mean control absorbance) × 100) and bar graphs were generated using GraphPad Prism 6 software. The same data were used to perform IC_{50} analysis. Thus, IC_{50} values were calculated in GraphPad Prism 6 using the "log (inhibitor) vs. normalized response" equation, which corresponds to a three-parameter logistic regression model (with the Hill slope fixed at -1). Data were normalized prior to curve fitting, and IC_{50} values are reported as best-fit estimates with 95% confidence intervals. The relationship between log concentration and viability was plotted as a line graph.

Wound Healing Assay

The wound closure assay was implemented on the L929 fibroblast cell line to evaluate cell migration and wound closure. Cells were seeded at 100,000 cells per well in 12-well plates, with eight different concentrations of FCL (1, 2, 5, 10, 50, 100, 1000 µg/mL, and control), each in triplicate. In the wound healing experiment, L929 cells were divided into experimental groups, including a control group without FCL treatment and groups treated with FCL at concentrations of 1, 2, 5, 10, 50, 100, and 1000 µg/mL. After formation of a confluent monolayer, a 100 µL yellow pipette tip was used to produce the scratch to create an artificial wound model (the average wound area in the initial scratches was calculated as 30,000 μm^2). The medium was aspirated. Following a PBS wash, the medium was replaced with fresh high-glucose culture medium[19]. The wound closure process was monitored at 0, 24, and 48 hours using an inverted microscope (Zeiss Axio Vert A1). The scratch area reduction was quantified, and wound closure area percentage calculated through analysis of images with ImageJ software[20]. For 24 hours: % wound closure=$[(A_0-A_{24})/A_0] \times 100$, for 48 hours: % wound closure=$[(A_0-A_{48})/A_0] \times 100$ (A_0=mean wound area at 0 hours A_{48}=mean wound area at 48 hours).

Statistical Analysis

IBM SPSS Statistics version 27 was employed for statistical analysis. Group means ± standard deviations are reported. One-way ANOVA and Duncan's post-hoc test were used to compare groups, considering p-values under 0.05 as statistically significant. Graphs were generated with the help of GraphPad Prism 6 software.

RESULTS

Wound Healing Findings in L929 Cells

Migration of L929 fibroblast cells in response to FCL treatment was evaluated through the scratch assay method (Figure 1). After 24 hours of incubation, wound closure rates in the control group and 1 µg/mL and 2 µg/mL FCL groups were calculated as 50.36%, 49.08%, and 50.85%, respectively. No statistically significant difference was found among these groups at 24 hours. In contrast, statistically significant increases in wound closure rates were observed at concentrations of 5, 10, 50, 100, and 1000 µg/mL FCL groups when compared to the control group at 24 hours. The closure rates at these concentrations

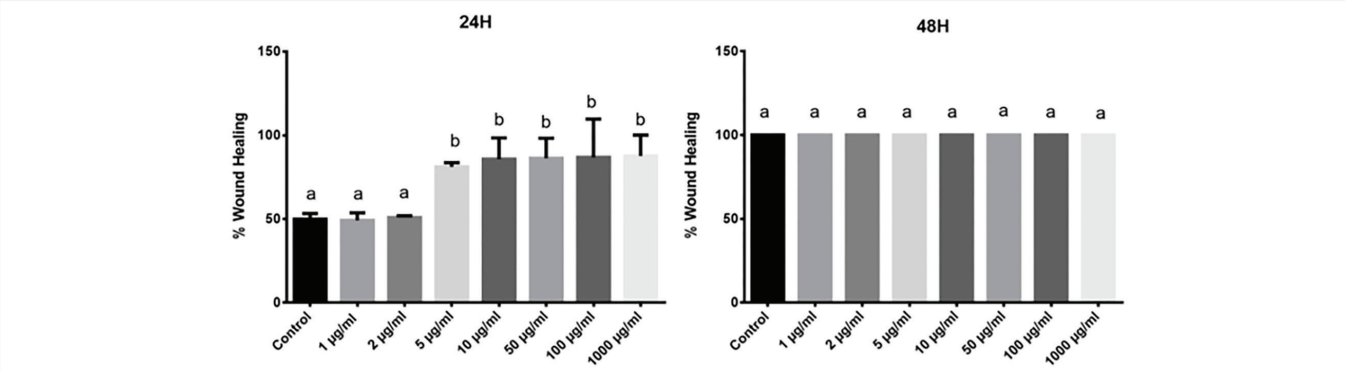

Figure 1. Effects of Ficus carica latex on the percentages of wound healing in L929 cells at 24 and 48 hours. The groups marked with the same letters (a,b) do not differ statistically significant from each other, whereas groups labeled with different letters indicate statistically significant differences ($p<0.05$). Data are expressed as mean ± standard deviation. Statistical significance was determined using one-way analysis of variance with Duncan's post-hoc test for each time point (24 and 48 hours) separately

were 81.05%, 85.72%, 86.23%, 86.74%, and 87.59%, respectively. After 48 hours of incubation, wound closure reached 100% in all groups. Wound closure images are given in Figure 2.

IC_{50} Findings of FCL on SH-SY5Y Cells

FCL was applied to SH-SY5Y human neuroblastoma cell cultures subjected to a concentration range of 1 to 1000 µg/mL, and cell viability was assessed by the MTT assay after 24 hours of incubation. As shown in Figure 3, viability rates were normalized and a dose-response curve was generated. The analysis revealed a decrease in cell viability with increasing FCL concentration, demonstrating a dose-dependent cytotoxic effect on SH-SY5Y cells. According to the 24-hour MTT data, the IC_{50} value of FCL was determined as 577.9 µg/mL.

MTT Findings of FCL on SH-SY5Y Cells

The results of the 24-hour MTT assay conducted on the SH-SY5Y cell line with various concentrations of FCL showed no statistically significant effect on cell viability at 1, 2, 5, 10, and 50 µg/mL concentrations. The cell viability rates at these concentrations were determined as 101.78%, 101.42%, 100.39%, 98.15%, and 95.09%, respectively. The average cell viability in 1-50 µg/mL concentration groups remained around 100%, and had no statistically significant differences compared with the control group. However, as shown in Figure 4, a statistically significant decrease in cell viability was detected at the concentration of 100 µg/mL, with the viability rate dropping to 75.85%. We found that the 100 µg/mL concentration had a statistically significant antiproliferative effect on SH-SY5Y cells. When exposed to the maximum concentration of 1000 µg/mL, cell viability was calculated as 40.67%, falling below the 50% threshold and indicating a marked cytotoxic effect of FCL. These findings demonstrate that FCL statistically significantly reduces cell viability in the SH-SY5Y cell line at 100 and 1000 µg/mL concentrations and that this effect occurs in a dose-dependent manner.

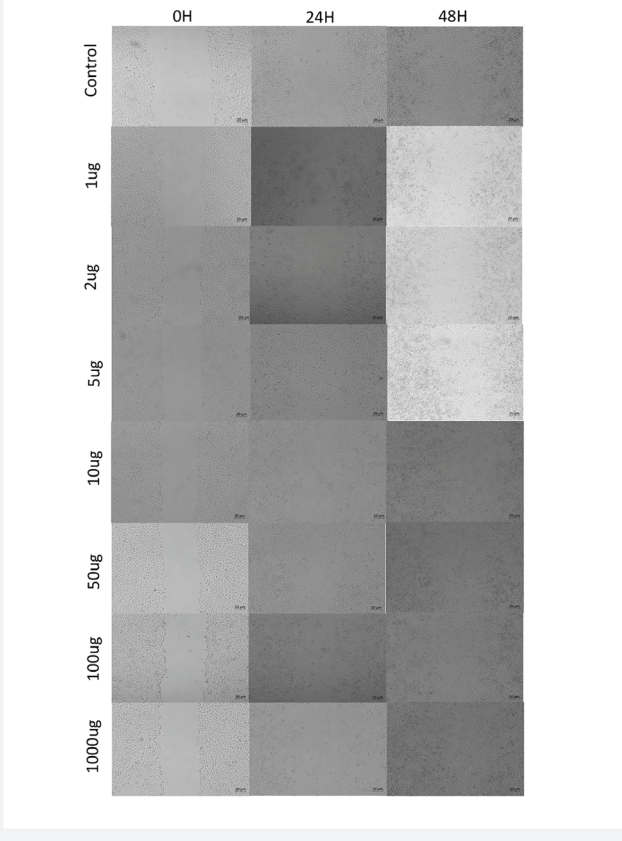

Figure 2. Effects of Ficus *carica* latex on wound healing in L929 cells at 24 and 48 hours

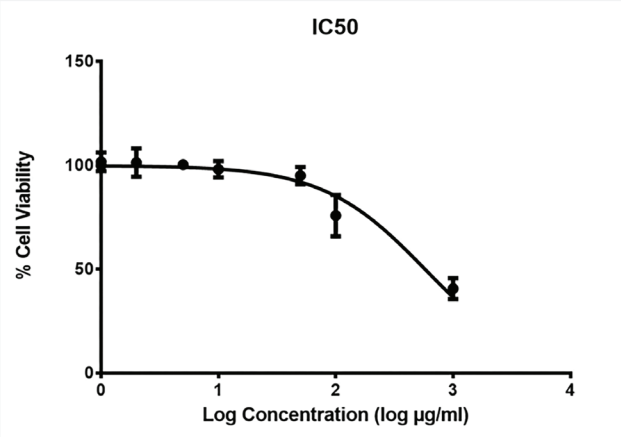

Figure 3. The effects of Ficus carica latex on SH-SY5Y neuroblastoma cells (IC_{50})

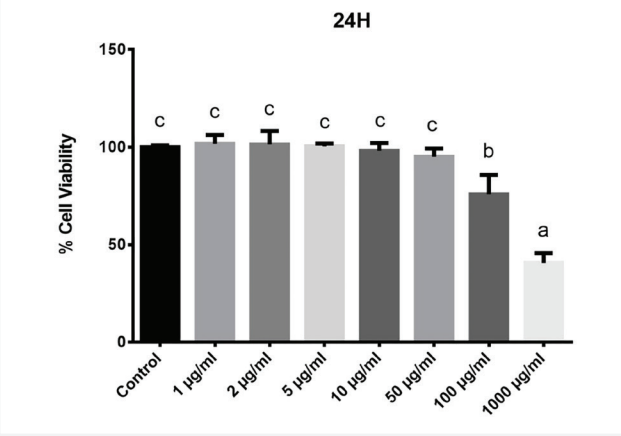

Figure 4. MTT results of Ficus carica latex on SH-SY5Y cell line after 24 hours of incubation. Statistical significance was determined using one-way analysis of variance with Duncan's post-hoc test for each time point 24 hours. In the figures, groups marked with the same letters (a,b,c) do not differ statistically significant from each other, whereas groups labeled with different letters indicate statistically significant differences ($p<0.05$)

DISCUSSION

Neuroblastoma is one of the most common malignant solid tumors in childhood, arising primarily from the adrenal glands and the sympathetic nervous system[1]. Despite advances in treatment, relapse and therapy resistance remain significant challenges, highlighting the need for new therapeutic approaches and natural product-based alternatives. In this context, many studies are being conducted to find cheaper drug alternatives (especially of plant origin) with fewer side effects in the treatment of neuroblastoma[21-25]. Some studies investigating the anticancer effects of FCL suggest that FCL exhibits antiproliferative effects. Therefore, in this study, we examined the antiproliferative effects of FCL on SH-SY5Y cells. In addition to the antiproliferative effects of FCL in the literature, FCL is widely used in traditional medicine, including Ayurveda, Unani, and Siddha, for wound healing and dermatological purposes. A study on a diabetic rat wound model reported that FCL accelerated wound healing by affecting the survival capacity of pathogenic bacteria and biofilm formation, as well as increasing β-defensin-1 and platelet endothelial cell adhesion molecule-1 expression and collagen production, thereby supporting the healing process[26]. In another study, topical application of FCL on pigeons infected with avian pox resulted in complete lesion regression and clinical healing comparable to 3% tetracycline ointment, demonstrating strong antiviral and healing effects[27]. Since FCL is used in traditional medicine for dermatological diseases, in this study we investigated the effects of FCL on wound healing for the first time in the literature. L929 human fibroblast cells are frequently preferred in biocompatibility, cytotoxicity testing, and *in vitro* wound healing models. Literature review reveals limited studies evaluating the effects of FCL on wound healing in the human fibroblast L929 cell line. For these reasons, we used the L929 cell line in our study, and our results showed that FCL's effect on promoting cell migration in L929 fibroblast cells is dose-dependent, with wound healing effects particularly at concentrations of 5 µg/mL and above at 24 hours. Moreover, we observed that at higher concentrations, this effect plateaued, showing no further dose-dependent increase. These findings suggest that FCL supports effective cell migration in the early phase of wound healing and that the concentration range of 5-10 µg/mL may be optimal in terms of both efficacy and low toxicity. In addition, in this study, the effects of FCL on the SH-SY5Y neuroblastoma cell line were investigated. A review of the literature reveals that the anticarcinogenic effects of FCL have been clearly demonstrated in many studies. Based on these properties, this study was conducted to investigate the potential effects of FCL on the SH-SY5Y cell line, which has not yet been studied in the literature. Our data showed that FCL exerts dose-dependent antiproliferative and cytotoxic effects on SH-SY5Y cells, with antiproliferative effect observed at concentrations of 100 and 1000 µg/mL. In addition, we observed that at a concentration of 1000 µg/mL, viability dropped below 50% and cytotoxic effects occurred at this concentration. At lower concentrations, no change in the proliferation of SH-SY5Y cells was observed. Other studies investigating the anticancer potential of FCL support our findings. This natural product has shown promising cytotoxic and antiproliferative effects in many different cancer cell lines. In one study, it has been shown that FCL decreased survival, inhibited DNA synthesis, causing cell cycle arrest at

the G_0/G_1 phase and activated apoptosis in U251 brain cancer cells[15]. In another study, FCL was shown to inhibit invasion in glioblastoma cell lines (U-138 MG, T98G, and U-87 MG) by inducing apoptosis through the regulation of Let-7d expression[28]. Additionally, treatment of MDA-MB-231 breast cancer cells with different concentrations of FCL (0.1%, 0.25%, 0.5%, and 1%) for 24 hours resulted in a reduction in cell proliferation[29]. A study on CaSki and HeLa cells reported that FCL plays a potential role in preventing the progression of cervical cancer by suppressing the proliferation of cervical cancer cells through the upregulation of tumor suppressor proteins p53 and pRb[16]. Studies in the literature show that FCL exhibits antiproliferative activity through many pathways. In general, FCL appears to reduce survival by decreasing DNA synthesis, arresting the cell cycle, upregulating tumor suppressor genes and inducing apoptosis. Many studies in the literature suggest that FCL is cytotoxic in cancer cells but not in healthy cells. Moreover, it is emphasized that when applied in certain formulations, FCL may be effective with fewer side effects. It has been reported that fig latex at 0.125 µg/mL exhibited cytotoxic effects on CaSki and HeLa cancer cells, whereas it did not cause toxicity in the normal HaCaT cell line[16]. In another study, fig latex suppressed proliferation of stomach cancer cells without causing toxicity, with an optimum inhibitory concentration of 5 mg/mL[14]. In a study conducted by Aysin et al.[30], they claimed that FCL induced cell death in a concentration-dependent manner, and high doses caused a more serious cytotoxic effect in cancer cells (A549, MCF-7, MDA-MB-231). The same study claimed that FCL showed less cytotoxicity in healthy cells (MRC-5 non-tumor lung cells) than cancer cells and FCL would have selective cytotoxicity to cancer[30]. In another study on the HT-29 colon cancer cell line, the bioavailability of FCL was enhanced by formulation in liposomal capsules, enabling controlled release and reducing toxic effects[31]. Moreover, FCL extracts have been shown to exhibit cytotoxic activity against MCF-7 breast cancer, HepG2 liver cancer, and HL-60 acute myeloid leukemia cell lines[17]. Animal model studies also support these findings. In a study on diabetic rats, application of fig latex reduced tumor size; hematocrit, hemoglobin, and erythrocyte parameters decreased, while platelet and leukocyte levels remained controlled compared to the cancer group. Moreover, no histopathological damage was detected in the liver and kidney of individuals treated with fig latex, indicating that latex can exert antitumor effects without systemic toxicity[32]. In studies conducted to determine the IC_{50} value of FCL, a separate investigation on colorectal cancer cells evaluated the effects of increasing concentrations of fig latex on HCT-116 and HT-29 cell proliferation; the 48-hour IC_{50} values were found to be 206 µg/mL and 182 µg/mL, respectively, and apoptosis was shown to be activated via PARP cleavage[33]. Additionally, in a study on HeLa cells, fig latex reduced viability in a dose-dependent manner even at low concentrations such as 2 µg/mL, with an IC_{50} of approximately 17 µg/mL[34]. IC_{50} values of FCL were found to be 1/26, 1/40, 1/45 in A549, MCF-7, MDA-MB-231 cancer cells, respectively, while this value was found to be 1/7 in MRC-5 non-tumor lung cells. This study suggested that FCL has less cytotoxicity in healthy cells than cancer cells[30]. In our study, we found the IC_{50} value of FCL in SH-SY5Y cells to be 577.9 µg/mL. It is understood from the literature that various cancer cell lines have been studied to elucidate the antiproliferative effects of FCL. However, the concentrations at which FCL exerts antiproliferative activity differ among many cell lines. For instance, while cytotoxic effects were observed in HeLa cells at 0.125 µg/mL, another study suggested that the optimum inhibitory concentration in gastric cancer cells was 5 mg/mL. In our study, we found that FCL at a concentration of 1000 µg/mL was cytotoxic in SH-SY5Y cells, whereas at 100 µg/mL it exhibited antiproliferative activity. Moreover, findings in the literature indicate that the IC_{50} value of FCL varies across different cell lines. Many studies have also demonstrated that the effects of FCL are dose-dependent. Furthermore, both our results and those reported in the literature show that FCL exerts varying levels of efficacy and cytotoxicity in different cell lines. Several studies have also shown that FCL may behave differently in cancer cells compared to normal cells. In our study, we observed that FCL at 100 µg/mL exerted antiproliferative effects on SH-SY5Y neuroblastoma cells, while the same concentration promoted wound healing in L929 cells. Based on our findings and existing literature, we suggest that the use of appropriate formulations or careful dose adjustment could enhance the efficacy of FCL while reducing its toxicity. In our study, FCL was evaluated on both L929 fibroblast and SH-SY5Y neuroblastoma cell lines. Our results indicate that concentrations of FCL 5 µg/mL and above promote wound healing in L929 cells, suggesting its potential as a component in future wound-healing formulations. On the other hand, its antiproliferative effect at a concentration of 100 µg/mL indicates that it may also be effective in the treatment of neuroblastoma. Furthermore, while the wound healing model in L929 cells provides new insights for the literature, the effects of FCL on the SH-SY5Y neuroblastoma cell line are demonstrated for the first time; thus, FCL contributes scientifically by revealing both its wound-healing potential and its antiproliferative effects on neuroblastoma cells, and the findings shed light on potential clinical applications, including wound-healing products and neuroblastoma therapy with careful dose control.

Study Limitations

This study presents important findings regarding the cytotoxic and wound healing effects of FCL; however, it has several limitations. Firstly, all experiments were conducted exclusively under *in vitro* conditions. The biological mechanisms

underlying the observed wound healing effects have not been fully elucidated. A limitation of the study is that we did not perform independent STR profiling in-house; however, all cells were obtained directly from ATCC, which authenticates lines prior to distribution, and were used only at early passages 12-15 to minimize variability. Moreover, we note that independent phytochemical profiling of FCL was not performed, which represents a limitation and will be addressed in future work. Another limitation of the study is that images of the cells were captured at 0, 24, and 48 hours. The complete closure of the wounds at 48 hours indicates that wound healing occurred at earlier stages. However, since no images were taken at earlier time points in our study, the exact timing of wound closure could not be determined with greater precision. This represents one of the limitations of our study. Additionally, although the SH-SY5Y cell line resembles human neuronal cells, it remains uncertain whether the observed cytotoxic effect would also occur in actual human brain cells. This uncertainty highlights the need for further research to ensure the safe use of FCL.

CONCLUSION

In this study, FCL was shown to statistically significantly promote wound healing even at concentrations of 5 µg/mL and above in L929 fibroblast cells, and this effect was supported by an increase in cell proliferation. On the other hand, evaluations on SH-SY5Y neuroblastoma cells revealed that FCL showed significant cytotoxic effects at a concentration of 1000 µg/mL, while it showed antiproliferative effects at a concentration of 100 µg/mL. Findings suggest that FCL might be effective as a treatment option for enhancing wound healing; however, its use on cancer cells such as neuroblastoma should be carefully dose-controlled. In addition, the proliferative properties of FCL in a healthy cell such as L929 and antiproliferative properties in SH-SY5Y cancer cells at a concentration of 100 µg/mL suggest that FCL may exhibit anticancer properties without damaging normal cells at certain concentrations.

Ethics

Ethic Committee Approval: There are no cells obtained from any living being in the study. The cells were purchased commercially. The active ingredients used were purchased and not extracted from plants. For these reasons, ethics committee approval is not required.

Informed Consent: Participant consent is not required.

Footnotes

Authorship Contributions

Concept: B.A., R.A.U., Design: B.A., Data Collection or Processing: B.A., A.A., Analysis or Interpretation: A.A., R.A.U., Literature Search: B.A., Writing: B.A., R.A.U.

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

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Exploring Prolonged Psychological Distress Induced by the COVID-19 Pandemic: A Public and Clinical Mental Health Perspective

COVID-19 Pandemisi Kaynaklı Uzamış Psikolojik Stres Durumunun İncelenmesi: Halk Sağlığı ve Klinik Perspektifiyle Ruh Sağlığı

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ABSTRACT

Aim: The coronavirus disease-2019 (COVID-19) pandemic has caused widespread psychological distress, with effects persisting into the post-pandemic period. However, there is limited evidence regarding psychological outcomes among patients and their relatives in clinical care settings in Türkiye. This study aimed to assess the prevalence and associated factors of psychological distress among individuals attending a gynecology clinic in Türkiye between 2024 and 2025.

