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NAMIK KEMAL MEDICAL JOURNAL

CONTENTS

ORIGINAL ARTICLES

NAMIK KEMAL MEDICAL JOURNAL

CONTENTS

ORIGINAL ARTICLE

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Predictive Factors for Bleeding Complications after Ultrasound-guided Percutaneous Native Kidney Biopsy

Ultrason Eşliğinde Yapılan Perkütan Nativ Böbrek Biyopsisi Sonrası Kanama Komplikasyonlarını Öngören Faktörler

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ABSTRACT

Aim: Kidney biopsy is the gold standard for the diagnosis of parenchymal diseases of the kidney. The most common complication after kidney parenchymal biopsy is bleeding. The aim of this study was to investigate patient-related factors that increase the risk of bleeding complications after percutaneous native renal parenchymal biopsy.

Materials and Methods: A total of 132 patients who underwent percutaneous native kidney parenchymal biopsy were included in the study. Demographic data, comorbidities, indications for kidney biopsy, anticoagulant and/or antiaggregant drug use, blood pressure, hemoglobin (HGB), platelet, international normalised ratio (INR), glomerular filtration rate (GFR), urea, creatinine values recorded in patient files before biopsy, development of bleeding complications after biopsy, need for intervention for bleeding, need for transfusion and length of hospital stay due to bleeding were retrospectively analysed.

Results: Bleeding complications occurred in 17 patients (12.9%) after biopsy. Of the 17 patients with bleeding, 5 (3.8%) were major bleedings. No patient required embolisation or surgical intervention. A statistically significant correlation was observed between pre-procedural antiaggregant and/or anticoagulant drug use, HGB levels, INR and development of bleeding complications. In the group without bleeding complications, mean pre-procedure HGB; 11.5±2.45 g/dL and INR; 1.05±0.1, while in the bleeding group, HGB; 10±1.99 mg/dL and INR; 1.12±0.12 ($p=0.013$, $p=0.009$, respectively). A history of anticoagulant and/or antiaggregant use was 29.4% in the patients with bleeding and 10.4% in the group without bleeding ($p=0.045$).

Conclusion: Our study draws attention to the necessity of determining new safe limits especially in terms of haemogram, INR levels, anticoagulant and/or antiaggregant drug use parameters, defining the high-risk patient group and taking appropriate precautions before biopsy in order to perform percutaneous native renal parenchymal biopsy with a lower bleeding risk.

Keywords: Kidney biopsy, hemorrhage, risk factors

ÖZ

Amaç: Böbrek biyopsisi, böbreğin parankimal hastalıkların tanısında altın standarttır. Böbrek parankim biyopsisi sonrası en sık görülen komplikasyon kanamadır. Bu çalışmanın amacı perkütan nativ böbrek parankim biyopsisi sonrası kanama komplikasyonu riskini artıran hastaya ait faktörleri araştırmaktır.

Gereç ve Yöntem: Perkütan nativ böbrek parankim biyopsisi yapılan toplam 132 hasta çalışmaya dahil edildi. Hasta dosyalarında biyopsi öncesi kaydedilen demografik veriler, yandaş hastalıklar, böbrek biyopsisi endikasyonları, antikoagülan ve/veya antiagregan ilaç kullanımı, kan basıncı, hemoglobin (HGB), trombosit, uluslararası normalleştirilmiş oran (INR), glomerüler filtrasyon hızı, üre, kreatinin değerleri ile biyopsi sonrası kanama

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komplikasyonu gelişimi, kanamaya müdahale gereksinimi, transfüzyon ihtiyacı ve kanama nedeniyle hastanede kalış süresi retrospektif olarak incelendi.

Bulgular: Biyopsi sonrasında 17 hastada (%12,9) kanama komplikasyonu meydana geldi. Kanaması olan 17 hastanın 5'i (%3,8) majör kanamaydı. Hiçbir hastada embolizasyon veya cerrahi müdahale gerekmedi. İşlem öncesi antiagregan ve/veya antikoagülan ilaç kullanımı, HGB düzeyleri, INR ve kanama komplikasyonu gelişimi arasında istatistiksel olarak anlamlı bir ilişki gözlendi. Kanama komplikasyonu olmayan grupta işlem öncesi ortalama HGB;11,5±2,45 g/dL ve INR; 1,05±0,1 iken, kanama grubunda HGB; 10±1,99 mg/dL ve INR; 1,12±0,12 saptandı (Sırasıyla p=0,013, p=0,009). Kanama saptanan hastalarda antikoagülan ve/veya antiagregan kullanım öyküsü %29,4 iken kanama olmayan grupta %10,4 saptandı (p=0,045).

Sonuç: Çalışmamız, perkütan nativ böbrek parankim biyopsisinin daha düşük kanama riskiyle uygulanabilmesi için biyopsi öncesi özellikle hemogram, INR düzeyleri, antikoagülan ve/veya antiagregan ilaç kullanımı parametreleri açısından yeni güvenli sınırların belirlenmesi, yüksek riskli hasta grubunun tanımlanması ve uygun önlemlerin alınması gerekliliğine dikkat çekmektedir.

Anahtar Kelimeler: Böbrek biyopsisi, kanama, risk faktörleri

INTRODUCTION

Kidney biopsy is the gold standard for diagnosing kidney parenchymal diseases[1]. Kidney biopsies can be performed using open surgical methods, laparoscopy, and percutaneous techniques guided by imaging. Among these, percutaneous kidney parenchymal biopsies are most commonly conducted under ultrasound (US) guidance[1].

In a large-scale study including percutaneous biopsies of transplanted kidneys and other organs, the highest rate of major bleeding was observed following native kidney biopsies[2]. Consistently, bleeding is also the most common complication after percutaneous native kidney biopsy[3,4]. Reported bleeding rates after percutaneous native kidney biopsy vary considerably in the literature due to the heterogeneity of study designs, different definitions of complications, and the predominance of single-centre experiences[4,5]. A meta-analysis by Poggio et al.[3] reported the incidence of perinephric hematoma as 11%. However, in studies where routine post-biopsy imaging was performed regardless of clinical symptoms, perinephric hematoma rates exceeding 80% have been reported[6,7]. Bleeding-related complications are often detected 12-24 hours after the procedure[4,8]. Various risk factors associated with the patient's characteristics, accompanying diseases, and medications (especially antiplatelet and anticoagulant agents), the centre, the technique of the procedure, and the equipment used in the procedure may lead to bleeding complications[8,9]. The aim of this study in our centre was to evaluate the incidence of bleeding complications and associated risk factors after native kidney biopsies performed despite standard pre-biopsy preparations.

MATERIALS AND METHODS

A total of 143 consecutive patients who underwent US-guided percutaneous kidney biopsy at the Nephrology Clinic of Muğla Training and Research Hospital between October 2021 and August 2024 were retrospectively reviewed. Patients under 18 years of age, those who underwent biopsy of a transplanted kidney, and those who had biopsies performed to investigate malignancy in solid kidney masses were excluded from the study. After applying the exclusion criteria, 132 patients who underwent native kidney parenchymal biopsy were included in the final analysis. Ten patients were excluded due to undergoing a biopsy of a transplanted kidney, and another was excluded due to a biopsy performed for a solid kidney mass.

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine and Health Sciences at Muğla University (decision no: 36, date: 27.02.2024).

Demographic information of the patients, comorbidities, use of anticoagulants and antiplatelet agents, pre-biopsy blood pressure, biochemical parameters [hemogram, blood urea nitrogen (BUN), glomerular filtration rate (GFR), creatinine, spot urine protein/creatinine ratio, prothrombin time (PT), international normalized ratio (INR)], indications for kidney biopsy, ultrasonographically measured kidney size, needle gauge, presence of bleeding complications, treatment applied in cases of bleeding and pathology results were recorded from patient files. Major bleeding complications are defined as those requiring treatment (blood transfusion, embolization, nephrectomy) or resulting in death, while minor bleeding complications are defined as those not requiring treatment.

At Muğla Training and Research Hospital, there is a standard procedure that must be followed for every patient who has been approved to undergo a kidney biopsy by the Interventional Radiology and Nephrology Departments. All percutaneous kidney biopsy procedures were performed by an interventional radiologist with 4 years of clinical experience in interventional radiology.

Pre-biopsy Preparation

Before the procedure, all patients were informed, and their consent was obtained. Patients taking antiplatelet agents or anticoagulants were referred to the relevant departments for risk evaluation prior to the biopsy.

If deemed appropriate, the medication may be discontinued; if not, a bridging therapy plan is discussed. For patients on antiplatelet therapy, the medication is stopped 5 days before the procedure and resumed 24 hours after. For those on warfarin, the patient is transitioned to low molecular weight heparin (LMWH) for bridging. Once an appropriate INR is achieved, the LMWH is discontinued 24 hours before the procedure and resumed 24 hours after.

One day prior to the procedure, patients' INR and platelet counts are checked. According to the guidelines published by the Cardiovascular and Interventional Radiological Society of Europe (CIRSE), interventional procedures are categorized into risk groups, with percutaneous kidney biopsies classified as high-risk[10]. It is routinely recommended to check INR, platelet count, and hematocrit levels before these procedures[10]. Before the procedure, the INR should be less than 1.5, and the platelet count needs to exceed 50,000/mm^3. In our department, having an INR below 1.5 and a platelet count above 50,000/mm^3 is considered an absolute requirement for performing a kidney biopsy.

One day prior to the biopsy procedure, all patients undergo an US evaluation of their kidneys to assess kidney size, parenchyma echogenicity, presence of hydronephrosis, and any cystic or tumoral formations, as well as anatomical variations. The specific kidney to be biopsied and the method of biopsy are determined.

Percutaneous Kidney Biopsy Procedure and Follow-up

All percutaneous kidney biopsies were performed under local anesthesia. Procedures were conducted using real-time US guidance (Toshiba Aplio 500, Toshiba Corporation, Tokyo, Japan). A 16-gauge 10 cm (22 mm tissue embedding distance, 17 mm sample collection chamber) fully automatic Tru-cut biopsy needle (Geotek Healthcare Products, Ankara, Türkiye) was employed consistently across all cases. The number of cores taken during biopsy is at least two and at most three. Patients were positioned prone during the procedure. The Tru-cut biopsy gun penetrates the kidney capsule from the lower pole of the kidney, and tissue samples are obtained using a tangential technique parallel to the capsule. At the end of the procedure, an US is performed to check for bleeding. Each patient is re-evaluated with US for bleeding control two hours after the procedure. All patients are monitored as inpatients in the nephrology clinic for 24 hours following the procedure. All patients are advised to rest in bed for the first six hours post-biopsy. Complete blood count monitoring is conducted for all patients eight hours after the procedure and the following morning. For patients with a decrease in HGB levels, a control US is performed the next day for bleeding assessment.

Statistical Analysis

All statistical analyses were performed utilizing IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., 2017, Armonk, NY). The chi-square test or Fisher's exact probability test (for tables with minimum expected values below 5) was employed to compare categorical variables between two groups. Variables were summarized using frequencies and percentages. The normality of numerical variables was evaluated using the Kolmogorov-Smirnov test ($n \geq 50$) or the Shapiro-Wilk test ($n < 50$). Since the data did not conform to a normal distribution, the Mann-Whitney U test, a non-parametric alternative, was used to compare the two groups with and without bleeding. To assess changes in HGB concentrations before and after the intervention, the Wilcoxon signed-rank test was applied. In addition, for the variables found to be statistically significant, univariate logistic regression analysis was performed to calculate the odds ratios (ORs). All hypothesis tests were performed at a significance level of 0.05; p-values below this level were considered statistically significant.

RESULTS

A total of 132 kidney biopsy patients who underwent the procedure between October 2021 and August 2024 were included in our study. The mean age of the patients was 49.2±17.4 years. Of the patients, 54% were male and 46% were female. The most common comorbidities were hypertension (HT) in 53%, diabetes mellitus in 23.5%, cardiovascular disease in 12.1%, and malignancy in 8.3%. The most frequent indication for biopsy was nephrotic syndrome, occurring in 30.3% of cases (Table 1).

Baseline laboratory and radiological data of the patients in accordance with the routine preparation before the biopsy procedure are presented in Table 2.

Bleeding complications occurred in 17 patients (12.9%). Of the 17 patients with bleeding, 5 (3.8%) were classified as having major bleeding (requiring blood transfusion). None of the patients with bleeding needed embolization or surgical intervention (Table 3).

A statistically significant correlation was observed between pre-biopsy HGB levels, INR, and the occurrence of bleeding complications. In the group without bleeding complications, the mean pre-biopsy HGB value was 11.5±2.45 g/dL, while in the bleeding group, it was 10±1.99 g/dL ($p=0.013$). The INR value in the non-bleeding group was 1.05±0.1, whereas in the bleeding group it was 1.12±0.12 ($p=0.009$).

Among the 5 patients with bleeding complications, a history of antiplatelet (2 patients on ASA) or anticoagulant (1 patient on warfarin, 1 patient on direct oral anticoagulant, and 1

Table 1. Demographical and medical historical characteristics of patients

Characteristic	n (%) or mean ± SD (min-max)
Age (years)	49.2±17.4 (16-85)
Gender, n (%)	
Male	71 (53.8%)
Female	61 (46.2%)
Comorbidities, n (%)	
Diabetes	31 (23.5%)
Hypertension	70 (53.0%)
Cardiovascular disease	16 (12.1%)
Malignancy	11 (8.3%)
Connective tissue disease	10 (7.5%)
Anticoagulant/antiplatelet use, n (%)	17 (12.8%)
Acetylsalicylic acid	7 (5.3%)
Clopidogrel	1 (0.7%)
Clopidogrel + acetylsalicylic acid	1 (0.7%)
LMWH	2 (1.5%)
Warfarin transitioned to LMWH	3 (2.2%)
Edoxaban	1 (0.7%)
Rivaroxaban	2 (2.2%)
Biopsy indications, n (%)	
Hematuria + non-nephrotic proteinuria	14 (10.6%)
Isolated proteinuria	20 (15.2%)
Unexplained kidney failure	32 (24.2%)
Nephritic syndrome	26 (19.7%)
Nephrotic syndrome	40 (30.3%)
Total number of patients	**132 (100%)**

LMWH: Low molecular weight heparin, SD: Standard deviation

patient on rivaroxaban) use was noted in 29.4% of cases. There was a statistically significant association between the use of antiplatelet/anticoagulant therapy and bleeding complications (p=0.045). No statistically significant association was found between bleeding complications and other variables, including patient sex, age, comorbid conditions, BUN, creatinine levels, GFR, platelet count, biopsy indication, and pre-biopsy blood pressure (Table 4).

ROC analysis identified the optimal INR threshold for predicting post-biopsy bleeding as ≥1.095 [area under the curve: 0.695; 95% confidence interval (CI): 0.551-0.840]. Using this cut-off, bleeding occurred significantly more often in patients with INR ≥1.095 (12/47, 25.5%) compared with INR <1.095 (5/85, 5.9%) (p=0.001). Binary logistic regression using the categorized INR variable showed an OR of 5.49 (95% CI: 1.80-16.75, p=0.003), indicating that INR ≥1.095 is independently associated with increased bleeding risk.

Table 2. Pre-biopsy clinical, biochemical and radiological measures

Measures	mean ± SD (min-max)
Blood pressure (mmHg)	
Systolic	127.8±19.9 (90-160)
Diastolic	78.2±11.5 (50-110)
Biochemical data	
BUN (mg/dL)	65.6±41.2 (12.6-227.5)
Creatinine (mg/dL)	2.25±2.03 (0.42-12.1)
GFR (mL/min/1.73 m²)	57±41.2 (4.9-147)
INR	1.06±0.11 (0.85-1.50)
HGB (g/dL)	11.4±2.45 (6.8-17.0)
Platelet (mm³)	259,167±99,146 (24,100-606,000)
Proteinuria (mg/g)	3,202.6±2,803.2 (85-14,260)
Size of the biopsied kidney	n (%)
≥10 cm	119 (90.2%)
<10 cm	13 (9.8%)
Total number of patients	132 (100%)

BUN: Blood urea nitrogen, GFR: Glomerular filtration rate, INR: International normalized ratio, HGB: Hemoglobin, SD: Standard deviation

Table 3. Post-procedural bleeding complications, need for transfusion after bleeding, follow-up due to bleeding

Variable	n (%)
Bleeding complications	
Yes	17 (12.9%)
Major	5 (3.8%)
Minor	12 (9.1%)
No	115 (87.1%)
Need for transfusion	
Yes	5 (3.8%)
No	127 (96.2%)
Follow-up, days	
No	9 (6.8%)
3 days	2 (1.5%)
4 days	2 (1.5%)
8 days	1 (0.76%)
10 days	2 (1.5%)
14 days	1 (0.76%)
Total number of patients	132 (100%)

Lower pre-biopsy HGB levels were also identified as a risk factor for bleeding, with a protective effect observed per unit increase in HGB (p=0.020; OR=0.751; 95% CI: 0.590-0.957) (Table 5). Due to the limited number of cases with major bleeding, multivariate regression analysis was not conducted, as it would not yield statistically reliable results and may risk overfitting. The slight discrepancy between the Mann-Whitney U test and

Table 4. Association of clinical, biochemical and radiologic factors with bleeding complications after percutaneous native kidney biopsy

Factors	Bleeding (-)	Bleeding (+)	p-value
Age (years, mean ± SD)	49.7±17.04	45.5±19.72	0.445
Gender, n (%)			0.551
Female	52 (85.2%)	9 (14.8%)	
Male	63 (88.7%)	8 (11.3%)	
Comorbidities, n (%)			-
Diabetes	30 (96.8%)	1 (3.2%)	0.074
Hypertension	62 (88.6%)	8 (11.4%)	0.597
Cardiovascular disease	13 (81.3%)	3 (18.8%)	0.434
Malignancy	10 (90.9%)	1 (9.1%)	1.000
Use of anticoagulant/antiplatelet drugs, n (%)			**0.045**
Use	12 (70.6%)	5 (29.4%)	
No use	103 (89.6%)	12 (10.4%)	
Blood pressure (mmHg)			-
Systolic	128.2±19.8	125.1±20.8	0.627
Diastolic	78.3±11.2	77.5±13.7	0.606
Biopsy indications, n (%)			0.605
Hematuria + non-nephrotic proteinuria	13 (92.9%)	1 (7.1%)	
Isolated proteinuria	16 (80.0%)	4 (20.0%)	
Unexplained kidney failure	29 (90.6%)	3 (9.4%)	
Nephritic syndrome	21 (80.8%)	5 (19.2%)	
Nephrotic syndrome	36 (90.0%)	4 (10.0%)	
Biochemical factors			-
BUN (mg/dL)	65.1±40.0	69.2±49.8	0.900
Creatinine (mg/dL)	2.17±1.89	2.83±2.86	0.386
GFR (mL/min/1.73 m²)	57.9±40.7	50.9±45.1	0.411
INR	1.051±0.101	1.128±0.120	**0.009**
Pre-biopsy HGB (g/dL)	11.5±2.45	10.0±1.99	**0.013**
Platelet (mm³)	262,784±100,070	234,706±91,671	0.110
Size of the biopsied kidney (cm)			0.374
≥10 cm	105 (88.2%)	14 (11.8%)	
<10 cm	10 (76.9%)	3 (23.1%)	

BUN: Blood urea nitrogen, GFR: Glomerular filtration rate, INR: International normalized ratio, HGB: Hemoglobin, SD: Standard deviation

Table 5. Univariate binary logistic regression analysis of factors associated with bleeding

Variable	OR	95% CI	p-value
INR ≥1.095	5.49	1.80-16.75	0.003
Pre-biopsy HGB	0.751	0.590-0.957	0.020
Use of anticoagulant / antiplatelet drugs	3.576	1.075-11.903	0.038

The odds ratio for INR may be unstable due to the narrow INR range and the limited number of bleeding events in our cohort. Due to the low number of major bleeding events (n=5), all regression analyses were performed for any bleeding (major + minor)

OR: Odds ratio, CI: Confidence interval, INR: International normalized ratio, HGB: Hemoglobin

logistic regression p-values is attributable to differences in statistical methodology (rank-based vs. model-based analysis). In patients with bleeding, hemoglobin levels decreased significantly after biopsy according to the Wilcoxon signed-rank test (p=0.017).

DISCUSSION

In our study, the overall rate of bleeding complications following US-guided percutaneous kidney biopsy was 12.9%, with major and minor complications occurring in 3.8% and 9% of cases, respectively. These findings are consistent with previously published studies for both major and minor bleeding rates.

A meta-analysis conducted by Poggio et al.[3] in 2020, which included 118,064 biopsies, reported a bleeding complication rate identified by hematoma of 11%, indicating that our overall bleeding rate is similar. However, the rate of requiring blood transfusion in that meta-analysis was 1.6%, which is lower than the corresponding rate in our study[2]. In other studies focusing on native kidney biopsies, major bleeding complication rates ranged from 1.1% to 8%[5,11-15]. The major bleeding rate observed in our study falls within this wide range and ranks approximately in the middle of the reported values[5,11-15].

In the study by Korbet et al.[12], cystoscopy was required in 0.18%, radiological embolization in 0.85%, and the mortality rate was 0.09%; in the study by Halimi et al.[5], angiographic intervention was needed in 0.4%, and nephrectomy was performed in 0.1%; in Waldo et al.'s[11] study, radiological interventions were required in 1.2%; and in the study by Lees et al.[16], embolization was necessary in 0.4%, with a mortality rate of 0.04%. In our study, all 5 patients who experienced major bleeding were treated with blood transfusions only, and no interventional or surgical treatments were necessary. There was no organ loss (nephrectomy) or mortality. The low number of patients may have reduced the likelihood of detecting complications such as mortality, nephrectomy, or bleeding requiring interventional procedures. More than 30 kidney biopsies, with an average of 35.2 patients per year, were performed at our center by an experienced interventional radiologist. This moderate-volume of procedures likely contributed to the positive outcomes observed in our study. These results are consistent with those reported by Tøndel et al.[15].

In our study, the majority of bleeding complications (94.1%) were observed within the first 24 hours following the procedure, with only a single case (5.9%) identified at 120 hours post-biopsy. In alignment with recommendations from the literature aimed at ensuring reliable complication detection[17,18], our institutional protocol mandates a minimum of 24 hours of post-procedural monitoring for all patients. We therefore believe that this practice enabled the timely identification of the vast majority of potential complications.

Numerous studies in the literature have explored the association between coagulation tests including PT, partial thromboplastin time (PTT), INR and bleeding time (BT)—and post-biopsy bleeding complications. Although PT, PTT, and INR are routinely assessed prior to kidney biopsy, several studies have shown that, within acceptable reference ranges, these parameters are not significantly correlated with bleeding risk after biopsy[16,19]. In Whittier et al.[20] cohort, patients with lower baseline HGB exhibited progressively longer BT, although prolonged BT was not independently predictive of transfusion requirements or major bleeding events. In contrast to this study, in the study by Korbet et al.[12], BT >7.5 min was found to be among the features that predicted any complication after kidney biopsy. In a recent review it was suggested that INR and PTT values should be within normal ranges before performing a kidney biopsy[1]. However, in this review, the findings related to BT were considered controversial[1], and as noted in the CIRSE guidelines, BT has largely fallen out of favor in modern clinical practice for assessing bleeding risk due to conflicting evidence regarding its utility[10]. In our study, BT values were not included in the pre-biopsy assessment protocol, and PTT was not analyzed, as none of the patients were receiving intravenous unfractionated heparin.

We identified a statistically significant relationship between bleeding complications following percutaneous native kidney biopsy and pre-biopsy INR, HGB levels, and the use of anticoagulants and/or antiplatelet agents. According to our institutional protocol, an INR below 1.5 was deemed adequate for proceeding with kidney biopsy[10]. However, a notable finding in our cohort was that 35% (6 out of 17) of patients who developed bleeding complications had an INR exceeding the laboratory upper limit of 1.2. Furthermore, 50% (5 out of 10) of all patients with an INR above 1.2 experienced post-biopsy bleeding. These observations suggest that using a more stringent INR threshold, specifically maintaining the INR below the laboratory reference upper limit of 1.2 - as proposed in the review by Schnuelle et al.[1] - may provide enhanced safety in minimizing bleeding risks following percutaneous kidney biopsy.

Although the association between INR and bleeding was statistically significant, the observed (OR=5.49) should be evaluated cautiously. The point estimate should be interpreted with caution because of sparse data. This extreme value likely reflects the limited number of bleeding complications and the narrow INR distribution in our cohort rather than a true clinical effect. All biopsies were performed only when INR <1.5, and most values were clustered around 1.0-1.2, which may have amplified the apparent risk in the univariate model. No outliers or data errors were identified, and the observed effect is more likely attributable to quasi-separation than to model misspecification. Accordingly, the INR-bleeding relationship should be regarded as an indicative trend rather than a precise quantitative estimate.

In our study, we observed that anemia heightened the risk of bleeding complications following percutaneous native kidney biopsy, a finding that aligns with other published studies[5,13,14,21,22]. In a study by Pombas et al.[14], HGB levels below 10 g/dL were identified as a risk factor for major complications after kidney biopsy. Similarly, a study by Aaltonen et al.[22], which included outpatient and kidney transplant patients, found a correlation between anemia and bleeding complications. In the French cohort study by Halimi et al.[5], anemia was shown to

increase the risk of major bleeding by 3.47 times. In a cohort study by Palsson et al.[13], when patients with HGB levels <10 g/dL were compared to those with ≥10 g/dL, 14.5% of the anemic group required blood transfusions after biopsy, while only 1.2% of the non-anemic group needed transfusion. Given the observed associations between anemia and increased bleeding risk in our study, consistent with findings from larger cohorts, we hypothesize that low HGB levels may serve as a potentially modifiable risk factor for post-biopsy bleeding complications. However, further large-scale prospective studies are warranted to confirm this relationship and to guide pre-biopsy risk stratification.

According to our pre-biopsy protocol, antiplatelet and anticoagulant medications were discontinued prior to the procedure. However, we found that a history of using these medications was still associated with bleeding complications. This suggests that past use of these drugs may contribute to bleeding risk, even if discontinued. Due to the small number of cases, both medication groups (antiplatelets and anticoagulants) were evaluated together. Therefore, we cannot provide separate evidence for antiplatelet or anticoagulant use. However, there are studies in the literature with conflicting results regarding the use of antiplatelet drugs, particularly aspirin, in kidney biopsy. Some studies have found that aspirin use during biopsy increases the risk of bleeding[9,23], while others have shown no such increase[2,16,24]. A meta-analysis published in 2023, evaluating the effect of aspirin on bleeding complications after kidney biopsy, concluded that aspirin does not elevate the risk of major bleeding[25].

Kidney biopsy complications are generally minimized by discontinuing antiplatelet medications, including non-steroidal anti-inflammatory drugs (NSAIDs), prior to the procedure. It is typically advised to stop these medications 7-10 days before the biopsy to reduce the risk of bleeding[4,26]. Only in emergency situations should aspirin (100 mg) be continued[1,2,16,27]. For anticoagulants, it is recommended that warfarin be stopped 5 days prior, conventional heparin at least 6 hours prior, and direct Xa inhibitors 72 hours before the procedure[1,28]. At our center, antiplatelet therapy was discontinued 5 days before biopsy, which is shorter than the 7-10 days recommended in some reviews[1,4]. This should be considered a potential limitation and confounder. Additionally, our patients are not routinely questioned about the use of commonly used NSAIDs which may have antiplatelet effects. In our study, the use of anticoagulants and antiplatelet agents, even when discontinued according to established protocols, remained a significant risk factor for bleeding complications. Further research, particularly in elective biopsies, designed to address the pharmacological mechanisms of these drugs is needed to establish safe therapeutic windows for treated patients.

Study Limitations

Numerous studies have sought to identify risk factors associated with bleeding complications following US-guided percutaneous native kidney biopsy. These investigations have explored a wide range of variables, including patient age, sex, kidney size, needle gauge, the number of needle passes, comorbid conditions (e.g., HT, amyloidosis), as well as pre-biopsy laboratory parameters such as serum creatinine, BUN, and estimated glomerular filtration rate (eGFR)[12,15,16,19,29-34]. In our study, all biopsies were performed using a 16-gauge biopsy needle, with 2-3 passes targeting the kidney cortex. Biopsies were not conducted in patients whose kidneys measured less than 90 mm in longitudinal diameter or in those with poor corticomedullary differentiation on ultrasonography. As needle gauge was standardized in all cases, its association with bleeding complications could not be evaluated. Furthermore, we found no statistically significant association between post-biopsy bleeding complications and demographic or clinical factors such as age, sex, kidney size, comorbidities, biopsy indications, histopathological diagnoses, or degree of proteinuria. Contrary to the findings reported by Lees et al.[16], Monahan et al.[19], Tøndel et al.[15], and Korbet et al.[12] our analysis did not reveal any correlation between serum creatinine, BUN or eGFR values and the occurrence of bleeding complications. The retrospective design, single-center setting, and relatively small sample size of our study may have limited its statistical power to detect potential associations.

CONCLUSION

In conclusion, US-guided percutaneous native kidney biopsy remains an essential diagnostic tool with an acceptable safety profile. In our study, bleeding complications were consistent with prior reports and mainly occurred within 24 hours without major adverse outcomes. Pre-biopsy INR, anemia, and prior use of anticoagulants or antiplatelet agents were significantly associated with bleeding risk, while other clinical and demographic factors showed no correlation. Considering the limitations of our study, larger prospective multicenter studies are needed to better define bleeding risk factors and optimize biopsy protocols to enhance patient safety.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine and Health Sciences at Muğla University (decision no: 36, date: 27.02.2024).

Informed Consent: The retrospective design is a single-center setting study.

Footnotes

Authorship Contributions

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

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Occupational Risks and Safety Practices of Auto Paint Workers: A Qualitative Study

Oto Boya İşçilerinin Mesleki Riskleri ve Güvenlik Uygulamaları: Nitel Bir Çalışma

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ABSTRACT

Aim: The aim of this study was to identify the occupational risks, working conditions, health problems, and protective practices related to worker health among auto paint workers.

Materials and Methods: This research was designed as a qualitative study using a phenomenological approach. A total of 13 auto paint workers participated in the study. Data were collected through face-to-face, semi-structured, and in-depth interviews.

Results: Participants primarily reported exposure to chemical hazards as their main occupational risk. In addition, long working hours and working in cold environments, particularly during the winter months, were identified as additional occupational risks. The most commonly reported health problems were respiratory diseases and dermatological conditions. The findings also revealed that occupational training activities and protective practices were inadequate. Furthermore, participants expressed concerns about the lack of new apprentices in industrial areas, emphasizing the potential shortage of skilled auto paint workers in the future.

Conclusion: The respiratory and dermatological health problems observed among auto paint workers were largely associated with chemical exposure and insufficient protective measures. These findings indicate a clear need for systematic interventions aimed at increasing risk awareness and ensuring the effective and consistent use of personal protective equipment.

Keywords: Employee, safety, auto paint, chemical, occupational

ÖZ

Amaç: Bu çalışmanın amacı, oto boyacıların mesleki risklerini, çalışma koşullarını, sağlık sorunlarını ve çalışan sağlığını korumaya yönelik uygulamalarını belirlemektir.

Gereç ve Yöntem: Araştırma, fenomenolojik yaklaşım temelinde tasarlanmış nitel bir çalışmadır. Çalışmaya toplam 13 oto boyacı dahil edilmiştir. Veriler, yüz yüze gerçekleştirilen yarı yapılandırılmış ve derinlemesine görüşmeler aracılığıyla toplanmıştır.

Bulgular: Katılımcılar, başta kimyasal maddeler olmak üzere çeşitli mesleki risklere maruz kaldıklarını belirtmiştir. Uzun çalışma saatleri ile özellikle kış aylarında soğuk ortamlarda çalışma, ek mesleki riskler olarak ifade edilmiştir. En sık bildirilen sağlık sorunları solunum sistemi hastalıkları ve deri rahatsızlıklarıdır. Bulgular, mesleki eğitim faaliyetlerinin ve koruyucu uygulamaların yetersiz olduğunu ortaya koymuştur. Ayrıca katılımcılar, sanayi bölgelerinde yeni çırakların yetişmemesinin gelecekte nitelikli oto boyacı eksikliğine yol açacağına ilişkin endişelerini dile getirmiştir.

Sonuç: Oto boyacılarda gözlenen solunum ve dermatolojik sağlık sorunlarının büyük ölçüde kimyasal maruziyet ve yetersiz koruyucu uygulamalarla ilişkili olduğu görülmüştür. Bu bağlamda, risk farkındalığını artıran ve kişisel koruyucu ekipman kullanımını teşvik eden sistematik müdahalelere ihtiyaç bulunmaktadır.

Anahtar Kelimeler: Çalışan güvenliği, güvenlik, oto boyası, kimyasal, mesleki

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INTRODUCTION

Automotive painting is a significant sector in Türkiye, encompassing the surface preparation and painting processes of both metal and non-metal motor vehicle parts. Due to its inherent characteristics, this sector is classified as hazardous under the Occupational Health and Safety Regulation for Workplaces[1]. The automotive industry, which is classified as a hazardous sector, ranks fourth among European countries in terms of production volume, with Türkiye being one of the leading producers. Approximately 60,000 workers are employed in this sector; however, occupational subcategories such as painting, bodywork, and assembly are not systematically distinguished in workforce statistics[2].

In large-scale enterprises, occupational health and safety (OHS) practices are actively implemented in accordance with legal regulations[3]. However, outside these enterprises, workers engaged in automotive repair, maintenance, and servicing activities who are often self-employed or employed in small-scale enterprises frequently have limited access to and inadequate implementation of protective OHS measures. The workforce in these enterprises typically ranges from one to five employees[4]. Due to their small size, these enterprises are classified as micro-workplaces, where OHS practices are often insufficient[3].

In small-scale automotive painting operations, spray painting is the most commonly used technique. In Türkiye, automobile painting is frequently performed in workshops located within industrial zones, often without the use of specialized spray booths. Instead, spray guns are commonly operated in open environments or, in some cases, within paint ovens. This practice of open spraying poses a substantial chemical hazard to both workers and the surrounding environment[2].

Automotive paints, which are widely used across various applications, generally consist of five main components: binders, solvents, fillers, additives, and pigments. In this sector, hazardous substances such as toluene, xylene, heptane, methanol, polyurethane, ethylbenzene, and benzene are extensively used. Volatile organic compounds (VOCs) present in paints—particularly solvents—are known to cause persistent and potentially fatal damage to the respiratory tract and mucous membranes[4-6]. Moreover, both acute and chronic exposure to these chemicals may result in systemic effects involving multiple organs and physiological systems[7,8].

The most significant health effects associated with VOC exposure include lung cancer, acute and chronic respiratory diseases, neurological toxicity, and irritation of the eyes and throat[3,8,9]. In addition, heavy metals such as chromium, lead, and mercury found in paints exert systemic toxic effects. Lead exposure can impair the central nervous system and disrupt hemoglobin synthesis, whereas chromium exposure may cause chrome ulcers on the skin and is strongly associated with lung cancer[10]. Furthermore, exposure to thinners and gasoline, which are frequently used in paint removal processes, further exacerbates these health risks[4].

The automotive sector represents a rapidly growing industry both in Türkiye and globally. In Türkiye alone, approximately 28 million vehicles are registered[11]. The increasing number of vehicles has expanded the workload in this sector, thereby intensifying labor demand across related fields and sub-sectors. Despite this growth, research focusing on OHS risks in the auto painting industry remains limited[5,8,9]. Existing studies indicate that auto painters often work under conditions that are detrimental to health, do not consistently adopt preventive measures, and place insufficient emphasis on periodic medical examinations[5,8].

Therefore, this study aims to systematically identify OHS risks in the auto painting sector, evaluate exposure to physical, chemical, and ergonomic hazards, and develop evidence-based preventive measures to reduce the risk of occupational accidents and work-related diseases.

MATERIALS AND METHODS

Study Type

This study was conducted using a qualitative phenomenological research design to explore the occupational exposures of auto painters working in an industrial site. Phenomenology is a qualitative research approach that enables individuals to express their perceptions, interpretations, perspectives, and emotions related to a specific phenomenon, thereby revealing their lived experiences. Rather than focusing on social norms or generalized attitudes, phenomenological research concentrates on phenomena that are recognized but not yet fully or deeply understood, with an emphasis on individual experiences and meanings attributed to those experiences. This approach facilitates an in-depth understanding of participants' lived experiences related to occupational exposure[12].

Permission was obtained from the Trakya University Non-Interventional Clinical Research Ethics Committee with (decision number: 2023.13/15, date: 11.09.2023). Informed consent was obtained from the participants, and throughout the research process, the principles of the Helsinki Declaration were adhered to. Participants were assured of the confidentiality of their identities and audio recordings.

Study Population and Sample

In qualitative research, purposive sampling is commonly employed to obtain rich and in-depth data. Because qualitative studies aim to explore specific experiences rather than achieve

statistical generalizability, sample size calculation is not required. Data collection continues until data saturation is achieved that is, when no new themes or insights emerge from the interviews. In this study, data saturation was reached after interviewing 13 auto painters working in the industrial site, at which point data collection was concluded[13].

Inclusion Criteria

Auto painters who were able to read, understand, and express themselves clearly and who voluntarily agreed to participate in the study were included.

Data Collection Tools: The semi-structured interview form was pilot-tested with three auto painters who were not included in the main study sample. The pilot interviews were conducted to assess the clarity, relevance, and flow of the questions. Based on feedback from the pilot process, minor revisions were made to improve wording and to expand selected questions related to occupational risks, preventive measures, and perceived health effects. The pilot and final versions of the interview form are provided as Supplementary File 1.

Data were collected using a Personal Information Form and a Semi-Structured Interview Form.

Personal Information Form: This form consisted of five questions addressing the participants' socio-demographic and occupational characteristics, including age, gender, education level, years of work experience, and weekly working hours.

Semi-Structured Interview Form: The semi-structured interview form was developed by the researchers based on a review of the relevant literature. The draft interview guide was reviewed by three field experts to ensure content validity and methodological appropriateness. The final interview form included seven open-ended questions focusing on working conditions and occupational exposures of auto painting workers. The interview questions related to working conditions and occupational exposures are presented in Table 1.

Research Procedure

The research was conducted between September and November 2023. Data were collected through face-to-face interviews using an in-depth interview technique by a researcher. The researcher holds a doctoral degree in OHS and has conducted studies in the field of employee health. Prior to data collection, participants were provided with information and their consent was obtained. Interviews were conducted when the paint workers felt suitable and ready for the interview, in the office section of the workshop. The interviews were recorded using an audio recording device. Participants were specifically informed that the data and recordings obtained from the study would only be used for scientific purposes. Individual, in-depth, and semi-structured face-to-face interviews were conducted until data saturation was reached, that is, until no new information or themes emerged. The duration of the interviews varied between 30-45 minutes depending on the responses provided.

Statistical Analysis

Descriptive analysis and content analysis were used to analyze the data. Audio recordings obtained during the interviews were transcribed verbatim. The transcribed data were then analyzed using content analysis, a widely used qualitative data analysis method.

Descriptive analysis was employed to organize and interpret the data obtained from interviews in a systematic manner. Within this approach, the findings were presented as closely as possible to their original form, and direct quotations from participants were included where appropriate to reflect their perspectives. Content analysis, in contrast, aimed to identify underlying concepts and relationships that could explain the data obtained from the participants. Data summarized and interpreted through descriptive analysis were further examined in depth using content analysis, during which concepts were derived from the data and relationships among these concepts were identified. The findings were subsequently organized into concepts, categories, and themes to enhance interpretability[13].

Table 1. Interview questions

When and how did you start this profession?
Did you receive any training before or after starting auto painting?
Do you have any standards for preventive measures and implementation guidelines to protect your health?
What are the occupational risks you are exposed to in this profession?
Do you think you have developed any health problems due to your profession? If yes, what is the reason for this belief, and what suggestions do you have?
Are you satisfied with your working conditions? (employee safety, working conditions, salary, training, health checks, etc.)
Have you experienced any work-related accidents in your profession? If yes, what was the cause and result of the accident? And what are your suggestions for the situation?

In this study, auto paint workers' perceptions of occupational risks were initially described using descriptive analysis and subsequently evaluated in terms of conceptual relationships through content analysis. Participants' direct quotations were included under relevant categories to support the findings[13].

To ensure internal consistency and reliability of the coding process, inter-coder agreement was calculated using the formula proposed by Miles and Huberman: $\Delta = C \div (C + D) \times 100$, where Δ represents the reliability coefficient, C denotes the number of coding agreements, and D represents the number of disagreements. An inter-coder agreement of at least 80% is considered acceptable[14]. In this study, inter-coder agreement among the researchers was calculated as 89%. In cases of disagreement, consensus was reached by considering the majority opinion.

SPSS version 21 was used for the analysis of quantitative data, and MAXQDA version 24 was utilized for qualitative data analysis.

RESULTS

The mean age of the participants was 45.07±8.44 (29-59 years), all of whom were male. The average years of work experience were 31.30±9.41 (8-42), and the mean weekly working hours were 77.07±13.15 (60-102). When examining the educational backgrounds of the auto painters, it was found that 76.9% had completed primary school (n=10), 15.4% had completed middle school (n=2), and 7.7% had completed high school (n=1), (Table 2).

Following the interviews conducted with auto paint workers, the analyzed statements were categorized under four main themes: occupational risk factors, employee safety, health issues, and concerns about the future (Table 3). Figure 1 shows the codes for these themes as word clouds.

Occupational Risks

Under this main theme, participants predominantly emphasized chemical risk factors among their occupational hazards. They reported frequent and direct use of substances such as paint, thinner, primer, and varnish, and expressed concern about the potential health effects of these chemicals. Participants also identified dust generated during surface preparation with sandpaper and the abrasive effects of sanding on the hands as additional risk factors. Furthermore, long working hours and exposure to cold working conditions particularly during the winter months were described as challenging aspects of their work.

"I" ve been a painter for about 30 years. We constantly use chemicals like paint, thinner, primer, and so on… all of these are poisonous." (P5)

Table 2. Descriptive characteristics of participants (n=13)

Participant Number	Age/ gender	Average years of working	Education level
P1	52/Male	40	Primary school
P2	55/Male	42	Primary school
P3	44/Male	31	Primary school
P4	34/Male	20	Middle school
P5	42/Male	29	Primary school
P6	45/Male	33	Primary school
P7	29/Male	14	Middle school
P8	47/Male	35	Primary school
P9	40/Male	27	Primary school
P10	50/Male	38	Primary school
P11	38/Male	8	High school
P12	59/Male	42	Primary school
P13	48/Male	36	Primary school

Table 3. Themes and subthemes

Main themes	Subthemes
Occupational risk factors	Chemicals and thermal conditions
Health issues	Respiratory system diseases and skin diseases
Employee safety	Working conditions, Protective measures, Work accidents
Concerns about the future	Lack of new workers

"We use paints knowing that they harm our health. What else can I do?" (P2)

"We used to paint cars inside the shop, but later industrial-type ovens were introduced, and now we paint inside an enclosed oven cabin. The space is completely closed, and the paint settles on us. I do not always wear overalls or an appropriate mask. I have health problems, and I am afraid." (P1)

"The most important materials in this job are paint, thinner, primer, and sandpaper. However, all of these are chemicals, and I do not know much about their effects on our health." (P11)

"Our region has a Mediterranean climate, but in winter it can get very cold. We work outside, especially during sanding, and sometimes I cannot feel my fingers." (P4)

"I especially dislike working in the cold during winter because my hands crack due to the cold." (P9)

"If we were civil servants, we might have regular leave. We work day and night, summer and winter." (P7)

Figure 1. Participants' work life word cloud

Health Issues

Auto paint workers frequently reported that the respiratory system and skin were the most adversely affected due to occupational exposure. Although the majority of participants acknowledged experiencing various health problems, none reported having received a formal diagnosis of an occupational disease. Moreover, participants appeared to remain largely indifferent toward adopting protective measures to safeguard their health. Auto paint workers particularly emphasized continuous exposure to chemicals, which they associated with serious health problems and, in some cases, work-related accidents.

"Sanding may seem simple, but it is actually very demanding. Our hands often bleed from sanding, and we do not even have fingerprints anymore." (P13)

"Every time I spray paint, my lungs react immediately. About five or six years ago, I was spraying paint and suddenly felt my breathing become blocked. I thought I was dying. I was taken to the hospital with difficulty, where I received emergency treatment, and it was determined that I had been poisoned by the paint." (P10)

"I learned later that paint is a highly carcinogenic substance. You start this profession at the age of ten without knowing this. Over time, health problems develop, but there is nothing you can do about it now. How can we quit this job at this age? Starting a new job is very difficult." (P2)

"Unfortunately, I developed lung cancer because of this profession. About ten years ago, my respiratory problems began. I tried to change my profession and even opened a fast-food shop for a year, but it did not work out, so I had to return to my previous job. I do not know how much longer I can continue. I am losing my health, and this makes me very sad." (P1)

"Thinner and sandpaper cause wounds on my hands." (P8)

"Preparing a car for painting requires constant bending and working in narrow spaces, which causes back and lower back pain." (P3)

"These chemicals are very harmful to both our skin and lungs. There are already cancer patients in my family, so I am afraid I will become one too." (P9)

Employee Safety

Auto paint workers are often directed toward this profession by their families after completing primary school in order to learn a trade. They typically begin working as apprentices at a very young age (11-12 years) and acquire occupational skills informally through master–apprentice relationships. It was observed that participants generally did not receive any formal vocational or OHS training. Consequently, their awareness of occupational risks appears to be limited. In parallel, the use of personal protective equipment was found to be highly inadequate.

"I started working in the industry at the age of 12, right after finishing primary school. My family was experiencing financial difficulties, so I could not continue my education." (P12)

"The painting process is very delicate, and ideally you should wear a mask and goggles, but I cannot say that I always use them… Some parts of this job should be done by robots, not humans." (P11)

"I cannot use protective equipment. We started this job when we were children, and back then our master did not use anything either. I never got used to it. Look at my hands now—they have eczema, which is very uncomfortable, but still I cannot use protective equipment." (P10)

"I know that not wearing gloves or a mask is harmful, but we are just not used to it. Our master never used any protective equipment, so I do not either." (P7)

"When we do oven painting, we definitely need to use a proper mask. However, good-quality masks are very expensive, so it is difficult for us to afford them. At least I started using the masks we had during the COVID-19 pandemic." (P8)

"After the painting process, I have difficulty breathing, so I especially wear a mask in the oven. However, I do not use it in the workshop because it is an open area." (P3)

"I started this job at the age of 11. I wish I had known some things beforehand, as I had painful experiences. In my early years, an acid container spilled on my finger by accident, and the wound on my index finger did not heal for months." (P13)

"I did not receive any training related to this profession. I learned everything from my master—whatever I saw and heard from him." (P12)

"If we had at least used masks and gloves when we first started this profession, I would not be sick at such a young age. Those entering the workforce should first be taught how to protect themselves. However, nobody starts with education here, and nobody knows how to protect themselves. Painting processes should be carried out in accordance with occupational health and safety regulations." (P1)

Fear of the Future

All participants expressed concern that new apprentices are no longer entering the profession, resulting in a lack of trained future masters. A shared concern was that within the next 10-15 years, the number of skilled masters in the sector will decline further, while those who remain will be required to work under increasing workloads. In addition, participants emphasized that health problems typically emerge between the ages of 40 and 50 in this profession, and therefore called for early retirement rights based on occupational wear and tear to be granted by the government.

"This job is very demanding and requires detailed skills, so learning it is not easy. However, apprentices are no longer coming, and in the future the number of people doing this job will decrease even further. There are only 17 auto painters in this industrial area, but approximately 17,000 vehicles in this district, so it is impossible for us to keep up. Moreover, because this is a tourist area, the number of vehicles doubles during the summer months." (P6)

"We earn well only when we work excessively, but we cannot always protect ourselves. We are losing our health because of overwork, and we want to be granted the right to early retirement." (P9)

"Considering that we work from morning until evening in this profession, our income is low. At the same time, we are losing our health, so what will our future look like?" (P3)

"There are no new masters being trained for the future, and the number of apprentices is very low. Unfortunately, this profession is disappearin." (P7)

"I am 55 years old, and this is the first time I have encountered someone who is interested in our problems. My request to you, doctor, is to please convey our statements to the authorities and the ministry. Please emphasize the need for education, support for the working environment, retirement rights, and especially the assignment of a physician to address our health problems." (P2)

DISCUSSION

The population of the district where the study was conducted is approximately 123,000, with an average of one vehicle for every two individuals[11]. However, due to tourism during the summer months, vehicle traffic in the region increases by approximately two to three times. Despite this substantial demand, there are only 17 auto painters working across the two industrial sites in the district. Through in-depth interviews with these auto painters, the present study examined occupational risks associated with auto painting. The most prominent finding is that workers are exposed to intensive chemical hazards, experience significant health problems, and that adequate OHS measures are not sufficiently implemented.

The findings indicate that auto painters frequently use painting materials as an inherent requirement of their work and commonly describe these substances as toxic. To facilitate spray application, paint fluidity is increased through the extensive use of solvents such as toluene, xylene, ethylbenzene, and benzene. During application, these solvents largely evaporate, as they are not incorporated into the final paint structure[4]. Several substances used in the automotive painting sector (e.g., benzene and talc) are classified as Group 1 carcinogens (carcinogenic to humans) by the International Agency for Research on Cancer, while others (e.g., toluene and certain solvents) are classified as Group 2B carcinogens (possibly carcinogenic to humans)[15].

In line with this classification, workers in the present study expressed particular concern regarding respiratory symptoms and the fear of developing lung cancer in the future. These concerns are consistent with findings reported in previous studies[16]. The literature also indicates that eye-related health problems are common among auto painters[5]. Acute exposure to chemicals such as toluene is known to irritate the mucous membranes of the respiratory tract, while chronic exposure may lead to fluid accumulation in the lungs and long-term respiratory damage[17-19].

Consistent with existing evidence, lung cancer has been identified as the most prevalent type of occupational cancer, followed by skin cancer, bladder cancer, and leukemia[20]. To mitigate these health risks, the use of personal protective equipment particularly appropriate respiratory protection—is essential. However, almost all participants in this study reported not using masks regularly, despite acknowledging the harmful effects of chemical exposure. This lack of personal protective equipment use not only increases the risk of respiratory diseases but also heightens the likelihood of systemic health problems. Therefore, the findings underscore the urgent need for improved OHS practices, including access to appropriate protective equipment, worker training, and regulatory enforcement in small-scale automotive painting workplaces.

It was determined in this study that not all painting processes are performed inside paint booths. Due to the cost of using paint booths operated by external companies, some master painters carry out certain vehicle painting procedures in open areas within their workshops. From an OHS perspective, automotive painting should be conducted exclusively in paint booths (also referred to as ovens) specifically designed for this purpose. Aromatic hydrocarbons such as toluene and benzene are heavier than air; toluene has a density approximately 3.2 times that of air, while benzene is 2.8 times heavier[21]. Conventional top-down ventilation systems pose additional risks, as they may carry harmful chemicals heavier than air directly into the worker's breathing zone. In contrast, bottom-up ventilation systems may fail to provide sufficient suction due to the large working area. Moreover, achieving optimal paint quality requires a constant temperature and a controlled environment[4,16]. Automotive paint booths are designed to meet these requirements by providing appropriate ventilation and environmental control.

However, findings from this study indicate that auto painters do not frequently use paint booths. When combined with inadequate ventilation and poor temperature regulation in the working environment, this significantly increases occupational exposure risks. Supporting this finding, a study conducted in Nigeria reported that spray painters were exposed to VOCs at levels far exceeding the permitted limit of 1.9 ppm, reaching up to 13.4 ppm[5]. Similarly, a study conducted in Türkiye found that indoor air measurements for both toluene and benzene among auto paint workers exceeded legal limit values. In one enclosed-space measurement, toluene concentration was reported as 203.87 mg/m³, and in another, 301.44 mg/m³, both surpassing the limit value of 192 mg/m³. Additionally, the same study reported that white blood cell counts among auto paint workers were 26.3% higher than reference values. Workers with elevated white blood cell levels also had significantly higher phenol values ($p=0.014 <0.050$), suggesting that paint products may lead to notable hematological changes[4]. Considerig these findings, it can be concluded that auto painters operate in environments that do not meet established standards and that their health is under serious threat.

Despite all workers reporting exposure to chemical substances and the auto painting sector being classified as hazardous, the majority stated that they do not use personal protective equipment and have not received any formal OHS training. Alarmingly, some workers reported using basic dust masks instead of gas-filtered respirators, which offer minimal protection. Such masks do not provide protection against toluene or benzene exposure. Furthermore, exposure to these chemicals can also occur through dermal absorption[22]. These substances readily interact with fat cells in the skin, making it essential to completely prevent skin contact during painting activities[23]. As observed in Image 1, workers do not use gloves during vehicle preparation or painting processes. This study particularly highlighted the high prevalence of skin-related health problems. In addition, the use of sandpaper may significantly increase skin irritation, while dust exposure during sanding can elevate the risk of respiratory diseases[24].

In a previous study, 92.5% of spray painters reported not using any personal protective equipment. Despite nearly half of these workers reporting a chemical taste in their mouths and even observing paint residues in their sputum immediately after painting, their continued failure to use protective equipment remains a serious concern[5].

Although workers expressed awareness of various occupational risks and reported experiencing health problems, it was found that auto painters do not undergo regular periodic medical examinations. Some participants indicated that they sought medical care only when health complaints became severe. This situation may be attributed to the absence of a workplace health unit within the industrial site and the workers' limited knowledge regarding OHS services. Despite acknowledging workplace hazards, their failure to adopt appropriate protective measures suggests insufficient or incorrect knowledge about occupational risks. In this context, professionally designed and effectively delivered training programs are needed to motivate workers and promote lasting positive behavioral change.

From an employee safety perspective, the lack of formal education further exacerbates occupational risks. Workers reported learning the profession solely through the master–apprentice relationship without any structured training. Although this relationship plays a critical role in vocational skill development, it should be supported by formal education programs. Workers must be able to recognize occupational hazards and implement appropriate OHS measures to protect both their own health and the environment[25]. Workplace observations revealed considerable disorder, as illustrated in Image 2, indicating the presence of multiple hazards, including electrical risks. However, such conditions were perceived as normal by the workers.

As emphasized during interviews, workers' reluctance to use protective equipment, particularly masks, was influenced by their masters' behaviors, as their mentors also did not use such equipment. The persistence of unsafe working conditions and behaviors among auto painters is thought to be associated with starting work at a very young age, insufficient education on occupational risks, and the absence of positive role models. Child labor is especially concerning in hazardous

Image 1. Vehicle preparation and painting process in the paint booth

Image 2. Photos related to the working areas of auto painters

work environments, as prolonged exposure combined with limited education increases the likelihood of early-onset health problems. While mentors play a crucial role in shaping apprentices' work attitudes and behaviors, the effects of negative mentoring on the development of professional responsibility and craftsmanship have not been adequately explored[26]. These findings not only reveal deficiencies in existing legal regulations and inspection mechanisms but also underscore the need for further research in this field.

Study Limitations

A primary limitation of qualitative research is the limited generalizability of its findings, as results are often context-specific and shaped by the social and cultural characteristics of the study setting. Accordingly, the findings of this study reflect the experiences of a relatively small group of auto paint workers employed at a single industrial site in Türkiye. Although the study yielded rich and in-depth information regarding the phenomenon under investigation, the time elapsed between some occupational experiences and the interviews may have introduced recall bias, potentially affecting the accuracy of participants' accounts.

Despite these limitations, this study has notable strengths. Previous research on OHS in the auto painting sector has largely concentrated on quantitative exposure measurements, whereas workers' lived experiences, perceptions of risk, and everyday coping strategies—particularly in small-scale industrial settings have remained insufficiently explored. In this respect, the present phenomenological study provides an in-depth understanding of auto paint workers' perspectives and fills an important gap in the literature. Although conducted at a single industrial site, the study offers valuable insights that can inform future research, policy development, and intervention strategies aimed at improving OHS practices in similar settings.

CONCLUSION

This study demonstrates that auto paint workers are exposed to hazardous chemical substances associated with respiratory, dermatological, and potentially cancer-related health risks, while the use of personal protective equipment and awareness of OHS measures remain inadequate. Addressing these challenges requires further research that integrates qualitative findings with environmental exposure measurements and structured educational interventions.

Expanding OHS services across all sectors, ensuring universal access to basic OHS training, and increasing the number of workplace health professionals—particularly occupational health nurses are essential steps toward improving worker protection. In addition, strengthening regular inspections and providing governmental support for the training of new artisans with a strong emphasis on occupational safety will contribute not only to the protection of workers' health but also to the sustainability of skilled craftsmanship in the auto painting sector.

Ethics

Ethics Committee Approval: Permission was obtained from the Trakya University Non-Interventional Clinical Research Ethics Committee with (decision number: 2023.13/15, date: 11.09.2023).

Informed Consent: Informed consent was obtained from the participants. Participants were assured of the confidentiality of their identities and audio recordings.

Footnotes

Authorship Contributions

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

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ORIGINAL ARTICLE

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Melatonin as a Radioprotective Agent: Assessing Its Efficacy in Preventing Lung Injury Induced by Radiation Therapy

Melatoninin Radyasyona Bağlı Akciğer Hasarına Karşı Doza Bağımlı Etkileri: Deneysel Bir Çalışma

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ABSTRACT

Aim: The purpose of our study was to investigate whether melatonin (MLT) can protect against radiation-induced lung injury (RILI) in a rat model, with particular emphasis on dose-dependent effects in preventing pneumonitis and pulmonary fibrosis following radiotherapy (RT).

