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EDITORIAL

EDİTÖRYAL

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The potential of artificial intelligence and machine learning algorithms in the field of cardiovascular diseases

Kardiyovasküler hastalıklar alanında yapay zeka ve makine öğrenimi algoritmalarının potansiyeli

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ABSTRACT

Artificial intelligence (AI) and machine learning (ML) is a form of intelligence that has a wide-reaching impact on various aspects of contemporary life, including the field of medicine. Artificial intelligence tools and technology are utilized for the early identification and diagnosis of severe or intricate heart conditions. The potential of artificial intelligence (AI) in cardiovascular medicine is significant. However, the major challenges hindering the development of AI applications in this field include the scarcity of diverse data and limited availability of huge datasets. These revolutionary digital technologies will play a significant role in shaping the future of cardiology.

Key Words: Artificial intelligence, machine learning, cardiovascular disease

ÖZ

Yapay zeka (AI) ve makine öğrenimi (ML), tıp da dahil olmak üzere modern yaşamın neredeyse her alanına dokunan bir zekadır. Yapay zeka araçları ve teknolojileri, ciddi veya karmaşık kalp sorunlarının erken risk tahmini ve teşhisine uygulanmaktadır. Kardiyovasküler tıpta yapay zekanın potansiyeli büyüktür, ancak sınırlı veri çeşitliliği ve birçok büyük veri tabanına erişim eksikliği, yapay zeka uygulamalarının geliştirilmesinin önündeki en büyük engeller arasındadır. Kardiyolojinin geleceği büyük ölçüde bu yenilikçi dijital teknolojilere dayanacaktır.

Anahtar Sözcükler: Yapay zeka, makine öğrenimi, kardiyovasküler hastalıklar

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Artificial intelligence (AI) is intelligence that affects nearly every aspect of modern life, including medicine. Clinical practice increasingly employs artificial intelligence tools and technology to assist individuals suffering from or at risk of developing heart disease. We use these novel techniques to detect and diagnose serious or complex heart diseases early.Furthermore, AI has the ability to improve medical image analysis, such as echocardiography and cardiac MRI scans. Clinical data like electrocardiograms (ECGs), echocardiograms, and medical imaging can apply machine learning algorithms to predict outcomes, stratify risks, and identify cardiovascular diseases [1].

One of the most common uses of machine learning (ML) in cardiology is to predict heart rhythm abnormalities. The most common clinically significant arrhythmia, atrial fibrillation (AF) carries a substantial risk of stroke, heart failure and death. Therefore, significant efforts are currently underway to enhance AF screening, early identification and treatment. Among these initiatives, AI technology

has shown therapeutic utility and potential. Artificial intelligence has enabled the prediction of AF using multivariable models as well as the improvement of 12-lead ECGs in sinus rhythm. Consumer-direct goods such as smartphones and watches use AI-based algorithms widely to identify AF through platoplethysmography, both actively and passively [2]. Recent studies show that artificial intelligence can provide useful prognostic evaluations and treatment recommendations.

With the rising prevalence of heart failure, it is crucial to ensure precise diagnosis and personalized treatment. Wearable gadgets and remote monitoring assisted by AI, allow for early identification of heart failure and better patient care. Machine learning systems can reduce needless hospitalizations for heart failure by identifying individuals who are more likely to experience cardiac decompensation after discharge than traditional risk assessments. Artificial intelligence uses a variety of data from these individuals, including ECGs, echocardiograms and electronic health records [3]. Kwon et al., demonstrated that an AI-enabled smart watch with a 2-lead ECG could diagnose heart failure with a lower ejection fraction with satisfactory accuracy [4]. Another relevant field is heart transplantation, where ML algorithms appear to be useful for predicting the likelihood of mortality, transplantation or transplant success in individuals on the waiting list.

Artificial intelligence has proven to be more effective than traditional risk scales in predicting cardiovascular disease risk in the general population, ranging from primary care to conventional electronic medical records. However, the findings vary depending on sample size, indicating that ML approaches perform better with larger samples. Medical experts can use AI to help diagnose coronary artery disease (CAD) by analyzing computed tomography and magnetic resonance images to assess the presence and severity of plaque in the coronary arteries. Medical professionals can also take advantage of the real-time feedback and guidance offered by AI algorithms during CAD procedures such as angioplasty and stent implantation [5]. AI methodologies, combined with the growing accessibility of big data, have already made notable progress in the fields of disease detection, risk assessment, phenotyping and clinical decision support for a range of cardiovascular diseases, including atherosclerotic heart disease, peripheral arterial disease, abdominal aortic aneurysm and carotid artery disease [6].

In the near future, artificial intelligence may bring about a paradigm shift in cardiovascular medicine. The potential for AI in cardiovascular medicine is enormous but the restricted diversity of data and lack of access to many huge databases are among the most significant barriers to the development of AI applications. The future of cardiology will be highly reliant on these revolutionary digital technologies. Despite this, we have not adequately addressed the ethical quandaries surrounding the adoption of AI technology in the real world.

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RESEARCH ARTICLE

ARAŞTIRMA

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Diagnostic Value of Ultrasonographic Septation in the Differentiation of Scrotal Hematocele and Hydrocele: A Single-Center, Retrospective Study

Skrotal Hematosel ve Hidrosel Ayırımında Ultrasonografik Septasyonun Tanısal Değeri: Tek Merkezli, Retrospektif Bir Çalışma

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ABSTRACT

Aim: Since the scrotum and testicles are superficial organs, they can be easily examined by palpation and sometimes transillumination. However, it is not possible to distinguish whether the fluid in the scrotum is hydrocele or hematocele. This study attempts to find an ultrasonographic distinction between hematocele and hydrocele based on a simple finding (septation) that every physician can recognize.

Methods: Patients who were admitted to the emergency department (ED) with complaints of scrotal pain, swelling or redness were divided into two groups: those whose complaints developed due to trauma and those whose complaints developed spontaneously. All included patients underwent scrotal US/Doppler by an emergency medicine specialist with US training.

Results: Of the 61 patients included in the study, 36 (59%) presented with scrotal trauma and 25 (41%) with non-traumatic complaints. While hematocele was detected in 8 (22.2%) and hydrocele was detected in 1 (2.8%) of 36 patients with a history of trauma, neither hematocele nor hydrocele was detected in 27 (75%). On the other hand, septation was detected in 6 of 8 hematocele patients (75%), but no septation was detected in 2 (25%) patients (p = 0.013). However, no signs of septation were found in any of the 28 patients who presented due to scrotal trauma and had no signs of hematocele. When patients with a history of scrotal trauma were categorized according to duration, no signs of septation were detected in 12 patients who had acute or scrotal trauma within 1 week, while signs of septation were detected in 6 of 24 patients (25%) with a history of scrotal trauma 1-4 weeks ago (p = 0.043).

Conclusion: When septation is seen on the US of a patient who presents to the ED with scrotal pain, swelling, or ecchymosis, hematocele must first be ruled out. The presence of septation is not useful in acute trauma for distinguishing hematocele from hydrocele, but it may be useful in distinguishing chronic hematocele from acute hematocele or hydrocele.

Key words: hematocele, hydrocele, septation, ultrasound, emergency department.

ÖZ

Amaç: Skrotum ve testisler yüzeysel organlar olduğundan dolayı palpasyonla ve bazen de transilluminasyonla kolaylıkla incelenebilir. Ancak bu yöntemlerle skrotumdaki sıvının hidrosel mi yoksa hematosel mi olduğunu ayırt etmek mümkün değildir. Bu çalışma, her hekimin tanıyabileceği basit bir bulguya (septasyon) dayanarak hematosel ve hidrosel arasındaki ultrasonografik ayrımı bulmaya çalışmaktadır.

Yöntem: Acil servise (AS) skrotal ağrı, şişlik veya kızarıklık şikayeti ile başvuran hastalar, şikayetleri travmaya bağlı ve şikayetleri spontan olarak gelişenler olmak üzere iki gruba ayrıldı. Çalışmaya dahil edilen tüm hastalara US/ Doppler eğitimini almış bir acil tıp uzmanı tarafından skrotal US/Doppler yapıldı.

Bulgular: Çalışmaya dahil edilen 61 hastanın 36'sı (%59) skrotal travma, 25'i (%41) ise travma dışı şikayetlerle başvurdu. Travma öyküsü olan 36 hastanın 8'inde (%22,2) hematosel, 1'inde (%2,8) hidrosel saptanırken, 27'sinde (%75) ne hematosel ne de hidrosel saptandı. Sekiz hematosel hastasının 6'sında (%75) septasyon saptanırken, 2'sinde (%25) septasyon saptanmadı (p=0,013). Öte yandan; skrotal travma nedeniyle başvuran ve hematosel bulgusu olmayan 28 hastanın hiçbirinde septasyon bulgusu rastlanmadı. Skrotal travma öyküsü olan hastalar, süreye göre gruplandırıldığında, akut veya 1 hafta içinde skrotal travma geçiren 12 hastanın hiçbirinde septasyon bulgusu rastlanmamışken, 1-4 hafta önce travma geçiren 24 hastanın 6'sında (%25) septasyon bulgusu saptanmıştır (p=0.043).

Sonuç: Acil servise skrotal ağrı, şişlik veya ekimoz şikayetiyle başvuran bir hastanın US'de septasyon görüldüğünde öncelikle hematosel dışlanmalıdır. Septasyon varlığı akut travmada hematoselin hidroselden ayrımında yararlı değildir, ancak kronik hematoselin akut hematosel veya hidroselden ayrımında yararlı olabilir.

Anahtar kelimeler: hematosel, hidrosel, septasyon, ultrason, acil servis.

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Introduction

Patients suffering from scrotal trauma generally complain of pain, nausea, vomiting, fainting, and swelling in the scrotum. On physical examination, significant tenderness, swelling, redness, and ecchymosis may be observed [1]. The most important evaluation method for patients with scrotal region trauma is US/Doppler evaluation in the supine position. In the ultrasonographic evaluation of a normal adult, the testicles are homogeneously echogenic, and their dimensions are around 5cm x 3cm x 2cm [2]. As a result of this examination, we can obtain clearer information about the pathological condition of the scrotal region, but sometimes the distinction between some scrotal pathological conditions, such as hematocele and hydrocele, may require experience and expertise.

While normally there is 0.5–1 ml of fluid between the testicle and these membranes to ensure the lubrication of the testicle, in hydrocele, this amount of fluid can reach 100–200 ml and sometimes much more [3]. Although hydrocele is the most common cause of scrotal swelling, occurring in 1 in every 10 children and 1 in every 100 adults, it is often considered a benign condition [4]. On the other hand, hematocele can develop secondary to trauma and cause serious consequences [1,4]. Fluid or blood may accumulate in the tunica vaginalis of the scrotum due to trauma and accidents such as kicking the testicle, falling, or riding a bicycle for a long time. Since the fluid will resorb on its own, it should not be drained unless necessary [5]. There is no need for urgent surgery in small hematoceles and hematomas as long as the tunica albuginea is intact. Sometimes, if the hematocele is large and tense enough to impair testicular blood flow, drainage may be necessary as it can cause compartment syndrome and thus ischemia of the testicle [2-4]. It is extremely important to detect testicular rupture, which can rarely develop after trauma, at an early stage. The rate of testicular salvage with operations performed in the first 72 hours is 90% [5,6]. Additionally, attention should be paid to child abuse. In this context, hematocele is a very important finding, as it can be a sign of serious pathologies.

The most valuable diagnostic method to distinguish hematocele from hydrocele is Doppler/ultrasound. In fact, to date, except for a few individual cases, no study has been found to distinguish these two important pathological conditions radiologically. According to our emergency department (ED) experience, we were able to observe that septation findings were detected more frequently among the ultrasonographic findings of hematocele cases compared to hydrocele cases. Based on this hypothesis, this study aims to make an ultrasonographic distinction between hematocele and hydrocele based on a simple finding (septation) that every physician can recognize.

Patients and Method

This study was carried out between November 15, 2022, and January 15, 2024, in the emergency department (ED) of Denizli Servergazi State Hospital treating approximately 200,000 ED patients annually. Patients who applied to the ED with complaints of scrotal pain, swelling, and redness were divided into two groups to find out the duration of ultrasonographic septations and to determine whether this formation occurs in acute situations or not:

Group 1: Patients whose symptoms develop due to trauma (those with a history of trauma in the last 4 weeks).

Group 2: Patients whose symptoms develop spontaneously.

After obtaining written consent forms, detailed medical histories were taken, and clinical examinations were performed for all patients included in the study. Then, scrotal US/Doppler was applied on all patients by an emergency medicine specialist with US/Doppler training using a GE LOGİQ 7 Ultrasound machine with 4C-M12L-9L probe. All patients were evaluated for all possible scrotal pathologies, and necessary intervention and treatment procedures were applied to cases in which pathological conditions such as epididymitis-orchitis, testicular torsion, etc. were detected by US/Doppler. For the definitive diagnosis and treatment of each patient with scrotal pathology, consultation was requested from the urology and radyology departments, and thus definitive diagnoses were made clinically and radyologically by a radyologist and a urologist.