Materials and Methods: A cross-sectional survey was conducted among 528 adults using a structured face-to-face questionnaire. Psychological distress was assessed using the validated Turkish version of the COVID-19 peritraumatic distress index. Socio-demographic characteristics were recorded. Descriptive statistics summarized the data, and univariate and multivariate logistic regression analyses were used to identify predictors of psychological distress. Results were visualized using forest plots.

Results: Among participants, 47% reported severe psychological distress. Female participants had significantly higher odds of distress than males [adjusted odds ratio (OR): 2.82, 95% confidence interval (CI): 1.66-4.78]. Among the 528 participants, 47% exhibited severe psychological distress as measured by the COVID-19 Peritraumatic Distress Index. Psychological distress was significantly more prevalent among women (62.0%) compared with men (36.2%). In multivariate logistic regression analysis, female sex remained a strong predictor of psychological distress (adjusted OR: 2.82, 95% CI: 1.66-4.78). Single individuals demonstrated higher odds of distress than married participants (adjusted OR: 1.92, 95% CI: 1.03-3.58). Participants residing in non-owned housing, such as rental or guest accommodations, had markedly higher risk of distress compared with those living in their own homes (adjusted OR: 3.87, 95% CI: 1.58-9.49). Employment status was also a significant determinant: unemployed participants experienced notably higher distress prevalence (62.1%) relative to employed individuals (37.3%), and unemployment emerged as an independent predictor of distress in the adjusted model. In contrast, education level and income did not demonstrate statistically significant associations with psychological distress after adjustment for other covariates.

Conclusion: Psychological distress remains prevalent among healthcare-seeking individuals in Türkiye during the post-pandemic period. Psychological distress remains a substantial and persistent issue among individuals seeking healthcare services in Türkiye during the post-pandemic period. Women, single individuals, those living in insecure housing conditions, and unemployed participants constitute high-risk groups requiring prioritized attention. These findings underscore the need for integrated and accessible mental health interventions across routine healthcare services. Embedding psychosocial support within general clinical practice may play a critical role in mitigating the long-term psychological consequences of the COVID-19 pandemic and addressing unmet mental health needs in vulnerable populations. Embedding psychosocial support services within general healthcare delivery may help address the ongoing psychological consequences of the pandemic.

Keywords: Adults, COVID-19 aftermath, distress, long-term effects, mental health impact

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ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) pandemisi, pandeminin sona ermesinden sonraki döneme kadar uzanan yaygın psikolojik strese neden olmuştur. Ancak, Türkiye'deki klinik bakım ortamlarında hastalar ve yakınları arasındaki uzun dönemli psikolojik sonuçlara ilişkin veriler sınırlıdır. Bu çalışma, 2024-2025 yılları arasında bir jinekoloji kliniğine başvuran bireylerde psikolojik stres yaygınlığını ve ilişkili faktörleri değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntem: Kesitsel tasarıma sahip bu araştırmada, yapılandırılmış yüz yüze anket yöntemiyle 528 erişkin bireyden veri toplanmıştır. Psikolojik stres, COVID-19 peritravmatik stres indeksinin Türkçe geçerli ve güvenilir formu kullanılarak değerlendirilmiştir. Katılımcıların sosyo-demografik özellikleri kaydedilmiştir. Veriler tanımlayıcı istatistiklerle özetlenmiş; psikolojik stres ile ilişkili faktörleri belirlemek amacıyla tek değişkenli ve çok değişkenli lojistik regresyon analizleri yapılmıştır. Bulgular orman grafikleri ile görselleştirilmiştir.

Bulgular: Katılımcıların %47'si ciddi düzeyde psikolojik stres bildirmiştir. Kadınların psikolojik stres yaşama olasılığı erkeklere göre anlamlı düzeyde yüksektir [düzeltilmiş olasılık oranları (OR): 2,82; %95 güven aralığı (GA): 1,66-4,78]. Bekar olmak (OR: 1,92; %95 GA: 1,03-3,58), kiralık veya misafir konutlarda yaşamak (OR: 3,87; %95 GA: 1,58-9,49) ve işsiz olmak (OR: 0,37; %95 GA: 0,24-0,92) diğer anlamlı belirleyiciler olarak saptanmıştır. Eğitim düzeyi ve gelir seviyesi, düzeltilmiş modelde psikolojik stres ile anlamlı ilişki göstermemiştir.

Sonuç: Pandemi sonrası dönemde, Türkiye'de sağlık hizmeti arayışında olan bireyler arasında psikolojik stres yaygınlığını sürdürmektedir. Kadınlar, işsiz bireyler ve güvencesiz barınma koşullarında yaşayanlar başta olmak üzere risk altındaki gruplara yönelik bütüncül ruh sağlığı müdahalelerine acil ihtiyaç vardır. Psikososyal destek hizmetlerinin genel sağlık hizmetlerine entegre edilmesi, pandeminin süregelen psikolojik etkilerinin azaltılmasına katkı sağlayabilir.

Anahtar Kelimeler: Erişkinler, COVID-19 sonrası dönem, psikolojik stres, uzun dönem etkiler, ruh sağlığına etkisi

INTRODUCTION

The coronavirus disease-2019 (COVID-19) pandemic emerged in late 2019, originating in China, and rapidly evolved into a global public health crisis, affecting millions of people worldwide. Beyond its direct physical health impact, COVID-19 has significantly challenged mental health and psychological resilience across global populations[1,2]. Public health measures implemented to mitigate viral transmission, such as lockdowns, social distancing, and quarantine, although essential, have substantially disrupted daily life, contributing to elevated levels of psychological distress, including anxiety, depression, and post-traumatic stress disorder symptoms[3-5].

A growing body of evidence highlights the adverse psychological consequences of the pandemic and the associated containment strategies. These include increased incidences of emotional disturbance, irritability, insomnia, anger, and emotional exhaustion[6-10]. Factors such as prolonged quarantine duration, fear of infection, social isolation, financial insecurity, and stigmatization have been consistently associated with worsening mental health outcomes[11]. Moreover, socio-demographic variables such as ethnicity, education level, employment status, and type of residence have been identified as key moderators of psychological distress during the pandemic[12-15].

In Türkiye, the psychological impact of COVID-19 has been similarly profound, particularly among patients and their relatives seeking care in private clinical settings. In these contexts, uncertainties related to health outcomes and access to care may further exacerbate stress and anxiety. Although several international multicenter studies have examined psychological distress in diverse populations[16-18], there remains a lack of data specifically focusing on private healthcare environments in Türkiye.

Although COVID-19 is no longer a dominant topic in public discourse or media, many individuals continue to experience residual psychological distress related to the pandemic and still attempt to protect themselves. Figure 1 illustrates COVID-19 case trends; however, it is important to note that no updated national data have been reported since 2024[19-22].

Given the heterogeneity in pandemic responses and socio-cultural contexts across countries, understanding localized psychological responses is crucial for developing effective, targeted mental health interventions. This study aims to address this gap by assessing the prevalence and severity of psychological distress among patients and their relatives attending a private clinic in Türkiye during the post-pandemic period. The findings are expected to provide valuable insight into the ongoing psychosocial impacts of the COVID-19 crisis in private healthcare settings.

MATERIALS AND METHODS

Selection and Description of the Cases

A face-to-face questionnaire was administered to 528 patients and their accompanying relatives attending a private gynecology clinic in Türkiye between January 2024 and January 2025. The study population consisted of adults aged 18 years and above. Participants were recruited consecutively as they presented to the clinic, regardless of the reason for their visit.

This study employed a non-probability sampling method. A post-hoc power analysis based on the observed difference in psychological distress prevalence between groups indicated a

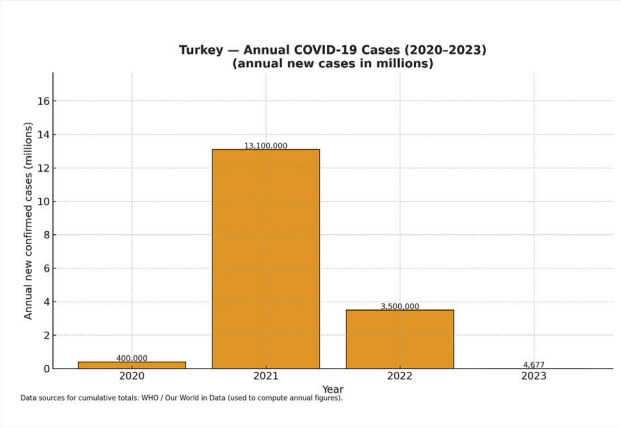

Figure 1. COVID-19 case trends from the onset of the pandemic to 2024

COVID-19: Coronavirus disease-2019, WHO: World Health Organization

statistical power (1-β) of 0.999 at a significance level of α: 0.05. This high power suggests that the study sample size was more than adequate to detect differences in distress prevalence with a medium effect size (Cohen's w ≈ 0.30).

Study Tool (Measurement)

Data were collected using a structured, face-to-face questionnaire administered by trained research personnel. The questionnaire consisted of two parts.

Part 1 included socio-demographic information: gender, age, educational level, marital status, type of residence, household income, employment status, and whether the respondent was a healthcare worker.

Part 2 included the COVID-19 Peritraumatic Distress Index (CPDI), developed by Qiu et al.[23] and validated for the Turkish population by Kocaay et al.[24]. The CPDI is a self-reported instrument comprising 24 items rated on a 5-point Likert scale (never, occasionally, sometimes, often, always). It assesses a range of symptoms experienced in the past week, including anxiety, depression, specific phobias, cognitive change, avoidance, compulsive behaviors, physical symptoms, and social functioning impairmets.

The CPDI was developed in accordance with the diagnostic criteria for stress-related disorders and specific phobias defined in the 11th revision of the International Classification of Diseases, and was reviewed by mental health professionals to ensure clinical relevance. Total scores range from 0 to 100. A score of 28-51 indicates mild to moderate distress, while a score ≥52 indicates severe distress[23].

Pilot testing was conducted with 10 participants to assess face validity and with an additional 10 participants to assess internal consistency. Cronbach's alpha values of 0.824 and 0.925 indicated good to excellent internal reliability of the instrument in the study population.

Technical Information

Data collection was conducted over a 12-month period, from January 2024 to January 2025. All participants provided written informed consent before participation. The questionnaire was administered once to each participant in a private setting to ensure confidentiality. Participants were instructed to respond based on their experiences within the preceding week.

Out of the total number of individuals approached at the private gynecology clinic, 528 completed the questionnaire, yielding a response rate of approximately 65%. Although the clinical setting predominantly serves female patients, the proportion of male respondents was relatively higher. This discrepancy is likely due to a higher refusal rate among female patients compared to their male accompanying relatives. All responses were anonymized prior to analysis to ensure the confidentiality and privacy of participants' data.

Ethical approval for the study was obtained from the Clinical Research Ethical Committee of T.C. University of Health Sciences Dışkapı Yıldırım Beyazıt Training and Research Hospital (approval no: 107/35, date: 22.03.2021).

Statistical Analysis

Descriptive statistics were used to summarize demographic characteristics and CPDI scores, and are presented as frequencies, percentages, means, and standard deviations.

Univariate logistic regression analyses were performed to examine associations between psychological distress (dependent variable) and independent variables including age, sex, education, and employment status. Crude odds ratios (OR) with 95% confidence intervals (CIs) were reported. Subsequently, multivariate logistic regression was performed to determine the adjusted associations between psychological distress and socio-demographic variables. Age, sex, marital status, residence type, educational level, employment status, and income were included in the multivariate model.

To address multicollinearity, educational level categories were collapsed into two groups: (1) primary and secondary, and (2) tertiary education.

The pooled results were graphically represented with a forest plot. All statistical analyses were conducted using IBM SPSS Statistics version 26 and R program.

RESULTS

A total of 528 respondents participated in this survey. Table 1 presents the sample characteristics. In the study, 58.1% of participants were male, 81.4% were married, 93.0%, residing in their own home, 62.1% had secondary education, 61.0% were employed, 71.8% reported medium to high income, and 11.0% were health personnel.

The overall prevalence of psychological distress related to the COVID-19 pandemic is detailed in Table 2. Females exhibited significantly higher distress levels (62.0%) compared to males (36.2%). Similarly, individuals living in non-owned residences (renting or as guests) reported 81.1% distress levels compared to those living in their own homes (44.4%). 66.3% of the single participants were also more likely to report distress than married individuals (42.6%). Notably, 57.4% of participants with primary or secondary education had a higher prevalence of distress compared to those with tertiary education (41.1%). Moreover, 62.1% of the unemployed individuals experienced higher levels of psychological distress than employed participants (37.3%).

Table 1. Distribution of socio-demographic variables among respondents (n=528)

	Mean/count	SD/percentage
Age	41.47	0.4
Sex		
Male	307	58.1
Female	221	41.9
Marital status		
Married	430	81.4
Single	98	18.6
Place of residence		
Own home	491	93.0
Other (rent, guest etc.)	37	7.0
Education		
Primary	10	1.9
Secondary	328	62.1
Tertiary	190	36.0
Employment		
Employed	322	61.0
Unemployed	206	39.0
Income		
Low	74	14.0
Medium	190	36.0
High	189	35.8
Very high	75	14.2
Health personnel		
Yes	58	11.0
No	470	89.0
Distress	51.54	11.4
Mild-moderate	280	53.0
Severe	248	47.0

SD: Standard deviation

Table 3 displays the results of univariate and multivariate logistic regression analyses. In the univariate models, sex, marital status, residence, and employment status were significantly associated with psychological distress.

After adjusting for multiple variables, including age, sex, marital status, type of residence, educational level, employment status, and income, the multivariate analysis identified several significant predictors:

Female participants had significantly higher odds of psychological distress compared to males (adjusted OR: 2.82, 95% CI: 1.66-4.78, p<0.001). Single individuals were more likely to report distress compared to married participants (adjusted OR: 1.92, 95% CI: 1.03-3.58, p=0.038). Participants living in rented or guest accommodations had higher odds of distress compared to those living in their own homes (adjusted OR: 3.87, 95% CI: 1.58-9.49, p=0.003). Unemployed participants had significantly greater odds of distress compared to employed individuals (adjusted OR: 0.37, 95% CI: 0.24-0.92, p=0.015).

In contrast, education level and income group were not statistically significant predictors in the adjusted model. For instance, individuals with primary or secondary education had similar distress levels to those with tertiary education (adjusted OR: 1.04, 95% CI: 0.61-1.79, p=0.880). Likewise, those with low-to-medium income did not differ significantly in distress levels from those with high-to-very high income (adjusted OR: 0.68, 95% CI: 0.46-1.02, p=0.061).

Figure 2 presents the adjusted OR and 95% CI for key socio-demographic variables associated with psychological distress during the post-pandemic period. The red vertical line at OR: 1 indicates the null effect. Variables with CI not crossing 1 are statistically significant predictors (employment, residence and sex).

DISCUSSION

This study contributes to the growing body of literature indicating that the psychological consequences of the COVID-19 pandemic have persisted well beyond its acute phase, particularly among individuals seeking care in clinical settings. Among patients and their relatives attending a private gynecology clinic in Türkiye, we observed a notably high level of psychological distress: nearly half (47%) reported severe symptoms based on the CPDI. These findings suggest that, for some individuals, the psychological burden of the pandemic remains unresolved.

Our results align with international studies conducted during the early stages of the pandemic. For instance, Qiu et al.[23]

Table 2. Prevalence of COVID-19-related psychological distress by socio-demographic characteristics of respondents (n=528)			
Factors	**Distress (count)**	**Total population**	**Prevalence of distress (in %)**
Sex			
Male	111	307	36.2
Female	137	221	62.0
Chi-square	34.432***		
Place of residence			
Own home	218	491	44.4
Other (rent, guest etc.)	30	37	81.1
Chi-square	18.587***		
Marital status			
Married	183	98	42.6
Single	65	430	66.3
Chi-square	18.102***		
Education			
Primary/secondary	109	190	57.4
Tertiary	139	338	41.1
Chi-square	12.885***		
Employment			
Employed	120	322	37.3
Unemployed	128	206	62.1
Chi-square	31.193***		
Income			
Low	36	74	48.6
Medium	77	190	40.5
Good	99	189	47.1
Very good	46	75	61.3
Chi-square	9.464**		
Health personnel			
Yes	31	58	53.4
No	217	470	46.2
Chi-square	1.098*		
Test statistic and p-value *p>0.05, **p=0.024, ***p<0.001 COVID-19: Coronavirus disease-2019			

reported that 35% of respondents in China experienced moderate to severe psychological distress during the initial outbreak. Similarly, Wang et al.[25] found that over 50% of participants reported moderate to severe psychological impact. Notably, our data were collected during 2024-2025 years after the global peak of the pandemic, highlighting the sustained nature of distress in certain populations, particularly those who remain engaged with healthcare institutions. This persistence may reflect the chronic course of pandemic-related stressors.

Compared to national-level data, our findings are equally significant. In one of the earliest Turkish studies during the pandemic, Özdin and Bayrak Özdin[17] found that 45.1% of participants reported anxiety symptoms and 23.6% reported depressive symptoms[26,27]. Our distress rates are even higher, likely due to the clinical nature of the sample and continued socio-economic challenges in Türkiye, including inflation, healthcare system strain, and ongoing fears related to new virus variants and access to care.

Gender emerged as a strong predictor of distress, with women exhibiting nearly three times higher odds of psychological distress than men (adjusted OR 2.82). This finding is consistent with both national and international research[17,27,28-30], suggesting that women have been disproportionately affected by the pandemic, likely due to increased caregiving responsibilities, precarious employment conditions, and heightened vulnerability to domestic violence and mental health disorders.

Employment status also played a key role in mental well-being. Unemployed individuals had significantly higher levels of psychological distress than employed individuals (adjusted OR: 0.37). This finding mirrors global trends, where job loss and financial uncertainty have consistently been identified

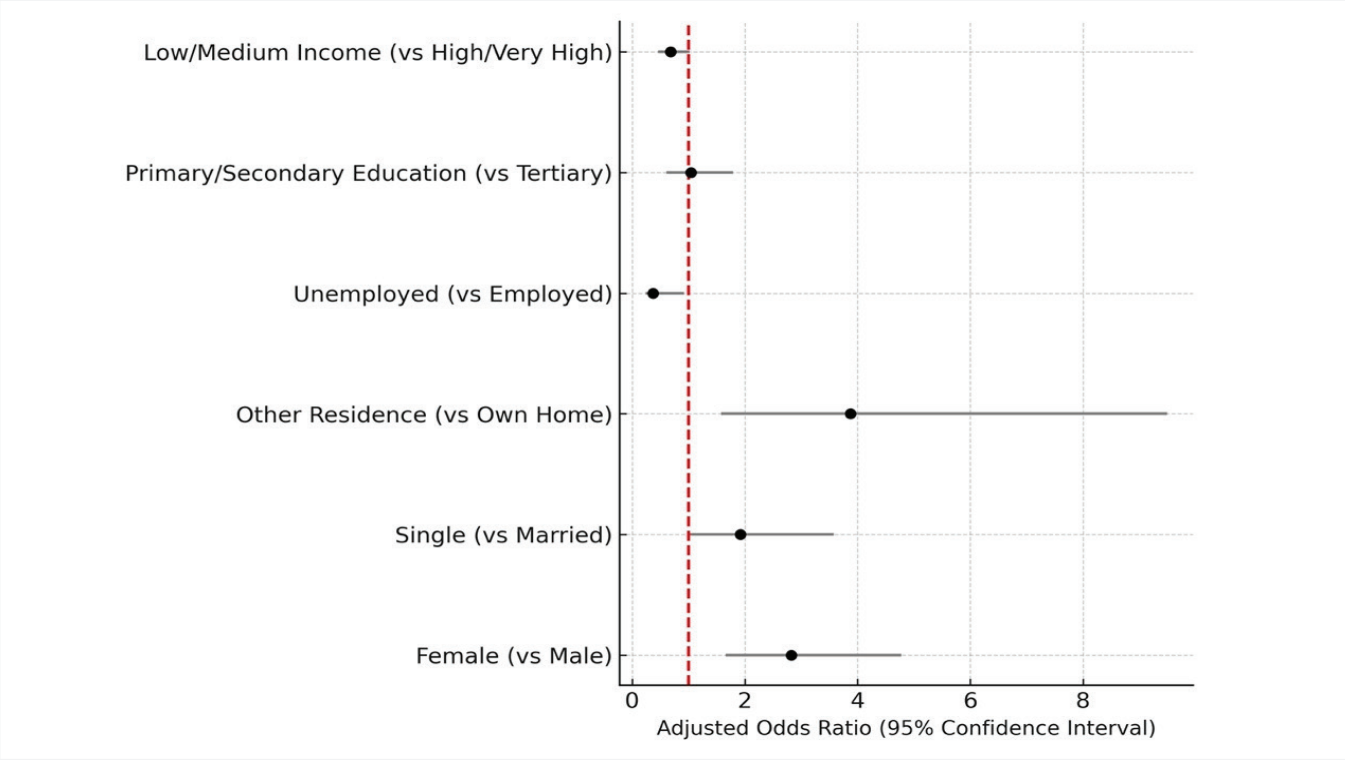

Figure 2. Forest plot of multivariate predictors of psychological distress

Table 3. Multivariate analysis of psychological distress during the post-pandemic period

Factors	Univariate		Multivariable model[a]	
	Crude OR (95% CI)	**p-value**	**Adjusted OR (95% CI)**	**p-value**
Age	-0.027 (8-0.092-0.038)	0.412	1.004 (0.990-1.017)	0.607
Sex				
Male	Reference			
Female	5.819 (3.319-8.319)	<0.001**	2.820 (1.662-4.782)	<0.001**
Marital status				
Married	Reference			
Single	-0.891 (-3.838-2.056)	0.553	1.924 (1.035-3.575)	0.038
Residence				
Own home	Reference			
Other	5.145 (1.450-8.839)	0.006*	3.872 (1.579-9.495)	0.003*
Education				
Tertiary	Reference			
Primary/secondary	-0.580 (-3.219-2.060)	0.666	1.042 (0.608-1.789)	0.880
Employment				
Employed	Reference			
Unemployed	4.908 (2.792-7.023)	<0.00**	0.366 (0.244-0.923)	0.015*
Income				
High-very high	Reference			
Low-medium	0.780 (-0.313-1.872)	0.161	0.684 (0.460-1.017)	0.061

*p<0.05, **p<0.001

[a]: Age and sex (n=528). R^2: 67.9%, CI: Confidence interval, OR: Odds ratio

as major predictors of mental health decline during the pandemic[28,29].