Materials and Methods: Treatment groups received MLT at a dose of 100 mg/kg, 50 mg/kg, and 5 mg/kg before 12 Gy RT in a single fraction. At the end of week 8, rats were sacrificed, and hematoxylin and eosin stained lung tissues were evaluated using a semi-quantitative scoring method based on the alveolar septal area.

Results: The mean alveolar septal area was 38+11.07 μm^2 in the control group. RT group displayed deteriorated alveolar space and increased alveolar septal thickening with a mean of 42.3+6.60 μm^2. 100 mg/kg group displayed the greatest septal thickening and the most alveolar space decrease with a mean of 49.0+12.37 μm^2. MLT 50 mg/kg and 5 mg/kg groups displayed preserved alveolar morphology and less septal degradation with a mean of 35.0+9.08 μm^2 and 31.1+5.73 μm^2 respectively. The alveolar septal area was significantly greater in the 100 mg/kg compared with the 50 and 5 mg/kg groups (p=0.034 and 0,003, respectively). This finding suggests that the 100 mg/kg MLT dose triggered greater profibrotic changes in lung tissues, leading to reduced alveolar space, potentially due to pro-oxidant effects at high doses.

Conclusion: These findings highlight the importance of MLT dosage in managing RILI <50 mg/kg doses seem to have potentially less alveolar septum degradatation offering protective effects against lung injury.

Keywords: Melatonin, oxidative stress, pneumonitis, radiation-induced lung injury, radioprotection, rat

ÖZ

Amaç: Çalışmamızın amacı, melatonin (MLT) tedavisinin, radyoterapi (RT) sonrasında oluşan pnömonit ve pulmoner fibrozis gibi radyasyon kaynaklı akciğer hasarını (RILI) engelleyip engellemeyeceğini araştırmak ve özellikle doz bağımlı etkilerini incelemektir.

Gereç ve Yöntem: Tedavi gruplarına 12 Gy RT'yi tek bir dozda uygulamadan önce sırasıyla 100 mg/kg, 50 mg/kg ve 5 mg/kg MLT verilmiştir. 8. haftanın sonunda sıçanlar feda edilmiş ve hematoksilin ve eozin ile boyanmış akciğer dokuları alveoler septum alanına dayalı yarı-quantitatif bir puanlama yöntemiyle değerlendirilmiştir.

Bulgular: Kontrol grubunda ortalama alveoler septum alanı 38+11,07 μm^2 bulunmuştur. RT grubunda alveoler boşlukta bozulma ve alveoler septum kalınlaşmasında artış gözlenmiş ve ortalama 42,3+6,60 μm^2 olmuştur. 100 mg/kg grubu, en büyük septum kalınlaşması ve alveoler boşlukta en fazla

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azalmayı göstermiştir ve ortalama 49,0+12,37 μm² bulunmuştur. MLT 50 mg/kg ve 5 mg/kg grupları ise alveolar morfolojiyi korumuş ve daha az septum bozulması göstermiştir, ortalama sırasıyla 35,0+9,08 μm² ve 31,1+5,73 μm² olmuştur. 100 mg/kg grubunun alveolar septum alanı, 50 ve 5 mg/kg gruplarına göre anlamlı şekilde daha büyük bulunmuştur (p=0,034 ve 0,003). Bu bulgu, 100 mg/kg MLT dozunun, yüksek dozda pro-oksidan etkiler nedeniyle, akciğer dokusunda daha fazla profibrotik değişikliklere yol açarak alveoler boşluğun azalmasına neden olduğunu göstermektedir.

Sonuç: Bu bulgular, RILI yönetiminde MLT dozajının önemini vurgulamaktadır. 50 mg/kg altı dozlar, alveolar septum bozulmasının daha az olmasını sağlayarak akciğer hasarına karşı koruyucu etki gösterebilir.

Anahtar Kelimeler: Melatonin, oksidatif stres, pnömonit, radyasyona bağlı akciğer hasarı, radyoproteksiyon, sıçan

INTRODUCTION

With 2.5 million cases in 2022, lung cancer is a major cause of mortality globally[1]. Approximately 60% of lung cancer patients undergo radiotherapy (RT) either as a standalone treatment or in combination with chemo/immunotherapy[2]. For non-small cell lung cancer, which is the most frequent kind, thoracic RT is recommended for most of the advanced stages[3]. Although RT remains a cornerstone treatment, it is associated with serious adverse effects. One of the complications is radiation-induced lung injury (RILI), which consists of pneumonitis and pulmonary fibrosis. The prevalence of RILI varies depending on the influence of factors such as previous surgeries, pre-existing lung conditions, concurrent use of lung-damaging agents and systemic comorbidities such as diabetes mellitus[4]. It has been reported that up to 30% of patients may experience radiation pneumonitis 1-6 months post-treatment[5,6]. For a higher dose of RT, the incidence rates of RILI may increase up to 40%[5].

The pathophysiology of RILI is a complex process and driven by inflammation. Following exposure to RT, lung undergoes a series of molecular events which cause acute and chronic pulmonary damage. Earliest responses include damage to the vascular endothelium. It leads to increased vascular permeability and infiltration of inflammatory cells into the lung parenchyma. These immune cells release pro-inflammatory cytokines like tumor necrosis factor-alfa, interleukin-6, and transforming growth factor-beta, which increase the inflammatory response and start fibrotic changes[7]. There is currently no established curative treatment of RILI. Eventhough steroids have been used to subside inflammation, serious side effects of steroids are a major problem. Therefore, it is necessary to find alternative therapeutic drugs which has fewer side effects.

Melatonin (MLT), a multifunctional hormone with powerful antioxidant properties, has gained a lot of interest for its potential as a radioprotective agent. It has been shown to reduce oxidative stress and stimulate cell survival by scavenging free radicals and modulating autophagy[8,9]. Although its protective effects appear to be dose dependent, the ideal dosage for lung protection after thoracic RT remains unclear[10]. Previous studies suggested inconsistent results on optimal dose for MLT, emphasizing the need for further research[11].

In our study, we aim to address the current gap in knowledge by systematically investigating the dose-dependent efficacy of MLT in mitigating RILI using an *in vivo* model. By evaluating histopathological changes of alveolar damage, we pursue to identify the minimum effective MLT dose necessary for pulmonary protection. Our findings may help guide future thoracic RT protocols for lung cancer patients.

MATERIALS AND METHODS

Rats

Our study had ethical approval from the Bezmialem Foundation University Experimental Application and Research Center (decision number: 2022-79, date: 27.01.2025) and the experiment was also conducted in the same center. A total of 45 adult male Sprague Dawley rats (200-300 g) were divided into 5 groups (n=9 per group). The rats were housed in standard rat cages at 23±2 °C temperature with 55±10% humidity. Twelve-hour day and night light periods were obtained. The rats were fed with ad-libitum rat chow and drinking water. The study is an animal experiment, and no patient data were used.

Melatonin

For treatment groups, synthetic MLT powder (N-acetyl-5-methyoxytryptamine, ≥98, Sigma-Aldrich, USA) was dissolved in ethanol and diluted in 0.9% saline solution to a concentration of 10 mg/mL.

Radiotherapy Technique

Before irradiation, the rats were anesthetized with 60-90 mg/kg intraperitoneal ketamine hydrochloride (Ketalar; EWL Eczacıbaşı Warner Lambert İlaç Sanayi ve Ticaret A.Ş., İstanbul) and 6-10 mg/kg xylazine hydrochloride (Rompun 2% Bayer Kimya San. Ltd. Şti., İstanbul, Türkiye). After anesthesia, the rats were laid on a foam tray with arms and legs stabilized with cotton bandages in a supine position. Then the simulated computed tomography was performed. In the Varian treatment planning system, the heart and lung volumes were contoured, and a 3D conformal plan was generated. An X-ray linear accelerator (Rapid Arc, Varian Medical Systems, Palo Alto, USA) which produces a 6-megavolt photon beam at 100 cm was used to irradiate the thoracic region with 12 Gy in 1 fraction at a dose rate of 300 monitor units. Elastic-gel bolus was used to maximize the cardiac point dose[12].

Treatment Groups

For the experiment, 5 groups of Sprague-Dawley rats were used. Each group had 9 adult male rats. Prior to sham irradiation, 1 cc of saline solution was administered to the control group. G2 group had 1 cc saline injection intraperitoneally 30 min before 12 Gy thoracic RT. For other groups, MLT was administered at a dose of 100, 50 and 5 mg/kg respectively[8,11]. Eight weeks later, histological changes in the lung were examined. Experimental design and workflow is depicted in Figure 1.

Histological Evaluation

Eight weeks later, rats were anesthetized with ketamine and then the animals were euthanized by exsanguination. After thoracotomy, the lung tissue was harvested and fixed for 24 hours in 10% neutral buffered formalin. Following standard tissue preparation, 5 µm transverse slices were obtained to display the lungs' alveoli and bronchial structure. Hematoxylin and eosin (H&E) was used to stain the obtained sections.

Lung samples were assessed with a standardized, semi-quantitative alveolar septal area-based scoring method which measures alveolar septum area with a rapid and automated algorithm designed to segment alveolar space images across entire tissue selection[13]. The extent of tissue damage was quantitatively evaluated using a mathematical segmentation-based image analysis approach. Five photographs taken at 10X magnification from the alveolar area of each tissue were analyzed with the Fiji program[14]. Through image segmentation, the alveolar space and alveolar wall was identified as distinct regions of interest. A numerical score was then calculated by determining the area ratio of the alveolar wall relative to the alveolar space. Segmentation relied on pixel color differences, as alveolar spaces exhibited more uniform coloration compared to the heterogeneous tones found in the alveolar walls.

In addition to H&E staining, a separate set of paraffin sections was stained with Masson's trichrome, which specifically highlights type I collagen and is commonly used to assess fibrotic areas.

Statistical Analysis

The Shapiro-Wilk test was used to analyze the variables' appropriateness for a normal distribution. The one-way analysis of variance with post-hoc Tukey's multiple comparison test (comparisons between all groups) was employed to compare the numerous independent groups. All statistical analyses were conducted using SPSS version 29 for Mac (IBM Corp. Armonk, NY), and a significance level of $p<0.05$ was accepted.

RESULTS

In the semi-quantitative evaluation of H&E-stained lung sections, the control group (G1) exhibited a normal lung parenchymal architecture characterized by uniformly thin alveolar septa and well-preserved alveolar spaces. The

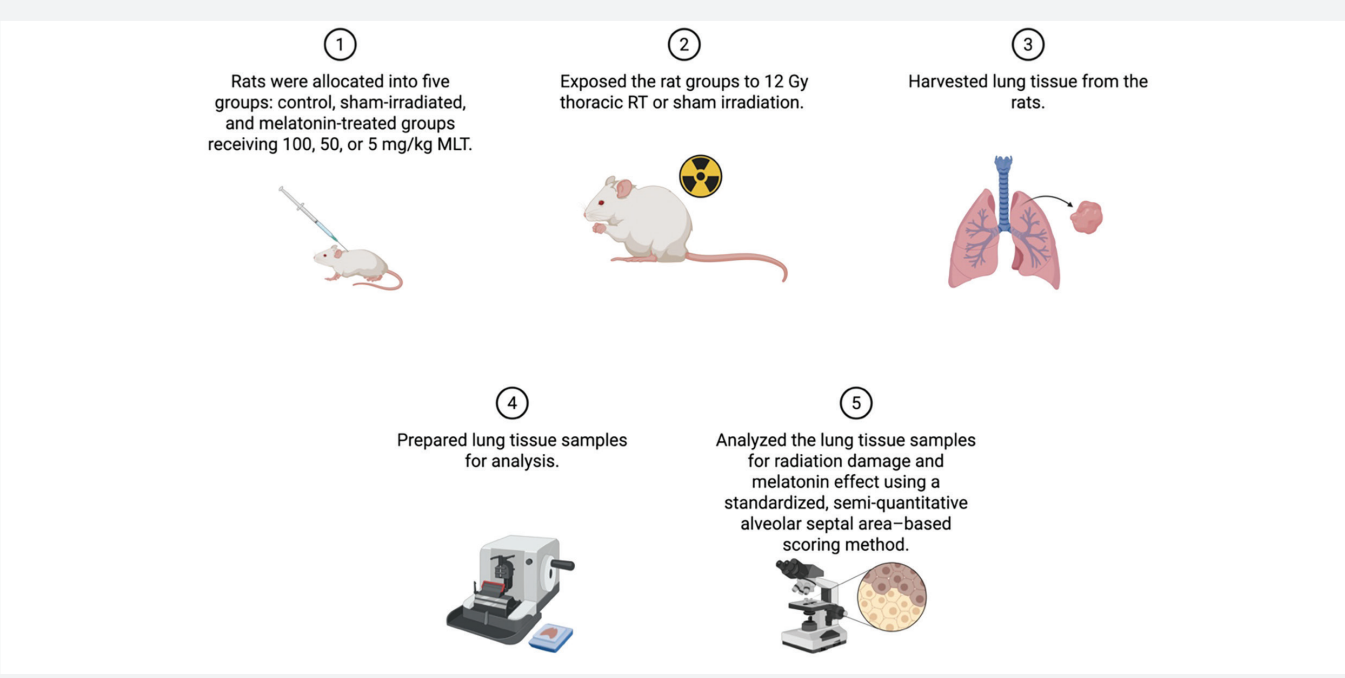

Figure 1. Experimental design and workflow of melatonin treatment and thoracic radiotherapy in a rat model of RILI

RILI: Radiation-induced lung injury, MLT: Melatonin, RT: Radiotherapy

alveoli were regularly shaped, evenly distributed, and free of structural distortion, collapse, or over-distension. The alveolar walls appeared delicate and continuous, with intact epithelial lining and no evidence of septal thickening, edema, inflammatory cell infiltration, or hemorrhage (Figure 2a). Quantitative assessment confirmed these observations, with a mean alveolar septal area of 38±11.07 μm² (Figure 2f). RT-only group (G2) demonstrated deteriorated alveolar space and increased alveolar septa thickening with a mean septal area of 42.3±6.60 μm², although the difference from control was not statistically significant (Figure 2b-f). MLT 50 mg/kg (G4) and 5 mg/kg (G5) groups showing preserved alveolar morphology and reduced septal thickening, with mean areas of 35.0±9.08 μm² and 31.1±5.73 μm², respectively (Figure 2d-f, Table 1). Even though the difference to the control group was not statistically significant, it suggests the antioxidant MLT application even in low doses has a protective effect against profibrotic changes in lung alveoli (Figure 2e-f).

In contrast, MLT 100 mg/kg group (G3) exhibiting the greatest septal thickening and the most alveolar space decrease, with mean septal area (49.0±12.37 μm²). Compared to this group, both the 50 and 5 mg/kg MLT groups had significantly smaller alveolar septal areas (Group 3 vs. 4 p=0.034; Group 3 vs. 5 p=0.003). These results suggest that high-dose MLT may promote profibrotic changes through potential pro-oxidant effects, while lower doses demonstrate protective effects.

Masson trichrome staining was used to evaluate fibrosis and associated morphological changes in the alveolar septa and bronchial structures of lung tissue. No marked difference was observed among the treatment groups in the extent of light green-stained areas, indicating comparable levels of type I collagen deposition and, consequently, fibrosis. Representative histological images illustrating morphological features were shown in Figure 3.

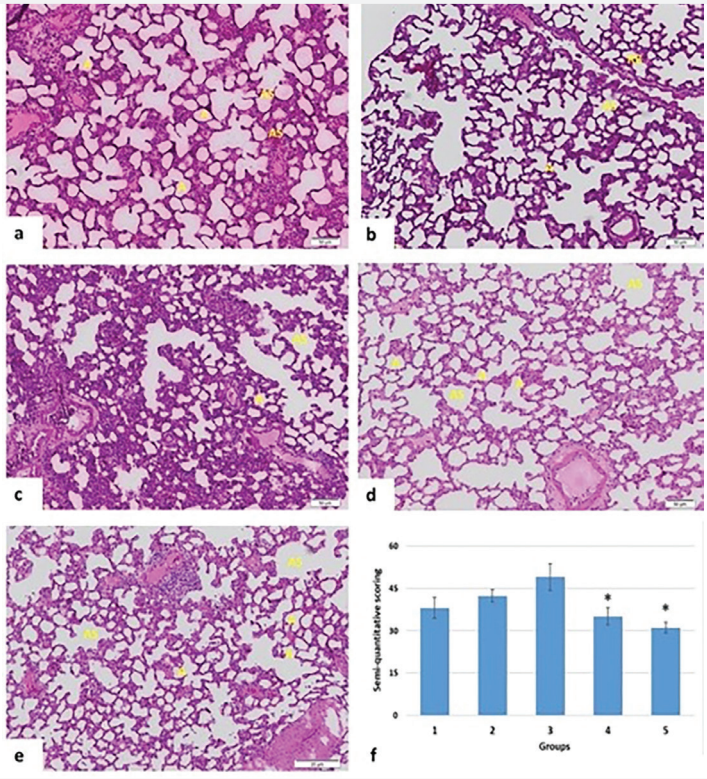

Figure 2. Representative hematoxylin and eosin-stained lung tissues (a-e) and semi-quantitative evaluation of alveolar septal area (f). (a) Control group showing normal lung parenchyma with thin alveolar septa and well-preserved alveoli (mean septal area: 38.0±11.07 μm²). (b) RT-only group demonstrating deteriorated alveolar space and increased alveolar septa thickening with a mean septal area of 42.3±6.60 μm², although the difference from control was not statistically significant. (c) MLT 100 mg/kg group (G3) exhibiting the greatest septal thickening and the most alveolar space decrease, with mean septal area (49.0±12.37 μm²). (d, e) MLT 50 mg/kg (G4) and 5 mg/kg (G5) groups showing preserved alveolar morphology and less septal degradation, with mean areas of 35.0±9.08 μm² and 31.1±5.73 μm², respectively. (f) Quantitative analysis showing significantly greater septal thickening in the 100 mg/kg group compared with the 50 and 5 mg/kg groups (p=0.034 and 0.003, respectively). 10X magnification

AS: Alveolar septal area, A: Alveoli, MLT: Melatonin, RT: Radiotherapy

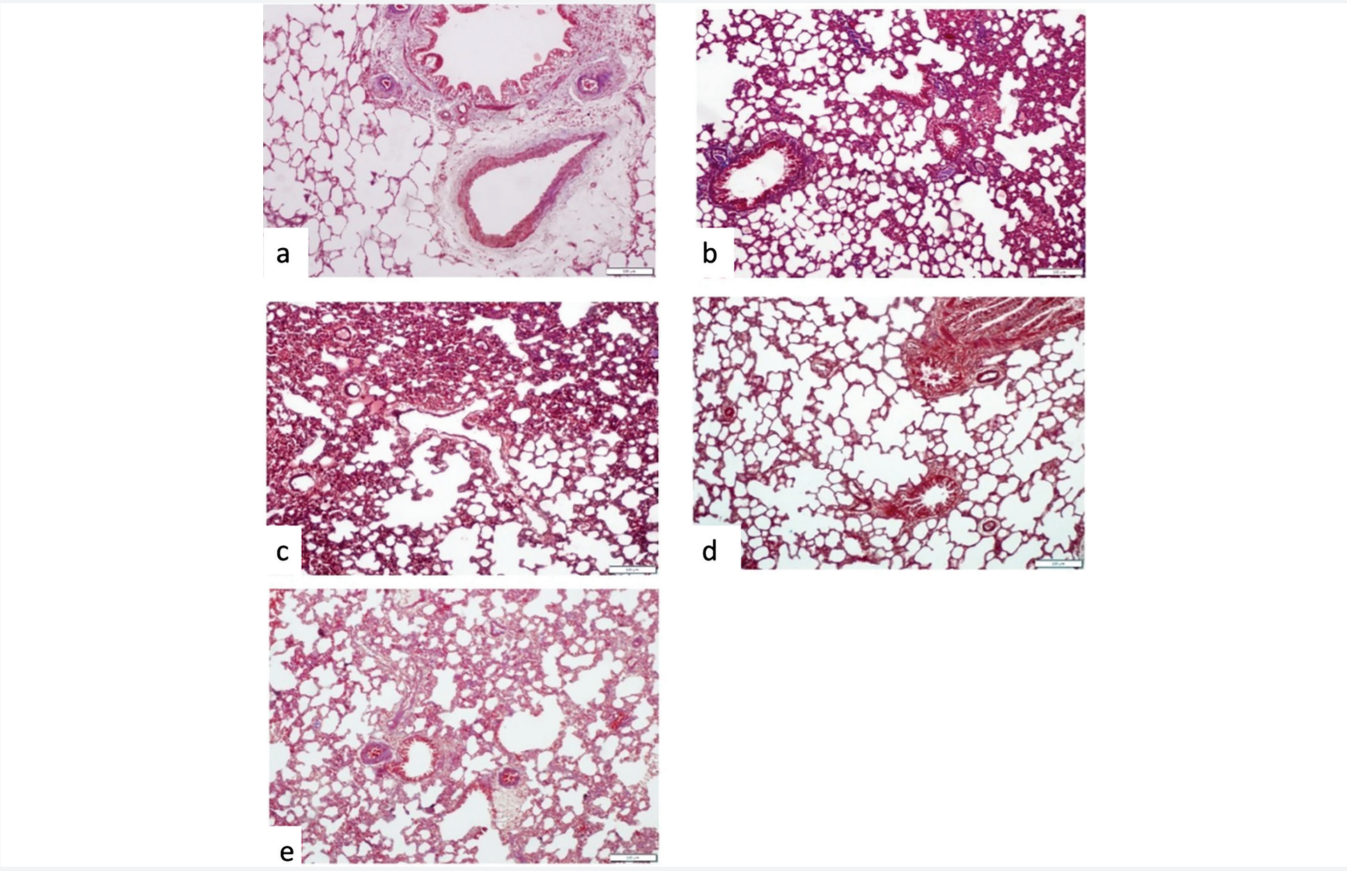

Figure 3. Representative histological images of lung tissues stained with Masson trichrome to evaluate fibrosis and morphological alterations in the alveolar septum and bronchial structures. (a) Group 1 (control), (b) Group 2 (RT only), (c) Group 3 (RT + MLT 100 mg/kg), (d) Group 4 (RT + MLT 50 mg/kg), and (e) Group 5 (RT + MLT 5 mg/kg). 10X magnification. Masson trichrome staining did not reveal a marked difference in fibrotic changes among the groups, with representative morphological appearances shown here

MLT: Melatonin, RT: Radiotherapy

Table 1. Mean alveolar septal areas of among control, radiation-only, and radiation plus MLT treatment groups and multiple comparisons among groups with post-hoc Tuckey test

Treatment groups	Mean of alveolar septal area (μm²) ± SD error	Comparisons among groups	Mean difference (μm) ± SD error	p-value	95% CI (lower bound-upper bound)
G1 (control)	38.07±3.69	G2	-4.27±4.31	0.85	-16.60 to 8.07
		G3	-10.96±4.61	0.14	-24.15 to 2.22
		G4	2.98±4.31	0.95	-9.35 to 15.31
		G5	6.97±4.31	0.49	-5.36 to 19.30
G2 (RT)	42.34±2.20	G1	4.27±4.31	0.85	-8.07 to 16.60
		G3	-6.69±4.61	0.59	-19.88 to 6.49
		G4	7.25±4.31	0.45	-5.08 to 19.58
		G5	11.24±4.31	0.08	-1.10 to 23.57
G3 (RT + 100 mg/kg MLT)	49.03±4.67	G1	10.96±4.61	0.14	-2.22 to 24.15
		G2	6.69±4.61	0.59	-6.49 to 19.88
		G4	13.94±4.61	0.034	0.76 to 27.13
		G5	17.93±4.61	0.003	4.75 to 31.12

Table 1. Continued

Treatment groups	Mean of alveolar septal area (μm²) ± SD error	Comparisons among groups	Mean difference (μm) ± SD error	p-value	95% CI (lower bound-upper bound)
G4 (RT + 50 mg/kg MLT)	35.09±3.02	G1	-2.98±4.31	0.95	-2.22 to 24.15
		G2	-7.25±4.31	0.45	-19.58 to 5.08
		G3	-13.94±4.61	0.034	-27.13 to -0.76
		G5	3.99±4.31	0.88	-8.35 to 16.32
G5 (RT + 5 mg/kg MLT)	31.10±1.91	G1	-6.97±4.31	0.49	-19.30 to 5.36
		G2	-11.24±4.31	0.08	-23.57 to 1.10
		G3	-17.93±4.61	0.003	-31.12 to -4.75
		G4	-3.99±4.31	0.88	-16.32 to 8.35

SD: Standard deviation, CI: Confidence interval, MLT: Melatonin, RT: Radiotherapy

DISCUSSION

RILI is one of the most common side effects of RT in lung cancer treatment. It typically begins with acute lung damage and inflammation, eventually progressing to lung fibrosis, which is a chronic, progressive and potentially fatal condition that severely impairs respiratory function. Currently, there is no curative treatment available for RILI. MLT has shown potential to mitigate RILI. While many studies have explored the impact of specific MLT doses on RILI, only a limited number have directly compared varying doses. To address this gap, the primary objective of our study is to find the lowest effective dosage for RILI. We evaluated the impact of RT and varying doses of MLT on lung morphology, focusing on changes in alveolar septal thickness and parenchymal integrity.

The control group served as the baseline, showcasing healthy lung parenchyma with thin alveolar septa and distinct respiratory bronchioles. The mean alveolar septal area of 38±11.07 μm² provided a clear reference for assessing the impact of RT and MLT treatment on lung tissue morphology. This group highlights the expected structural integrity of lung tissue under normal conditions, serving as a crucial comparison point. The RT-only group exhibited moderate morphological changes, characterized by deteriorated alveolar space and thickened alveolar septa, with a mean of 42.3±6.60 μm². Although the difference compared to the control group was not statistically significant, this numerical increase suggests an early tendency toward profibrotic or other pathological changes. The lack of a statistically significant difference between the RT-only and control groups is likely attributable to the relatively low radiation dose and the 8-week evaluation period, which were chosen to capture early lung injury rather than established fibrosis. These findings, while subtle, highlight the potential of RT to induce morphological alterations in lung tissue. Previous studies have shown that radiation can lead to inflammation and subsequent architectural distortion, with various inflammatory mediators contributing to these processes[15-17].

The MLT treatment groups demonstrated significant benefit in terms of alveolar septal preservation, with the MLT 50 mg/kg and 5 mg/kg groups displaying normal alveolar morphology and less septal degradation, reflected by mean alveolar septal areas of 35.0±9.08 μm² and 31.1±5.73 μm², respectively, compared to 42.3±6.60 μm² in the RT-only group. These findings are consistent with previous reports indication that MLT exerts protective effects on lung tissues by preserving alveolar architecture and mitigating structural damage under pathological conditions[11,17]. In bleomycin-induced lung injury models, MLT administration reduced collagen deposition and improved structural integrity, suggesting a role in counteracting fibrotic remodeling[18]. Similarly, MLT has been shown to suppress inflammatory cell infiltration and provide structural protection in lungs exposed to chronic lipopolysaccharide[19]. These studies and our findings emphasize MLT's multifaceted role in lung protection by reducing oxidative stress, inflammation and structural deterioration.

Interestingly in the MLT-treated groups, a clear dose-dependent effect on lung morphology was observed. The 100 mg/kg MLT group exhibited the most significant pathological changes, including marked alveolar space reduction and thickened alveolar septa, with a mean alveolar septal area of 49.0±12.37 μm². This finding suggests that high-dose MLT may have pro-oxidant effects, potentially exacerbating alveolar septal thickening. In contrast, the 50 mg/kg and 5 mg/kg MLT groups displayed near-normal alveolar septal thickness, with mean values of 35.0±9.08 μm² and 31.1±5.73 μm², respectively. Notably, the differences between these lower doses and the 100 mg/kg group were statistically significant, emphasizing

the adverse effects of high-dose MLT. This finding is consistent with prior experimental studies demonstrating that high doses of MLT may exert paradoxical pro-oxidant, pro-inflammatory, or tissue-damaging effects, potentially through mitochondrial dysfunction and dysregulation of redox homeostasis, thereby attenuating its radioprotective benefits at higher dose ranges[20,21]. Given the absence of molecular oxidative stress or inflammatory biomarkers, this finding should be interpreted cautiously and regarded as a preliminary observation rather than definitive evidence of a pro-oxidant or profibrotic effect of high-dose MLT. Furthermore, the low-dose groups exhibited trends suggesting a protective effect against alveolar septal thickening, as their mean alveolar septal areas were slightly lower than the control group, though these differences were not statistically significant.

The evaluation of alveolar septal thickening in lung tissues following radiation exposure is a complex process that can yield varying results depending on the methodologies employed. In our study, Masson trichrome staining did not reveal significant differences in fibrosis among the treatment groups, despite the semi-quantitative evaluation indicating dose-dependent morphological changes. It is essential to consider the sensitivity of the staining method and the specific stages of fibrosis being assessed. The limitations of Masson trichrome staining in detecting early or subtle fibrotic processes may contribute to this discrepancy, as the staining technique is primarily designed to highlight collagen deposition rather than the nuanced cellular and molecular changes that precede overt fibrosis[22]. The study by Farhood et al.[23] emphasizes that while traditional histopathological methods may not capture early fibrotic changes, other techniques such as molecular assessments could provide deeper insights into the underlying processes. For instance, the modulation of inflammatory cytokines and signaling pathways by agents like MLT has been shown to play a critical role in mitigating RILI, suggesting that the absence of detectable morphological differences might not reflect a lack of biological activity but rather the early stages of fibrotic processes that are not yet fully developed[16]. Furthermore, the use of advanced imaging techniques, such as micro-CT, has been proposed as a means to detect early RILI, potentially offering a more sensitive approach to evaluating lung tissue changes over time[24]. Moreover, the findings from the study by Dogan highlight the importance of understanding the temporal dynamics of lung injury post-radiation, as early histological changes may not manifest as significant architectural distortion until later stages[15]. This aligns with the notion that the assessment of alveolar septal thickening should incorporate a variety of methodologies, including both histological and molecular techniques, to provide a comprehensive understanding of the tissue response to radiation and treatment interventions[13].

Study Limitations

Although the Sprague-Dawley rat model allows controlled evaluation of RILI, the findings may not be fully generalizable to clinical practice due to interspecies differences and the use of a single-fraction 12 Gy thoracic irradiation, which does not mirror conventional fractionated RT. Second, lung injury was assessed at a single late time point (8 weeks), precluding evaluation of early inflammatory changes or long-term fibrotic progression. Third, histopathological assessment was limited to semi-quantitative alveolar septal area analysis and Masson's trichrome staining, without accompanying molecular or biochemical markers of oxidative stress, inflammation, or fibrosis, which restricts mechanistic interpretation, particularly regarding dose-dependent MLT effects.

CONCLUSION

In conclusion, low-dose MLT (5 and 50 mg/kg) preserved alveolar morphology and limited septal thickening, indicating a protective effect against early radiation- and age-related profibrotic changes in lung tissue. Conversely, high-dose MLT 100 mg/kg) was associated with increased alveolar septal thickening and reduced alveolar spaces, suggesting a possible pro-oxidant and profibrotic effect at higher doses. Despite these dose-dependent histomorphometric differences, Masson trichrome staining demonstrated no significant differences in collagen deposition among groups, indicating the absence of established fibrosis at this stage.

Ethics

Ethics Committee Approval: Our study had ethical approval from the Bezmialem Foundation University Experimental Application and Research Center (decision number: 2022-79, date: 27.01.2025) and the experiment was also conducted in the same center.

Informed Consent: The study is an animal experiment, and no patient data were used.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.C., K.Y., P.A., M.Ü., Ş.A.E., F.Ö.D., Concept: K.Y., S.Ö., M.Ü., Ş.A.E., Design: E.C., P.A., S.K., Data Collection or Processing: K.Y., P.A., S.Ö., S.K., F.Ö.D., Analysis or Interpretation: E.C., Literature Search: S.Ö., F.Ö.D., Writing: E.C., G.E.Ş.

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

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ORIGINAL ARTICLE

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Polydatin Attenuates Isoproterenol-induced Pathological Cardiac Hypertrophy via *miR-214/FOXO3/NFAT* Axis

Polidatinin İzoproterenol-indüklenen Patolojik Kardiyak Hipertrofiyi *miR-214/FOXO3/NFAT* Ekseni Üzerinden Azaltıcı Etkisinin İncelenmesi

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ABSTRACT

Aim: Pathological cardiac hypertrophy precedes heart failure and alters myocardial structure, function, and molecular signaling. miRNAs, particularly *miR-214*, regulate key remodeling and hypertrophic pathways. Although polydatin has reported cardioprotective effects, whether these involve miRNA-mediated mechanisms is unclear. This study investigates the potential role of the *miR-214*/*Forkhead box O3* (*FOXO3*)/nuclear factor of activated T-cells (*NFAT*) axis in mediating polydatin's effects.

Materials and Methods: Male Wistar rats were divided into three groups: Control, isoproterenol (ISO), 5 mg/kg/day, s.c.), and ISO + polydatin (100 mg/kg/day, oral). Following four-weeks treatment, cardiac hypertrophy was assessed by measuring heart weight, heart weight/body weight ratio, and heart weight/tibia length ratio. For molecular analyses, the expression levels of miR-1, *miR-214*, and miR-133b, as well as the mRNA levels of *FOXO3*, *NFAT*, atrial natriuretic peptide (*ANP*), and brain natriuretic peptide (*BNP*), were measured by real-time polymerase chain reaction.

Results: Morphological parameters were significantly increased in the ISO group compared with controls ($p<0.001$) and were markedly suppressed by polydatin ($p<0.05$). Among the miRNAs, only *miR-214* was significantly upregulated by ISO ($p=0.006$), and this effect was attenuated by polydatin ($p=0.030$). ISO downregulated *FOXO3* ($p=0.027$) and upregulated *NFAT* ($p<0.001$), effects that were reversed by polydatin. Expression of fetal gene markers *ANP* ($p=0.029$) and *BNP* ($p=0.002$) was markedly elevated in the ISO group, but these increases were abolished with polydatin treatment ($p>0.05$ vs. control).

Conclusion: Polydatin demonstrates cardioprotective effects against ISO-induced pathological cardiac hypertrophy. These effects involve not only morphological improvements but also molecular regulation via the *miR-214*/*FOXO3*/*NFAT* axis and suppression of the fetal gene program. These findings suggest that polydatin may have potential as a pharmacological agent for preventing pathological cardiac remodeling.

Keywords: Polydatin, cardiac hypertrophy, *miR-214*, *Forkhead box O3* (*FOXO3*), nuclear factor of activated T-cells (*NFAT*)

ÖZ

Amaç: Patolojik kardiyak hipertrofi, kalp yetersizliğinin erken bir habercisidir ve miyokardın yapısını, işlevini ve moleküler sinyal yollarını değiştirir. miRNA'lar, özellikle *miR-214*, yeniden şekillenme ve hipertrofi ile ilişkili temel yolları düzenler. Polidatinin kardiyoprotektif etkileri daha önce bildirilmiş olsa da, bu etkilerin miRNA-bağımlı mekanizmalarla gerçekleşip gerçekleşmediği belirsizdir. Bu çalışma, polidatinin etkilerinde *miR-214*/*Forkhead box O3* (*FOXO3*)/nükleer faktör aktivasyon T-hücreleri (*NFAT*) ekseninin olası rolünü araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Erkek Wistar sıçanları üç gruba ayrıldı: Kontrol, İzoproterenol (ISO); 5 mg/kg/gün, cilt altı) ve ISO + polidatin (100 mg/kg/gün, oral). Dört haftalık tedavi sonrası kalp ağırlığı, kalp ağırlığı/vücut ağırlığı oranı ve kalp ağırlığı/tibia uzunluğu oranı ölçülerek kardiyak hipertrofi değerlendirildi. Moleküler analizler için miR-1, *miR-214* ve miR-133b düzeyleri ile *FOXO3*, *NFAT*, atriyal natriüretik peptit (*ANP*) ve beyin natriüretik peptidi (BNP) mRNA düzeyleri gerçek zamanlı polimeraz zincir reaksiyonu yöntemiyle ölçüldü.

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Bulgular: Morfolojik parametreler, ISO grubunda kontrol grubuna kıyasla anlamlı düzeyde arttı (p<0,001) ve bu artışlar polidatin tedavisi ile belirgin şekilde baskılandı (p<0,05). İncelenen miRNA'lar arasında yalnızca *miR-214* düzeyi ISO grubunda anlamlı olarak yükseldi (p=0,006), polidatin tedavisi ise bu artışı azalttı (p=0,030). ISO uygulaması *FOXO3* ekspresyonunu baskıladı (p=0,027) ve *NFAT* ekspresyonunu arttırdı (p<0,001). Polidatin ise bu değişiklikleri tersine çevirdi. Fetal gen belirteçleri olan *ANP* (p=0,029) ve *BNP* (p=0,002) düzeyleri ISO grubunda anlamlı şekilde yükselirken, polidatin tedavisi sonrasında bu artışlar ortadan kalktı ve düzeyler kontrol grubundan farklı bulunmadı (kontrole göre p>0,05).

Sonuç: Polidatin, ISO kaynaklı patolojik kardiyak hipertrofiye karşı kardiyoprotektif etkiler göstermektedir. Bu etkiler yalnızca morfolojik düzelmelerle sınırlı değildir; aynı zamanda *miR-214*/*FOXO3*/*NFAT* ekseni üzerinden moleküler düzenlemeyi ve fetal gen programının baskılanmasını da içerir. Bulgular, polidatinin patolojik kardiyak yeniden şekillenmenin önlenmesinde potansiyel bir farmakolojik ajan olabileceğini göstermektedir.

Anahtar Kelimeler: Polidatin, kardiyak hipertrofi, *miR-214*, *Forkhead box O3* (*FOXO3*), nükleer faktör aktivasyon T-hücreleri (*NFAT*)

INTRODUCTION

Cardiac hypertrophy is defined as an adaptive response of the myocardium to an increased hemodynamic load, tissue injury, or a pathophysiological stimulus[1,2]. Initially, it emerges as a compensatory mechanism aiming to preserve cardiac function by reducing oxygen consumption, balancing systolic wall stress, and enhancing ejection performance[3]. However, when this process becomes chronic, it leads to major cellular and molecular changes, including a marked increase in protein synthesis in cardiomyocytes, reorganization of sarcomeres, alterations in the cytoskeletal structure, a metabolic shift from oxidative phosphorylation to glycolysis, and reactivation of the fetal gene expression program[4,5]. Over time, this adaptive response progresses to pathological remodeling, driven by the development of perivascular and interstitial fibrosis, cellular reprogramming, and sustained activation of signaling pathways such as calcineurin-nuclear factor of activated T-cells (*NFAT*)[6,7]. The resulting structural and functional alterations eventually exceed the compensatory capacity of the heart, leading to progressive cardiac dysfunction and, ultimately, heart failure. Therefore, a comprehensive understanding of the molecular mechanisms underlying pathological hypertrophy plays a critical role in the development of targeted and rational therapeutic strategies for the prevention and treatment of heart failure[8].

MiRNAs are small non-coding RNA molecules, 18-25 nucleotides in length, that play a critical role in the post-transcriptional regulation of gene expression[9]. By interacting with complementary sequences in the 3' untranslated region of protein-coding mRNAs, they induce translational repression or mRNA degradation[10,11]. In this way, miRNAs fine-tune cellular protein synthesis and contribute to the regulation of fundamental biological processes[12]. Recent studies have demonstrated that miRNAs occupy central roles in physiological processes such as cell proliferation, differentiation, and cell cycle control[13,14]. However, dysregulation of their expression levels can drive pathological processes such as cardiomyocyte hypertrophy, fibrosis, and apoptosis[15,16]. In the cardiovascular system, specific miRNAs such as miR-1, miR-133, and *miR-214* have been shown to play important roles in the pathogenesis of diseases including pathological left ventricular hypertrophy, myocardial ischemia, and heart failure[9,17-20]. Among these, *miR-214*, which is highly conserved across species, is abundantly expressed in the heart and has emerged as a potent epigenetic regulator. Initially identified in tumorigenesis and cancer progression[21], *miR-214* has more recently been shown to exert key effects in cardiac hypertrophy and heart failure[22-24]. Experimental models demonstrate that inhibition of *miR-214* significantly reduces pressure overload-induced hypertrophic responses, whereas transgenic mice with cardiomyocyte-specific overexpression of *miR-214* exhibit pronounced hypertrophic phenotypes[23,25]. Consistently, *miR-214* has been found to be significantly upregulated in both human heart failure patients and hypertrophic mouse hearts[24,26]. In angiotensin II (Ang II)-induced hypertrophy models, *miR-214* expression was markedly elevated; its overexpression aggravated hypertrophy, whereas its inhibition attenuated hypertrophic responses both *in vitro* and *in vivo*[27]. Collectively, these findings suggest that *miR-214* is a strong regulator of cardiac hypertrophy and that its inhibition may confer cardioprotective benefits[25]. The biological effects of miRNAs are mediated through their direct mRNA targets[28]. In this context, *miR-214* has been identified as a critical upstream regulator of the *sirtuin 3* (*SIRT3*)/*Forkhead box O3 (FOXO3)* signaling axis. Recent evidence demonstrates that *miR-214* directly targets *SIRT3*, whose suppression leads to impaired deacetylation and reduced stabilization of *FOXO3*[27]. Consequently, elevated *miR-214* levels are associated with the functional attenuation of *FOXO3* signaling. The *FOXO* family regulates multiple fundamental processes including cell growth, differentiation, apoptosis, oxidative stress response, and energy metabolism[29,30]. Within the cardiovascular system, *FOXO* proteins serve as protective barriers against pathological hypertrophy[28,31]. Numerous studies have shown that activation of *FOXO* exerts anti-hypertrophic effects and plays key roles in reducing oxidative stress, preserving mitochondrial function, and maintaining cellular homeostasis[32,33]. The regulatory role of *FOXO* is directly linked to the *NFAT* signaling pathway, which is frequently activated in cardiomyocytes[34]. Upon activation through the calcium/calcineurin pathway, *NFAT* translocates into the nucleus and promotes the expression of hypertrophic marker genes such as atrial natriuretic peptide (*ANP*), and brain natriuretic peptide (*BNP*)[30]. Thus, *NFAT* functions as a potent transcription factor driving cardiac hypertrophy[34]. Conversely,

FOXO counteracts excessive *NFAT* activation, thereby preventing pathological cardiomyocyte growth[7,29,35]. This establishes an antagonistic balance between *FOXO* and *NFAT*: when *FOXO* is active, hypertrophic gene expression is restrained, whereas suppression of *FOXO* amplifies *NFAT*-mediated hypertrophic responses[34].

In recent years, the therapeutic potential of natural products has been increasingly investigated, with particular attention given to the protective effects of polyphenolic compounds on the cardiovascular system[36]. One such natural product is polydatin, a resveratrol glucoside isolated from Polygonum cuspidatum, which is also naturally found in foods such as red wine and grapes[37]. Numerous studies have demonstrated that polydatin possesses a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective effects[38]. Experimental findings have revealed that polydatin exerts notable benefits on the cardiovascular system[39,40]. Specifically, it has been reported to exert preventive effects against Ang II-induced cardiac hypertrophy, protect cardiomyocytes from ischemia-reperfusion injury, and alleviate cardiac dysfunction in diabetic models[39-42]. In addition, polydatin has been shown to inhibit the calcineurin-*NFAT* signaling pathway, thereby decreasing hypertrophic gene expression and directly influencing the process of cardiac remodeling[43]. However, despite these cardioprotective and antioxidant activities, the precise mechanisms by which polydatin improves cardiac remodeling and the extent of these effects remain incompletely understood[39].

Based on this background, the primary aim of the present study was to investigate the role of the *miR-214*/*FOXO3*/*NFAT* signaling pathway in an isoproterenol (ISO)-induced cardiac hypertrophy model and to determine whether polydatin modulates this axis. Furthermore, we sought to evaluate whether polydatin modulates the *miR-214*-mediated signaling axis in the same model, thereby clarifying its potential cardioprotective mechanisms during cardiac hypertrophy and remodeling. In this way, the study aims to contribute both to the understanding of the molecular basis of pathological cardiac hypertrophy and to the identification of polydatin as a potential therapeutic candidate.

MATERIALS AND METHODS

Experimental Animals and Tissue Source

Cardiac tissues were obtained from eight-week-old male Wistar rats (n=12) provided by the Trakya University Laboratory Animal Research Unit as part of a previously approved project. All experimental procedures, including animal housing and treatments, were carried out in compliance with institutional ethical standards and were approved by the Trakya University Animal Experiments Local Ethics Committee (protocol number: TÜHADYEK-2024/08, date: 05.11.2025). Cardiac tissues became available for the present study following the completion of that project, thereby avoiding additional animal use.

The original project protocol involved three experimental groups: Control, ISO, 5 mg/kg/day, s.c.), and ISO + polydatin (100 mg/kg/day, oral gavage), with treatments administered for four weeks. During the experimental period, animals were kept under standard laboratory conditions (22±1 °C, 55% relative humidity, 12-hour light/dark cycle) with ad libitum access to food and water. Solutions were freshly prepared in 0.9% saline on a daily basis. Body weights were monitored weekly throughout the experimental period. The experimental design, treatment duration, and dosing regimen were established following a comprehensive review of the relevant literature[39,44].

Morphological Analysis of the Hearts

At the end of the four-week treatment period, body weight, heart weight, lung weight, and tibia length of the animals were measured to evaluate the presence of cardiac hypertrophy. To provide a more accurate assessment of morphological changes, the following ratios were calculated: heart weight/body weight, heart weight/tibia length, lung weight/body weight, and lung weight/tibia length. These parameters served as indices of structural alterations and hypertrophy.

Determination of mRNA Expression Levels

The mRNA expression levels of *FOXO3*, *NFAT*, *ANP*, and *BNP* genes in cardiac tissue were determined using quantitative real-time polymerase chain reaction (RT-qPCR). First, total RNA was isolated from left ventricular tissues using the mirVana™ miRNA Isolation Kit (Thermo Fisher Scientific, USA). The purity and concentration of the isolated total RNA were measured by evaluating the A260/280 ratio with a microplate reader (Tecan Infinite m1000 Pro Microplate Reader with Tecan Quant Plate). Subsequently, 300 ng/µL of total RNA from each sample was converted into cDNA using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. RT-qPCR analysis was then performed with gene-specific primers (*FOXO3*, *NFAT*, *ANP*, *BNP*), cDNA samples, and SYBR Select Master Mix (Thermo Fisher Scientific, USA) on the 7500 Fast Real-Time PCR system (Applied Biosystems, USA). The PCR conditions consisted of an initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of 95 °C for 15 seconds, 60 °C for 15 seconds, and 72 °C for 15 seconds. Cycle threshold (Ct) values obtained during PCR amplification were used to evaluate gene expression levels. Relative gene expression changes were calculated using the $2^{-\Delta\Delta Ct}$ method, with *glyceraldehyde-3-phosphate dehydrogenase* serving as the reference gene for normalization. All samples were analyzed in technical duplicates. The results were expressed as relative fold changes compared to the Control group. The primer sequences used in the reactions are provided in Table 1.

Determination of miRNA Expression Levels

Total RNA, including small RNAs, was isolated from left ventricular tissue samples using the mirVana™ miRNA Isolation Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. The purity and concentration of the isolated RNA were determined with a Tecan Infinite M1000 Pro Microplate Reader with Quant Plate. For specific reverse transcription of target miRNAs, cDNA synthesis was performed using the TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, USA) together with gene-specific stem-loop primers. The synthesized cDNAs were subsequently amplified by RT-qPCR using TaqMan™ Universal PCR Master Mix and TaqMan MicroRNA Assays (Thermo Fisher Scientific, USA) on the 7500 Fast Real-Time PCR System (Applied Biosystems, USA), following the manufacturer's instructions. The probe sets used contained specific sequences for the experimentally investigated miRNAs: miR-1 (assay ID: 002222), *miR-214* (assay ID: 000517), and miR-133b (assay ID: 002247). Ct values obtained during the PCR cycles were used to calculate the expression levels of the target miRNAs. Relative expression changes were analyzed using the $2^{-\Delta\Delta Ct}$ method, with U6 small nuclear RNA (assay ID: 001973) serving as the reference gene for normalization. All samples were analyzed in technical duplicates. The results were expressed as relative fold changes compared to the Control group.

Statistical Analysis

The normality of the data distribution was assessed using the Shapiro-Wilk test. Group differences were analyzed using ANOVA, followed by Tukey-honestly significant difference post hoc test for pairwise comparisons. This approach was applied to analyze miRNA, mRNA, and morphological parameters, as well as *ANP* and *BNP* expression levels. Experimental data were presented as mean ± standard error of the mean (SEM). A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

Table 1. Primer sequences used for RT-qPCR analysis

Gene region	Primer sequences
FOXO3	F: 5- CTTCAAGGATAAGGGCGACAGCA -3 R: 5- GCTCTTGCCAGTCCCTTCGTT -3
NFAT	F: 5- CGAGGACGGGGCACCAAC -3 R: 5- AGTAGATGGAGGCGGGTCTACATT -3
ANP	F: 5- ATTGGAGCAAATCCCGTATACAGT -3 R: 5- GCTTCATCGGTCTGCTCGCTCA-3
BNP	F: 5- CTGCTCCTGCTTTTCCTTAATCTGT -3 R: 5- AGCTGTCTCTGAGCCATTTCC -3
GAPDH	F: 5-GCAGCCCAGAACATCATCCCT-3 R: 5-CATGCCAGTGAGCTTCCCGTT-3

FOXO3: Forkhead box O3, NFAT: Nuclear factor of activated T-cells, *ANP:* Atrial natriuretic peptide, *BNP: Brain natriuretic peptide, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase*, RT-qPCR: Real-time polymerase chain reaction

RESULTS

At the end of the four-week treatment period, cardiac hypertrophy was evaluated based on heart weight, heart weight/body weight ratio, heart weight/tibia length ratio, as well as lung weight and lung weight/tibia length ratio. In rats with ISO-induced cardiac hypertrophy, heart weight, heart weight/body weight ratio, and heart weight/tibia length ratio were significantly higher than the Control group ($p<0.001$). In contrast, polydatin administration markedly reduced these parameters and significantly attenuated the hypertrophic effects of ISO ($p<0.05$) (Figure 1). These findings indicate that ISO induced a pathological concentric type of cardiac hypertrophy by increasing myocardial mass, whereas polydatin substantially mitigated this hypertrophic response, exerting a cardioprotective effect.

Furthermore, no statistically significant differences were observed among groups in terms of lung weight or lung weight/tibia length ratio ($p>0.05$) (Figure 1). This suggests that ISO-induced hypertrophy was not accompanied by pulmonary edema or advanced heart failure. Thus, although pronounced cardiac hypertrophy was established in this experimental model, secondary effects of cardiac dysfunction at the pulmonary level were not evident. Overall, these results demonstrate that ISO successfully induced an experimental model of pathological cardiac hypertrophy, while polydatin provided significant morphological protection against this process.

miRNAs are critical regulators of pathological cardiac hypertrophy. In this study, we evaluated the expression of miR-1, *miR-214*, and miR-133b, which have previously been implicated in hypertrophic processes. The findings revealed significant differences among groups specifically in the expression levels of *miR-214* ($p=0.007$). In the ISO-treated group, *miR-214* expression was significantly higher compared with the Control group ($p=0.006$). This increase strongly supports the close association of *miR-214* with pathological cardiac hypertrophy, as previously reported in the literature. In contrast, polydatin treatment significantly reduced *miR-214* expression compared with the ISO group ($p=0.030$), suggesting that one of the mechanisms by which polydatin attenuates cardiac hypertrophy may involve the regulation of *miR-214*. On the other hand, no significant differences were observed among groups in the expression levels of miR-1 and miR-133b ($p=0.860$ and $p=0.554$, respectively) (Figure 2). This indicates that miR-1 and miR-133b did not undergo notable alterations during the development of ISO-induced cardiac hypertrophy and did not contribute meaningfully to hypertrophy pathogenesis under the conditions of this study.

Furthermore, polydatin treatment did not exert a significant effect on these two miRNAs. Overall, these results suggest that the cardioprotective effects of polydatin are not mediated through miR-1 or miR-133b, but rather by modulating the expression of miRNAs directly linked to pathological hypertrophy, particularly *miR-214*. This highlights the possibility that the beneficial effects of polydatin on cardiac hypertrophy are molecularly associated with the regulation of *miR-214*.

Since miRNAs exert their biological functions by regulating target gene expression, we next examined the impact of altered *miR-214* levels on genes associated with cardiac hypertrophy. Specifically, we evaluated the mRNA expression of *FOXO3* and *NFAT*, which are downstream effectors functionally regulated within the *miR-214-SIRT3* signaling pathway and are central regulators of hypertrophic remodeling. The analyses revealed significant differences among groups in *NFAT* and *FOXO3* expression levels ($p<0.001$ and $p=0.006$, respectively). In the ISO-treated group, *FOXO3* expression was markedly decreased ($p=0.027$), while *NFAT* expression was significantly increased compared with the Control group ($p<0.001$). These findings indicate that ISO suppresses anti-hypertrophic mechanisms via *FOXO3*, while simultaneously activating hypertrophic signaling through *NFAT* during the development of cardiac hypertrophy. Polydatin treatment reversed these changes, reducing *NFAT* levels ($p<0.001$) and increasing *FOXO3* expression ($p=0.005$) compared with the ISO group. Moreover, no significant differences were observed between the Control and ISO + POL groups in terms of *NFAT* and *FOXO3* expression ($p>0.05$), suggesting that polydatin largely restored gene expression to control levels (Figure 3). These results demonstrate that the cardioprotective effects of polydatin are mediated not only through the regulation of *miR-214* but also via the modulation of hypertrophy-related transcription factors such as *FOXO3* and *NFAT*, which are established targets of *miR-214*.

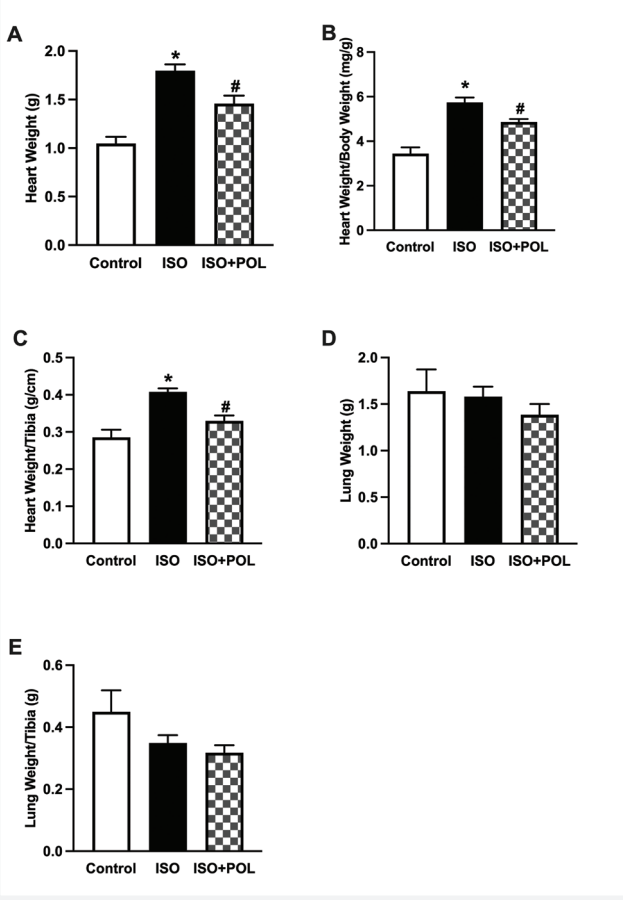

Figure 1. Effects of polydatin treatment on isoproterenol-induced pathological cardiac hypertrophy. The evaluated parameters include heart weight (A), heart weight/body weight ratio (B), heart weight/tibia length ratio (C), lung weight (D), and lung weight/tibia length ratio (E). Values are presented as mean ± SEM (n=4 for Control, ISO, and ISO + POL groups). *$p<0.001$ vs. Control group; #$p<0.05$ vs. ISO group

ISO: Isoproterenol, POL: Propranolol, SEM: Standard error of the mean

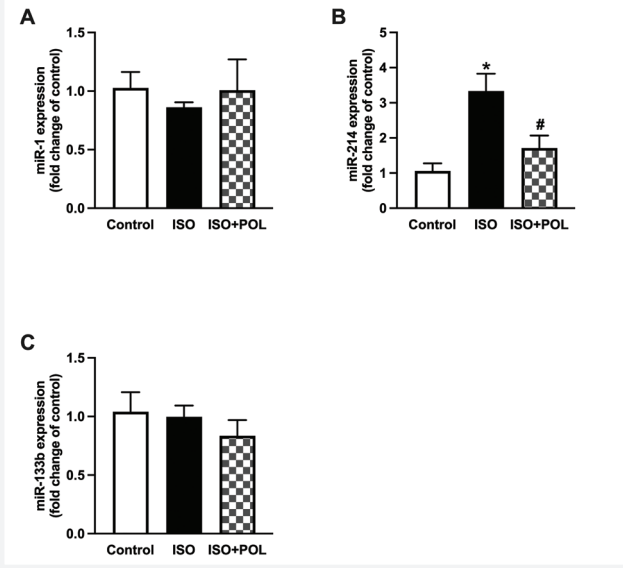

Figure 2. Effects of four-week polydatin treatment on the expression levels of cardiac hypertrophy-related miRNAs: (A) miR-1, (B) miR-214, and (C) miR-133b. Changes in the expression levels of these miRNAs were evaluated in the isoproterenol-induced pathological cardiac hypertrophy model following polydatin administration. Data are presented as mean ± SEM (n=4 for Control, ISO, and ISO + POL groups; each sample analyzed in duplicate). *$p<0.05$ vs. Control group; #$p<0.05$ vs. ISO group

ISO: Isoproterenol, POL: Propranolol, SEM: Standard error of the mean

Consistent with the increase in *NFAT* expression, the expression levels of *ANP* and *BNP* established markers of cardiac hypertrophy were significantly elevated in the ISO group (p=0.029 and p=0.002, respectively). These findings indicate that ISO-induced pathological cardiac hypertrophy involves the reactivation of the fetal gene program. Polydatin treatment markedly suppressed these alterations, significantly reducing *ANP* and *BNP* expression and restoring them to levels comparable with those of the Control group (p>0.05) (Figure 3). This result demonstrates that polydatin exerts a regulatory effect not only at the transcription factor level by inhibiting *NFAT* activation, but also on its downstream targets, the hypertrophic markers. Thus, polydatin attenuates hypertrophic signaling mediated by *NFAT* and prevents the reactivation of the fetal gene program, thereby reinforcing its cardioprotective effect.