Patients during their stay in the ED were given detailed information about the procedure, their safety was ensured, and preparations were made for all kinds of emergencies.

Criteria for inclusion in the study:

- Patients over 18 years old who were admitted to the ED with complaints of scrotal pain, swelling, and redness.

Exclusion criteria:

- Patients with disease in the scrotal area (e.g., testicular tumors).

- Patients with a history of scrotal area surgery

- Patients under 18 years of age

- Patients who did not agree to participate in the study

Statistics and analysis

In the analysis of the collected data, descriptive statistics (number, percentage, mean, and standard deviation), the Kolmogorov-Smirnov analysis method for the suitability of a normal distribution, the t-test for the analysis of parametric binary variables, the Mann-Whitney U test for non-parametric ones, the analysis of variance for the analysis of more than two parametric variables, and the Kruskal-Wallis test were used for non-parametric ones. In statistical analysis, $p< 0.05$ will be considered significant, and the data were analyzed using the SPSS-22 (Statistical Package for Social Sciences) program.

Results

During the period of the study, a total of 68 patients were admitted to the ED with scrotal trauma, pain, swelling, and redness. These patients were given detailed information about our study while the necessary interventions were being performed. While 61 of the 68 patients admitted to the ED agreed to participate in the study and signed the consent form, 7 patients were not included in the study because they did not give written consent. The median age of the patients included in the study is 34(19-68), and the average age is 37.7 ±13.9. In terms of presenting complaints, 36 of 61 patients (59%) presented with scrotal trauma and 25 (41%) with non-traumatic complaints. US/Doppler was performed on all 61 patients included in the study (Figure 1). According to US/Doppler results, the most common causes of scrotal pain, swelling, and redness were epididymo-orchitis and varicocele, while the least common causes were orchitis and Mid. ureteral stone, respectively [13 (21.3%), 12 (19.7%), 1 (1.6%) and 1 (1.6%)] (Table 1). Hematocele was detected in 8 (22.2%) and hydrocele was detected in 1 (2.8%) of 36 patients with a history of trauma, while neither hematocele nor hydrocele was detected in 27 (75%) (Table 2). While septation was detected in 6 of 8 patients (75%), no septation was detected in 2 (25%) patients (Figure 2). According to this distribution, septation findings were detected more in patients with hematocele, and the difference was found to be statistically significant ($p = 0.013$). On the other hand, no signs of septation were detected in any of the 28 patients who presented due to scrotal trauma and had no signs of hematocele. (Table 2).

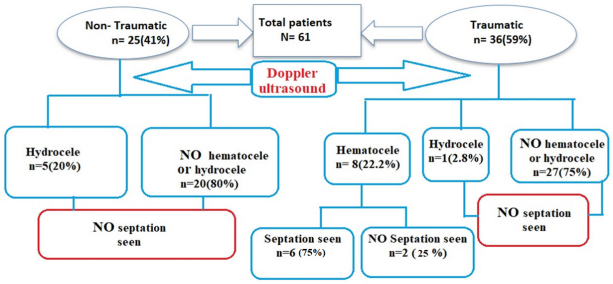

Figure 1. Algorithmic distribution of patients participating in the study.

Table 1. Doppler/US results of the patients included in the study.

Doppler findings	Traumatic group n	Non-traumatic group n	Total n(%)
Testicular torsion	9	2	11(18)
Epididymitis	2	5	7(11.5)
Orchitis	0	1	1(1.6)
Epididymo-orchitis	9	4	13(21.3)
Varicocele	5	7	12(19.7)
Hematocele	8	0	8(13.1)
Hydrocele	1	5	6(9.8)
Strangulated inguinal hernias	2	0	2(3.3)
Mid. ureteral stone	0	1	1(1.6)
Total	36	25	61(100.0)

When the patients with a history of scrotal trauma were categorized according to duration, no signs

of septation were detected in 12 patients who had acute or scrotal trauma within 1 week, while signs of septation were detected in 6 of 24 patients (25%) with a history of scrotal trauma 1-4 weeks ago. The difference between both groups in terms of trauma history was statistically significant (p = 0.043) (Table 3).

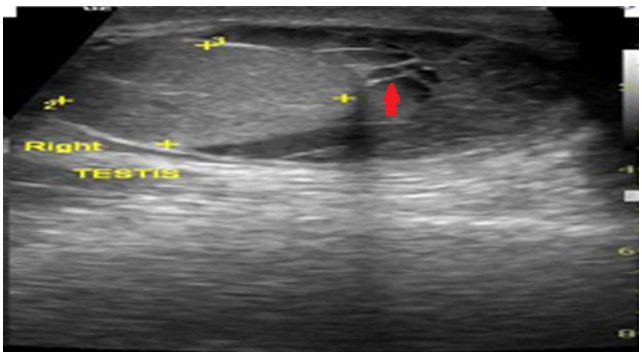

Figure 2. This US image is belong to 21-year-old patient who had a history of blunt trauma 18 days ago. A moderate-grade septal hydrocele with echo in the right testicle (red arrow).

On the other hand, while hydrocele was detected in 5 of 25 non-traumatic patients (20%), neither hematocele nor hydrocele were detected in the remaining 20 patients, and no signs of septation were observed in any patient in this group.

Table 2. The relationship between Doppler/US findings and septation.

Doppler/US findings	Non-traumatic group	Traumatic group	P*	Septation (+)	Septation (-)	P*
Hematocele	0	8	0.115	6	2	0.013
Hydrocele	5	1		0	6	
No hematocele or hydrocele	20	27		0	47	
Total	25	36		6	55	

* Pearson Chi-square was used.

Table 3. The relationship between the presence of septation and trauma time.

Septation status	≤ 1 week	> 1 week	p*
Septation seen	0	6	0.043
No septation seen	12	18	
Total	12	24	

* Pearson Chi-square was used.

Discussion

Hydrocele is the most common non-traumatic cause of scrotal swelling, affecting 1 in every 10 children and 1 in every 100 adults [7,8]. In adult men and older people, hydrocele may occur due to reasons such as trauma to the scrotum, inflamatory diseases of the testicles and their appendages, testicular tumors, varicocele surgeries, and after radiotherapy. Unilateral hydrocele occurs in 70% of kidney transplant patients. Testicular torsion (sudden rotation of the testicular around itself) can cause reactive hydrocele in 20% of patients and may mask testicular torsion that requires urgent intervention [9],. On the other hand, hematocele, which occurs when blood accumulates between the tunica vaginalis leaves, is common after blunt trauma [10,11]. Large hematoceles (increasing the volume of the affected testicle > 3 times) can cause compression of the blood vessels, mimicking torsion [11,12]. In this case, urgent surgery may be required to ensure testicular perfusion. Unlike a hydrocele, a hematocele has increased echogenicity, and septa can often be seen within it [13]. It should be remembered that if the hematocele is large, it will be difficult for the US to detect tunica albuginea rupture [14].

In patients who presented to the ED with complaints of acute scrotal pain, scrotal redness, or swelling, the most common diagnoses were epididymo-orchitis and testicular torsion, while the least common diagnoses were orchitis and mid-ureteral stones. In a review article by Velasquez et al., they reported that the most common infective cause of acute scrotal pain is epididymitis and epididymo-orchitis, while the most common ischemic cause is testicular torsion. These results are parallel to the study titled "Acute scrotal pain" conducted by Burgher et al. and are also compatible with our study [10,15].

In this study, according to the US results of 36 traumatized patients, hematocele or hydrocele was not detected in 27 patients (75%), while hematocele was detected in 8 patients (22.2%) and hydrocele was detected in 1 patient (2.8%). None of the individuals with pathological diseases other than hydrocele and hematocele had septation. Conversely, in 6 out of 8 patients (75%) who developed hematocele, septation findings were found; in 2 patients (25%), no septation was found. This difference was statistically significant (p=0.013). In a study by Cunningham, three patients who had scrotal swelling for 2 days, a year, and many years, respectively, were shown

to eventually develop hematocele, although their clinical history and physical findings were atypical [16]. All of these lesions showed an essentially anechoic mass crossed by thick, irregular septa. It has also been observed that the size and number of septations in an acute case decrease within 10 days. In a case report published by Bickle et al., it was emphasized that hematocele was associated with increased echolocation and separation [17]. These results are parallel to the results of the recently published study by Sood et al. Sood et al.'s study titled "Post-traumatic ruptured scrotal collection: pyocele or hematocele?" In the study, it was stated that hematocele was associated with increased echogenicity and septation [18].

On the other hand, Symeonidis et al. In a case report they published, a young patient who presented with a complaint of long-term swelling in the scrotal region was observed to have a thick and irregular septation along with a complex hydrocele in the left hemiscrotum on US, and it was determined during surgery that the assumed "complex hydrocele" was actually a multicystic testicular tumor [19].

Although no significant case-based study has been found explaining the relationship between hematocele and hydrocele and ultrasonographic septation findings, the results of the above-mentioned studies and case reports seem to be compatible with the results of our study. According to the results of our study, while no signs of septation were detected in hydrocele cases, septation was observed in most (n = 6) hematocele cases (n = 8). It was observed that all cases with septation findings had a history of trauma at least 1 week ago, and it was not detected in patients with a history of acute trauma or trauma within 1 week. A statistically significant difference was detected between septation formation and hematocele formation history (p = 0.043). It is thought that the sign of septation may be seen, especially in chronic hematocele and complicated hydrocele (hematocele and pyocele) findings [20]. In a study conducted by Patil et al., it was observed that septation findings were seen in chronic and complicated hydrocele cases, as well as being detected more frequently in hematocele cases [21]. These results are also compatible with the results obtained in our study. When the fluid accumulated in the scrotum contains high amounts of protein or cholesterol, hydrocele becomes complex, and septation symptoms begin to develop. Thus, ultrasonographically, separation and loculations may occur in cases of hematocele or complex hydrocele.

Limitations: Since most of the patients did not have a previous scrotal US, the results obtained could not be compared with previous results. Therefore, it has made it difficult to determine whether the detected pathological events are acute or not. On the other hand, since this study was conducted in the ED, patients could not be called for control. As a result, it was unable to gather data regarding the progression of scrotal pathological diseases.

Conclusions: When septation is seen on the US of a patient who presents to the ED with scrotal pain, swelling, or ecchymosis, hematocele must first be ruled out. If septation is observed, it should be considered that hematocele is not an acute event. In other words, the presence of septation is not useful in acute trauma for distinguishing hematocele from hydrocele, but it may be useful in distinguishing chronic hematocele from hydrocele. We think that this finding can be easily recognized by emergency physicians who do not have advanced training in the US.

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RESEARCH ARTICLE

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Is an unresected subtalar joint a cause of failure in tibiotalocalcaneal arthrodesis with a nail?

Çivi ile tibiotalokalkaneal artrodezde subtalar eklemin rezeke edilmemiş olması başarısızlık nedeni midir?

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ABSTRACT

Aim: In this study, we aimed to evaluate clinical and functional outcomes of tibiotalocalcaneal arthrodesis (TTCA) using intramedullary nailing (IMN) without cartilage resection of the subtalar (ST) joint.

Methods: 22 ankles of 21 patients (one patient was bilateral), who underwent TTCA using IMN without cartilage resection of the ST joint in our clinic between January 2013 and December 2022 were retrospectively analyzed. Data including demographic and clinical characteristics of the patients, etiology, Kellgren-Lawrence classification, postoperative fusion rate, fusion status and complications were recorded. The European Quality of Life 5 Dimensions (EQ-5D) scores were also noted.

Results: Ten patients were male and 11 were female. The mean age was 54.7±14.9 years. The mean follow-up was 46.4±19.1 months. The mean best health status score of the EQ-5D (EQ-VAS) was 73.9±16.5. Tibiotalar (TT) fusion occurred in all patients, while ST fusion was not achieved in ten patients. Four of these patients underwent secondary procedures, while no intervention was planned for the other six patients because they were asymptomatic.

Conclusions: This study has demonstrated that unresected subtalar articular cartilage leads to a significant rate of ST fusion failure. Nevertheless, the possibility of partial fusion or fibrous fusion at the reaming site is thought to be the reason for the high rate of asymptomatic patients.

Key Words: Tibiotalocalcaneal, arthrodesis, intramedullary nailing, osteoarthritis, subtalar joint resection

ÖZ

Amaç: Bu çalışmanın amacı, subtalar (ST) eklemde kıkırdak rezeksiyonu yapılmadan intramedüller çivileme (İMÇ) ile tibiotalokalkaneal artrodezin (TTKA) klinik ve fonksiyonel sonuçlarını değerlendirmektir.

Yöntemler: Ocak 2013- Aralık 2022 tarihleri arasında kliniğimizde ST eklemin kıkırdak rezeksiyonu yapılmaksızın İMÇ ile TTKA gerçekleştirilen 21 hastanın 22 ayak bileği (bir hasta bilateral) retrospektif olarak incelendi. Hastaların demografik ve klinik özellikleri, etiyolojileri, Kellgren-Lawrence sınıflandırması, ameliyat sonrası kaynama oranı, füzyon durumu ve komplikasyonları kaydedildi. Avrupa Yaşam Kalitesi 5 Boyut (EQ-5D) skorları da not edildi.