Supporting this, a meta-analysis by Salari et al.[8] confirmed that unemployment significantly elevated the risk of anxiety and depression during the COVID-19 crisis.

Another important factor was housing status. Participants living in their own homes reported lower distress levels than those in rented or guest accommodations. While less frequently explored in the literature, housing stability likely contributes to a greater sense of security and control, both of which are recognized as protective factors for mental health[30].

In contrast to earlier studies, our results did not identify educational level as a significant predictor of psychological distress in the adjusted models. Although higher education is generally considered protective, offering better access to information and coping strategies, our findings support the growing recognition that structural vulnerabilities such as employment status, housing, and marital status may be more influential in long-term crises[31,32].

The long-term persistence of psychological symptoms is a point of concern. Emerging studies suggest that pandemic-related distress may become chronic, particularly among individuals without prior psychiatric diagnoses[33]. Vindegaard and Benros[4] have emphasized the critical need for sustained mental health monitoring and support even as infection rates decline.

Overall, our findings underscore the importance of integrating mental health services into both public and private healthcare systems. Despite the availability of national mental health hotlines and support services in Türkiye, our data reveal a significant unmet need for on-site psychological assessment and intervention in private outpatient settings. Given the sensitive and emotionally complex nature of gynecological and reproductive health services, these settings represent a key opportunity for identifyng and addressing distress.

The rationale for conducting this study was grounded in the continued observation that, although COVID-19 is no longer a dominant topic in public discourse or media, many individuals still experience residual psychological distress and maintain protective behaviors. As noted in the introduction, the absence of updated national data since 2024 has hindered comprehensive public monitoring of the pandemic's long-term psychological impact. However, clinical impressions and anecdotal reports indicate that pandemic-related distress remains a relevant issue across diverse population groups. The results of our study confirm this concern, with nearly half of the participants exhibiting severe psychological distress despite the temporal distance from the peak of the pandemic. These findings emphasize the need for regular mental health screening and support services to be integrated into all healthcare settings, both public and private, as part of a broader strategy to address ongoing mental health consequences of the COVID-19 crisis.

Study Limitations

This study has several limitations that should be considered when interpreting the findings. First, the study was conducted in a single clinical setting, which may limit the generalizability of the results to the broader population. Although the sample included both patients and their relatives, the findings reflect individuals who are already engaged with the healthcare system and may not represent the experiences of those who do not seek medical care.

Second, the cross-sectional design of the study does not allow for causal inferences or assessment of changes in psychological distress over time. Longitudinal follow-up would be necessary to evaluate the persistence or evolution of psychological symptoms in the post-pandemic period.

Third, the findings of this study are based on a non-probability sample. While this sampling strategy was chosen for its practicality and suitability to the research objectives, it limits the statistical generalizability of the results to the entire target population. The potential impact of this limitation on the interpretation of the findings should be acknowledged when considering the study's implications.

Fourth, the use of self-reported data, particularly for the CPDI, may be subject to response bias, including underreporting or overreporting due to social desirability or recall limitations. While the instrument demonstrated good internal consistency, it does not replace clinical diagnosis, and no structured psychiatric interviews were conducted.

Fifth, the lack of access to updated national COVID-19 data after 2024 limits the ability to contextualize findings within current epidemiological trends. As such, ongoing pandemic-related psychological stress may not be directly attributable to case numbers but rather to residual socio-economic and emotional impacts.

Finally, some potentially relevant variables, such as previous psychiatric history, social support levels, or vaccination status, were not assessed, which could have further enriched the analysis of psychological distress predictors.

CONCLUSION

The psychological burden of the COVID-19 pandemic remains substantial among patients and caregivers in private clinical settings in Türkiye. Tailored mental health support, especially for women, the unemployed, and individuals with insecure housing, is essential. Future research should focus on long-term psychological outcomes and the development of context-

specific strategies that bridge public and private healthcare systems.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the Clinical Research Ethical Committee of T.C. University of Health Sciences Dışkapı Yıldırım Beyazıt Training and Research Hospital (approval no: 107/35, date: 22.03.2021).

Informed Consent: All participants provided written informed consent before participation.

Footnotes

Authorship Contributions

Data Collection or Processing: M.F.T., Analysis or Interpretation: B.K.B., Literature Search: B.K.B., Writing: B.K.B., M.F.T.

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

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The Relationship Between Cardiorespiratory Function and Disease Severity, Pain and Fatigue Parameters in Fibromyalgia Syndrome

Fibromiyalji Sendromunda Kardiyorespiratuvar Fonksiyonun Hastalık Şiddeti, Ağrı ve Yorgunluk Parametreleri ile İlişkisi

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ABSTRACT

Aim: The objective of the present study is to examine the effects of fibromyalgia on cardiorespiratory and autonomic nervous system functions, as well as quality of life, in fibromyalgia patients.

Materials and Methods: The study included 39 women aged 25-50 years with fibromyalgia syndrome and 39 women without fibromyalgia as a control group. Fibromyalgia impact questionnaire, pain score with visual analogue scale, widespread pain index, symptom severity scale, fibromyalgia severity scale, Beck depression inventory, fatigue severity scale, 6-minute walk test and 24-hour blood pressure monitoring with Holter device were applied to all participants.

Results: The comparative analysis revealed that patients suffering from fibromyalgia exhibited a diminished 6-minute walking distance, with values recorded at 492.26±53.95 meters, significantly lower than the control group's mean of 550.13±44.56 meters ($p<0.05$). While heart rate recovery index was comparable, fibromyalgia patients had a higher prevalence of non-dipper blood pressure patterns (26.9% vs. 11.5%, $p<0.05$). Higher fibromyalgia severity scale scores were associated with greater fatigue severity scale ($r=0.619$, $p<0.001$) and Beck depression inventory scores ($r=0.457$, $p<0.001$), and inversely correlated with 6-minute walking distance ($r=-0.444$, $p<0.001$). The fibromyalgia impact questionnaire scores were correlated with fatigue severity scale scores ($r=0.717$, $p<0.001$) and Beck depression inventory scores ($r=0.541$, $p<0.001$), but not with 6-minute walking distance ($r=-0.069$, $p=0.675$).

Conclusion: The study found that women with fibromyalgia have impaired physical capacity and autonomic nervous system function compared to those without the condition. This highlights the importance of periodically assessing circadian blood pressure rhythms in people with fibromyalgia. Furthermore, the study suggests that fatigue and depressed mood are more impactful for individuals with fibromyalgia than physical limitations.

Keywords: Fibromyalgia syndrome, cardiorespiratory, ambulatory blood pressure, 6-minute walk test, autonomic dysfunction, non-dipping blood pressure

ÖZ

Amaç: Bu çalışmanın amacı, fibromiyaljinin kardiyorespiratuvar ve otonom sinir sistemi fonksiyonları ile yaşam kalitesi üzerindeki etkilerini incelemektir.

Gereç ve Yöntem: Çalışmaya 25-50 yaş arası fibromiyalji sendromu tanısı almış 39 kadın ve kontrol grubu olarak 39 fibromiyaljisi olmayan kadın dahil edildi. Tüm katılımcılara fibromiyalji etki anketi, görsel analog skala ile ağrı skoru, yaygın ağrı indeksi, semptom şiddet skalası, fibromiyalji şiddet skalası, Beck depresyon envanteri, yorgunluk şiddet skalası, 6 dakikalık yürüme testi ve Holter cihazı ile 24 saatlik kan basıncı takibi uygulandı.

Bulgular: Karşılaştırmalı analiz, fibromiyalji hastalarının 6 dakikalık yürüme mesafesinde azalma olduğunu ve 492,26±53,95 metre olarak kaydedilen ortalama mesafenin, kontrol grubunun 550,13±44,56 metrelik ortalamasından önemli ölçüde düşük olduğunu ortaya koymuştur ($p<0,005$). Kalp hızı

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toparlanma indeksi benzer olsa da, fibromiyalji hastalarında non-dipper kan basıncı paterninin görülme sıklığı daha yüksekti (%26,9'a karşı %11,5, p<0,05). Daha yüksek fibromiyalji şiddet skalası puanları, daha yüksek yorgunluk şiddet skalası (r=0,619, p<0,001) ve Beck depresyon envanteri puanları (r=0,457, p<0,001) ile ilişkiliyken, daha düşük şiddet daha iyi 6 dakikalık yürüme mesafesi (r=-0,444, p<0,001) ile ilişkiliydi. Fibromiyalji etki anketi puanları, yorgunluk şiddet skalası (r=0,717, p<0,001) ve Beck depresyon envanteri puanları (r=0,541, p<0,001) ile ilişkiliydi, ancak 6 dakikalık yürüme mesafesi (r=-0,069, p=0,675) ile ilişkili değildi.

Sonuç: Çalışma, fibromiyaljisi olan kadınların, sağlıklı olanlara kıyasla fiziksel kapasite ve otonom sinir sistemi fonksiyonlarında bozulma olduğunu ortaya koymuştur. Bu durum, fibromiyaljisi olan kişilerde sirkadiyen kan basıncı ritimlerinin periyodik olarak değerlendirilmesinin önemini vurgulamaktadır. Ayrıca çalışma, fibromiyaljisi olan bireylerde yorgunluk ve depresif ruh halinin fiziksel kısıtlılıklardan daha etkili olduğunu göstermektedir.

Anahtar Kelimeler: Fibromiyalji sendromu, kardiyorespiratuvar, ambulatuvar kan basıncı, 6 dakikalık yürüme testi, otonom disfonksiyon, kan basıncında düşüş olmaması

INTRODUCTION

Fibromyalgia syndrome (FMS), a rheumatic disorder, is characterized by widespread chronic pain, sleep disturbances, stiffness, fatigue, and various symptoms[1]. The symptoms of FMS profoundly affect daily activities and quality of life[2]. Although the exact cause of FMS, which affects a significant proportion of the general population, is not fully understood, it is thought to be related to increased sensitivity in the central nervous system and inadequate pain inhibition pathways[3]. A significant proportion of FMS patients have autonomic nervous system dysfunction, particularly in stressful situations. This imbalance is caused by a disturbance of the harmony between the sympathetic and parasympathetic nervous systems. Studies on the autonomic nervous system in individuals with FMS have reported varying results. Some studies on FMS patients report sympathetic hyperactivity and decreased parasympathetic activity, while others report autonomic system inhibition, characterized by suppression of both systems[4].

FMS patients have several cardiovascular autonomic abnormalities. These include blunted autonomic reactivity to acute stress, changes in baroreflex sensitivity, and increased arterial stiffness[5]. Studies show that FMS patients have a non-dipper blood pressure (BP) pattern, which is when the drop in BP at night is less than 10%[6]. Even if not hypertensive, this pattern is associated with target organ damage and increased risk of cardiovascular morbidity[7]. FMS has been shown to be an independent predictor of a non-dipper BP pattern, suggesting a disrupted circadian rhythm of BP[6]. The 6 minute walk test (6MWT) is a simple, safe, and low-cost test for assessing functional capacity in various conditions, including FMS[8]. Studies have shown that FMS patients walk shorter distances on the 6MWT compared to healthy individuals[9]. Furthermore, FMS patients report higher pain intensity and perceived exertion during the 6MWT, suggesting that increased pain and exertion during physical activity exacerbates functional limitations in FMS[10]. However, studies have reported conflicting results regarding the relationship between functional capacity and quality of life in FMS[8,11-14]. Autonomic dysregulation plays a central role in explaining the complex nature of symptoms and functional limitations in FMS[4]. The objective of this study is to assess cardiorespiratory function parameters, such as functional capacity (6MWT), cardiovascular autonomic function [heart rate recovery (HRR) index and dipper/non-dipper pattern characteristics according to ambulatory BP monitoring], and to investigate the relationship between functional capacity, fatigue, depression, and fibromyalgia symptom parameters in patients with FMS. This holistic approach is critical for better understanding the complex interplay between symptoms, functional limitations, and autonomic dysregulation in FMS patients. The findings may contribute to the development of new FMS diagnosis and treatment strategies.

MATERIALS AND METHODS

This study was conducted in the Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Trakya University in order to investigate the relationship between cardiopulmonary function in FMS patients and the basic parameters of the disease, such as disease severity, pain, and fatigue. Participants consented to participate in the study in accordance with the Declaration of Helsinki, as evidenced by their signatures on the informed consent form. Approval of the Trakya University Faculty of Medicine Scientific Research Ethics Committee was obtained for the study (protocol no: TÜTF-BAEK 2021/248, approval number: 12/02, date: 31.05.2021). The study group was 39 female patients diagnosed with FMS, according to the 2016 American College of Rheumatology (ACR) criteria[15]. A control group of 39 individuals without FMS and not meeting the 2016 ACR criteria was also included. Exclusion criteria were as follows: age <25 years and >50 years, musculoskeletal problems that may interfere with ambulation, uncontrolled arrhythmia, unstable ischemia, severe valvular stenosis and congenital heart disease, metabolic problems (hypo/hyperkalemia, hypovolemia); severe cardiomyopathy; active pericarditis/myocarditis; recent history of thrombophlebitis/embolism; active infection; malignancy; unstable angina; or myocardial infarction in the last month. In addition, subjects with a pre-assessed resting heart rate >120 beats/min, systolic BP >180 mmHg and/or diastolic BP >100 mmHg before the 6MWT were excluded.

Evaluation of Sociodemographic Characteristics

A comprehensive documentation of the participants' demographic and health-related information was conducted, encompassing age, height, weight, body mass index (BMI), marital status, educational background, occupational status, presence of comorbidities, and the medications related to these conditions.

Pain Intensity Assessment

The intensity of their pain was evaluated using the visual analog scale, wherein patients were instructed to indicate their pain levels on a 10-centimeter line.

Symptom Severity Assessment

Widespread Pain Index

Widespread pain is characterized by the presence of pain in a minimum of four of the five areas that have been designated as relevant. It is important to note that pain in the jaw, chest, and abdomen is not considered to be confined to the widespread pain group. For each area, areas where continuous pain has been felt in the last seven days are marked. The score ranges from 0 to 19. Conversely, elevated scores are indicative of the presence of pain[15].

Symptom Severity Scale

The scale is evaluated in two groups, A and B. The total score obtained from items belonging to these groups is calculated. In group A, all items including fatigue, waking up without rest, cognitive findings, and somatic symptoms in the last week are scored between 0-3 (maximum score: 9). In group B, headache, lower abdominal pain-cramps, and depression in the last 6 months are evaluated (maximum score: 3). Consequently, the maximum attainable score for the symptom severity scale (SSS) is 12[15].

Fibromyalgia Severity Scale

The widespread pain and symptom severity scales are utilized to ascertain whether a patient has FMS. Scores falling below 12 on these scales do not suggest the presence of the condition. A widespread pain index (WPI) of ≥7 and a SSS of ≥5, or alternatively a WPI of 4-6 and a SSS of ≥9, are indicative of FMS. The severity of the disease increases in proportion to the scores obtained. The existence of concomitant painful disorders does not preclude a diagnosis of FMS[15].

Assessment of Quality of Life and Functional Status

The fibromyalgia impact questionnaire (FIQ), which has been demonstrated to be both valid and reliable in Turkish by Sarmer et al.[16], was utilized to assess the quality of life and functional status in individuals diagnosed with FMS[17]. The scale in question encompasses ten distinct features, including physical function, general well-being, incapacity to attend work, difficulties experienced at the workplace, pain, fatigue, morning fatigue, stiffness, anxiety, and depression. With the exception of the subjective experience of well-being, low scores are indicative of recovery or a lesser degree of impact from the disease. A total FIQ score of <39 is considered as mildly affected, ≥39 to <59 as moderately affected, and ≥59 as severely affected[18].

Fatigue Severity Assessment

The fatigue levels of the participants were measured using the fatigue severity scale (FSS)[19]. The validity and reliability of this scale was previously demonstrated in Turkish by Gencay-Can and Can[20]. The scale comprises nine questions. Each question is scored on a scale ranging from 1 to 7, with 1 representing "strongly disagree" and 7 representing "strongly agree". The scores from each question are then aggregated to obtain a total score, which is subsequently calculated as the mean of these values. The attainment of elevated scores is indicative of the presence of fatigue[19].

Depression Assessment

The beck depression inventory (BDI) was utilized to assess the severity of depression in the study participants[21]. The BDI comprises a total of 21 questions. In the scale, patients are invited to select the sentence that best reflects their experience. Each item is composed of four sentences. The severity of each condition is then assigned a point value, with the most mild condition receiving a score of 0 and the most severe condition receiving a score of 3. The maximum score that can be attained is 63. Scores of 0-13 are indicative of no depression, 14-24 points indicate moderate depression, and scores of over 25 points are suggestive of severe depression[21,22].

Assessment of Cardiorespiratory Capacity and Heart Rate Recovery Index

In the present study, the 6MWT was utilized as a means to evaluate cardiorespiratory capacity, owing to its simplicity and the minimal equipment requirements. A comprehensive evaluation encompassing BP, heart rate, and oxygen saturation was conducted prior to and following the administration of the test. The participants were requested to walk as quickly as possible for a period of six minutes along a 30-meter straight corridor, and the total distance covered was meticulously recorded in meters[23]. Furthermore, the discrepancy between the heart rate at the conclusion of the test and that recorded one minute later was documented as the HRR index[24]. Abnormal HRR was defined as a decrease of 12 beats per minute or less from peak exercise heart rate[25].

Ambulatory Blood Pressure Measurement

The 24-hour evaluation was performed with BP Holter devices. The hours between 6am and 10pm were considered daytime and 10pm and 6am were considered nighttime. The devices were programmed to measure BP at 30 min intervals during daytime and 60 min intervals at night. When evaluating the ambulatory blood pressure (ABP) measurements of the participants, it was taken into consideration that the percentage of valid measurements during the day was above 70%. Those whose mean nighttime systolic BP decreased by 10% or more compared to the mean daytime value were defined as dipper, and those who decreased less were defined as non-dipper. A 24-hour average of systolic BP ≥130 mmHg and/or diastolic BP ≥80 mmHg was considered hypertensive[26].

Sample Size Calculation

The power analysis using G*Power revealed that 80% power could be achieved with 95% confidence if the study included 78 participants (39 in each group).

Statistical Analysis

The statistical analysis of the data was conducted utilizing the SPSS 21.0 software. Continuous variables are expressed as the mean ± standard deviation, while categorical variables are presented as numbers and percentages. The chi-squared test was utilized for the purpose of comparing qualitative data, while the Student t-test and Mann-Whitney U test were employed for the purpose of comparing quantitative data. Spearman's correlation analysis was utilized to evaluate the bilateral relationships between the variables. The results were evaluated with a 95% confidence interval and a significance level of $p<0.05$.