DISCUSSION

ISO administration induced pathological concentric cardiac hypertrophy by increasing ventricular wall thickness, whereas polydatin markedly attenuated this hypertrophic response. At the molecular level, ISO was found to increase *miR-214* expression, suppress *FOXO3*, and activate *NFAT*, whereas polydatin reversed these alterations, thereby exerting a cardioprotective effect. Furthermore, elevated *ANP* and *BNP* levels indicated that ISO reactivated the fetal gene program, which was suppressed by polydatin. Taken together, these findings suggest that polydatin confers protection against cardiac hypertrophy by modulating a proposed *miR-214*/*FOXO3*/*NFAT* signaling axis, with *SIRT3* included as an intermediate node based on established literature (Figure 4).

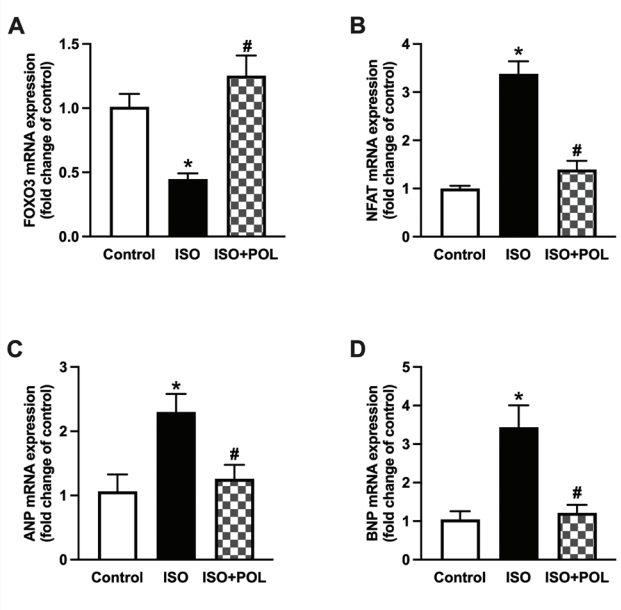

Figure 3. Changes in mRNA expression levels of *FOXO3* and *NFAT*, which are associated with miR-214, as well as the fetal genes *ANP* and *BNP*. (A) *FOXO3*, (B) *NFAT*, (C) *ANP*, and (D) *BNP* mRNA expression levels. Data are presented as mean ± SEM (n=4 for Control, ISO, and ISO + POL groups; each sample analyzed in duplicate). *p<0.05 vs. Control group; #p<0.05 vs. ISO group

FOXO3: FOXO3: Forkhead box O3, NFAT: Nuclear factor of activated T-cells, ANP: Atrial natriuretic peptide, BNP: Brain natriuretic peptide, POL: Propranolol, SEM: Standard error of the mean

Morphometric analysis confirmed the development of pathological hypertrophy in the ISO group, as indicated by marked increases in heart weight indices, while polydatin significantly blunted these effects. This finding is consistent with previous studies reporting that ISO induces cardiac remodeling through β-adrenergic stimulation, leading to increased ventricular wall thickness and hypertrophy[44-46]. The significant reduction of these parameters following polydatin treatment highlights the cardioprotective potential of this compound. This result is in line with earlier research suggesting that polydatin mitigates myocardial injury through its antioxidant, anti-inflammatory, and anti-apoptotic properties[39,42]. Thus, the results of our study reveal that polydatin confers protection not only at the molecular level but also at the structural level, as reflected by organ weight measurements. Another noteworthy aspect of the results is the absence of significant changes in lung weight and lung weight/tibia length ratio despite ISO administration. Notably, the preservation of lung parameters indicates the absence of pulmonary congestion, suggesting that the ISO protocol employed in this study models early to mid-stage pathological cardiac hypertrophy rather than decompensated heart failure. The concordance between robust cardiac hypertrophic remodeling and preserved lung indices supports the interpretation that the observed molecular and structural alterations precede the development of overt hemodynamic congestion. This indicates that the hypertrophic response induced by ISO did not progress to pulmonary edema or advanced heart failure. Therefore, while the model accurately represents pathological hypertrophy, it does not capture the transition to overt heart failure. In this regard, it should be acknowledged that the ISO protocol used in the present study is pharmacologically established to model early to mid-stage pathological cardiac hypertrophy rather than advanced heart failure. Similarly, the literature has reported that ISO predominantly mimics early hypertrophic changes without reproducing long-term cardiac dysfunction[44,45]. Overall, our findings support the validity of ISO as a model of cardiac hypertrophy and demonstrate that polydatin provides meaningful structural protection within this experimental framework. The present study was designed as a focused, exploratory investigation aimed at elucidating molecular and structural alterations associated with pathological cardiac

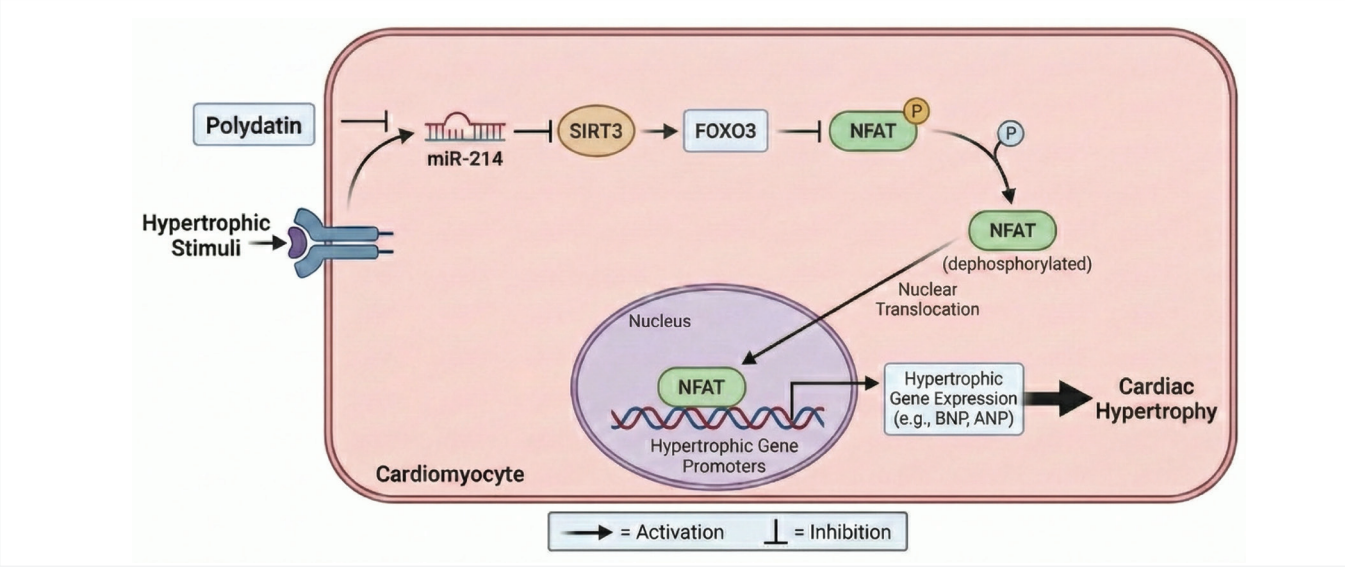

Figure 4. Proposed mechanism illustrating the modulatory effect of polydatin on pathological cardiac hypertrophy via the miR-214/*SIRT3/FOXO3/NFAT* signaling axis in cardiomyocytes. Hypertrophic stimuli upregulate miR-214 expression, which suppresses *SIRT3*, leading to impaired *FOXO3* deacetylation and reduced functional stability. The consequent decrease in *FOXO3* activity relieves its inhibitory control over *NFAT*, facilitating *NFAT* dephosphorylation and nuclear translocation. Activated *NFAT* accumulates in the nucleus and binds to hypertrophy-associated gene promoters, thereby inducing the expression of fetal genes such as *ANP* and *BNP* and promoting the hypertrophic phenotype. Polydatin treatment attenuates this signaling cascade by inhibiting miR-214, restoring the *SIRT3/FOXO3* signaling axis, limiting *NFAT* activation, and consequently suppressing hypertrophic gene transcription. Arrows denote activation, whereas blunt-ended lines indicate inhibitory interactions

SIRT3: *Sirtuin 3*, *FOXO3*: *Forkhead box O3*, *NFAT*: Nuclear factor of activated T-cells, T-cells, BNP: Brain natriuretic peptide, ANP: Atrial natriuretic peptide

hypertrophy, rather than providing a comprehensive functional or electrophysiological characterization. Accordingly, the findings should be interpreted within the framework of a preliminary study that provides a molecular basis for future investigations incorporating expanded experimental groups and functional cardiac assessments.

Expression profiling of miR-1, *miR-214*, and miR-133b revealed that only *miR-214* was significantly upregulated in ISO-induced hypertrophy. The significant upregulation of *miR-214* in the ISO group supports previous reports linking this miRNA to hypertrophy and heart failure[20,27]. This increase highlights the role of *miR-214* in regulating processes such as cardiac remodeling and fibrosis. The observation that polydatin treatment significantly suppressed *miR-214* expression suggests that its cardioprotective effect is at least partly mediated through *miR-214* regulation. In contrast, no significant changes were observed in the expression of miR-1 and miR-133b. Although previous studies have reported potential associations of these miRNAs with cardiac hypertrophy and myocardial remodeling[9,47,48], the lack of changes in our study implies that their involvement may be context-dependent or more prominent in other hypertrophy models. Indeed, several reports have demonstrated significant alterations in miR-1 and miR-133b in pressure overload or ischemia-reperfusion models, suggesting that the regulation of these miRNAs may vary depending on the pathological stimulus or stage of disease. The absence of significant effects of polydatin on these two miRNAs further indicates that polydatin may act selectively through miRNAs more strongly linked to the hypertrophic response, particularly *miR-214*. Taken together, our findings suggest that the protective effects of polydatin against cardiac hypertrophy are not mediated by miR-1 or miR-133b, but rather by modulating the expression of miRNAs directly associated with pathological hypertrophy, such as *miR-214*.

To our knowledge, this study provides initial evidence linking polydatin treatment to the modulation of *miR-214* expression in an experimental model of cardiac hypertrophy. While polydatin has previously been shown to inhibit hypertrophic signaling pathways such as calcineurin-*NFAT*[43], our findings suggest that its cardioprotective effects may also involve upstream regulation at the miRNA level, particularly *miR-214*.

Analysis of *FOXO3* and *NFAT*, which are downstream effectors functionally regulated by *miR-214* signaling, showed that ISO

suppressed *FOXO3* and increased *NFAT* expression. Previous studies have reported that *FOXO3* is a key transcription factor activating anti-hypertrophic gene programs, and its downregulation facilitates the development of hypertrophy[31,34]. Conversely, *NFAT* has been shown to be activated via calcineurin-dependent signaling pathways, promoting the expression of hypertrophic genes[34]. Our findings are consistent with this evidence, indicating that ISO promotes hypertrophy by disrupting the *FOXO3*/*NFAT* balance. Polydatin treatment restored *FOXO3* expression and reduced *NFAT* levels back to control values, suggesting that the cardioprotective effect of polydatin is mediated not only through *miR-214* regulation but also by modulating critical transcription factors that are functionally regulated downstream of *miR-214* signaling. It should be noted that miRNAs classically exert their regulatory effects predominantly through translational repression rather than direct mRNA degradation. However, increasing evidence indicates that miRNA-mediated downregulation of target transcripts can also occur via mRNA destabilization, particularly in chronic pathological settings. In the present 4-week ISO model, the observed reduction in *FOXO3* mRNA may therefore reflect sustained or indirect regulation of *FOXO3* expression downstream of *miR-214* signaling, rather than acute translational inhibition alone. Moreover, given that *FOXO3* was assessed at the transcript level, these findings should be interpreted as indicative of coordinated pathway suppression rather than direct evidence of miRNA-mRNA binding or degradation.

Consistent with the increased *NFAT* activity, we observed significantly elevated expression of *ANP* and *BNP*, established markers of cardiac hypertrophy, in the ISO group. Reactivation of the fetal gene program is considered one of the molecular hallmarks of pathological cardiac hypertrophy[30], and our results support this concept. Importantly, polydatin treatment restored *ANP* and *BNP* expression to levels comparable with the Control group, demonstrating that this compound regulates not only transcription factors but also their downstream targets. Similarly, other natural compounds have also been reported to exert protective effects against cardiac hypertrophy by suppressing the fetal gene program[40,43,49]. Taken together, these findings are consistent with modulation of the *miR-214*/*FOXO3*/*NFAT* axis, whereby both hypertrophic signaling and the reactivation of the fetal gene program are suppressed. These results suggest that polydatin exerts cardioprotective effects against pathological cardiac hypertrophy in this experimental model.

Study Limitations

The present study should be interpreted as a preliminary, hypothesis-driven investigation aimed at elucidating a specific molecular signaling axis rather than providing a comprehensive functional characterization of pathological cardiac hypertrophy. The sample size was modest, which may limit the precision of certain estimates. In addition, only a single dose and treatment duration of polydatin were evaluated; therefore, potential dose- or time-dependent effects could not be assessed. The limited number of experimental groups reflects a design focused on pathway interrogation rather than exhaustive phenotypic stratification. At the molecular level, analyses were restricted to transcript-level measurements; thus, the absence of protein-level validation and assessment of *NFAT* and *FOXO3* nuclear translocation constitutes an important limitation. Furthermore, although our findings support a coordinated regulation of the *miR-214*/*FOXO3*/*NFAT* pathway by polydatin, *SIRT3* was incorporated as an intermediate regulator based on established mechanistic evidence from prior studies. Direct mechanistic validation (e.g., luciferase reporter assays or genetic manipulation of intermediate nodes) was beyond the scope of the present study. Accordingly, the proposed signaling axis should be interpreted as a hypothesis-driven model supported by converging gene expression profiles and established literature[27], warranting future causal investigation. Finally, the lack of electrocardiography or other functional cardiac assessments precludes conclusions regarding electrophysiological alterations and overall cardiac performance, and future studies incorporating functional and electrophysiological endpoints will be required to further substantiate these findings.

CONCLUSION

Polydatin exerted pronounced cardioprotective effects in an ISO-induced hypertrophy model, acting at both structural and molecular levels. Morphologically, it significantly attenuated ventricular wall thickening and the hypertrophic increase in myocardial mass. At the molecular level, ISO was shown to increase *miR-214* expression, suppress *FOXO3*, and activate *NFAT*, leading to elevated *ANP* and *BNP* levels. These findings support the involvement of a *miR-214*/*FOXO3*/*NFAT*-associated signaling axis and the reactivation of the fetal gene program in the progression of pathological hypertrophy. Polydatin treatment reversed these changes by reducing *miR-214* expression, restoring *FOXO3* levels, and suppressing *NFAT* activation. Consequently, the hypertrophic markers *ANP* and *BNP* were maintained at levels comparable to controls. Thus, the cardioprotective effects of polydatin extend beyond its antioxidant and anti-inflammatory properties, encompassing a multilayered mechanism involving miRNA regulation and modulation of transcription factors. Overall, this study identifies polydatin as a strong experimental candidate against pathological cardiac hypertrophy and introduces the *miR-214/FOXO3*/*NFAT* axis as a promising molecular target for the development of therapeutic strategies. Nevertheless, further studies using more comprehensive *in vivo* models, different dosing protocols, and long-term follow-up are required to translate these findings into clinical applications.

Ethics

Ethical Committee Approval: This study was approved by the Trakya University Animal Experiments Local Ethics Committee (protocol number: TÜHADYEK-2024/08, date: 05.11.2025).

Informed Consent: All experimental procedures, including the housing and treatment of animals, were conducted in accordance with institutional ethical standards.

Footnotes

Authorship Contributions

Concept: B.E.Y., Design: B.E.Y., M.Y., Data Collection or Processing: B.E.Y., M.Y., K.B., M.A., Analysis or Interpretation: B.E.Y., Literature Search: B.E.Y., M.Y., Writing: B.E.Y., M.Y., K.B., M.A.

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

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ORIGINAL ARTICLE

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The Effect of Botulinum Toxin Application to the Muscles of the Upper Extremities on Forearm Muscle Thickness and Motor Recovery in Patients with Hemiplegia

Hemiplejili Hastalarda Üst Ekstremite Kaslarına Botulinum Toksin Uygulamasının Ön Kol Kas Kalınlığı ve Motor İyileşme Üzerine Etkisi

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ABSTRACT

Aim: The aim of this study was to investigate the effects of botulinum toxin injection on upper extremity muscle thickness and motor recovery in stroke patients.

Materials and Methods: The study was conducted retrospectively. Twenty stroke patients (11 males, 9 females) who received botulinum toxin injections into the forearm muscles of the upper extremity were included in the study. Muscle thickness measurements, brunnstrom motor recovery stages, and modified Ashworth scale scores were assessed before and one month after botulinum toxin injection.

Results: There were no significant change in the brunnstrom upper extremity and hand stages before or after botulinum toxin application ($p>0.05$). No significant change in forearm muscle thickness was observed in measurements taken from the proximal third of the volar surface of the forearm before or after the injections ($p>0.05$). A significant decrease in spasticity levels was observed in wrist flexor group and finger flexor group muscles after botulinum toxin application ($p=0.001$).

Conclusion: The study shows that botulinum toxin reduces spasticity but has no effect on brunnstrom motor recovery and muscle thickness.

Keywords: Stroke, botulinum toxin injection, spasticity, muscle thickness, brunnstrom motor recovery stage

ÖZ

Amaç: Bu çalışmanın amacı, inme hastalarında botulinum toksin enjeksiyonunun üst ekstremite kas kalınlığı ve motor iyileşme üzerine etkilerini araştırmaktır.

Gereç ve Yöntem: Çalışma retrospektif olarak yürütülmüştür. Üst ekstremite ön kol kaslarına botulinum toksin enjeksiyonu uygulanan yirmi inme hastası (11 erkek, 9 kadın) çalışmaya dahil edilmiştir. Kas kalınlığı ölçümleri, brunnstrom motor iyileşme evreleri ve modifiye Ashworth Skalası skorları, botulinum toksin enjeksiyonundan önce ve bir ay sonra değerlendirilmiştir.

Bulgular: Botulinum toksin uygulamasından önce ve sonra brunnstrom üst ekstremite ve el evrelerinde anlamlı bir değişiklik görülmemiştir ($p>0,05$). Enjeksiyonlardan önce veya sonra ön kolun volar yüzeyinin proksimal üçte birinden alınan ölçümlerde ön kol kas kalınlığında anlamlı bir değişiklik gözlenmedi ($p>0,05$). Botulinum toksin uygulamasından sonra el bilek fleksör grubu ve el parmak fleksör grubu kaslarının spastisite düzeylerinde anlamlı bir azalma gözlendi ($p=0,001$).

Sonuç: Çalışma, botulinum toksinin spastisiteyi azalttığını ancak brunnstrom motor iyileşmesi ve kas kalınlığı üzerinde bir etkisi olmadığını göstermektedir.

Anahtar Kelimeler: İnme, botulinum toksin enjeksiyonu, spastisite, kas kalınlığı, brunnstrom motor iyileşme evresi

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INTRODUCTION

Stroke is the second leading cause of death worldwide. It poses a significant risk of disability among survivors. It is particularly prevalent in developing countries[1]. There are significant changes in muscle tone in patients after a stroke. Spasticity, which is defined as an increase in muscle tone depending on speed, is one of the most common conditions[2]. Spasticity that occurs during patients' recovery from a stroke can help maintain limb functionality when it is at reasonable levels. However, if it becomes severe, it can lead to a deterioration in motor activity and limitations in daily life participation, as well as the development of various physical impairments[3]. There are many treatment options for spasticity. Botulinum toxin (BoNT-A) injection treatment is one of the preferred options[4]. When treating spasticity, the clinical presentation and functional impairments must be carefully evaluated in order to develop an appropriate management strategy. Treatment goals should be determined based on the patient's current clinical status and functional capacity[2]. Opinions differ regarding the effects of BoNT-A injections on motor function during stroke rehabilitation.

A systematic review found that BoNT-A injections combined with post-stroke rehabilitation had a limited effect on motor function. Nevertheless, it has been suggested that BoNT-A could help to improve motor function[5]. A exploratory meta-analysis study involved 47 patients from two randomised trials who received BoNT-A injections for post-stroke spasticity. The study showed that reducing spasticity in the arm was significantly associated with improvement in motor function[6]. Shaw et al.[7] reported that BoNT-A type A did not significantly improve upper extremity motor function one month after treatment for post-stroke spasticity. Another meta-analysis study, compiled from a limited number of studies investigating the effects of BoNT-A type A injections on muscle elasticity, muscle thickness, and structure, revealed a significant improvement in muscle elasticity as assessed by ultrasound in the short term. Furthermore, no significant difference was found in muscle structure (muscle thickness, pennation angle) in the short term. However, a decrease in normalized muscle volume was observed in the long term[8]. In a study by Picelli et al.[9] examining the ultrasonographic features of the medial gastrocnemius muscle following BoNT-A treatment in post-stroke patients, no significant change in muscle thickness was observed after one month. However, some studies indicate a decrease in muscle mass in the early stages after BoNT-A injections. A study in rats found that a decrease in gastrocnemius muscle mass began in the second week after BoNT-A injections[10]. In a prospective study conducted on cerebral palsy patients who received BoNT-A injections for the first time, a decrease in gastrocnemius muscle volume was observed in all follow-ups at 4 weeks, 13 weeks, and 25 weeks[11]. Another study by Tok et al.[12] evaluated the medial gastrocnemius muscle on the hemiplegic side and observed a reduction in muscle thickness two months after treatment. Similarly, Yi et al.[13] observed a decrease in medial gastrocnemius muscle thickness for up to 12 weeks following BoNT-A injection in children with spastic hemiplegic cerebral palsy.

The use of BoNT-A in the treatment of spasticity has been investigated in systematic review and meta-analysis studies containing many studies. However, these generally focus on the effectiveness of the treatment[14-16]. To our knowledge, no study has examined the effect of BoNT-A application on upper extremity forearm muscle thickness and motor recovery in stroke patients. The aim of this study was to investigate the early effects of BoNT-A injections on upper extremity forearm muscle thickness and motor recovery in stroke patients.

MATERIAL AND METHODS

This retrospective cross-sectional study was conducted in accordance with the Ethical Principles of the Declaration of Helsinki and was approved by the Bolu Abant Izzet Baysal University Clinical Research Ethics Committee (approval number: 2023/429, date: 19.12.2023). This study was registered as a clinical trial on ClinicalTrials.gov.

Selection and Description of the Cases

Patient charts were scanned to identify patients who were hospitalised for neurological rehabilitation at the Bolu Abant Izzet Baysal University Physical Medicine and Rehabilitation Training and Research Hospital between 1 January 2022 and 1 December 2023 and who received BoNT-A injections treatment. Twenty patients who met these criteria were identified and included in the study. All of the patients included were also receiving neurological rehabilitation.

Study inclusion criteria were as follows: patients with hemiplegia due to first stroke who had BoNT-A injections for spasticity in the upper extremity muscles (flexor carpi ulnaris, palmaris longus, flexor carpi radialis, pronator teres, flexor digitorum superficialis, flexor digitorum profundus); patients aged between 30 and 75; and patients who had been at least 3 months post-stroke. No upper limit has been set for the duration of the disease. Exclusion criteria were having a neurological disease other than stroke, malignancy, and having a preexisting musculoskeletal deformity or disease in the upper extremity of the patient who had received BoNT-A injections.

The patients received neurological rehabilitation including range of motion, strengthening, balance-coordination, and stretching exercises during the period between BoNT-A injections and a one-month follow-up. Rehabilitation sessions were held five days a week, with each session lasting one hour.

All of these patients gave informed consent before the BoNT-A injections.

Evaluation Parameters

Patient data were retrospectively retrieved from the hospital's medical record system.

Patients' demographic data, the names of the upper extremity muscles injected with BoNT-A, and the injection doses were recorded.

Information on the brunnstrom motor recovery stage of the upper extremity and hand, the spasticity level of the upper extremity muscles according to the modified Ashworth scale (MAS), and the thickness of the forearm muscles measured by ultrasound was obtained from medical records kept before and one month after BoNT-A injections. The reason for using the first month evaluation data is to examine early effects, as this is the period when the effect of BoNT-A is most pronounced.

Primary Outcomes

Brunnstrom Stage: It was used to evaluate motor recovery in the upper extremity, hand and lower extremity, and also to indicate which motor level the patient is at. The brunnstrom stage consists of six stages, each representing progressively improving movement patterns. Higher scores indicate better motor recovery[17].

Muscle thickness measurements were performed using a Falcon 2101 EXL 2003 ultrasound device equipped with an 8 MHz linear probe, both before and one month after BoNT-A application.

In patients who received BoNT-A injections into the musculus (m) palmaris longus, m. pronator teres, m. flexor carpi radialis, m. flexor carpi ulnaris, m. flexor digitorum profundus, and m. flexor digitorum superficialis, muscle thickness was measured using ultrasound from the proximal one-third of the volar side of the forearm. Measurements were taken before and one month after the application. The forearm muscles were at rest during the measurements. Muscle thickness was recorded by measuring the vertical distance between the subcutaneous tissue and the radius and ulna bone borders on the ultrasound image. Two measurements were taken, radial and ulnar side, and recorded in millimeters (Figure 1). Ultrasound measurements showed good to excellent intra-rater reliability (intraclass correlation coefficient= 0.89; 95% confidence interval: 0.80-0.94).

Secondary Outcomes

MAS: It involves scoring the amount of resistance that specific muscle groups offer to passive movement by manually moving the extremity. A 5-stage scale has been defined to rate resistance during passive muscle tension. Higher scores indicate greater levels of spasticity[18].

Statistical Analysis

For statistics the SPSS 23 program was used. The normality of the data was assessed using the Shapiro-Wilk test. The mean, standard deviation, frequency, median, minimum and maximum values were recorded. The Paired Samples t-test was used to compare normally distributed data, while the Wilcoxon Signed-Rank test was used to compare non-normally distributed data. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In this retrospective cross-sectional study, 20 patients (11 males, 9 females) were included. The mean age of the patients was 60 years. Other demographic data are presented in Table 1. The muscles injected with BoNT-A and the mean dosages were indicated in the Table 2.

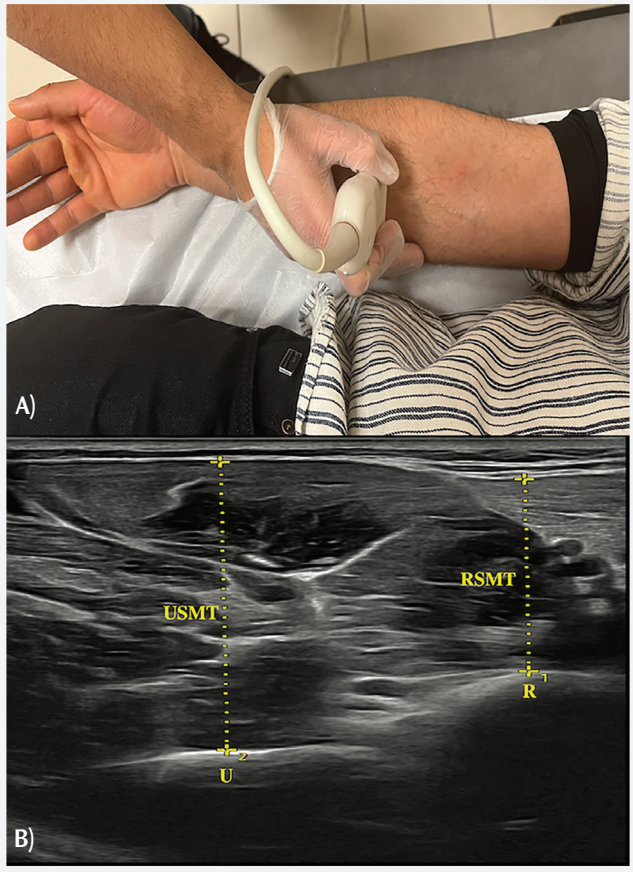

Figure 1. Images of the ultrasound scanning protocol

A) A linear ultrasound probe was placed in the proximal one-third of the volar side of the forearm for transverse scanning, B) To measure muscle thicknesses (RSMT, USMT), the callipers were placed between the bony cortex and the superficial muscle fascia

R: Radius; U: Ulna; RSMT: Radial side muscle thickness; USMT: Ulnar side muscle thickness

Muscle thickness measurements, brunnstrom stages, and MAS scores were assessed before and one month after BoNT-A injections.

There was no significant change in the brunnstrom upper extremity stage before or after BoNT-A application (p>0.05). Similarly, the brunnstrom hand stage did not show any significant change (p>0.05) (Table 3).

Table 1. Demographic data of patients

	Mean ± SD (min-max)
Age (years)	60.0±8.58 (37-73)
Duration of disease (months)	59.10±59.36 (6-215)
	Frequency [n (%)]
Gender	
Male	11 (55%)
Woman	9 (45%)
Marital status	
Married	17 (85%)
Single	3 (15%)
Affected side	
Right	10 (50%)
Left	10 (50%)
Etiology of CVE	
Thromboembolism	14 (70%)
Hemorrhagic	6 (30%)

CVE: Cerebrovascular event, SD: Standard deviation

No significant change in muscle thickness was observed in measurements taken from the proximal third of the ulnar side of the volar surface of the forearm before or after the injections (p=0.198). Similarly, no significant change in muscle thickness was observed in measurements taken from the proximal third of the radial side of the volar surface of the forearm (p=0.775) (Table 3).

Wrist Flexor Group Muscles: A significant decrease in spasticity levels was observed in this muscle group after BoNT-A application (p=0.001).

Finger Flexor Group Muscles: Similarly, a significant decrease in spasticity levels was observed in this muscle group after treatment. (p=0.001) (Table 3).

Table 2. Muscles injected with botulinum toxin and dosages

	Patients injected, n	Dosage IU (mean ± SD)
Flexor carpi ulnaris	20	26±2.05
Palmaris longus	18	24.7±1.18
Flexor carpi radialis	20	29.5±1.54
Pronator teres	20	30±0
Flexor digitorum superficialis	20	26.2±2.20
Flexor digitorum profundus	20	26.2±2.20

IU: International unit, SD: Standard deviation

Table 3. Comparison of the forearm muscle thickness, motor recovery and spasticity of patients before and one month after a botulinum toxin injections

	Before botulinum toxin application	After botulinum toxin application	Mean diffrence	(95% CI)	p-value*
	Mean ± SD	Mean ± SD			
Muscle thickness of the forearm/mm					
Ulnar side (mm)	32.2±4.8	31.6±4.4	0.76	-0.29-1.82	0.198
Radial side (mm)	17.6±3.5	17.4±3.0	0.12	-0.75-1.00	0.775
	Median (min-max)	Median (min-max)	p-value**		
Motor recovery					
Brunnstrom upper extremity	3 (2-5)	3 (2-5)	0.157		
Brunnstrom hand	3 (2-4)	3 (2-4)	0.083		
Spasticity					
MAS of the wrist flexor group muscles	3 (1-4)	3 (1-4)	0.001		
MAS of the finger flexor group muscles	4 (1-4)	3 (1-4)	0.001		

*The Paired Samples t-test was applied, **Wilcoxon Signed-Rank test was applied, mm: Millimeters, MAS: Modified Ashworth scale, CI: Confidence interval, SD: Standard deviation

DISCUSSION

This study found that injecting BoNT-A into the upper extremity muscles of stroke patients significantly reduced spasticity after a 1-month follow-up, but did not significantly affect motor recovery or muscle thickness.

The use of BoNT-A the treatment of post-stroke spasticity is widely accepted in the literature[19,20]. A study by Sun et al.[14] demonstrated that BoNT-A was superior to placebo in reducing upper or lower limb spasticity after stroke. In a study conducted by Varvarousis et al.[19], which evaluated lower limb spasticity (using MAS) and gait pattern (10-metre walk test) after stroke, it was observed that BoNT-A reduced the degree of lower limb spasticity. In this recent study, a significant decrease was observed in wrist and finger flexor spasticity levels, as assessed by MAS, in patients treated with BoNT-A.

Studies investigating the effects of BoNT-A on motor function have produced mixed results. While some studies indicate that BoNT-A has a positive effect on improving motor function[21,22], others suggest that it has no significant effect[23,24]. In a study by Lim et al.[25] comparing the effects of BoNT-A injections in subacute and chronic stroke patients, no change was observed in brunnstrom stages in either group, while an improvement was observed in Fugl-Meyer scores. In a study by Devier et al.[26], an improvement in motor function was observed in patients receiving rehabilitation alongside BoNT-A treatment for chronic upper extremity spasticity following a stroke. In a study by Çelebi et al.[27] investigating the effects of ultrasound-guided BoNT-A injections on pain, functionality, spasticity, and range of motion in patients with upper extremity spasticity following a stroke, motor function was evaluated using the Fugl-Meyer scale at the second week and third month of treatment. It was observed that motor function had improved significantly In a study by Hesse et al.[28] investigating the effects of BoNT-A injections treatment for upper extremity spasticity within 12 weeks of a stroke, no significant change in motor function values was observed when evaluated using the modified Rankin Scale and the Motor Function Assessment scale. In Hesse et al.[28] study examining the effect of early BoNT-A treatment on finger flexors six weeks after subacute stroke, arm motor function was evaluated using the Fugl-Meyer scale, and no significant change was observed. In our study, no significant change in motor function recovery was observed. This result is consistent with some previous studies and shows that BoNT-A has a limited effect on motor function. As the patients in this study were chronic stroke patients, no change may have been observed in their Brunnstom motor recovery stages after BoNT-A. Furthermore, brunnstrom staging may not be the optimal method for assessing motor recovery after BoNT-A application. Although frequently used to assess motor recovery, it may be insufficient in capturing minor functional changes and fine motor movements outside of synergy in the patient. More detailed scales such as Fugl-Meyer may yield different results.

The effect of BoNT-A on muscle thickness has been investigated mostly in lower extremity muscles, and there are limited studies in the literature. No significant changes were observed in the ultrasonographic features of the muscle 4 weeks after BoNT-A injections into the gastrocnemius medialis and lateralis muscles due to spastic equinus in stroke patients[11]. However, there are studies showing that the thickness of the gastrocnemius muscle decreases 2 months after BoNT-A administration in stroke patients and approximately 4 weeks after BoNT-A administration in patients with cerebral palsy[12,13]. However, no significant change in muscle thickness was observed in our study due to BoNT-A. The main difference between this study and other studies is the evaluation of the upper extremity muscles. While upper limb muscles are subjected to short bursts of low-intensity activity throughout the day, lower limb muscles require stronger contractions to maintain body balance[29]. The lower limb muscle architecture has a wide pennate angle, allowing for greater force generation[30]. However, age-related sarcopenia has been found to be more severe in lower extremity muscles than in upper extremity muscles[31]. These differences between upper and lower limb muscles may have differentiated the effect of BoNT-A injections on muscle thickness.

Repeated BoNT-A administration has been shown to cause muscle volume reduction and fibrosis over time in the masseter muscle and in experimental animals[32,33]. Ultrasonographic examinations in stroke patients revealed decreased muscle thickness in the spastic extremity compared to the contralateral muscles[34]. Another study found that spastic muscles were thinner than non-spastic muscles, but no significant differences were found in muscle and fascial properties between the spastic flexor carpi ulnaris with and without BoNT-A injections[35].

A review has indicated that repeated BoNT-A injections can cause permanent muscle atrophy and negatively impact BoNT-A long-term effectiveness. Therefore, it has been emphasized that further studies examining the muscle properties of BoNT-A-treated muscles are needed, and that this monitoring can be achieved especialy through ultrasound, a non-invasive method[36]. In summary, the present study adds to the limited evidence on early changes in muscle thickness and motor recovery at the 1-month follow-up BoNT-A treatment in stroke patients.

Study Limitations

One of the limitations of this study is that the number of patients is low, and the etiology is not uniform. The small sample size and the retrospective nature of the study limit the generalizability of the findings. Due to the limited sample

size, the study may have been underpowered to detecting small effects, especially changes in muscle thickness. The short follow-up period (1 month) and the application of only a single dose of BoNT-A injection do not allow the evaluation of the long-term effects of the treatment. Due to the retrospective design of the study, there was insufficient data available on patient evaluations from the second or third month. This is one of the study's limitations, as it does not reflect the medium- and long-term effects of BoNT-A. Therefore, long-term studies with larger samples, prospective designs and repeated injections are needed to evaluate the long-term effects of BoNT-A more accurately. In addition to evaluating muscle thickness using ultrasound, we suggest evaluating muscle volume using magnetic resonance imaging in future studies.

CONCLUSION

The study shows that BoNT-A reduces spasticity after a 1-month follow-up, significant changes in motor recovery and muscle thickness were not observed.

Ethics

Ethical Committee Approval: The Ethical Principles of the Declaration of Helsinki and was approved by the Bolu Abant Izzet Baysal University Clinical Research Ethics Committee (approval number: 2023/429, date: 19.12.2023).

Informed Consent: This retrospective cross-sectional study.

Footnotes

Authorship Contributions

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

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ORIGINAL ARTICLE

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Determination of Seizure Recurrence Frequency and Factors Increasing the Risk of Seizure Recurrence in Patients Presenting with a First and Single Seizure

İlk ve Tek Nöbet ile Başvuran Hastalarda Nöbet Tekrar Sıklığının Saptanması ve Nöbet Tekrar Riskini Artıran Faktörlerin Belirlenmesi

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ABSTRACT

Aim: Approximately 10% of the population experiences a seizure at some point in their lives. However, the prevalence of epilepsy in the general population is around 3%. It is important to predict which individuals presenting with a first-time seizure are at high risk of recurrence and who should be started on antiepileptic medication.

Materials and Methods: This study included 140 patients over the age of 18 who presented to the emergency department or Neurology outpatient clinic for the first time with a complaint of fainting, starting from January 2022. Demographic characteristics, neurological examination findings, electroencephalography (EEG), and brain magnetic resonance imaging (MRI) data were obtained from the hospital information system. Patients were contacted via their registered phone numbers to inquire about seizure recurrence after the first episode, and outpatient follow-up records were reviewed to identify those diagnosed with epilepsy.

Results: A total of 140 patients were included in the study. The mean age of the patients was 56.17±19.45 years, and 74 (52.9%) were female. Twenty-three patients (16.4%) were newly diagnosed with epilepsy. A statistically significant relationship was found between medication use and loss of consciousness with seizure recurrence [(p=0.006), (p=0.022)]. No statistically significant difference was found between seizure recurrence and gender, history of febrile convulsions, family history of epilepsy, history of head trauma, brain MRI findings, or EEG results (p>0.05).

Conclusion: Considering the challenges of a long-term treatment process, the decision to initiate antiepileptic drugs is important for clinicians. Knowing the rate of epilepsy development after a first seizure and which patient groups are at risk of recurrence can assist clinicians in making this difficult decision.

Keywords: First seizure, seizure recurrence, epilepsy

ÖZ

Amaç: Toplumun yaklaşık %10'u yaşamının bir döneminde nöbet geçirmektedir. Buna karşın toplumda epilepsi görülme oranı %3 civarındadır. İlk defa nöbet şikayeti ile başvuran bireylerin hangilerinde nöbet tekrar riskinin yüksek olduğu ve hangilerine nöbet önleyici ilaç başlanması gerektiğinin öngörülmesi önem taşımaktadır.

Gereç ve Yöntem: Bu çalışmaya Ocak 2022 tarihinden itibaren acil servis veya Nöroloji polikliniğine ilk defa bayılma şikayeti ile başvuran 18 yaş üstü 140 hasta dahil edildi. Hastaların demografik özellikleri, nörolojik muayene bulguları, elektroensefalografi (EEG), beyin manyetik rezonans görüntüleme (MRG) bilgilerine hastane bilgi sistemi üzerinden ulaşıldı. Kayıtlı telefon numarası aranarak ilk nöbet sonrası nöbet tekrarı olup olmadığı sorgulandı ve poliklinik takipleri incelenerek epilepsi tanısı alanlar kayıt altına alındı.

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Bulgular: Çalışmaya toplam 140 hasta dahil edildi. Hastaların ortalama yaşı 56,17±19,45 yıldı, 74'ü (%52,9) kadındı. Hastaların 23'ü (%16,4) yeni epilepsi tanısı aldı. İlaç kullanımı ve bilinç kaybı ile nöbet tekrarı arasında istatistiksel olarak anlamlı bir fark saptandı [(p=0,006), (p=0,022)]. Cinsiyet, febril konvülsiyon, ailede epilepsi ve kafa travması öyküsü, Beyin MRG ve EEG bulguları ile nöbet tekrarı arasında istatistiksel olarak anlamlı bir fark saptanmadı (p>0,05).

Sonuç: Uzun yıllar sürecek tedavi sürecinin zorlukları göz önünde bulundurulduğuna nöbet önleyici ilaç başlama kararı klinisyenler için önem taşımaktadır. İlk nöbet sonrası epilepsi hastalığı gelişme oranlarının ve hangi hasta grubunun nöbet tekrarı açısından riskli olduğunun bilinmesi bu zor kararın verilmesinde klinisyene yardımcı olur.

Anahtar Kelimeler: İlk nöbet, nöbet tekrarı, epilepsi

INTRODUCTION

An epileptic seizure refers to temporary signs and/or symptoms caused by abnormal excessive neuronal activity in the brain, whereas epilepsy is defined as a disorder characterized by the brain's enduring predisposition to generate epileptic seizures. The diagnosis of epilepsy is made when a person experiences at least two unprovoked (or reflex) seizures occurring more than 24 hours apart, or one unprovoked (or reflex) seizure with a probability of further seizures similar to the general recurrence risk (at least 60%) over the next 10 years. Although the prevalence of epilepsy in the general population is around 3%, approximately three times as many individuals experience a seizure at least once in their lifetime. Some individuals who present with an epileptic seizure are diagnosed with epilepsy, while a larger portion does not experience another seizure. The clinician's task here is to identify the group at risk of seizure recurrence and to decide whether treatment should be initiated in appropriate patients[1,2].

Deciding whether to initiate treatment in a patient presenting with their first-ever seizure is one of the most challenging issues in neurological practice. The first step in evaluating this patient group is to obtain a detailed history from the patient and any witnesses to differentiate the event from other possible conditions. Information provided by a witness can be particularly guiding for the clinician. One of the most critical parameters in deciding on treatment is whether the patient has a previous history of seizures. A significant portion of patients, when questioned in detail, report that the current event was not their first, and that they have previously experienced absence or myoclonic seizures. Although epilepsy is a clinical diagnosis, brain magnetic resonance imaging (MRI) and electroencephalography (EEG) are helpful both diagnostically and therapeutically. The clinician must weigh the challenges of long-term therapy—including potential stigmatization, medication side effects, and lifestyle limitations—against the risk of seizure recurrence. Knowing which patients are at higher risk for recurrence makes this difficult decision easier[3,4].

This study aims to identify factors that increase the risk of seizure recurrence in individuals presenting with first-ever seizure.

MATERIAL AND METHODS

This retrospective observational study included 140 patients over the age of 18 who presented to the emergency department or neurology outpatient clinic with a first-ever seizure between January 2022 and June 2023. Outpatient and emergency department records were retrospectively reviewed through the hospital information management system.

Collected data included demographic characteristics (age, sex, comorbidities, medication use), family history of epilepsy, history of head trauma, febrile convulsions, previous syncope, neurological examination findings, presence of convulsions during the event, urinary or fecal incontinence, tongue biting, postictal confusion, ictal crying, and EEG and brain MRI findings.

Patients were contacted by their registered phone numbers, and verbal consent was obtained to inquire about seizure recurrence after the index event. In addition, hospital system records were reviewed to identify subsequent diagnoses such as epilepsy or non-epileptic conditions [(e.g., syncope, hypoglycemia, cerebrovascular disease, psychogenic non-epileptic seizures (PNES)].

At the time of inclusion, all patients had experienced a first-ever unprovoked seizure and had not yet been diagnosed with epilepsy. During follow-up, the diagnosis of epilepsy was determined in accordance with the 2014 International League Against Epilepsy (ILAE) criteria—either after a second unprovoked seizure (recurrence) or after the first seizure if clinical and paraclinical features indicated a high probability (≥60%) of recurrence, such as epileptiform EEG abnormalities or structural brain lesions on MRI. This approach allowed inclusion of both patients with early risk-based epilepsy diagnosis and those in whom the diagnosis was established after recurrence, avoiding circularity in outcome assessment.

The timing of epilepsy diagnosis (immediately after the index seizure vs. after recurrence) could not be retrospectively determined in all cases due to limitations in record documentation.

The mean follow-up period after the first seizure was 14.2±5.6 months (range, 6-24 months). Patients who could not be contacted or had a follow-up duration of less than six months

were excluded from the analysis. Further exclusion criteria included a previous diagnosis of epilepsy, a history of seizures prior to the index event, age under 18 years, refusal to provide verbal consent during the telephone interview, and incomplete data.

The primary endpoint was seizure recurrence, defined as any subsequent unprovoked seizure occurring ≥24 hours after the index event. Recurrence was ascertained independently of diagnostic labels.

Patients with a previously documented seizure or epilepsy diagnosis were excluded to establish a "first-ever seizure" cohort. However, during follow-up, some participants retrospectively reported earlier unrecognized events (e.g., brief auras, nocturnal spells) that had not been medically evaluated at presentation. These cases were classified as "presenting as first-ever seizures," consistent with prior epidemiological studies.

Associations between demographic characteristics, clinical findings, EEG and brain MRI results, and seizure recurrence were evaluated.

Sensitivity and specificity analyses for individual risk factors were not performed because the retrospective design and incomplete temporal documentation could introduce circularity between diagnostic findings and recurrence outcomes. Instead, risk factor associations were assessed descriptively.

Ethical approval for this study was obtained from the Ethics Committee of the University of Health Sciences Bursa Yüksek İhtisas Training and Research Hospital (protocol no: 2011-KAEK-25 2023/06-07, date: 14.06.2023). The study was conducted in accordance with the Declaration of Helsinki.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard deviation, median (range) and/or interquartile range for numerical variables, and as frequency (n) and percentage (%) for categorical variables. The Kolmogorov-Smirnov test was used to assess the normality of the distribution. Levene's test was applied to evaluate the homogeneity of variances. Fisher's exact test was used to analyze associations between categorical variables. All statistical analyses were performed using two-tailed tests, and a p-value<0.05 was considered statistically significant. Results were presented as means ± standard deviations or percentages, as appropriate.

RESULTS

A total of 140 patients were enrolled in the study. The mean age was 56.17±19.45 years, and 74 patients (52.9%) were female. Eight patients (5.7%) had a history of febrile convulsions, four (2.9%) had a family history of epilepsy, 17 (12.1%) had a history of head trauma, and one (0.7%) reported cannabis use. The most common comorbidities were cerebrovascular disease (n=24, 17.1%) and coronary artery disease (n=22, 15.7%). Seventy-four patients (52.9%) were receiving medication for comorbid conditions. A witness was present in 86 cases. Loss of consciousness occurred in 85 patients (60.7%), convulsions in 66 (47.1%), tongue biting in 16 (11.4%), and postictal confusion in 47 (33.6%). Seizure recurrence occurred in 23 patients (16.4%) during the follow-up period

On cranial imaging, 22 patients (15.8%) had periventricular ischemia, 26 (18.6%) had cerebrovascular disease, 11 (7.9%) had intracranial mass lesions, 2 (1.4%) had mesial temporal sclerosis, 4 (2.9%) had hydrocephalus, and 65 (47.8%) had normal findings. Epileptiform abnormalities were detected on EEG in 14 patients. Twenty-three patients (16.4%) were diagnosed with epilepsy, 2 (1.4%) cerebrovasculer disease, and 2 (1.4%) with PNES (Table 1). All patients who ultimately met the diagnostic criteria for epilepsy experienced seizure recurrence during follow-up. This overlap reflected the natural progression of the disorder rather than a diagnostic dependency between recurrence and epilepsy. Among them, 19 were started on levetiracetam, 3 on valproic acid, and 1 on carbamazepine.

Table 1. Clinical and demographic characteristics of the patients

Parameter	Value
Age (years)*	56.17±19.45
Gender#	
Male	66 (47.1%)
Female	74 (52.9%)
Comorbidities#	
Cerebrovascular disease	24 (17.1%)
Coronary artery disease	22 (15.7%)
Chronic obstructive pulmonary disease	14 (10.0%)
Atrial fibrillation	10 (7.1%)
Malignancy	8 (5.7%)
Hypothyroidism	7 (5.0%)
Cannabis use#	1 (0.7%)
Regular medication use for comorbidities#	74 (52.9%)
History of syncope#	39 (27.9%)
History of febrile convulsions#	8 (5.7%)
Family history of epilepsy#	4 (2.9%)
History of head trauma#	17 (12.1%)
Witnessed seizure#	86 (61.4%)
Patients with first seizure as GTCS#	37 (26.4%)
Loss of consciousness	85 (60.7%)
Convulsions	66 (47.1%)

Table 1. Continued	
Parameter	**Value**
Tongue biting	16 (11.4%)
Abrasion/ecchymosis	15 (10.7%)
Symptoms/signs#	
Urinary incontinence	27 (19.3%)
Fecal incontinence	3 (2.1%)
Postictal confusion	47 (33.6%)
Ictal cry	11 (7.9%)
Cranial imaging findings#	
Periventricular ischemia	22 (15.8%)
Cerebrovascular disease	26 (18.6%)
Space-occupying lesion	11 (7.9%)
Mesial temporal sclerosis	2 (1.4%)
Arachnoid cyst	0 (%0)
Hydrocephalus	4 (2.9%)
Normal	65 (47.8%)
Final diagnoses#	
Newly diagnosed epilepsy¹	23 (16.4%)
Conversion disorder	2 (1.4%)
Syncope	7 (5.0%)
Hypoglycemia	2 (1.4%)
Hyperosmolar coma	1 (0.7%)
Cerebrovascular disease	2 (1.4%)
Hepatic encephalopathy	1 (0.7%)
Panic attack	1 (0.7%)
Epileptiform anomaly on EEG	14 (20.3%)
Seizure recurrence	23 (16.4%)

*Mean ± standard deviation, #n (%), ¹Diagnosis established according to ILAE criteria after diagnostic evaluation and follow-up, not solely based on recurrence, GTCS: Generalized tonic-clonic seizure, EEG: Electroencephalography, ILAE: International League Against Epilepsy

All patients diagnosed with epilepsy experienced recurrence during follow-up; however, this finding should be interpreted with caution due to the small sample size.

No statistically significant associations were found between seizure recurrence and sex, comorbidities, family history of epilepsy, history of head trauma, history of febrile convulsions, or brain MRI and EEG findings (Table 2). However, analysis of presenting symptoms and medication use revealed statistically significant associations between seizure recurrence and both lack of regular medication use (p=0.006) and loss of consciousness at presentation (p=0.022). Seizure recurrence was more common among those not using medication and those presenting with loss of consciousness (Table 3).

DISCUSSION

When examining the adult age group, it has been observed that individuals diagnosed with seizures are most frequently between the ages of 30 and 40[5]. This age group is considered one of the most active periods in life in terms of marriage, having children, military service, obtaining a driver's license, and career development. Additionally, the stigmatization of individuals diagnosed with epilepsy and the decrease in their quality of life remain significant issues. Considering the importance of this age group in a person's life, it becomes clearer how crucial it is for the clinician to make a decision regarding the diagnosis of epilepsy and the initiation of treatment in patients presenting with a first and single seizure. In this study, high-risk groups in terms of seizure recurrence were investigated, and data were provided to guide clinicians in deciding whether to initiate treatment. The higher mean age in our cohort (56.2 years) likely reflects the inclusion of patients presenting to the emergency department, where seizure onset in older adults is often secondary to cerebrovascular or metabolic causes.

The mean follow-up period after the first seizure was 14.2±5.6 months (range: 6-24 months). This follow-up duration aligns with prior cohort studies reporting that approximately 53% of seizure recurrences occur within the first 6 months after the initial event, and recurrence risks plateau around 40% by 2 years[6]. Because the study design was retrospective and based primarily on hospital records and telephone interviews, seizure recurrence data may be underestimated, particularly for patients who did not return for follow-up or sought care at other institutions.

Although the prevalence of epilepsy in the general population is around 3%, the lifetime risk of experiencing a single seizure is approximately 8-10%. When a patient presents with a first-time epileptic seizure, the primary step should be establishing an accurate diagnosis. While EEG and brain MRI are used to support the diagnosis, epilepsy remains primarily a clinical diagnosis. Epileptic seizures can sometimes be confused with non-epileptic events. Differential diagnosis is essential to prevent unnecessary long-term use of antiepileptic drugs in individuals presenting with a first seizure. At this stage, the history provided by the patient and any witnesses is extremely important. Witnessed seizures facilitate diagnosis. In outpatient practice, video recordings made with mobile phone cameras are often helpful. The most valuable diagnostic clue is direct observation of the seizure by a neurologist[7].