Bulgular: Hastaların 10'u erkek, 11'i kadın olup, ortalama yaşı 54.7±14.9 yıl idi. Ortalama takip süresi 46.4±19.1 ay idi. EQ-5D'de en iyi sağlık skoru ortalaması 73.9±16.5 idi. Hastaların tümünde tibiotalar (TT) füzyon görülürken, on hastada ST füzyon elde edilemedi. Bu hastaların dördünde sekonder prosedürler uygulanırken, diğer altı hastada asemptomatik olması sebebiyle girişim planlanmadı.

Sonuç: Bu çalışma subtalar eklem kıkırdağının rezeke edilmemesinin ciddi oranda ST füzyon başarısızlığına neden olduğunu göstermiştir. Buna rağmen, hastaların yüksek oranda asemptomatik olmalarının nedeni olarak, oyma bölgesindeki kısmi füzyon ya da fibröz füzyon olasılığı düşünülmektedir.

Anahtar Kelimeler: Tibiotalokalkaneal, artrodez, intramedüller çivileme, osteoartrit, subtalar eklem rezeksiyonu

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INTRODUCTION

Tibiotalocalcaneal arthrodesis (TTCA) was first described by Lexer [1] in 1906 and the first case of TTCA with a metal intramedullary nail (IMN) into the ankle was reported by Adams [2] in 1948. It is an effective salvage procedure for severe deformity and instability of the hindfoot and ankle. It is usually indicated in failed ankle fusion, failed total ankle arthroplasty, and cases of severe bone loss due to conditions such as Charcot's disease , rheumatoid arthritis, severe post-traumatic osteoarthritis, and tuberculosis arthroplasty [3-7]. The main goal of arthrodesis is to relieve pain, correct deformity, and achieve a plantigrade, stable foot [8]. TTCA with IMN is advantageous in terms of alignment, length, and stability [7,8]. In addition, this procedure requires less soft tissue dissection and offers early mobilization with full weight-bearing [9,10].

Preparation and stabilisation of both joints are essential steps in TTCA surgery. Although open surgical cartilage resection is used to prepare the joint, soft tissue problems remain the main challenge. Therefore, alternative percutaneous and arthroscopic procedures have been developed to reduce soft tissue problems [5,11]. TTCA without ST cartilage resection is the other alternative procedure [9,10,12,13]. In this procedure, the ST cartilage is debrided by just reaming. TTCA with or without cartilage resection of the ST joint has advantages and disadvantages. However, TTCA with cartilage resection of both the ankle and the ST joint is in terms of superior due to its rapid and high fusion rate. Furthermore, stabilising the ST joint can reduce deformity and increase stability in patients with neuropathic arthropathy, severe deformity, and severe post-traumatic osteoarthritis. However, ankle cartilage resection alone with TTCA is superior in terms of shorter operative time and less soft tissue injury [9].

Cartilage resection of the ST joint is critical in TTCA, but additional open surgical procedures may lead to more soft tissue problems and longer surgical time [9]. Especially considering that most patients with indications for TTCA have an increased risk of infection and soft tissue problems, it may be a crucial challenge for these patients. Limited studies in the literature use TTCA without cartilage resection of the ST joint [9,12,13]. The arthrodesis mechanism is based on the reamerisation, and it was put forward complete or incomplete fusion is obtained with this technique. However, studies have shown optimistic results that do not adequately reflect potential complications [9,12,13]. Therefore, further literature is needed to determine the necessity of subtalar joint cartilage resection. The current study has asked about the clinical outcomes and complications associated with tibiotalocalcaneal arthrodesis using intramedullary nails unresected subtalar cartilage and how these outcomes compare to traditional subtalar cartilage resection techniques.

MATERIALS AND METHODS

Study design and study population

This single-center, retrospective study was conducted at a tertiary care hospital's Department of Orthopedics and Traumatology between January 2013 and December 2022. Patients who underwent TTCA with IMN in our clinic were analyzed. Those who underwent TTCA with unresected ST cartilage and were followed for more than 1 year were included. Surgery was indicated for various reasons in 21 patients, including post-traumatic arthropathy (13 patients), neuropathic arthropathy (five ankles of four patients), rheumatoid arthritis (one patient), primary osteoarthritis (one patient), failed total ankle arthroplasty (one patient), and failed TTCA (one patient). Patients who were lost to follow-up and resected ST joint cartilage were excluded. Finally, a total of 22 ankles of 21 patients were enrolled. One patient was bilateral. Preoperative Kellgren-Lawrence osteoarthritis grades were grade 3 in four patients and grade 4 in 18 patients. Data, including demographic and clinical characteristics of the patients, etiology, Kellgren-Lawrence classification, postoperative fusion rate and complications, were recorded. In addition, the European Quality of Life 5 Dimensions (EQ-5D) scores were noted. A written informed consent was obtained from each patient. The study was approved by the Institutional Ethics Committee (2021/191) and conducted in accordance with the principles of the Declaration of Helsinki.

Surgical technique

All patients were prepared in the supine position and administered cefazolin 1 g intravenously and 30 minutes before tourniquet inflation. The ankle was operated using an anterior approach. The ankle joint cartilage was resected over the tibiotalar (TT) joint surface using an osteotome, while the ST joint surface was preserved. The foot was placed in the plantigrade position with 5°external rotation and 5° valgus. A plantar heel incision was made. Under fluoroscopy guidance, an intramedullary guide wire was inserted into the calcaneus, talus, and tibia. After the insertion of the IMN, the distal part of the joint was fixed with two locking screws and the proximal part was fixed with two locking screws. The wound was closed, a short leg cast was applied to all patients for the first 15 days postoperatively, and then an elastic bandage was applied.

The patients were followed at weeks 2, 6, 12 and every three months after that. During the follow-up visit, anteroposterior (AP) and lateral ankle X-rays were obtained. Both TT and ST joint fusion were evaluated on AP and lateral radiographs. Fusion was defined as trabeculation crossing the fracture at three cortices on X-rays. CT was used for suspected cases after radiographic evaluation. Delayed fusion is defined as fusion that occurs later than 6 months postoperatively, although no or incomplete fusion is observed on a CT scan within 6 months postoperatively. Successful arthrodesis was defined as the lack of pain on palpation and weight-bearing during clinical examination.

Statistical analysis

Descriptive data were expressed as mean ± standard deviation (SD), median (min-max) for continuous variables, and number and frequency for categorical variables. As the group's sample size was less than 50, the Shapiro-Wilk test was used to check for normality. The Mann-Whitney U test was used when the data did not follow a normal distribution, and the Student t-test was used to analyse the difference between the measurements of the two groups. P values less than 0.05 were considered statistically significant.

RESULTS

Of the 21 patients, 10 were male, and 11 were female. The mean age was 54.7±14.9 (range, 18 to 78) years, and the mean body mass index (BMI) was 28.2±3.1 kg/m2. Six patients (27%) were smokers. The indications for surgery were as follows: post-traumatic arthropathy (13 patients), neuropathic arthropathy (five ankles of four patients), rheumatoid arthritis (one patient), primary osteoarthritis (one patient), failed total ankle arthroplasty (one patient) and failed TTCA (one patient). A synthetic graft was used in 14 cases, while a synthetic graft combined with an autograft was used in eight cases for the ankle joint. The baseline demographic and clinical characteristics of the patients, Kellgren-Lawrence classification, postoperative fusion rates, fusion status, and complications are shown in Table 1.

Table 1- Descriptive data of the patients

		n:21
Age, mean±SD (min-max)		54.7±14.9 (18.0-78.0)
Sex, n(%)	Female	11(52.4)
	Male	10(47.6)
BMI, mean±SD (min-max)		28.2±3.1 (20.0-33.3)
		n:22
Side, n(%)	Right	12(54.5)
	Left	10(45.5)
Follow-up, mean±SD (min-max)		46.4±19.1 (12.0-86.0)
Etiology, n(%)	Post-traumatic	13 (59.1)
	Neuropatic	5 (22.7)
	OA	1 (4.5)
	RA	1 (4.5)
	Failed TAA	1 (4.5)
	Failed TTCA	1 (4.5)
Fusion rate, n(%)	TT	22 (100.0)
	ST	12 (54.5)
Fusion Time (week), mean±SD (min-max)	TT (n:22)	18.4±5.5 (12.0-30.0)
	ST (n:12)	25.7±8.3 (16.0-40.0)
Kellgren Lawrence	Stage 3	4(18.2)
	Stage 4	18(81.8)

SD: Standart deviation, BMI: Body mass index, min: minimum, max: maximum, TTCA: Tibiotalocalcaneal arthrodesis; TT: Tibiotalar; ST: Subtalar; RA: Rheumatoid Arthritis; OA: Osteoarthritis; TAA: Total Ankle Arthroplasty, TTCA: Tibiotalocalcaneal arthrodesis.

The mean follow-up was 46.4±19.1 (range, 12 to 86) months. The mean EQ-5D score was 0.82±0.17. The mean best health status score (EQ-VAS) was

73.9±16.5. The means of EQ-5D subscales were: mobility 1.72±0.63, self-care 1.54±0.80, usual activities 1.59±0.73, pain/discomfort 1.50±0.59, anxiety/depression1.27±0.45 and EQ-5D Index 0.82±0.17. In addition, TT fusion was achieved in all patients (100%), and ST fusion was in 12 patients (54.5%). Six delayed ST fusions occurred. The mean time for TT and ST fusion was 18.4±5.5 weeks and 25.7±8.3 weeks, respectively.

Four patients have no complication. There were six delayed ST fusions. Spontaneous delayed fusion occurred between 30 and 40 weeks in three of them, one of them spontaneously fused after the screw breakage (Case#17). However, the other three required secondary procedures for fusion. One patient received antibiotics for a superficial infection (Case#3). The other had a deep infection. After the patient was treated with nail removal and antibiotic treatment, fusion was obtained at 36 weeks (Case#12-L). The last delayed fusion occurred at 32 weeks after dynamization (Case#7). In ten of the cases, a lack of ST fusions was observed. There was no difference in the EQ-5D score based on ST fusion status (Table 2). Four of them experienced hardware failure, including three cases of intramedullary nail (IMN) failure and one case of screw breakage. Additionally, three patients developed deep infections. Six of the patients with ST nonfusion were observed because they were asymptomatic (Table 3). Two cases of nail irritation were occurred and implants were removed. Additionally, three implants (Cases 4,12,16) were removed due to deep infection and one implant (Case#1) was removed due to nail breakage (Table 3). Although the left side of case 12 underwent spontaneous fusion following treatment of the infection, the other two patients with deep infection required one further surgery for subtalar arthrodesis, but this was declined (Cases 4 and 16). One of the three IMN breakages was observed because the patient was asymptomatic (Case#8). The hardware was removed in the other two patients. However, only one of the patients underwent revision surgery with screws (Case#12-R), as the other patient declined the additional intervention for subtalar arthrodesis (Case#1) (Table 3).

DISCUSSION

In the present study, we evaluated clinical and functional outcomes of TTCA unresected ST joint cartilage. The results of the current study showed that all patients achieved TT fusion, while a high rate of failed ST fusion was observed. Although the rate of failed ST fusion is high, secondary surgical interventions were required less frequently than expected because the majority of patients were asymptomatic.

Table 2- Comparison of EQ-5D outcomes according to ST fusion status

	ST Fusion (+) (n:10)	ST Fusion (-) (n:12)	p
Mobility	1.66±0.65	1.80±0.63	0.604[1]
Self-Care	1.50±0.80	1.60±0.84	0.758[1]
Usual Activities	1.50±0.67	1.70±0.82	0.581[1]
Pain/ Discomfort	1.50±0.67	1.50±0.52	0.851[1]
Anxiety/ Depression	1.17±0.39	1.40±0.51	0.232[1]
EQ-5D Index	0.82 ±0.15	0.78±.0.17	0.586[2]
EQ-VAS	75.8 ±16.7	71.0 ±13.7	0.546[1]

1 Independent T-test, 2 Mann-Whitney U Test
ST: Subtalar, EQ-5D: European Quality of Life 5 Dimensions (EQ-5D) score, VAS: Visual Analogue Scale

External fixators, IMNs, plates, and cannulated screws are usually used for fixation during TTCA. The main advantage of external fixators is that they can be used in case of chronic osteomyelitis and bone defects. However, the main disadvantages include prolonged duration of treatment, pin tract infection, and being painful and uncomfortable for the patient. In addition, nonfusion or malfusion can be seen in the osteotomy site in the tibia in patients undergoing bone transport [14]. Cannulated screws can be utilized, particularly in patients without neuropathy and bone defect, although these screws are biomechanically less effective than other tools [14]. Plates can be combined with grafts in case of bone defects and to provide augmentation in patients with a previous history of IMN. However, this method is associated with soft tissue injury and fibulectomy, requiring larger soft tissue dissection. Several studies comparing plates and IMN have concluded that both methods are similar biomechanically, although plates seem to provide more stable fixation [14]. Also, IMN can be used in patients with neuropathic arthropathy and severe deformity [14]. Previous studies have

Table 3- Summary of cases who performed TTCA.