RESULTS

In the present study, a total of 78 subjects participated, comprising 39 women diagnosed with FMS who were undergoing outpatient care at the Department of Physical Therapy and Rehabilitation, Trakya University Faculty of Medicine Hospital, and 39 female volunteers without FMS, ranging in age from 25 to 50 years. Demographic data of the study participants are shown in Table 1. When demographic characteristics were compared, no statistically significant difference was found between the two groups in terms of age and BMI averages ($p>0.05$), while statistically significant differences were found between marital status, educational status, and occupational groups ($p<0.05$). 24.4% of FMS patients used duloxetine, 6.4% used pregabalin. A comparison of the two groups in terms of reported comorbidities showed no statistically significant difference between the FMS and control groups. The distribution of comorbidities and medications for these comorbidities is shown in Table 2. The comparison of the symptom parameters of the FMS group and the control group is demonstrated in Table 3. Statistically significant differences were identified in all of the evaluated parameters ($p<0.05$). The statistical significance values of the difference between the mean 6-minute walking distance (6MWD) and HRR index of the FMS and control groups are shown in Table 4. A total of 16 subjects (41%) in the FMS group and 12 subjects (30.8%) in the control group exhibited abnormal HRR. While the mean 6MWD in the FMS group was statistically significantly lower than the control group ($p<0.05$), no statistically significant difference was found between the two groups in terms of both the mean HRR index and the number of patients with normal and abnormal HRR index ($p>0.05$). After the 24-hour ABPM, 11 people in the FMS group and 6 in the control group were found

Table 1. Demographic characteristics of participants

		FMS group (n=39)		Control group (n=39)		
		n	%	n	%	p
Age (years)	Mean ± SD (min-max)	41.74±6.00 (28-50)		38.95±6.64 (25-50)		0.055*
BMI (kg/m²)	Mean ± SD (min-max)	27.02±5.02 (18.80-40.10)		25.47±5.00 (18.00-39.10)		0.149***
Marital status	Married	35	89.7	26	66.7	**0.028****
	Single	4	10.3	13	33.3	
Educational background	Primary school	14	35.9	9	23.1	**0.002****
	Middle school	3	7.7	0	0	
	High school	12	30.8	4	10.3	
	University	10	25.6	26	66.7	
Occupational status	Housewife	18	46.2	7	17.9	**0.003****
	Employee	18	46.2	32	82.1	
	Retired	3	7.7	0	0	

*: Student's t-test, **: Chi-square test, ***: Mann-Whitney U test, level of significance set at $p<0.05$ BMI: Body mass index, FMS: Fibromyalgia syndrome, SD: Standard deviation, min: Minimum, max: Maximum,

Table 2. Distribution of comorbidities and medications of participants

		FMS group (n=39)		Control group (n=39)		
		n	%	n	%	p*
Comorbidities	Hypertension	10	25.6	3	7.7	0.068
	Diabetes mellitus	4	10.3	0	0	0.115
	Thyroid diseases	3	7.7	0	0	0.240
	Asthma	3	7.7	0	0	0.240
	Other comorbidities	6	15.4	1	2.6	0.108
Medications	Antihypertensive	10	25.6	3	7.7	0.068
	Antidiabetic	4	10.3	0	0	0.115
	Medicines for thyroid diseases	3	7.7	0	0	0.240
	Asthma medicines	1	2.6	0	0	1.000
	Antidepressants	1	2.6	0	0	1.000
	Other medications	3	7.7	2	5.1	1.000

*: Chi-square test, level of significance set at p<0.05, FMS: Fibromyalgia syndrome

Table 3. Comparison of symptom parameters between FMS and control groups

	FMS group Mean ± SD (n=39)	Control group Mean ± SD (n=39)	p*
VAS pain score (0-10)	4.49±1.89	0.97±1.06	**0.000**
Widesprain pain index (0-19)	8.44±4.33	1.97±1.61	**0.000**
Symptom severity scale (0-12)	7.08±2.39	3.28±1.26	**0.000**
Fibromyalgia severity scale (0-31)	15.51±5.31	5.25±2.24	**0.000**
Fibromyalgia impact questionnaire (0-100)	46.30±18.86	16.64±8.14	**0.000**
Fatigue severity scale (0-7)	5.37±1.44	3.16±1.31	**0.000**
Beck depression inventory (0-63)	13.77±8.41	7.46±6.20	**0.001**

*: Mann-Whitney U test, level of significance set at p<0.05, FMS: Fibromyalgia syndrome, VAS: Visual analog scale, SD: Standard deviation

Table 4. Comparison of 6-minute walking distance and HRR index between FMS and control groups

	FMS group Mean ± SD (min-max) (n=39)	Control group Mean ± SD (min-max) (n=39)	p*
6-minute walking distance (m)	492.26±53.95 (350-570)	550.13±44.56 (485-648)	**0.000**
HRR index (beat)	14.33±20.41 [(-33)-83]	18.89±20.79 [(-18)-85]	0.294

*: Mann-Whitney U test, level of significance set at p<0.05, FMS: Fibromyalgia syndrome, HRR: Heart rate recovery, SD: Standard deviation, min: Minimum, max: Maximum

to have hypertension. When these people were redistributed to their groups, 21 (26.9%) in the FMS group and 9 (11.5%) in the control group were found to have hypertension. The FMS group had a higher number of hypertensive patients than the control group (p<0.05). A comparison was made of the numbers of normal dipper and non-dipper individuals in the FMS group and the control group according to the data obtained as a result of 24-hour ABPM. The results showed that 82.1% of the individuals in the FMS group (32 patients) and 59% of the individuals in the control group (23 patients) showed a non-dipper pattern. The probability of being a non-dipper in the FMS group was higher than in the control group (p<0.05). In the 24-hour average systolic BP, a decrease in nighttime systolic BP was observed, with an average reduction of 5.29±6.30% in the FMS group and 6.82±7.3% in the control group. A statistical analysis revealed no significant difference between the groups (p>0.05). A correlation analysis was conducted on the symptom parameters of participants in the FMS group. The SSS score was correlated with the FSS score. There was a positive relationship between the fibromyalgia severity (FS) scale score and the FSS and BDI scores, as well as an inverse relationship with the 6MWD. The FIQ score was correlated with the FSS and BDI scores, but not the 6MWD score. The correlation analysis is displayed in Table 5.

DISCUSSION

The study examined the effects of fibromyalgia on cardiorespiratory and autonomic nervous system functions, as well as quality of life, in women with FMS. FMS patients exhibited a reduced 6MWD compared to the control group,

Table 5. Correlation of the symptom parameters of the participants in the FMS group, with FSS, BDI, and 6MWD

		Fatigue severity scale	Beck depression inventory	6-minute walking distance
Widesprain pain index	**r**	0.018	0.161	0.162
	p	0.912*	0.328*	0.326*
Symptom severity scale	**r**	**0.665**	0.289	-0.042
	p	**0.000***	0.074*	0.801*
Fibromyalgia severity scale	**r**	**0.619**	**0.457**	**-0.444**
	p	**0.000***	**0.000***	**0.000***
Fibromyalgia impact questionnaire	**r**	**0.717**	**0.541**	-0.069
	p	**0.000***	**0.000***	0.675*

*: Spearman correlation analysis, level of significance set at p<0.05, FMS: Fibromyalgia syndrome, FSS: Fatigue severity scale, BDI: Beck depression inventor, 6MWD: Six-minute walking distance

suggesting potential cardiovascular implications. While HRR was comparable, fibromyalgia patients had a higher prevalence of non-dipper BP patterns. Higher FS was associated with greater fatigue and depression, and lower severity with better walking distance. The correlation between symptom severity and fatigue was statistically significant. However, there was no correlation between symptom severity and depression. The investigation revealed no correlation between WPI and FSS scores, BDI, or 6MWD. The impact of fibromyalgia was associated with severity of fatigue and depression, but not 6MWD. The study found that FMS affects exercise capacity and well-being, as well as the cardiorespiratory system, particularly through effects on the autonomic nervous system, and symptom relationships are complex, emphasizing the multifaceted nature of the disease. The study demonstrated that individuals diagnosed with FMS exhibited diminished exercise capacity in comparison to individuals without FMS. This finding aligns with the findings of previous research and is associated with the severity of fibromyalgia[27-29]. The investigation further highlighted the impact of FMS on the autonomic nervous system, observing elevated arterial BP[4]. In healthy people, a decrease in BP at night, known as the "dipping pattern", is expected due to the circadian rhythm of BP. This rhythm is controlled by the autonomic nervous system, especially the sympathetic nervous system[26,30]. However, FMS patients often show a "non-dipping pattern", which is associated with cardiovascular risks[31,32]. Inal et al.[6] found an association between FMS and non-dipping BP, with a higher frequency in FMS patients than in healthy individuals, suggesting an additional risk factor. The study included both normotensive and hypertensive individuals and found that the majority had a non-dipping pattern in both groups, although the frequency was higher in the FMS group compared to the control group, consistent with previous research[6]. It is known that patients with FMS are at a higher risk for cardiovascular disease than healthy people[33,34]. Inadequate levels of moderate-to-high-intensity physical activity are blamed for this, in particular[33]. However, the non-dipper pattern may also contribute to the increased cardiovascular risk in FMS patients[7,31,35]. This pattern has been associated with cardiovascular morbidity in both normotensive and hypertensive individuals[7,31]. This association appears to be independent of traditional risk factors, such as office and 24-hour ABP levels[35]. Therefore, determining if FMS patients have a non-dipper pattern by performing ABP monitoring in addition to one-time BP checks may help reduce cardiovascular disease risk. Large-scale studies examining the relationship between cardiovascular risk and the non-dipper pattern in fibromyalgia patients would be helpful in this regard. The decline in heart rate following exercise at the anticipated rate has been demonstrated to be predominantly associated with the reactivation of the parasympathetic nervous system[5]. This phenomenon serves as an indicator of optimal autonomic nervous system regulation, thereby contributing to the maintenance of physiological balance and well-being. Abnormal HRR index, defined as a 12-beat drop or less in heart rate per minute after exercise, predicts mortality independent of workload and heart rate changes during exercise[25]. In addition to studies suggesting that FMS patients have a reduced chronotropic response to exercise and slower HRR compared to healthy individuals[36,37], there are also authors who, in parallel with our study, have found that FMS patients are similar to individuals without FMS in terms of HRR index[38]. Bardal et al.[38], who found similar results to our study, evaluated HRR index after a submaximal exercise test as in our study. It is hypothesized that the discrepancy in results between the studies is attributable to variations in the utilized test protocols. As previously indicated by Bardal et al.[38], in instances where a protocol of HRR index assessment is implemented, ensuring the maintenance of comparable absolute loading during the HRR period, FMS patients may show a narrower drop in the rate of post-exercise heart rate due to their lower aerobic capacity. Whereas in both the Bardal et al.[38] study and the current study, participants were at rest during the HRR period. The contradictory findings observed in this study reflect the intricate nature of autonomic nervous system dysfunction. While extant studies generally report sympathetic hyperactivity and decreased parasympathetic activity in FMS patients, some authors report autonomic system inhibition, characterized by suppression of both the sympathetic and parasympathetic systems[4]. This finding

suggests that the HRR index may be a misleading metric for evaluating the autonomic function of FMS patients. Numerous studies have demonstrated the negative impact of FMS on quality of life[39-41]. The FIQ is a specific questionnaire that measures all aspects of FMS. It is the most commonly used quality of life scale in studies conducted with FMS patients[42]. Our study found a moderate impact on quality of life when considering the mean score of the FMS group. FIQ scores were associated with fatigue and depression, which supports previous studies[43,44]. Conversely, the absence of correlation between the extensive distribution of pain and FSS scores, in addition to BDI scores, demonstrates the complex nature of symptom relations in FMS. As Martinez et al.[45] observed, fluctuations in pain levels during the day can complicate the discernment of relationships between FMS symptoms. Additionally, while fibromyalgia patients walked shorter distances than patients without fibromyalgia, no correlation was found between the 6MWD and the total FIQ score. The literature contains conflicting results regarding the existence of this correlation. Some studies found no association[11,12], while others reported weak to moderately significant associations[8,13,14]. These discrepancies may be due to the multifaceted nature of FMS and the sensitivity of the measurement tools. In patients with FMS, fatigue and depressed mood may have a greater impact than diminished physical capacity. Some patients may develop high willpower or adaptive strategies to perform daily activities, leading them to perceive a better quality of life even if their physical performance is low[11]. The 6MWD and FIQ provide different yet complementary information. Combining objective performance tests and subjective questionnaires is essential for understanding potential discrepancies between a patient's functional capacity and their perception of disease burden.

Study Limitations

The cross-sectional nature of our study, which examined the relationship between FMS clinical parameters and the cardiovascular system, constitutes a significant limitation. The modest size of the FMS population in the study area, in conjunction with the presence of hypertensive and normotensive individuals within the groups, serves to limit the generalizability of the results. Another limitation of the study is the heterogeneous nature of the groups. The groups included participants with comorbidities that could affect the autonomic nervous system, such as hypertension, diabetes, and thyroid disease, as well as participants who used medications such as antihypertensives and antidepressants. Similar levels of comorbidities in both groups mitigate the negative impact of this limitation on subsequent results. Subsequent studies are planned to enhance the patient population in our study by incorporating new patients and to promote greater homogeneity within the group.

CONCLUSION

This study investigated the effects of fibromyalgia on cardiorespiratory and autonomic nervous system functions, as well as quality of life, in women. The findings suggest that women with fibromyalgia have poorer physical capacity compared to unaffected women. The autonomic nervous system assessment revealed that the HRR index, which relates to the parasympathetic nervous system, was similar between the two groups. However, the "non-dipper" pattern, which relates to the sympathetic nervous system and was determined by 24-hour ABP monitoring, occurred more frequently in women with fibromyalgia. These results imply a potential cardiovascular risk in FMS patients and highlight the intricate nature of the autonomic nervous system. In consideration of the impact on quality of life and psychological factors, it has been observed that fatigue and depressive symptoms have the capacity to exacerbate the impact of fibromyalgia on patients. However, a similar relationship was not observed with functional capacity. In conclusion, it is crucial to consider circadian BP rhythms along with office BP measurements when evaluating patients with FMS. FMS is a multifaceted disorder involving physical limitations, autonomic imbalance, and psychological distress. The impact of FMS on patients' lives may not be directly related to physical performance. This is important to understand and to improve FMS patients' lives.

Ethics

Ethics Committee Approval: Approval of the Trakya University Faculty of Medicine Scientific Research Ethics Committee was obtained for the study (protocol no: TÜTF-BAEK 2021/248, approval number: 12/02, date: 31.05.2021).

Informed Consent: Participants consented to participate in the study in accordance with the Declaration of Helsinki, as evidenced by their signatures on the informed consent form.

Footnotes

Authorship Contributions

Concept: İ.K., N.T., H.Ö., D.D.K., Design: İ.K., N.T., H.Ö., D.D.K., Data Collection or Processing: İ.K., N.T., H.Ö., Analysis or Interpretation: İ.K., N.T., H.Ö., D.D.K., Literature Search: İ.K., N.T., H.Ö., D.D.K., Writing: İ.K., N.T., H.Ö.

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ORIGINAL ARTICLE

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A Retrospective Analysis of Anti-nuclear Antibody Test Results Sent to the Medical Microbiology Laboratory of Uşak Training and Research Hospital and Compatibility with the Immunoblot ANA Profile Test Concordance

Uşak Eğitim ve Araştırma Hastanesi Tıbbi Mikrobiyoloji Laboratuvarı'na Gönderilen Antinükleer Antikor Testi Sonuçlarının ve İmmünblot ANA Profil Testi ile Uyumunun Retrospektif Analiz

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ABSTRACT

Aim: In our study, we retrospectively analyzed samples sent from various clinics with antinuclear antibody indirect immunofluorescence (ANA-IIF) test requests, aiming to evaluate the distribution of ANA-IIF patterns and the concordance between ANA-IIF and anti-extractable nuclear antigens (anti-ENA) immunoblot test results.

Materials and Methods: A total of 6.980 samples submitted with ANA-IIF test requests from various clinics to the Medical Microbiology Laboratory of Uşak Training and Research Hospital between January 1, 2023, and December 31, 2024, were retrospectively evaluated due to suspected autoimmune disease. The ANA-IIF test results of these samples, along with the anti-ENA immunoblot test results—if performed—were assessed. The anti-ENA immunoblot test panel includes the detection of autoantibodies against the following antigens: dense fine speckled 70 (DFS70), histone, nucleosome, Sjögren's syndrome antigen-A, Sjögren's syndrome antigen-B, Mi-2, Ku, Ro-52, Scl-70, PM-Scl100, centromere protein-B, Sm, nRNP/Sm, proliferating cell nuclear antigen, Jo-1, M2, and ribosomal P protein.

Results: ANA positivity was detected in 34.1% (2,380/6,980) of the samples, with various patterns and titers. The most common patterns were fine speckled (AC-4, 22.23%), DFS (AC-2, 14.12%), and nucleolar (AC-8, AC-9, AC-10; 10%). The highest concordance between ANA and ENA results was observed in centromere (AC-3) and topoisomerase I-like (AC-29) patterns. In the immunoblot test, DFS70 and Mi-2 antigens were the most frequently detected targets.

Conclusion: DFS70 autoantibodies were found to be prevalent among samples with suspected autoimmune disease. Therefore, panels including DFS70 and Mi-2 antigens are valuable; however, further studies incorporating clinical data are necessary to better clarify their diagnostic relevance and appropriate usage.

Keywords: Antinuclear antibody, anti-ENA immunoblot, DFS70, Mi-2

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ÖZ

Amaç: Çalışmamızda antinükleer antikor indirekt immünofloresan (ANA-İİF) test istemiyle çeşitli kliniklerden gönderilen numuneleri retrospektif olarak tarayarak, ANA-İİF patern dağılımı, ANA-İİF ile anti-ekstrakte edilebilir antijen (anti-ENA) immünoblot testleri arasındaki uyumu değerlendirmeyi hedefledik.

Gereç ve Yöntem: Otoimmün hastalık şüphesiyle Uşak Eğitim ve Araştırma Hastanesi Tıbbi Mikrobiyoloji Laboratuvarı'na 01.01.2023-31.12.2024 tarihleri arasında ANA-İİF test istemiyle çeşitli kliniklerden gönderilen 6980 numune verisi retrospektif olarak değerlendirmeye alınmıştır. Bu numunelerin ANA-İİF testleri ve beraberinde çalışıldıysa anti-ENA immünoblot test sonuçları değerlendirilmiştir. Anti-ENA immünoblot test panelinde yoğun ince benekli 70 (DFS70), histon, nükleozom, Sjögren sendromu antijeni-A, Sjögren sendromu antijeni-B, Mi-2, Ku, Ro-52, Scl-70, PM-Scl100, sentromer protein-B, Sm, nRNP/Sm, proliferasyon hücre nükleer antijeni, Jo-1, M2, Ribozomal P antijenlerine karşı otoantikorlar araştırılmaktadır.

Bulgular: Numunelerin %34,1'inde (2380/6980) çeşitli patern ve titrelerde ANA pozitifliği saptanmıştır. En sık gözlenen paternler sırasıyla; ince benekli (AC-4, %22,23), DFS (AC-2, %14,12) ve nükleoler (AC-8, AC-9, AC-10; %10) paternlerdir. ANA ile ENA test sonuçları arasında en yüksek uyum sentromer (AC-3) ve topoizomeraz I-benzeri (AC-29) paternlerde gözlenmiştir. İmmunblot testinde en sık saptanan otoantikor hedefleri DFS70 ve Mi-2 antijenleridir.

Sonuç: Otoimmün hastalık şüphesiyle gönderilen örneklerde DFS70 otoantikorlarının belirgin oranda yer aldığı görülmüştür. Bu nedenle, DFS70 ve Mi-2 antijenlerini içeren test panelleri değerli olmakla birlikte, klinik verilerin de dahil edildiği daha kapsamlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Antinükleer antikor, anti-ENA immünoblot, DFS70, Mi-2

INTRODUCTION

Autoimmune diseases are conditions in which the state of "immunological tolerance" is disrupted, leading the organism to develop autoantibodies against its own antigens. In the United States of America (USA), the prevalence of autoimmune diseases has been reported to be around 7-8%. In industrialized countries, this rate is estimated to be approximately 5%[1]. A report from the USA also indicates that the national expenditure on autoimmune diseases is steadily increasing[2]. Autoimmune diseases are broadly classified into two categories: systemic and organ-specific. Systemic autoimmune diseases include conditions such as systemic lupus erythematosus (SLE), rheumatoid arthritis, juvenile idiopathic arthritis, scleroderma, systemic sclerosis (SSc), Sjögren's syndrome, polymyositis (PM), and dermatomyositis (DM). Autoantibodies play a crucial role in the diagnosis, treatment, and monitoring of these diseases. Antinuclear antibodies (ANA), which are commonly assessed in the diagnosis of autoimmune diseases, comprise a large group of autoantibodies targeting various antigens within the cell nucleus[1]. However, since the term "ANA" does not encompass autoantibodies directed against cytoplasmic and mitotic structures, the term "anti-cell antibody" is now preferred. To promote the use of this inclusive terminology and to standardize result reporting, the International Consensus on ANA Patterns (ICAP) has introduced the (AC) code system[3]. In the diagnosis of ANA, the gold standard method is the indirect immunofluorescence (IIF) assay. The ideal substrate used in this method is Hep-2 cells, which are derived from human laryngeal carcinoma. These cells are preferred because they express a wide range of nuclear, cytoplasmic, and mitotic structures, allowing for the detection of autoantibodies when present. In cases with a positive ANA result, the presence of specific autoantibodies should be investigated by testing for antibodies against extractable nuclear antigens (ENA). For this purpose, enzyme-linked immunosorbent assay (ELISA) or immunoblotting techniques may be employed. In certain clinical conditions, the ANA-IIF test may yield false-negative results. In such cases, especially when clinical suspicion remains high, anti-ENA testing can still be considered[1]. In our study, patient samples submitted to our laboratory with a request for ANA-IIF testing due to suspected autoimmune disease were retrospectively analyzed. The aim was to evaluate the distribution of ANA patterns and to assess the concordance between the results of the ANA-IIF test and the anti-ENA immunoblot test, which was either requested simultaneously or added reflexively.

MATERIALS AND METHODS

Sample Selection

In this study, data from 6980 samples submitted to the Medical Microbiology Laboratory of Uşak Training and Research Hospital between 01.01.2023 and 31.12.2024 with a request for ANA-IIF testing due to suspected autoimmune diseases were retrospectively analyzed. The study was approved by the Uşak University Faculty of Medicine Scientific Research Ethics Committee (decision no: 321-321-20, date: 15.02.2024).

ANA-IIF Assay

For the ANA-IIF test, samples were processed using the IIF assay kit (Euroimmun, Germany) with HEp-20-10 cells and monkey liver substrate at a 1:100 screening dilution, following the manufacturer's instructions. Evaluation was performed visually under a Eurostar III fluorescence microscope (Euroimmun AG, Lübeck, Germany) at 200× and 400× magnification. Reporting was conducted in accordance with ICAP standards, including the corresponding AC codes. Cells exhibiting staining in the nuclear region were reported along with the corresponding

positivity titer. In contrast, patterns showing no nuclear staining but displaying cytoplasmic and/or mitotic staining were reported as "ANA negative, see comment", with the observed pattern described in the comment section.

Anti-ENA Immunoblot Assay

Detection of anti-ENA was performed using the immunoblot method with the EUROLINE ANA profile test kit including Mi-2, Ku, dense fine speckled 70 (DFS70) (Euroimmun, Germany). This kit detects autoantibodies against the following antigens: DFS70, histone, nucleosome, Sjögren's syndrome antigen(SS)-A, SS-B, DNA helicase (Mi-2), DNA binding nuclear protein (Ku), SS (Ro-52), topoisomerase-1 (Scl-70), histidine tRNA synthetase (PM-Scl100), centromere protein-B (CENP B), Smith antigen nuclear ribonucleoprotein (nRNP/Sm), proliferating cell nuclear antigen (PCNA), cytoplasmic histidyl tRNA synthetase (Jo-1), pyruvate dehydrogenase complex-E2 (M2), and ribosomal P. Since both ANA-IIF and ELISA tests are used in our laboratory for the detection of double-stranded DNA autoantibodies (anti-dsDNA), the results of these antibodies were not included in the study.