Although making a diagnosis may seem simple with detailed questioning, this is not always possible in practice. Studies have shown that about 20-30% of adults diagnosed with epilepsy are misdiagnosed. This rate is even higher in patients with

Table 2. Analysis of variables according to seizure recurrence

Variable	Seizure recurrence-Yes, n (%)	Seizure recurrence-Yes, n (%)	Total, n (%)	p-value (Fisher's exact test)
Gender				
Male	8 (12.1)	58 (87.9)	66 (47.1)	>0.05
Female	15 (20.3)	59 (79.7)	74 (52.9)	
Cerebrovascular disease				
Yes	3 (12.5)	21 (87.5)	24 (17.1)	>0.05
No	20 (17.2)	96 (82.8)	116 (82.9)	
Coronary artery disease				
Yes	3 (13.6)	19 (86.4)	22 (15.7)	>0.05
No	20 (16.9)	98 (83.1)	118 (84.3)	
Chronic obstructive pulmonary disease				
Yes	1 (7.1)	13 (92.9)	14 (10.0)	>0.05
No	22 (17.5)	104 (82.5)	126 (90.0)	
Atrial fibrillation				
Yes	2 (20.0)	8 (80.0)	10 (7.1)	>0.05
No	23 (17.7)	107 (82.3)	130 (92.9)	
Malignancy				
Yes	1 (12.5)	7 (87.5)	8 (5.7)	>0.05
No	24 (17.6)	112 (82.4)	136 (94.3)	
Hypothyroidism				
Yes	1 (14.3)	6 (85.7)	7 (5.0)	>0.05
No	24 (17.1)	116 (82.9)	140 (100)	
Cannabis use				
Yes	0 (0%)	1 (100)	1 (0.7)	>0.05
No	25 (17.9)	117 (82.1)	142 (99.3)	
Regular medication use for comorbidities				
Yes	15 (20.3)	59 (79.7)	74 (52.9)	>0.05
No	8 (12.7)	55 (87.3)	63 (45.0)	
Febrile convulsions				
Yes	0 (0%)	8 (100)	8 (5.7)	>0.05
No	25 (18.0)	114 (82.0)	139 (99.3)	
Head trauma history				
Yes	2 (11.8)	15 (88.2)	17 (12.1)	>0.05
No	23 (17.6)	108 (82.4)	131 (87.9)	

Percentages were calculated based on available data for each variable; missing data were not imputed. No correction for multiple comparisons was applied, and results should be interpreted as exploratory

drug-resistant epilepsy. Epileptic seizures may be confused with PNES, syncope, transient ischemic attacks, panic attacks, hypoglycemia, sleep disorders, and similar conditions. Among these, PNES is the most commonly confused condition. In approximately 10% of patients investigated for drug-resistant epilepsy, PNES has been identified. Even in patients diagnosed with status epilepticus, the rate of PNES has been reported as 8.1%, meaning that about 1 in 10 such presentations may actually be PNES. Across all age groups, PNES is most frequently diagnosed in individuals aged 15-29. In this group, the rate of PNES in those diagnosed with status epilepticus can reach up to 20%. PNES is a frequent mimic of epilepsy and remains an important differential diagnosis, particularly in young adults and women. Although only two cases were identified in our cohort, awareness of PNES is essential to prevent misdiagnosis and unnecessary treatment. In our study, we observed that patients presenting to the emergency department with syncope complaints received various diagnoses including epilepsy,

Table 3. Analysis of variables according to seizure recurrence

Variable	Seizure recurrence-Yes, n (%)	Seizure recurrence-No, n (%)	Total, n (%)	p-value (Fisher's exact test)
Drug use				p=0.006
No	16 (27.6)	42 (72.4)	58 (43.9)	
Yes	19 (22.4)	66 (77.6)	85 (56.1)	
Loss of consciousness				p=0.022
No	7 (10.0)	63 (90.0)	70 (50.0)	
Yes	16 (18.8)	69 (81.2)	85 (60.7)	
Convulsion				>0.05
No	9 (13.8)	56 (86.2)	65 (47.1)	
Yes	5 (31.3)	11 (68.8)	16 (11.4)	
Abrasion/ecchymosis				>0.05
No	16 (16.2)	83 (83.8)	99 (70.7)	
Yes	6 (23.1)	20 (76.9)	26 (18.6)	
Tongue biting				>0.05
No	18 (15.3)	100 (84.7)	118 (84.3)	
Yes	4 (25.0)	12 (75.0)	16 (11.4)	
Urinary incontinence				>0.05
No	19 (16.4)	97 (83.6)	116 (82.9)	
Yes	4 (14.8)	23 (85.2)	27 (19.3)	
Fecal incontinence				>0.05
No	23 (17.2)	110 (82.8)	133 (97.5)	
Yes	0 (0.0)	3 (100)	3 (2.5)	
Postictal confusion				>0.05
No	13 (16.0)	68 (84.0)	81 (57.9)	
Yes	10 (21.3)	37 (78.7)	47 (33.6)	
Ictal cry				>0.05
No	21 (17.6)	98 (82.4)	119 (85.0)	
Yes	2 (18.2)	9 (81.8)	11 (7.9)	
Witnessed seizure				>0.05
No	5 (15.6)	27 (84.4)	32 (22.9)	
Yes	18 (20.9)	68 (79.1)	86 (61.4)	

Percentages were calculated based on available data for each variable; missing data were not imputed. No correction for multiple comparisons was applied, and results should be interpreted as exploratory. Percentages may not sum to column totals due to missing data.

cerebrovascular disease, PNES, panic attacks, hyperosmolar coma, hypoglycemia, and hepatic encephalopathy. The key point is that while epilepsy should always be considered in patients presenting with syncope, other potential causes must not be overlooked[8-11].

Upon questioning patients presenting with a first seizure, it was found that approximately 41% had a previous seizure history; among them, half had experienced five or more seizures. In about 28% of this group, convulsive seizures were identified upon further questioning. Initial events that were non-convulsive, involved behavioral changes, or occurred in individuals with lower socioeconomic status were associated with delayed medical attention[12]. In our study, among patients presenting to the emergency department with a first-time complaint of syncope, 35.8% were found—upon more detailed questioning by the clinician—to have a history of prior syncope. A critical point to remember is that without detailed questioning, patients often do not report a prior history of seizures. This is especially true for seizures presenting as absence or myoclonic types, which represent higher-risk groups in this context. A history of prior seizures plays a decisive role in the decision to initiate antiepileptic treatment. Therefore, questioning both patients and their relatives in this regard is essential in neurological practice and should not be overlooked. Although we aimed to include only patients with first-ever seizures, retrospective interviews during follow-up revealed that 35.8%

reported possible prior unrecognized episodes. Similar findings have been reported in previous studies[12]. This highlights the difficulty of distinguishing truly first-ever seizures from cases presenting as first events, particularly in retrospective designs and emergency-based cohorts. We acknowledge this as a limitation that may introduce baseline heterogeneity.

In a large-scale study involving individuals experiencing a first seizure, 33.6% of those diagnosed with epilepsy had epileptiform abnormalities on EEG, and 49.3% had pathological findings on brain MRI[13]. In our study, 14 out of 23 patients (60.9%) diagnosed with epilepsy showed epileptiform abnormalities on EEG. While 47.8% of the patients had normal brain MRI findings, others exhibited findings such as periventricular ischemia, hydrocephalus, and space-occupying lesions. The presence of epileptiform abnormalities on EEG has been shown to be the most significant predictor for seizure recurrence after an acute symptomatic seizure. Moreover, patients with abnormal brain imaging and epileptiform EEG findings are known to have a high risk of seizure recurrence[14]. In our study, however, we did not find a statistically significant relationship between brain MRI or EEG findings and seizure recurrence.

A central methodological concern in first-seizure research is the potential conflation of the diagnostic label with the outcome. In our study, seizure recurrence was the prespecified primary endpoint, whereas the diagnosis of epilepsy followed an ILAE-based, multifactorial clinical assessment (history/semiology, etiologic context, EEG, and MRI). To avoid circularity, we clarify that recurrence itself was not used to establish the diagnosis. Nevertheless, because some patients who ultimately fulfilled ILAE criteria later experienced recurrence, an apparent overlap emerged between the diagnostic label and the outcome. This overlap reflects the high pre-test probability embedded in ILAE criteria (e.g., one unprovoked seizure with >60% predicted risk of further seizures) rather than incorporation of the outcome into the diagnosis. In the revised Results, we now report the timing of diagnostic decisions relative to recurrence and provide subgroup summaries to make this separation explicit.

In our study, all patients diagnosed with epilepsy after the first seizure experienced recurrence during follow-up. This rate, although higher than typically reported (50-60%), may reflect the selection of a high-risk subgroup with concurrent EEG and MRI abnormalities and the small sample size of the cohort.

The absence of a statistically significant association between EEG or MRI findings and seizure recurrence in our cohort may have several explanations. First, the timing of EEG and MRI was variable, and in many cases, EEG was not obtained within the first 24-48 hours, when epileptiform abnormalities are most likely to be detected. Second, some lesions observed on MRI may not be epileptogenic, whereas patients with normal imaging may still face a high risk of recurrence due to subtle cortical abnormalities not visible on conventional MRI[15,16]. Third, the relatively small sample size of our study may have limited the statistical power to detect such associations. Previous studies have shown that performing EEG within 16 hours after a seizure identifies epileptiform discharges in more than 50% of cases, while delays beyond this period markedly reduce diagnostic yield[17].

Evidence also indicates that seizure recurrence risk is highest in the early period, with approximately 27% of patients experiencing a second seizure within 6 months, 36% within 1 year, and 43% within 2 years[18]. Thus, our mean follow-up of 14 months likely captured a substantial proportion of recurrence events, although the limited follow-up period may have prevented identification of longer-term associations.

In our study, seizure recurrence was significantly associated with loss of consciousness at the initial event and with the absence of regular medication use for comorbid conditions. The first finding aligns with previous literature, as loss of consciousness often reflects a generalized seizure or impaired awareness, both of which are linked to a higher risk of recurrence[19]. The second finding may seem unexpected, since some drugs are known to lower the seizure threshold. In our cohort, however, "medication use" referred to the ongoing treatment of chronic comorbidities such as hypertension, diabetes mellitus, or cardiovascular disease, rather than to antiseizure therapy. Adherence to such treatments often entails more frequent healthcare contact, which may allow earlier recognition and management of underlying risk factors, thereby reducing recurrence risk[20]. By contrast, the absence of regular follow-up may result in unrecognized conditions or poorer overall health control, indirectly contributing to higher recurrence rates.

After establishing an accurate diagnosis, the next step is to decide whether to initiate antiepileptic treatment. The general consensus among clinicians is to refrain from starting therapy after a first single seizure, unless specific risk factors are present. In selected patients with epileptiform EEG abnormalities or structural brain lesions on MRI, however, early treatment may be justified.

The risk of seizure recurrence is highest within the first two years. Patients with neurological deficits, abnormal neuroimaging, epileptiform EEG findings, nocturnal seizures, or seizures related to prior brain injury are at increased risk. In contrast, individuals with no structural abnormalities on MRI and no epileptiform activity on EEG have an estimated recurrence risk of only 10-20%, and in such cases observation without immediate treatment is generally recommended[14].

For acute symptomatic seizures, the two-year recurrence risk has been reported as approximately 32%[21]. When considering antiepileptic therapy after a first event, clinicians must balance

the potential benefits of reducing recurrence against the risks of adverse effects and the burden of long-term treatment. Knowledge of recurrence rates and the identification of high-risk patient subgroups are therefore essential to guide clinical decision-making and support individualized management strategies.

Study Limitations

This study has several limitations. First, the relatively higher mean age of our cohort compared with population-based studies likely reflects the recruitment of older patients from an emergency-department setting. The retrospective design carries inherent risks of incomplete data, as information was obtained from medical records and telephone interviews. Patients lost to follow-up or treated elsewhere may have experienced unrecorded recurrences, leading to underestimation of recurrence rates. Second, the mean follow-up of 14 months may not have captured late recurrences occurring beyond two years after the initial seizure. Third, EEG and brain MRI were not consistently performed within optimal time frames; delayed EEGs and non-epileptogenic MRI lesions may have limited detection of relevant abnormalities.

Although recurrence was not used as a diagnostic criterion, all patients diagnosed with epilepsy experienced recurrence, which may have created an apparent association between the two. Some participants later reported previously unrecognized events, blurring the distinction between truly first-ever and first-presenting seizures. Moreover, "medication use" referred to chronic comorbidity treatment rather than antiseizure therapy, which may limit generalizability. Finally, the relatively small sample size reduced statistical power, particularly for subgroup analyses. Larger, prospective studies with standardized diagnostic timing and longer follow-up are needed to validate these results.

CONCLUSION

In this retrospective cohort of adults presenting with a first-ever seizure, seizure recurrence occurred in a substantial proportion of patients within the first year of follow-up. Loss of consciousness at presentation and the absence of regular medication use for comorbid conditions were significantly associated with recurrence. These findings underscore the importance of meticulous history-taking, including witness accounts, in the initial evaluation of first-ever seizures. Recognition of clinical features suggestive of higher recurrence risk may aid clinicians in individualized decision-making regarding follow-up intensity and treatment initiation. Regular healthcare contact and comprehensive management of comorbidities may also play a protective role and should be considered as part of holistic patient care.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ethics Committee of the University of Health Sciences Bursa Yüksek İhtisas Training and Research Hospital (protocol number: 2011-KAEK-25 2023/06-07, date: 14.06.2023).

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

Footnotes

Authorship Contributions

Concept: N.B.P., D.Y., B.S., M.Y., Design: N.B.P., D.Y., B.S., M.Y., Data Collection or Processing: N.B.P., D.Y., B.S., M.Y., Analysis or Interpretation: N.B.P., D.Y., M.Y., Writing: N.B.P., D.Y., B.S.

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ORIGINAL ARTICLE

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Cross Platform Blood Transcriptomics Identifies a Two-*gene* Classifier for Coronary Artery Disease Detection

Koroner Arter Hastalığının Saptanmasında Platformlar Arası Kan Transkriptomi Analiziyle İki Genli Bir Sınıflandırıcının Belirlenmesi

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ABSTRACT

Aim: Coronary artery disease (CAD) remains a major global health burden, and currently available blood biomarkers lack sensitivity for early detection. This study aimed to identify and validate circulating mRNA and long non-coding RNA (lncRNA) biomarkers of CAD and to develop a predictive transcriptomic model.

Materials and Methods: We analyzed plasma RNA-seq data from stable CAD patients and healthy controls (*GSE208194*) and validated findings in an independent peripheral blood microarray cohort (*GSE113079*). Differential expression was assessed using a threshold of $|\log^2$ fold-change$| \geq 1$ and false-discovery rate <0.05. Enrichment analysis of gene ontology and Kyoto Encyclopedia of Genes and Genomes pathways was performed. A predictive model was constructed using logistic regression model-penalized logistic regression, with hyperparameters tuned within a nested cross-validation framework, and evaluated in the independent validation cohort.

Results: A total of 182 transcripts (177 mRNAs, 5 lncRNAs) were differentially expressed, with 91% down-regulated in CAD. Enrichment analysis revealed coordinated dysregulation of ribosomal biogenesis, cytoplasmic translation, mitochondrial oxidative phosphorylation, and p53/NF-κB inflammatory pathways. Cross-platform validation confirmed 85 transcripts, indicating a robust expression signature. The predictive model selected two genes, *NEUROD2* and *RPS27*, achieving an external area under the curve of 0.820 in the validation cohort.

Conclusion: Blood transcriptomic profiling identifies a reproducible CAD-associated expression signature and supports a concise two-gene classifier reflecting inflammatory and metabolic stress-related pathways. These findings provide a framework for the further development of blood-based transcriptomic assays and warrant validation in larger, diverse populations to define clinical utility in cardiovascular risk assessment.

Keywords: Coronary artery disease, transcriptome, biomarkers, RNA, logistic models

ÖZ

Amaç: Koroner arter hastalığı (KAH) küresel ölçekte önemli bir sağlık yükü olmaya devam etmektedir ve mevcut kan biyobelirteçleri erken tanı için yeterli duyarlılığa sahip değildir. Bu çalışmanın amacı, KAH ile ilişkili dolaşımdaki mRNA ve uzun kodlamayan RNA (lncRNA) biyobelirteçlerini tanımlamak ve doğrulamak, ayrıca öngörücü bir transkriptomik model geliştirmektir.

Gereç ve Yöntem: Stabil KAH hastaları ve sağlıklı kontrollerden elde edilen plazma RNA-sekanslama verileri (*GSE208194*) analiz edilmiş ve bulgular bağımsız bir periferik kan mikrodizi kohortunda (*GSE113079*) doğrulanmıştır. Diferansiyel gen ekspresyonu $|\ |\log^2$ kat değişimi$| \geq 1$ ve yanlış keşif oranı $<0,05$ eşikleri kullanılarak değerlendirilmiştir. Gen ontolojisi ve Kyoto Genler ve Genomlar Ansiklopedisi yolakları için zenginleştirme analizleri yapılmıştır. Öngörücü model, lojistik regresyon modeli ile cezalandırılmış lojistik regresyon kullanılarak oluşturulmuş, hiperparametreler iç içe çapraz doğrulama çerçevesinde ayarlanmış ve bağımsız doğrulama kohortunda değerlendirilmiştir.

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Bulgular: Toplam 182 transkript (177 mRNA, 5 lncRNA) diferansiyel olarak eksprese bulunmuş ve bunların %91'i KAH'de aşağı regüle edilmiştir. Zenginleştirme analizleri ribozomal biyogenez, sitoplazmik translasyon, mitokondriyal oksidatif fosforilasyon ve p53/NF-κB enflamatuvar yolaklarında eşgüdümlü düzensizlikleri ortaya koymuştur. Platformlar arası doğrulama 85 transkripti teyit ederek sağlam bir ekspresyon imzasına işaret etmiştir. Öngörücü model iki geni (*NEUROD2* ve *RPS27*) seçmiş ve doğrulama kohortunda 0,820'lik harici eğri altında kalan alan değerine ulaşmıştır.

Sonuç: Kan transkriptomik profillemesi, KAH ile ilişkili, tekrarlanabilir bir gen ekspresyon imzası ortaya koymakta ve enflamasyon ile metabolik stres süreçlerini yansıtan iki genli, yalın bir sınıflandırıcının kullanılabileceğini göstermektedir. Bu bulgular, kan temelli transkriptomik biyobelirteçlerin geliştirilmesine yönelik önemli bir temel sağlamaktadır. Ancak klinik uygulamadaki değerinin ortaya konabilmesi için daha geniş ve farklı özelliklere sahip hasta popülasyonlarında doğrulanması gerekmektedir.

Anahtar Kelimeler: Koroner arter hastalığı, transkriptom, biyobelirteçler, RNA, lojistik modeller

INTRODUCTION

Coronary artery disease (CAD) remains a major global health problem, responsible for high morbidity and mortality despite preventive and therapeutic advances. Cardiovascular diseases cause over 20 million deaths annually[1] with ischemic heart disease alone accounting for about 9 million[2]. Despite decades of advances in prevention and therapy, CAD continues to be a leading cause of morbidity and mortality globally[3]. CAD arises from atherosclerosis, a chronic inflammatory process triggered by lipid deposition, endothelial dysfunction, and immune activation[4]. These mechanisms, including vascular inflammation and thrombosis, drive its clinical manifestations. However, many individuals harbor subclinical CAD undetected until acute coronary events ocur[5].

Current blood-based biomarkers mainly reflect risk factors or myocardial injury rather than subclinical atherosclerosis. Low-density lipoprotein cholesterol and other lipids miss many at-risk patients[4], while C-reactive protein is nonspecific and troponins rise only after myocardial damage[6]. Imaging techniques like coronary computed tomography (CT) or angiography offer definitive diagnosis but are costly or invasive[7]. Hence, new non-invasive biomarkers reflecting early pathobiological changes are needed.

Transcriptomic profiling enables genome-wide assessment of mRNA and non-coding RNA expression in blood, capturing systemic molecular alterations[8]. Studies have shown distinct blood gene-expression signatures in CAD. The 23-gene Corus CAD score accurately predicted obstructive CAD and adverse outcomes in validation cohorts[9-11]. Long non-coding RNAs (lncRNAs) also regulate lipid metabolism and inflammation and are stable in circulation[4,5,12]. For example, *ANRIL* at the chromosome 9p21 locus links genetic risk to CAD[13], while lncRNAs such as *H19*, *MIAT*, and *MALAT1* are elevated in acute myocardial infarction[14,15].

Nonetheless, most studies lack replication, cross-platform validation, or inclusion of non-coding RNAs. Many derive from single cohorts with limited generalizability[7,8]. The Corus score included only mRNAs and excluded diabetics[9,11], while newer efforts only recently began profiling lncRNAs systematically[16]. Moreover, predictive modeling translating transcriptomic data into clinical tools remains rare, with few classifiers validated beyond the Corus model[9,11,17].

This study aims to identify and validate blood-based mRNA and lncRNA biomarkers for CAD to build a robust diagnostic classifier. By integrating both RNA types and validating across independent populations, it seeks a comprehensive transcriptomic signature to enhance early, noninvasive CAD detection and risk assessment.

MATERIALS AND METHODS

Datasets

This study was a retrospective bioinformatics analysis of two publicly available GEO transcriptomic datasets on CAD. The discovery dataset, *GSE208194*, comprised RNA-seq data from circulating cell-free RNA (cfRNA) in plasma from 59 patients with stable CAD (4 months post-acute event) and 30 healthy controls, sequenced on an Illumina NovaSeq 6000. The validation dataset, *GSE113079*, was a microarray-based transcriptome of PBMCs from 93 CAD patients and 48 controls (Agilent Human lncRNA+mRNA array v4.0), covering genome-wide mRNA and lncRNA expression. *GSE208194* reflects circulating RNA released from tissues, whereas *GSE113079* captures immune-cell transcript levels, providing an independent cohort and platform for validation.

Data Acquisition and Preprocessing

All data were obtained from GEO using the GEOquery package (v2.66.0) in R.[18] for the discovery RNA-seq dataset (*GSE208194*), we downloaded the processed gene-level expression matrix provided by the data submitters, reported as transcripts per million (TPM). While raw sequencing reads for this dataset are available via the Sequence Read Archive (SRA), a gene-level raw count matrix is not included in the GEO Series record. As this study represents a secondary analysis of publicly available data, downstream analyses were therefore conducted using the supplied TPM-based expression matrix rather than reprocessing raw FASTQ files.

Gene identifiers were mapped from Ensembl IDs to official gene symbols using GENCODE v33 human gene annotation[19]. Analyses were restricted to protein-coding genes and annotated lncRNAs. To ensure robust detection and reduce noise from low-abundance transcripts, features with TPM ≥1 in at least 90% of samples were retained. Remaining TPM values were log-transformed [$\log^2$ (TPM + 1)] to stabilize variance. Quality control was performed by inspection of sample-level expression distributions and principal component analysis (PCA).

For the validation dataset *GSE113079* (Agilent one-color microarray), we downloaded the processed expression matrix provided by the submitters, consisting of background-corrected, $\log^2$-transformed probe intensities. As described in the original publication[20], the arrays were quantile-normalized in GeneSpring GX prior to submission. Probe identifiers were mapped to gene symbols using the platform annotation file (*GPL20115*). Analyses were restricted to genes represented in both datasets, enabling direct cross-platform validation. All microarray samples (93 CAD, 48 control) passed quality control in the original study and were retained for downstream analyses.

Differential Expression Analysis

In the discovery plasma RNA-seq dataset (*GSE208194*), differential expression between CAD and control groups was analyzed using the limma package (v3.62.2)[21]. The analysis was performed on $\log^2$ (TPM + 1)–transformed gene-level expression values, with disease status (CAD vs. control) specified as the primary model term. Because all samples were generated within a single sequencing run and no additional technical covariates were reported in the GEO record, no further adjustment variables were included in the linear model.

For each gene, limma's linear modeling framework with empirical Bayes moderation was applied to obtain moderated log-fold changes and associated statistics, improving variance estimation and statistical stability. Genes were considered differentially expressed if they met both an absolute $\log^2$ fold-change threshold of ≥1.0 and a Benjamini–Hochberg false-discovery rate (FDR) <0.05, ensuring selection of transcripts with both statistical support and biologically meaningful effect sizes. The resulting differentially expressed genes (DEG) were retained as candidate circulating RNA biomarkers for CAD. Volcano plots were used to visualize the overall distribution of effect sizes and significance levels and to verify that the selected thresholds captured the most prominent mRNA and lncRNA signals.

Validation in Independent Cohort

To validate the RNA-seq findings, we analyzed the *GSE113079* PBMC microarray dataset as an independent cohort. A similar differential expression analysis was performed using limma on the microarray gene expression matrix. For each gene, a linear model was fitted with group (CAD vs. control) as the contrast of interest, followed by empirical Bayes moderation to obtain moderated statistics. The larger sample size and normalization of the original dataset helped minimize potential batch effects, which were further checked using PCA plots showing no evident batch structure. Differential expression was defined by an FDR <0.05, consistent with the RNA-seq criteria. Genes were considered validated if they were significantly differentially expressed in the microarray cohort (FDR <0.05) with a concordant direction of change between datasets. This cross-platform validation ensured that identified mRNA and lncRNA biomarkers were reproducible across independent cohorts and technologies. Genes not represented on the microarray (e.g., certain lncRNAs) were excluded, and the validated gene set was used for subsequent analyses. In addition to statistical significance and concordant direction of effect, cross-platform effect-size concordance was quantified by calculating Pearson correlations of $\log^2$ fold-change estimates between the discovery RNA-seq and validation microarray datasets. Correlations were computed separately for (i) the validated gene set and (ii) the full set of discovery DEG that were represented on the microarray platform.

Functional Enrichment Analysis

To interpret the biological significance of the DEG, we performed gene ontology (GO) and pathway enrichment analyses using the clusterProfiler package (v4.14.6)[22]. Significantly up- and down-regulated genes were tested for enrichment in GO biological process (BP), molecular function, cellular component, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway categories. Enrichment was assessed using a one-tailed hypergeometric test, with the background defined as all genes expressed above the filtering threshold in the RNA-seq dataset to control for detection bias. Multiple testing correction was applied using the Benjamini-Hochberg procedure, and terms with FDR <0.05 were considered significant. Results were summarized with enrichment fold changes (gene ratios) and adjusted p-values. Visualization of the top enriched GO terms and KEGG pathways was done using clusterProfiler (v4.16.0)[23]. Since lncRNAs are not directly annotated to GO/KEGG terms, enrichment primarily reflected the differentially expressed mRNAs. The results were interpreted in the context of CAD-related processes, including ribosomal biogenesis, oxidative phosphorylation, RNA processing, and DNA damage response.

Predictive Modeling

Using the discovery plasma RNA-seq dataset, we built a predictive model to test whether the identified transcriptomic biomarkers could distinguish CAD patients from controls.

An L1-penalized logistic regression model (LASSO) was applied, suitable for high-dimensional data and automatic variable selection by shrinking some coefficients to zero. To prevent feature-selection leakage, differential expression analysis was not performed globally prior to modeling. Instead, DEG identification was repeated independently within each training fold during cross-validation, and only fold-specific DEGs were used as candidate predictors. Expression data for candidate biomarkers were standardized to zero mean and unit variance within each training fold to ensure strict separation between training and assessment data. The binary outcome was CAD status. All modeling steps were implemented in R using the fastml[24] framework, which enforces guarded resampling and leakage-safe workflows, together with glmnet[25] for penalized regression.

We employed a nested cross-validation design, consisting of an outer stratified 10-fold cross-validation loop for unbiased performance estimation and an inner repeated 10-fold cross-validation (5 repeats) loop for hyperparameter tuning. Within each outer training fold, a grid search across α (0-1, step 0.05) and λ values was conducted to identify the optimal penalty parameters. This ensured that DEG selection, preprocessing, and hyperparameter optimization were confined entirely to training data in each resampling iteration. The optimal model consistently corresponded to $\alpha = 1$, indicating pure LASSO regularization.

Model discrimination was evaluated using ROC curves and area under the curves (AUCs) computed on held-out outer test folds using the pROC package[26]. Overall cross-validated performance was summarized by pooling predictions from all outer test folds, and 95% confidence intervals (CIs) were obtained using DeLong's method. The optimal decision threshold was determined by maximizing Youden's J statistic, from which sensitivity, specificity, accuracy, and predictive values were calculated.

To assess biomarker stability, we performed 1,000 bootstrap resamples of the discovery dataset, refitting the final leakage-corrected LASSO model for each resample using the optimal α and λ. The frequency with which each gene retained a nonzero coefficient was recorded to quantify selection stability. Features appearing in more than 50% of resamples were considered robust predictors. This stability analysis was conducted independently of cross-validation to characterize feature robustness rather than predictive performance. All modeling procedures adhered to TRIPOD guidelines[27], with explicit safeguards against data leakage at all stages of feature selection, model tuning, and evaluation.

Statistical Analysis

All data processing and analyses were performed in R (version 4.4.2). Throughout the analysis, reproducibility and transparency were maintained by setting random seeds for cross-validation (to ensure consistent partitioning) and by documenting all code steps. All statistical tests were two-tailed, and a significance level of 0.05 was used unless otherwise specified. The Methods were written adhering to STROBE[28] and REMARK[29] guidelines for observational transcriptomic analyses and biomarker evaluations, ensuring that the study can be replicated and built upon by other researchers.

RESULTS

Unsupervised Clustering of Samples

PCA of the discovery data (*GSE208194*) revealed distinct clustering of CAD patients versus controls. In the Figure 1A, PC1 (32 %) and PC2 (15 %) together capture roughly 47 % of the total variance. Most CAD samples cluster on the negative side of PC1, whereas control samples are enriched on the positive side, producing a clear but not complete group separation. The overlap near the PC1 origin indicates some heterogeneity within both cohorts, yet the overall pattern supports the existence of a disease-associated blood-transcriptomic signature. Similarly, a Uniform Manifold Approximation and Projection (UMAP) analysis reinforced the clustering pattern (Figure 1B). Most CAD samples project to the lower-left sector of the two-dimensional map, whereas control samples are enriched in the upper-right, producing two loose aggregations along the UMAP-1 axis. Although a noticeable subset of samples from each group intermingle in the central region, the overall distribution points to systematic transcriptomic differences between CAD and controls, justifying downstream differential-expression analyses.

Differentially Expressed mRNAs

Using limma moderated analysis, we identified a robust set of genes that were significantly differentially expressed between CAD patients and controls (Table S1). In total, 182 transcripts met the significance criteria ($|\log^{FC}| \geq 1$ and p-adjusted <0.05), of which 177 were protein-coding mRNAs and 5 were lncRNAs. Among these 182 DEGs, the majority (approximately 91%) showed lower expression in CAD relative to controls, while about 9% were up-regulated in CAD. In other words, 166 genes were down-regulated and 16 were up-regulated in CAD patients (Figure 2). This overall trend of suppressed gene expression in CAD blood is consistent with previous transcriptomic studies of CAD patients[7].

In our transcriptome analysis of CAD samples, the most strongly upregulated transcripts were mitochondrial-encoded oxidative phosphorylation genes. For example, *MT-ND1* (NADH dehydrogenase subunit 1) was markedly induced ($\log^{FC} = 1.712$, p-adjusted <0.001), as were *MT-ND6* ($\log^{FC} = 1.661$, p-adjusted <0.001), *MT-ND5* ($\log^{FC} = 1.624$, p-adjusted <0.001) and *MT-ND3*

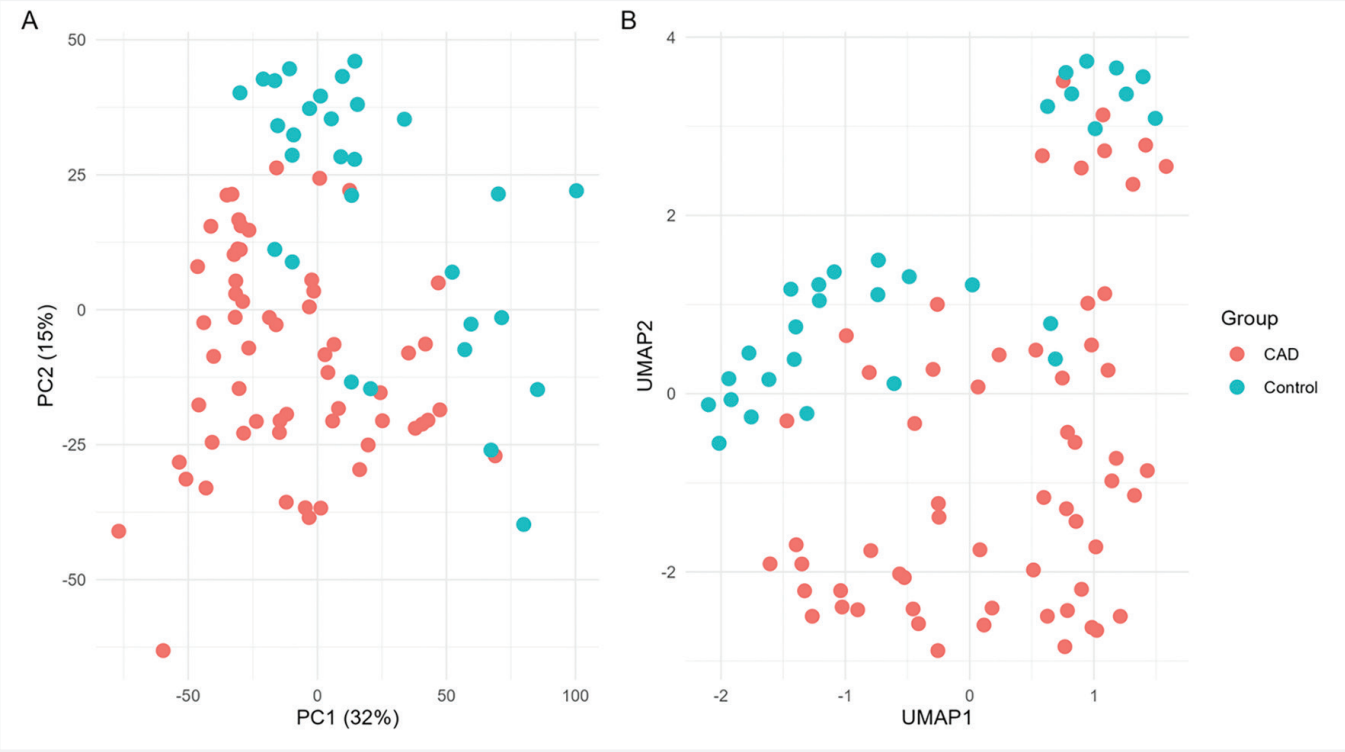

Figure 1. Unsupervised dimensionality-reduction of whole-blood transcriptomes from CAD patients and healthy controls (A) PCA, (B) UMAP
CAD: Coronary artery disease, PCA: Principal-component analysis, UMAP: Uniform Manifold Approximation and Projection

(log^{FC} =1.604, p-adjusted <0.001). These genes encode core subunits of mitochondrial Complex I (NADH dehydrogenase)[6]. Complex I is an essential component of the electron transport chain for adenosine triphosphate (ATP) production, and its dysfunction is known to increase mitochondrial reactive oxygen species and trigger inflammation and vascular remodeling in atherosclerotic cardiovascular disease[30]. Thus, the coordinated upregulation of multiple Complex I subunit genes in CAD samples may reflect altered mitochondrial activity. In support of this, other mitochondrial transcripts (e.g. *MT-CYB* for Complex III and *MT-CO2/MT-CO3* for Complex IV) were also modestly elevated. Overall, these results suggest that CAD is associated with enhanced expression of respiratory chain components, potentially as a compensatory response or in relation to increased oxidative stress in disease.

Conversely, several genes involved in mitochondrial biogenesis and function were among the most downregulated. Notably, *NDUFAF2* (a Complex I assembly factor) was significantly decreased (log^{FC} =-1.275, p-adjusted <0.001), as was *COA6* (a cytochrome c oxidase/Complex IV assembly factor; log^{FC} =-1.325, p-adjusted <0.001) and *TOMM5* (a subunit of the translocase of outer mitochondrial membrane; log^{FC} =-1.231, p-adjusted <0.001). Loss of *NDUFAF2* is known to impair Complex I assembly and cause mitochondrial dysfunction with increased oxidative stress[31], which can exacerbate endothelial injury and inflammation. Reduced *COA6* levels would likely hinder Complex IV maturation, further compromising electron transport. In addition, the endogenous retroviral envelope gene *ERV3-1* was strongly suppressed (log^{FC} =-1.485, p-adjusted <0.001); *ERV3* has been implicated in immune regulation (including autoimmunity)[32], so its downregulation might reflect altered innate immune signaling in CAD. Together, the downregulated genes indicate a pattern of mitochondrial compromise, with disrupted assembly of multiple respiratory complexes and import machinery, which is consistent with theories that mitochondrial dysfunction and resultant reactive oxygen species contribute to CAD pathogenesis[30,31].

Differentially Expressed lncRNAs

In addition to mRNAs, five lncRNAs showed significant differential expression between CAD and controls ($|log^{FC}| \geq 1$, FDR <0.05): *LINC03031*, *CCDC26*, *MAST4-AS1*, *MIR663AHG*, and *PCED1B-AS1*. *LINC03031* was upregulated (log^{FC} =1.182, p<0.001) and *MAST4-AS1* downregulated (log^{FC} =-1.361, p<0.001), representing novel, previously unreported candidates in cardiovascular disease. *CCDC26* was upregulated (log^{FC} =1.094, FDR <0.001) and has been identified as an oncogenic lncRNA[33] and one of the most elevated plasma lncRNAs in CAD[34] possibly

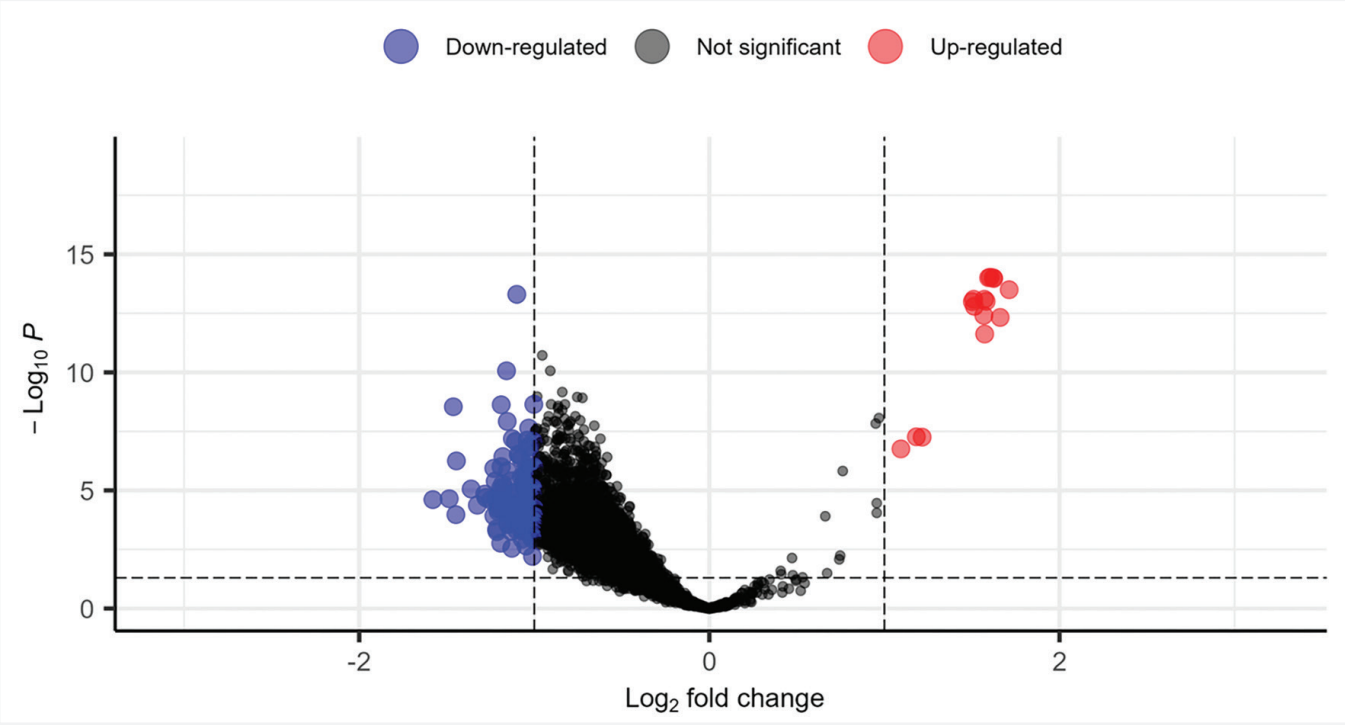

Figure 2. Volcano plot summarizing DEGs between CAD and control groups. The plot highlights the significant DEGs (|logFC | ≥1 and p-adjusted <0.05) in color, with the horizontal axis showing logFC (CAD/Control) and the vertical axis showing -log10(P). Notably, the distribution of points underscores the predominance of down-regulated genes in CAD (left side) compared to up-regulated genes (right side)

DEGs: Differentially expressed genes, CAD: Coronary artery disease

influencing vascular proliferation and inflammation. *PCED1B-AS1* was downregulated (log^{FC} =-1.072, p<0.001); known to promote cell proliferation via MAPK signaling[35], its reduction here may reflect suppressed growth signaling. *MIR663AHG* was also downregulated (log^{FC} =-1.579, p<0.001). As the host gene of miR-663a, which exerts anti-inflammatory effects[36], *MIR663AHG* repression may reduce vascular protection and favor pro-atherogenic signaling.[37] Collectively, these lncRNAs, particularly upregulated *CCDC26* and downregulated *PCED1B-AS1* and *MIR663AHG*, highlight biological pathways related to proliferation, hypoxia, and inflammation. Their stability and detectability in blood suggest value as noninvasive biomarkers for CAD detection, risk stratification, and therapy monitoring. Further validation and mechanistic studies are warranted to clarify their functional roles in atherosclerosis.

Pathway- and Gene Ontology Enrichment of DEGs

To identify biological pathways altered in CAD, we performed KEGG and GO enrichment analyses on DEGs (Figure 3). Seventeen KEGG pathways passed the 5% FDR threshold (Figure 4A). The top hit was "Ribosome" (hsa03010; 32/102 DEGs, p<0.001), followed by "coronavirus disease-19" and "thermogenesis," both sharing ribosomal genes. Mitochondrial pathways, including oxidative phosphorylation (15/102 genes, p<0.001) and diabetic cardiomyopathy, were also enriched, indicating up-regulated respiratory-chain activity (e.g., *NDUFA9, ATP5F1B*). Nucleotide excision repair, spliceosome, and RNA polymerase modules showed similar enrichment, suggesting intensified nucleic-acid maintenance. GO, BP confirmed enrichment in cytoplasmic translation, ribosome biogenesis, oxidative phosphorylation, and ATP synthesis (all p<0.001). p53-mediated apoptotic signaling further suggested DNA-damage responses. Cellular-component terms were dominated by ribosomal subunits and mitochondrial complexes I, IV, and ATP synthase, while spliceosomal complexes (U1, U2, U4/U6-U5) pointed to enhanced post-transcriptional activity. The top molecular-function term was "structural constituent of ribosome" (34/157 DEGs, p<0.001), followed by transporter and RNA-binding activities. These enrichments indicate a metabolic-inflammatory shift in CAD blood, characterized by heightened protein synthesis, mitochondrial energy production, and RNA processing, consistent with chronic immune activation and oxidative stress in atherosclerosis. Ribosomal and mitochondrial pathways are thereby highlighted as potential therapeutic targets.

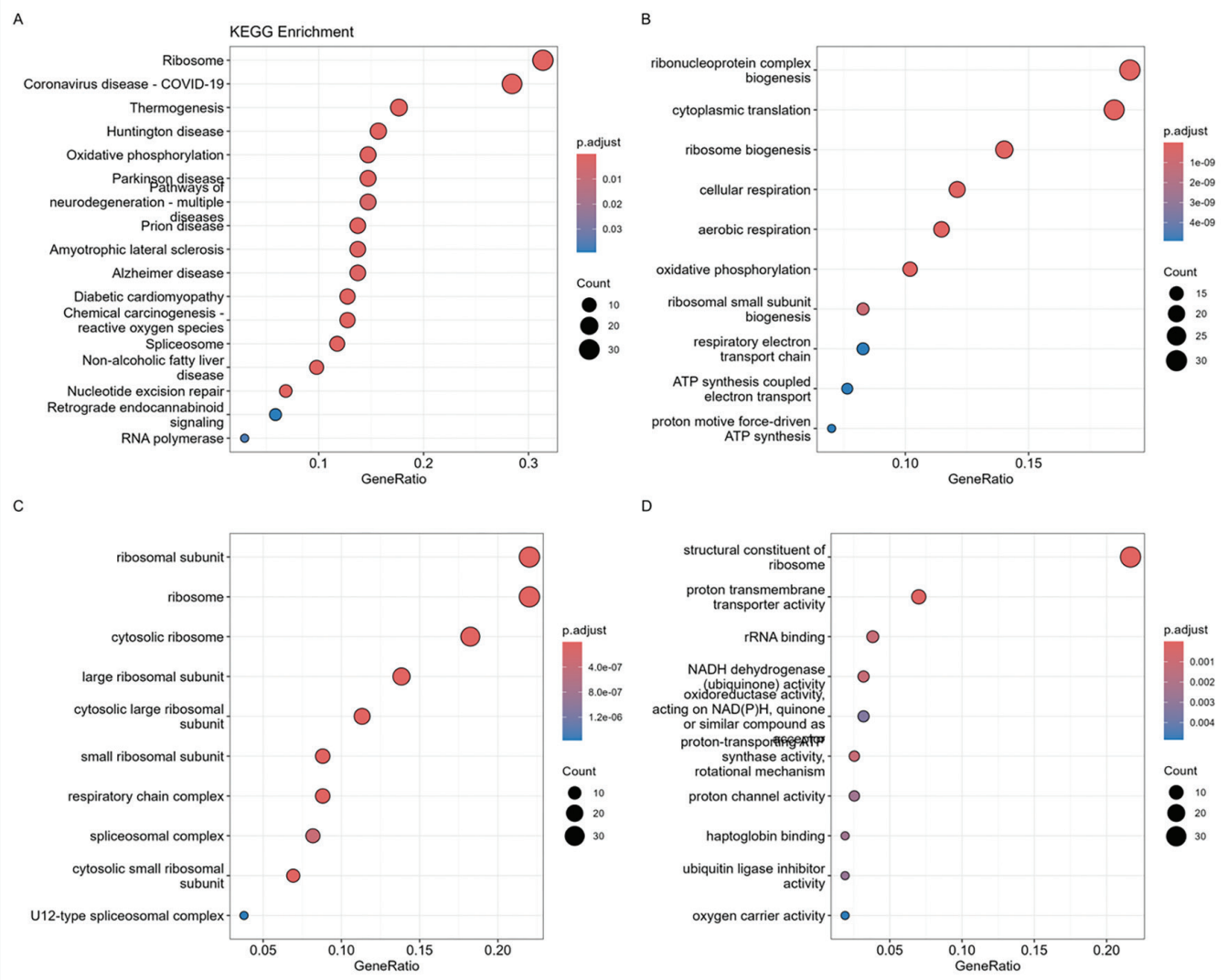

Figure 3. Functional enrichment of CAD-associated transcripts. Dot-plots display the top enriched terms for A) KEGG pathways, B) Gene ontology-biological process, C) Gene ontology-cellular component and D) Gene ontology-molecular function

CAD: Coronary artery disease, KEGG: Kyoto Encyclopedia of Genes and Genomes, ATP: Adenosine triphosphate

Validation Phase Results

In the validation phase, 85 genes were confirmed as differentially expressed, supporting the discovery-phase findings (Table S2). Effect-size concordance across platforms was quantified by correlating $\log^{FC}$ estimates between the discovery RNA-seq and validation microarray cohorts. For the validated genes (n=85; p-adjusted <0.05 in the validation cohort with concordant direction), effect sizes were strongly correlated (r=0.671; 95% CI 0.535-0.774; p<0.001). In contrast, when considering the full set of discovery DEGs represented on the microarray platform (n=1116), cross-platform correlation was weak and not statistically significant (r=0.0368; 95% CI -0.0219 to 0.0953; p=0.219), indicating that reproducible effect-size agreement is concentrated within the validated subset rather than across all discovery DEGs.

Eighty-three encoded proteins, while two were lncRNAs (*LINC03031* and *PCED1B-AS1*). Validation fold-changes were attenuated relative to discovery (median | $\log^{FC}$ | =0.39 *vs.* 1.07), a pattern consistent with cross-cohort and cross-platform replication. Most genes retained high statistical significance: 71 of 85 (84%) had adjusted $p<10^{-3}$, 47 (55%) $<10^{-6}$, 32 (38%) $<10^{-9}$, and 19 (22%) $<10^{-12}$, demonstrating robust reproducibility. *LINC03031* showed stronger up-regulation ($\log^{FC}$ = +1.385 vs +1.182), whereas *SRSF10*, *ARPC4-TTLL3*, and *RPS27* reproduced marked down-regulation. Other highly significant transcripts (*GNLY, CNOT6L, RPL7, TOMM5*) reinforced pathways related to ribosome biogenesis, mitochondrial function, and immune activation. Overall, gene-level changes were directionally consistent and statistically robust, though quantitative effect sizes diminished across cohorts.

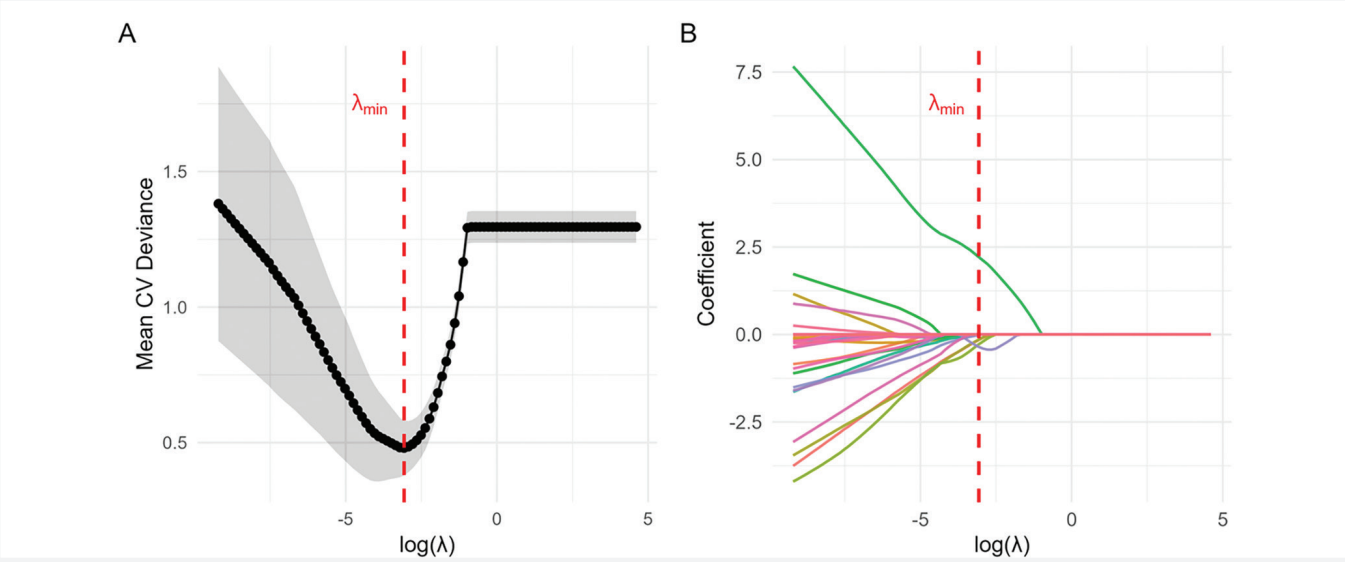

Figure 4. Regularized logistic regression model performance and coefficient profiles. (A) Cross-validation MSE plotted against the logarithm of the regularization parameter log(lambda). (B) Coefficient trajectories across the range of log(lambda) values MSE: Mean squared error, KEGG: Kyoto Encyclopedia of Genes and Genomes, GO: Gene ontology, ATP: Adenosine triphosphate, NADH: Nicotinamide adenine dinucleotide + hydrogen, COVID-19: Coronavirus disease-2019

Predictive Model Performance

The final predictive model was based on regularized logistic regression with LASSO penalization. Using leakage-controlled evaluation, the pooled cross-validated ROC AUC from the outer cross-validation loop was 0.975 (95% CI: 0.942-1.000), indicating strong discrimination under strict resampling conditions. This estimate was derived exclusively from held-out test folds, ensuring unbiased performance assessment.

When applied to the independent validation cohort (*GSE113079*), the model achieved an ROC AUC of 0.820 (95% CI: 0.737-0.906) (Figure 5), demonstrating preservation of predictive performance across platforms and biological compartments. At the Youden-optimized threshold, sensitivity =0.882, specificity =0.688, accuracy =0.823, balanced accuracy =0.785, positive predictive value =0.845, and negative predictive value =0.750.

Only two genes, *NEUROD2* and *RPS27*, retained non-zero coefficients in the final model (Figure 4B). Bootstrap stability analysis (1,000 resamples) showed consistent selection of *NEUROD2* in 99.9% and *RPS27* in 51.0% of runs, exceeding the predefined 50% stability threshold (Figure 6). Other transcripts appeared in fewer than 35% of resamples. These results indicate that predictive performance is driven by a minimal, stable two-gene signature rather than diffuse multigene effects.

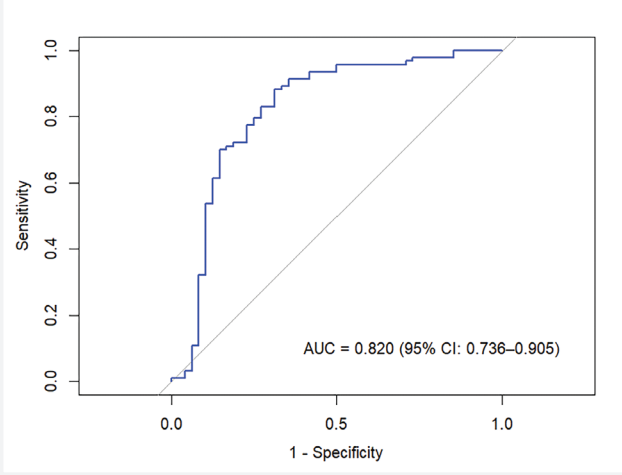

Figure 5. Receiver-operating-characteristic curve of the two-gene LASSO classifier in the validation cohort

AUC: Area under the curve, CI: Confidence interval

DISCUSSION

This study identified distinct blood transcriptomic signatures associated with CAD. Differential-expression analysis revealed coordinated dysregulation of mRNAs and lncRNAs, with enrichment in ribosomal biogenesis, oxidative phosphorylation, and inflammatory-stress pathways (p53, TNF/NF-κB). A concise

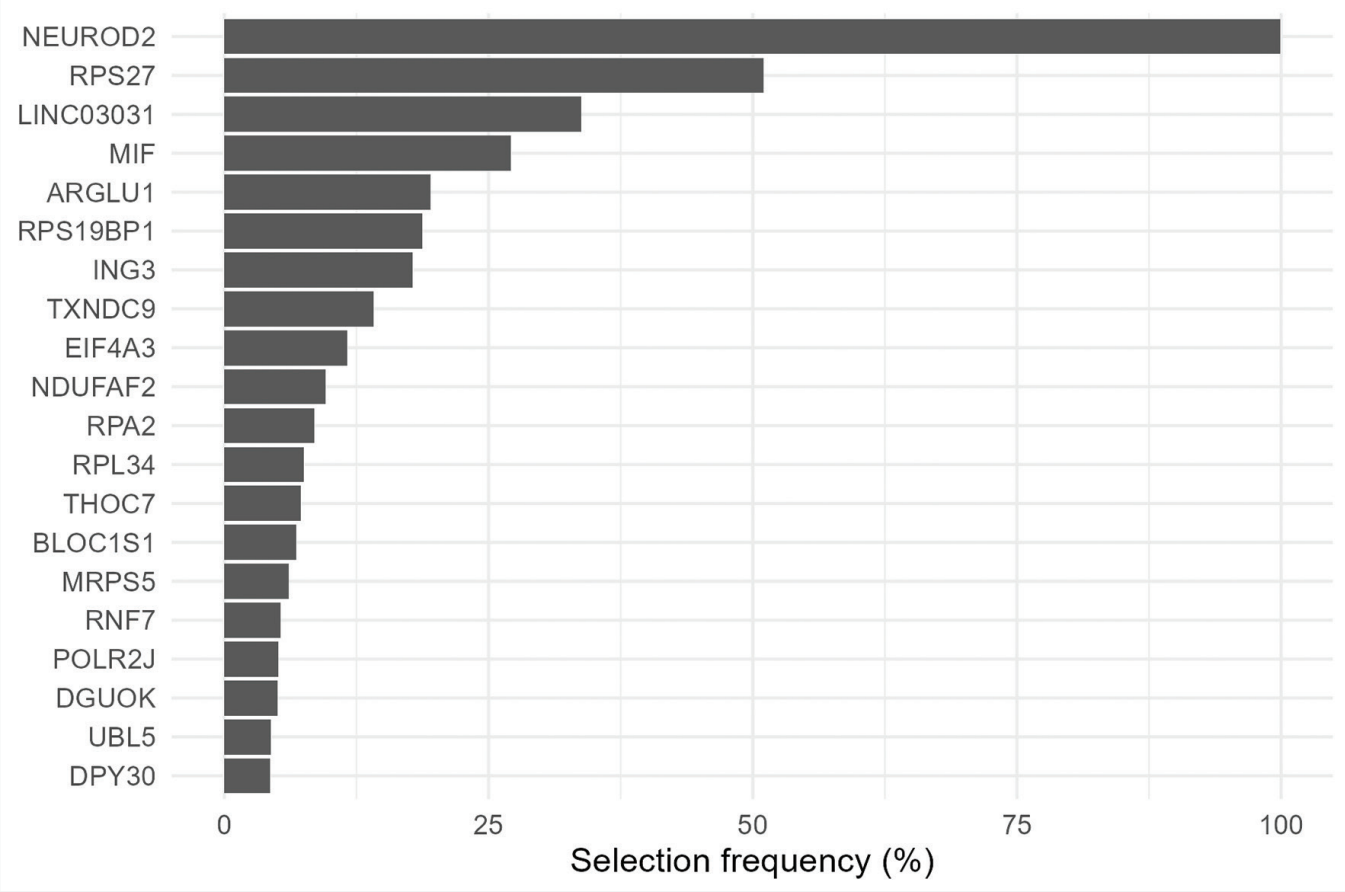

Figure 6. Top 20 features ranked by selection frequency across 1000 bootstrap resamples using the regularized logistic regression model at optimal alpha and lambda

AUC: Area under the curve, CI: Confidence interval

two-gene signature (*NEUROD2*, *RPS27*) accurately discriminated patients from controls, while *LINC03031* and *PCED1B-AS1* emerged as reproducible lncRNA markers, all validated across platforms. *NEUROD2*, previously recognized as a neuronal transcription factor, was recently found elevated in stable CAD[34] and implicated in macrophage inflammation via protein kinase D and *NLRP3/NF-κB*[38]. *RPS27*, a ribosomal protein, modulates p53 and NF-κB signaling[39,40], indicating its role in immune activation. *LINC03031*, largely uncharacterized, was strongly upregulated, representing a new CAD-linked transcript, while *PCED1B-AS1*, known from cancer biology, was downregulated, potentially reflecting suppressed hypoxia or growth-factor signaling[41]. These findings extend prior transcriptomic diagnostic efforts such as Corus CAD[11], PREDICT[42], and later multi-gene models[34,43]. In contrast to these larger gene panels, which typically comprise 12-23 transcripts and were optimized within narrowly defined clinical populations, the present study demonstrates that a highly compact two-gene signature can retain robust discriminatory performance when externally validated across cohorts, platforms, and biological compartments. Our results confirm *NEUROD2* as a reproducible CAD marker and highlight ribosomal-stress involvement through *RPS27*, defining a minimal two-gene panel with comparable diagnostic accuracy. The extreme parsimony of this model reduces model complexity, limits overfitting risk, and facilitates assay standardization, supporting feasibility for low-cost, qPCR-based implementation. Clinically, this compact panel could complement existing tests by offering a low-cost, qPCR-based assay for early or atypical CAD detection. Because it captures inflammatory and stress-response activity, it may yield information beyond lipid or imaging data.