Case #	Age	Sex	Side	BMİ	Etiology	TT Fusion (week)	ST Fusion (week)	Complication	Treatment of Complications	Follow-up (month)
1	18	F	L	25	Post-traumatic	16	-	IMN Breakage	Implant Removal	37
2	51	F	L	29	Post-traumatic	20	-	ST nonfusion	Observation	69
3	42	M	R	28	Post-traumatic	20	40	Superficial Infection and Delayed Fusion	Antibiotic treatment	44
4	62	M	R	27	Neuropathic	28	-	Deep Infection	Implant Removal + Spacer	33
5	52	F	R	28	Neuropathic	12	-	ST nonfusion	Observation	54
6	38	F	R	33	Post-traumatic	17	24	-	-	60
7	56	F	R	20	Post-traumatic	16	32	Delayed Fusion	Dynamisation	57
8	58	F	L	32	RA	20	-	IMN Breakage	Observation	58
9	55	F	L	29	OA	12	16	Irritation	Implant removal	48
10	56	F	R	29	Post-traumatic	16	-	ST nonfusion	Observation	49
11	54	M	L	33	Post-traumatic	12	-	ST nonfusion	Observation	46
12	24	F	R	27	Neuropathic	20	-	IMN Breakage	Revision arthrodesis with screw	86
	24	F	L	27	Neuropathic	12	36	Deep Infection Delayed Fusion	Implant Removal	74
13	57	M	R	30	Neuropathic	12	20	-	-	26
14	66	F	R	33	Failed TAA	20	20	Irritation	Implant Removal	55
15	74	F	L	29	Failed TTCA	24	30	Delayed Fusion	Observation	30
16	78	M	L	27	Post-traumatic	30	-	Deep Infection	Implant Removal + Spacer	36
17	68	M	R	28	Post-traumatic	26	34	Screw Breakage Delayed Fusion	Observation	57
18	48	M	L	28	Post-traumatic	16	20	-	-	55
19	60	M	L	27	Post-traumatic	20	36	Delayed Fusion	Observation	12
20	60	M	R	27	Post-traumatic	12	16	-	-	13
21	72	M	R	25	Post-traumatic	24	-	ST nonfusion	Observation	21

TTCA: Tibiotalocalcaneal arthrodesis; TT: Tibiotalar; ST: Subtalar; F: Female; M: Male; RA: Rheumatoid Arthritis; OA: Osteoarthritis; TAA: Total Ankle Arthroplasty, TTCA: Tibiotalocalcaneal arthrodesis, IMN:

shown that IMN is associated with less nonfusion than other methods [15]. The main advantage of IMN is that it does not require wide soft tissue resection.

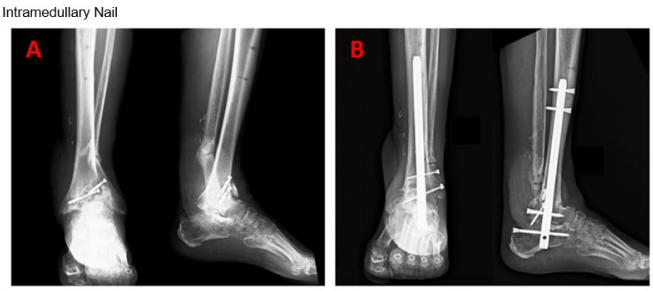

Figure 1. A: Anteroposterior and lateral X-ray images of post-traumatic ankle osteoarthritis. B: Anteroposterior and lateral X-ray images at postoperative 24 months.

The main goal of any fixation method is to achieve sufficient compression and alignment. Research has shown that intramedullary nailing (IMN) is the most effective method for minimizing soft tissue damage. In conventional TTCA with IMN, the ankle and subtalar joints are removed to create a suitable environment for fusion [16-18]. This procedure is more reliable for achieving fusion as optimal bone fusion requires aligning the two cancellous bone surfaces and ensuring stability and compression. However, this method is associated with more soft tissue damage and a longer operation duration. Additionally, it may increase the risk of infection and soft tissue disorders or exacerbate pre-existing conditions. The most preferred approach for ankle arthrodesis with IMN involves preparing both the ankle and ST joint. This method is associated with

high fusion rates in both joints [11,16-18,19]. Both approaches use the same surgical method for the ankle, resulting in comparable success rates for the fusion of the TT joint. Furthermore, a study found a nonfusion rate of 13%, and nonfusion occurred in the ankle in two and the ST joint in four patients [19]. In another study, the fusion rate was 96.6%, and only one patient experienced nonfusion in the TT joint [16]. Furthermore, a study showed a fusion rate of 90%, with nonfusion occurring in the ST joint in two patients and the TT joint in one patient [17]. In this study, all patients underwent the same open procedures for the TT joint, resulting in TT fusion. Although a low rate of soft tissue problems is expected when the ST joint is not resected, four infections (three deep and one superficial) were observed at the surgical site of TT fusion.

Apart from the conventional technique, TTCA can be performed without resecting the ST joint or with partial resection. This method causes less soft tissue damage and has a shorter operation duration. However, it is important to consider the possibility of fusion developing in this site, as the ST joint is not resected. During drilling for IMN fixation, partial debridement and autografting have already occurred in the ST joint. In this respect, a similarity can be drawn with arthrodesis through the use of arthroscopic and percutaneous partial debridement. No additional arthroscopic or percutaneous procedures were performed for ST joint debridement in this study. To achieve fusion with the percutaneous technique, it is necessary to eliminate the remaining cartilage and obtain a bone repair response. Several factors can contribute to the destruction of remaining cartilage, including depletion of synovial fluid, immobilization, compression-induced cartilage destruction, and synovitis in patients with rheumatoid arthritis [20]. The arthrodesis mechanism of the unresected subtalar joint technique is based on reaming, and it has been suggested that this technique achieves complete or incomplete fusion. In cases where percutaneous arthrodesis is performed, even without radiographic evidence of bone fusion, many of these patients have reported incomplete bone fusion or fibrous fusion [21]. In the present study, the ST fusion rates are lower than in the literature (% 54.5). Although there was a high rate of nonfusion, there was little need for secondary interventon. This could be attributed to partial bone fusion or fibrous fusion in asymptomatic patients. The current study demonstrates that there was no difference in the EQ-5D score based on ST fusion status. Similarly, it has been reported that although the nonfusion rate in hallux rigidus patients undergoing percutaneous metatarsophalangeal arthrodesis was almost 20%, all patients were asymptomatic [21]. Based on a systematic literature review, it was found that nearly a quarter of nonfusion joints in minimally invasive arthrodesis of the first metatarsophalangeal joint were asymptomatic, while 5.5% were symptomatic [22].

Literature reveals a limited number of studies performing unresected ST joint cartilage, and these studies have shown results similar to those of the standard approach [9]. In a case series, Mulhern et al. [9] found a fusion rate of 91.2% at six months and 100% at nine months. Although the authors reported no rate for ST joint separately, either advanced osteoarthritis or partial fusion/stable pseudoarthrosis was seen in all patients. Only one patient underwent revision surgery using the classical approach, because the patient reported postoperative ST joint pain. Furthermore, the authors concluded that ankle arthrosis was not affected by the absence of ST joint preparation. Therefore, they suggest that TTCA can be performed without ST joint resection for all patients, as both methods resulted in similar clinical outcomes. In another study, the ankle fusion rate was 100%, whereas no fusion was observed in the ST joint for two patients. Despite these results, some authors have reported limited success and a more cautious approach [23]. Gross et al. [13] reported fusion rates of 86% for TT and 74% for ST in their study. Although the fusion rate of the TT joint seems to be compatible with previous studies, the lower fusion rate of the ST joint can be attributed to insufficient compression and the lack of ST joint resection. Therefore, the authors began performing percutaneous resection of the ST joint after completing the study. Moore et al. [12] recommended ST joint resection only for patients with preoperative movement of the ST joint.

Several studies have shown that both methods significantly improve functional and pain scores

[11, 16-18, 23]. The standard approach resulted in pain relief and improved quality of life for 79% of patients [17], while the method of unresected ST joint cartilage had a patient satisfaction rate of 92% [23]. However, both methods are associated with complications, including infection, nonfusion, and residual deformity [9, 13, 16, 17]. In a systematic review, 22% of patients who underwent the standard approach required reoperation [18]. Another study reported a reoperation rate of 30% in patients who underwent unresected ST joint cartilage surgery [13]. In our study, the mean EQ-VAS was 73.9±16.5. This study has demonstrated that unresected subtalar articular cartilage is associated with a significant rate of complications. These included 10 cases of ST nonfusion, 6 cases of delayed fusion and 2 cases of nail irritation. Four fusion-related complications were due to infection. Despite the high rate of complications, only eight of the cases required reoperation. Furthermore, there was no difference in the EQ-5D score according to ST joint fusion status. Therefore, these findings suggest that partial bony or fibrous fusion may improve functional status.

This study has several limitations. First, it is a retrospective study without information on preoperative functional status. Therefore, the improvement in patients' clinical status couldn't be compared. Lacking of a control group is another limitation. Although radiographic evidence of fusion is required for the presence of fusion, this may not be sufficient to differentiate partial fusion from complete fusion, as partial fusion can be achieved in the ST joint. However, complete fusion can be achieved [9]. The main strength of this study is its long-term follow-up; fusion was also assessed with pain.

Conclusion: In conclusion, the current study has shown that unresected subtalar articular cartilage is associated with a significant incidence of ST fusion failure, which makes us think about the results of existing studies examining unresected ST articular cartilage. The cause(s) of the lower-than-expected rate of symptomatic patients, despite the high rate of ST fusion failure, should be investigated in further studies.

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Effects of Collagen Coating, Fetal Bovine Serum Concentration, Differentiation Agents, and Neurotoxin Application on In Vitro Modeling of Parkinson's Disease Using SH-SY5Y Cell Culture

SH-SY5Y Hücrelerinin Kültürlenmesinde Kollajen Kaplama, Fetal Sığır Serum Konsantrasyonu, Diferansiyasyon Ajanları ve Nörotoksin Uygulamasının Parkinson Hastalığının İn vitro Modellemesine Etkileri

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ABSTRACT

Objective: This study aims to optimize SH-SY5Y culture conditions to develop precise in vitro disease models for Parkinson's disease (PD) research. It seeks to investigate the effects of various factors such as collagen coating, fetal bovine serum (FBS) concentration, differentiation agents, and neurotoxin treatments on cellular behavior and disease modeling.

Materials and Methods: The human neuroblastoma SH-SY5Y cell line was cultured in DMEM/F12 supplemented with heat-inactivated FBS, penicillin-streptomycin, and L-glutamine. Collagen coating was applied to assess its impact on cell differentiation, while the ideal cell density and serum ratio for generating neurite-like cells were determined through experimentation. The MTT assay was employed to evaluate the cytotoxic effects of paraquat, while dopamine levels were quantified using ELISA. Gene expression was analyzed via real-time qPCR. Immunofluorescence staining and neurite length measurements were conducted to validate the PD model and assess cellular morphology.

Results: Cells cultured at a density of $5x10^3$ cells/cm^2 with collagen and 2% FBS exhibited characteristics of dopaminergic neurons upon exposure to retinoic acid. Conversely, paraquat treatment induced neurotoxicity, resulting in decreased dopamine levels and impaired neurite outgrowth.

Conclusion: This study investigated the optimization of SH-SY5Y cell culture conditions for PD modeling. Key findings include optimal cell density, FBS concentration, and beneficial effects of collagen coating. Additionally, an effective paraquat neurotoxicity protocol has been established, providing a solid framework for future research on neuronal differentiation and degeneration.

Key words: Parkinson's disease, SH-SY5Y cells, neuronal differentiation, neurotoxicity, in vitro modeling

ÖZ

Amaç: Bu çalışma, Parkinson hastalığı (PH) araştırmaları için doğru in vitro hastalık modelleri geliştirmek amacıyla SH-SY5Y kültür koşullarını optimize etmeyi amaçlamaktadır. Kollajen kaplama, fetal sığır serum (FSS) konsantrasyonu, diferansiyasyon ajanları ve nörotoksin tedavileri gibi çeşitli faktörlerin hücresel davranış ve hastalık modellemesi üzerindeki etkilerini araştırmayı hedeflemektedir.

Yöntem: İnsan nöroblastoma SH-SY5Y hücre hattı, ısı ile inaktive edilmiş FSS, penisilin-streptomisin ve L-glutamin ile desteklenmiş DMEM/F12 içinde kültürlenmiştir. Hücre diferansiyasyonu üzerindeki etkisini değerlendirmek için kollajen kaplama uygulanmış, ideal hücre yoğunluğu ve serum oranı ise deneysel olarak belirlenmiştir. Parakuat'ın sitotoksik etkilerini değerlendirmek için MTT testi kullanılmış, dopamin seviyeleri ELISA ile ölçülmüştür. Gen ekspresyonu gerçek zamanlı qPCR ile analiz edilmiştir. Parkinson modelini doğrulamak ve hücresel morfolojiyi değerlendirmek için immünofloresan boyama ve nörit uzunluğu ölçümleri yapılmıştır.