Statistical Analysis

Descriptive statistics were used to summarize the data. The number and percentage of each ANA-IIF and anti-ENA immunoblot result were calculated, and the findings were presented in tables. Ninety-five percent confidence intervals (CIs) for proportions were calculated using SPSS version 27. The Wilson method was applied for patterns with sufficient sample size, while the Clopper-Pearson (exact) method was used for patterns with small sample sizes or rare events. The agreement between ANA and ENA test results was evaluated using Cohen's kappa (κ) coefficient, based on a 2×2 contingency table constructed from the positive and negative results of both tests. Cohen's kappa was calculated using the following formula:

$$\kappa=(P_o-P_e)/(1-P_e),$$

where P_o represents the observed agreement and P_e denotes the expected agreement by chance. Interpretation of κ values was based on the widely accepted Landis and Koch classification: κ<0.00 = poor, 0.00-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial, and 0.81-1.00 = almost perfect agreement. In addition, the chi-square (χ^2) test was performed to assess the statistical association between ANA and ENA positivity. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 27 (IBM Corp., Armonk, NY, USA).

RESULTS

In our study, 6980 samples submitted to the Medical Microbiology Laboratory of Uşak Training and Research Hospital with a request for ANA testing were retrospectively analyzed. Among the patient samples examined, ANA-IIF positivity was detected in 34.1% (2380/6980) at various patterns and titers. The distribution of positive results according to pattern type is as follows: single patterns 73.70% (1754/2380), double patterns 23.28% (554/2380), and triple patterns 3.02% (72/2380). Among those with a single pattern: nuclear 55.17% (1313/2380), cytoplasmic 13.19% (314/2380), and mitotic 5.34% (127/2380). The most frequently reported pattern among samples with single pattern positivity was "fine speckled (AC-4)", accounting for 22.23% (529/2380) of all positive samples. This was followed by the "DFS (AC-2)" and "nucleolar (AC-8, 9, 10)" patterns. The most commonly reported cytoplasmic and mitotic patterns were "ANA-negative, cytoplasmic fine speckled (AC-20)" and "ANA-negative, intercellular bridge (midbody)", respectively. Dual patterns were observed in 23.28% (554/2380) and triple patterns in 3.02% (72/2380) of the positive samples. The numerical data and proportions of other reported positive patterns are presented in Table 1. Among the 6980 samples, anti-ENA immunoblot testing was performed on 4069 samples due to concurrent or reflex test requests. Of these 4069 samples, 1994 were reported as ANA-IIF positive, while 2075 were ANA-IIF negative. The ANA patterns associated with the antigens included in the anti-ENA immunoblot test used in our laboratory, along with the concordance and CIs between ANA-ENA tests, are presented in Table 2. The patterns showing the highest concordance between ANA and ENA were "centromere (AC-3)" and "topoisomerase I-like (AC-29)", followed by "pleomorphic PCNA (AC-13)" and "ANA-negative cytoplasmic reticular (AC-21)" patterns. Among the 1994 ANA-IIF-positive samples tested with the anti-ENA immunoblot testing, 1106 were found to be positive for anti-ENA antibodies and were therefore considered ANA-ENA compatible. Of the 2075 samples with a negative ANA-IIF test and a concurrent anti-ENA immunoblot test request, 1883 were found to be negative and were assessed as ANA-ENA compatible. ANA-ENA concordance rates for patients with concurrent ANA-IIF and anti-ENA immunoblot test requests are presented in Table 3. A total of 4069 serum samples were evaluated for the compatibility between ANA and ENA test results. A significant association was observed between ANA and ENA positivity [χ^2 (1, n=4069)=1267.5, p<0.001, φ=0.56]. Cohen's kappa coefficient was 0.46 (95% CI: 0.42-0.50), indicating a moderate level of agreement between the two tests. While the chi-square test demonstrated a statistically significant relationship, the kappa value suggested that the overall concordance between ANA and ENA results was moderate, implying that these two assays are not fully interchangeable in diagnostic evaluation. Among the 4069 samples tested for anti-ENA, a total of 1917 autoantibodies were detected, including cases of multiple autoantibody positivity observed in all samples that were either ANA-IIF test positive or negative. All autoantibodies

Table 1. Distribution of samples with single pattern positivity (n=1754) in the anti-nuclear antibody indirect immunofluorescence (ANA-IIF) test

AC code	Pattern name	n (%)	AC code	Pattern name	n (%)	AC code	Pattern name	n (%)
Nuclear		1313 (55.17)	**Cytoplasmic**		314 (13.19)	**Mitotic**		127 (5.34)
AC-1	Homogenous	96 (4.03)	**AC-15**	Cytoplasmic fibrillar linear	15 (0.63)	**AC-24**	Centorosome	26 (1.09)
AC-2	Dense fine speckled	336 (14.12)	**AC-16**	Cytoplasmic fibrillar filamentous	33 (1.39)	**AC-25,26**	Spindle fibers	49 (2.06)
AC-3	Centromere	42 (1.77)	**AC-17**	Cytoplasmic fibrillar segmental	2 (0.08)	**AC-27**	Intercellular bridge (midbody)	52 (2.19)
AC-4	Fine speckled	529 (22.23)	**AC-15,16,17**	Cytoplasmic fibrillar	44 (1.85)	**AC-28**	Mitotic chromosomal	0 (0)
AC-5	Coarse speckled	22 (0.93)	**AC-18**	Cytoplasmic discrete dots	4 (0.17)			
AC-6	Multiple nuclear dots	6 (0.25)	**AC-19**	Cytoplasmic dense fine speckled	11 (0.46)			
AC-7	Few nuclear dots	7 (0.29)	**AC-20**	Cytoplasmic fine speckled	126 (5.29)			
AC-8,9,10	Nucleolar	238 (10)	**AC-21**	Cytoplasmic reticular	56 (2.35)			
AC-11,12	Nuclear membrane	17 (0.71)	**AC-22**	Cytoplasmic polar speckled	14 (0.59)			
AC-13	Pleomorphic PCNA	17 (0.71)	**AC-23**	Cytoplasmic rods and rings	9 (0.38)			
AC-14	Pleomorphic CENP-F	0 (0)						
AC-29	Topoisomerase-I like	3 (0.13)						

AC: Anti-cell, N: Number, P: Percentage, PCNA: Proliferating cell nuclear antigen, CENP-F: Centromere protein-F

Table 2. Autoantibodies corresponding to antigens in the extractable nuclear antigens (ENA) immunoblot test, corresponding antinuclear antibody indirect immunofluorescence (ANA-IIF) patterns, and ANA-ENA concordance rates

AC code*	Pattern name	Associated antigen	Anti-ENA test performed (n)	ANA-ENA concordant (n)	ANA-ENA concordance (%)	95% CI
Positive single patterns			1450	736	50.76	0.48-0.53
Nuclear			1213	711	58.62	0.56-0.61
AC-1	Homogenous	Nükleosome, histone	83	31	37.35	0.28-0.48
AC-2	Dense fine speckled	DFS70	311	216	69.45	0.64-0.74
AC-3	Centromere	CENP-B	40	40	100	0.91-1.00
AC-4	Fine speckled	SS-A, SS-B, Mi-2, Ku	494	342	69.23	0.65-0.73
AC-5	Coarse speckled	nRNP/Sm, Sm	21	14	66.67	0.45-0.83
AC-8, 9, 10	Nucleolar	PM/Scl-100	225	49	21.78	0.17-0.28
AC-13	Pleomorphic PCNA	PCNA	17	16	94.12	0.73-0.99
AC-29	Topoisomerase-I like	Scl-70	3	3	100	0.29-1.00
Cytoplasmic			163	25	15.3	0.11-0.22
AC-19	Cytoplasmic dense fine speckled	Ribosomal P	7	1	14.29	0.00-0.58
AC-20	Cytoplasmic fine speckled	Jo-1	68	3	1.47	0.01-0.12
AC-21	Cytoplasmic reticular	M2	28	21	75	0.57-0.87
Positive double patterns			476	317	66.60	0.62-0.71
Positive triple patterns			68	53	77.94	0.67-0.86
Total			1994	1106	55.47	0.53-0.58

*Only ANA-IIF patterns (1841/1994) corresponding to the antigen in the anti-ENA immunoblot test are listed in the table.

AC: Anti-cell, ANA: Antinuclear antibody, ENA: Extractable nuclear antigen, PCNA: Proliferating cell nuclear, CENP-B: Centromere protein-F, CI: Confidence interval, SS-AA: Sjögren syndrome antigen-A, SS-B: Sjögren syndrome antigen-B, DFS70: Dense fine speckled 70, PM: Polymyositis

Table 3. The ANA-ENA concordance rate according to ANA-IIF positivity in patients with concurrent antinuclear antibody indirect immunofluorescence (ANA-IIF) and anti-extractable nuclear antigen (ENA) immunoblot test requests (n=4069)

	ENA positive	ENA negative	Total
ANA positive	1106 (55.5%-85.2%)	888 (44.5%-32.0%)	1994 (100%-49.0%)
ANA negative	192 (9.3%-14.8%)	1883 (90.7%-68.0%)	2075 (100%-51.0%)
Total	1298 (100%)	2771 (100%)	4069 (100%)

Values in each cell are presented as n (row %, column %)

ANA: Antinuclear antibody, ENA: Extractable nuclear antigen, IIF: Indirect immunofluorescence

Table 4. Antigen distribution of autoantibodies in samples that tested positive in the anti-ENA immunoblot test (n=1917)

	DFS70	Mi-2	Ro-52	PM-Scl100	Ku	SS-A	M2	Scl-70	Jo-1	RNP	SS-B	Centromere	Histone	PCNA	Ribosomal P	Nükleosome	Sm	Total
Number (n)	336	238	215	174	133	126	125	91	86	83	73	68	60	43	31	23	12	1917
Percent (%)	17.5	12.4	11.2	9.1	6.9	6.6	6.5	4.8	4.5	4.3	3.8	3.6	3.1	2.3	1.6	1.2	0.6	100

ENA: Extractable nuclear antigen, DFS70: Dense fine speckled 70, SS-A: Sjögren syndrome antigen-A, PCNA: Proliferating cell nuclear antigen, PM: Polymyositis

Table 5. Anti-ENA immunoblot results in ANA-negative (n=4600) samples

	Not requested	Negative	Ro-52	Jo-1	PM-Scl100	M2	Mi-2	Others	Total
Number (n)	2525	1883	39	33	22	16	15	67	4600
Percent (%)	54.9	40.9	0.9	0.7	0.5	0.3	0.3	1.5	100

ANA: Antinuclear antibody, ENA: Extractable nuclear antigen, PM: Polymyositis

identified in the anti-ENA tests are listed in Table 4. Among the 4600 samples that tested negative for ANA, 2525 did not have a request for anti-ENA testing. For the remaining 2075 samples, anti-ENA immunoblot testing was simultaneously requested by the clinicians along with the ANA test. Of these, 1883 (90.75%) were also negative for anti-ENA. However, in 192 samples (9.25%), various autoantibodies were detected as positive despite a negative ANA-IIF result. The numerical data and proportions of the top five autoantibodies identified in ANA-negative but anti-ENA-positive samples are presented in Table 5.

DISCUSSION

The frequency of ANA positivity may vary considerably depending on the patient population and referral criteria. Previous studies evaluating ANA test results from patients referred for suspected systemic autoimmune diseases have reported positivity rates ranging from 15.4% to 41.2%[4-8]. The larger number of patients in our sample compared to other studies from our country in the literature increases the reliability of the results. In our cohort, the ANA positivity rate was 34.1%, which is consistent with these earlier findings. Among the ANA-positive samples, 55.17% exhibited a nuclear staining pattern, 13.19% showed a cytoplasmic pattern, and 5.34% displayed a mitotic pattern. These results align with previous studies. For example, Stinton et al.[9] identified nuclear patterns in 1102 (40.5%) and cytoplasmic patterns in 402 (14.8%) of 2724 ANA-IIF-tested samples. Similarly, Karakeçe et al.[4] reported the distribution of nuclear, nucleolar, mitotic, and cytoplasmic patterns as 56.2%, 16.2%, 14%, and 13.6%, respectively. The proportion of cytoplasmic staining among ANA-positive results has been reported to range from 6.4% to 21%[10]. In our study, the rate of isolated cytoplasmic staining was 13.19%, which also falls within this range. In studies involving patients tested for suspected systemic autoimmune diseases, the most common ANA-IIF pattern has been the speckled/granular type (AC-4, 5), followed by the homogeneous pattern (AC-1)[11,12]. The speckled pattern is seen in a wide range of autoimmune disorders, including SLE, SSc, mixed connective tissue disease, myositis, and Sjögren's syndrome. The homogeneous pattern is also frequently observed in SLE, RA, juvenile chronic arthritis, and Sjögren's syndrome. Aras et al.[13] reported the dense fine speckled pattern (AC-2) as the most frequent in their analysis of ANA tests from various clinics. Similarly, in a retrospective review of 3330 ANA-IIF results, Arslan and Togay[14] found the speckled (AC-4, 5), dense fine speckled (AC-2), and nucleolar (AC-8, 9, 10) patterns to be the most prevalent, with rates of 30.3%, 21.7%, and 19%, respectively. Our findings are in line with this, with the fine speckled (AC-4), dense fine speckled (AC-2), and nucleolar (AC-8, 9, 10) patterns being

the most common in our study as well, observed in 22.23%, 14.12%, and 10% of cases, respectively. It is well established that ANA positivity can be found in up to 20% of healthy individuals, and nearly half of these cases are linked to anti-DFS70 antibodies[15]. The dense fine speckled pattern has also been observed in various non-autoimmune conditions, including chronic inflammatory diseases, cancer, human immunodeficiency virus (HIV) infection, alopecia areata, and atopic dermatitis[16]. Takeichi et al.[17] demonstrated in their study that DFS70 autoantibodies trigger proinflammatory cytokines in keratinocytes, thereby establishing a link between these antibodies and inflammation. Notably, studies without clinical preselection tend to report this pattern more frequently. Our results, which showed the dense fine speckled pattern in 14.12% (n=336) of patients, are consistent with this observation and support previous findings in the literature. In two previous studies evaluating the concordance between ANA-IIF and anti-ENA immunoblot tests, the highest agreement was observed with the centromere (AC-3) pattern, with concordance rates of 92% and 77.77%, respectively[13,14]. Similarly, in our study, this pattern showed the highest concordance, with a rate of 100%. The antigens associated with this pattern include CENP-A, CENP-B, CENP-C, and less commonly, CENP-D[1]. Among these, only CENP-B is included in the anti-ENA immunoblot test panel used in our laboratory. The perfect concordance observed suggests that the current panel is largely sufficient for detecting the autoantibody responsible for the centromere pattern in ANA-IIF. Interestingly, our study also demonstrated 100% concordance for the topoisomerase I-like staining pattern (AC-29) and 94.12% for the pleomorphic PCNA pattern (AC-13), which is higher than previously reported rates. This may be attributed to the high specificity of the associated autoantibodies. The concordance rate for the dense fine speckled (AC-2) pattern in our cohort was 69.45%. Previous studies have reported concordance rates of 85% and 37.98% for this pattern[13,14]. Gurbuz et al.[18] determined the concordance rate for the dense fine speckled (AC-2) pattern in the ANA-IIF test to be 81.9% in their study examining DFS-positive cases. When comparing the anti-ENA immunoblot test results of the 221 samples showing this pattern, they stated that they could not detect the DFS70 autoantibody in 40 samples, and that five different autoantibodies were detected in 7 of these. This variability supports the idea that the AC-2 pattern may be associated with a broader spectrum of autoantibodies and not exclusively with DFS70. In our study, the most frequently detected autoantibody among the samples tested with the anti-ENA immunoblot was anti-DFS70, identified in 17.5% of cases (336/1917). Similarly, in another study using an immunoblot panel that included DFS70, this autoantibody was also the most common, with a frequency of 20.65%[19]. The second most frequent antibody in our cohort was anti-Mi-2. Initially identified in 1976 in a patient with DM, anti-Mi-2 was originally considered a biomarker for DM. However, subsequent studies have shown its potential utility as a marker for PM as well[20]. While ANA positivity alone is a relatively weak biomarker for DM/PM, the presence of specific ENA antibodies can be more informative. In our study, the third most frequently detected antibody was anti-Ro-52, which is the most commonly observed ENA type in PM/DM and plays a valuable role in differential diagnosis[1]. The relatively high frequency of anti-Mi-2 and anti-Ro-52 in our results highlights the potential diagnostic benefit of incorporating disease-specific myositis panels concurrent with standard ENA immunoblot assays in routine clinical practice, particularly for the evaluation of systemic autoimmune diseases. Among the 2075 samples that underwent simultaneous ANA-IIF and anti-ENA immunoblot testing, 1883 (90.74%) were negative by both methods, while 192 (9.25%) were ANA-negative but positive for at least one autoantibody in the ENA panel. In ANA-negative samples, the most frequently detected autoantibodies were anti-Ro-52, anti-PM-Scl100, and anti-Jo-1. Notably, Gür Vural et al[7]. identified the same top three antibodies in ANA-negative samples in their study[19]. Ro-52 and SS-A autoantibodies are associated with Sjögren's syndrome, while the Jo-1 autoantibody is linked to inflammatory myopathies. Since SS-A, Ro-52, Jo-1, and ribosomal P antigens are not adequately expressed in Hep-2 cells, they may lead to false-negative results in ANA-IIF tests. Therefore, it is recommended to perform an anti-ENA immunoblot test even when the ANA-IIF test is negative, especially in cases with high clinical suspicion[1]. This may explain the detection of anti-Ro-52 and anti-Jo-1 in ANA-negative cases. However, further studies from multiple centers using different commercial kits and larger sample sizes are needed to clarify the behavior of anti-PM-Scl100 antibodies.

Study Limitations

One of the limitations of our study is the insufficient number of certain patterns within the sample population, which restricted the assessment of ANA-ENA concordance for those specific patterns. Another limitation is the inability to perform advanced characterization in some patterns, such as nucleolar (AC-8, 9, 10), nuclear membrane (AC-11, 12), and cytoplasmic fibrillar patterns (AC-15, 16, 17).

CONCLUSION

In conclusion, anti-ENA immunoblot testing should be reserved for specific patient groups when performed together with ANA-IIF, since routine testing of all patients may lead to unnecessary healthcare expenses. Our study identified high frequencies of anti-DFS70 and anti-Mi-2 antibodies. We believe that ENA panels containing these specificities, as well as disease-focused myositis antibody panels, may offer valuable support in the

diagnosis of autoimmune diseases. However, further studies with broader sample sizes and integrated clinical data are warranted to evaluate their full diagnostic utility.

Ethics

Ethics Committee Approval: The study was approved by the Uşak University Faculty of Medicine Scientific Research Ethics Committee (decision no: 321-321-20, date: 15.02.2024).

Informed Consent: Retrospective study.

Footnote

Authorship Contributions

Concept: B.G., Design: B.G., H.H.K., Data Collection or Processing: B.G., Analysis or Interpretation: B.G., H.H.K., Literature Search: B.G., H.H.K., Writing: B.G., H.H.K,

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CASE REPORT

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Lung Adenocarcinoma and Hyperamylasemia Associated with Paraneoplastic Syndrome: A Case Report

Akciğer Adenokarsinomu ve Paraneoplastik Sendrom ile Birlikte Görülen Hiperamilazemi

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ABSTRACT

Paraneoplastic syndromes are systemic manifestations arising from tumor-associated biochemical or immunologic effects, occurring independently of direct tumor invasion. While common paraneoplastic syndromes such as hypercalcemia and hyponatremia are frequently observed in lung cancer, hyperamylasemia is a rare phenomenon, particularly associated with metastatic lung adenocarcinoma. A 67-year-old male presented with complaints of fatigue and shortness of breath. Laboratory investigations revealed elevated serum amylase (1330 U/L). The patient had no abdominal symptoms, and imaging showed no pancreatic pathology. Chest computed tomography identified a spiculated lesion in the left upper lobe of the lung, and biopsy confirmed a diagnosis of lung adenocarcinoma. Additional evaluations found no other cause for the amylase elevation, leading to a diagnosis of paraneoplastic hyperamylasemia. Following chemotherapy with carboplatin and paclitaxel, a significant reduction in serum amylase levels was observed, alongside partial tumor regression. This case highlights hyperamylasemia as a rare paraneoplastic syndrome in lung adenocarcinoma. Unexpected biochemical abnormalities in cancer patients should be evaluated as potential paraneoplastic syndromes associated with malignancy and closely monitored. In this case, the decrease in amylase levels following chemotherapy supports a paraneoplastic etiology.