An important aspect of this study is the biological distinction between the discovery and validation samples. Plasma cfRNA represents a heterogeneous mixture of extracellular transcripts released from multiple tissues, including vascular, cardiac, and immune-related cell types, whereas PBMC transcriptomes predominantly reflect gene expression within circulating immune cells. Prior work has demonstrated that cfRNA captures broad, systemic disease-associated transcriptional

programs rather than signals confined to a single tissue or cell type[44]. Accordingly, replication of key signals across biologically distinct blood-derived RNA sources supports the robustness and generalizability of the identified transcriptomic patterns, while necessarily limiting precise inference about tissue or cellular origin. This reduced spatial resolution limits biological specificity for localized processes, such as coronary plaque-restricted pathology. The observed signals are therefore most consistent with systemic transcriptional programs that are hypothesis-consistent with known features of CAD, including chronic vascular inflammation, immune activation, mitochondrial stress, and altered protein synthesis, rather than direct evidence of plaque-localized mechanisms.

At the same time, the biological nature of the validated signals warrants careful interpretation. Several prominent components of the signature, particularly ribosomal and mitochondrial transcripts, are consistent with generalized inflammatory and metabolic stress responses rather than being uniquely specific to CAD. Ribosomal proteins such as *RPS27* have been shown to participate in immune activation and stress-related signaling pathways across diverse disease contexts, reflecting broad host response programs rather than tissue-restricted pathology[45]. Accordingly, the presence of such transcripts suggests engagement of systemic immune and stress-associated processes. The validated non-coding transcripts are therefore most plausibly interpreted as reflecting regulatory programs related to cellular stress adaptation rather than disease-specific markers. These features are hypothesis-consistent with established models of atheroinflammation, in which sustained immune activation, mitochondrial dysfunction, and translational stress contribute to vascular injury and disease progression, but they do not provide direct evidence of plaque-localized or CAD-specific molecular mechanisms.

These considerations indicate that the identified transcriptomic signature is most appropriately interpreted as reflecting systemic inflammatory and stress-related processes rather than a plaque-specific molecular fingerprint or a disease-specific marker uniquely distinguishing CAD from other inflammatory conditions. Prior work has shown that plasma cfRNA captures broad, multi-tissue transcriptional programs and has limited spatial resolution with respect to tissue and lesion specificity[44]. Accordingly, the present findings are hypothesis-consistent with an association between the observed signature and overall atheroinflammatory burden in CAD, but they do not provide direct evidence of plaque-localized pathology or anatomic coronary stenosis. From a clinical perspective, these features suggest that such a signature, if validated in prospective studies, may be more appropriately considered for applications such as blood-based screening or risk stratification rather than as a definitive diagnostic of obstructive coronary disease. Potential use cases may include identifying individuals with elevated systemic inflammatory activity in whom further cardiovascular evaluation may be warranted, or complementing established risk factors and imaging modalities. These proposed applications remain speculative and require dedicated clinical validation beyond the scope of the present study.

Future validation efforts should focus on three key areas: (i) prospective evaluation in larger and more diverse populations to establish generalizability and reference ranges; (ii) assessment of specificity relative to other cardiovascular and systemic inflammatory diseases; and (iii) testing integrated models combining transcriptomic markers with established clinical predictors to quantify incremental diagnostic and prognostic value. Mechanistic studies should define their roles in vascular inflammation and remodeling, while integrated multi-omic and prospective evaluations will be critical for clinical translation.

Study Limitations

This study has several limitations. Although the findings were validated across independent cohorts and platforms, sample sizes were modest and relatively homogeneous, warranting confirmation in larger and more diverse populations. The use of biologically distinct compartments, plasma cfRNA in discovery and PBMCs in validation, strengthens robustness but complicates interpretation of tissue specificity and cellular origin. Although raw RNA-seq reads for the discovery cohort are available in the SRA, a gene-level raw count matrix was not provided as part of the GEO Series record; reprocessing FASTQ files to generate counts would constitute a new primary analysis and was beyond the scope of this secondary study. Gene-expression analyses were not adjusted for clinical covariates, leaving potential residual confounding. In addition, the identified transcriptomic signals may reflect general inflammatory or metabolic stress rather than CAD-specific processes, and specificity relative to other cardiovascular or systemic inflammatory diseases remains untested. Finally, the cross-sectional design precludes causal inference, and prospective clinical validation is required to establish diagnostic utility and define optimal clinical applications.

CONCLUSION

We identified and cross-validated a concise two-gene blood transcriptomic signature, *NEUROD2* and *RPS27*, that distinguishes patients with CAD from controls with robust discriminatory performance (nested cross-validated AUC=0.975; external AUC=0.820) across independent cohorts and platforms. This mRNA pair reflects ribosome- and mitochondria-associated inflammatory and metabolic stress pathways highlighted by enrichment analyses, complementing

established biomarkers rather than replacing them. Although *LINC03031* and *PCED1B-AS1* were not retained in the final predictive model, their reproducible dysregulation supports the broader relevance of lncRNAs in CAD-associated transcriptional alterations. Collectively, these findings provide a biologically coherent and technically validated framework for the further development of a blood-based transcriptomic assay, pending confirmation in larger, ethnically diverse populations and prospective studies to define clinical utility and application.

Ethics

Ethical Committee Approval: Ethics approval was not required because this study was based exclusively on publicly available de-identified secondary datasets from the GEO database. No new human participants were recruited, and no identifiable personal or clinical data were accessed.

Informed Consent: This study was a retrospective bioinformatics analysis of two publicly available GEO transcriptomic datasets on CAD.

Data Availability Statement

The data that support the findings of this study are publicly available in the NCBI GEO database. The associated GSE numbers are as follows: *GSE208194* and *GSE113079*. These datasets can be accessed directly via the following link: NCBI GEO database. For any additional information or specific data requests, please contact the corresponding author.

Data Deposition

All codes are available on GitHub repository: https://github.com/selcukorkmaz/blood-transcriptome-cad

Footnotes

Authorship Contributions

Concept: B.E.Y., S.K., Design: B.E.Y., S.K., Data Collection or Processing: S.K., Analysis or Interpretation: B.E.Y., S.K., Literature Search: B.E.Y., S.K., Writing: B.E.Y., S.K.

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

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Supplementary File: https://d2v96fxpocvxx.cloudfront.net/37eae217-e8b5-4f55-976f-35df98003e83/content-images/7a123a81-927b-4901-9cc5-91fbcce6b3fa.pdf

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ORIGINAL ARTICLE

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The Mediating Role of Child's Quality of Life in the Relationship between Mother's Illness Anxiety and Child's Illness Anxiety

Annenin Hastalık Kaygısı ile Çocuğun Hastalık Kaygısı Arasındaki İlişkide Çocuğun Yaşam Kalitesinin Aracılık Rolü

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ABSTRACT

Aim: This study aimed to investigate how a child's quality of life influences the connection between a mother's and child's levels of illness anxiety.

Materials and Methods: Online data was collected from 302 mother-child pairs with children aged 10-15. The scale for the Assessment of Illness Behavior was applied to assess the mothers' level of illness anxiety. The Pediatric Quality of Life Inventory was applied to assess children's quality of life, and the Childhood Illness Attitude scale was applied to assess children's level of illness anxiety. To determine participant eligibility based on study criteria, mothers completed a sociodemographic questionnaire. The study employed Process Macro to analyze the mediating effect.

Results: The analysis revealed a significant association between maternal and child illness anxiety, with the child's quality of life serving as a partially significant mediating factor in this relationship.

Conclusion: Considering the study findings, it can be stated that the child's quality of life is an important determinant in the relationship between maternal and child illness anxiety. Therefore, the results highlight the importance for professionals working on the treatment of illness anxiety in children to also focus on the quality-of-life variable during intervention.

Keywords: Anxiety, child, illness behavior, mothers, quality of life

ÖZ

Amaç: Bu çalışma, çocuğun yaşam kalitesinin anne ve çocuğun hastalık kaygısı düzeyleri arasındaki bağlantıyı nasıl etkilediğini incelemeyi amaçlamaktadır.

Gereç ve Yöntem: 10-15 yaş arası çocukları olan 302 anne-çocuk çiftinden çevrimiçi veri toplanmıştır. Annelerin hastalık kaygısı düzeyini değerlendirmek için Hastalık Davranışını Değerlendirme ölçeği uygulanmıştır. Çocukların yaşam kalitesini değerlendirmek için Pediatrik Yaşam Kalitesi Envanteri, çocukların hastalık kaygısı düzeyini değerlendirmek için ise Çocukluk Çağı Hastalık Tutumu ölçeği uygulanmıştır. Çalışma kriterlerine göre katılımcıların uygunluğunu belirlemek için anneler sosyodemografik bir anket doldurmuştur. Çalışmada, aracılık etkisini analiz etmek için Process Macro kullanılmıştır.

Bulgular: Yapılan analizler, anne ve çocuk hastalık kaygısı arasında anlamlı bir ilişki olduğunu ortaya koymuştur ve çocuğun yaşam kalitesi bu ilişkide kısmen anlamlı bir aracı faktör olarak rol oynamaktadır.

Sonuç: Çalışma bulguları göz önüne alındığında, çocuğun yaşam kalitesinin anne ve çocuk hastalık kaygısı arasındaki ilişkide önemli bir belirleyici olduğu söylenebilir. Bu nedenle, sonuçlar çocuklarda hastalık kaygısının tedavisi üzerinde çalışan profesyonellerin müdahale sırasında yaşam kalitesi değişkenine de odaklanmasının önemini vurgulamaktadır.

Anahtar Kelimeler: Kaygı, çocuk, hastalık davranışı, anne, yaşam kalitesi

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INTRODUCTION

Health anxiety is defined as a condition characterized by the catastrophic misinterpretation of bodily sensations, coupled with an associated fear or worry about having, or potentially developing, a serious illness. This condition often leads to reassurance-seeking behaviors, such as frequent consultations with physicians or extensive online health-related searches[1,2].

Over the past few years, health anxiety was assumed to be a condition exclusive to adulthood; however, recent research has shown that children may also experience health anxiety[3,4]. Given that maternal mental health is a significant predictor of children's psychological well-being[5], it is crucial to examine the relationship between maternal health anxiety and health anxiety symptoms in children.

Models explaining health anxiety and those discussing its etiology suggest that maternal health anxiety may contribute to the development of health anxiety in children[6,7]. For instance, the cognitive-behavioral theory of health anxiety emphasizes that environmental learning plays a significant role in the development of health anxiety[6]. According to ecological systems theory, the family is considered the primary environmental system influencing a child's behavior and development[8]. Supporting the perspectives of these theories, research on anxiety suggests that having anxious parents may contribute to higher levels of anxiety in children[9]. Similarly, there is a growing body of evidence indicating a relationship between maternal and child health anxiety[10,11]. While the international literature highlights a diverse range of studies focusing on the relationship between maternal and child illness anxiety, research examining this relationship within the context of Turkish culture remains limited.

Mental health problems are also known to be associated with quality of life[12]. Quality of life is defined as a multidimensional concept that encompasses physical, emotional, and psychological well-being, as well as overall life satisfaction[13]. A review of studies focusing on the relationship between quality of life and illness anxiety indicates that as individuals' illness anxiety increases, their quality-of-life decreases; however, it is also noteworthy that research in this area remains limited[14,15].

It has been observed that the mother's anxiety level is also related to the children's quality of life[16,17]. The mother's increased anxiety level may lead to behavioral consequences that reduce quality of life. In the context of illness anxiety, behaviors such as frequent hospital visits and repeated internet searches can reduce quality of life. When evaluated from the perspective of the cognitive-behavioral theory discussed above, the child may learn by observing these behaviors in their mother. As a result of this learning, the child's quality of life may be affected.

The literature includes studies that separately examine the effect of parental anxiety on child anxiety[18], the impact of parental illness anxiety on child illness anxiety[11], and the relationship between parental anxiety and the child's quality of life[16], all reporting significant findings regarding these variables. However, there is a lack of research investigating the relationships among these variables through mediation hypotheses, specifically focusing on the mediating role of the child's quality of life in the effect of parental anxiety on child anxiety.

Designed to address the gaps identified in literature, this study aims to examine the mediating effect of child quality of life in the relationship between maternal and child illness anxiety. Accordingly, it is anticipated that this research will make significant contributions to both national and international literature. Moreover, as the first national study conducted following the adaptation of the Childhood Illness Attitude scale (CIAS) into Turkish, it is expected to provide important contributions to the national literature. Within this context, the primary hypothesis of the study has been formulated as: The child's quality of life has a significant mediating effect in the relationship between maternal and child illness anxiety.

MATERIALS AND METHODS

Case Selection and Description

The study sample consisted of a total of 302 child participants, including 155 girls and 147 boys, aged between 10 and 15 years, along with their mother. Participants were recruited through social media platforms using a convenience sampling method. Inclusion criteria required that the child be between 10 and 15 years of age and that both the child and the mother provided informed consent to participate in the study. When determining the exclusion criteria for the study, anxiety was considered as a spectrum; therefore, it was assumed that any diagnoses of anxiety or related disorders in participants could affect the results. Consequently, based on self-reports, the absence of an ongoing psychiatric diagnosis and treatment in both the mother and the child was established as an exclusion criterion.

A total of 452 individuals initially participated in the study. However, 30 children were excluded for not meeting age criteria, and 20 mothers and 12 children were excluded due to receiving ongoing psychiatric treatment. Additionally, 78 children, whose mothers had consented to participate, declined to take part in the study, and 10 mothers could not be reached due to incorrect contact information. Therefore, the final sample consisted of 302 mothers and 302 children.

Sociodemographic Form

This form was developed by the researcher to collect information about participant characteristics and was administered to the mothers. It includes questions regarding the child's date

of birth, gender, number of siblings, grade level, academic performance, and the presence of any ongoing psychiatric diagnoses under treatment. The form also collects information about the mothers' income level, educational background, and any psychiatric diagnoses they may have under treatment.

The Scale for the Assessment of Illness Behavior

This 25-item scale, originally developed by Rief et al.[19] and adapted into Turkish by Güleç[20], was used to assess mothers' illness-related behavioral tendencies associated with health anxiety. The scale for the Assessment of Illness Behavior (SAIB) is designed to evaluate behavioral manifestations of illness concern, such as excessive health monitoring, preoccupation with bodily symptoms, and frequent medical consultations, rather than health anxiety as a cognitive or affective construct[19]. In line with cognitive behavioral models of health anxiety, such illness related behaviors are considered observable expressions of underlying health anxiety and may play a role in the intergenerational transmission of illness related fears through modeling processes[21]. Accordingly, in the present study, SAIB scores were conceptualized as indicators of the behavioral component of maternal health anxiety.

The SAIB is a 4-point Likert-type scale, with responses ranging from 0= Completely true, to 3= Completely untrue. Higher scores indicate lower levels of illness behavior, whereas lower scores reflect higher levels of illness behavior. The internal consistency coefficient of the Turkish version was reported as .81,[20] and in the current study, Cronbach's alpha was also 0.81.

The Pediatric Quality of Life Inventory

The Pediatric Quality of Life Inventory (PedsQL), developed by Varni et al.[22] was used to assess the quality of life of child participants. The scale includes self-report forms tailored to different age groups. In this study, the Turkish-adapted versions of the forms for ages 8-12 and 13-18 were used[23,24]. The 23-item scale uses a 5-point Likert scale (0= Never to 4= Almost always) and is scored by converting responses to a 0-100 scale (0=100, 1=75, 2=50, 3=25, 4=0). The total score is calculated by dividing the sum of the item scores by the number of items. Higher scores indicate a better perceived quality of life. The internal consistency coefficient for the Turkish version was 0.86 for children aged 8-12 and 0.82 for those aged 13-15. In the present study, Cronbach's alpha was found to be 0.86.

The Childhood Illness Attitude Scale

The CIAS, developed by Wright and Asmundson[4] and adapted into Turkish by Aktan et al.[25] was used to assess health anxiety levels in children. The Turkish version of the scale consists of 28 items, 2 of which are excluded from scoring and serve only to gather additional information. The remaining 26 items are rated on a 3-point Likert scale (1= Never to 3= Often). Higher scores indicate higher levels of health anxiety in children. The scale does not have a specific cut-off score. The internal consistency coefficient for the Turkish version was reported as 0.86[25], while in this study, it was found to be 0.87.

Data Collection Procedure

Initially, a research proposal was prepared and submitted to the Ethics Committee of Işık University. After receiving ethical approval (decision no: 2022/04, date: 11.04.2022), data collection commenced. The data were collected online using Google Forms to administer the scales. Participants and their parents were provided with information about the study's purpose, and informed consent was obtained. In the first phase, the mothers were asked to complete the Sociodemographic Information Form and the SAIB. In the second phase, child participants were asked to complete the PedsQL and the CIAS. To prevent common method bias during the response process, the scales were completed at different times for the mother and child. Children were assured that their responses would not be shared with their parents.

Statistical Analysis

IBM SPSS 25 statistical software was used to conduct the data analyses. Initially, skewness and kurtosis values were examined to assess whether the data followed a normal distribution. Subsequently, reliability analyses were performed to determine the Cronbach's alpha coefficients of the scales, and frequency analyses were conducted to examine the sociodemographic characteristics of the sample. Pearson correlation analysis was employed to investigate the relationships between variables. To test the study's main hypothesis regarding the mediating effect of child quality of life in the relationship between maternal and child illness anxiety, the direct effects of parental anxiety on child anxiety, parental anxiety on child quality of life, and child quality of life on child anxiety were first analyzed. Thereafter, the mediating role of child quality of life in this relationship was examined using the PROCESS Macro version 4.3 for SPSS.

RESULTS

The variables' normal distributions, as well as their means and standard deviations, are displayed in Table 1.

In the next step, the correlations between the variables were examined, and the results are summarized in Table 2. The findings revealed a weak but significant negative correlation between scores on the SAIB and the CIAS ($r=-.270$, $p<0.001$). Additionally, a moderate negative correlation was found between CIAS scores and the PedsQL scores ($r=0-.445$ $p<0.001$). Finally, the relationship between maternal health anxiety (SAIB scores) and children's quality of life (PedsQL scores) was not significant ($r=0.111$, $p=0.053$). According to Baron and Kenny[26], for mediation analysis to be conducted, all variables must be

statistically significant with each other. However, according to Hayes, while this is important, it is not a requirement for conducting mediation analysis[27].

To investigate the mediating role of children's quality of life in the relationship between maternal and child health anxiety, the PROCESS Macro was applied. In the present study, the mediating effect was tested using Hayes' Model 4[27] with 5,000 bootstrap resamples and a 95% confidence interval. The results of the mediation analysis are presented in Table 3, and the hypothesized model is illustrated in Figure 1.

As shown in Table 3, path a, representing the relationship between the SAIB scores and the PedsQL scores, was not statistically significant. Looking at the path b values in Table 3, PedsQL scores significantly and negatively predict CIAS scores. Examining the effect of SAIB scores on CIAS scores (path c), it is observed that SAIB scores significantly and negatively predict CIAS scores. Considering the results for SAIB scores, it was found that maternal health anxiety positively predicts children's health anxiety. When the mediating variable (PedsQL) was included in the model, the direct effect of maternal health anxiety on children's health anxiety (path c') was found to be significantly negative, with SAIB scores significantly negatively predicting CIAS scores. In conclusion, based on the SAIB scores, it was determined that maternal health anxiety significantly and positively predicts children's health anxiety severity.

As shown in Table 3, the analysis results indicate that the child's quality of life mediates the relationship between maternal and child health anxiety. The effect between the dependent and independent variables, initially -0.2228, decreased to -0.1842 when the mediating variable was included in the model, but it remained significant. It was concluded that the child's quality of life plays a partial mediating role. The proportion of the mediating effect in the total effect was found to be 15%.

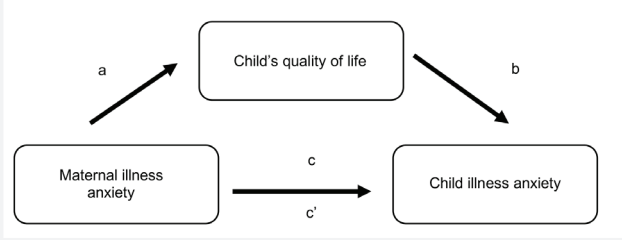

Figure 1. Mediation model of the study

Table 1. Normality analysis results, mean and standard deviation scores

Scales	Skewness	Kurtosis	Mean	SD
SAIB[a]	-0.038	-0.158	37.17	10.396
PedsQL[b]	-0.637	0.303	73.15	12.698
CIAS[c]	-0.088	-0.523	52.80	8.584

SAIB[a]: The Scale for the Assessment of Illness Behavior, PedsQ[b]: The Pediatric Quality of Life Inventory, CIAS[c]: The Childhood Illness Attitude scale, SD: Standard deviation

Table 2. Correlation matrix

	1	2	3
SAIB[a]	-		
PedsQL[b]	0.111	-	
CIAS[c]	-0.270**	-0.445**	-

**p<0.001

SAIB[a]: The Scale for the Assessment of Illness Behavior, PedsQ[b]: The Pediatric Quality of Life Inventory, CIAS[c]: The Childhood Illness Attitude scale

Table 3. Mediation analysis results

	B	SE	β	p	t	95% Confidence interval	
						Lower	Upper
Path a	0.1362	0.0701	0.1115	0.0529	1.9431	-0.0017	0.2741
Path b	-0.2839	0.0341	-0.4199	0.0000	-8.3157	-0.3511	-0.2167
Path c (total)	-0.2228	0.0459	-0.2698	0.0000	-4.8537	-0.3131	-0.1325
Path c' (direct)	-0.1842	0.0417	-0.2230	0.0000	-4.4162	-0.2662	-0.1021
	B		SE			Lower	Upper
Mediation	-0.0387			0.0209		-0.0825	-0.0011

B: Unstandardized regression coefficient; SE: Standard error; β: Standardized regression coefficient

DISCUSSION

This study aimed to examine the mediating role of children's quality of life in the relationship between maternal and child health anxiety. Although existing literature explores and validates the relationship between maternal-child health anxiety[11,28], and the connection between quality of life and health anxiety levels[14,15], there is a lack of studies specifically addressing the variables of maternal health anxiety, child health anxiety, and child quality of life simultaneously. Additionally, since the CIAS scale, which assesses health anxiety in children, has only recently been introduced to the Turkish literature, there are no studies examining these relationships. Therefore, it is considered important to examine the relationships between the mentioned variables in order to enhance children's psychological functionality and contribute to both national and international literature.

The first finding indicated that maternal health anxiety significantly and positively predicts children's health anxiety. This result is consistent with the literature suggesting that maternal psychopathology is a risk factor for the development of psychopathology in children[5,9], as well as studies that have identified a relationship between maternal and child health anxiety[11,28]. A recent review by Rask et al.[10] suggested that the relationship between maternal and child health anxiety can be explained through both social learning and genetic perspectives. Based on these findings, it is recommended that specialists working with children who have health anxiety examine the role of maternal health anxiety in these symptoms and its impact on the treatment process.

In another step of the study, the direct effect of maternal illness anxiety on children's quality of life was examined. Although this effect did not reach statistical significance (p=0.0529), some sources consider this level to be marginally significant[29]. A review of the literature indicates that studies specifically investigating this relationship are limited, with most focusing instead on the effects of general parental anxiety on children's quality of life. Reporting findings similar to our study, Thorgaard et al.[30] compared children whose mothers had a diagnosis of rheumatoid arthritis with children of healthy mothers and found no significant differences in quality of life between the experimental and control groups. The same study also reported that children of mothers with high illness anxiety exhibited more symptomatic complaints and attended more hospital visits. However, a study focusing on children with cerebral palsy and their parents demonstrated that maternal anxiety levels had a significantly negative effect on children's quality of life[17]. Considering these inconsistencies and the limited number of studies in literature, it is evident that future research on parental health anxiety and its impact on children is of considerable importance.

Another finding of the study indicates that children's quality of life significantly and negatively predicts child illness anxiety. A review of the literature reveals that most studies have focused on adults, consistently reporting a negative association between quality of life and illness anxiety[14,15]. Studies involving children have predominantly aimed to assess the quality of life of those with developmental disorders, chronic illnesses, or physical disabilities[31-34]. While no studies directly examining the relationship between illness anxiety and quality of life in children were identified, research has demonstrated associations between children's mental health problems and their quality of life[35,36]. Considering these findings, the observed negative relationship between quality of life and child illness anxiety highlights the potential importance of incorporating interventions aimed at improving quality of life into the treatment of illness anxiety in children.

Literature reviews reveal that the relationship between children's quality of life and their mothers' health anxiety has also been explored[30]. In one study, when comparing children with healthy mothers to those whose mothers had a diagnosis of rheumatoid arthritis, no significant difference was found in the quality-of-life scores of children whose mothers had severe health anxiety[30]. On the other hand, in a study showing the opposite result, it was found that the life satisfaction of adolescents with highly stressed parents was affected within a year[37]. Given that a marginal significance was found between the two variables in this study, along with the contradictory results in the literature, it is believed that further investigation of this relationship in different populations and larger samples would be valuable.

The mediation analysis revealed that the child's quality of life partially mediates the relationship between maternal and child illness anxiety. As one of the few studies to investigate the indirect role of quality of life in this context, it provides valuable insights for clinicians. The findings suggest that incorporating interventions aimed at enhancing children's quality of life into treatment modules for illness anxiety could be particularly beneficial. The results reveal that when assessing children's health anxiety, it is clinically critical to focus not only on the level of symptoms but also on the emotional, social, and physical functioning dimensions of quality of life. In this context, the mediating effect of the child's quality of life provides concrete clinical guidance for parent-focused interventions: reducing maternal anxiety may not only directly affect the child's health anxiety but also create an indirect protective effect by improving the child's daily functioning. Therefore, adding components to intervention programs that aim to regulate parents' cognitive distortions about illness, reduce the burden of caregiving, and improve the quality of mother-child interaction can contribute significantly at the clinical level to reducing children's health anxiety by enhancing their quality of life. Consequently, this

study may guide professionals by highlighting strategies that help strengthen children throughout the psychotherapy process.

Study Limitations

Despite the strengths of this study, several limitations should be acknowledged. For instance, the mediating effect of children's quality of life on the relationship between maternal and child illness anxiety accounted for only 15% of the total effect. Consequently, other potential mediators were not examined and should be addressed in future research. A study examining the relationship between maternal and child psychopathologies found that emotion regulation[38] mediates this relationship, while another study found that mindful awareness[39] mediates this relationship. Future research may consider including these variables. The sample comprised a non-clinical population; however, no formal psychiatric assessments or structured interviews were conducted. Data on psychiatric conditions were obtained solely through self-report, with exclusion criteria based on participants' responses. Additionally, the lack of information regarding participants' other physical, neurological, or chronic conditions constitutes a further limitation.

Although mothers and children completed the measures at separate times, the online data collection setting may still entail contextual factors that could influence responses, and this should be considered when interpreting the results.

Although the sample size was adequate for the planned analyses, the use of a convenience sampling method limits the generalizability of the findings. This approach was preferred due to practical constraints in reaching mother-child dyads that met the study's inclusion and exclusion criteria. Participants recruited through social media may differ from the broader population in ways that could introduce selection bias, and this should be taken into account when interpreting the results. Online recruitment, however, allowed access to participants from diverse geographical regions, which provided some practical advantages despite the inherent limitations of convenience sampling.

Another limitation of the study is that demographic variables such as the child's age, the mother's educational level, and socioeconomic status were not included as covariates in the mediation model. These factors may be associated with both health anxiety and quality of life, and their absence should be considered when interpreting the findings.

CONCLUSION

In conclusion, the findings of this study are believed to make a valuable contribution to the literature on health anxiety in children and to future research in this area. It is hoped that studies like this, which can guide experts in both preventive and therapeutic interventions for health anxiety, will increase in the future.

Ethics

Ethical Committee Approval: Initially, a research proposal was prepared and submitted to the Ethics Committee of Işık University. After receiving ethical approval (decision no: 2022/04, date: 11.04.2022), data collection commenced.

Informed Consent: Participants and their parents were provided with information about the study's purpose, and informed consent was obtained.

Footnotes

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

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Evaluation of the Diagnostic Performance of Large Language Models in Distinguishing Pulmonary Embolism and Pulmonary Artery Sarcoma

Pulmoner Emboli ve Pulmoner Arter Sarkomunu Ayırt Etmede Büyük Dil Modellerinin Tanısal Performansının Değerlendirilmesi

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ABSTRACT

Aim: Pulmonary embolism (PE) and pulmonary artery sarcoma (PAS) present with similar clinical symptoms but differ significantly in pathology and treatment. Accurate differentiation is critical yet challenging in clinical practice. This study aimed to evaluate the diagnostic efficacy of large language models (LLMs) in distinguishing PE from PAS, and to explore their potential as clinical decision support tools.

Materials and Methods: Eighteen cases with confirmed diagnoses of PE or PAS were assessed using three LLMs: DeepSeek V3, Gemini Flash 2.5, and ChatGPT-4o. Models were provided with basic clinical data, followed by advanced imaging and treatment information. The Role-Goal-Context framework was applied to standardize the input prompts.

Results: DeepSeek V3 achieved the highest accuracy in detecting PE during the preliminary diagnostic phase (88.9 %), while Gemini Flash 2.5 performed best in identifying PAS in the conclusive phase (22.2 %). ChatGPT-4o yielded more stable results under conditions of limited data availability.

Conclusion: LLMs show promise as supportive tools in differentiating PE from PAS when guided by structured prompts and expert oversight. Their utility is limited in rare or data-deficient scenarios. A hybrid model involving human expertise and LLM integration appears most effective in enhancing diagnostic precision and clinical decision-making.

Keywords: Pulmonary embolism, pulmonary artery sarcoma, large language models, diagnostic accuracy, clinical decision support

ÖZ

Amaç: Pulmoner emboli (PE) ve pulmoner arter sarkomu (PAS) benzer klinik semptomlarla ortaya çıkar, ancak patoloji ve tedavi açısından önemli farklılıklar gösterir. Klinik uygulamada doğru ayırıcı tanı koymak çok önemlidir, ancak zordur. Bu çalışma, PE ile PAS'ı ayırt etmede büyük dil modellerinin (LLM) tanısal etkinliğini değerlendirmek ve klinik karar destek araçları olarak potansiyellerini araştırmak amacıyla yapılmıştır.

Gereç ve Yöntem: PE veya PAS tanısı doğrulanmış 18 olgu, DeepSeek V3, Gemini Flash 2.5 ve ChatGPT-4o olmak üzere üç LLM kullanılarak değerlendirilmiştir. Modeller, temel klinik verilerle beslendikten sonra ileri görüntüleme ve tedavi bilgileriyle beslenmiştir. Giriş komutlarını standartlaştırmak için Rol-Hedef-Bağlam çerçevesi uygulanmıştır.

Bulgular: DeepSeek V3, ön tanı aşamasında PE'yi tespit etmede en yüksek doğruluğu elde ederken (%88,9), Gemini Flash 2.5 kesin aşamada PAS'ı tanımlamada en iyi performansı gösterdi (%22,2). ChatGPT-4o, sınırlı veri kullanılabilirliği koşullarında daha istikrarlı sonuçlar verdi.

Sonuç: LLM'ler, yapılandırılmış komutlar ve uzman gözetimi ile yönlendirildiğinde, PE'yi PAS'tan ayırt etmede destekleyici araçlar olarak umut vaat etmektedir. Nadir veya veri eksikliği olan senaryolarda kullanışlılıkları sınırlıdır. İnsan uzmanlığı ve LLM entegrasyonunu içeren hibrit bir model, tanı doğruluğunu ve klinik karar vermeyi geliştirmede en etkili görünmektedir.

Anahtar Kelimeler: Pulmoner emboli, pulmoner arter sarkomu, büyük dil modelleri, tanı doğruluğu, klinik karar desteği

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INTRODUCTION

Acute pulmonary embolism (PE) is a common and potentially lethal result of venous thromboembolic disease (VTE). D-dimer, a plasmin-derived degradation product, has a high sensitivity and negative predictive value in the diagnosis of VTE. As imaging modalities in the diagnosis of PE, lower extremity venous Doppler ultrasonography and echocardiography, as well as pulmonary computed tomography angiography (CTA) and scintigraphy, magnetic resonance angiography (MRA), and conventional pulmonary angiography. However, pulmonary CTA is the most crucial and widely utilized imaging modality for the diagnosis of suspected PE, with high sensitivity and specificity. A central filling defect in a vessel encircled by contrast material is one of the direct CT findings of acute PE[1].

However, pulmonary artery sarcoma (PAS) should also be considered in the differential diagnosis because it appears as a defect filling with a similar appearance to PE. PAS is a rare and malignant tumor that develops within the inner or middle layer of the pulmonary artery, with an estimated incidence rate ranging from 0.001-0.03%, with a male-to-female ratio of 1:2. It mainly occurs in middle-aged people, with an average onset age of approximately 50 years. The survival period of PAS is approximately 1.5 months for those who did not undergo surgery timely. If there is a suspicion of PAS in the presence of a lesion that does not improve or persists despite PE treatment, it should be evaluated with positron emission tomography/computed tomography (PET/CT). PAS typically presents with an indolent onset, and its clinical symptoms resemble those of PE. Common manifestations of PAS include exertional dyspnea, chest pain, cough, hemoptysis, fatigue, fever, anemia, weight loss, increased erythrocyte sedimentation rate, and absence of hypercoagulability. The level of D-dimer elevates in patients with PE whereas, in the patients with PAS is usually within the normal range[2-4].

As recent developments, large language models (LLMs) can be used in many areas due to their ability to deeply analyze natural language context, create human-like, consistent, and fluent texts, demonstrate content awareness in information-intensive contexts, and contribute to decision-making and problem-solving processes by understanding instructions[5,6].

This study aimed to evaluate the potential of LLMs in medical decision support processes and, in particular, to comparatively analyze the preliminary and final diagnosis performances of different models and to investigate the ability to make differential diagnosis over PE and PAS cases included using literature data. 18 real medical cases published in the literature were selected[7-18], and for each case, a preliminary diagnosis was obtained from the models by providing only basic clinical information. Then, final diagnoses were collected with an extended dataset including advanced imaging results, laboratory data, and treatment information. Three different platforms were used as LLMs: ChatGPT-4o (OpenAI), Gemini Flash 2.5 (Google DeepMind), and DeepSeek V3 (DeepSeek-AI). Each model was evaluated using fixed and identically structured prompts.

MATERIALS AND METHODS

Study Population

This study reviewed the National Institutes of Health library (https://pubmed.ncbi.nlm.nih.gov) for literature on PE and PAS from 1999 to 2024. Publications that comprised cases with confirmed diagnoses by CT, PET/CT, and histology were chosen at random and included in the evaluation. This study uses data from current articles to perform screening and research utilizing LLM.

Inclusion and Exclusion Criteria

Cases were included if they met the following criteria: (1) the diagnosis of either PE or PAS was confirmed by imaging (e.g., CT angiography, PET-CT) and/or histopathological examination; (2) sufficient clinical details (such as symptoms, D-dimer levels, imaging results, and treatment approach) were available in the case description to construct the diagnostic prompts; (3) the case was published in a peer-reviewed journal between 1999 and 2024. Nine patients presented as a case series in an article were included in the study[19].

Exclusion Criteria

(1) Review articles or editorials without detailed case data, (2) duplicate cases published in more than one article, and (3) cases lacking either a confirmed final diagnosis or the clinical/imaging information required for the two-stage prompt design.

Study Protocols

The study comprised publications that identified the type of article, the age and gender of the patients, and the country to which the item belonged. The D-dimer level as a laboratory test, the diagnostic examinations conducted (echocardiography, Doppler US, CT angiography, MRA, PET/CT), the duration of the disease, the presence of accompanying conditions, the reason for the complaint at the time of application, the onset of the symptoms, and the initial preliminary diagnosis of the patients and the final diagnosis as a result of the techniques performed were all categorized. According to the article data, the location of the PE observed in the CTA and the following treatment strategy (medical, interventional, or surgical) were assessed, as were the PAS size, type, localization, extension, presence of recurrence, and outcome (day) found in these individuals.

Large Language Models

A two-stage evaluation process was adopted for each case included in the study. In the first stage, only the basic clinical

history and initial evaluation findings of the patient were presented to the model to obtain a preliminary diagnosis. In the second stage, detailed information such as advanced imaging results, laboratory data, and treatment process for the same case was presented to the model to obtain a final diagnosis. Thanks to this two-stage structure, the models' diagnostic thinking levels, the way they use information, and their ability to produce meaning in a clinical context were comparatively analyzed.

Three different LLM (ChatGPT-4o, Gemini Flash 2.5, and DeepSeek V3) were evaluated separately for each case using fixed prompt structures. Model outputs were collected manually, and each diagnosis was compared with the final diagnosis of the relevant case stated in the literature.

Prompt Design

The quality of interactions with the LLM is directly dependent on the structure of the prompts used. Prompts are instructions given to the LLM to perform a task. By creating effective prompts, researchers and practitioners can increase the accuracy and relevance of the responses the model provides[6]. In this study, a prompt was created and used with the Role-Goal-Context (RGC) framework.

The RGC framework offers a structured methodology that stands out in terms of clarity and direction. This framework is based on a clear definition of the role the model should adopt, the goal it is expected to achieve, and the contextual information that affects this process. This makes it easier for the model to focus on the task and produce more meaningful, context-sensitive outputs[20,21]. This framework suggests that a prompt should consist of three basic elements:

Role: Specifies the area of expertise in which the model is expected to act.

Goal: Clearly states the specific task the model is expected to answer.

Context: Provides the case information and guiding context needed for the model to produce the answer.

The RGC structure is recommended to increase both consistency and task focus, especially in medical LLM applications. In this context, the prompt structure used in the study was designed in two stages. For each case, first the "preliminary diagnosis prompt", which includes only basic clinical information, and then the "final diagnosis prompt", which includes advanced examinations and treatment processes, was used. Both prompts were prepared to preserve the same RGC structure. The model was asked to produce only a single-sentence, non-explanatory diagnostic response. The prompt used for preliminary diagnosis is given below.

Because no exemplars, demonstrations, or labelled example cases were provided in the prompts, the prompting approach corresponds to a zero-shot setup. This choice was made to *(i)* keep the prompting conditions identical across models, *(ii)* better reflect a first-pass clinical decision support use case, and *(iii)* reduce the risk of implicit guidance from in-prompt examples.

"You are a pulmonary embolism specialist and have in-depth knowledge in the diagnosis and treatment of this disease. You are asked to analyze the following case and make a diagnosis. The following case includes a patient's clinical history and initial evaluation results. Based on these data, make a single sentence for the patient's probable preliminary diagnosis. Make a short sentence for the preliminary diagnosis without explanation." Based on this prompt, the following explanation can be made for the RGC frame.

Role: *You are a pulmonary embolism specialist and have in-depth knowledge of the diagnosis and treatment of this disease.*

Objective: *Analyze the following case and make a diagnosis.*

Context: *The following case includes a patient's clinical history and initial evaluation results. Based on this data, make a single sentence for the patient's probable preliminary diagnosis. Make a short sentence for the preliminary diagnosis without explanation.*

The final diagnosis prompt was created as follows: *"After adding advanced imaging, laboratory, and treatment information about the same case, in light of this information, state the patient's final diagnosis in a single sentence. Make a final diagnosis with a short sentence without explanation."*

This framework allowed for the objectification of the evaluation process and the standard comparison of the responses of various models to the diagnosis procedure. Furthermore, maintaining the prompt language was intended to lessen the impact of bias in model comparisons. For every situation, a different workspace was made.

Assessment and Analysis

The evaluations were made by an experienced radiologist (13 years of clinical experience), and each diagnostic result was classified into three categories:

- If the preliminary diagnosis obtained from LLM according to the given prompts (PE and thrombotic conditions), and the final diagnosis is PAS, labeled as "Exact Match";
- If the preliminary diagnosis in the article for LLM is preliminary diagnosis (PE and thrombotic conditions), and the final diagnosis is PE and thrombotic conditions, labeled as "Approximate Match";
- If the LMM diagnosis and the final diagnosis are unrelated, labeled as "No Match".

The obtained data were systematically tabulated, and the diagnostic accuracy rates were statistically analyzed. This approach provided the opportunity to objectively evaluate the reliability and applicability of LLMs in the context of clinical decision support systems.

The workflow visualizing the process steps and evaluation process followed in the study is presented (Figure 1).

Used LLMs and Access Method

In this study, model outputs were comparatively evaluated using three different LLMs (ChatGPT-4o, Gemini Flash 2.5, and DeepSeek V3) in the diagnosis processes for PE cases. The models were tested directly through user interfaces via open-access platforms; no external intervention, parameter adjustment, or special fine-tuning process was applied. With this method, the raw performance of the models was measured in a natural interaction environment.

The models were accessed via their official web user interfaces not via an application programming interface (API). Because web interfaces may not expose an exact API model identifier and may be updated over time, we report the model labels shown in the interface and the access dates to support reproducibility: ChatGPT-4o [(OpenAI; accessed on 06.07.2025)], Gemini Flash (Google; interface label shown as "Gemini Flash 2.5" at time of access on [(06.07.2025)], and DeepSeek V3 (DeepSeek; accessed on [(07.07.2025)]. Each case was evaluated in a new, empty session to prevent cross-case context leakage.

Statistical Analysis

A statistical package program (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.) was used to analyze the data obtained from the literature. Descriptive analysis was used for demographic data, chi-square test for categorical data; independent sample T-test was used for normally distributed data in comparisons of binary groups, and Mann-Whitney U test was used for non-normally distributed data. Statistical significance level was accepted as $p<0.05$.

Ethical Considerations

Since this study utilized publicly available case data extracted from previously published literature and did not involve any direct patient contact or identifiable personal information, ethics committee approval was not required in accordance with institutional and international research guidelines.

RESULTS

Of the scanned articles, 77.8% (n=14) were case reports, 22.2% (n=4) were original articles. The most cases were reported in 2024 (n=6), 2018 (n=5).

Female gender (n=10, 55.6%) was the most common. The mean age of the patients was 49.94±13.67 years, and PAS was most frequently detected in the 40-49 and 50-59 age groups (n=5, 27.8%).

The country reporting PAS the most was China (n=11, 61.1%), followed by Türkiye (n=2, 11.1%) and Iran, Brazil, Taiwan, Germany, and the USA (n=1, 5.6%) with equal frequency.

The mean duration of complaints was 67.39±137,85 days, and the mean outcome was 173,89±293,34 days. 38.9% (n=7) of the cases had other accompanying diseases, the most common being post-COVID status (n=2, 11.1%), followed by ARDS, diabetes mellitus, hypertension, pneumonia, and TB (n=1, 5.6%).

The mean D-dimer level in the patients was 596.06±875.78 mg/L 11.1% (n=2) of the cases had a history of anticoagulant drug use, and DMA heparin was used.

Of the patients, 77.8% (n=14) had dyspnea, 16.7% (n=3) syncope, fever, and cough; 11.1% (n=2) experienced palpitations and exhaustion; 44.4% (n=8) experienced chest discomfort; and 27.8% (n=5) experienced hemoptysis. Of those 5.6% (n=1) experienced symptoms that worsened with exercise, the previous day, and the past two hours, while 11.1% (n=2) experienced symptoms that worsened throughout the previous two days.

Echocardiography was performed on 88.9% (n=16) of the patients, Dopple US on 22.2% (n=4), pulmonary CT angiography on 100% (n=18), magnetic resonance imaging (MRI) on 16.7% (n=3), PET/CT on 22.2% (n=4), and MRI on 100% (n=18).

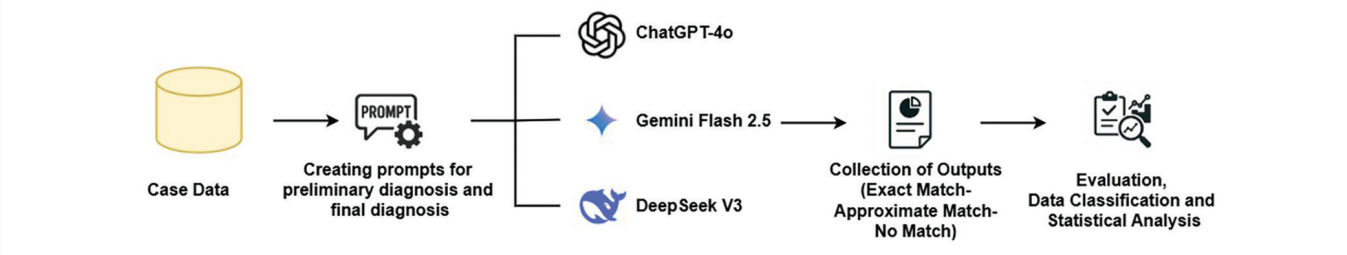

Figure 1. Workflow diagram showing the steps of the operations carried out within the scope of the study

The detected PE localization was mostly at the trifurcation level in 50% (n=9), main-right pulmonary artery level in 16.7% (n=3), main pulmonary artery level in 11.1% (n=2), and right main pulmonary artery level in 5.6% (n=1).

Among the histopathological types of PAS, intimal-mural was the most common (66.7%, n=12), while leiomyosarcoma was the second most common histopathological diagnosis in 11.1% (n=2). Histopathologically, the most common PAS localization was at the trifurcation (61.1%, n=11), main-right pulmonary artery (16.7%, n=3), and main pulmonary artery and right pulmonary artery (11.1%, n=2) were other regions. The intimal-mural type was mostly observed in females (n=7) (Table 1). PAS mostly extended to the pulmonary valve (11.1%, n=2), while aorticopulmonary valve extension to the aorta and right ventricle was reported equally frequently (5.6%, n=1) (Table 1).

The mean three-dimensional (anteroposterior, transverse, craniocaudal) diameters of the PAS in the articles included in the study (n=4, 22.22%) were 3.48±1.86 x 6.18±4.07 x 7.63±8.34 mm. Dimension information was not available in 13 of the articles.

The most preferred method in terms of PE-PAS treatment approach was 38.9% (n=7) surgery, 22.2% (n=4) medical-surgical, 11.1% (n=2) interventional, and 5.6% (n=1) interventional-surgical. The approach was not specified in four cases. Among interventional and surgical methods, the most common approach was surgical (27.8%, n=5); pulmonary endarterectomy, biopsy, right pneumectomy, transcatheter thrombolysis, and inferior vena cava filter, thrombectomy (5.6%, n=1) were other approaches.

The most likely diagnosis for both genders was PE (72.2%, n=13); however, only one male case (n=1) considered the possibility of PAS as a preliminary diagnosis (Table 2).

Comparing literature and LLM data for the preliminary diagnosis of PE, DeepSeek V3 (n=16, 88.89%), ChatGPT-4o (n=15, 83.33%), and Gemini Flash 2.5 (n=12, 66.67%) were the most successful LLM approaches (see Figures 2,3; Table 3).

Table 1. Distribution of PAS histopathological types according to gender

Histopathological type	Gender	
	Male (n,%)	Female (n,%)
Intimal-mural	5 (62.5)	7 (70.0)
Luminal	0	1 (10.0)
Undifferentiated sarcoma	0	1 (10.0)
Leiomyosarcoma	1 (12.5)	1 (10.0)
Rhabdomyosarcoma	1 (12.5)	0
Renal clear cell high-grade sarcoma	1 (12.5)	0
Total	8 (100)	10 (100)

PAS: Pulmonary artery sarcoma

Table 2. Distribution of preliminary diagnoses by gender

Preliminary diagnosis	Gender	
	Male (n,%)	Female (n,%)
PTE	5 (62.5)	8 (80.0)
Pulmonary stenosis	1 (12.5)	0
Chronic thromboembolic pulmonary hypertension	1 (12.5)	0
Pulmonary artery sarcoma	1 (12.5)	0
Pulmonary artery aneurysm	0	1 (10.0)
Malignancy and PTE	0	1 (10.0)
Total	8 (100)	10 (100)

PTE: Pulmonary thromboembolism

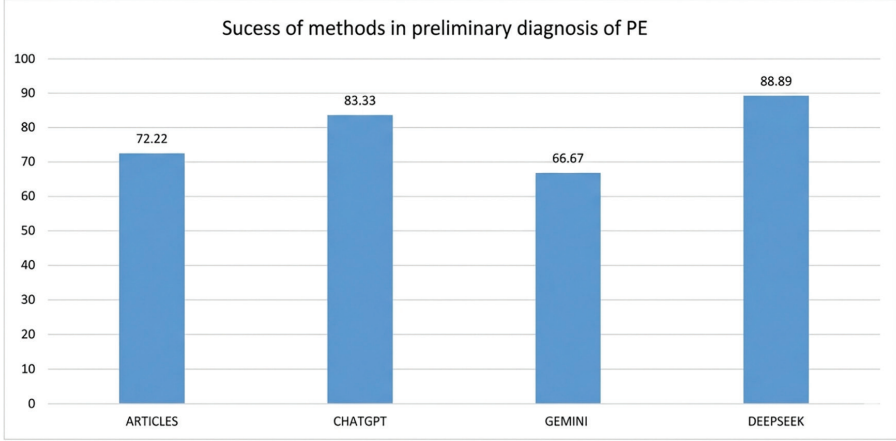

Figure 2. Success distribution of literature and LLM methods in the preliminary diagnosis of PE
LLM: Large language models, PE: Pulmonary embolise

The most successful LLM method for the preliminary diagnosis of PE and the final diagnosis of PAS as a result of the provided case information was Gemini Flash 2.5 (n=4, 22.22%), ChatGPT-4o (n=3, 16.67%), and DeepSeek V3 (n=2, 11.11%) (Figure 4).

DISCUSSION

PAS lesions are continuous, with rounded, bulged, or lobulated surfaces that protrude in the direction of blood flow. They form as tumor tissue accumulates, grows, and invades the surrounding tissues. Regarding filling deficiencies in the pulmonary trunk and pulmonary arteries, the computed tomography pulmonary angiography (CTPA) results for PE and PAS are comparable; nevertheless, the characteristics of each are different. When seen against the blood flow in CT scans, PE shows as cup-like formations. This may be the result of friction caused by blood flow at the surface clot, which is undergoing dissolution by the fibrinolytic system of the blood. Necrosis and bleeding may also occur in PAS, and contrast-enhanced CT can significantly enhance the signals from the blood vessels supplying the PAS tumor, which originates from the pulmonary arteries involved. As a result, the tumor signals in CTPA images are markedly intensified. In contrast, the emboli in patients with PE appear as filling defects with relatively uniform intensities[3]. In this study, the highest PAS detection success of LLMs was observed in Gemini Flash 2.5 (n=4/18, 22.22%).

The World Health Organization classifies PAS into 2 types: wall sarcoma, which is mainly smooth muscle sarcoma, and intimal sarcoma. The most common pathological type of PAS is undifferentiated sarcoma (34%), followed by fibrosarcoma (21%), smooth muscle sarcoma (20%), rhabdomyosarcoma (6%), mesenchymal histiocytoma (6%), intrachondral sarcoma (4%), angiosarcoma (4%), osteosarcoma (3%), and malignant fibrous histiocytoma (2%)[4]. Consistent with the literature, in the current study, the most frequently intimal-mural types were observed.

In this study, Gemini Flash 2.5 had the highest success rate for PAS final diagnosis, and DeepSeek V3 had the highest success rate for PE preliminary diagnosis based on the results of LLM approaches employing literature data. However, when prompts and disease information were supplied, no LMM technique was able to identify PAS as the initial tentative diagnosis. PE was taken into consideration initially, then infectious causes, and chronic thromboembolic pulmonary hypertension. On the other hand, based on the evidence surrounding the condition, there is research that suggests PAS as the initial tentative diagnosis[22]. This shows that although learning techniques, such as deep LLM, are thought to provide support and help in establishing the diagnosis in terms of time and cost, the importance of the human factor in diagnosis and the need for clinical management.

A common characteristic of the cases where model errors were concentrated was that they included individuals who resembled PE but did not have PET/CT (except for cases where PAS was suspected) and whose D-dimer levels were within normal ranges. This implies that LLMs show bias when faced with incomplete data and have poor clinical prediction in uncommon circumstances. Furthermore, notable variations in diagnostic success were noted between the models employed; ChatGPT-4o yielded more accurate results, particularly in cases with limited clinical findings and inadequate imaging support, whereas DeepSeek V3 and Gemini Flash 2.5 models demonstrated lower accuracy rates in comparable cases.