Bulgular: $5x10^3$ hücre/cm^2 yoğunluğunda kültürlenmiş hücreler, kollajen ve %2 FSS ile retinoik asit maruziyetinde dopaminerjik nöron özellikleri sergilemiştir. Bununla birlikte, parakuat tedavisi nörotoksisiteye neden olmuş, dopamin seviyelerinde azalma ve nörit büyümesinde bozulma gözlenmiştir.

Sonuç: Bu çalışma, PH modellemesi için SH-SY5Y hücre kültürü koşullarınının optimizasyonu araştırmıştır. Temel bulgular arasında optimal hücre yoğunluğu, FSS konsantrasyonu ve kolajen kaplamanın faydalı etkileri yer almaktadır. Ek olarak, nöronal farklılaşma ve dejenerasyon konusunda gelecekteki araştırmalar için sağlam bir çerçeve sağlayan etkili bir parakuat nörotoksisite protokolü oluşturulmuştur.

Anahtar kelimeler: Parkinson hastalığı, SH-SY5Y hücreleri, nöronal diferansiyasyon, nörotoksisite, in vitro modelleme

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Introduction

The SH-SY5Y cell line, derived from the SK-N-SH cell line isolated from a 4-year-old neuroblastoma patient's bone marrow [1], exhibits a range of human-specific proteins and protein isoforms absent in rodent primary cultures. Known for producing neuroblastic (N-type) cells capable of substrate adhesion (S-type) and transdifferentiation [2], SH-SY5Y cells contain a small fraction of S-type cells despite originating from three consecutive subclones of N-type cells. Several differentiation protocols have been developed to enhance SH-SY5Y as a neuronal cell culture model [3]. Differentiation induces cells into the G0 and G1 phases, leading to synchronized cell cycles and the formation of a homogeneous neuronal population [2], accompanied by specific events such as neuritic structure formation and synaptophysin-positive synapse development [4].

Retinoic acid (RA) addition to the culture medium is a well-established method for SH-SY5Y differentiation, known for its growth inhibitory and differentiation-inducing properties [5]. Typically, RA is applied at 10 μM for 3-5 days in serum-free or low-serum media [4], although protocol variations exist.

Simultaneous stimulation of adhesion and growth factor receptors is emphasized for SH-SY5Y differentiation [6]. The influence of the extracellular matrix (ECM) on neuroblastoma differentiation is evident, with ECM composition changes inducing morphological differentiation [7]. This underscores the role of ECM-derived signals in guiding neuroblastoma differentiation, particularly given the impact of ECM stiffness and biophysical properties on neuroblastoma processes [8]. Moreover, the overlap between signaling pathways involved in the biophysical cross-relationship and RA-mediated neuritogenesis suggests a potential influence of mechano-transductive signals from the ECM on neuroblastoma differentiation [9].

Collagen, fibronectin, and laminin, ECM components, are widely employed in cell culture to enhance cell adhesion. Collagen, in particular, affects cortical progenitor cell proliferation and differentiation, promoting neuronal fate while inhibiting early progenitor cell proliferation [10].

Establishing a reliable human neuron culture method is vital for accurately modeling the human nervous system and studying neurodegenerative diseases. This study aims to determine the optimal RA/collagen/low-serum combination to stimulate adhesion and growth factor receptors simultaneously in SH-SY5Y cells and to establish an in vitro model of PD using paraquat, a neurotoxin.

Materials and Methods

Cell culture

The human neuroblastoma SH-SY5Y cell line (ATCC, Rockville, MD, USA) was acquired from the SAP Institute (Ankara, Turkey). Cells were cultured in a 1:1 mixture of DMEM/F12 supplemented with 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, 1% (v/v) L-glutamine and maintained in a humidified CO_2 (5%) incubator at 37°C. Upon seeding, cells were allowed to proliferate for 24 hours until reaching 80–90% confluency, with medium renewal every other day.

Investigation of the effect of collagen during differentiation of SH-SY5Y cells

Some of the six-well plates were coated with 5 μg/cm2 collagen (A10483-01, Gibco) following the manufacturer's instructions, while others were left uncoated. Subsequently, cells were seeded into the wells at a density of $5x10^4$ cells/cm^2. After a 24-hours incubation at 37°C with 5% CO_2, cells in the wells were treated with RA (10 μM) (R2625, Sigma-Aldrich) supplemented medium containing 2% FBS. The medium was refreshed every other day. Cell images were captured under an inverted microscope (Zeiss Axio) on days 0, 3, and 6.

Determination of optimum cell density and serum ratio in the generation of neurite-like differentiated-SH-SY5Y (d-SH-SY5Y) cells

Six-well plates were coated with collagen, and cells were seeded into the wells at densities of $5x10^3$, $1x10^4$, and $5x10^4$ cells/cm^2. Following a 24-hours incubation at 37°C with 5% CO^2, cells were treated with medium containing varying proportions of FBS (1% and 2%) and RA (10 μM). The medium was refreshed every other day. On day 6, cell images were captured under an

inverted microscope (Zeiss Axio).

Determination of concentration and time-dependent effects of paraquat on d-SH-SY5Y cell viability by MTT assay

SH-SY5Y cells (passages 20 to 45) were subcultured into 96-well plates at a density of 5x10³ cells/cm². Cells were treated with medium supplemented with 10 µM RA for 6 days to enhance the dopaminergic phenotype and induce differentiation into neurite-like cells. d-SH-SY5Y cells were exposed to different concentrations of paraquat (0, 250, 500, 1000, and 2000 µM) for 24 hours. Subsequently, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) (SE2039502-Serva) solution was added to the wells at a final concentration of 5 mg/ml, followed by further incubation for 4 hours at 37°C, 5% CO^2. The supernatants were removed, and the pellets were dissolved in 200 µl/well of DMSO. The solubilized blue crystals were measured colorimetrically at 570 nm and 690 nm using a microplate reader (Bio-tek Instruments, Inc.). Cell viability percentage was calculated relative to the colorimetric intensity of control cells.

Enzyme-Linked ImmunoSorbent Assay (ELISA)

The Human Dopamine (DA) ELISA Kit (MBS045009; MyBioSource, San Diego, CA), with a sensitivity of 1.0 pg/mL, was employed to quantify the concentrations of DA in (d)-SH-SY5Y cells following treatment with RA and RA+Paraquat, adhering strictly to the guidelines provided by the manufacturer. Absorbance of samples was recorded at 450 nm. The experimental procedure was conducted in triplicate for validation purposes.

Neurite length measurements

Images were analyzed using ImageJ software, and neurites were manually traced using the ImageJ Plug-In NeuronJ. The cumulative neurite length from all cells and the cell count from the DAPI channel were utilized to calculate the relative neurite length. Additionally, the numbers of NeuN+ cells were determined from images of (d)-SH-SY5Y cells. Three independent cell culture experiments were conducted, and at least three different images were captured for each experiment. At least 50 neurites per condition were quantified for length from the images.

Real-Time Quantitative Polymerase Chain Reaction

Real-time qPCR analyses were conducted following previously established protocol (Bucolo et al., 2012), with slight modifications. Each reaction for the gene of interest was performed in a final volume of 10 µL, comprising 3 µL of cDNA, 0.4 µL of Milli-Q water, 5 µL of SensiFAST SYBR No-ROX master mix (Bioline, Australia), and 0.8 µL of corresponding forward and reverse primers (5 µM, Sigma-Aldrich, Castle Hill, NSW, Australia) to achieve a final primer concentration of 400 nM. The sequences of the primers for the RT-PCR were as follows: NeuN Forward primer (5′-3′): CTACAGCGACAGTTACGGCA, Reverse primer (5′-3′) ATGGTCCGAGAAGGAAACGG; GAPDH Forward primer (5′-3′) CAGCCTCAAGATCATCAGCA, Reverse primer (5′-3′) TGTGGTCATGAGTCCTTCCA.

Immunofluorescence staining of (d)-SH-SY5Y cells with Anti-Tyrosine Hydroxylase antibody

To validate the establishment of an in vitro Parkinson's model, SH-SY5Y cells were seeded in collagen-coated 6-well plates at a density of 5x10³ cells/cm², differentiated with RA treatment for 6 days, and then treated with paraquat (1000 µM) for 24 hours. Following fixation with paraformaldehyde for 15 minutes, cells were blocked with phosphate-buffered saline (PBS) containing 0.1% Tween-20 (v/v) and 5% bovine serum albumin for 30 minutes at room temperature. (d)-SH-SY5Y cells were immunostained with NeuN antibody (GeneTex (GTX133127) at a dilution of 1:1000, which labels dopaminergic/noradrenergic neurons. After overnight incubation at 4°C, cells were incubated with secondary antibodies (Alexa Fluor 594 anti-rabbit IgG, Cell Signaling Technologies (8889) at a dilution of 1:250, along with DAPI at room temperature. Cells were observed under a fluorescent microscope at 40X magnification. In order to provide a semi-quantitative analysis of staining intensities, we utilized ImageJ v1.51 (n = 10 images per experimental condition were analyzed for NeuN-IR).

Statistics

Data were derived from a minimum of three independent experiments. Mean values are presented, with error bars indicating the standard error of the mean (SEM). Statistical comparisons between the control group and the treated groups were performed using an unpaired two-tailed Student's t-test for parametric data, or the Mann-Whitney U test for non-parametric data. For multiple group comparisons, one-way ANOVA was utilized, followed by Tukey's HSD post hoc test for parametric data with equal variance, or the Games-Howell post hoc test for parametric data with unequal variance, to conduct pairwise comparisons between the groups. Statistical analysis was performed using the IBM SPSS version 21.0 software package. Statistical significance was set at $p < 0.05$ as appropriate.

Results

Effect of collagen, cell density and serum ratio in forming neuron-like SH-SY5Y cells

The impact of collagen on the differentiation of SH-SY5Y cells was investigated. Enhanced adhesion of (d)-SH-SY5Y cells was observed in collagen-coated wells compared to uncoated wells, resulting in improved cell differentiation. However, at high cell densities, cells were found to be overcrowded, resulting in reduced cell viability by the 6th day (Fig 1A). Therefore, future SH-SY5Y differentiation protocols should be conducted in collagen-coated wells. Furthermore, optimization of the fetal bovine serum (FBS) concentration in the medium and cell seeding density per well were prioritized.

$5x10^3$ cells/cm2

Treatment with medium containing 1% FBS: Although there were cells that adhered and formed neurite-like connections, it was observed that most of the cells lost their adhesion and became suspended.

Treatment with medium containing 2% FBS: It was observed that 80-90% of the cells formed neurite-like connections with neighboring cells, and the cell density per well was appropriate.

$1x10^4$ cells/cm2

Treatment with 1% FBS-containing medium: It was determined that there were less adherent cells compared to the $5x10^3$ cells/cm² group, that the adherent cells did not form neurite-like structures, and that the suspended cells were more.

Treatment with medium containing 2% FBS: It was observed that the cells provided neurite outgrowth, but the cell density in the well was too high.

$5x10^4$ cells/cm²

Treatment with medium containing 1% FBS: It was observed that almost all of the cells were suspended.