Keywords: Hyperamylasemia, lung adenocarcinoma, paraneoplastic syndrome

ÖZ

Paraneoplastik sendromlar, kanserin doğrudan invazyonu olmaksızın, tümörle ilişkili biyokimyasal veya immünolojik etkiler sonucu ortaya çıkan sistemik belirtilerdir. Hiperkalsemi ve hiponatremi gibi yaygın paraneoplastik sendromlar akciğer kanserinde sık görülürken, hiperamilazemi nadir bir fenomen olup özellikle metastatik akciğer adenokarsinomunda izlenebilir. Altmış yedi yaşında erkek hasta, halsizlik ve nefes darlığı şikayetleriyle başvurdu. Yapılan laboratuvar incelemelerinde serum amilaz yüksekliği (1330 U/L) tespit edildi. Hastanın abdominal şikayeti yoktu ve görüntülemelerde pankreasa ait patoloji izlenmedi. Toraks bilgisayarlı tomografide sol akciğer üst lobunda spiküle kenarlı lezyon saptandı; biyopsi ile akciğer adenokarsinomu tanısı doğrulandı. Ek incelemeler sonucunda amilaz yüksekliğine başka bir neden bulunamadı ve paraneoplastik hiperamilazemi tanısı düşünüldü. Hastaya uygulanan karboplatin ve paklitaksel ile kemoterapi sonrası serum amilaz seviyelerinde belirgin düşüş gözlendi ve tümörde kısmi gerileme sağlandı. Bu olgu, akciğer adenokarsinomunda nadir görülen bir paraneoplastik sendrom olan hiperamilazemiyi vurgulamaktadır. Kanserli hastalarda beklenmeyen biyokimyasal anormallikler maligniteye bağlı paraneoplastik sendromlar olarak değerlendirilmeli ve izlenmelidir. Bu olguda kemoterapi ile amilaz seviyesindeki düşüş paraneoplastik etiyolojiyi desteklemektedir

Anahtar Kelimeler: Hiperamilazemi, akciğer adenokarsinom, paraneoplastik sendrom

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INTRODUCTION

Paraneoplastic syndromes are systemic manifestations that arise without direct tumor invasion, due to biochemical substances secreted by tumor cells or an immune response to the tumor. These syndromes can be the first sign of disease in some types of cancer and can play a significant role in early diagnosis. Lung cancers, particularly non-small cell lung cancer of the adenocarcinoma type, can be associated with various paraneoplastic syndromes[1-3]. Although hypercalcemia and hyponatremia are common paraneoplastic syndromes in lung cancer patients, hyperamylasemia is quite rare and is seldom reported in non-pancreatic tumors[4,5]. Hyperamylasemia associated with non-pancreatic organs is rarely reported, particularly in lung cancer cases. This article presents a patient with lung adenocarcinoma who presented with elevated serum amylase. Despite no pancreatic pathology in the patient, the high serum amylase level was evaluated as paraneoplastic hyperamylasemia. Given the limited number of similar cases in the literature, this case aims to raise awareness about the diagnosis, differential diagnosis, and management of paraneoplastic hyperamylasemia.

CASE REPORT

A 67-year-old male patient presented with increasing fatigue, weakness, and shortness of breath over the past months. The patient had a 45 pack-year smoking history but no alcohol use. Physical examination was within normal limits; there was no abdominal tenderness, and no complaints of abdominal pain. Routine biochemical tests revealed elevated serum amylase level (1330 U/L). The patient was admitted for further evaluation. Due to the absence of abdominal pain history and no pathology in the pancreas on abdominal ultrasound and computed tomography (CT) examinations, a pancreatic cause was not considered (Figure 1,2).

Thoracic CT examination revealed a 6 cm lesion with spiculated margins in the left upper lobe of the lung. Tru-cut biopsy from this lesion resulted in a diagnosis of lung adenocarcinoma (Figure 3-5). Fluorine-18 fluorodeoxyglucose positron emission tomography/CT showed a primary lesion in the left upper lobe with maximum standardized uptake value (SUV_{max}) value of 10.2 and 6.6 cm in size, as well as multiple metastatic lesions measuring 1.5-2 cm in both lung parenchyma (Figure 6).

To exclude other possible causes of hyperamylasemia, ultrasonography of the salivary glands and polyethylene glycol precipitation tests for macroamylasemia were performed. Both examinations were evaluated as normal. Spot urine and 24-hour urine amylase levels were found to be high, suggesting that the amylase elevation was due to overproduction. In the differential diagnosis, no abnormality was detected in the patient's kidney and thyroid function tests to exclude other causes of hyperamylasemia. Plasma and urine catecholamine levels, liver function tests, and alfa-fetoprotein values were within normal limits for the differential diagnosis of other causes of hyperamylasemia such as pheochromocytoma and hepatocellular cancer, and no pathology was seen in the relevant organs on imaging. The patient's performance status was evaluated as 1, and oxygen dependency was present. The patient, with negative driver mutations, was started on a three-month chemotherapy regimen with weekly carboplatin and

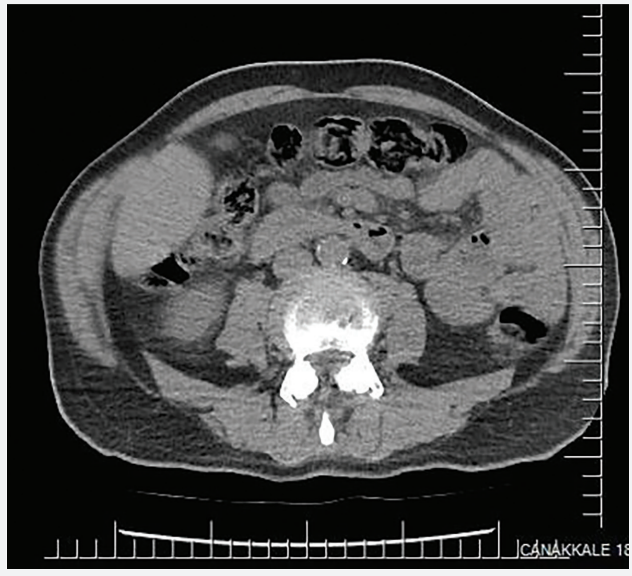

Figure 1. Normal pancreatic CT image at diagnosis
CT: Computed tomography

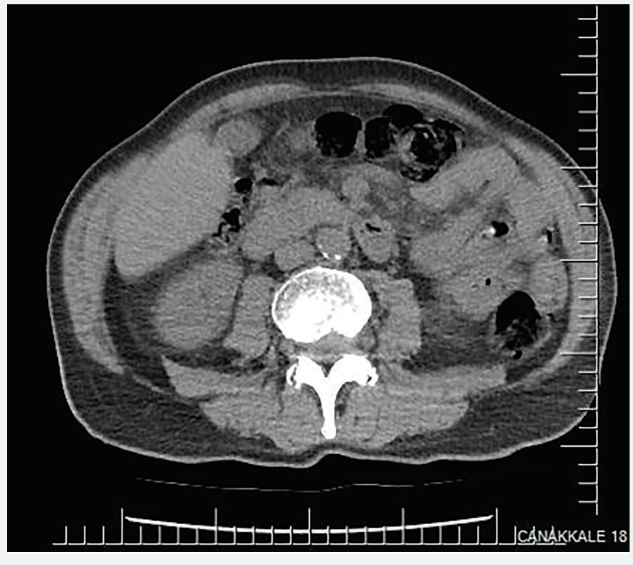

Figure 2. Normal pancreatic CT image at diagnosis
CT: Computed tomography

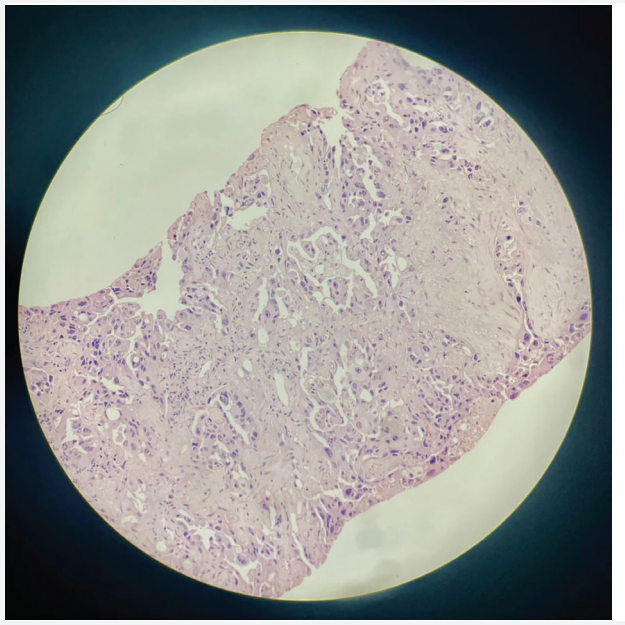

Figure 3. Pathological biopsy image of lung adenocarcinoma at diagnosis (hematoxylin-eosin x10)

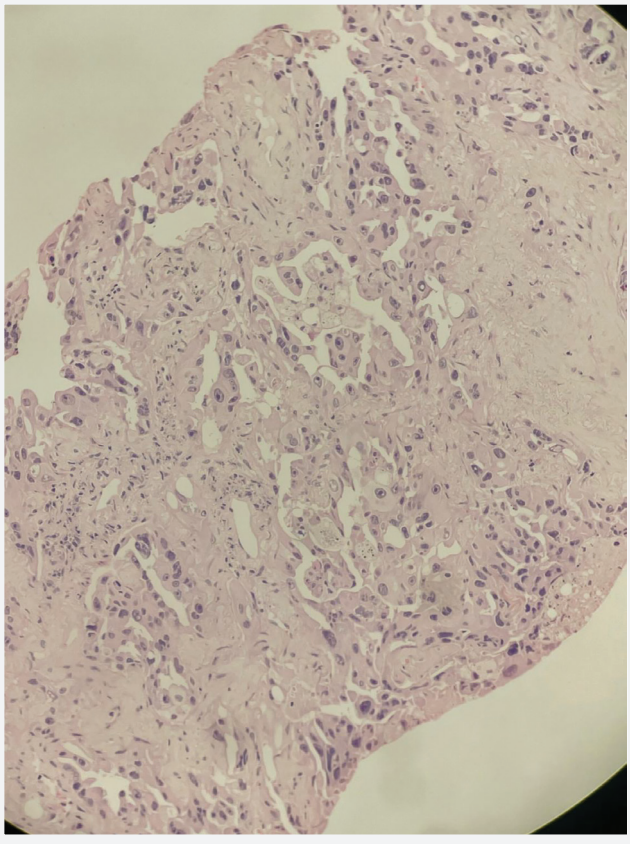

Figure 4. Pathological biopsy image of lung adenocarcinoma at diagnosis (hematoxylin-eosin ×20)

paclitaxel. Serum amylase levels were monitored weekly during the treatment. Post-treatment controls after three months showed that the primary lesion in the lung had shrunk to 3 cm, the SUV_{max} value decreased to 5.8, and there was significant

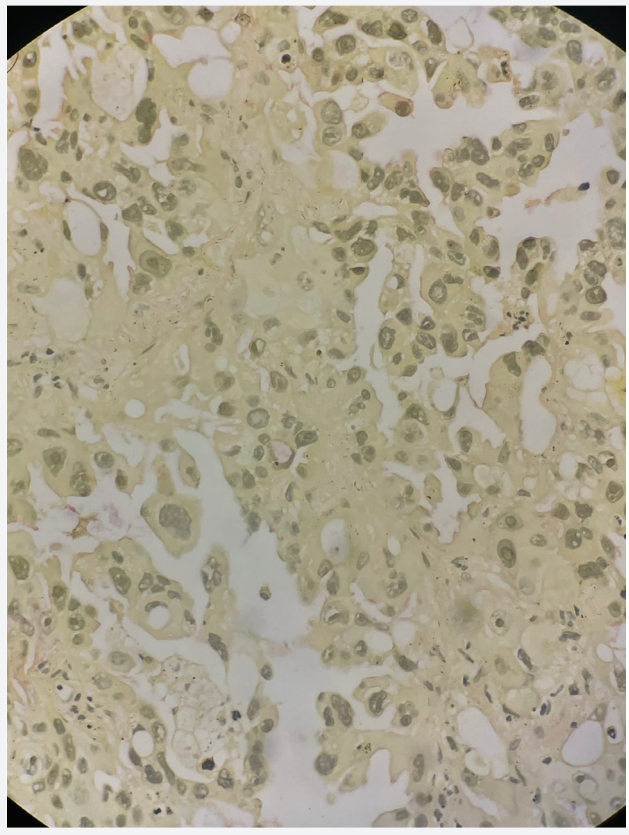

Figure 5. Pathological biopsy image of lung adenocarcinoma at diagnosis (mucin stain ×40)

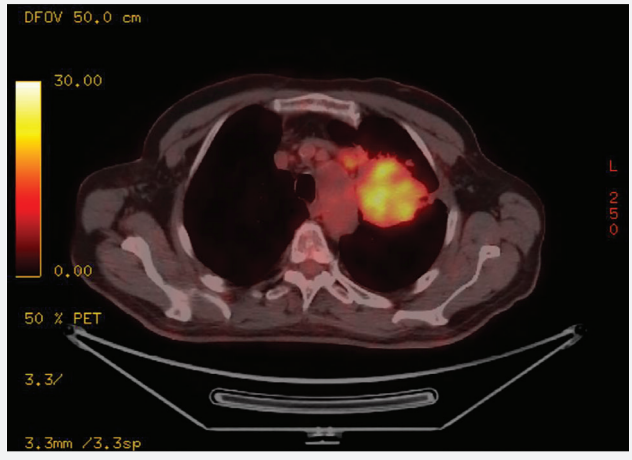

Figure 6. PET-CT image at diagnosis (04/04/2024) showing the primary lesion in the lung

PET-CT: Positron emission tomography-computed tomography

regression in metastatic lesions (Figure 7). The serum amylase value, which was 1330 U/L before treatment, dropped to 173 U/L at the end of treatment (Figure 8).

Patient Perspective

The patient stated that increasing fatigue and shortness of breath in recent months made daily activities difficult and negatively affected quality of life. Initially, the patient thought these symptoms were due to smoking, but sought medical help as complaints worsened. The diagnosis of lung cancer and the report of high serum amylase levels were unexpected and challenging for both the patient and family. The patient was explained that cancer can cause some side effects called paraneoplastic syndromes, and that amylase elevation could be part of this syndrome. The decrease in serum amylase levels and tumor sizes during weekly chemotherapy treatment increased the patient's faith in the treatment and strengthened hopes for the future. The patient understood the importance of closely monitoring amylase levels during treatment and expressed that this process provided great confidence in terms of health. Informed consent was obtained from the patient.

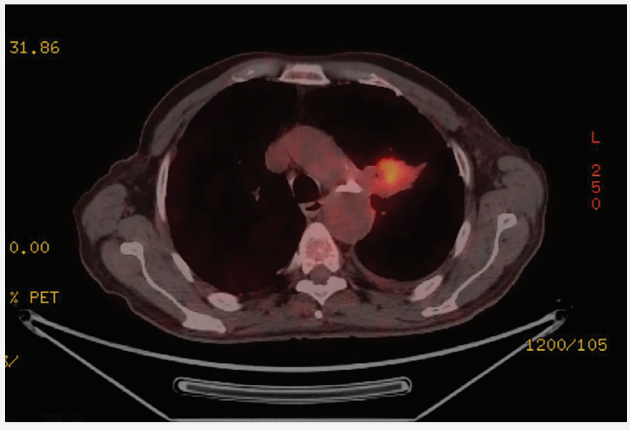

Figure 7. PET-CT image after treatment (20/09/2024) showing the responding lesion in the lung

PET-CT: Positron emission tomography-computed tomography

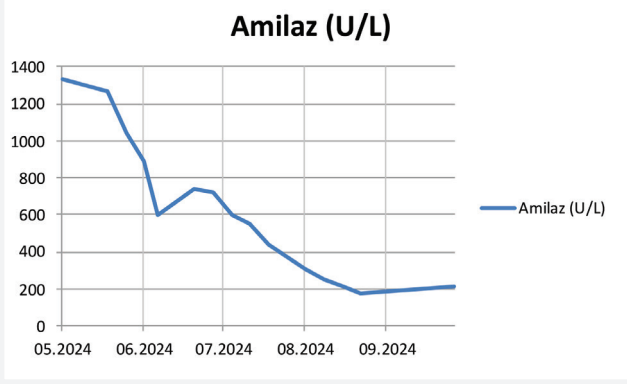

Figure 8. Change in amylase levels with treatment

DISCUSSION

Paraneoplastic syndromes are systemic manifestations in cancer patients resulting from biochemical or immunological changes related to the tumor, often due to substances secreted by tumor tissue. These syndromes are important indicators that can complicate or delay diagnosis in the early stages of cancer. Lung adenocarcinoma is a type of cancer that can be associated with paraneoplastic syndromes. While common paraneoplastic syndromes include hypercalcemia, hyponatremia, Cushing's syndrome, and neurological disorders, hyperamylasemia is a rarer finding[3,6]. In reported cases in the literature, associations with other paraneoplastic syndromes have also been seen; cases involving the salivary glands or pancreas, amyloidosis, hypercalcemia, Cushing's syndrome, hyperglycemia, hypocalcemia, elevation in liver enzymes, etc., have been reported. In our reported case, there was no additional paraneoplastic pathology, and even in similar cases reported in the literature, amylase values as high as in our case were rarely encountered[7-10]. Hyperamylasemia is usually associated with pancreatic diseases, but it can also occur in tumors of non-pancreatic organs. In some cancers like lung adenocarcinoma, especially in metastatic disease, elevated serum amylase levels can emerge as a paraneoplastic phenomenon. In the literature, there are limited case reports of hyperamylasemia associated with lung cancer, and the pathophysiology of this syndrome is not fully understood. However, in most of these cases, amylase levels increase without pathological conditions in the salivary glands or pancreas[5,11,12].

This case demonstrates that in a patient diagnosed with lung adenocarcinoma, serum amylase levels were high without any pathology in the pancreas or salivary glands, and this was accepted as a paraneoplastic effect of lung adenocarcinoma. The significant decrease in amylase levels in response to treatment supports this hypothesis. Paraneoplastic hyperamylasemia usually occurs in advanced stages of cancer, and monitoring biochemical abnormalities during treatment can provide important information about the patient's response to therapy. This case emphasizes the importance of recognizing the paraneoplastic effects of malignant diseases like lung adenocarcinoma and incorporating such biochemical changes into clinical management.

CONCLUSION

This case demonstrates that hyperamylasemia developing in a patient with lung adenocarcinoma, in the absence of any pancreatic or salivary gland pathology, may represent a rare manifestation of a paraneoplastic syndrome. Paraneoplastic

hyperamylasemia is considered one of the indirect biochemical effects of malignancies and, although uncommon, should be taken into account during the diagnosis and follow-up of patients with lung cancer.

In our patient, a marked decrease in amylase levels was observed following the administration of carboplatin and paclitaxel therapy, suggesting the chemosensitivity of paraneoplastic hyperamylasemia. The reduction in amylase levels was consistent with the partial regression observed in the tumor, indicating that this biochemical marker may be useful for monitoring treatment response. This case highlights that unexpected biochemical alterations in patients with lung adenocarcinoma may be associated with paraneoplastic syndromes and underscores the importance of regular monitoring of biochemical parameters during treatment. Recognition of paraneoplastic syndromes and follow-up of their biochemical markers may support the diagnostic process and provide clinical benefit in assessing treatment response.

Ethics

Informed Consent: Informed consent was obtained from the patient.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.Y., Concept: V.Ç., Design: V.Ç., Data Collection or Processing: V.Ç., S.C., G.U., Analysis or Interpretation: V.Ç., S.C., G.U., Literature Search: V.Ç., Writing: V.Ç., S.C., G.U.

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

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CASE REPORT

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Nam Kem Med J 2025;13(4):439-443

Long-lasting Steroid Tapering Scheme in the Management of Relapsed Atezolizumab-induced Grade 3 Pneumonitis: A Case Report

Tekrarlayan Atezolizumab İlişkili Pnömonit Yönetiminde Uzun Süreli Steroid Azaltma Şeması: Olgu Raporu

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ABSTRACT

Immune checkpoint inhibitors, like atezolizumab, have significantly improved survival outcomes in cancer patients, including those with extensive-stage small cell lung cancer (SCLC). However, their use is associated with potentially severe immune-related adverse events, including pneumonitis, which can be life-threatening. We present a case of a 52-year-old male patient with SCLC who developed grade 3 pneumonitis during atezolizumab maintenance therapy. The patient, a chronic smoker, developed symptoms of dry cough and shortness of breath after the third maintenance dose. Radiologic imaging showed bilateral consolidation and ground-glass opacities. A diagnosis of atezolizumab-induced pneumonitis was confirmed, and treatment with high-dose methylprednisolone (1 mg/kg/day) was initiated, leading to clinical improvement. Following discharge, the patient experienced a relapse of symptoms while tapering steroids, prompting a steroid dose increase. After resolution of acute symptoms, the long-lasting steroid tapering regimen was used, ultimately resulting in the successful discontinuation of steroids. This case highlights the challenges of managing immune checkpoint inhibitor-induced pneumonitis, especially when it relapses, and underscores the importance of a personalized steroid tapering protocol. A tailored approach, combined with vigilant monitoring, can effectively manage this complication, emphasizing the need for individualized treatment strategies to balance immunotherapy efficacy and toxicity risks. Further research is necessary to optimize steroid tapering protocols and better understand long-term outcomes of immunotherapy-related pneumonitis.