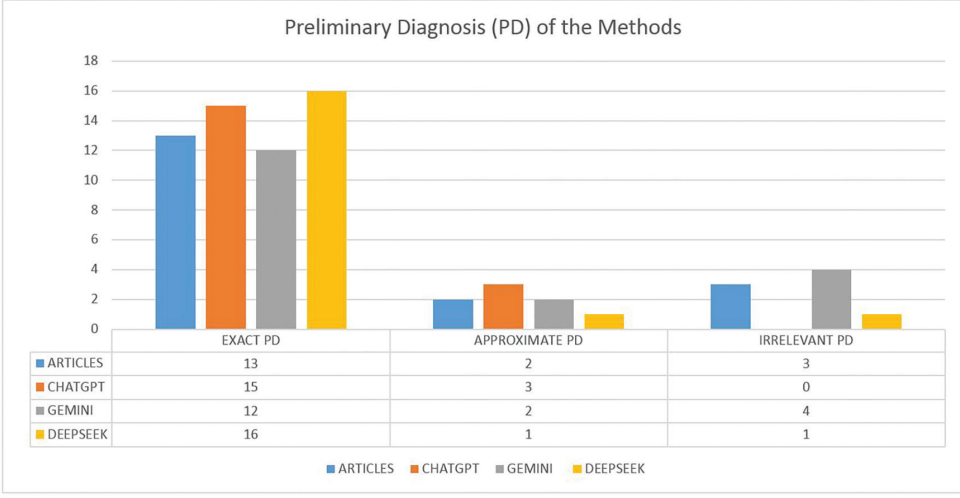

Figure 3. Distribution of LLM methods in the detection of PE preliminary diagnosis
LLM: Large language models, PE: Pulmonary embolise

Table 3. Matching and distribution of LLM methods in preliminary and final diagnosis

	CASE	CHATGPT (preliminary diagnosis)	Final diagnosis	Matching
ChatGPT-4o	CASE 1[2]	Pulmonary embolism	Pulmonary artery sarcoma	EXACT MATCH
	CASE 2[18]	Pulmonary embolism	Acute massive pulmonary embolism	APPROXIMATE MATCHING
	CASE 3[19]	Subsegmental pulmonary embolism	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 4[19]	Subacute or chronic pulmonary embolism	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 5[19]	Pulmonary embolism due to chronic thromboembolic pulmonary hypertension	Pulmonary embolism complicated by thrombosis of the main pulmonary artery and right pulmonary artery due to chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 6[19]	Pulmonary embolism	Massive pulmonary embolism	APPROXIMATE MATCHING
	CASE 7[19]	Chronic thromboembolic pulmonary hypertension	Bilateral pulmonary thromboembolism complicated by chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 8[22]	Pulmonary embolism	Massive pulmonary embolism	APPROXIMATE MATCHING
	CASE 9[7]	Pulmonary embolism	Massive pulmonary embolism	APPROXIMATE MATCHING
	CASE 10[17]	Pulmonary embolism compatible with bilateral pulmonary artery embolism	Bilateral massive pulmonary embolism complicated by chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 11[4]	Chronic thromboembolic pulmonary hypertension	Pulmonary artery sarcoma	EXACT MATCH
	CASE 12[9]	Pulmonary embolism	Pulmonary artery sarcoma	EXACT MATCH
	CASE 13[10]	Pulmonary embolism	Pulmonary arterial hemorrhage due to right interlobar pulmonary artery pseudoaneurysm	NO MATCH
	CASE 14[11]	Chronic pulmonary embolism	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 15[13]	Acute pulmonary embolism	Submassive acute pulmonary embolism	APPROXIMATE MATCHING
	CASE 16[12]	Pulmonary embolism	Central pulmonary embolism	APPROXIMATE MATCHING
	CASE 17[14]	Pulmonary hypertension assive pulmonary embolism compatible with a large filling defect in the main pulmonary artery	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 18[16]	Recurrent or persistent pulmonary thromboembolism.	Chronic thromboembolic pulmonary embolism causing pulmonary hypertension	APPROXIMATE MATCHING

Table 3. Continued

	CASE	Gemini (preliminary diagnosis)	Final diagnosis	Matching
Gemini Flash 2.5	CASE 1[2]	Pulmonary embolism	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 2[18]	Pulmonary hypertension	Acute proximal pulmonary embolism	APPROXIMATE MATCHING
	CASE 3[19]	Pulmonary hypertension	Pulmoner arteriyel hipertansiyon	NO MATCH
	CASE 4[19]	Acute or chronic pulmonary embolism	Pulmonary artery sarcoma	EXACT MATCH
	CASE 5[19]	Septic pulmonary embolism due to chronic thromboembolic pulmonary hypertension or infective endocarditis	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 6[19]	Acute massive pulmonary embolism	Acute massive pulmonary embolism	APPROXIMATE MATCHING
	CASE 7[19]	Pulmonary hypertension	Chronic thromboembolic pulmonary hypertension	NO MATCH
	CASE 8[22]	Pulmonary tuberculosis	Chronic pulmonary embolism resulting from pulmonary tuberculosis and showing findings compatible with lung infection	APPROXIMATE MATCHING
	CASE 9[7]	Pulmonary embolism	Acute pulmonary embolism	APPROXIMATE MATCHING
	CASE 10[17]	Pulmonary embolism with chronic dyspnea and acute deterioration after COVID-19	Pulmonary artery embolism	APPROXIMATE MATCHING
	CASE 11[4]	Pulmonary embolism	Atypical pulmonary embolism or primary pulmonary artery sarcoma	EXACT MATCH
	CASE 12[9]	Pulmonary embolism or cardiopulmonary pathology	Atypical pulmonary embolism or primary pulmonary artery sarcoma	EXACT MATCH
	CASE 13[10]	Pulmonary embolism	Saccular dilatation of the right pulmonary artery without significant intraluminal filling defect or obstruction, accompanied by moderate right pleural effusion, is an atypical pulmonary artery pathology.	NO MATCH
	CASE 14[11]	Pulmonary embolism	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 15[13]	Acute pulmonary embolism	Acute pulmonary embolism	APPROXIMATE MATCHING
	CASE 16[12]	Pulmonary embolism	Acute pulmonary embolism	APPROXIMATE MATCHING
	CASE 17[14]	Chronic thromboembolic pulmonary hypertension	Pulmonary artery sarcoma (PAS)	EXACT MATCH
	CASE 18[16]	Chronic thromboembolic pulmonary hypertension or recurrent pulmonary embolism	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING

Table 3. Continued

	CASE	DeepSeek (preliminary diagnosis)	Final diagnosis	Matching
DeepSeek V3	CASE 1[2]	Pulmonary embolism	Pulmonary artery sarcoma (PAS)	EXACT MATCH
	CASE 2[18]	Acute pulmonary embolism	Massive pulmonary embolism	APPROXIMATE MATCHING
	CASE 3[19]	Pulmonary embolism	Acute submassive pulmonary embolism	APPROXIMATE MATCHING
	CASE 4[19]	Pulmonary embolism	Chronic pulmonary embolism	APPROXIMATE MATCHING
	CASE 5[19]	Pulmonary embolism	pulmonary embolism	APPROXIMATE MATCHING
	CASE 6[19]	Acute pulmonary embolism	Acute pulmonary embolism	APPROXIMATE MATCHING
	CASE 7[19]	Massive pulmonary embolism	Massive pulmonary embolism	APPROXIMATE MATCHING
	CASE 8[22]	Pulmonary tuberculosis	Pulmonary tuberculosis	NO MATCH
	CASE 9[7]	Acute pulmonary embolism	Massive bilateral pulmonary embolism	APPROXIMATE MATCHING
	CASE 10[17]	Bilateral multiple pulmonary embolism	Acute right heart failure and pulmonary hypertension due to bilateral multiple pulmonary embolism	APPROXIMATE MATCHING
	CASE 11[4]	Chronic thromboembolic pulmonary hypertension	Pulmonary artery sarcoma (PAS)	EXACT MATCH
	CASE 12[9]	Pulmonary Embolism	Acute massive pulmonary embolism	APPROXIMATE MATCHING
	CASE 13[10]	Acute Pulmonary Embolism	Right pulmonary artery aneurysm	NO MATCH
	CASE 14[11]	Pulmonary Embolism	Chronic thromboembolic pulmonary hypertension	NO MATCH
	CASE 15[13]	Acute Pulmonary Embolism	Acute pulmonary embolism	APPROXIMATE MATCHING
	CASE 16[12]	Acute Pulmonary Embolism	Possible pulmonary embolism	APPROXIMATE MATCHING
	CASE 17[14]	Pulmonary Embolism	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING
	CASE 18[16]	Pulmonary embolism refractory to anticoagulant therapy	Chronic thromboembolic pulmonary hypertension	APPROXIMATE MATCHING

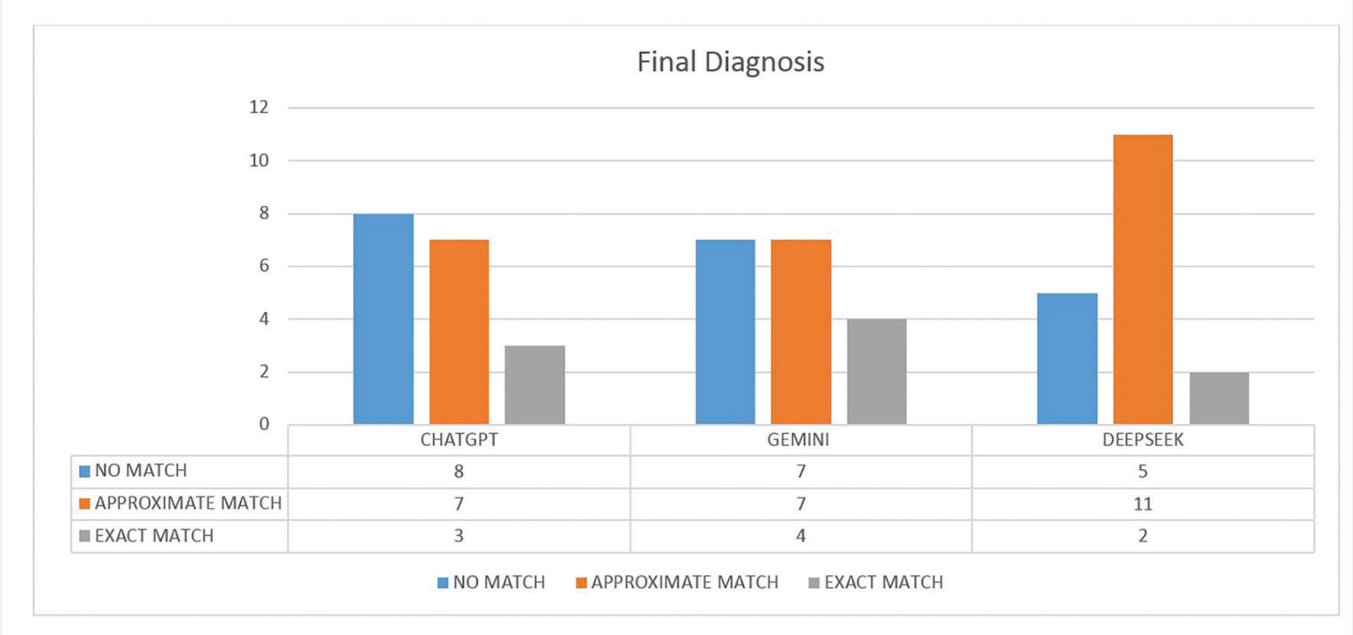

Figure 4. Final diagnosis (PAS) distribution in LLM methods
PAS: Pulmonary artery sarcoma, LLM: Large language models

Research in the literature makes a solid argument for medical practitioners using LLMs to assist clinical practice. Due to the possibility of patient injury, this use of LLMs also entails serious ethical concerns. It may become ethically required to use these technologies in clinical practice if these hazards are reduced by sufficient and trustworthy quality control procedures[5]. However, before this process, data processing methods must be transparent and auditable, and decision-making mechanisms must be structured in line with the principle of responsibility[23].

Despite their outstanding ability to generate responses based on vast amounts of data, LLMs have serious inherent limits when it comes to clinical decision-making. These limitations include a propensity to draw conclusions that defy medical common sense, poor performance in open-ended and ambiguous clinical scenarios, a high degree of confidence in their responses that does not always translate into accuracy, and a failure to perform well on rare conditions or those that are not sufficiently represented in the training data. These issues imply that LLMs should be applied cautiously and moderately in actual clinical settings, as they are caused by the models' rigid thinking and excessive dependence on previously observed patterns[24]. Furthermore, free and open-source models have greater performance limitations than premium and closed-source models, which often have better accuracy rates. As such, model selection should be carefully considered based on the context of use. It should be noted that LLMs are periodically updated by their developers, which may change their diagnostic behavior and output consistency over time. Therefore, reproducibility of results between different time points or model versions may not be guaranteed. LLMs could be used as auxiliary tools in clinical decision support systems, but only with the advice of an expert.

Technical proficiency alone is insufficient to integrate LLMs into clinical applications; a multidisciplinary strategy encompassing ethical principles, legal responsibilities, and regulatory approval procedures is also necessary. Legal liability may emerge when LLM-based decision support systems are used, especially in the healthcare industry, to safeguard patient privacy and consent rights. It is also well known that certain models incorporate user input and cues to enhance the model itself, which raises further moral and legal concerns around patient data privacy.

In a similar study, potential high-risk factors and precautions in cancer were investigated using literature data and LLM. 59 articles were included in the review and were categorized as quantitative studies on LLMs, chatbot-focused studies, and qualitative discussions on LLMs on cancer. Quantitative studies emphasize the advanced capabilities of LLMs in the field of natural language processing, while chatbot-focused articles reveal their potential in clinical support and data management. Qualitative research emphasizes the broader impacts of LLMs, including risks and ethical issues. As the results of the study, Quantitative studies suggest that LLMs may contribute to advancements in diagnostics and patient care, while chatbot-focused studies, particularly on ChatGPT-4o, indicate their potential utility in clinical support and patient communication. Conversely, qualitative analyses reveal concerns about ethics, data privacy, and the need for tailored models. The integration of LLMs in cancer research and healthcare presents a promising avenue for improving patient care[23].

Although LLMs provide valuable contributions in specific clinical contexts, their effectiveness may be restricted in rare diseases or cases with missing data. This circumstance demonstrates that artificial intelligence-only solutions are insufficient for decision support procedures. As a result, according to a comprehensive survey done across Europe, independent experts continue to obtain the greatest accuracy rates. However, models like GPT-4 can yield impressive outcomes, particularly in certain domains like organ estimates. In the study conducted by Saban et al.[25], it was emphasized that hybrid approaches using human expertise and artificial intelligence together can be one of the most effective methods in justifying imaging requests. Our study similarly demonstrates that LLMs should only be considered as supportive tools in clinical decision support processes and that final decisions should be made under expert supervision. Hybrid human-AI approaches, where LLMs are used together with expert supervision, are considered to be the most appropriate strategy for increasing diagnostic accuracy and safely optimizing clinical decision support processes.

Study Limitations

Data leakage is a potential limitation of our design because all evaluated cases were extracted from previously published literature. Therefore, some case narratives or key phrases may have appeared in the training corpora of the evaluated LLMs, and model outputs could partly reflect memorisation rather than clinical reasoning. To mitigate this risk, we provided only structured clinical summaries without author names, journal titles, or direct quotations, and we did not use any retrieval or browsing features. Nevertheless, the possibility of partial exposure cannot be fully excluded; thus, our results should be interpreted as reflecting performance in a real-world setting where models may have prior exposure to published case reports. Future work should include unpublished or prospectively collected cases, and/or synthetic cases with altered details to more rigorously quantify memorisation effects.

Additionally, the relatively small number of cases (n=18) and the single-run evaluation per case should be considered exploratory; future studies with larger, prospectively collected datasets and repeated runs per model to assess output stability are warranted.

CONCLUSION

The diagnoses given by the models were compared with human expert opinions and case results in the literature, and their potential in terms of accuracy, clinical consistency, and decision support systems was interpreted. In this context, the study presents an interdisciplinary evaluation questioning the usability of LLMs in diagnostic processes based on structured information in the field of health. Although artificial intelligence systems and LLM can be used to evaluate and improve the effectiveness of the diagnosis and treatment process, PAS should be considered in the differential diagnosis of PE cases that do not improve despite treatment, in addition to the patient's clinical history and current laboratory data.

Ethics

Ethical Committee Approval: Since this study utilized publicly available case data extracted from previously published literature and did not involve any direct patient contact or identifiable personal information, ethics committee approval was not required in accordance with institutional and international research guidelines.

Declaration on the use of Artificial Intelligence (AI): During the preparation of this work the author(s) used QuillBot AI in order to improve the readability and language of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Footnotes

Authorship Contributions

Concept: H.S., Design: H.S., M.A.Ş., Data Collection or Processing: H.S., Analysis or Interpretation: H.S., M.A.Ş., Literature Search: H.S., M.A.Ş., Writing: H.S., M.A.Ş.

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ORIGINAL ARTICLE

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Delirium in the Coronary Intensive Care Unit: Predictors and Prognostic Impact on Mortality

Koroner Yoğun Bakım Ünitesinde Deliryum: Belirleyiciler ve Mortalite Üzerindeki Prognostik Etkisi

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ABSTRACT

Aim: Delirium is a frequent acute neuropsychiatric condition in intensive care units and is associated with increased morbidity and mortality. However, prognostic factors among cardiac patients with delirium in the coronary intensive care unit (CICU) remain insufficiently defined. This study aimed to evaluate clinical characteristics and mortality-associated factors in delirious CICU patients.

Materials and Methods: In this single-center retrospective cohort study, 62 patients diagnosed with delirium during CICU hospitalization at Tekirdağ Namık Kemal University Hospital between January and December 2024 were analyzed. Demographic, clinical, laboratory, and treatment-related variables, as well as in-hospital and 6-month mortality outcomes, were evaluated.

Results: The mean age was 76.6±10.5 years, and 58.1% were female. Prolonged mechanical ventilation (p=0.012), respiratory tract infection (p=0.038), and central venous catheter use (p=0.036) were significantly associated with adverse in-hospital outcomes. Elevated blood urea nitrogen (BUN) (p=0.034), creatinine (p=0.007), lactate (p=0.012), decreased pH (p=0.026), and prolonged CICU stay (p=0.001) were significantly associated with in-hospital mortality. Six-month mortality was significantly associated with depression history (p=0.002), nursing home residence (p=0.002), morphine use (p=0.002), central venous catheterization (p=0.011), heart failure (p=0.003), elevated BUN (p=0.003), creatinine (p=0.007), lactate (p=0.012), and mechanical ventilation duration (p=0.049).

Conclusion: Among delirious CICU patients, mortality is closely associated with organ dysfunction markers, infection burden, invasive interventions, and psychosocial vulnerability. Delirium appears to reflect systemic clinical instability, underscoring the importance of comprehensive risk assessment and multidisciplinary management.

Keywords: Delirium, coronary intensive care, mortality, retrospective study

ÖZ

Amaç: Deliryum, yoğun bakım ünitelerinde sık görülen ve mortalite ile morbidite artışıyla ilişkili akut nöropsikiyatrik bir durumdur. Ancak koroner yoğun bakım ünitesinde (KYBÜ) deliryum gelişen kardiyak hastalarda mortalite ile ilişkili prognostik faktörler yeterince tanımlanmamıştır. Bu çalışmada, KYBÜ'de deliryum gelişen hastaların klinik özellikleri ve mortalite ile ilişkili faktörlerin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Tek merkezli, retrospektif tasarlanan bu araştırmaya, Tekirdağ Namık Kemal Üniversitesi Araştırma ve Uygulama Hastanesi KYBÜ'de Ocak-Aralık 2024 arasında yatarak izlenmiş ve deliryum tanısı konmuş 62 hasta dahil edilmiştir. Sosyodemografik veriler, laboratuvar bulguları, risk faktörleri, tedavi yaklaşımları, yatış süreleri, mortalite ve advers olaylar geriye dönük olarak analiz edilmiştir.

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Bulgular: Hastaların ortalama yaşı 76,6±10,5 yıl olup %58,1'i kadındı. Uzamış mekanik ventilasyon süresi (p=0,012), solunum yolu enfeksiyonu (p=0,038) ve santral venöz kateter kullanımı (p=0,036) hastane içi advers sonuçlarla anlamlı ilişkili bulundu. Yüksek kan üre azotu (BUN) (p=0,034), kreatinin (p=0,007), laktat (p=0,012), düşük pH (p=0,026) ve uzamış KYBÜ yatış süresi (p=0,001) hastane içi mortalite ile anlamlı ilişkiliydi. Altı aylık mortalite ise depresyon öyküsü (p=0,002), bakım evi öyküsü (p=0,002), morfin kullanımı (p=0,002), santral venöz kateterizasyon (p=0,011), kalp yetersizliği (p=0,003), yüksek BUN (p=0,003), kreatinin (p=0,007), laktat (p=0,012) ve mekanik ventilasyon süresi (p=0,049) ile ilişkili bulundu.

Sonuç: KYBÜ'de deliryum gelişen hastalarda mortalite; organ fonksiyon bozukluğu göstergeleri, enfeksiyon yükü, invaziv girişimler ve psikososyal kırılganlık faktörleri ile yakından ilişkilidir. Deliryum, sistemik klinik instabilitenin bir göstergesi olarak değerlendirilmelidir ve kapsamlı risk değerlendirmesi ile multidisipliner yönetim yaklaşımları önem taşımaktadır.

Anahtar Kelimeler: Deliryum, koroner yoğun bakım, mortalite, retrospektif çalışma

INTRODUCTION

Delirium is an acute mental status disorder observed in 20-50% of patients in intensive care units (ICUs)[1] and in up to 80% of those undergoing mechanical ventilation[2-4]. In cardiac surgery ICUs, the reported incidence of delirium ranges from 11.4% to 55%, depending on the diagnostic tool and study design used[2,5]. The association of delirium with increased mortality[3], prolonged hospital stays[6], extended mechanical ventilation duration[7], and elevated healthcare costs[8] underscores its critical importance in determining patient prognosis. Therefore, prevention, early detection, and effective treatment of delirium are of vital importance[5].

For many years, the diagnosis and management of delirium in both adult and pediatric ICU patients have been inadequately addressed[9-11]. However, in recent years, increased awareness of delirium has emerged in ICUs due to the development of more refined diagnostic criteria and the growing body of research on therapeutic interventions[12,13]. Nevertheless, knowledge regarding the impact of delirium on acute coronary syndrome (ACS) patients—especially those in cardiac intensive care units (CICUs)—remains limited[14]. Recent studies emphasize that delirium is a common comorbidity among CICU patients, significantly affecting survival outcomes and healthcare resource utilization[15]. Similarly, in cardiac surgery patients, delirium has been associated with higher mortality rates, prolonged hospitalization, reduced functional independence, and greater healthcare costs. Moreover, it has been identified as a strong predictor of 10-year mortality[16-18].

In this study, we aimed to retrospectively analyze the sociodemographic and clinical characteristics, contributing factors for delirium development, treatment approaches, and hospital length of stay among CICU patients who developed delirium and were consulted to the psychiatry department during their hospitalization. Additionally, by examining in-hospital, and 6-month mortality rates, this study goes beyond merely describing the prevalence and clinical features of delirium in critically ill cardiovascular patients in the CICU. It also aims to reveal the prognostic significance of delirium in this unique clinical setting. Ultimately, we seek to provide a more comprehensive insight into the impact of delirium in the context of cardiac intensive care.

MATERIALS AND METHODS

This study was conducted in the CICU of Tekirdağ Namık Kemal University Training and Research Hospital and was designed as a retrospective, descriptive, single-center study. It was approved by the Clinical Research Ethics Committee of Tekirdağ Namık Kemal University Faculty of Medicine on 31.12.2024, with the decision number 2024.326.12.09, and carried out in accordance with the ethical standards outlined in the Declaration of Helsinki.

The study included patients who were diagnosed with delirium during their CICU admission within the past year. Delirium diagnosis was established following psychiatric consultation based on clinical evaluation in accordance with the diagnostic and statistical manual of mental disorders, fifth edition criteria. Inclusion criteria were defined as the development of delirium during CICU hospitalization and the presence of complete clinical documentation in the hospital information management system.

Patients who died in the postoperative period after major cardiac surgery, individuals whose delirium diagnosis was ruled out by a psychiatrist, and those with pre-existing severe psychiatric disorders (e.g., schizophrenia, bipolar disorder, dementia) were excluded. Postoperative mortality may be driven by surgical complexity, transfusion requirements, and perioperative complications and could therefore confound the association between delirium and outcomes in non-surgical CICU patients. Severe psychiatric disorders were excluded due to diagnostic ambiguity in retrospective delirium assessment and the potential for misclassification.

The collected data included patients' demographic and clinical characteristics, laboratory parameters, cardiac risk factors (e.g., hypertension, diabetes mellitus, hyperlipidemia, smoking), predisposing and precipitating factors for delirium, medical treatments and medications administered, vital signs, invasive procedures performed, and admission diagnoses to the CICU. Additionally, CICU and total hospital length of stay, in-hospital adverse events, and in-hospital, and 6-month mortality rates were analyzed.

"In-hospital adverse events" were defined as any clinical complications that occurred during hospitalization and

negatively affected the patient's clinical course, resulting in additional treatment needs, prolonged length of stay, or increased risk of mortality—such as infections, catheter-related complications, respiratory failure, or hemodynamic instability.

Statistical Analysis

In descriptive statistics, for numerical variables, the number of observations (n), mean, standard deviation, median, interquartile range, minimum, and maximum values were calculated. For categorical variables, frequency (n) and percentage (%) distributions were reported. For continuous variables, the independent samples t-test was applied when data were normally distributed; otherwise, the Mann-Whitney U test was used.

Predictors of clinical outcomes were evaluated using univariable logistic regression analysis. Owing to the limited sample size and the low number of events, multivariable logistic regression analysis was not conducted. In the binary logistic regression models, the dependent variables were defined as in-hospital adverse events, in-hospital mortality, and 6-month mortality. Statistical significance was established at a two-sided p-value of <0.05. All statistical analyses were performed using SPSS software, version 24.0.

RESULTS

Baseline Characteristics

Data from a total of 62 patients were included in the study. Among the participants, 58.1% were female (n=36) and 41.9% were male (n=26). The mean age was 78.42±10.46 years for females and 74.15±10.46 years for males (p=0.114). The average length of hospital stay was 12.35±8.72 days, while the mean CICU stay was 6.61±4.85 days. Baseline characteristics of the study population was presented in Table 1.

Table 1. Baseline characteristics of the study population

Age, years	76.6±10.4
Sex, male, n (%)	26 (41.9)
Comorbidities, n (%)	
Hypertension	48 (77.4)
Diabetes mellitus	21 (33.9)
Coronary artery disease	39 (62.9)
Ischemic stroke	12 (19.4)
Malignancy	5 (8.1)
Cause of admission, n (%)	
Acute coronary syndrome	27 (43.5)
Arrhythmia	11 (17.7)
Heart failure	24 (38.7)
Laboratory parameters	
Hemoglobin, g/dL	11.2±1.6
Creatinine, mg/dL	1.3±0.6
ALT, U/L	220 (3-383)

ALT: Alanine aminotransferase

Impact of Infection and Mechanical Ventilation on Length of Hospital Stay

When examining the relationship between length of stay in both the CICU and the hospital and the factors predisposing and precipitating delirium, it was found that hospital stay was significantly prolonged in cases with longer duration of mechanical ventilation and the presence of respiratory system infections (p=0.012 and p=0.038, respectively). CICU length of stay was also significantly longer in the presence of other types of infections (p=0.026) (Table 2).

Predictors of Adverse Events and Mortality

A comprehensive analysis revealed several variables that were significantly associated with adverse clinical outcomes, including in-hospital, and 6-month mortality (Table 3). Specifically, length of ICU stay (p=0.002), presence of non-respiratory infections (p<0.001), elevated pCO_2 levels (p=0.031), history of smoking (p=0.031), and the presence of a central venous catheter (p=0.036) were significantly associated with the occurrence of adverse events and increased mortality risk.

Regarding in-hospital mortality, significant associations were observed with prolonged CICU stay (p=0.001), blood urea nitrogen (BUN)/(CRE) creatinine ratio (p=0.014), inotrope administration (p=0.018), acidemia (pH level) (p=0.026), elevated BUN (p=0.034), hypertension (p=0.040), total parenteral nutrition (TPN), gastrointestinal infections, history of depression, and residence in a nursing facility (each p=0.045).

For 6-month mortality, significant associations encompassed morphine use, TPN, history of depression, and nursing home residence (each p=0.002), as well as BUN (p=0.003), heart failure (p=0.003), serum CRE (p=0.007), fever (p=0.007), diuretic use (p=0.008), central venous catheterization (p=0.011), lactate level (p=0.012), coronary insufficiency (p=0.041), mechanical ventilation duration, and cerebrovascular disease (each p=0.049).

DISCUSSION

This study identifies a strong association between delirium and mortality in patients admitted to the CICU. Hypoxia, systemic inflammation, neurotransmitter imbalances, and organ dysfunction play major roles in the pathophysiology of delirium[1,3]. Among elderly patients in particular, the frequency and severity of delirium are critical factors that are associated with prognosis[19].

Table 2. Statistically significant variables associated with predisposing and precipitating factors for delirium in relation to LOS

Factor	n0	n1	Mean LOS, days (0)	Mean LOS, days (1)	Dif. (days)	p
Mechanical ventilation duration (hospital)	50	12	10.84	18.67	+7.83	0.011*
Infection-respiratory system (hospital)	53	9	11.23	19.00	+7.77	0.038*
Infection-other (CICU)	59	3	6.14	16.00	+9.86	0.025*

*p<0.05 was considered statistically significant, (0) indicates absence of the factor; (1) indicates presence of the factor. Mean LOS values are presented in days
LOS: Length of stay, CICU: Cardiac intensive care unit

Table 3. Significant variables associated with mortality and adverse outcomes

Variable	Adverse events (p)	In-hospital mortality (p)	6-month mortality (p)
ICU length of stay	0.002*	0.001*	
Infection-other	0.003*	<0.001*	
pCO_2	0.031*		
Smoking history	0.031*		
Central venous catheter	0.036*	0.021*	0.011*
BUN/creatinine ratio		0.014*	
Inotropes		0.018*	
pH		0.026*	
BUN		0.034*	0.003*
Hypertension		0.040*	
TPN		0.045*	0.002*
Infection-GIS		0.045*	
History of depression		0.045*	0.002*
Nursing home residency		0.045*	0.002*
Morphine			0.002*
Heart failure			0.003*
Creatinine			0.007*
Fever			0.007*
Diuretics			0.008*
Lactate			0.012*
Coronary insufficiency			0.041*
Duration of mechanical ventilation			0.049*
Cerebrovascular disease			0.049*

*p<0.05 was considered statistically significant, ICU: Intensive care unit, TPN: Total parenteral nutrition, GIS: Gastrointestinal system, BUN: Blood urea nitrogen

The study population was predominantly elderly, with a mean age of 76.6 years, which aligns with the well-established association between advanced age and increased vulnerability to delirium. In elderly cardiac patients, reduced physiological reserve, multimorbidity, and polypharmacy contribute to heightened susceptibility to acute cognitive disturbances.

No statistically significant difference in mean age was observed between female and male patients (p=0.114), indicating that age distribution was comparable between genders.

The high prevalence of hypertension, coronary artery disease, and diabetes mellitus reflects a typical elderly cardiac intensive care population. Likewise, the predominance of ACS and heart failure as admission diagnoses confirms that this cohort represents a clinically characteristic CICU setting. Therefore, the findings of the present study primarily apply to an elderly, multimorbid cardiac intensive care population.

In our study, variables such as mechanical ventilation duration, presence of infection, use of central venous catheters, and biochemical indicators of organ dysfunction (BUN, CRE, BUN/CRE ratio, pH, lactate) were significantly associated with both short- and long-term mortality. These findings are consistent with previously reported evidence demonstrating a significant association between delirium and increases mortality among CICU patients[14,15] as well as with results from multicenter studies. For instance, one study reported that in elderly CICU patients, delirium was associated with prolonged hospital stays and increased 30-day and 6-month mortality rates[20].

The significant association of infections (particularly non-respiratory and gastrointestinal) and mortality underscores the clinical relevance of infection-related complications in CICU patients. Central venous catheterization was significantly associated not only with all types of mortality but also with adverse in-hospital events. The literature similarly emphasizes that central venous catheters and other invasive procedures associated with an increased risk of delirium. One study demonstrated that such interventions are independent predictors of delirium incidence[21]. These findings likely reflect both the severity of the underlying illness and the susceptibility to procedure-related complications.

The significant association of depression and nursing home residency with long-term mortality highlights the potential relevance of psychosocial factors in prognostic assessments[17,18]. In our study, both depression and nursing home residency emerged as significant predictors of in-hospital and 6-month mortality. These findings indicate that, in addition to conventional clinical and procedural risk factors, social vulnerability and pre-existing mental health conditions may substantially influence short- and intermediate-term outcomes among patients admitted to the CICU who develop delirium.

The observed association between morphine use and increased 6-month mortality should be interpreted with caution. In the CICU setting, opioid administration often reflects greater disease severity, advanced cardiac conditions, or increased symptom burden, rather than a direct causal effect. In addition, opioids may contribute indirectly to adverse outcomes through side effects such as sedation, impaired attention, and reduced level of consciousness, which are recognized risk factors for delirium. Therefore, morphine use may represent both a marker of illness severity and a potential contributor to a more vulnerable clinical state associated with poorer long-term outcomes.

The selection of in-hospital and 6-month mortality as outcome measures was intended to reflect both early and intermediate-term prognostic associations related to delirium. In-hospital mortality is commonly used in intensive care research as an early outcome indicator associated with acute disease severity and in-hospital complications. Six-month mortality, however, provides a broader perspective on sustained clinical outcomes beyond the acute phase and has been reported to reflect longer-term prognostic patterns in critically ill and cardiac intensive care populations with delirium. These follow-up intervals are consistent with prior studies evaluating delirium-related outcomes in intensive care settings[3,20].

The strong association between CICU length of stay and mortality suggests that delirium may be associated with a more complicated clinical course, potentially contributing to prolonging hospitalization. Delirium has been associated with delays in treatment delivery, interruptions in diagnostic procedures, and a general slowdown in the recovery process[6,7]. These findings are consistent with previous studies and highlight the potential value of multidisciplinary strategies focused on prevention and early recognition of delirium to reduce both mortality and morbidity[12,14].

Non-pharmacological interventions play a central role in delirium prevention and management, particularly in critically ill populations. Multicomponent strategies—including early mobilization, maintenance of normal sleep-wake cycles, adequate pain control, orientation protocols, and optimization of the ICU environment—have been emphasized in recent critical care guidelines as effective approaches to reduce delirium risk and support recovery[13]. In the CICU, the implementation of proactive, multidisciplinary non-pharmacological prevention protocols may contribute to improved clinical outcomes by mitigating delirium-related complications and supporting overall recovery.

Study Limitations

This study has several limitations. First, its single-center, retrospective design limits the generalizability of the findings. Reliance on retrospective chart reviews and psychiatric consultation notes for the diagnosis of delirium, without the routine use of standardized screening instruments, may have introduced diagnostic variability and limited detection sensitivity.

Second, delirium subtypes (hypoactive vs. hyperactive) were not differentiated. Given the consultation-based sampling strategy, hypoactive delirium cases—being less clinically overt—may have been underrecognized and underrepresented.

Third, the exclusion of postoperative deaths and patients with severe pre-existing psychiatric disorders may have reduced the representativeness of the study population and potentially underestimated the overall burden of delirium in broader CICU settings. However, these exclusions were implemented to minimize diagnostic ambiguity and confounding factors related to surgical complexity and baseline psychiatric symptomatology.

Furthermore, a key limitation of our study is the relatively small sample size, particularly within certain subgroups where the number of patients was very limited, which may reduce the statistical power, increase the risk of type II error, and necessitate cautious interpretation of the observed associations and significance levels. Additionally, the relatively small sample size may have limited the statistical power of subgroup analyses, and variability in treatment approaches may further complicate interpretation of the results.

Prospective, multicenter studies with standardized delirium assessment tools and uniform treatment protocols are needed to confirm and expand upon these findings. Notably, a prospective study addressing these limitations is currently underway at our institution. Future studies with prospective, multicenter designs and standardized diagnostic and treatment protocols will be instrumental in overcoming these limitations.

CONCLUSION

Delirium represents a serious clinical concern associated with both short- and long-term mortality in CICU patients. Early diagnosis, vigilant monitoring of risk factors, and timely therapeutic interventions may reduce mortality. Close surveillance of delirium risk factors and the implementation of preventive strategies have the potential to significantly improve patient outcomes.

Ethics

Ethical Committee Approval: It was approved by the Clinical Research Ethics Committee of Tekirdağ Namık Kemal University Faculty of Medicine on 31.12.2024, with the decision number 2024.326.12.09, and carried out in accordance with the ethical standards outlined in the Declaration of Helsinki.

Informed Consent: This study was retrospective, descriptive, single-center study.

Footnotes

Authorship Contributions

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

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ORIGINAL ARTICLE

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Evaluation of Children and Adolescents Receiving Transcranial Magnetic Stimulation Treatment at a University Hospital

Bir Üniversite Hastanesinde Transkraniyal Manyetik Stimülasyon Tedavisi Alan Çocuk ve Ergenlerin Değerlendirilmesi

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ABSTRACT

Aim: This study presents the first clinical data from Türkiye on the use of transcranial magnetic stimulation (TMS) in treating psychiatric disorders among children and adolescents. This study aimed to evaluate clinical outcomes and the safety of TMS in treatment-resistant child and adolescent psychiatric cases retrospectively.

Materials and Methods: Medical records of 23 patients who received TMS between April 2015 and October 2024 at the Department of Child and Adolescent Psychiatry, Pamukkale University Faculty of Medicine, were reviewed. Demographic and clinical variables-including age, sex, diagnosis, comorbidities, medication use, applied TMS protocol, and Clinical Global Impression-Severity (CGI-S) and Improvement (CGI-I) scores were analyzed.

Results: The mean age was 15.65±1.64 years, and 69.6% were male. The most frequent diagnoses were obsessive-compulsive disorder [(OCD); 39.1%] and depressive disorder (30.4%). Diagnosis-specific protocols targeted distinct brain regions and frequencies. Among patients with available CGI-I data (n=18), marked improvement was observed in 38.9% of cases, mild improvement in 38.9%, and no significant change in 22.2%. Among patients with depression, 60% showed marked improvement, compared with 25% of those with OCD. Mild, transient side effects (headache, visual dimming) occurred in 13% of patients.

Conclusion: TMS appears to be a safe and well-tolerated adjunctive treatment option and was associated with clinical improvement in a proportion of children of children and adolescents with partially or fully treatment-resistant psychiatric disorders. These findings represent the first national data on pediatric TMS use in Türkiye and underscore the need for larger, prospective, and controlled studies to confirm its efficacy and optimize treatment protocols.

Keywords: Transcranial magnetic stimulation, adolescent, depressive disorder, obsessive-compulsive disorder, treatment resistance

ÖZ

Amaç: Bu çalışma, çocuk ve ergen psikiyatrik bozukluklarının tedavisinde transkraniyal manyetik stimülasyon (TMS) kullanımına ilişkin Türkiye'de bildirilen ilk klinik verileri sunmaktadır. Bu araştırmada, tedaviye dirençli çocuk ve ergen psikiyatri olgularında TMS uygulamalarının etkinlik ve güvenilirliğinin retrospektif olarak değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Pamukkale Üniversitesi Tıp Fakültesi Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Anabilim Dalı'nda Nisan 2015-Ekim 2024 tarihleri arasında TMS uygulanan 23 olgunun tıbbi kayıtları incelenmiştir. Yaş, cinsiyet, tanı, ek tanılar, ilaç kullanımı, uygulanan TMS protokolü ile Klinik Global İzlem-Şiddet (KGİ-Ş) ve KGİ- İyileşme (KGİ-İ) puanlarını içeren demografik ve klinik değişkenler değerlendirilmiştir.

Bulgular: Olguların yaş ortalaması 15,65±1,64 yıl olup, %69,6'sı erkeklerden oluşmaktaydı. En sık görülen tanılar obsesif-kompulsif bozukluk [(OKB); %39,1] ve depresif bozukluk (%30,4) idi. Tanıya özgü protokoller, farklı beyin bölgelerini ve frekanslarını hedef almıştır. CGI-I verileri mevcut olan hastalar arasında (n=18), olguların %38,9'unda belirgin iyileşme, %38,9'unda hafif düzeyde iyileşme gözlenmiş, %22,2'sinde ise anlamlı bir değişiklik gözlenmemiştir. Depresyon tanılı olguların %60'ında belirgin iyileşme saptanırken, OKB olgularında bu oran %25 olarak bulunmuştur. Yan etki oranı %13 olup, gözlenen yan etkiler hafif ve geçici nitelikte (baş ağrısı, geçici görme kararması) seyretmiştir.

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Sonuç: TMS, çocuk ve ergenlerde kısmen veya tamamen tedaviye dirençli psikiyatrik bozukluklarda güvenilir, iyi tolere edilen ve potansiyel olarak etkili tamamlayıcı bir tedavi seçeneği olarak görünmektedir. Bu çalışma, Türkiye'de pediatrik TMS uygulamalarına ilişkin bildirilen ilk ulusal verileri sunmakta olup, etkinliğin doğrulanması ve tedavi protokollerinin optimize edilmesi amacıyla geniş örneklemli, prospektif ve kontrollü araştırmalara ihtiyaç olduğunu göstermektedir.

Anahtar Kelimeler: Transkraniyal manyetik stimülasyon, ergen, depresif bozukluk, obsesif-kompulsif bozukluk, tedavi direnci

INTRODUCTION

The global prevalence rate of mental disorders in children and adolescents increases with age, reaching 6.8% for ages 5-9, 12.4% for ages 10-14, and 13.96% for ages 15-19[1]. These disorders not only impair individual and familial functioning but are also associated with serious social consequences such as high school dropout rates, reduced economic productivity, legal problems, suicide, and homelessness[2,3]. In recent decades, significant advances have been made in the field of psychopharmacology for the treatment of these disorders. However, pharmacological monotherapy fails to achieve adequate therapeutic effects in some cases, and concerns regarding side-effect profiles and long-term safety have increased the demand for alternative and integrative treatment modalities[4].

Transcranial magnetic stimulation (TMS) is a non-invasive neurostimulation technique that modulates brain tissue activity through the application of magnetic fields[5]. Its repetitive form, known as repetitive TMS (rTMS), has the capacity to induce neural changes that persist beyond the stimulation period[6]. The therapeutic effects are generally achieved through cumulative sessions, during which neuroplasticity-based lasting alterations are induced within cortical networks[7,8]. Depending on the applied frequency, high-frequency (>5 Hz) protocols exert excitatory effects, whereas low-frequency (≤1 Hz) protocols produce inhibitory effects[9,10].

In adult populations, the therapeutic efficacy of TMS has been extensively investigated across various psychiatric disorders, including major depressive disorder (MDD), obsessive-compulsive disorder (OCD), bipolar disorder, and schizophrenia. Randomized controlled trials have demonstrated the efficacy of TMS, particularly in treatment-resistant MDD and OCD, leading to its approval by the US Food and Drug Administration (FDA) for these indications[11,12].

In contrast, studies investigating the use of TMS in child and adolescent psychiatry remain limited. This scarcity can be attributed to factors such as developmental differences in neuroplasticity, uncertainties regarding safety and tolerability, ethical constraints, and methodological challenges in achieving adequate sample sizes[13-15]. Nevertheless, recent research has suggested that TMS may contribute to clinical improvement across a range of diagnoses, including MDD, OCD, autism spectrum disorder (ASD), Tourette's disorder, and attention-deficit/hyperactivity disorder and is generally considered a safe intervention[15-18]. As a result of these advances, the use of specific TMS devices in adolescents aged 15 years and older with depressive disorder has recently been approved by the FDA[19].

TMS protocols vary according to the targeted anatomical region and stimulation frequency, depending on the psychiatric disorder being treated. The left dorsolateral prefrontal cortex (DLPFC) is commonly targeted in depressive and anxiety disorders. In contrast, the right DLPFC or the supplementary motor area (SMA) are preferred in OCD and tic disorders[17]. For ASD interventions, stimulation sites have included not only the DLPFC but also social cognition-related networks such as the medial prefrontal cortex (mPFC) and temporoparietal regions[20]. However, in pediatric populations, the standardization of parameters such as stimulation site, dosage, and session number has not yet been achieved. The existing literature reports substantial heterogeneity among protocols and a lack of consensus regarding standardized procedures[16-18,20].

Systematic data on the use of TMS in the field of child and adolescent psychiatry in Türkiye are minimal, and no studies specifically focusing on this topic have been identified in the national literature. This gap highlights the importance of sharing clinical experiences within the country, both to contribute to the establishment of a national database and to inform international efforts in protocol development. In this context, the present study aims to retrospectively examine pediatric cases that received TMS treatment at the Department of Child and Adolescent Psychiatry, Pamukkale University Faculty of Medicine, and to evaluate the observed patterns of clinical response in light of the existing literature.

MATERIALS AND METHODS

This descriptive study retrospectively examined cases that underwent TMS treatment at the Department of Child and Adolescent Psychiatry, Pamukkale University Faculty of Medicine Hospital, between April 1, 2015, and October 1, 2024.

The study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (decision number: 17, approval date: 02.10.2024).

Participants

A total of 24 children and adolescents who received TMS treatment during the specified period were identified. One case was excluded from the study because the treatment protocol was discontinued after the first session (this patient, diagnosed with ASD, was unable to adapt to the clinical setting and exhibited marked psychomotor agitation). Consequently, a total of 23 children and adolescents were included in the final analysis.

The study included cases aged between 12 and 18 years who had completed clinical evaluations both before and after TMS treatment and met the diagnostic criteria for at least one psychiatric disorder according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. Five cases for which Clinical Global Impression-Severity, (CGI) data were unavailable were excluded only from the analyses of clinical treatment response. Pharmacological treatment regimens remained stable throughout the TMS intervention period.

Data Collection

Demographic and clinical data, including participants' age, sex, primary diagnosis, comorbid conditions, medication use, TMS treatment protocol, targeted brain region, and scores on the CGI-Severity (CGI-S) and CGI-Improvement (CGI-I) scales, were retrospectively obtained from patient medical records.

Clinical Global Impression Scale

CGI consists of three general measures. The CGI-S illness severity measure is rated on a scale from 1 (normal, not ill at all) to 7 (the most severely ill among patients). If the patient has not been evaluated, a score of "0" is assigned. CGI-S is rated at admission (CGI-S adm) and at discharge (CGI-S dis). The overall CGI improvement measure is rated from 1 (very much improved) to 7 (much worse). Again, "0" means "not evaluated." CGI-I was only rated at discharge. The third measure, called the CGI-efficacy index, was not evaluated in the current study[21,22].

TMS Treatment

rTMS was administered at the rTMS unit of Pamukkale University Psychiatry Hospital by a psychiatric nurse certified in TMS application, using a Neuro-MS/D device (Neurosoft Ltd., Russia) equipped with an angled figure-of-eight coil.

During the first session, the motor threshold was determined. For this purpose, stimuli of gradually increasing intensity were delivered approximately 5 cm lateral to the vertex along the interaural line, and involuntary finger movements were observed on the contralateral side. If no visible muscle contraction was elicited at the coil position used to determine the threshold, the coil was slightly adjusted within negligible distances to optimize the response. rTMS sessions were conducted according to diagnosis-specific target brain regions and frequency parameters. Clinical status and potential adverse effects were evaluated before and after each session. The specific protocols applied are summarized below:

Depression Protocol (Left DLPFC): Stimulation was delivered at 120% of the individual motor threshold with a frequency of 10 Hz. A total of 75 trains, each consisting of 40 pulses, were administered with an inter-train interval of 11 seconds, resulting in 3,000 pulses per session. The average duration of each session was approximately 18.3 minutes.

Tic Disorder Protocol (Bilateral SMA): Stimulation was delivered at 120% of the individual motor threshold with a frequency of 1 Hz. A total of 20 trains, each consisting of 60 pulses, were administered with an inter-train interval of 2 seconds, resulting in a total of 1,200 pulses per session. The average session duration was approximately 20 minutes.

OCD and Auditory Hallucination Protocols [mPFC; Orbitofrontal Cortex (OFC); Left Temporoparietal Junction: stimulation was administered at 110% of the individual motor threshold with a frequency of 1 Hz. Each session consisted of a single train comprising 1,000 pulses, with an inter-train interval of 1 second. The average session duration was approximately 16.4 minutes.

All protocols were planned to include 20 sessions, conducted three times per week. Clinical status and potential adverse effects were evaluated before and after each session.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics, version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were reported as mean ± standard deviation for continuous variables and as frequencies and percentages for categorical variables. The distributions of categorical variables, such as sex, diagnostic group, presence of comorbidity, treatment type, and applied TMS protocol, were examined using the chi-square test; when more than 20% of the expected cell frequencies were below five, Fisher's exact test was applied. Clinical severity was assessed using the CGI-S scale, whereas post-TMS clinical improvement was classified according to scores on the CGI-I scale. Comparisons of clinical response rates (marked or very marked improvement) between diagnostic groups were also conducted using the chi-square or Fisher's exact test as appropriate. A p-value <0.05 was considered statistically significant.

RESULTS

Of the participants, 16 were male (69.6%), and 7 were female (30.4%), with ages ranging from 12 to 18 years (mean =

15.65±1.64 years). Nine patients (39.1%) received TMS as outpatients, 10 (43.5%) during inpatient treatment, and 4 (17.4%) received TMS during both inpatient and outpatient treatment periods.

The Diagnostic Distribution of the Sample (n=23) was as Follows: 39.1% OCD (n=9); 30.4% depressive disorder (n=7), 13.0% Tourette's disorder (n=3), 8.7% psychotic disorder (n=2), 4.3% bipolar disorder (n=1), and 4.3% tic disorder (n=1). Comorbid psychiatric diagnoses were present in 13 participants (56.5%).

All cases received rTMS treatment as an adjunct to their ongoing pharmacotherapy. Among the 21 patients for whom medication data were available, all were found to be receiving multiple psychotropic medications. Treatment protocols were determined based on the primary diagnosis and, in some cases, tailored to target specific primary symptoms. The specific protocols applied are detailed in Table 1.

DLPFC (n=8): A total of eight patients (34.7%) received a high-frequency (10 Hz) protocol targeting the left DLPFC. Of these, seven had a primary diagnosis of depressive disorder, while one patient with psychotic disorder received stimulation targeting treatment-resistant negative/depressive symptoms.

OCD Protocol (n=12): Twelve patients (52.2%) underwent a low-frequency (1 Hz) protocol targeting the mPFC (n=6), OFC (n=5), or SMA (n=1). Among these, nine had a primary diagnosis of OCD, one had bipolar disorder (targeting OCD symptoms), and two had Tourette's disorder (targeting OCD symptoms).

Tic Protocol (n=2): Two patients (8.7%) received a low-frequency (1 Hz) bilateral SMA protocol. One of these had Tourette's disorder (targeting tics), and the other was diagnosed with tic disorder.

Auditory Hallucination Protocol (n=1): One patient (4.4%) with psychotic disorder received a low-frequency (1 Hz) protocol targeting the left temporoparietal junction for treatment-resistant auditory hallucinations (Table 1).

Examination of the participants' CGI-S scores revealed that clinical severity levels ranged from 4 to 7. Specifically, 4.4% (n=1) of participants were rated as moderately ill, 30.4% (n=7) as moderately ill, 30.4% (n=7) as markedly ill, and 34.8% (n=8) as severely ill. These findings indicate that, overall, the sample exhibited moderate-to-severe levels of clinical symptomatology (Table 2).

Based on CGI-I scores among patients with available outcome data (n=18), 38.9% (n=7) of participants showed marked improvement, 38.9% (n=7) showed mild improvement, and 22.2% (n=4) demonstrated no clinically significant change. These results indicate that following TMS treatment, a substantial proportion of participants experienced either marked or partial clinical improvement (Table 3). Given the very small sample sizes within certain diagnostic subgroups, these percentages should be interpreted with caution.

Among patients diagnosed with depressive disorder, 60.0% (n=3) showed marked improvement, 20.0% (n=1) showed mild improvement, and 20.0% (n=1) exhibited no change. In the OCD group, 25.0% (n=2) demonstrated marked improvement, 37.5% (n=3) mild improvement, and 37.5% (n=3) no change. Among those diagnosed with Tourette's disorder, 50.0% (n=1) showed marked improvement and 50.0% (n=1) mild improvement. In patients with psychotic disorders (treatment-resistant auditory hallucinations and treatment-resistant negative/depressive symptoms), mild improvement was observed in 100.0% (n=2). The patient diagnosed with tic disorder showed marked improvement (100.0%, n=1).

Overall, adverse effects were reported in three participants (13%). Two participants experienced mild headache and one reported transient visual dimming.

DISCUSSION

In this retrospective study, 23 children and adolescents who received rTMS treatment were evaluated in terms of their clinical characteristics, stimulation protocols, and treatment

Table 1. Target brain regions, stimulation protocols, and clinical indications of rTMS treatment

Target brain region	Stimulation protocol (frequency)	Diagnostic indication	n	%	Number of sessions
Left DLPFC	High-frequency rTMS (10 Hz)	Depressive disorder	8	34.7	20
mPFC	Low-frequency rTMS (1 Hz)	OCD	6	26.1	20
OFC	Low-frequency rTMS (1 Hz)	OCD	5	21.7	20
Bilateral SMA	Low-frequency rTMS (1 Hz)	Tic disorders/Tourette syndrome	2	8.7	20
Bilateral SMA	Low-frequency rTMS (1 Hz)	OCD	1	4.3	20
Left temporoparietal junction	Low-frequency rTMS (1 Hz)	Treatment-resistant auditory hallucinations	1	4.3	20

DLPFC: Dorsolateral prefrontal cortex, mPFC: Medial prefrontal cortex, OFC: Orbitofrontal cortex, SMA: Supplementary motor area, OCD: Obsessive-compulsive disorder, rTMS: Repetitive transcranial magnetic stimulation

Table 2. Distribution of clinical global impression (CGI) scores

	n	%
CGI-S		
4 (moderately ill)	1	4.4
5 (markedly ill)	7	30.4
6 (severely ill)	7	30.4
7 (among the most extremely ill patients)	8	34.8
Total	23	100
CGI-I		
2 (much improved)	7	38.9
3 (minimally improved)	7	38.9
4 (no change)	4	22.2
Total	18	100

CGI-S: Clinical Global Impression-Severity, CGI-I: Clinical Global Impressionimprovement, CGI-I scores were available for 18 patients; percentages were calculated based on these available data; n: Number of cases, %: Percentage

Table 3. CGI-I scores by diagnosis

Diagnosis	CGI-I score	n	%
Depressive disorder	2 (much improved)	3	60.0
	3 (minimally improved)	1	20.0
	4 (no change)	1	20.0
OCD	2 (much improved)	2	25.0
	3 (minimally improved)	3	37.5
	4 (no change)	3	37.5
Tourette syndrome	2 (much improved)	1	50.0
	3 (minimally improved)	1	50.0
Psychotic disorder	3 (minimally improved)	2	100.0
Tic disorder	2 (much improved)	1	100.0

CGI-I: Clinical Global Impression-Severity, OCD: Obsessive-compulsive disorder, n: Number of cases; %: Percentage. Percentages for diagnostic subgroups with very small sample sizes should be interpreted with caution

outcomes. The majority of participants presented with moderate-to-severe psychiatric disorders, with OCD and depressive disorder being the most common diagnoses. TMS protocols were tailored according to diagnosis-specific target brain regions and stimulation frequencies. Following treatment, approximately 39% of cases demonstrated marked clinical improvement, while reported adverse effects were mild and transient in nature.

The literature indicates that rTMS has increasingly been utilized in the treatment of various psychiatric disorders among children and adolescents; however, it is most commonly regarded as an adjunctive therapeutic option for treatment-resistant cases, particularly in depressive disorders where pharmacotherapy has yielded inadequate or only partial responses[15,23,24]. The ability of rTMS to modulate cortical excitability and neuronal plasticity offers a novel therapeutic avenue for treatment-resistant cases in which pharmacological interventions are ineffective. Indeed, recent clinical studies conducted on adolescents diagnosed with treatment-resistant MDD and OCD have consistently demonstrated that TMS administration yields significant clinical improvements in this population despite ongoing pharmacotherapy[23-26]. All cases included in our study consisted of patients who exhibited moderate-to-severe clinical symptoms and had shown insufficient response to ongoing psychopharmacological treatments. The recorded improvements reflected in the CGI scores were consistent with findings reported in the existing literature. This observation supports the potential benefit of TMS as a therapeutic option in treatment-resistant cases.

The TMS protocols applied in our study were designed to target brain regions identified in the literature as disorder-specific. For instance, in cases of depressive disorder, high-frequency (10 Hz) stimulation was directed to the left DLPFC, consistent with the general principle of enhancing cortical excitability in this region, which has been frequently shown to exhibit hypoactivity in treatment-resistant depression[15,16] . Activation of the DLPFC has been shown to produce significant clinical improvement in depressive symptoms through multiple neurobiological mechanisms, primarily by modulating limbic-prefrontal connections associated with emotional regulation, motivation, and executive functioning[27].

In contrast, low-frequency (1 Hz) stimulation was applied to the mPFC and OFC regions in patients diagnosed with OCD. The selection of these regions and stimulation frequency was aimed at reducing the hyperactivity within the cortico-striato-thalamo-cortical circuit, which is known to play a central role in the pathophysiology of OCD[7,15,17]. Given the inhibitory effects of low-frequency (1 Hz) stimulation on cortical activity, the 1 Hz protocol applied to the mPFC and OFC can be considered to target the neurophysiological basis of compulsive behaviors by suppressing excessive activation within this circuit[9]. In one OCD case, low-frequency (1 Hz) stimulation was applied to the bilateral SMA, based on findings reported in previous studies in the literature[28,29]. Considering that standardized stimulation protocols have not yet been established in the child and adolescent population and that stimulation parameters have been reported heterogeneously across studies, the targeting of different brain regions in our study reflects clinicians' ongoing efforts to develop disorder-specific, circuit-based treatment protocols[16,18]. This variability also indicates that a universally accepted "gold standard" protocol with proven efficacy for this age group has yet to be established.

In our study, adverse effects were observed in 13% (n=3) of cases, consisting of mild headache (n=2) and transient visual dimming (n=1). No adverse events requiring discontinuation of treatment were observed. In addition, no serious side effects were detected after the intervention.

Recent meta-analyses have characterized TMS as a generally well-tolerated neuromodulation technique in children and adolescents, with no reports of serious adverse effects. An umbrella review conducted by Santos et al.[18] summarized more than 40 randomized controlled trials on the use of rTMS and Transcranial Direct Current Stimulation in pediatric populations and reported a rate of serious adverse events below 1%. Similarly, Gallop et al.[16], in their systematic review including 19 clinical studies, reported that the most frequently observed adverse effects of rTMS were transient headache, dizziness, and discomfort at the stimulation site, emphasizing that these effects were generally mild and short-lived. Findings summarized by El-Shahawy et al.[17] also indicate that rTMS applications in child and adolescent populations exhibit a safety profile comparable to that observed in adults, with no reports of serious adverse effects such as seizures, syncope, or long-term neurological complications. Our findings are consistent with the general safety profile reported in the literature. Although, in theory, increased neurophysiological sensitivity has been suggested in children and adolescents due to heightened brain plasticity, current evidence indicates that TMS is a safe and well-tolerated intervention when administered under appropriate clinical conditions and stimulation parameters. Nevertheless, given that long-term safety data remain limited, prospective follow-up studies are warranted.

Study Limitations

Several limitations should be considered when interpreting the findings of this study. First, as the study was retrospective and descriptive in design, the observed clinical improvements cannot be directly attributed to TMS treatment. However, the consistency of our results with the meta-analytic evidence discussed above reinforces their clinical relevance. The absence of a control group represents another limitation, as it precludes evaluation of potential placebo effects or the contribution of concurrent psychopharmacological treatments. Nonetheless, the fact that TMS was primarily administered to patients with treatment-resistant and severe clinical presentations supports the clinical significance of the improvements observed.

The relatively small sample size (n=23) and the heterogeneous diagnostic distribution limit the possibility of conducting statistical comparisons and reduce the generalizability of the findings. In addition, clinical response was assessed solely using the CGI scales. Due to the retrospective design, diagnosis-specific standardized rating scales (e.g., Yale-Brown Obsessive Compulsive scale for OCD or Children's Depression Rating Scale-Revised depressive disorders) were not systematically available. Future prospective studies should incorporate disorder-specific measures to allow a more precise evaluation of treatment response. The observational and subjective nature of CGI-based evaluations does not capture corresponding changes at the neuropsychological or neurophysiological level. For these reasons, the current findings should be regarded as preliminary data. Prospective, controlled studies with larger sample sizes conducted in Türkiye will be essential to more clearly delineate the efficacy and safety profile of TMS in children and adolescents.

CONCLUSION

To the best of our knowledge, this study provides the first clinical data from Türkiye regarding the use of TMS in child and adolescent psychiatry. Our findings suggest that TMS may represent a feasible and well-tolerated adjunctive treatment option, particularly for patients showing partial resistance to pharmacotherapy. The observed rates of partial or marked improvement in depressive and obsessive-compulsive symptoms also highlight the potential clinical relevance of circuit-based neuromodulation approaches in this population.