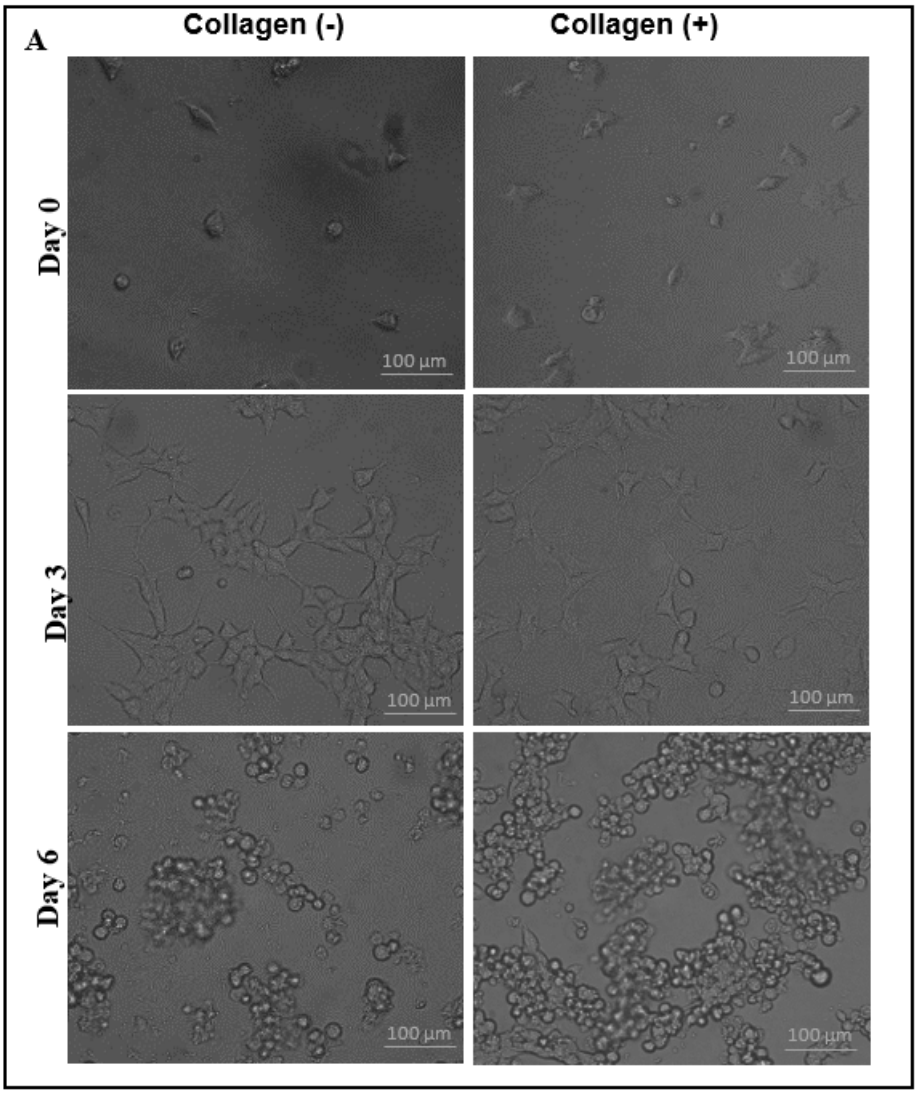
Treatment with medium containing 2% FBS: Compared to the cell group at the same density treated with medium containing 1% FBS, it was determined that the cells had a better tendency to adhere to the ground, but the cell density was higher.

As a result, it was determined that $5x10^3$ cells/cm² density and medium containing 2% FBS were suitable for SH-SY5Y differentiation. It was decided to meet these conditions in future studies (Fig 1B).

Effect of paraquat on (d)-SH-SY5Y cell viability

Paraquat decreased (d)-SH-SY5Y cell viability in direct proportion to concentration and exposure time. There was no statistically significant difference in the viability rates of (d)-SH-SY5Y cells applied 250 or 500 µM paraquat for 24 hours, compared to control (Table 1). However, a statistically significant reduction in cell viability was observed in (d)-SH-SY5Y cells treated with 1000 or 2000 µM paraquat compared to the control (Table 1).

According to ISO 10993-5 standards, four qualitative classification groups were based on to determine the cytotoxic effect levels of chemicals tested in vitro. According to this standard, it is stated that the total cell concentration of highly cytotoxic substances causes cell death above 50%, while 50-79% of substances with moderate cytotoxic effect, 80-89% of substances with mild cytotoxic effect, and 90% of non-cytotoxic substances. It has been stated that it provides cell viability equal to or more than 90% (Iso and STANDARD 2009).



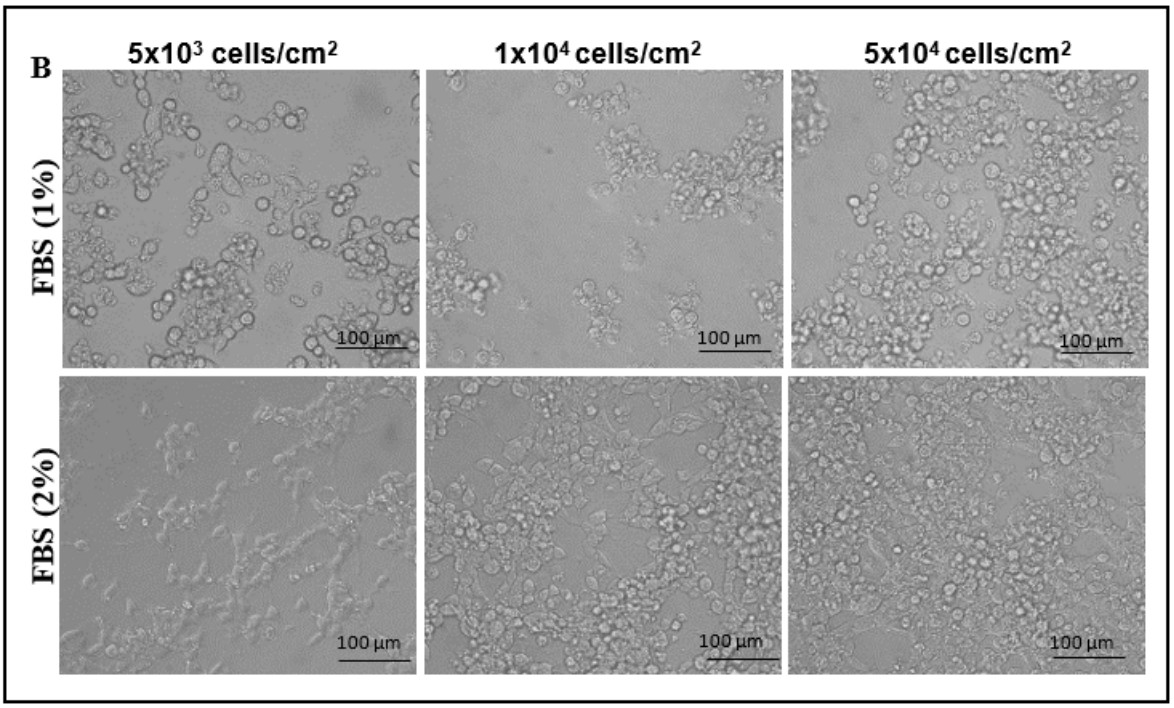


Figure 1: Photomicrographs of SH-SY5Y cells treated with RA in collagen-coated and uncoated wells on days 0, 3, and 6 (A), cultured with different cell density and FBS ratios (B) (20X magnification) (Scale bar: 100 µm).

The 24-hour application of 1000 µM paraquat concentration was determined to be the application that significantly reduced cell viability compared to the control, but did not show a high toxic effect on the cells. Further studies were continued with this application.

Table 1. MTT results of paraquat at 24 hours in each dilution compared to control group

	24 hours		
Groups	Mean of Cell Viability (%)	SD	p value
Control	100	4	-
250 µM Paraquat	98	5	0.950
500 µM Paraquat	95	4	0.445
1000 µM Paraquat	69	2	0.001*
2000 µM Paraquat	50	3	0.001*

SD: standart deviation, *p values compared to control group.

Changes of DA, NeuN and Neurite lengths in (d)-SH-SY5Y cells in response to RA and Paraquat exposure

As presented in Table 2, there was a significant increase in DA concentration in the RA+ group, indicating that RA treatment supports the function of dopaminergic neurons. Conversely, application of paraquat to (d)-SH-SY5Y cells has led to a decrease in the elevated dopamine levels.

The presence of RA, a known neurogenic soluble factor, facilitated the emergence of well-developed neurites. The mean neurite length in the RA-differentiated group showed a significant increase compared to the control group. Paraquat treatment, on the other hand, caused a significant reduction in mean neurite length compared to the RA group only (Table 2).

The study investigated whether RA and paraquat-induced changes in neurite outgrowth correspond with alterations in neuronal marker expression. qRT-PCR analysis demonstrated that the level of NeuN, a neuronal marker, expression in cells treated with both RA and Paraquat was lower than that observed in cells treated solely with RA (Table 2). Immunofluorescence analysis of NeuN exhibited variations consistent with those observed in neurite length and NeuN mRNA expression. RA administration led to robust NeuN immunostaining. However, in the (d)-SH-SY5Y group treated with 1000 µM paraquat for 24 hours following 6 days of RA application, a discernible decrease in NeuN expression level was observed compared to the group receiving only RA (Table 2 and Fig 2).

Discussion

The study's findings encompass the optimization of cell seeding density within SH-SY5Y cell culture conditions aimed at PD modeling. Moreover, it elucidates several critical aspects concerning the necessity of differentiation factors, FBS within the culture medium, collagen for substrate adhesion, and neurotoxic agents for inducing degeneration.

PD is a chronic, progressive disorder characterized by both motor and non-motor symptoms, as well as the loss of dopaminergic neurons in the substantia nigra pars compacta region of the brain and the formation of intracellular inclusion bodies known as alpha-synuclein. It is the second most common neurodegenerative disease worldwide after Alzheimer's disease, with an estimated prevalence of approximately 1% among individuals over the age of 55 [11–13].

In the context of PD models, numerous studies have established the importance of optimizing various parameters such as cell density, differentiation protocols, and serum concentrations to enhance neuronal differentiation and model disease pathologies accurately [14–17]. However, inconsistencies in the literature regarding the optimal conditions for cell differentiation and the precise role of extracellular matrix components, like collagen, highlight a gap in understanding [18–21]. Additionally, the variable effects of FBS concentration on cell proliferation and differentiation, as well as the specific impact of neurotoxins like paraquat on dopaminergic neurons, necessitate further investigation. This study addresses these gaps by systematically optimizing the cell density and FBS concentration for SH-SY5Y cell differentiation, elucidating the effects of collagen coating on cell adhesion and differentiation, and establishing a neurotoxin treatment protocol that balances cytotoxicity and relevance to PD pathology. This research not only corroborates existing findings but also provides new insights into the precise conditions required for reproducible and accurate neuronal differentiation and degeneration modeling in vitro, thereby contributing to the resolution of previously

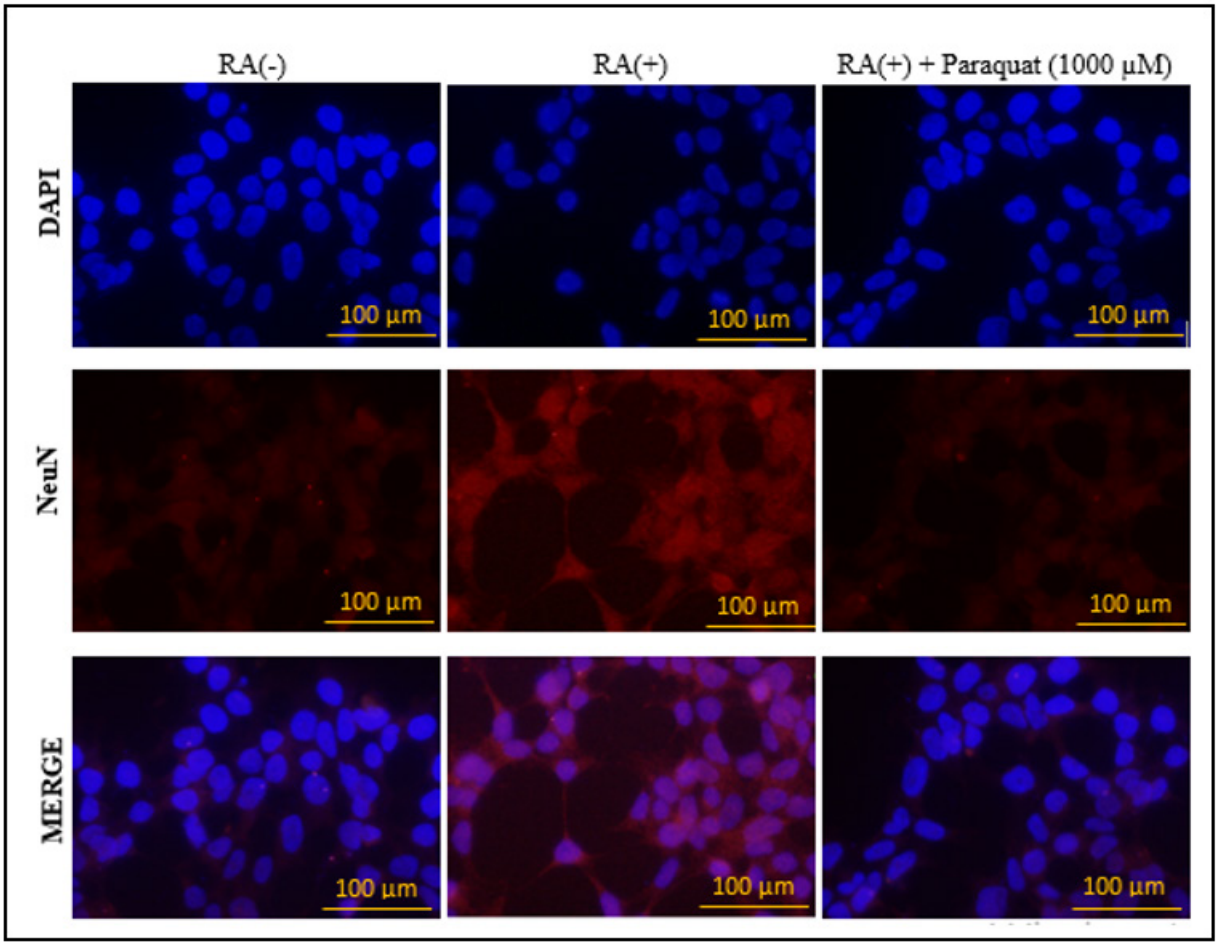


Figure 2: Representative images of NeuN and DAPI immunofluorescence in undifferentiated, retinoic acid differentiated and retinoic acid+paraquat treated SH-SY5Y cells. (40X magnification) (Scale bar: 100 µm).