Keywords: Lung cancer, immune-related pneumonitis, atezolizumab

ÖZ

Atezolizumab gibi immün kontrol noktası inhibitörleri, yaygın evre küçük hücreli akciğer kanseri (SCLC) olanlar da dahil olmak üzere kanser hastalarında sağkalım sonuçlarını önemli ölçüde iyileştirmiştir. Bununla birlikte, kullanımları, yaşamı tehdit edebilen pnömonit de dahil olmak üzere potansiyel olarak ciddi bağışıklık ile ilişkili yan etkilerle ilişkilidir. Bu yazıda, atezolizumab idame tedavisi sırasında grade 3 pnömonit gelişen 52 yaşında SCLC'li (SCLC) erkek hasta sunulmuştur. Kronik sigara içicisi olan hastada üçüncü idame dozundan sonra kuru öksürük ve nefes darlığı semptomları gelişmiştir. Radyolojik görüntülemede bilateral konsolidasyon ve buzlu cam opasiteleri görüldü. Atezolizumab kaynaklı pnömoni tanısı doğrulandı ve yüksek doz metilprednizolon (1 mg/kg/gün) tedavisi başlanarak klinik iyileşme sağlandı. Taburcu edildikten sonra, hasta steroidleri azaltırken semptomlarda bir nüksetme yaşamış ve bu da steroid dozunun artırılmasına neden olmuştur. Akut semptomların çözülmesinden sonra, uzun süreli steroid azaltma rejimi kullanılmış ve sonuçta steroidlerin başarılı bir şekilde kesilmesiyle sonuçlanmıştır. Bu olgu, özellikle nüks ettiğinde immün kontrol noktası inhibitörüne bağlı pnömoniti yönetmenin zorluklarını vurgulamakta ve kişiselleştirilmiş bir steroid azaltma protokolünün öneminin altını çizmektedir. Dikkatli izleme ile birlikte özel bir yaklaşım, immünoterapi etkinliği ve toksisite risklerini dengelemek için bireyselleştirilmiş tedavi stratejilerine duyulan ihtiyacı vurgulayarak bu komplikasyonu etkili bir şekilde yönetebilir. Steroid azaltma protokollerini optimize etmek ve immünoterapiyle ilişkili pnömonitin uzun vadeli sonuçlarını daha iyi anlamak için daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Akciğer kanseri, immünoterapi ilişkili pnömonit, atezolizumab

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INTRODUCTION

Cancer cells carry neoantigens resulting from genetic and epigenetic alterations that can potentially be recognized by the immune system. However, this immune response usually fails due to resistance mutations in tumor cells, tumor-induced local immunosuppression, weakened T-cell signaling and increased immune self-tolerance. Immune checkpoint inhibitors are new anti-cancer therapeutic agents whose efficacy in various cancer types has been proven by many studies[1].

Immunotherapies can have a wide spectrum of side effects due to the strengthening of the immune response. The skin, liver, gastrointestinal tract, lung and endocrine system can be attacked. Pneumonitis is one of the relatively rare but lethal side effects of this group of drugs. However, little is known about the clinical and radiologic features of checkpoint inhibitor-induced lung disease.

The standard first-line treatment for extensive stage small cell lung cancer (SCLC) has been platinum chemotherapy with etoposide for many years[2]. Given for more than 20 years, there has been limited progress, despite response rates of 60 to 65%, with a median overall survival of about 10 months[3,4]. SCLC has high mutation rates. Because of this feature, the addition of immune checkpoint inhibitors to treatment has been shown to positively contribute to survival in the IMpower133 clinic study[5]. We herein report successful management of a patient with SCLC of grade 3 pneumonitis related atezolizumab olarak düzeletelim geçen her yerde treatment.

CASE REPORT

A 52-year-old male patient with extensive stage SCLC was admitted to medical oncology outpatient clinic with complaints of dry cough and shortness of breath lasting for three days while he was on Atezolizumab maintenance therapy (he was taken 3rd maintenance dose before 10 days ago). He was previously performed Atezolizumab 1200 mg/21 days in combination with platinum - etoposide for 4 cycles, and then his treatment was switched to maintenance Atezolizumab 1200 mg/21 days treatment upon after partial response. The patient had been a smoker for more than 30 years and was in otherwise good health with no other signs of disease. Physical examination revealed decreased breath sounds bilaterally, velcro type rales on the auscultation of the right side of the lung, the heart rate 115/min, the blood pressure 115/80 mmhg, the respiratory rate 18 per minute, without fever and the oxygen saturation 85% while ambient air. Electrocardiogram showed no findings except sinus tachycardia. The peripheral blood sampling verified that white blood cell, neutrophil and monocyte counts were normal and increased C-reactive protein level. Influenza A-B and coronavirus disease-2019 rapid tests and two blood cultures were negative. chest computed tomography (CT) showed patchy distributed bilateral consolidation and ground-glass opacities (Figure 1). After initial rapid work-up, patient was hospitalised and 1mg/kg methylprednisolone was initiated due to high suspicion of immunotherapy related pneumonitis. Few days after corticosteroid treatment, the patient's symptoms tended to improve, the acute-phase response was declined, oxygen saturation recovered and dyspnea resolved. After a period of one week with no sign of disease, the patient was discharged with a steroid tapering scheme (8 mg dose reduction every 5 days), pneumocystis carinii pneumonia (PCP) prophylaxis and calcium and vitamin D replacement.

Twenty days after discharge, while taking 20 mg/day methylprednisolone, the patient re-presented to our outpatient clinic with the complaint of shortness of breath. The patient with elevated acute phase response and dyspnea was hospitalized and chest CT and angiography were performed. No pulmonary embolism was observed. As radiologic images were compatible with lobar pneumonia (Figure 2), IV antibiotic therapy covering pseudomal infection was initiated. Chest CT was repeated after 48-72 hours with no acute phase reactants response and no growth in blood and sputum cultures. Methylprednisolone was increased again to 40 mg dose with the concern of recurrence of pneumonitis due to ground glass opacity and increased bilateral infiltrations on imaging. After receiving methylprednisolone 40 mg/5 days, clinical and laboratory response was observed. Antibiotic treatment was discontinued. 40 mg methylprednisolone was completed to 7 days and the patient was discharged with a slow dose reduction scheme (4 mg every 10 days). In outpatient follow-up, corticosteroids were safely discontinued with slow dose reduction and atezolizumab was permanently discontinued. The patient continues follow-up without treatment for SCLC.

DISCUSSION

This case highlights the complexities of managing immune checkpoint inhibitor-related pneumonitis, particularly in the context of relapsed pneumonitis. While immune checkpoint inhibitors like atezolizumab have revolutionized the treatment landscape for cancer, their potential for causing severe immune-related adverse events, such as grade 3-4 pneumonitis, requires careful and often prolonged management. The success of the steroid tapering regimen in this patient underscores the need for personalized and adaptive therapeutic strategies to balance the benefits of immunotherapy with the risks of such life-threatening complications.

According to the Common Terminology Criteria for Adverse Events version 5, immunotherapy-associated pneumonitis that affects daily vital activities and/or is life-threatening in addition to the need for oxygen is evaluated as grade 3-4[6]. European Society for Medical Oncology (ESMO) and National

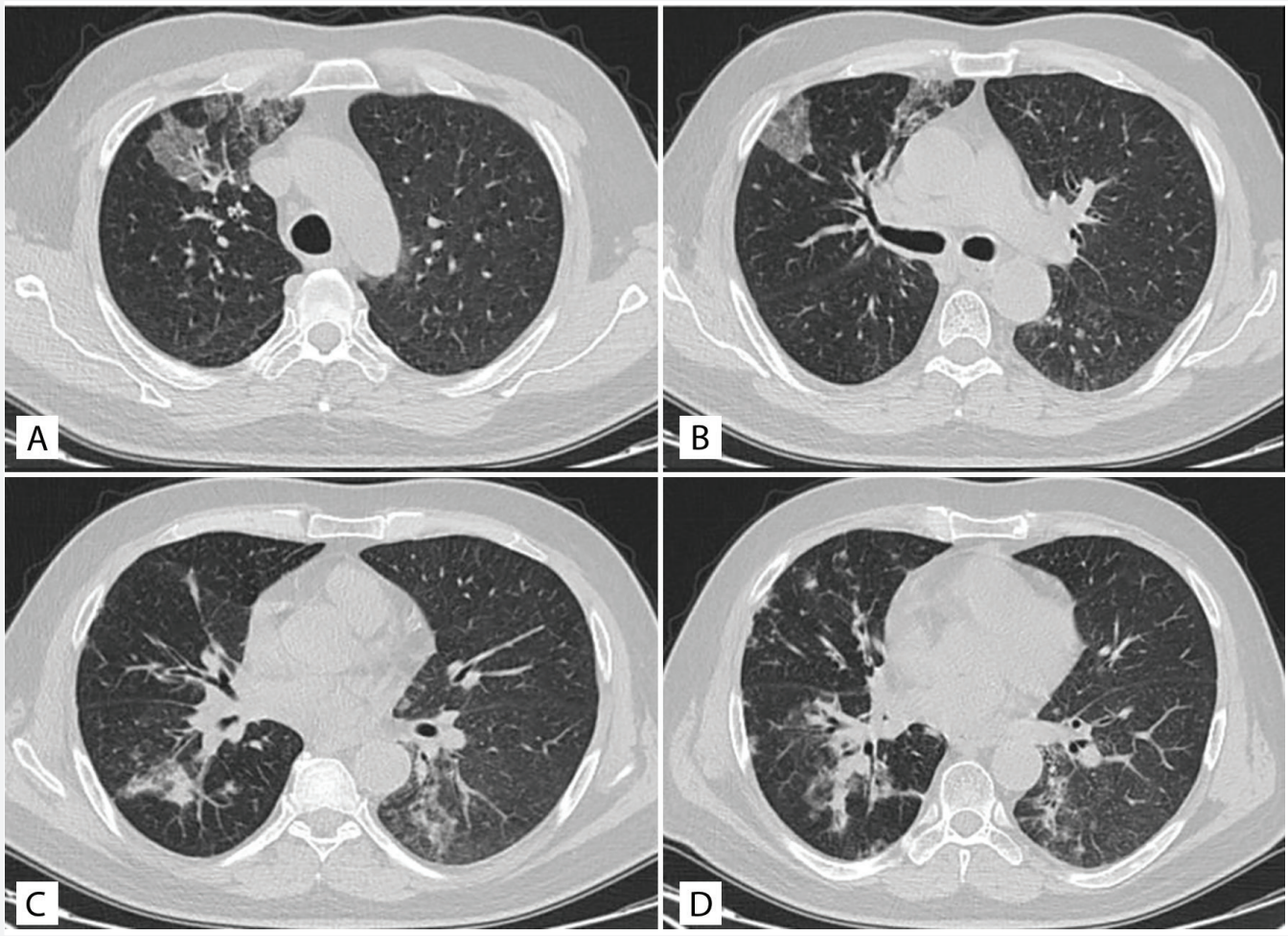

Figure 1. Multifocal ground-glass areas in both lung parenchyma
A) Upper lung slice B) Slice at carina level C, D) Slices at post-arch

Comprehensive Cancer Network guidelines recommend that patients with grade 3-4 pneumonitis should be hospitalized for management[7,8]. Glucocorticoids treatment and holding immunotherapy constitute the backbone of management. In addition, in terms of differential diagnosis, a rapid work up for pseudomonal pneumonia, PCP infection, disease progression, and vascular thromboembolic diseases should be performed. Blood culture, nasal swabs, sputum culture, metabolic and biochemical panels are usually performed on the inpatient setting. Bronchoalveolar lavage may be useful for microorganism production or disease invasion assessment. Finally, it is recommended to add antibiotherapy covering the pseudomonal pneumonia and PCP infection.

In studies performed before the prevalent use of immunotherapies in different cancer types, the risk of pneumonitis was reported to be higher in NSCLC and RCC and in combined immunotherapies than in melanoma[9]. Immunotherapies, which are now indicated for almost every type of cancer, need to be evaluated for these fatal side effects. Grade 3-4 pneumonitis was seen in 3.1% of patients in durvalumab condensation immunotherapy[10], which is the new standard treatment in early-stage SCLC, while it was seen in 0.5% patients in the IMpower133 study evaluating patients receiving atezolizumab in extensive stage SCLC.

Although the optimal dose reduction and duration for grade 3-4 pneumonitis responsive to corticosteroid therapy is not clear, ESMO guidelines recommend steroid dose reduction for at least 6-8 weeks. If symptoms or imaging studies worsen significantly during tapering, we will return to the last well-tolerated dose of prednisone (or equivalent) for two weeks before continuing tapering. In cases of grade 4 pneumonitis, immunotherapy is completely discontinued, while in grade 2 cases, immunotherapy can usually be restarted. For grade 3 cases, there is no certainty and patient characteristics such

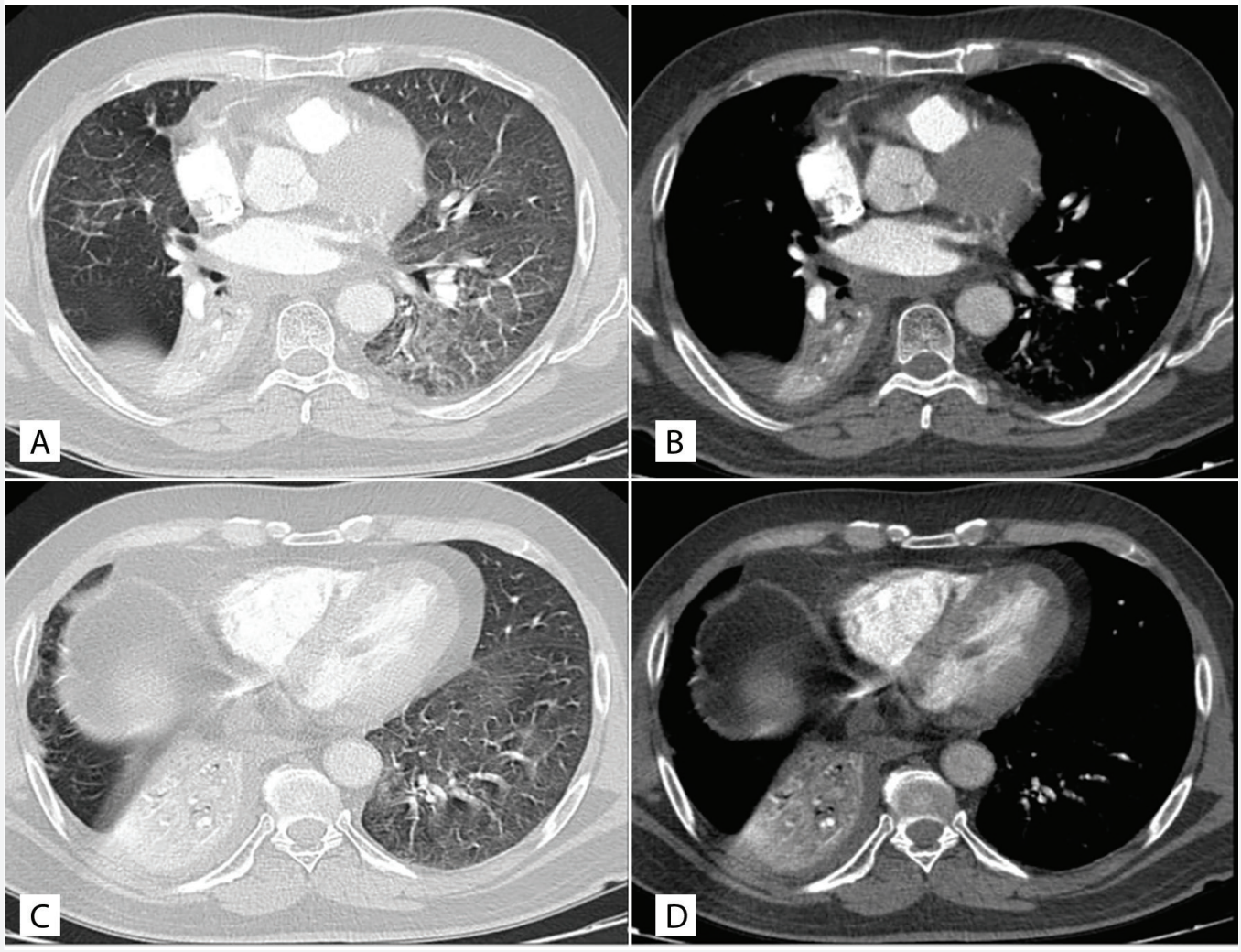

Figure 2. Consolidation with air bronchograms in the lower lobe of the right lung
A-D) Slices at air bronchograms level

as the course of pneumonitis, response to treatment and life expectancy need to be taken into account.

CONCLUSION

A tailored steroid tapering regimen, coupled with appropriate monitoring, proved effective in managing this life-threatening complication. Despite the risks associated with immune checkpoint inhibitors, careful management can allow for favorable outcomes, emphasizing the importance of individualized steroid treatment strategies and close follow-up. Further studies are needed to refine the optimal approach for steroid tapering and to better understand the long-term implications of immunotherapy-related pneumonitis.

Ethics

Informed Consent: The patient provided written informed consent for the publication of this article.

Footnotes

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

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REVIEW

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Metachronous Multiple Primary Lung Cancer: A Case Report and Review of the Literature

Metakron Çoklu Primer Akciğer Kanseri: Bir Olgu Sunumu ve Literatür Derlemesi

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ABSTRACT

Multiple primary lung cancers (MPLC) may develop either synchronously or metachronously. While synchronous MPLC involves the simultaneous occurrence of histologically distinct tumors, metachronous MPLC arises over time following the treatment of an initial lung cancer. To highlight the diagnostic and therapeutic challenges, we present a rare case of triple metachronous primary lung cancer, accompanied by a systematic review of case reports and reviews published between 2010 and 2024, focusing on clinical, pathological, and radiological aspects. In the presented case, three distinct histopathological subtypes of primary lung cancer developed over an eight-year period. Genetic analysis revealed TP53 and PTEN mutations, potentially contributing to metachronous tumor development. The literature emphasizes the importance of timely imaging and histological confirmation in distinguishing metachronous tumors from recurrence or metastasis. This case represents one of the rare reports of triple metachronous primary lung cancer in the literature and underscores that the diagnostic process necessitates the integrated use of radiological, histopathological, and molecular methods. High clinical vigilance is essential for identifying MPLC in patients with a previous history of lung cancer, and aggressive surveillance strategies—such as low-dose computed tomography—and a multidisciplinary approach are critical for improving patient outcomes.

Keywords: Multiple primary lung cancer, metachronous lung cancer, lung adenocarcinoma, squamous cell carcinoma, small cell lung carcinoma

ÖZ

Çoklu primer akciğer kanserleri (ÇPAK), senkron ya da metakron şekilde gelişebilir. Senkron ÇPAK, histolojik olarak farklı tümörlerin eş zamanlı ortaya çıkmasını ifade ederken; metakron ÇPAK, ilk akciğer kanserinin tedavisini takiben zaman içinde gelişir. Tanısal ve tedaviye ilişkin zorlukları vurgulamak amacıyla, üçlü metakron primer akciğer kanseri olgusunu ve 2010-2024 yılları arasında yayımlanan olgu sunumları ile derlemeleri klinik, patolojik ve radyolojik açıdan inceleyen sistematik bir derlemeyi sunmaktayız. Sunulan olguda, sekiz yıl içinde üç farklı histopatolojik alt tipte primer akciğer kanseri gelişmiştir. Genetik analizde TP53 ve PTEN mutasyonları saptanmış olup, bu mutasyonların metakron tümör gelişimine katkıda bulunabileceği düşünülmektedir. Literatürde, metakron tümörlerin nüks veya metastazdan ayırt edilmesinde zamanında yapılan görüntüleme ve histolojik doğrulamanın önemi vurgulanmaktadır. Bu olgu, literatürde nadiren bildirilen üçlü metakron primer akciğer kanseri olgularından biri olup, tanı sürecinin radyolojik, histopatolojik ve moleküler yöntemlerin bütüncül kullanımıyla yürütülmesi gerektiğini göstermektedir. Akciğer kanseri öyküsü olan hastalarda yüksek klinik farkındalık esastır ve düşük doz bilgisayarlı tomografi gibi agresif izlem stratejileri ile multidisipliner bir yaklaşım, hasta sonuçlarının iyileştirilmesi açısından büyük öneme sahiptir.

Anahtar Kelimeler: Çoklu primer akciğer kanseri, metakron akciğer kanseri, akciğer adenokarsinomu, yassı hücreli karsinom, küçük hücreli akciğer karsinomu

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INTRODUCTION

Lung cancer continues to represent the leading cause of cancer-related mortality worldwide. Based on the latest GLOBOCAN 2022 estimates, approximately 2.5 million new cases and 1.8 million deaths were reported globally in that year[1]. Key risk factors for lung cancer include tobacco smoke exposure, environmental carcinogens, and genetic predisposition[2]. Patients with lung cancer may present with multiple primary tumors either simultaneously [synchronous multiple primary lung cancer (MPLC)] or sequentially following the treatment of the initial tumor (metachronous MPLC). This phenomenon has emerged as a significant clinical challenge, with the classification and management of these tumors remaining complex[3]. Crucially, distinguishing whether a subsequent tumor is an independent primary malignancy or a recurrence/metastasis of the initial tumor is essential for determining staging, therapeutic strategies, and prognosis.

The first documented case of two distinct primary lung cancers was reported by Beyreuther[4] in 1924. In 1975, Martini and Melamed[5] proposed clinicopathological criteria for the diagnosis of MPLC. Over time, these criteria have been refined and integrated into clinical guidelines. Lung tumors arising at different times are classified as metachronous if they exhibit distinct histologies or, in cases of similar histology, if there is no evidence of systemic metastasis between tumors over a period of four years or longer[6]. In this report, we present a rare case of triple metachronous primary lung cancer in a long-term smoker, characterized by three distinct histopathological subtypes: adenocarcinoma, squamous cell carcinoma, and small cell carcinoma, diagnosed at different time intervals. The second primary lung cancer was detected four years after the initial diagnosis, followed by a third tumor four years later. In addition to this unique case presentation, a comprehensive literature review was performed through PubMed, MEDLINE, and Scopus databases to identify case reports and reviews published between 2010 and 2024. Articles were evaluated for clinical, pathological, and radiological findings, emphasizing diagnostic innovations and management strategies in MPLC. This integrated analysis aims to highlight both the diagnostic and therapeutic challenges of metachronous MPLC and to provide a concise synthesis of recent evidence in this field.

A 63-year-old male patient was referred to our clinic in May 2012 due to a pulmonary mass. The patient had an 80 pack-year smoking history and had worked as an office clerk for 30 years. There was no family history of lung cancer, and the patient had no chronic illnesses.