Nevertheless, further well-designed prospective studies conducted in Türkiye are needed to better clarify the efficacy of TMS and to determine optimal stimulation parameters in pediatric populations. Although the high neuroplasticity characteristic of children and adolescents may enhance the therapeutic potential of TMS, the interaction between neuromodulation effects and neurodevelopmental processes remains incompletely understood. Future studies should therefore address efficacy, safety, and ethical considerations in larger pediatric samples.

Ethics

Ethical Committee Approval: The study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (decision number: 17, approval date: 02.10.2024).

Informed Consent: This descriptive study retrospectively examined.

Footnotes

Authorship Contributions

Concept: Ö.B., B.K.B., A.B., Design: Ö.B., B.K.B., M.A.T., A.B., Data Collection or Processing: A.P., B.K.B., Analysis or Interpretation: Ö.B., M.A.T., A.B., B.K.B., Literature Search: Ö.B., A.P., M.A.T., A.B., Writing: Ö.B., A.P., B.K.B.

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ORIGINAL ARTICLE

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Morphological and Biomechanical Determinants of Persistent Sac Expansion Following Endovascular Aortic Repair: A Longitudinal Analysis Focused on Endotension and Non-shrinking Sacs

Endovasküler Aort Tamiri Sonrası Kalıcı Kese Genişlemesinin Morfolojik ve Biyomekanik Belirleyicileri: Endotension ve Küçülmeyen Keselere Odaklanan Boylamsal Bir Analiz

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ABSTRACT

Aim: The fundamental objective of endovascular aortic repair (EVAR) is the effective exclusion and subsequent shrinkage of the aneurysm sac. Nevertheless, a clinical challenge persists in a subset of patients who exhibit progressive sac enlargement despite the total absence of detectable endoleaks, a phenomenon known as endotension or type V endoleak. This study investigates the morphological, biomechanical, and systemic determinants of persistent sac expansion following EVAR.

Materials and Methods: A longitudinal analysis was performed on 41 consecutive patients with a minimum 18-month follow-up. We evaluated anatomical metrics via thin-slice computed tomography angiography, biochemical markers including high-sensitivity C-reactive protein (hs-CRP), and systemic comorbidities. Multivariable logistic regression was employed to identify independent predictors of adverse sac dynamics.

Results: Sac expansion (≥5 mm) was observed in 14.6% of the cohort during the follow-up period. Multivariable analysis demonstrated that a preoperative sac diameter exceeding 60 mm [odds ratio (OR): 3.24] and an increased mural thrombus burden (OR: 2.86) were the strongest independent biomechanical predictors of persistent enlargement. Patients with adverse sac behavior frequently exhibited elevated hs-CRP levels and a higher prevalence of systemic conditions such as obesity, chronic obstructive pulmonary disease, and hypothyroidism

Conclusion: Post-EVAR sac expansion is a multifactorial systemic phenotype influenced by landing zone quality, mural thrombus biomechanics, and host inflammatory status. These findings suggest that in endotension cases, the transmission of pulsatile energy through the mural thrombus may maintain sac pressurization even without a visible flow channel. Recognizing persistent expansion as a distinct phenotype necessitates a shift in post-EVAR surveillance from a binary endoleak-focused approach toward a more comprehensive assessment of longitudinal sac dynamics and biomechanical risk factors.

Keywords: EVAR, aneurysm sac expansion, endotension, mural thrombus biomechanics, , hs-CRP, longitudinal analysis

ÖZ

Amaç: Endovasküler aort tamiri (EVAR) sonrası başarının temel ölçütü anevrizma kesesinin küçülmesidir. Ancak bazı olgularda, herhangi bir endoleak saptanmamasına rağmen kesenin büyümeye devam ettiği "endotension" tip V endoleak fenotipi gözlenmektedir. Bu çalışma, EVAR sonrası kalıcı kese genişlemesine yol açan morfolojik, biyomekanik ve sistemik faktörleri boylamsal bir perspektifle incelemeyi amaçlamaktadır

Gereç ve Yöntem: Çalışma kapsamında, EVAR uygulanan ve en az 18 ay takip edilen 41 ardışık hastanın verileri retrospektif olarak analiz edilmiştir. Bilgisayarlı tomografi anjiyografi üzerinden alınan anatomik ölçümler; yüksek duyarlılıklı C-reaktif protein (hs-CRP) düzeyi gibi biyokimyasal parametreler ve komorbidite profilleri ile karşılaştırılarak, kese genişlemesini öngördüren faktörler multivaryant lojistik regresyon modeli ile belirlenmiştir.

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Bulgular: Takip süresince hastaların %14,6'sında ≥5 mm kese genişlemesi saptanmıştır. Yapılan analizlerde, 60 mm üzerindeki preoperatif kese çapı [olasılık oranı (OR): 3.24] ve yüksek mural trombüs yükü (OR: 2.86), kalıcı kese büyümesinin en güçlü bağımsız biyomekanik belirleyicileri olarak öne çıkmıştır. Genişleme gösteren hastalarda sistemik inflamasyonun bir göstergesi olan hs-CRP düzeylerinin belirgin şekilde yüksek olduğu ve bu hastalarda kronik obstrüktif akciğer hastalığı, obezite ve hipotiroidi gibi sistemik durumların daha sık görüldüğü tespit edilmiştir.

Sonuç: Post-EVAR kese genişlemesi, sadece teknik bir yetersizlik değil; landing zone kalitesi, mural trombüs biyomekaniği ve hastanın enflamatuvar profilinin etkileşimiyle ortaya çıkan sistemik bir fenotiptir. Görünür bir sızıntı olmasa dahi, mural trombüsün basınç iletimini kolaylaştırması kesenin gerilmesine neden olabilmektedir. Bu durum, takip süreçlerinde endoleak varlığından ziyade kese hacmi ve biyomekanik risk faktörlerine odaklanan kişiselleştirilmiş stratejilerin önemini vurgulamaktadır.

Anahtar Kelimeler: EVAR, anevrizma kese genişlemesi, endotension, mural trombüs biyomekaniği, hs-CRP, landing zone kalitesi

INTRODUCTION

Endovascular aortic repair (EVAR) has fundamentally transformed the therapeutic landscape for abdominal aortic aneurysms and selected dissections, offering a minimally invasive alternative with superior perioperative morbidity and recovery profiles compared to open surgical repair[1-3]. The traditional paradigm of EVAR success relies on the effective exclusion of the aneurysmal sac from systemic pressure, typically manifesting as sac shrinkage or stabilization over time[3].

Despite technical advancements, long-term durability is frequently jeopardized by persistent sac expansion[3,4]. While endoleaks-categorized into well-defined subtypes based on their source are considered the primary drivers of expansion, clinical practice has revealed a paradoxical subgroup of patients[4]. These individuals demonstrate progressive sac enlargement despite the total absence of detectable endoleaks on high-resolution serial imaging, a phenomenon known as "endotension" or type V endoleak[5,6]. This phenomenon suggests that sac pressurization may persist through occult mechanisms, including graft porosity, transmission of pulsatile energy through the mural thrombus, or micro-collateral flow[5,6].

Furthermore, emerging evidence indicates that the host's systemic environment encompassing chronic inflammation, collagen metabolism disorders (e.g., fibrillin gene defects), and metabolic dysregulation may critically influence the failure of sac regression[6,7]. This study aims to move beyond binary endoleak classifications to explore the integrative morphological, biochemical, and biomechanical determinants of persistent sac expansion in a well-defined longitudinal cohort.

MATERIALS AND METHODS

Study Design and Population

This retrospective observational study was conducted at the Department of Cardiovascular Surgery, a tertiary referral center. We screened 41 consecutive patients who underwent primary endovascular stent-graft implantation for aortic aneurysm between January 2009 and February 2013. The study protocol received Maltepe University Faculty of Medicine Clinical Research Ethics Committee (approval number: AN023, date: 27.11.2013) and all participants provided written informed consent.

Patients were eligible for inclusion if they were treated with endovascular stent-graft implantation for primary aortic pathology and possessed comprehensive preoperative, perioperative, and postoperative clinical data. A strict minimum follow-up duration of 18 months with standardized imaging assessments was mandatory, alongside voluntary consent for medical record analysis. Conversely, the study excluded patients presenting with congenital aortic malformations, penetrating aortic ulcers, or those requiring emergency EVAR under hemodynamically unstable conditions that precluded standardized preoperative imaging. Furthermore, patients were excluded if they possessed severe concomitant valvular stenosis or regurgitation that could significantly alter systemic aortic hemodynamics, or if they had incomplete follow-up records.

Imaging Protocol and Definitions

Preoperative and postoperative assessments were performed using contrast-enhanced computed tomography angiography (CTA) with thin-slice acquisitions (≤1.5 mm). In the postoperative period, serial imaging was conducted according to a standardized surveillance protocol, with contrast-enhanced CTA performed at 3, 6, 12, and 18 months, provided that clinical conditions permitted intravenous contrast administration. In addition, Doppler ultrasonography was routinely used for interim evaluation at 9 months. In patients in whom intravenous contrast administration was contraindicated, follow-up assessments were performed using Doppler ultrasonography and/or non-contrast-enhanced computed tomography to ensure continuity of sac diameter measurements. Aneurysm sac diameter was defined as the maximal transverse diameter measured perpendicular to the centerline of flow. Persistent sac expansion was strictly defined as a ≥5 mm increase in the maximal sac diameter relative to the first postoperative baseline measurement. Endotension

was classified as persistent sac expansion in the absence of any detectable type I-IV endoleak on serial arterial- and delayed-phase CTA.

Clinical and Biochemical Assessment

In addition to standard anatomical metrics (neck angulation, thrombus distribution), we evaluated systemic comorbidities including obesity (body mass index), spirometry results, presence of systemic hypertension, complete blood count and thyroid function tests. Inflammatory status was quantified using high-sensitivity C-reactive protein (hs-CRP) and the neutrophil-to-lymphocyte ratio.

Statistical Analysis

Statistical analyses were performed using specialized software (SPSS version 25.0). Continuous variables were assessed for normality using the Shapiro-Wilk test and expressed as mean ± standard deviation or median with interquartile range. Categorical variables were reported as frequencies.

To identify predictors of sac expansion, we employed a rigorous two-stage modeling approach. Initially, univariable analysis (Student's t-test, Mann-Whitney U, or chi-square/Fisher's exact test as appropriate) was used to screen potential risk factors. Variables demonstrating clinical relevance or statistical significance ($p<0.10$) were then entered into a multivariable logistic regression model using a backward stepwise elimination method. This was done to determine independent associations and calculate odds ratios (ORs) with 95% confidence intervals. To account for the dynamic nature of sac changes, longitudinal data were further analyzed using generalized estimating equations to evaluate the impact of time-dependent variables on sac diameter. A p-value<0.05 was considered statistically significant.

RESULTS

Patient Characteristics

Between January 2009 and February 2013, a total of 41 patients who underwent EVAR for aortic aneurysm or aortic dissection met the inclusion criteria and were included in the final analysis. All patients completed a minimum follow-up duration of 18 months, with serial imaging available for longitudinal assessment of aneurysm sac behavior.

The study cohort consisted predominantly of male patients and represented a typical EVAR population with advanced age and a high prevalence of cardiovascular risk factors. Baseline demographic characteristics and comorbid conditions were comparable across the cohort and are summarized in Table 1.

Sac Dynamics and Procedural Outcomes

Technically successful stent-graft deployment was achieved in all 41 cases, with no immediate type I or III endoleaks detected on completion angiography. However, the 18-month longitudinal follow-up revealed that aneurysm sac behavior is

Table 1. Demographic and clinical characteristics of the study cohort

Variable	Patients (n=41) / value
Age (years), mean ± SD	68.59±6.68
Gender, n (%)	
Female	11 (26.8%)
Male	30 (73.2%)
Body mass index (kg/m²), median (range)	27.13 (22.91-39.54)
Comorbidities, n (%)	
Hypertension	19 (46.3%)
Diabetes mellitus	12 (29.3%)
Hyperlipidemia	13 (31.7%)
Coronary artery disease	9 (22.0%)
Carotid artery disease	10 (24.2%)
Chronic obstructive pulmonary disease	13 (31.7%)
Smoking status, n (%)	
Never smoked	6 (14.6%)
Former smoker	21 (51.2%)
Current smoker	14 (34.1%)
Laboratory and vital signs	
Pulse (bpm), median (range)	81 (65-121)
Systolic blood pressure (mmHg), median (range)	130 (100-185)
Diastolic blood pressure (mmHg), median (range)	85 (60-110)
Creatinine (mg/dL), median (range)	1.1 (0.8-1.5)
Fasting blood glucose (mg/dL), median (range)	100 (90-305)
HbA1c (%), median (range)	5.5 (4.0-8.2)
LDL cholesterol (mg/dL), mean ± SD	131.12±29.24
HDL cholesterol (mg/dL), median (range)	40 (28-90)
Triglycerides (mg/dL), mean ± SD	403.1±122.96
hsCRP (mg/L), median (range)	2.17 (0.8-4.10)
Hematocrit (%), mean ± SD	39.90±5.11
Neutrophil-to-lymphocyte ratio, median (range)	1.60 (1.10-2.90)

BMI: Body mass index, COPD: Chronic obstructive pulmonary disease, bpm: beats per minute, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, hsCRP: high-sensitivity C-reactive protein, SD: Standard deviation, EVAR: Endovascular aortic repair, systolic and diastolic blood pressure as well as pulse rate values represent mean measurements obtained from repeated outpatient assessments and ambulatory blood pressure monitoring during the 18-month follow-up period. All laboratory parameters reflect averaged results from serial analyses performed preoperatively and at 3, 9, 12, and 18 months after EVAR

a complex, multifactorial process that cannot be adequately explained by endoleak classification alone. Persistent sac expansion, defined as an increase of >5 mm, was recorded in 6 patients (14.6%), highlighting that expansion can occur despite apparently complete aneurysm exclusion. This observation underscores the inherent limitations of a purely binary success–failure paradigm, as the absence of traditional endoleaks did not guarantee sac stability or shrinkage in our cohort. Anatomical and procedural characteristics of the study population are summarized in Table 2.

Endoleak Patterns and the Endotension Phenotype

A distinct subgroup of patients (n=2, 4.9%) developed progressive sac enlargement in the total absence of any detectable endoleak on serial CTA, consistent with the phenomenon of endotension (type V endoleak). In our study, endotension emerged as a clinically meaningful postoperative phenotype with potential implications for long-term outcomes, rather than a rare incidental finding. Conversely, persistent collateral flow (Arc of Riolan) was identified as a driver in two other expansion cases, specifically linked to patent lumbar arteries where retrograde filling was confirmed in delayed-phase imaging.

Our findings demonstrate a significant dissociation between endoleak status and sac stability; while some patients with documented low-flow endoleaks showed stable or regressing diameters, others without any detectable leak exhibited continued expansion. This reinforces the concept that endoleak presence alone is an insufficient surrogate marker for aneurysm stability, requiring clinicians to interpret imaging findings within the broader context of longitudinal sac dynamics and systemic forces.

Shared Features and Independent Predictors

A comparative analysis revealed that adverse sac behavior was associated with specific systemic and anatomical factors. Patients with expanding sacs shared common features such as elevated hs-CRP levels (mean 3.8 mg/L), uncorrected hypothyroidism, chronic anemia, and persistent diastolic hypertension. Furthermore, clinical features suggestive of subclinical connective tissue vulnerability, potentially related to fibrillin associated matrix fragility, were more frequently observed in the expansion group; however, no molecular confirmation was available, and this observation should be interpreted as hypothesis-generating.

In multivariable analysis (Figure 1), a larger preoperative sac diameter (>60 mm; OR: 3.24, p = 0.031) and a high mural thrombus burden (OR: 2.86, p = 0.042) emerged as the strongest independent predictors of persistent post-EVAR sac expansion, whereas procedural factors and the brand of the graft were not significantly associated with adverse sac behavior. Independent predictors identified in multivariable logistic regression analysis are summarized in Table 3. These results reinforce the concept that post-EVAR sac dynamics are predominantly driven by pre-existing anatomical and biological factors rather than isolated procedural or imaging-defined findings.

Table 2. Anatomical and procedural characteristics of the study population

Variable	Value (n=41)	p-value*
Aortic pathology, n (%)		
Infrarenal abdominal aortic aneurysm	39 (95.1%)	0.64
Thoracic aortic aneurysm	2 (4.9%)	0.64
Preoperative anatomical metrics		
Maximum aneurysm sac diameter (mm), mean ± SD	58.4±8.2	0.018
Proximal neck length (mm), mean ± SD	22.1±5.4	0.011
Proximal neck angulation (degrees), mean ± SD	32.5±12.1	0.032
Presence of mural thrombus, n (%)	28 (68.3%)	0.041
Procedural parameters		
Stent-graft main body diameter (mm), mean ± SD	28.2±3.1	0.27
Stent-graft oversizing (%), mean ± SD	15.4±4.2	0.19
Total procedure time (min), mean ± SD	115±25	0.48
Fluoroscopy time (min), mean ± SD	18.4±6.5	0.56
Contrast medium volume (mL), mean ± SD	95±30	0.61

*p-values refer to the association between each variable and the presence of persistent sac expansion and/or any type of endoleak during follow-up, SD: Standard deviation, EVAR: Endovascular aortic repair, the majority of patients were treated for infrarenal abdominal aortic aneurysms, with thoracic aortic aneurysms representing a small minority of cases. Preoperative imaging demonstrated moderate aneurysm size and generally acceptable proximal neck anatomy, although mural thrombus was frequently observed. Procedural parameters reflected standard EVAR practice with appropriate stent-graft sizing and oversizing, and no excessive procedural or fluoroscopic durations were recorded

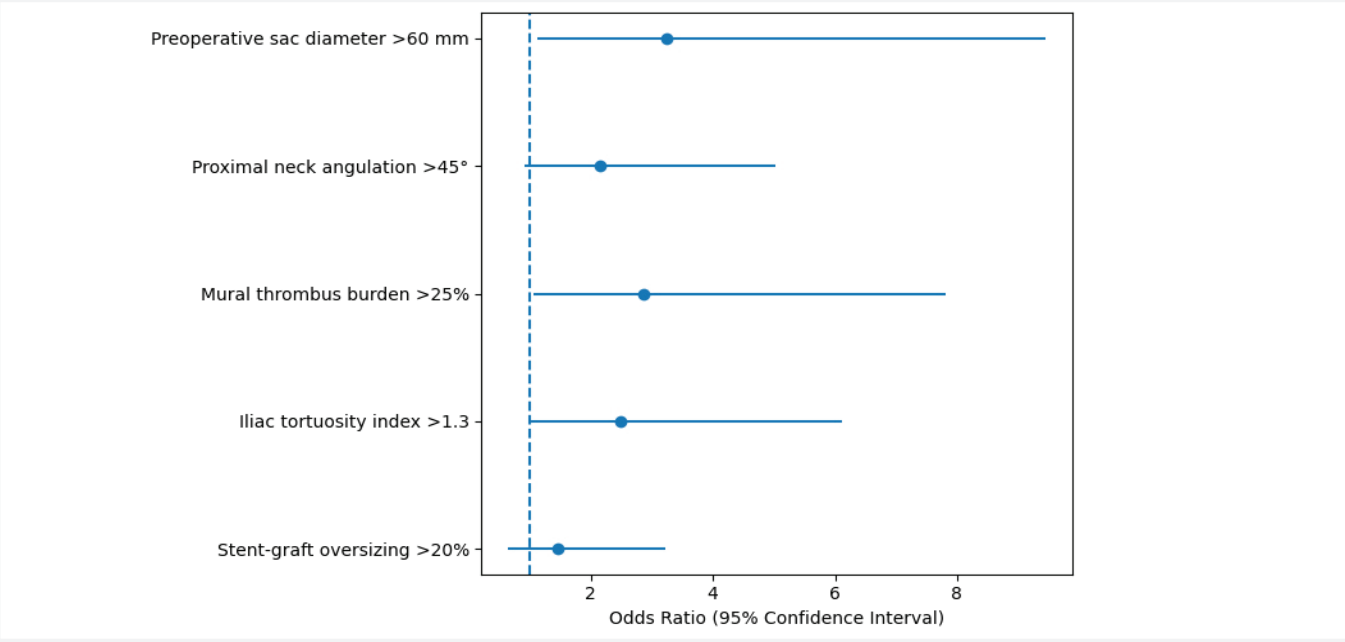

Figure 1. Multivariable predictors of persistent sac expansion after EVAR Forest plot illustrating odds ratios and 95% confidence intervals for variables included in the multivariable model. Increased preoperative sac diameter, mural thrombus burden, and iliac tortuosity index were associated with adverse sac behavior, whereas procedural oversizing was not a significant determinant. The vertical dashed line represents the null effect (odds ratio=1)

EVAR: Endovascular aortic repair

Table 3. Multivariable logistic regression analysis of factors associated with persistent sac expansion after

Variable	Odds ratio	95% Confidence interval	p-value**
Anatomical factors			
Preoperative sac diameter >60 mm	3.24	1.12-9.45	0.031*
Proximal neck angulation >45°	2.15	0.92-5.02	0.078
Mural thrombus burden >25%	2.86	1.05-7.82	0.042*
Iliac tortuosity index >1.3	2.48	1.01-6.12	0.047*
Procedural factors			
Stent-graft oversizing >20%	1.45	0.65-3.22	0.365
Follow-up findings			
Presence of type II endoleak	1.22	0.48-3.10	0.672
Presence of type I/III endoleak†	N/A	N/A	N/A

**Variables with p < 0.10 in univariable analysis were entered into the multivariable logistic regression model using backward stepwise elimination, *Statistically significant (p<0.05), †Type I and type III endoleaks were excluded from the regression model because no such events were observed in this cohort

Illustrative Case Scenarios of Adverse Sac Behavior Guiding Post-EVAR Clinical Decision-making

Case 1: Type II Endoleak with Progressive Sac Enlargement Leading to Open Conversion

A 67-year-old female patient with diabetes mellitus and collagen tissue disease underwent EVAR for an infrarenal abdominal aortic aneurysm with a preoperative sac diameter of 69.7x107.1mm (Figure 2A). After the EVAR procedure follow-up imaging demonstrated limited sac regression, measuring 69.6x106.4 mm at 6 months and 69.5x107.0 mm at 12 months. At the 18-month CTA, the sac diameter increased to 69.6x110.9 mm, corresponding to a cumulative 3-mm expansion (Figure 2B). The Doppler ultrasonography demonstrated persistent pulsatile flow within the sac, and contrast-enhanced CTA confirmed a type II endoleak originating from lumbar arteries (Figure 2C).

Given the documented sac enlargement exceeding guideline-recommended thresholds and ongoing sac pressurization, an endovascular attempt at lumbar artery embolization using occluder devices was performed but proved unsuccessful due to extensive and complex collateral flow. As sac pressurization persisted despite endovascular intervention, open surgical conversion was undertaken, resulting in definitive resolution.

Case 2: Early Type II Endoleak with Moderate Sac Expansion Managed with Intensified Surveillance

A 69-year-old male patient underwent EVAR for an infrarenal abdominal aortic aneurysm with a preoperative sac diameter of 60 mm. At the 6-month follow-up, contrast-enhanced CTA demonstrated a type II endoleak, with the aneurysm sac measuring 63 mm, corresponding to a 3-mm increase in diameter (Figure 3A). Detailed imaging analysis identified retrograde sac perfusion through lumbar arteries supplied via collateral flow from the arc of Riolan, indicating a mesenteric–lumbar collateral pathway as the source of the endoleak (Figure 3B, C).

Given the moderate degree of sac enlargement, the early timing after EVAR, and the absence of rapid expansion or high-risk features, a strategy of repeated imaging surveillance was adopted rather than immediate reintervention, with a low threshold for secondary intervention should further sac expansion be observed.

The moderate degree of sac enlargement, early post-EVAR timing, and identification of a collateral-driven type II endoleak without high-risk features supported a management strategy of intensified imaging surveillance rather than immediate reintervention, with a low threshold for secondary intervention should further sac expansion occur.

Case 3: Endotension Phenotype with Minimal Sac Growth Managed Conservatively

A 71-year-old male patient with hypertension and chronic obstructive pulmonary disease underwent EVAR for an infrarenal abdominal aortic aneurysm with a baseline sac diameter of 58mm. Serial CTA demonstrated 58 mm at 6 months, 59 mm at 12 months, and 60 mm at 18 months, reflecting a total sac enlargement of 2 mm over 18 months (Figure 4A, B). No demonstrable endoleak was identified on repeated imaging, and no graft-related complications were observed. Given the low expansion rate, absence of endoleak (Figure 4C), and stability over time, the patient was classified as having an endotension phenotype and managed with close radiological surveillance rather than reintervention, in accordance with guideline-based recommendations.

Case 4: Minimal Sac Enlargement with Type II Endoleak Managed Conservatively

A 73-year-old female patient with hypertension and obesity underwent EVAR for an aneurysm with a baseline sac diameter of 62 mm. Follow-up imaging demonstrated 62 mm at 6 months and 63 mm at 12 months, corresponding to a 1-mm increase over one year. CTA revealed

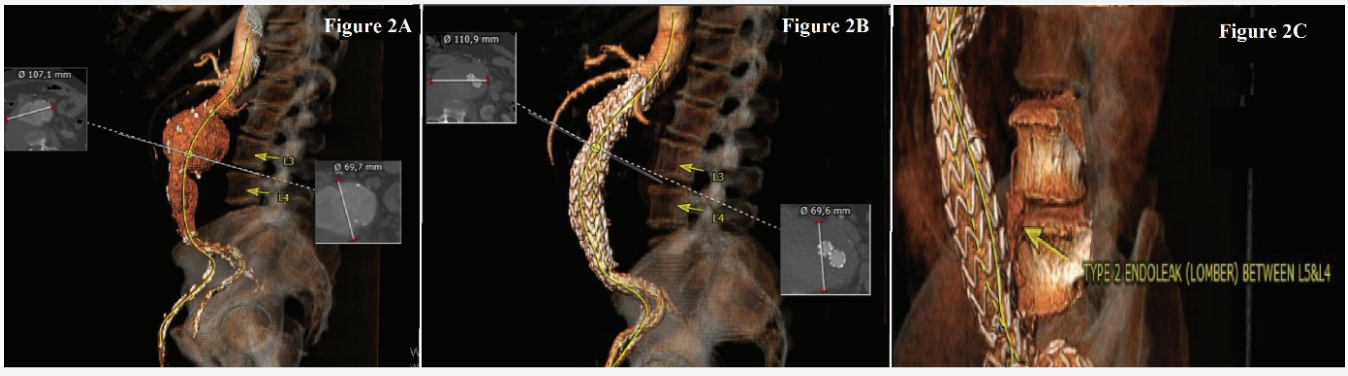

Figure 2. Serial computed tomography angiography demonstrating adverse sac behavior associated with type II endoleak

Figure 2A. Baseline contrast-enhanced computed tomography angiography of a 67-year-old female patient with diabetes mellitus and collagen tissue disease demonstrating an infrarenal abdominal aortic aneurysm prior to endovascular aortic repair. The preoperative aneurysm sac dimensions measured 69.7×107.1 mm; Figure 2B. Follow-up computed tomography angiography obtained at 18 months after endovascular aortic repair demonstrates adverse sac behavior characterized by interval sac enlargement. While early follow-up showed limited sac regression, with measurements of 69.6×106.4 mm at 6 months and 69.5×107.0 mm at 12 months, the sac diameter increased to 69.6×110.9 mm at 18 months, corresponding to an overall expansion of approximately 3 mm; Figure 2C. Contrast-enhanced computed tomography angiography and Doppler ultrasonography demonstrate persistent pulsatile flow within the aneurysm sac, with contrast opacification originating from lumbar arterial branches, consistent with a type II endoleak. These findings explain the failure of sac regression and ongoing sac pressurization despite technically successful endograft deployment

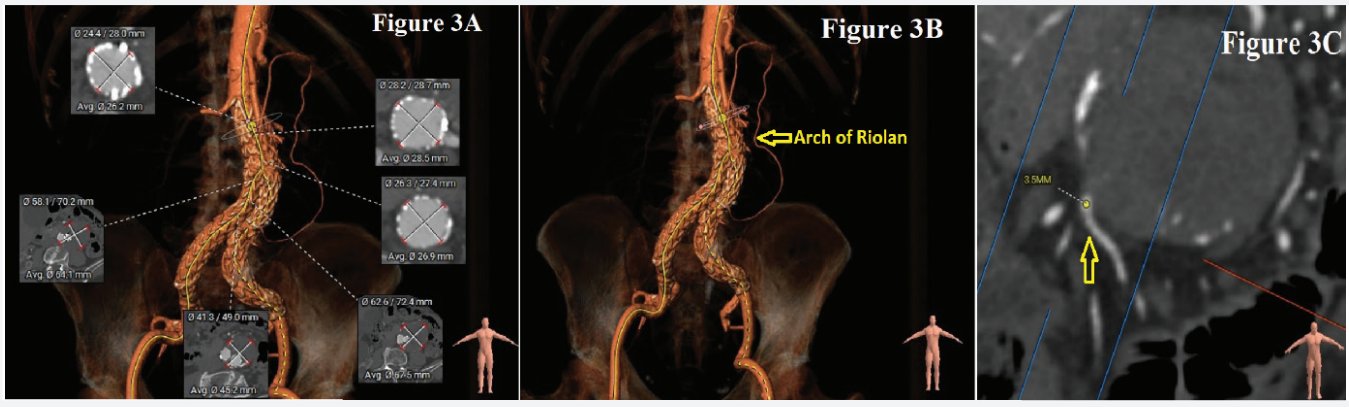

Figure 3. Type II endoleak mediated by mesenteric-lumbar collateral flow via the arc of riolan

Figure 3A. Baseline three-dimensional volume-rendered computed tomography angiography following endovascular aortic repair in a 69-year-old male patient demonstrates appropriate endograft deployment and exclusion of the infrarenal abdominal aortic aneurysm. The preoperative aneurysm sac diameter measured 60 mm. Multiplanar reconstructions and cross-sectional measurements illustrate the spatial relationship between the endograft and the aneurysm sac without evidence of proximal or distal sealing failure at this stage; Figure 3B. computed tomography angiography obtained at the 6-month follow-up reveals a type II endoleak with interval sac enlargement to 63 mm, corresponding to a 3-mm increase in diameter. Volume-rendered imaging demonstrates retrograde filling of lumbar arteries supplied via collateral flow from the arc of Riolan, highlighting a mesenteric–lumbar collateral pathway as the source of persistent sac perfusion. Proximal and distal graft fixation zones remain intact; Figure 3C. Axial contrast-enhanced computed tomography angiography confirms contrast opacification within the aneurysm sac adjacent to a lumbar arterial branch, consistent with retrograde low-pressure filling. The measured contrast column within the collateral vessel further supports the diagnosis of a lumbar artery-mediated type II endoleak without evidence of high-pressure systemic inflow

a type II endoleak originating from lumbar arteries. Given the minimal degree of sac enlargement, potential measurement variability, and absence of rapid progression, the patient was managed with conservative, very close follow-up, without immediate reintervention (Figure 5A, B).

Case 5: Accelerated Late Sac Expansion Following Aorto-uni-iliac EVAR and Distal Type I Endoleak Prompting Open Conversion

A 75-year-old male patient underwent EVAR for an infrarenal abdominal aortic aneurysm with a preoperative maximum sac diameter of 96.4 mm. Due to anatomical considerations, the procedure was performed using an aorto-uni-iliac stent-graft configuration, combined with a femoro–femoral crossover bypass to maintain bilateral lower limb perfusion. Early postoperative imaging confirmed appropriate graft positioning and satisfactory exclusion of the aneurysm sac. During follow-up, the aneurysm sac initially appeared stable; however, at the 6-month evaluation, interval sac enlargement was detected, raising concern for persistent sac pressurization.

Given imaging findings (Figure 6A, B) suggestive of retrograde pelvic inflow, and the assumption that the left internal iliac artery was chronically occluded, an endovascular occlusion of the left internal iliac artery using an occluder device was performed (Figure 6C). Serial contrast-enhanced CTA suggested a high-pressure endoleak originating from the distal sealing zone near the right common iliac artery, consistent with a distal type I endoleak (Figure 6D). As the aneurysm sac diameter exceeded 101 mm (Figure 6B), and given the progressive nature of expansion despite secondary endovascular intervention, a decision was made to proceed with open surgical conversion.

Despite this intervention, subsequent imaging demonstrated continued and progressive sac enlargement. Intraoperatively, the aneurysm sac was found to be pressurized. An attempt at mechanical banding of the distal iliac neck failed to achieve a meaningful reduction in intra-sac pressure. Upon opening the sac, a relatively fresh thrombus was encountered (Figure 7), within which the trajectory of active endoleak flow could be traced circumferentially around the right common iliac artery, confirming the distal sealing zone as the source of persistent sac filling. Consequently, the endograft material was completely explanted (Figure 7), and definitive repair was achieved by performing an aortobifemoral bypass, resulting in successful exclusion and decompression of the aneurysm sac.

DISCUSSION

The present study demonstrates that aneurysm sac behavior following EVAR is governed by an intricate interplay of anatomical, mechanical, and systemic factors[7]. Type II endoleaks represent the most frequently encountered endoleak subtype after EVAR, with an overall reported incidence ranging

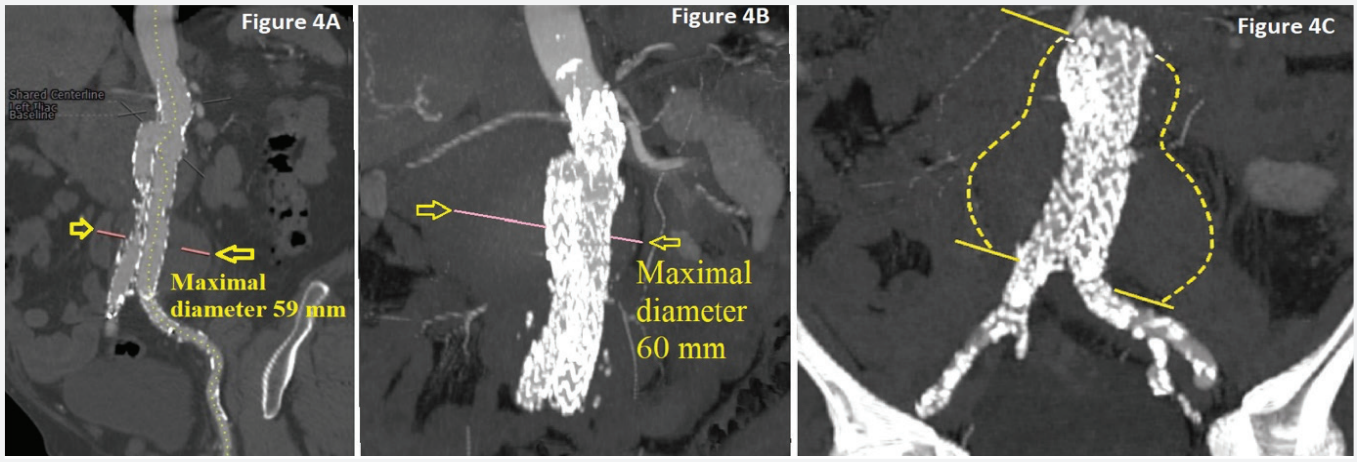

Figure 4. Type V endoleak (endotension)

A 71-year-old male with comorbidities underwent endovascular aneurysm repair for a 58 mm infrarenal abdominal aortic aneurysms. Over 18 months, the sac enlarged by 2 mm (Figure 4A, B) without detectable endoleak (Figure 4C). Due to the slow growth, stable graft, and signs of endotension, guideline-based surveillance was preferred over reintervention; Figure 4A. This coronal computed tomography angiography image shows the abdominal aortic aneurysm sac following endovascular aneurysm repair. The image is labeled with the maximal diameter of 59 mm, a 1 mm increase from baseline. Yellow dashed centerlines track the stent graft, and a double-headed arrow measures the aneurysm's maximal width. There is no visible endoleak, and the stent graft appears well-positioned. This scan has been done at the 12-month follow-up admission; Figure 4B. This follow-up computed tomography angiography, obtained 18 months after endovascular aortic repair, demonstrates a further 1 mm increase, with the aneurysm sac reaching 60 mm in maximal diameter. Again, no endoleak is identified despite the progressive but slow sac expansion over time. The stent graft remains intact with no evidence of migration or structural compromise; Figure 4C. This axial image highlights the absence of contrast extravasation, confirming no visible endoleak. Dashed yellow lines delineate the area between the endograft and the outer aneurysm sac, consistent with endotension—a condition where the aneurysm expands without a detectable leak. Yellow arrows mark the reference boundaries. The stable graft position and lack of complications support the diagnosis of an endotension phenotype

between 20% and 40% among EVAR-treated patients and accounting for nearly half of all identified endoleaks[8]. These endoleaks commonly originate from retrograde collateral flow through branches such as the inferior mesenteric artery, lumbar arteries, median sacral artery, or accessory renal arteries[8]. Importantly, type II endoleaks may remain clinically silent or radiographically occult during early follow-up and are not infrequently detected only after aneurysm sac enlargement becomes evident[8]. Consequently, in cases of continued or progressive sac expansion despite technically successful stent-graft implantation, type II endoleak is often the first and most plausible diagnosis considered in clinical practice. Consistent with these observations, type II endoleaks were not uncommon in our study cohort and constituted a substantial proportion of patients exhibiting adverse sac behavior, underscoring their continued clinical relevance in post-EVAR surveillance. One of the most important insights of the present study is the identification of a distinct subgroup of patients who developed progressive aneurysm sac enlargement in the absence of any detectable endoleak. The presence of this phenotype underscores the critical importance of prolonged follow-up and careful longitudinal assessment, as extended surveillance allows for a more accurate characterization of the factors contributing to endotension[9]. In this context, the true incidence of endotension reported in the literature may be underestimated, potentially reflecting artificially low rates driven by relatively short follow-up durations rather than a genuine rarity of the condition[10]. Accordingly, cohorts with extended imaging surveillance, such as the present study population, are particularly valuable for elucidating the determinants and natural history of endotension-related adverse sac behavior. Rather than representing a rare event, endotension in our cohort emerged as a meaningful postoperative phenotype. Several mechanisms may explain this persistent pressurization, including the transmission of pulsatile forces through the stent-graft material, microleakage below the resolution of conventional imaging, and the biomechanical effects of mural thrombus[9-11]. Our results support the concept that anatomical factors, particularly those related to landing zone quality and thrombus morphology, play a central role in determining these dynamics.

The role of persistent lumbar artery inflow in type II endoleaks deserves particular attention. While type II leaks are often considered benign, our findings in two cases suggest that

lumbar arteries can act as high-pressure conduits reinforced by systemic paraspinal circulation. This persistent inflow prevents the expected fibrotic transformation of the sac[11]. Furthermore, the association between elevated hs-CRP and sac growth suggests that a systemic inflammatory state may keep the mural thrombus in a "fluid" state, facilitating the transmission of systemic pressure even in the absence of a visible flow channel[12,13].

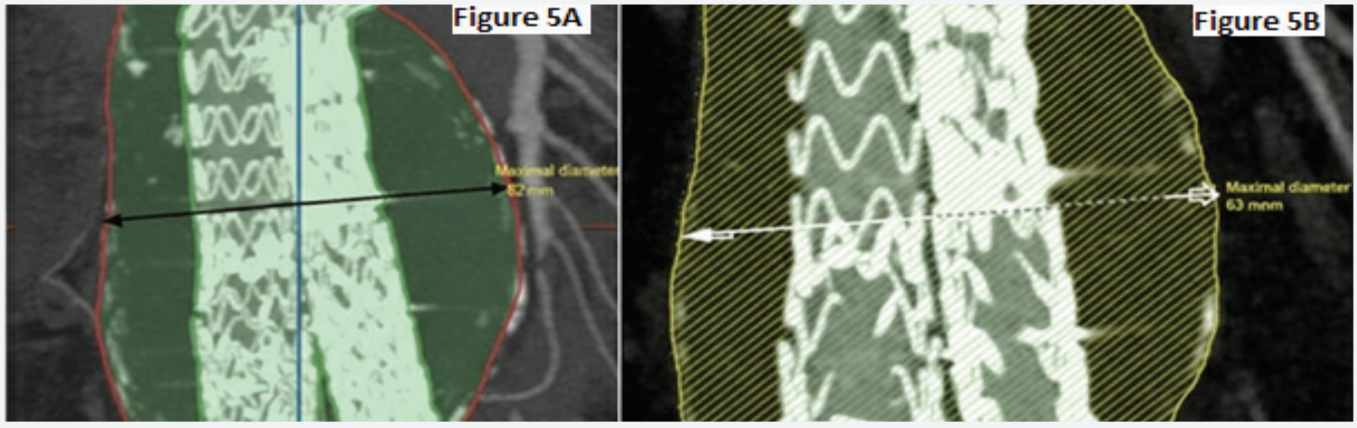

Figure 5. Minimal sac enlargement associated with lumbar artery–mediated type II endoleak managed conservatively

Figure 5A. Contrast-enhanced computed tomography angiography obtained during follow-up in a 73-year-old female patient with hypertension and obesity demonstrates an infrarenal abdominal aortic aneurysm treated with endovascular aortic repair. Three-dimensional reconstruction and cross-sectional measurement indicate a baseline aneurysm sac diameter of 62 mm, with preserved endograft position and intact proximal and distal sealing zones. No significant sac deformation or graft-related complication is evident; Figure 5B. Computed tomography angiography performed at 12 months after endovascular aortic repair demonstrates a minimal increase in aneurysm sac diameter to 63 mm, corresponding to a 1-mm enlargement over one year. Shaded segmentation highlights the aneurysm sac boundaries, and contrast opacification adjacent to lumbar arterial branches is consistent with a type II endoleak originating from lumbar arteries. Given the minimal degree of sac enlargement, potential measurement variability inherent to serial imaging, and absence of rapid progression, these findings supported a strategy of conservative management with very close imaging surveillance, without immediate reintervention

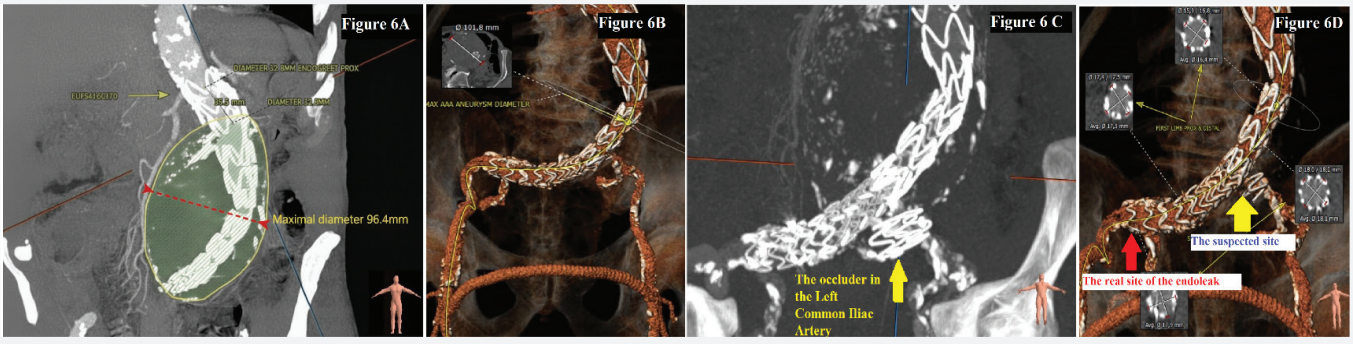

Figure 6. Imaging findings of accelerated sac expansion and distal type I endoleak after aorto-uni-ılıac endovascular aortic repai

Figure 6A. Baseline contrast-enhanced computed tomography angiography demonstrates the infrarenal abdominal aortic aneurysm treated with an aorto-uni-iliac stent-graft configuration. The aneurysm sac is clearly delineated, with an initial maximum diameter of 96.4 mm, and satisfactory early exclusion is observed; Figure 6B. Follow-up computed tomography angiography reveals progressive aneurysm sac enlargement, exceeding 101 mm, despite apparently preserved graft position. These findings indicate persistent sac pressurization and prompted further investigation for a high-pressure endoleak source; Figure 6C. Three-dimensional reconstructed imaging illustrates endovascular occlusion of the left common iliac artery using an occluder device, performed under the assumption of retrograde pelvic inflow contributing to sac pressurization; Figure 6D. Subsequent computed tomography angiography identifies a high-pressure distal Type I endoleak originating from the right iliac sealing zone, resulting in direct systemic pressurization of the aneurysm sac and explaining the continued rapid sac expansion despite secondary endovascular intervention.

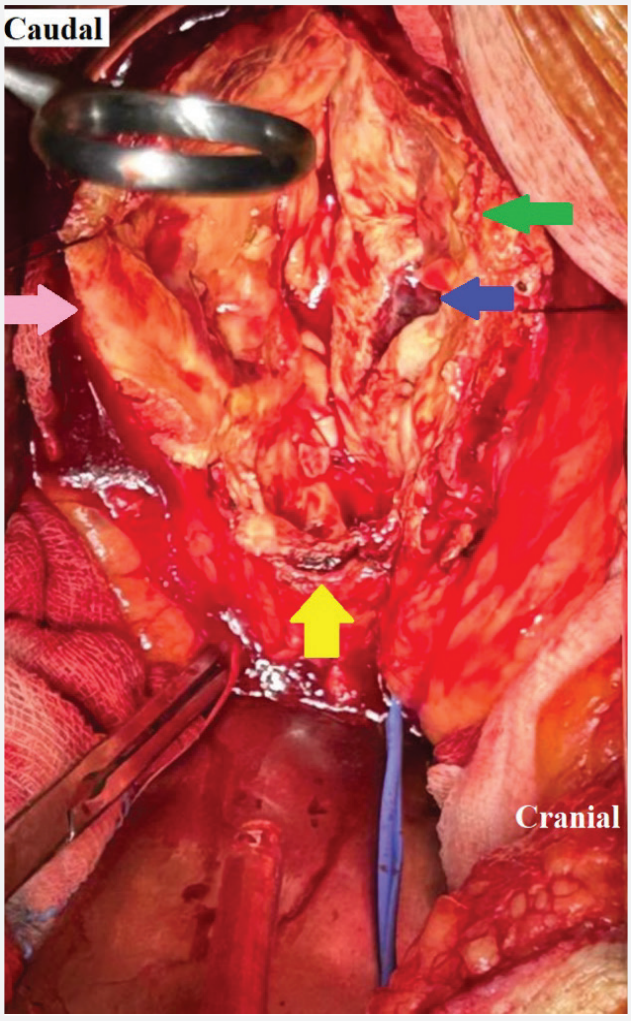

Figure 7. Intraoperative image demonstrating the site of a distal type I endoleak originating from the right common iliac artery landing zone. The blue arrow indicates relatively fresh thrombus formation within the aneurysm sac, allowing visualization of the endoleak flow trajectory, while the yellow arrow denotes the proximal landing zone. On the endoleak side, high-velocity jet flow and turbulent intra-sac hemodynamics contributed to increased intimal injury, resulting in focal irregularity and fragility of the aneurysm sac wall, as indicated by the green arrow. In contrast, the region marked by the pink arrow demonstrates a smoother, more uniform sac wall morphology with preserved structural integrity, consistent with effective sealing and subsequent thrombus organization followed by fibrotic remodeling

The failure of banding during open conversion in our series provides a critical clinical lesson. It suggests that in the setting of advanced sac expansion or endotension, the biomechanical failure is often circumferential or material-related[12]. Simple mechanical constriction of the neck may be insufficient to counteract the transmitted forces, making total graft excision a more definitive, albeit invasive, necessity[13]. This reinforces the need to view sac expansion, regardless of endoleak status, as a warning signal warranting intensified surveillance and individualized decision making[13,14]. Beyond anatomical determinants, persistent sac expansion after EVAR may also reflect a systemic vascular phenotype characterized by chronic inflammation, impaired arterial compliance, and altered extracellular matrix remodeling. Patients with adverse sac dynamics in our cohort more frequently exhibited elevated hs-CRP levels, obesity, chronic obstructive pulmonary disease, hypothyroidism, and diastolic hypertension. Although these variables did not consistently remain independent predictors in multivariable analysis, their clustering suggests that systemic inflammatory burden and vascular wall vulnerability may contribute to sustained intra-sac pressurization[14]. Obesity and chronic obstructive pulmonary disease, in particular, are well recognized to promote low-grade systemic inflammation, endothelial dysfunction, and increased arterial stiffness, mechanisms that may plausibly facilitate transmission of pulsatile forces to the excluded aneurysm sac[15-17]. Taken together, these observations support the concept that failure of sac regression should not be interpreted solely as a graft-related phenomenon but rather as the interaction between local biomechanical conditions and host-related biological susceptibility[18-20]. In this context, persistent sac expansion should not be interpreted solely as a local graft-related phenomenon but rather as the downstream manifestation of an unfavorable anatomical–biological milieu. This integrated perspective may help explain why sac regression fails to occur in certain patients despite technically successful EVAR and underscores the importance of incorporating systemic patient characteristics into postprocedural risk stratification and surveillance strategies.

Finally, the integration of metabolic and genetic factors reveals that sac behavior is a systemic phenotype. Hypothyroidism and anemia likely impair the metabolic pathways required for vascular wall remodeling, while diastolic hypertension provides a constant stressor that prevents sac contraction[18]. In patients with underlying connective tissue weakness, such as fibrillin defects, these forces are amplified[19]. Recognizing persistent sac expansion as a distinct post-EVAR phenotype may enable more refined risk stratification and contribute to improved selection of patients requiring secondary intervention by supporting surveillance strategies that integrate longitudinal sac behavior with anatomical risk markers and systemic inflammatory status, rather than relying exclusively on conventional endoleak classification[1,20-22]. In this context, persistent aneurysm sac expansion should be interpreted as a clinically meaningful indicator of adverse postprocedural dynamics reflecting the combined influence of anatomical configuration, biomechanical forces, and host-related biological susceptibility. Accordingly, longitudinal sac monitoring may represent a more robust marker of procedural

durability than endoleak status alone. This perspective is consistent with evidence from large randomized trials such as EVAR-1 (1), DREAM (21), and OVER (22), which established the central role of aneurysm sac dynamics as a surrogate marker of long-term outcomes after EVAR. Incorporation of sac behavior into postprocedural surveillance frameworks may therefore facilitate more individualized follow-up strategies and support timely decision-making regarding secondary intervention in selected patients.

Study Limitations

This study has several limitations that should be acknowledged. First, its retrospective design inherently limits causal inference and may be subject to selection bias. Second, the study was conducted at a single center with a relatively limited sample size, which may restrict the generalizability of the findings to broader populations or different EVAR platforms.

Additionally, direct measurement of intra-sac pressure was not performed, and conclusions regarding biomechanical mechanisms are therefore inferential rather than mechanistically proven. Advanced imaging modalities capable of detecting microleakage or dynamic pressure transmission were not routinely available during the study period.

Nevertheless, the study benefits from a well-defined cohort, standardized imaging follow-up, and complete longitudinal data without loss to follow-up. The homogeneity of procedural technique and surveillance protocols may, in fact, strengthen internal validity by reducing confounding variability.

CONCLUSION

Persistent aneurysm sac expansion after EVAR represents a complex and clinically relevant phenomenon that extends beyond conventional endoleak classification. Our findings demonstrate that sac behavior is influenced by a constellation of anatomical and procedural factors and should be interpreted as an independent marker of post-EVAR success or failure.

Routine follow-up strategies should place greater emphasis on longitudinal sac dynamics rather than reliance on endoleak status alone. Recognizing endotension and related sac expansion phenotypes may improve patient selection for secondary interventions and contribute to more personalized post-EVAR management strategies.

Future studies incorporating advanced imaging, biomechanical modeling, and direct pressure measurements are warranted to further elucidate the mechanisms underlying persistent sac expansion and to optimize long-term outcomes following EVAR.

Ethics

Ethical Committee Approval: The study protocol received Maltepe University Faculty of Medicine Clinical Research Ethics Committee (approval number: AN023, date: 27.11.2013).

Informed Consent: All participants provided written informed consent.

Acknowledgement

This study was originally conceived and designed by the author (Lütfi Çağatay Onar MD) as a medical specialty thesis in 2009. Ethical approval for the study was obtained in November 2013, and the work was formally completed as a thesis in 2014. During the thesis phase, the author received academic supervision from Professor Dr. Uğur Filizcan. While Professor Dr. Filizcan was not involved in the technical aspects of the manuscript preparation, and therefore is not listed among the authors of the present article, the author gratefully acknowledges his invaluable clinical guidance and contributions to patient management and postoperative follow-up, which were conducted in accordance with contemporary clinical guidelines at the time of the study.

Footnotes

Authorship Contributions

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Triple Tendon Transfer with Flexor Carpi Radialis in Radial Nerve Palsy

Radyal Sinir Paralizisinde Fleksör Karpi Radialis ile Üçlü Tendon Transferi

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ABSTRACT

Aim: This study aims to evaluate the clinical outcomes of our case series involving finger extension restoration using the flexor carpi radialis (FCR) tendon in patients with radial nerve palsy.

Materials and Methods: We retrospectively evaluated patients who underwent tendon transfers using FCR for radial nerve palsy between January 2016 and December 2021. Nineteen patients (15 males, 4 females) were included. Data on the side of injury, gender, age, etiology, history of nerve repair, and time of admission were recorded. Functional outcomes were assessed by comparing preoperative and postoperative Quick-DASH and Bincaz scores.

Results: Postoperative Quick-DASH scores showed statistically significant improvement ($p<0.001$). The mean functional loss score decreased from 31.82 ± 14.82 preoperatively to 16.03 ± 7.97 postoperatively. Analysis of Bincaz scores using the Wilcoxon signed-rank test also demonstrated significant functional recovery ($p<0.001$).

Conclusion: Tendon transfers remain a reliable strategy for restoring motor function when radial nerve repair is not feasible. While various techniques exist, each presents distinct advantages and disadvantages. Surgeons should adopt a case-specific approach tailored to the patient's occupational and functional requirements.

Keywords: Radial neuropathy, flexor carpi radialis, tendon transfer

ÖZ

Amaç: Bu çalışmanın amacı radyal sinir paralizili hastalarda fleksör karpi radialis (FCR) tendonu kullanılarak parmak ekstansiyonunun yeniden sağlanmasına yönelik olgu serimizin klinik sonuçlarını değerlendirmektir.

Gereç ve Yöntem: Çalışmada, Ocak 2016 ile Aralık 2021 tarihleri arasında kliniğimizde radyal sinir felci nedeniyle FCR kullanılarak yapılan tendon transferlerinin sonuçları değerlendirilmiştir. Çalışmaya 19 hasta (15 erkek, 4 kadın) dahil edilmiştir. Olguların taraf, cinsiyet, yaş, yaralanma etiyolojisi, sinir tamiri yapılıp yapılmadığı ve başvuru zamanı kayıt altına alınmıştır. Fonksiyonel değerlendirme cerrahi öncesi ve sonrası Quick-DASH skoru ve Bincaz skorlarıyla karşılaştırmalı olarak yapılmıştır.

Bulgular: Hastaların Quick-DASH skorlarında, operasyon sonrası istatistiksel olarak ileri düzeyde anlamlı bir iyileşme kaydedilmiştir ($p<0,001$). Preoperatif dönemde $31,82\pm14,82$ olan ortalama fonksiyonel kayıp skoru, postoperatif dönemde $16,03\pm7,97$ seviyesine gerilemiştir. Bincaz skorları Wilcoxon işaretli sıralar testi ile analiz edilmiş ve değişim istatistiksel olarak anlamlı bulunmuştur ($p<0,001$).

Sonuç: Tendon transferleri, özellikle radyal sinir onarımının mümkün olmadığı durumlarda motor fonksiyonun yeniden sağlanması açısından güvenilir bir yöntemdir. Farklı teknikler bulunmakla birlikte her birinin kendine özgü avantaj ve dezavantajları vardır. Cerrahlar, hastaların mesleki ve fonksiyonel gereksinimlerini dikkate alarak olguya özgü bir yaklaşım benimsemelidir.

Anahtar Kelimeler: Radyal sinir paralizisi, fleksör karpi radialis, tendon transferi

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INTRODUCTION

The radial nerve is the most frequently injured nerve in the upper extremity[1] typically resulting from direct trauma rather than indirect mechanisms[2]. Although primary nerve repair remains the gold standard of treatment, successful reinnervation is not always achieved, necessitating palliative reconstructive options. In cases where nerve repair or conservative management fails to restore function, tendon transfer may be considered[1].

Loss of radial nerve function leads to significant grasping impairment due to the loss of wrist extension, finger metacarpophalangeal (MCP) extension, and thumb extension[3]. Although most tendon transfers are performed successfully according to standard protocols, the optimal combination of donor tendons for radial nerve palsy remains a subject of ongoing debate[4].

The flexor carpi radialis (FCR) is primarily used for finger extension[5], though some surgeons prefer the flexor carpi ulnaris (FCU), which possesses twice the tensile strength[6]. In addition to these tendons, both of which have a relatively short course, the flexor digitorum superficialis (FDS) is also an alternative. Regarding thumb restoration, transferring the palmaris longus (PL) to the rerouted extensor pollicis longus (EPL) is a frequent preference for providing abduction and extension[7], though abductor pollicis longus (APL) and extensor pollicis brevis (EPB) transfers are also viable considerations.