Table 2. Results on changes in dopamine (DA) concentration, neurite length, mRNA expression level, and integrated density of NeuN in SH-SY5Y cells in response to retinoic acid (RA) and paraquat exposure

	RA (-)			RA (+)			RA (+) + Paraquat		
	Mean	SD	p value	Mean	SD	p value	Mean	SD	p value
The concentration of DA (µg/ml)	2	1	-	8	1	0.001*	5	1	0.062#
Mean neurite length (µm)	17	0.5	-	30	0.7	0.001*	20	0.6	0.001#
NeuN mRNA expression (normalized to GAPDH)	1	0.5	-	6	0.8	0.001*	3	0.4	0.002#
NeuN integrated density (arbitrary unit)	10	1	-	29	2	0.001*	10	1	0.001#

Mean: Absorbance (OD), SD: standart deviation, *p values compared to control group, #p values compared to RA (+) group.

conflicting data in the literature.

In this study firstly, the impact of collagen coating on SH-SY5Y cell differentiation was investigated. It was observed that collagen-coated wells promoted enhanced cell adhesion and improved differentiation of SH-SY5Y cells, corroborating previous studies highlighting the importance of extracellular matrix components in neuronal differentiation [22].

FBS, essential for neuronal cell proliferation, differentiation, and maturation, is a vital component of cell culture media, sourced from fetal bovine blood. However, the exact amount of FBS required during the differentiation of SH-SY5Y cells into neurons varies [23]. Deviating from the optimal FBS concentration poses risks; inadequate levels may compromise cell viability and hinder differentiation, while excessive

levels can impede neuronal differentiation by maintaining an undifferentiated state. Additionally, variations in FBS concentration can lead to inconsistent experimental outcomes. Alongside FBS concentration, cell density during neuronal differentiation is critical for orchestrating cellular interactions and signaling pathways essential for functional neuron development [24]. Optimal cell density fosters effective cell-cell communication, promotes neurite outgrowth, and supports synaptic connection establishment, thereby influencing the efficiency and fidelity of neuronal differentiation processes. Deviations from optimal cell density can disrupt these interactions, impairing neuronal maturation and functionality [25]. Hence, meticulous control and optimization of both FBS concentration and cell density are imperative for achieving reproducible outcomes in neuronal differentiation studies conducted in vitro.

Findings to determine the optimal cell density and serum ratio for neurite-like formation in SH-SY5Y cells revealed that a density of 5 x 10^3 cells/cm^2, medium containing 2% FBS, and 6 days of RA application optimally supported neurite growth. These findings are consistent with previous reports indicating the critical role of serum concentration in neuronal differentiation [26] and demonstrating the effect of 2% FBS concentration on neurite elongation in SH-SY5Y cells [27].

The establishment of an optimal neurotoxin treatment protocol, balancing toxicity and relevance to disease pathology, is crucial for the development of a neurodegenerative cell model. The cytotoxic effects of paraquat on (d)-SH-SY5Y cell viability were also assessed in this study. Exposure to paraquat resulted in a decrease in cell viability proportional to concentration and duration; however, it was determined that the most effective neurotoxicity in (d)-SH-SY5Y cells was induced by a 24-hours application of paraquat at a concentration of 1000 µM, which did not exhibit a high degree of toxicity on the cells. These findings are in line with previous studies demonstrating the neurotoxic effects of paraquat [28].

Additionally, changes in DA levels and NeuN expression in response to RA and paraquat exposure were examined. RA treatment led to a significant increase in both DA concentration and NeuN expression. However, exposure to paraquat resulted in attenuation of the RA-induced elevation in dopamine and NeuN levels; this suggests a potential neurotoxic effect on dopaminergic neurons. These findings are consistent with previous studies showing that paraquat causes a decrease in DA level and NeuN expression in neuronal cells [29,30].

Lastly, alterations in neurite lengths further corroborated the neurotoxic effects of paraquat and the neurogenic properties of RA. RA treatment facilitated neurite outgrowth, while paraquat exposure resulted in a significant reduction in neurite length. These findings highlight the potential utility of the SH-SY5Y model established by paraquat administration for studying neuronal development and neurodegenerative processes in PD. In summary, the findings of this study emphasize the complex interaction between environmental toxins and endogenous factors to build a proper and reliable in vitro PD model. Additionally, they offer valuable insights into optimizing SH-SY5Y cell culture conditions for modeling PD.

Limitations

It is important to acknowledge several limitations in this study. Firstly, the use of a single cell line, SH-SY5Y, may limit the generalizability of the findings to other cell types. Future studies incorporating a broader range of cell lines and environmental factors are warranted to address these limitations and enhance the translational relevance of these findings.

Conclusion

In conclusion, this study optimized the culture conditions of SH-SY5Y cells to enhance their utility in PD modeling. We established that a cell density of 5 x 10^3 cells/cm^2, 2% FBS, and six days of RA treatment support optimal neuronal differentiation. Collagen coating significantly enhanced cell adhesion and differentiation. Additionally, a 24-hours exposure to 1000 µM paraquat effectively induced dopaminergic neuron degeneration, aligning with PD pathogenesis. These findings resolve inconsistencies in the literature and establish a robust framework for future research, offering valuable insights for

optimizing in vitro models to study neuronal differentiation and neurodegeneration in PD.

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Ethics Committee Approval: The current study has no study with human and human participants. The study is not subject to ethics committee approval. Ethics Committee Approval is not required for cell culture studies.

ORCID and Author contribution: **F.G.K. (0000-0002-7248-7933)** was responsible for formulating the hypothesis, analyses and writing the report.

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Comprehensive Retrospective Analysis of Inguinal Hernias and Our Experiences with Special Hernias

Kasık Fıtıklarının Kapsamlı Retrospektif Analizi ve Özel Fıtıklardaki Deneyimlerimiz

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ABSTRACT

Aim: This study aims to review specific types of inguinal hernias over the past three years and analyze the surgical decision-making processes based on recent literature.
Materials and Methods: A retrospective review was conducted of 1,159 patients who underwent inguinal hernia surgery at Servergazi State Hospital between January 1, 2021, and December 31, 2023. Data analyzed included patient demographics, hernia types, locations, surgical methods, and complications. Special hernias, including Amyand's, Littre's, and Richter's hernias, were specifically identified and reviewed.
Results: Among the 1,159 patients, 113 were female (9.7%) and 1,046 were male (90.3%). The average age was 47.8 years for females and 58.6 years for males. Hernia types included 838 inguinal (72.3%), 267 femoral (23.1%), and 54 recurrent (4.6%). Specific hernias identified included 4 cases of Amyand's hernia (0.34%), 2 cases of Littre's hernia (0.17%), and 1 case of Richter's hernia (0.08%). Surgical approaches consisted of 83.7% open surgery and 16.3% laparoscopic procedures, with meshplasty performed in 96.5% of cases. Complications were infrequent: seroma in 3.9%, hematoma in 1.8%, wound infection in 2.8%, and recurrence in 0.4%. Specific hernias required additional procedures such as appendectomy for Amyand's hernia and diverticulectomy for Littre's hernia, with no complications reported during follow-up.
Conclusion: Inguinal hernias often contain omental tissue, but special hernias like Amyand's (appendix), Littre's (Meckel's diverticulum), and Richter's (intestinal wall) necessitate thorough examination. Amyand's hernia, which can range from incidental findings to acute appendicitis, benefits from mesh repair and appendectomy if needed. Littre's hernia requires diverticulectomy in addition to hernia repair. Richter's hernia, presenting with varied symptoms, should be managed with direct inspection and appropriate repair. The increasing use of minimally invasive techniques may lead to a higher incidence of Richter's hernia. Tailoring surgical strategies to the type of hernia ensures optimal outcomes.

Key words: Amyand's hernia, Littre's hernia, Richter's hernia, Inguinal hernia

ÖZ

Amaç: Bu çalışma inguinal herni nedeniyle son üç yılda opere edilen hastalardaki özel fıtıkları gözden geçirmek ve güncel literatüre dayalı cerrahi yönetimini ve karar alma süreçlerini analiz etmeye amaçlamaktadır.
Yöntem: 1 Ocak 2021 ile 31 Aralık 2023 tarihleri arasında Servergazi Devlet Hastanesi'nde kasık fıtığı ameliyatı geçiren 1159 hastada retrospektif olarak incelendi. Hastaların demografik verileri, fıtık tipleri, yerleşim yerleri, cerrahi yöntemler, hastanede kalış süreleri ve komplikasyonlar analiz edildi. Amyand, Littre ve Richter gibi özel fıtıklar tanımlandı ve incelendi.
Bulgular: 1159 hasta arasında 113'ü kadın (%9.7) ve 1046'sı erkekti (%90.3). Kadınlarda ortalama yaş 47.8, erkeklerde ise 58.6 idi. Fıtık tipleri arasında 838 inguinal (%72.3), 267 femoral (%23.1) ve 54 nüks herni (%4,6) idi. Belirlenen spesifik fıtıklar dört Amyand (%0,34), iki Littre (%0,17) ve bir Richter (%0,08) herni idi. Cerrahi yaklaşımlar %83,7 açık ve %16,3 laparoskopikti; vakaların %96,5'inde greftli onarım yapılmıştı. Komplikasyonlar oranları düşüktü; %3.9'unda seroma, %1,8'inde hematom, %2,8'inde yara enfeksiyonu ve %0,4'ünde nüks herni görüldü. Spesifik fıtıklar, Amyand için apendektomi ve Littre için divertikülektomi ile birlikte greftli onarım tercih edilmişti. Richter hernisine mesh plug uygulanmıştı. Spesifik fıtıkların 10.gün ve birinci ay takiplerinde hiçbir komplikasyon bildirilmedi.
Sonuç: İnguinal hernilerde fıtık kesesi içerisinde sıklıkla omental doku bulunur, ancak Amyand (apandisit), Littre (Meckel divertikülü) ve Richter (bağırsak duvarı) gibi özel fıtıklar dikkatli inceleme gerektirir. Herni kesesi içerisinde saptanan dokuya göre tedavi stratejisi değişmektedir. Greftli veya anatomik onarım cerrahın peroperatif bulgularına göre karar vermesi gereken bir durumdur.

Anahtar kelimeler: Amyand fıtığı, Littre fıtığı, Richter fıtığı, inguinal herni

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Introduction

Inguinal hernia is the most common type of hernia and represents a significant condition within the field of general surgery, with over 20 million hernia repairs performed worldwide annually [1–3]. The primary risk factor for the development of an inguinal hernia is a reduction in collagen synthesis. Additional risk factors include advanced age, male gender, connective tissue disorders, significant weight loss, and obesity. Conditions that elevate intra-abdominal pressure, such as heavy lifting, persistent coughing, constipation, and difficulty urinating, are also considered predisposing factors for inguinal hernia [1, 2]. An indirect hernia occurs when protrusion happens through the internal inguinal ring, while direct hernias emerge from the posterior wall of the inguinal canal, specifically within the Hesselbach triangle. Direct hernias typically have fewer complications due to their larger necks. Reducible inguinal hernias are often characterized by an inguinal bulge, with contents that can either spontaneously return to the abdomen or do so under pressure. Complications arise when the hernia contents become trapped or incarcerated, with strangulation leading to compromised blood supply, which can result in obstruction, bowel necrosis, and perforation [1–5]. The lifetime incidence of inguinal hernia is 27–43% in men and 3–6% in women. Inguinal hernias are almost always symptomatic, with surgery being the primary treatment option. Although a small percentage of patients may be asymptomatic, approximately 70% of these individuals will undergo surgery within 5 years [1–5]. Additionally, there are specific types of hernias that are named based on the shape of the hernia or the organ involved, rather than their anatomical location. These include Richter's hernia (RH), which involves the incarceration of a portion of the intestinal wall; Littre's hernia (LH), characterized by the presence of Meckel's diverticulum within the hernia sac; Amyand's hernia (AH), where the appendix vermiformis is involved in the inguinal hernia; and Lassus hernia, defined by the compression of the ovary into the inguinal hernia [6–11]. The aim of our study is to review specific hernia cases among patients who underwent inguinal hernia surgery in the past three years and to discuss the surgical decision-making process during this period, supported by relevant literature.

Material and Methods

The study was designed retrospectively. Records from the hospital automation system of 1,159 patients who underwent surgery for inguinal hernia between January 1, 2021, and December 31, 2023, at the General Surgery Department of Servergazi State Hospital were examined. The analysis included patients' ages, genders, types of hernias, hernia localizations, types of surgeries (elective vs. emergency), surgical methods, hospital stays, and complications. Only patients with complete and verified record-archive information were included in the analysis. Patients who had surgeries for other types of hernias (e.g., umbilical, epigastric, incisional) at our hospital were excluded from the study. The results will be presented using descriptive statistics, including averages, minimum-maximum values, and percentages. Special hernias in the study were defined as those involving the appendix vermiformis, Meckel's diverticulum, or a single-layer intestinal wall within the hernia sac. Ethical approval for the study was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (E-60116787-020-501276).