Computed tomography (CT) imaging revealed a mass measuring 5.0×3.5 cm in the right upper lobe. A biopsy performed under CT guidance was consistent with non-small cell lung cancer (NSCLC). Clinical staging, conducted using transbronchial needle biopsy and F-18 fluorodeoxyglucose positron emission tomography/CT (^{18}F-FDG PET), was determined as cT2N0M0. The patient underwent a right upper and middle lobectomy. Pathological examination revealed well-differentiated adenocarcinoma (Figure 1) with a pathological stage of pT2N0. Molecular analysis based on next-generation sequencing (NGS) was performed on the primary lung adenocarcinoma specimen to evaluate potential hereditary or somatic predispositions. The targeted panel included 54 cancer-related genes and 26 microsatellite loci. The analysis revealed pathogenic variants in the *TP53* gene (c.734G>T, p.G245V) and the *PTEN* gene (c.380G>T, p.G127V). Both mutations were classified as Tier 2C alterations, indicating potential clinical significance. Adjuvant treatment with four cycles of cisplatin and vinorelbine combination therapy was administered. The patient was subsequently placed under routine follow-up.

Four years after the initial diagnosis, in October 2016, follow-up thoracic CT and ^{18}F-FDG PET/CT imaging revealed a lobulated, malignant mass lesion measuring 27×27 mm in the posterobasal segment of the left lower lobe of the lung (maximum standardized uptake value: 8.5). No distant metastases were observed. The patient underwent wedge resection of the left lower lobe. Pathological examination revealed squamous cell carcinoma (Figure 2) (TTF-1 negative, p63 positive) with a pathological stage of pT1cN0. The patient was followed up without adjuvant therapy.

Four years after the second primary tumor, in January 2020, thoracic CT revealed an endobronchial lesion extending from the left lower lobe to the secondary carina. A punch biopsy was performed. Pathological examination revealed findings consistent with small cell carcinoma (Figure 3), characterized by p63 negativity, synaptophysin positivity, chromogranin positivity, and CD56 positivity. PET-CT imaging demonstrated limited-stage disease, and concurrent curative chemoradiotherapy was administered. Follow-up CT scans showed a complete response to therapy. The patient has been under surveillance for the past four years without evidence of malignancy (Table 1).

DISCUSSION

MPLC can present either synchronously (simultaneously) or metachronously (at different times)[7]. According to the criteria for synchronous lung cancer defined by Martini and Melamed[5], synchronous tumors are secondary tumors observed concurrently with a primary tumor but with a different histology. If the tumors share the same histology, they must be located in different segments or lobes.

The definition of metachronous MPLC includes secondary tumors histologically distinct from the primary tumor or tumors of the same histology arising after a tumor-free

interval of at least two years, located in a different lobe, and without extrapulmonary metastasis at the time of diagnosis[8].

Antakli et al.[9] made modifications to Martini and Melamed's[5] criteria, adding a definition for metachronous lung tumors with the same histology as the primary tumor. These revised criteria require the presence of two or more of the following: anatomical distinction from the primary tumor, association with a premalignant lesion, different DNA ploidy, and absence of systemic metastasis or mediastinal spread. The rate of developing a second primary lung cancer after curative treatment of NSCLC is reported to be 1-2% per patient per year, whereas this rate rises to 6% per patient per year after curative treatment of SCLC[10]. Retrospective studies indicate that the median interval between the primary and new lung cancer is 38 to 48 months, with approximately two-thirds of the tumors sharing the same histology[11]. Triple primary lung cancer is extremely rare, even within the MPLC category. This case represents one of the few reports of triple primary lung cancer with three distinct histological types[12,13].

Smoking is a major etiological factor in the development of metachronous multiple lung cancers[2]. While the development of metachronous tumors in patients who continue smoking after the initial diagnosis is not surprising, the occurrence of metachronous tumors many years later in patients who quit smoking raises questions about the persistent epigenetic effects of smoking. Carcinogens in cigarette smoke are known to cause genetic damage, leading to mutations in tumor suppressor genes such as *TP53* and *PTEN*. TP53 mutations impair tumor suppressor function, inhibit apoptosis, and disrupt DNA repair mechanisms, leading to genetic instability and cellular malignant transformation, and are therefore widely recognized as markers of poor prognosis in various malignancies, including NSCLC[14]. PTEN mutations activate the PI3K/AKT/mTOR pathway, promoting cellular proliferation, growth, and metastatic potential. The coexistence of both mutations supports tumor heterogeneity, allowing the development of independent clones and potentially triggering the formation of metachronous tumors[15]. In our case, despite smoking cessation at the time of initial diagnosis, the development of two additional primary lung tumors four and eight years later may be attributed to the persistent mutagenic effects of smoking. The coexistence of TP53 and PTEN mutations in this patient may explain the mechanisms underlying the emergence of three distinct metachronous primary tumors.

Approximately two-thirds of metachronous lung cancers are resectable, and about one-third of these patients undergo limited resections[6]. In metachronous MPLC, the American College of Chest Physicians recommends surgical treatment as the first choice when the tumor is detected in its early stages, as surgical resection can provide long-term survival[16]. One of the greatest challenges in treating a second primary lung cancer is determining the feasibility of surgical resection. Patients who have undergone prior lung cancer resection may not be suitable candidates for a second lobectomy or pneumonectomy due to limited pulmonary reserves[17].

The use of high-resolution CT has significantly increased the detection of small lung cancers, which correlates with the rising incidence of MPLC[18,19]. According to the 8th edition of the TNM classification for NSCLC, a second tumor in the same lobe is categorized as T3, tumors in different ipsilateral lobes as T4, and tumors in the contralateral lung as M1[20]. As a result, most MPLC cases are reclassified as stage III or IV, which are

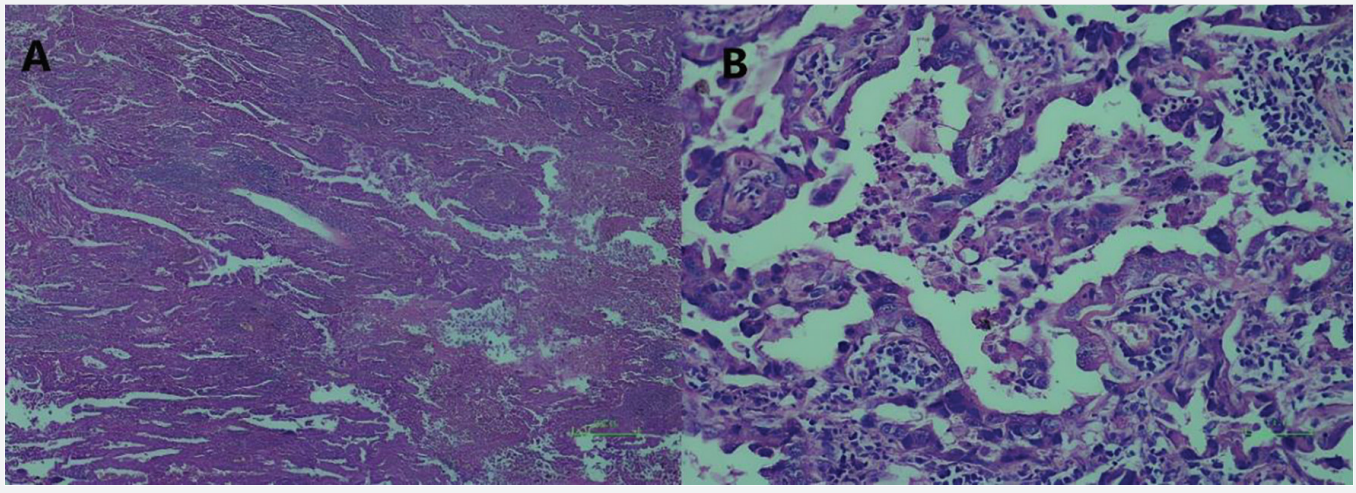

Figure 1. (A) Tumoral infiltration exhibiting lepidic and papillary growth patterns (hematoxylin and eosin ×40) (B) Tumoral infiltration forming papillary structures with fibrovascular cores, composed of cells with large nuclei and prominent nucleoli (hematoxylin and eosin ×200)

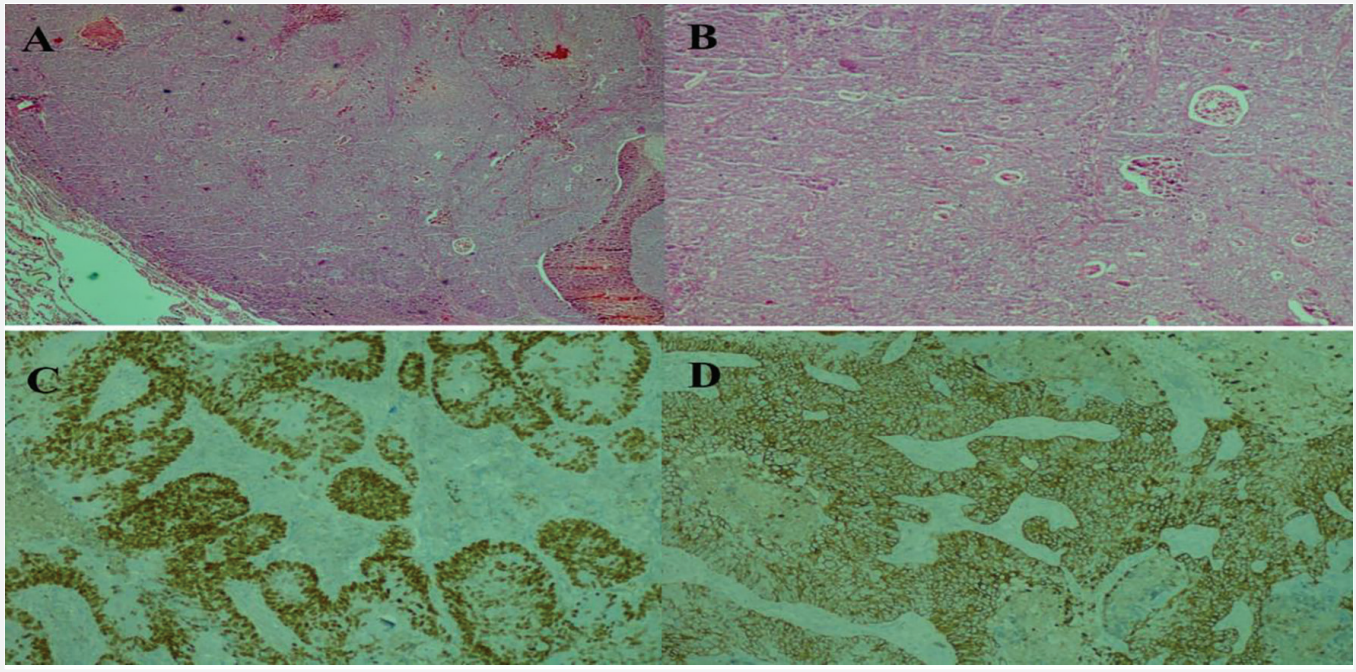

Figure 2. (A) Tumoral infiltration in a solid pattern invading the lung parenchyma (hematoxylin and eosin ×40) (B) Tumoral infiltration in a solid pattern, composed of cells with vesicular nuclei, prominent nucleoli, and eosinophilic cytoplasm (hematoxylin and eosin ×200) (C) Nuclear positivity with p63 immunohistochemistry (×200) (D) Cytoplasmic positivity with CK5 immunohistochemistry (×200)

Table 1. Characteristics of the patient's multiple primary lung tumors

Histological type	Well-differentiated adenocarcinoma	Squamous cell carcinoma	Small cell carcinoma
Year of diagnosis	2012	2016	2020
Tumor location	Right upper lobe	Posterobasal segment of the left lower lobe	Central part of the left lung
Tumor size (radiological)	50x35 mm	27x27 mm	40x30 mm
Disease Stage (based on the 8th TNM staging system)	pT2N0	pT1cN0	Limited-stage disease
Curative treatment	Right upper lobectomy	Wedge resection of the left lower lobe	Concurrent chemoradiotherapy

typically associated with poorer prognoses and are managed with systemic therapies such as chemotherapy or radiotherapy. However, van Bodegom et al.[21] reported significantly better survival outcomes in MPLC patients compared to those with advanced-stage single tumors. Similarly, Pairolero et al.[22] demonstrated more favorable prognoses in patients with multiple primary tumors compared to those with local recurrence or metastatic disease. A comprehensive meta-analysis by Jiang et al.[23] confirmed that MPLC patients show superior 3- and 5-year overall survival rates compared to those with intrapulmonary metastases. The same analysis concluded that histological type (similar or different) and laterality (unilateral or bilateral) were not significantly associated with differences in overall survival[23].

Furthermore, the prognosis of metachronous NSCLC has been specifically evaluated in dedicated surgical cohorts. A meta-analysis by Hamaji et al.[16] revealed a five-year overall survival rate of 46% following resection of a second metachronous NSCLC, underscoring the potential benefit of surgical intervention in patients with good performance status who are eligible for resection. Supporting this, Haraguchi et al.[24] found that patients with the same histologic subtype in both primary tumors had significantly improved outcomes, reporting five-year survival rates of 71% versus 47% (p=0.0174). These findings suggest that histological similarity may serve as a positive prognostic indicator in metachronous NSCLC and reinforce the rationale for aggressive surgical approaches in well-selected patients. Interestingly, despite the presence

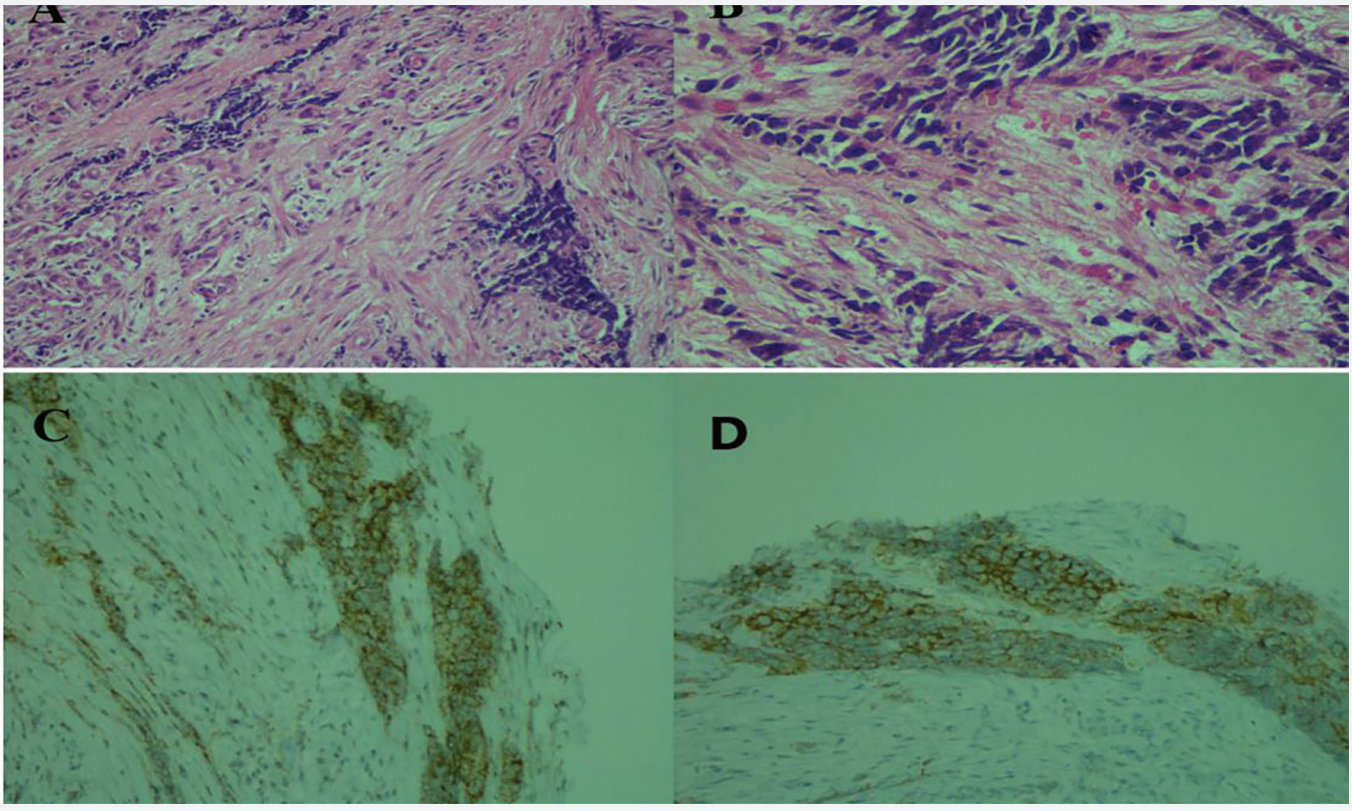

Figure 3. (A) Tumoral infiltration with crush artifacts, small hyperchromatic nuclei (hematoxylin and eosin ×100) (B) Tumoral infiltration with crush artifacts, small hyperchromatic nuclei, and nuclear irregularities (hematoxylin and eosin ×400) (C) Cytoplasmic positivity with CD56 immunohistochemistry (×400) (D) Cytoplasmic positivity with synaptophysin immunohistochemistry (×400)

of three distinct histologic subtypes in our case, the patient exhibited a favorable long-term outcome. This may reflect the potential influence of early detection, individualized treatment planning, and close radiologic follow-up in modifying the expected prognostic trajectory.

In conclusion, the prognosis of MPLC—both synchronous and metachronous—is considerably better than that of patients with intrapulmonary metastases. Despite the tumor-free interval in metachronous cases, no significant survival difference was noted between synchronous and metachronous MPLC in existing analyses[23]. When new suspicious pulmonary lesions arise during surveillance of patients with a prior lung cancer history, multidisciplinary evaluation is essential. Nevertheless, hesitation by clinicians to perform re-biopsies—due to procedural risks such as anticoagulation needs—and patient anxiety regarding recurrence, treatment delays, or invasive procedures continue to pose challenges in timely diagnosis and management of metachronous MPLC.

Despite the diagnostic challenges, advanced imaging technologies and multidisciplinary strategies significantly enhance the detection accuracy of metachronous MPLC. Such integrative approaches, as demonstrated in our case, enable the timely differentiation of second primary tumors from recurrences or metastases, allowing patients to benefit from curative interventions and improved long-term outcomes.

The follow-up of metachronous MPLC remains controversial, particularly in patients who have undergone previous curative resections. However, the American Association for Thoracic Surgery (AATS) guidelines emphasize the need for aggressive surveillance strategies in this population[25]. These include annual low-dose computed tomography (LDCT) screening up to age 79, and more intensive imaging every six months during the first 2-3 years post-resection, when recurrence risk is highest. Such structured monitoring protocols may facilitate earlier detection of new malignancies, particularly in high-risk individuals, and ultimately contribute to improved survival outcomes.

Understanding the molecular profile of metachronous MPLC is equally essential for developing personalized surveillance and therapeutic strategies. Genomic analysis using NGS can identify actionable somatic mutations such as TP53 and PTEN, which are associated with an increased risk of recurrence. These findings may support more aggressive treatment planning and

closer follow-up algorithms in genetically predisposed patients.

Finally, integration of radiological, pathological, and molecular data remains pivotal in enhancing diagnostic precision, informing prognosis, and optimizing clinical management of patients with MPLC.

CONCLUSION

Metachronous MPLC represents a complex and diagnostically challenging clinical entity that necessitates a comprehensive multidisciplinary approach. In patients with a history of lung cancer, the emergence of new suspicious pulmonary lesions during follow-up should raise clinical suspicion for metachronous MPLC. This case, involving three distinct histopathological subtypes over an 8-year period, contributes uniquely to the limited literature by illustrating the natural history and management complexities of triple metachronous MPLC. Our report emphasizes the need for high clinical vigilance and the critical role of serial imaging and histological confirmation in distinguishing new primary tumors from recurrence or metastasis. Whenever feasible, molecular analysis of the initial tumor using NGS should be performed to assess for potential hereditary or somatic predispositions. Notably, in our case, concurrent TP53 and PTEN mutations were identified, which may indicate an increased risk for metachronous tumor development and further underscores the importance of integrating molecular profiling into individualized follow-up strategies. Early detection and aggressive curative interventions, including surgical resection when appropriate, may improve clinical outcomes in selected patients diagnosed with MPLC. Histopathological confirmation remains essential to guide optimal therapeutic decision-making. While regular follow-up is universally important, the AATS recommends annual LDCT until the age of 79 in long-term lung cancer survivors, with semiannual imaging during the first 2-3 years after resection due to peak recurrence risk. However, these recommendations are not personalized and may not fully address the needs of patients with high-risk molecular profiles. In this context, more frequent surveillance—every 3 months during the first 2 years, followed by 3-6 month intervals during years 3 to 5—may facilitate timely identification of new lesions. Notably, despite our patient harboring high-risk prognostic factors such as somatic TP53 and PTEN mutations and three distinct histologic subtypes, the case demonstrated an unexpectedly favorable long-term clinical outcome. This observation suggests that intensified surveillance algorithms could be beneficial in selected high-risk MPLC populations. Prospective studies are warranted to validate whether personalized follow-up strategies improve early detection and overall survival in such patients. This case highlights the potential value of individualized surveillance strategies that integrate radiologic and molecular evaluation to enhance early detection, inform prognosis, and improve overall survival and quality of life in patients with MPLC.

Footnotes

Authorship Contributions

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

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Sadettin Çiftçi
Savaş İzzetoğlu
Selami Gürkan
Selma Tekin
Semih Aşıkovalı
Semih Çakmak
Semiha Cömertoğlu
Serap Baysal
Sercan Ergün
Serdar Kamil Çepni
Şeref Alpsoy
Serhat Örün
Serkan Yazıcı
Sevil Sadri
Şevket Barış Morkavuk
Sibel Yurt
Sonat Pınar Kara
Soner Kına
Suat Batar
Suna Ömerbaşoğlu
Tacettin İnandı
Tuba Özgöçer
Tuğba Nurcan Yüksel
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