The aim of this study is to evaluate the clinical results of triple tendon transfer using the FCR for finger extension.

MATERIALS AND METHODS

This study retrospectively evaluated the results of tendon transfers performed for radial nerve palsy at our clinical center between January 2016 and December 2021. Nineteen patients (15 males, 4 females) who underwent triple tendon transfer using the FCR were included ethical approval was obtained from the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (approval number: E-46048792-050.01.04-350165, date: 22.09.2023).

A power analysis conducted via G*Power software, based on literature-derived effect sizes of 0.8 (Cohen), 80% power, and a 0.05 margin of error, indicated a minimum required sample size of n=15.

Surgical Technique

Under axillary anesthesia and tourniquet control, patients were placed in the supine position following standard surgical preparation and administration of 2 g of cefazolin prophylaxis. A longitudinal incision was made along the dorsal forearm. To restore wrist extension, the pronator teres (PT) was harvested with its periosteum from the radial attachment (Figure 1A), and tenorrhaphy to the extensor carpi radialis brevis (ECRB) tendon was performed using the Pulvertaft weave technique (Figure 1B).

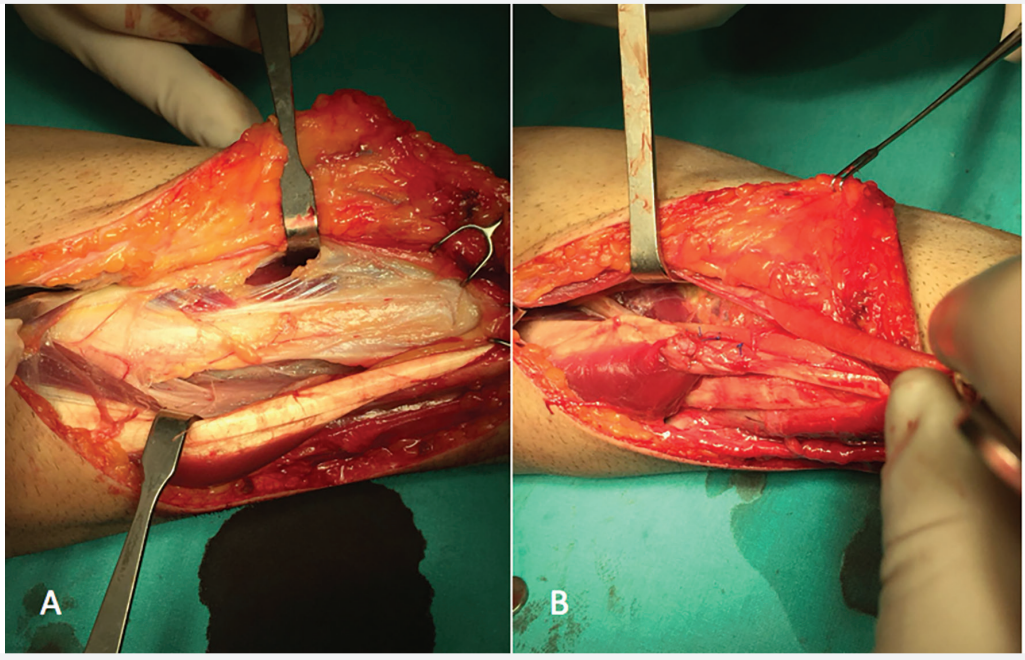

Figure 1. Pronator teres (PT) to extensor carpi radialis brevis (ECRB) tendon transfer. A) Harvesting of the PT with its periosteum from the radius B) PT-to-ECRB tendon transfer

Finger extension was restored by harvesting the FCR tendon at the wrist, which was then routed proximally and transferred to the extensor indicis proprius (EIP), extensor digitorum communis (EDC 2-5), and extensor digiti minimi (EDM) tendons via the Pulvertaft method (Figures 2A-C). For thumb extension, the EPL was rerouted volarly and transferred to the PL tendon (Figures 3A-C); in five patients where the PL was absent, the fourth FDS 4 was utilized as the donor (Figure 4). Skin closure was performed using 5/0 polyvinylidene fluoride sutures.

Postoperatively, a long-arm plaster splint was applied. Patients received wound care every two days, and sutures were removed during the second postoperative week. Controlled mobilization was initiated at four weeks, with full functional hand utilization permitted at two months. Follow-up examinations were conducted monthly for the first three months and quarterly thereafter for the first year. The mean follow-up duration was 32 months (range: 24 to 52 months).

Tendon transfers were evaluated using standard methods in patient follow-ups. Outcome measures included goniometric quantification of the range of motion in the MCP and interphalangeal joints, as well as wrist flexion, extension, and ulnar/radial deviation. Functional assessment was performed using Quick-DASH and Bincaz scoring systems preoperatively and at a minimum of two years post-treatment(Tables 1, 2)[8].

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics v26.0. The conformity of variables to normal distribution was examined using the Shapiro-Wilk method. Since the data did not show normal distribution and were in an ordinal categorical structure, the Wilcoxon signed-rank test was used to analyze the differences between preoperative and postoperative measurements. Descriptive data are presented as mean ± standard deviation for continuous variables and as number (n) and percentage (%) for categorical variables. The significance level was accepted as $p<0.05$.

RESULTS

Analysis of the demographic data for the 19 patients included in the study revealed a mean age of 34.53±15.13 years (range: 8 to 64 years). The majority of the sample consisted of male patients (78.9%, n=15), while female patients accounted for 21.1% (n=4). Regarding the distribution of the surgical site, a predominance of the left side (57.9%, n=11) was observed.

Two patients underwent surgery during the acute period, while 17 were operated on during the chronic period. The FDS of the ring finger was utilized in the five patients lacking a PL. Neurorrhaphy was performed in two cases: one involving a schwannoma and the other an acute stab wound. PT-to-ECRB transfer for wrist extension was omitted in three patients, two of whom presented with posterior interosseous nerve (PIN) lesions and one with nerve entrapment. All cases achieved

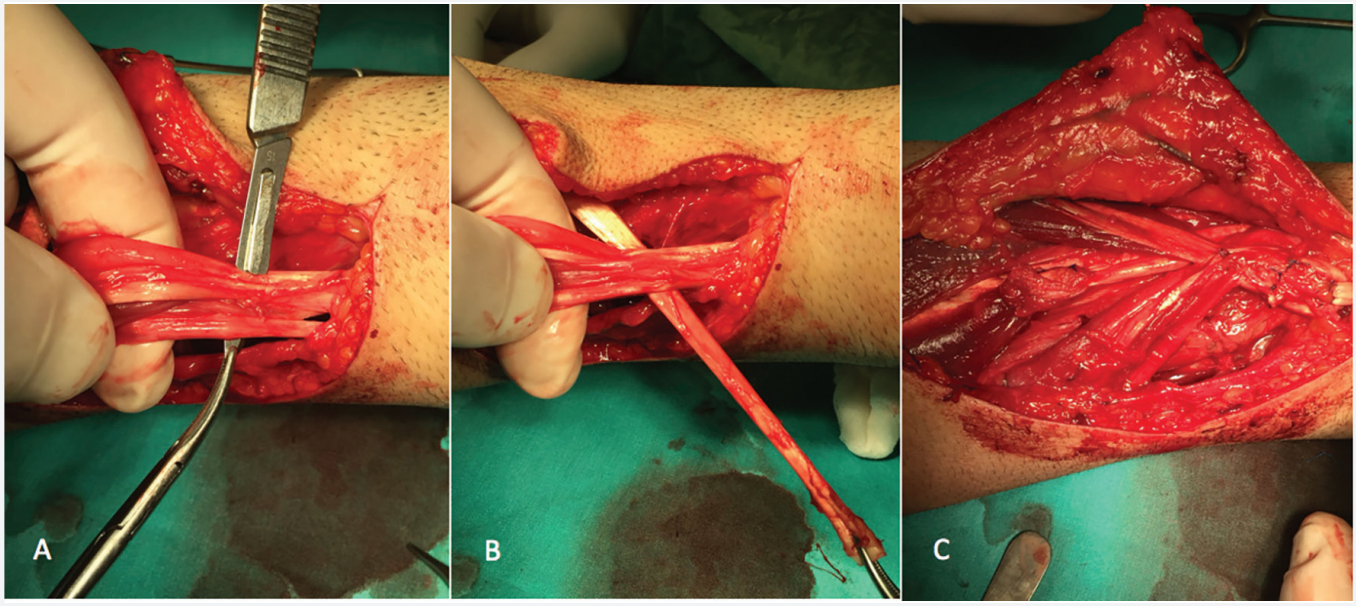

Figure 2. Flexor carpi radialis (FCR) transfer to the extensor digitorum communis (EDC 2-5), extensor indicis proprius, and extensor digiti minimi tendon transfer. A) Exposure of the finger extensors. B) Weaving of the FCR tendon through the finger extensors C) Final configuration using the Pulvertaft technique

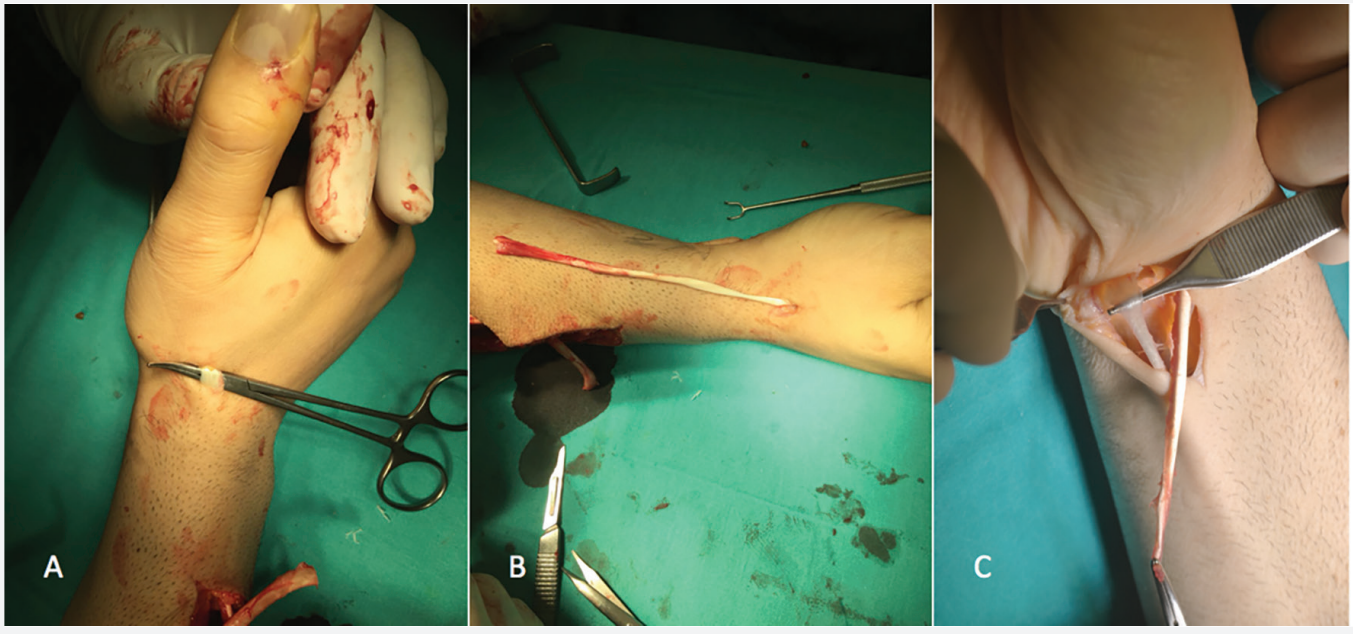

Figure 3. Palmaris longus to extensor pollicis longus (EPL) tendon transfer. A) Exposure of the EPL tendon B) Release of the EPL C) Volar rerouting of the EPL

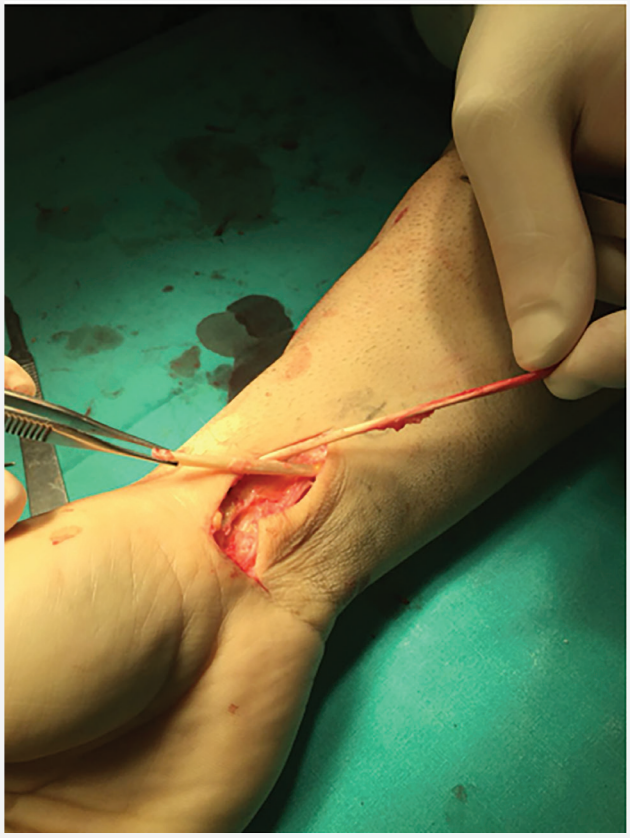

Figure 4. Fourth flexor digitorum superficialis to extensor pollicis longus tendon transfer

Table 1. Bincaz criteria

Points	3	2	1	0
Wrist extension		0-29°	0-29°	<0°
MCP joint extension		Full	Loss <10°	Loss >10°
First web space opening		>39°	0-39°	<30°
Patient satisfaction	Excellent	Good	Fair	Bad

MCP: Metacarpophalangeal

Table 2. Bincaz scoring system

Outcome	Score
Excellent	≥8
Good	6-7
Fair	4-5
Poor	≤3

primary healing without wound complications. Detailed descriptive data are presented in Table 3.

Significant postoperative improvement was recorded in the Quick-DASH scores used to evaluate functional status ($p<0.001$). The mean functional loss score, which was 31.82 ± 14.82 in the preoperative period, regressed to 16.03 ± 7.97 postoperatively (Table 4). Results of the ordinal categorical analysis using Bincaz scoring demonstrate the categorical levels of functional recovery. This transition between preoperative and postoperative measurements was analyzed using the Wilcoxon signed-rank test, and the change was found to be statistically significant ($p<0.001$; Table 5).

Table 3. Patient characteristics and surgical procedures

ID	Age	Sex	Side	Type of injury	Neurorrhaphy	Status	Wrist extension	Finger extension	Thumb
1	54	M	Right	Humeral shaft fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP	EPL-PL
2	33	M	Left	Humeral shaft fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-PL
3	15	M	Left	Forearm fracture (PIN lesion)	-	Chronic	-	FCR-EDC (2-5), EIP, EDM	EPL-PL
4	27	M	Right	Humeral shaft fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EDM	EPL-PL
5	39	M	Left	Firearm injury	-	Acute	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-PL
6	34	M	Right	Soft tissue tumor	+	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-FDS4
7	44	M	Left	Forearm fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EDM	EPL-PL
8	31	F	Left	Humeral shaft fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-PL
9	50	M	Left	Humeral shaft fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-FDS4
10	35	M	Right	Neural entrapment	-	Chronic	-	FCR-EDC (2-5), EIP, EDM	EPL-PL
11	23	F	Right	Schwannoma	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-PL
12	29	M	Left	Stab injury	+	Acute	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-PL
13	8	M	Left	Brachial plexus injury	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-PL
14	64	F	Right	Humeral shaft fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-FDS4
15	20	M	Right	Stab injury	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-FDS4
16	31	M	Left	Humeral shaft fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-PL
17	25	M	Left	Humeral shaft fracture	-	Chronic	PT-ECRB	FCR-EDC (2-5), EIP, EDM	EPL-PL
18	64	M	Right	Humeral shaft fracture	-	Chronic	PT-ECRL	FCR-EDC (2-5), EIP, EDM	EPL-PL
19	30	F	Left	Forearm fracture (PIN lesion)	-	Chronic	-	FCR-EDC (2-5), EIP, EDM	EPL-FDS4

PT: Pronator teres, ECRB: Extensor carpi radialis brevis, ECRL: Extensor carpi radialis longus, FCR: Flexor carpi radialis, EDC: Extensor digitorum communis, EIP: Extensor indicis proprius, EDM: Extensor digiti minimi, EPL: Extensor pollicis longus, PL: Palmaris longus, FDS4: Fourth flexor digitorum superficialis tendon, PIN: Posterior interosseous nerve

Table 4. Comparison of Quick-DASH scores

Measurement	Preoperative	Postoperative	p-value*
Quick-DASH score	31.82±14.82	16.03±7.97	0.001

*Wilcoxon signed-rank test, p<0.05 considered statistically significant

Table 5. Comparison of Bincaz functional scores

Bincaz score	Preoperative n (%)	Postoperative n (%)	p-value*
Poor	8 (47.1%)	0 (0%)	
Fair	5 (29.4%)	1 (5.3%)	
Good	4 (23.5%)	13 (68.4%)	0.001
Excellent	0 (0%)	5 (26.3%)	
Total	17* (100%)	19 (100%)	

*Wilcoxon signed-rank test, p<0.05 considered statistically significant

DISCUSSION

The results of our study indicate that triple tendon transfer utilizing the FCR for radial nerve lesions yields highly satisfactory functional outcomes. Radial nerve injuries typically result from direct cuts, trauma, or fractures. The incidence of radial nerve damage following humeral fractures is reported at 11.8%[9], establishing the radial nerve as the most frequently injured nerve associated with long bone fractures[7]. In our patient cohort, 63% of nerve injuries were attributable to fractures of the humerus or forearm.

Treatment options for radial nerve injury encompass two fundamental methodologies. The initial modality entails nerve repair, employing a range of techniques, namely primary, delayed primary, and secondary neurorrhaphy. Furthermore, in exceptional instances, neurolysis may be undertaken. Additionally, neuroplastic procedures, commonly employing sural nerve transplantation, are often utilized to rectify secondary nerve defects. The second methodology involves tendon transfer, a procedure specifically targeted at the forearm to restore lost functionality by redirecting and reattaching a muscle-tendon unit to another tendon or bone. Although treatments for radial nerve palsy have demonstrated successful results, there is currently no consensus regarding the most effective method or combination of treatments[6,10,11].

Tendon transfer planning after nerve repair can be determined according to Seddon's nerve regeneration rule (1-2 mm per day)[12]. Considering the expected recovery time, it is appropriate to apply tendon transfer after 12 weeks[10]. Other indications for which tendon transfer can be predicted include: severe injuries that cannot be repaired, late admissions exceeding 10 months, lack of electromyography response 6 months after nerve repair, severe atrophy/scarring, and high-level injuries such as brachial plexus lesions. In cases where the radial nerve defect exceeds 10 cm and primary repair or grafting is precluded by extensive

scar tissue, immediate tendon transfer should be considered a viable reconstructive strategy[13-15].

Tendon transfers to restore the radial nerve motor functions are basically evaluated in three main sections: wrist, thumb, and fingers[4]. While there is general consensus on the use of the PT as a motor muscle for wrist extension, it is also possible to use a variety of other muscles, such as the finger flexors, wrist flexors, and even the brachioradialis, with preservation of internal innervation[4]. To facilitate early recovery of grasp and eliminate the necessity of external splinting, an alternative approach known as "internal splinting", involving the early transfer from the PT to the ECRB during nerve repair, has been suggested. The process of radial nerve regeneration remains unimpeded by the implementation of this end-to-side transfer technique[15].

The posterior column of the thumb contains three muscles: APL, EPB, and EPL. Various options arise when planning a tendon transfer for the thumb[4,8]. Primarily, thumb column extension and retroposition are required, while avoiding radial deviation. The same transfer can be applied for the EDC and EPL. However, this alone limits the separation from the first commissure; thus, a separate tendon transfer for the APL and EPB can be performed to correct this[5]. We utilize a similar technique to that suggested by Scuderi, where the EPL is transferred to the PL, thereby preventing thumb adduction[4,16].

For finger extension, the FCU or FCR can be selected. One or more FDS tendons are also options. The FCU provides a force of 2 kg, which is close to the finger extensors; however, its excursion distance is 3.3 cm, which is less than the EDC excursion distance (4-5 cm). The FCR also has a shorter excursion distance than the EDC, yet its excursion distance is greater than that of the FCU, although it possesses a significantly lower force (0.8 kg)[4,8,17]. The use of FCU is controversial because it is the strongest flexor of the wrist; its absence can weaken wrist flexion and ulnar deviation while causing radial deviation[4]. In addition to aesthetic concerns, the grip strength decreases, affecting essential functions such as hammering and throwing.

Furthermore, the FCR can be obtained through a smaller incision, whereas the FCU requires an incision involving approximately two-thirds of the forearm[8]. While the FCR can be routed through the interosseous membrane, the FCU is transferred subcutaneously, which causes a visible bulge around the ulna[8]. In low-level radial nerve injuries, such as a PIN injury, the use of the FCU may result in severe radial deviation since the extensor carpi radialis longus (ECRL) is intact[5,18].

Brand et al.[17] and Tsuge and Adachi[19] described combinations of tendon transfers without the use of the FCU, suggesting PT to ECRB, FCR to EDC, and PL to EPL. We preferred FCR to EIP, EDC (2-5), and EDM transfer in our cases, and our results were sufficient according to Quick-DASH and Bincaz scores. Functional daily life requires 30° of extension, 5° of flexion, 10° of radial deviation, and 15° of ulnar deviation[11]; all cases met these criteria. No instances of weakness associated with radial or ulnar deviation were observed. According to the Bincaz scoring, five of 19 cases were excellent, 13 were good, and one was fair. Furthermore, the PL to EPL transfer provided favorable thumb abduction and prevented separation issues within the first commissure. We believe that the preservation of the FCU contributed to these positive functional scores. Supporting this, literature states that MCP joint range of motion is more important for grasp function than the strength of the transferred muscle[8].

Karabeg[10] evaluated the use of FCR and FCU in 40 cases with radial nerve injury following war-related injuries. The Zachary score was 92.25% in the FCR group and 82.20% in the FCU group ($p<0.05$). Tajima scores also favored the FCR group ($p=0.024$). This difference was attributed to better preservation of finger extension in the 2nd to 5th digits. In a comparative study by Kumar et al.[8], 10 patients achieved excellent or good results, three were fair, and two were poor according to Bincaz criteria. In three cases with high radial nerve palsy, radial deviation was attributed to adhesions between the ECRL and ECRB, suggesting routine intraoperative inspection. In cases where the FCU was used for finger extension, patients reported difficulty opening large jar lids and holding plates. Furthermore, due to limited ulnar deviation, difficulties were noted in performing the "dart-throwing motion," which is essential in certain professions.

This study demonstrates that favorable results are obtained by preferring FCR transfer for finger extension across various injury types. Since the majority of our patients utilize their hands for physically demanding tasks, we believe that the preservation of the FCU was effective in achieving high patient satisfaction.

Study Limitations

The limitations of the study include the relatively small sample size and the absence of a control group undergoing other transfers, such as FCU or FDS for the wrist.

CONCLUSION

Tendon transfers are a reliable method for restoring radial nerve motor functions, particularly in cases where the nerve is irreparable. Although a wide variety of tendon transfer techniques exist, every technique presents distinct advantages and disadvantages in practice. The surgeon must determine the specific technique on a case-by-case basis according to the patient's occupational and functional requirements.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Tekirdağ Namık Kemal University Non-Interventional

Clinical Research Ethics Committee (approval number: E-46048792-050.01.04-350165, date: 22.09.2023).

Informed Consent: The study is a retrospective study.

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Fotnootes

Authorship Contributions

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

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CASE REPORT

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Spontaneous Gastric Perforation in a Young Male with Anorexia Nervosa, Binge-eating/Purging Type: A Case Report

Tıkınırcasına Yeme/Arınma Tipi Anoreksiya Nervosa Tanılı Genç Bir Erkekte Spontan Gastrik Perforasyon: Bir Olgu Sunumu

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ABSTRACT

Bulimia nervosa (BN) is an eating disorder characterized by recurrent binge eating and compensatory behaviors. Diagnostic crossover to anorexia nervosa (AN) often occurs in chronic cases. We report a 29-year-old male with a ten-year BN history who presented with mild dyspepsia and a single episode of black-colored vomiting. Physical examination was unremarkable. However, laboratory tests revealed hypokalemia and renal dysfunction. Imaging demonstrated a 1-cm gastric perforation that required emergency surgery. Psychiatric evaluation confirmed AN-binge-eating/purging type (AN-b/p). Following surgery, combined therapy with fluoxetine and topiramate achieved clinical stabilization, promoted weight gain, and eliminated purging behaviors. This case underscores that gastric perforation, although rare, is a potentially life-threatening complication of AN-b/p. In eating disorders, altered pain perception may blunt symptom awareness, enabling serious complications to progress silently. Multidisciplinary collaboration is essential for effective treatment and recovery.

Keywords: Eating disorders, bulimia nervosa, anorexia nervosa, binge-eating/purging type, gastric dilatation, gastric perforation, male, emergency surgery

ÖZ

Bulimia nervoza (BN), tekrarlayan tıkınırcasına yeme atakları ve telafi edici davranışlarla karakterize bir yeme bozukluğudur. Kronik olgularda sıklıkla anoreksiya nervoza (AN) tanısına geçiş görülebilir. On yıllık BN öyküsü olan 29 yaş erkek hasta, hafif dispepsi ve bir kez siyah renkli kusma şikayeti ile başvurdu. Fizik muayenesi belirgin bir bulgu vermemekle birlikte, laboratuvar testlerinde hipokalemi ve böbrek fonksiyon bozukluğu saptandı. Görüntülemede 1 cm'lik mide perforasyonu tespit edilerek acil cerrahi yapıldı. Psikiyatrik değerlendirme, AN-tıkınırcasına yeme/arınma tipi (AN-b/p) tanısını doğruladı. Ameliyat sonrası fluoksetin ve topiramat tedavisi ile klinik stabilizasyon, kilo artışı ve arıtma davranışlarının sona ermesi sağlandı. Bu olgu, mide perforasyonunun nadir olmakla birlikte AN-b/p'nin potansiyel olarak hayatı tehdit eden bir komplikasyonu olduğunu vurgulamaktadır. Yeme bozukluklarındaki bozulmuş ağrı algısı, şiddetli komplikasyonların hafif veya belirsiz semptomlar altında fark edilmeden ilerlemesine yol açabilir. Etkili tedavi ve iyileşme için multidisipliner iş birliği gereklidir.

Anahtar Kelimeler: Yeme bozuklukları, bulimia nervoza, anoreksiya nervoza, tıkınırcasına yeme/arınma tipi, gastrik dilatasyon, gastrik perforasyon, erkek, acil cerrahi

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INTRODUCTION

Bulimia nervosa (BN) is a severe eating disorder (ED) characterized by recurrent episodes of binge eating followed by maladaptive weight-control behaviors such as self-induced vomiting, misuse of laxatives or diuretics, fasting, or excessive exercise[1,2]. BN affects approximately 1-1.5% of women and 0.5-1% of men, accounting for a substantial share of disability-related years of life lost[3]. Failure to recognize and diagnose BN in a timely manner, particularly in men, markedly increases the likelihood of medical emergencies.

BN rarely presents alone; it frequently coexists with mood and anxiety disorders, with comorbidity rates reported as high as 70%[4]. The repetitive vomiting and purging behaviors characteristic of BN substantially increase the risk of systemic complications, including electrolyte disturbances, renal impairment, cardiomyopathy, and gastrointestinal (GI) injury[5]. BN is a well-recognized cause of GI symptoms. Acute gastric dilatation, necrosis, and spontaneous perforation, though rare, are potentially fatal complications. In the past century, fewer than 40 adult cases have been reported, most commonly in association with binge-purge episodes[6]. Mortality in BN exceeds 30%, and septic hemorrhagic shock, which often results from delayed diagnosis, is the leading cause of death[6].

Diagnostic crossover between BN and anorexia nervosa (AN) has been well documented in individuals with longstanding ED[1,7]. Persistent binge eating alongside a significantly low body mass index (BMI) is classified as AN-binge-eating/purging type (AN-b/p) according to DSM-5-TR[8]. Such transitions are often underrecognized in male patients, leading to delayed diagnosis and an increased risk of medical complications[9].

This case report highlights the clinical course and significance of a rare yet serious GI complication in a patient with a ten-year history of BN who fulfills the diagnostic criteria for AN-b/p. By assessing various clinical findings and associated risks, this report aims to enhance early diagnosis and optimize medical management strategies in EDs.

CASE REPORT

History and Physical Examination

A 29-year-old male presented to the emergency department with a three-day history of dyspepsia and a single episode of black-colored vomiting. He reported frequent episodes of self-induced vomiting to a sensation of gastric fullness, which offered him temporary relief. He had no history of abdominal pain, recent surgery, trauma, or endoscopic procedures. He has had a diagnosis of BN for the past ten years. Anthropometric measurements revealed a height of 160 cm and a weight of 40 kg (BMI: 15.6 kg/m²). His psychiatric history revealed prior treatment with fluoxetine (60-80 mg/day), sertraline (100-150 mg/day), and olanzapine (2.5-5 mg/day) at different times. These treatments provided limited benefit, likely due to inconsistent adherence, premature discontinuation, and irregular clinical follow-up. For the past six months, he has been consistently taking fluoxetine at a stable dose of 40 mg/day.

On admission, his vital signs were: body temperature 36 °C, blood pressure 95/57 mmHg, heart rate 120 bpm, respiratory rate 18 breaths/min, and SpO_2 97%. Abdominal examination revealed no signs of guarding or rebound tenderness, and rectal examination showed an empty ampulla.

Laboratory and Radiologic Findings

Laboratory investigations revealed leukocytosis (white blood cell: 12.52×10^3/μL), mild anemia (hemoglobin: 11.3 g/dL), and elevated inflammatory markers (C-reactive protein: 9 mg/L). Renal function tests showed markedly elevated urea (133 mg/dL) and creatinine (3.02 mg/dL) levels. Electrolyte analysis indicated hyponatremia (Na: 132 mEq/L) and significant hypokalemia (K: 2.57 mEq/L). Arterial blood gas analysis demonstrated alkalemia (pH: 7.51) with metabolic alkalosis (bicarbonate: 59 mEq/L) and a mildly elevated lactate level (2.2 mmol/L). Urinalysis was positive for ketones. The patient's laboratory results are presented in Table 1. Abdominal radiography revealed diffuse radiolucent air densities in the left upper and lower quadrants, while chest radiography was unremarkable with no evidence of subdiaphragmatic free air (Figure 1). Contrast-enhanced abdominal computed tomography demonstrated gastric dilatation on the coronal plane (Figure 2) and an approximately 1 cm perforation on the posterior wall of the gastric fundus, accompanied by contrast extravasation on the axial plane (Figure 3).

Mental Status Examination

On mental status examination, the patient's attention was intact, mood was mildly depressive with an appropriate and reactive affect, and no psychotic features or suicidal ideation were observed. Sleep was disturbed, while psychomotor activity remained within normal limits. The patient demonstrated a tendency to rationalize purging behavior, and insight was partially preserved. The clinical presentation was considered consistent with AN-b/p, according to DSM-5-TR criteria[1].

Psychiatric evaluation revealed that the patient was actively engaging in maladaptive eating behaviors. Despite prior therapeutic interventions, the patient had not adhered to consistent follow-up or pharmacotherapy, leading to a chronic pattern of disordered behavior. During the stabilization period, fluoxetine 40 mg/day was continued, and trazodone 25 mg at bedtime was introduced for sleep, with good tolerance.

Table 1. The patient's laboratory results at admission

Laboratory parameters	Results	Normal range
Hemogram		
Leukocytes (10^3/µL)	12.52	4-10
Neutrophils (10^3/µL)	9	2-7
Lymphocytes (10^3/µL)	2.12	0.4-7
Hemoglobin (10^3/g/dL)	11.3	12-16
Biochemistry		
Alanine aminotransferase (U/L)	18	0-50
Aspartate aminotransferase (U/L)	25	10-50
Urea (mg/dL)	133	12-43
Creatinine (mg/dL)	3.02	0.7-1.2
C-reactive protein (mg/L)	9	0-5
Sodium (mEq/L)	132	134-145
Potassium (mEq/L)	2.57	3.3-5.2
Chloride (mEq/L)	67	98-107
Calcium (mg/dL)	8.91	8.4-10.2
Albumin (g/L)	37	35-52
Amylase (U/L)	61	29-100
Lipase (U/L)	34	13-60
International normalized ratio	0.9	0.8-1.2
Blood gas		
pH	7.51	7.35-7.45
Bicarbonate (mmol/L)	59.6	22-26
Lactate (mmol/L)	2.2	0.5-2
Urinalysis		
Leukocytes	1	0-5
Erythrocytes	1	0-2
Ketones	++	Negative

Follow-up and Clinical Outcome

The patient underwent emergency laparotomy, and the gastric perforation was repaired primarily. His postoperative course remained stable, and he was discharged on the ninth day with complete surgical resolution. The patient was assessed as being at moderate risk for refeeding syndrome, and nutritional management was coordinated by the dietitian and internal medicine team with close monitoring of electrolyte levels.

Postoperatively, no recurrence of purging behaviors was observed, and treatment adherence was good. The fluoxetine dose was revised to 60 mg/day, and topiramate was added and titrated to 50 mg/day. Following the initiation of topiramate, the patient demonstrated a notable decrease in vomiting urges and a more regular pattern of food intake. The patient's BMI reached 19.3 kg/m², and he returned to work.

DISCUSSION

This case report presents the clinical course and management of gastric perforation in a patient with AN-b/p. The patient exhibited a diagnostic transition from BN to AN. Although EDs are primarily psychiatric conditions, they are often accompanied by severe medical complications. Our case involved a young male patient who presented with only mild dyspeptic symptoms. He exhibited no abdominal pain or signs of guarding that would suggest an acute abdomen. The unexpected finding of a fundic perforation underscores the importance of heightened clinical vigilance for rare but potentially life-threatening complications of AN-b/p. Gastric perforation can occur in AN—especially the AN-b/p type—due to chronic gastric hypomotility and delayed emptying, even without obvious symptoms[10,11].

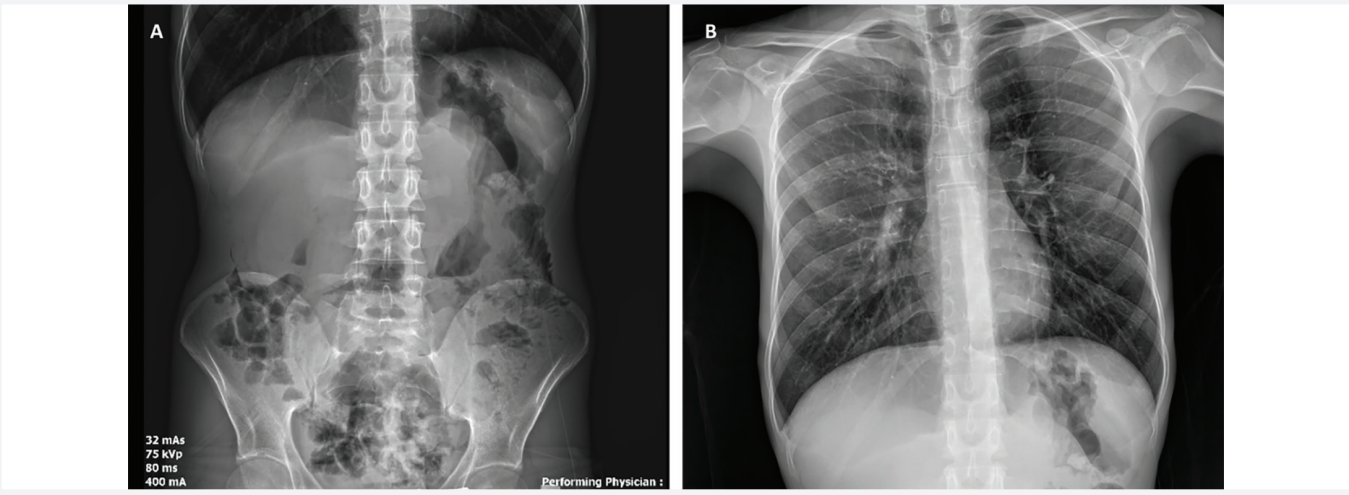

Figure 1. Radiographic findings: (A) Abdominal radiograph demonstrates diffuse radiolucent air densities in the left upper and lower quadrants, (B) Normal chest radiograph

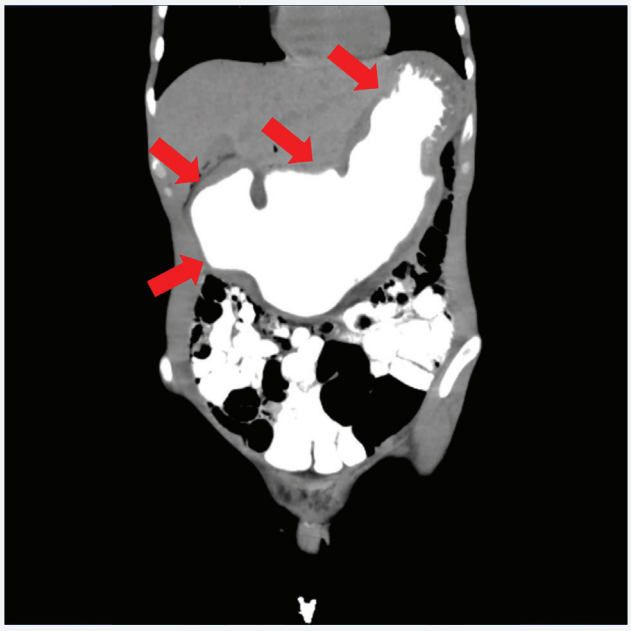

Figure 2. Abdominal computed tomography demonstrates gastric dilatation in the coronal plane (arrow)

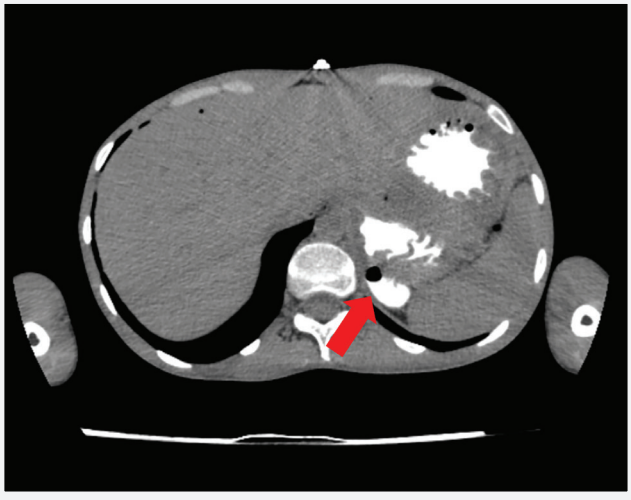

Figure 3. Abdominal computed tomography demonstrates a perforation in the posterior wall of the gastric fundus, with associated contrast extravasation and free intraperitoneal air in the axial plane (arrow)

EDs have been associated with a wide range of GI symptoms. Postprandial fullness and abdominal bloating are reported in the majority of individuals with EDs. Additionally, over half experience symptoms such as abdominal pain, gastric distension, early satiety, and nausea[12]. GI manifestations of EDs include esophageal symptoms, delayed gastric motility, functional dyspepsia, constipation, irritable bowel syndrome, rectal prolapse, hepatic failure, and even superior mesenteric artery syndrome[13]. BN also leads to complications specific to binge-purge behaviors, including recurrent vomiting and laxative misuse. Compensatory behaviors such as repeated vomiting and laxative abuse have been shown to cause electrolyte imbalances (e.g., hypokalemia, hypochloremic alkalosis), GI mucosal injury, oral pathologies, and hormonal-metabolic dysfunctions. These behaviors have also been associated with severe outcomes including esophageal rupture, gastric perforation, parotid gland hypertrophy, and even esophageal cancer[13]. In individuals with AN and low BMI, even isolated purging behaviors may provoke severe GI complications and significantly increase mortality[14]. In EDs, severe complications may develop silently, even in the absence of overt clinical symptoms. Impaired pain perception allows such complications to progress unnoticed. The pathophysiology of AN has been proposed to involve deficits in interoception, reflecting a reduced capacity to perceive and regulate internal physiological states[15]. Supporting this notion, empirical studies have shown that individuals with AN exhibit altered subjective responses to interoceptive cues, including pain[16]. Additionally, patients with AN often present with elevated levels of alexithymia, which is associated with difficulties in identifying and articulating emotions[17].

Acute gastric dilatation, a potentially fatal complication, has been reported in individuals engaging in binge-eating behavior, particularly when surgical management is delayed[18]. It has been shown that BN can rarely result in gastric perforation[6,19]. Gastric dilatation resulting from binge eating, combined with elevated intragastric pressure during vomiting, can lead to vascular insufficiency, dilation, and mucosal necrosis in the gastric fundus[20]. These factors are considered to predispose to gastric perforation. In our case, the sensation of bloating and dyspepsia preceding the vomiting episode may indicate acute gastric dilatation and act as a trigger for the compulsive purging behavior frequently observed in BN. The black-colored vomitus may further support the presence of chronic purging behavior and possible mucosal injury. Prompt recognition and timely intervention are essential to prevent severe complications. Acute gastric dilatation and rupture have also been reported in AN patients without prior binge episodes, potentially resulting from altered gastric compliance and ischemia[21].

Recent epidemiological studies estimate the prevalence of BN in men to be approximately 0.5-1%, highlighting that a substantial proportion of cases—especially among young adult males—remain undiagnosed or untreated[3].

From a psychiatric standpoint, the patient's history includes multiple trials of antidepressants and antipsychotics. However, inconsistent follow-up and poor treatment adherence have resulted in a chronic behavioral pattern. Factors including

irregular treatment attendance, premature discontinuation, and comorbid impulsivity have been reported to reduce remission rates in EDs[4]. At admission, the patient was consistently taking fluoxetine, providing stability for psychopharmacological management. During the postoperative period, pharmacological treatment for AN-b/p included a combination of fluoxetine (60 mg/day) and topiramate (50 mg/day). The use of fluoxetine in AN is off-label, with some studies suggesting potential benefits, particularly after weight restoration[22]. Topiramate is effective in controlling purging behaviors and impulsivity. A randomized, double-blind, placebo-controlled trial showed it significantly reduces binge eating and self-induced vomiting in BN patients[23]. Pharmacological management in AN-b/p requires caution because of malnutrition-related risks. Although selective serotonin reuptake inhibitors and topiramate are commonly employed, their use must be carefully monitored and individualized for each patient[10].

This case underscores the crucial need for a high index of suspicion for severe GI complications in patients with EDs, especially those presenting with the AN-b/p subtype. Despite presenting without overt abdominal symptoms, our patient developed gastric perforation—a life-threatening condition likely triggered by chronic purging and underlying gastric hypomotility. This case underscores the need to remain vigilant for severe somatic complications, even in patients with EDs who present with only mild or non-specific GI symptoms. Furthermore, the case exemplifies the complexity of managing EDs with psychiatric and medical comorbidities, emphasizing the importance of a multidisciplinary approach. Early recognition, timely surgical intervention, and coordinated psychiatric follow-up including evidence-based pharmacological treatment are essential for reducing morbidity and preventing recurrence. Clinicians should be aware that EDs can present with atypical features and carry the risk of abrupt, fatal complications even in young patients who may remain undiagnosed or undertreated.

CONCLUSION

This case additionally emphasizes the value of detailed case reporting for rare but potentially fatal complications in EDs, particularly among underrepresented groups such as male patients. By documenting the clinical course, diagnostic challenges, and successful multidisciplinary management, this report provides a reference for early recognition and intervention strategies. It reinforces the need for heightened awareness among clinicians, not only for timely surgical management but also for coordinated psychiatric care, thereby contributing to improved patient outcomes and guiding future clinical practice.

Ethics

Informed Consent: Written informed consent was obtained from the patient for the publication of this case report and any accompanying data.

Footnotes

Authorship Contributions

Concept: İ.A., H.İ.E., M.T.K., Design: İ.A., M.T.K., Data Collection or Processing: H.İ.E., M.T.K., Analysis or Interpretation: İ.A., H.İ.E., Literature Search: H.İ.E., M.T.K., Writing: İ.A.

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

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LETTER TO THE EDITOR

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Nam Kem Med J 2026;14(2):228-230

Maxillary Sinus Cholesterol Granuloma: An Unanticipated Diagnosis in a Paediatric Case

Maksiller Sinüs Kolesterol Granülomu: Pediatrik Olguda Beklenmedik Bir Tanı

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Keywords: Maxillary sinus, cholesterol granuloma, histopathology
Anahtar Kelimeler: Maksiller sinüs, kolesterol granülomu, histopatoloji

To the Editor,

Cholesterol granuloma (CG) is typically linked with chronic mid ear disease and is an uncommon histopathological finding in the maxillary sinus (MS). It consists of granulation tissue formation, intensive cholesterol crystal aggregates, which lead to foreign body giant cell reaction. Althought exact pathogenesis is unclear however, it is frequently linked to a history of trauma, sinusitis, rhinitis, and paranasal sinus surgery, particularly since these conditions can result in localized bleeding[1]. Cholesterol is thought to originate from the cell membranes of erythrocytes that are damaged during bleeding, leading to its crystallization due to poor drainage[2]. This condition is more frequently observed in mid-aged individuals. However, we present an unusual paediatric patient of CG in the MS, highlighting its uncommon occurrence and the significance of discriminative diagnosis.

Twelve years old girl was referred to otolaryngology clinic with a 5-year history of nasal obstruction that had exacerbated over the last month following a viral upper respiratory tract infection. Her symptoms included increasing clear nasal discharge, postnasal drip, and headache, predominantly in the left anterior and frontal regions. She reported no additional systemic or neurological complaints. On examination, grade 3 adenoid vegetation was observed, along with mucopurulent discharge progressing from the left osteomeatal complex to the nasopharynx via the middle meatus, and tenderness in the left maxillary region. She was initially diagnosed with acute rhinosinusitis and was began on antibiotic therapy, analgesics, and decongestants.

Despite 10 days of treatment, the patient's symptoms did not improve. On follow-up examination, adenoid vegetation persisted, but the previously noted purulent discharge from the left osteomeatal complex and left maxillary tenderness were no longer observed. Consequently, further investigation was warranted to differentiate the cause of the headache. Contrast-enhanced nasopharyngeal and cranial magnetic resonance imaging revealed complete opacification of the left MS, along with an additional oval-shaped, denser opacity measuring 2x1.4 cm located at the sinus floor, separated from the MS opacity. There was no significant bone erosion, and no intra-

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or extracranial pathology involving other anatomical structures was observed. The patient underwent adenoidectomy and MS surgery.

Both the MS lesion and the adenoidectomy specimens were sent for pathological examination. Preoperative diagnosis of the MS lesion was sinonasal polyp. The patient remained hospitalized for one day postoperatively. The patient's symptoms had subsided by the end of the first postoperative week, and her follow-up care is ongoing.

Gross examination revealed a polypoid lesion, 2x1.4 cm in diameter, with a brown-tan cut surface. Histopathologic analysis showed respiratory epithelium-lined tissue with numerous subepithelial cholesterol crystals, surrounded by multinucleated giant cells and macrophages. Destructed erythrocytes, hemosiderin-laden histiocytes, oedema, plasma cells, lymphocytes, and fibrotic changes were also present (Figure 1). The adenoidectomy sample was diagnosed as reactive lymphoid hyperplasia with associated chronic inflammation.

CG is a histological diagnosis characterized by granulation tissue including foreign body giant cells and numerous cholesterol clefts. While CG mostly detected in the mastoid bone and mid ear, CG in the MS is an uncommon lesion, especially in children. To our knowledge, this patient is the fifth case of CG presenting as an antrochoanal polyp in paediatric cases[1-3]. Potential aetiological causes of MS CG include impaired drainage, interrupted ventilation, and bleeding into the sinus, leading to destruction of red blood cells and cholesterol collection from erytrocytes' membranes. Surgical excision is recommended treatment. Differential diagnoses for CG include mucoceles, allergic/inflammatory polyps, sinus cysts, and tumors, with

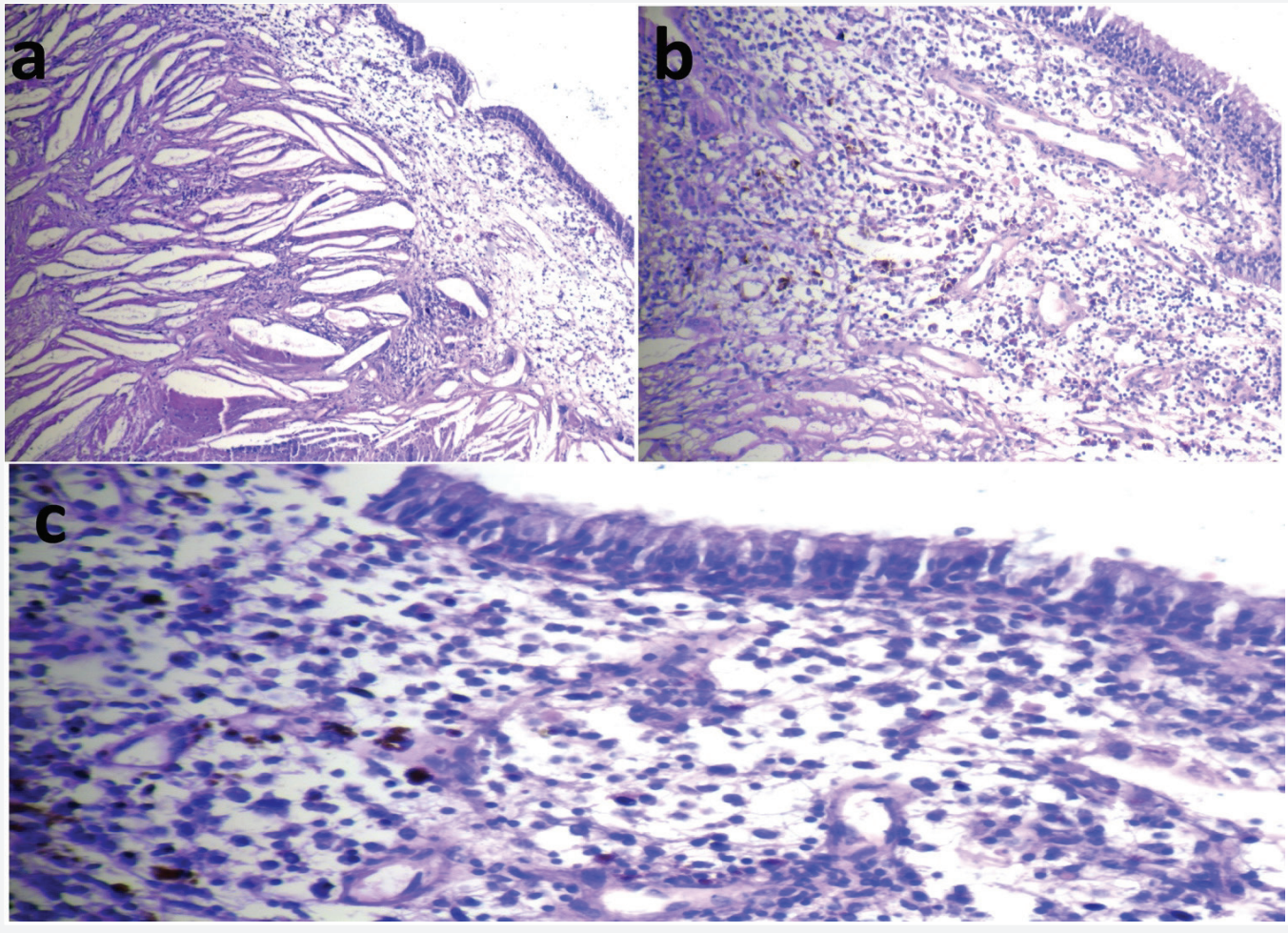

Figure 1. Histopathologic examination of the lesion showed respiratory epithelium-lined tissue with numerous subepithelial cholesterol crystals, surrounded by multinucleated giant cells and macrophages hematoxylin and eosin [(H&E), x100] (A). Destructed erythrocytes, hemosiderin-laden histiocytes, oedema, plasma cells, lymphocytes, and fibrotic changes are seen (H&E, x100; H&E, x200) (B, C).

histopathological analysis being crucial for a definitive diagnosis[3].

Footnotes

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LETTER TO THE EDITOR

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Nam Kem Med J 2026;14(2):231-232

Novel Indices for Vascular Inflammation and Risk Assessment

Vasküler Enflamasyon ve Risk Değerlendirmesi için Yeni Endeksler

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To the Editor,

We read with great interest the article by Aydın et al.[1] examining the prognostic value of the pan-immune-inflammation value in predicting saphenous vein graft (SVG) patency after coronary artery bypass surgery. Their findings underscore the critical role of systemic inflammation in the development of graft failure, and we congratulate the authors on highlighting this emerging biomarker.

We thank the authors for their important contribution. We would like to add a point about body composition. Body mass index (BMI) is still often used in risk prediction. However, BMI does not show body fat distribution or muscle mass. This can cause underestimation of risk in people who are not overweight but still have metabolic issues[2]. Visceral fat, not total weight, drives inflammation and vascular problems. The triglyceride-glucose (TyG) index is a simple marker. It indicates insulin resistance and is associated with an increased risk of heart disease, stroke, and atherosclerosis[3]. Inflammation may explain part of this link[4]. Another combined index, called chemotherapy-induced toxicity (CTI) (C-reactive protein-triglyceride-glucose index), combines TyG and inflammation markers. High CTI levels are associated with a higher risk of heart attacks, stroke, and death[5]. CTI gives a better view of overall cardiometabolic stress. The body roundness index (BRI) is another helpful measure. It uses waist and height to estimate body fat pattern. BRI works better than BMI for predicting metabolic syndrome and artery stiffness[6]. People with high BRI levels also show more inflammation[7]. This makes BRI more reliable than BMI for cardiovascular risk[7]. Additionally, the importance of combining inflammatory and nutritional indices in vascular risk prediction has been further supported by recent findings by Luo et al.[8] In their large-scale analysis of elderly adults, they found that both the systemic inflammatory response index (SIRI) and the geriatric nutritional risk index were independently associated with stroke risk. Notably, SIRI accounted for a significant portion of the relationship between dietary factors and stroke, highlighting the importance of systemic inflammation in vascular outcomes. Recently, we evaluated the prognostic value of the BRITISH ratio, which we newly defined, with a particular focus on its relationship with thyroid function and body composition[9]. Therefore, the BRITSH ratio can be used to assess prognosis in terms of coronary artery disease in all patients, not only those with hypothyroidism.

We believe future studies should use these indices TyG, CTI, BRI, BRITSH and SIRI to improve risk assessment. They are easy to calculate and can detect hidden risk. This can lead to better monitoring, especially after bypass surgery. Collectively, these findings support the notion that fat quantity alone is insufficient to capture cardiovascular risk - fat quality and localization, alongside metabolic and inflammatory context, are critical determinants. As the authors of the current study rightfully emphasize the role of systemic immune-inflammatory activation in SVG disease.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Ç.K., F.K., Concept: Ç.K., F.K., Design: Ç.K., F.K., Data Collection or Processing: Ç.K., F.K., Analysis or Interpretation: Ç.K., F.K., Literature Search: Ç.K., F.K., Writing: Ç.K., F.K.

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ERRATUM

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Yılmaz B, Esenkal D, Delen B, Dal S, Çelik SZ, Ürer Z, et al. Apoptotic and antifungal effects of *Lactobacillus paracasei* postbiotics on HepG2 cells against *Candida* spp. Nam Kem Med J. 2026;14:86-94

The mistake in the Turkish and English titles was made inadvertently by the author.

The English and Turkish titles on page 86 of the article were corrected by the author as shown below.

Incorrect part in the English title

Apoptotic and Antifungal Effects of *Lactobacillus paracasei* Postbiotics on HepG2 Cells Against *Candida* spp.

Correct part in the English title

Apoptotic Effects on HepG2 Cells and Antifungal Activity Against *Candida* spp. of *Lactobacillus paracasei* Postbiotics

Incorrect part in the Turkish title

Lactobacillus paracasei Postbiyotiklerinin HepG2 Hücrelerinde Apoptotik ve *Candida* spp.

Türlerine Karşı Antifungal Etkileri

Correct part in the Turkish title

Lactobacillus paracasei Postbiyotiklerinin HepG2 Hücrelerinde Apoptotik Etkisi ve *Candida* spp.'ye Karşı Antifungal Aktivitesi

Incorrect part in the "Cite this article as"

Yılmaz B, Esenkal D, Delen B, Dal S, Çelik SZ, Ürer Z, et al. Apoptotic and antifungal effects of *Lactobacillus paracasei* postbiotics on HepG2 cells against *Candida* spp. Nam Kem Med J. 2026;14(1):86-94

Correct part in the "Cite this article as"

Yılmaz B, Esenkal D, Delen B, Dal S, Çelik SZ, Ürer Z, et al. Apoptotic effects on HepG2 cells and antifungal activity against *Candida* spp. of *Lactobacillus paracasei* postbiotics. Nam Kem Med J. 2026;14(1):86-94