Results

Between January 1, 2021, and December 31, 2023, the Department of General Surgery at Servergazi State Hospital conducted 1,159 procedures for patients with inguinal hernias. Of these patients, 113 were female (9.7%) and 1,046 were male (90.3%). The mean age of female patients was 47.8 years (range 32–75 years), while the mean age of male patients was 58.6 years (range 18–96 years). The distribution of hernia types was as follows: 838 patients (72.3%) had inguinal hernias, 267 patients (23.1%) had femoral hernias, and 54 patients (4.6%) had recurrent hernias. In terms of hernia location, 705 patients (60.9%) had right inguinal hernias, 330 patients (28.5%) had left inguinal hernias, and 124 patients (10.6%) had bilateral hernias.

Surgical approaches included elective procedures for 1,102 patients (95.1%) and emergency surgeries for 57 patients (4.9%) due to suspected

strangulation. Of the total procedures, 970 (83.7%) were performed using open surgery, while 189 (16.3%) were laparoscopic. Meshplasty was performed in 1,118 patients (96.5%), and suture repair was conducted in 41 patients (3.5%). The average hospital stay was 1.7 days. Postoperative complications included seroma in 3.9% of patients, hematoma in 1.8%, wound infection in 2.8%, and hernia recurrence in 0.4% (Table 1).

Table 1. The demographic and surgical data of the 1159 patients

Variables	Data
Mean age (min-max)	
Female	47.8 (32-75)
Male	58.6 (18-96)
Gender n (%)	
Female	113 (%9.7)
Male	1046 (%90.3)
Hernia type n (%)	
Inguinal	838 (%72.3)
Femoral	267 (%23.1)
Recurrent	54 (%4.6)
Hernia site, n (%)	
Right	705 (%60.9)
Left	330 (%28.5)
Bilateral	124 (%10.6)
Operation timing, n (%)	
Elective	1102 (%95.1)
Urge	57 (%4.9)
Operation type, n (%)	
Open	970 (%83.7)
Laparoscopy	189 (%16.3)
Repair Technique, n (%)	
Mesh	1118 (%96.5)
Suture	41 (3.5)
Special hernia	7 (%0.6)
Length of hospitalization,	
Mean (min-max)	1.7 (1-6)
Complications, n (%)	
Seroma	46 (%3.9)
Hematoma	21 (%1.8)
Wound infection	33 (%2.8)
Recurrence	5 (%0.4)

Specific hernias were identified in 7 patients (0.6% of all procedures): 4 male patients (mean age 42.4 years) had Amyand's hernia (AH) (0.34%), 2 patients had Littre's hernia (LH) (0.17%), and 1 female patient had Richter's hernia (RH) (0.08%) (Table 2 and Figure 1). Patients with AH and LH underwent appendectomy and diverticulectomy, respectively, in addition to graft repair. Histological examination of appendectomy specimens revealed acute appendicitis in one case, while no ectopic tissue was found in the diverticulum of the LH cases. In the case of RH, intestinal perfusion was assessed as adequate, and the hernia was successfully reduced and repaired with a mesh plug. The average hospital stay for these specific hernia cases was 3.57 days. During hospitalization, patients received intravenous cefazolin sodium every 8 hours for antibiotic prophylaxis and were prescribed a 5-day course of oral cefuroxime upon discharge. No complications were observed during the 10-day and 1-month postoperative follow-up visits.

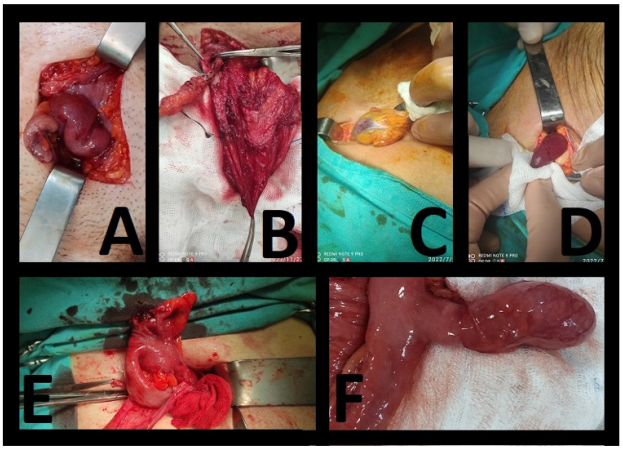

Fig 1: Peroperatif images of special hernias. A & B: Amyand's hernia (Appendix vermiformis in the hernia sac), C & D: Richter's hernia (intestinal wall in the femoral hernia sac), E & F: Littre's hernia (Meckel diverticula in hernia sac)

Discussion

Abdominal hernia is characterized by the protrusion of intra-abdominal organs through a defect in the abdominal wall for any reason. Hernias commonly occur in the groin area, with the omentum frequently found within the hernia sac. Rarely, the appendix vermiformis (Amyand's hernia), Meckel's diverticulum (Littre's hernia), and a segment of the intestinal wall (Richter's hernia) may be located within the hernia sac [1, 4, 5].

Amyand's hernia (AH) refers to the presence of the appendix vermiformis within the inguinal hernia sac. First described by Claudius Amyand, a surgeon for King George II, in 1735, AH was documented in this context in the literature [8, 9, 12, 13]. The

Table 2. Characteristics, demographic and surgical data of the special hernia patients.

Patient No	Age	Gender	Localization	Hernia type	Timing of surgery	Surgical treatment	Hospital day	Pathology
1	37	Male	Right	Amyand	Elective	Appendectomy, mesh herniographi	3	Normal appendix vermiformis
2	44	Male	Right	Amyand	Urge	Appendectomy, mesh herniographi	4	Acute appendicitis
3	54	Male	Right	Amyand	Urge	Appendectomy, anterior mesh repair	3	Normal appendix vermiformis
4	22	Male	Right	Amyand	Elective	Appendectomy, mesh herniographi	3	Normal appendix vermiformis
5	46	Female	Right	Littre	Urge	Meckel diverticule excision, mesh herniographi	4	Meckel diverticula
6	55	Male	Right	Littre	Elective	Meckel diverticule excision, mesh herniographi	4	Meckel diverticula
7	73	Female	Left	Richter (Femoral)	Urge	Reduction, mesh plug	4	-

Table 3. Losanoff Amyand hernia classification and management

Clasification	Description	Management
Type	Normal appendix in an inguinal hernia sac	Reduction, appendectomy in young, mesh hernioplasty
Type 2	Acute appendicitis within an inguinal hernia sac, no abdominal sepsis	Appendectomy, primary repair of hernia, no mesh replacement
Type 3	Acute appendicitis within an inguinal hernia sac, peritoneal sepsis	Laparotomy, appendectomy, primary repair of hernia, no mesh replacement
Type 4	Acute appendicitis within an inguinal hernia, related or unrelated abdominal pathology	Manage as types 1 to 3 hernia, investigate or treat second pathology as appropriate

true incidence of AH is difficult to determine, with rates in retrospective inguinal hernia series ranging from 0.14% to 1.3%. Appendicitis within an inguinal hernia is even rarer, occurring at a rate of 0.07%–0.13% according to large retrospective studies [8, 12, 13]. AH can occur across all age groups, from newborns to the elderly [12]. It is more common in men than in women, with a male-to-female ratio of approximately 7:1 [8]. In elective cases, the presence of the appendix in the hernia sac is often incidental. In contrast, emergency cases present with various symptoms such as lower right quadrant pain, a tender and irreducible groin lump, skin edema and erythema, and scrotal pain [8]. In our study, AH was detected in two cases during elective surgery and in two cases during emergency surgery due to incarcerated inguinal hernia. Losanoff et al. [14] classified AH into four subtypes based on clinical symptoms and the status of the appendix (Table 3). Singal et al. [15], using the Rikki modification of Losanoff's classification, added incisional hernias with protruding vermiform appendix as a fifth type of AH. According to this classification, three cases in our study were type 1, and one case was type 2. Since no patient exhibited signs of acute appendicitis during surgery, all cases underwent appendectomy and mesh hernioplasty. One case was subsequently reported to have acute appendicitis in the pathology report, and no complications were noted during the postoperative period.

Meckel's diverticulum is a congenital true intestinal diverticulum arising from the antimesenteric border of the small intestine, containing all bowel tissue layers, and is the most common gastrointestinal malformation [6, 7, 16–18]. Littre's hernia (LH) refers to any hernia containing a Meckel's diverticulum (MD) and is named after French surgeon Alexis de Littré, who first reported it in the 1700s. LH has a slightly higher incidence in females, with a ratio of 1.2:1. Femoral hernias are more common in females, while inguinal

hernias are more common in males. LH is exceedingly rare, with an estimated incidence of 0.09% of strangulated or incarcerated hernias in the literature [17]. Treatment for LH involves both hernia repair and removal of the Meckel's diverticulum [18]. In our study, one male and one female patient had LH. The female patient underwent emergency surgery for an incarcerated right inguinal hernia with signs of mechanical bowel obstruction, while the male patient had an electively reducible inguinal hernia. Both patients received open surgical diverticulectomy and mesh repair, with pathological examination of the diverticula showing no ectopic mucosal tissue.

Richter's hernia can occur at any typical hernia site but is more likely to present in small hernia rings with rigid fascial defects. Common locations include the inguinal canal (12%–36%), femoral ring (36%–88%), and abdominal wall incision (4%–25%). Less common types include umbilical, obturator, supravesical, spigelian, triangle of Petit, and sacral foramen hernias [19]. Richter's hernia is more prevalent in elderly patients, particularly those aged 60–80 [20]. The incidence of RH has recently increased with the rise of minimally invasive surgeries [20–22]. The clinical presentation can vary depending on the degree of bowel obstruction and includes cramp-like abdominal pain, nausea, vomiting, and sometimes swelling [10, 19, 20]. RH requires surgical intervention, with direct inspection being necessary to assess bowel viability. Preliminary attempts at manual reduction should be avoided. In our study, a 73-year-old patient with left groin pain and swelling underwent surgery for suspected strangulation. There was no ischemia observed in the bowel wall during the operation, and the hernia was reduced and repaired with a mesh plug.

Limitations: This study has several potential limitations. Notably, the limitations include the small sample size, the relatively short follow-up period, and, most significantly, the exclusion of hernias other than inguinal hernias. The specific hernia rates that might be observed if all types of hernias were included could differ from those reported in this study.

Conclusion: The content of the hernia is primarily composed of omental tissue. However, before reducing the hernia, it is crucial to open the hernia sac and inspect it for specific types of hernias. Treatment strategies for specific inguinal hernias vary depending on their contents. Amyand's hernia (AH) can present with a wide range of clinical manifestations, from being an asymptomatic incidental finding during elective hernioplasty to causing acute appendicitis with perforation and abscess formation within an incarcerated hernia. The Losanoff and Basson classification system provides valuable guidance for surgical decision-making. In cases involving appendicitis, prosthetic mesh can be used if the surgical field is relatively clean. The choice between mesh or anatomical repair for Littre's hernia depends on perioperative findings. Prophylactic diverticulectomy is recommended as Meckel's diverticulum may contain ectopic tissue. Additionally, it is anticipated that the incidence of Richter's hernia will increase with the rise in minimally invasive surgeries.

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RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

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Evaluation of iliococcygeal fixation procedure for pelvic organ prolapse: preoperative properties and short term postoperative outcomes

Pelvik organ prolapsusu için iliokoksigeal fiksasyon prosedürünün değerlendirilmesi: preoperatif özellikler ve kısa dönem postoperatif sonuçlar

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ABSTRACT

Aim: The aim of this study was to evaluate the short-term outcomes of patients who underwent iliococcygeal fixation (ICF) for the surgical treatment of pelvic organ prolapse (POP) and to assess the safety and feasibility of the ICF procedure.

Patients and Methods: Our study is retrospectively done with the data of 50 POP patients who underwent ICF in our clinic within the dates 01.04.2022 and 31.03.2024. A total of 44 women were found to be eligible for follow-up. Demographic data and pre- and post-operative clinical data at 3 months were assessed.

Results: According to the findings of our study, the difference between the median duration of surgery in NSD and CS patients is statistically significant, and the duration of surgery is longer in NSD patients (80 minutes (70-85) vs 60 minutes (50 -67,5), (p<0,05)). Only one recurrence, one hematoma and infection, and one bladder injury had occurred.

Conclusion: According to our study, ICF is a safe, durable and surgically feasible procedure that can be done concomitantly with other procedures. Thus, we strongly advocate this procedure until the ideal mesh is found since it avoids many serious complications of SSLF.

Key Words: Pelvic organ prolapse, sacrospinous ligament fixation, iliococcygeal fixation

ÖZ

Amaç: Bu çalışmada; pelvik organ proplapsusu (POP) nedeniyle, cerrahi tedavisi için iliokoksigeal fiksasyon (ICF) uyguladığımız hastaların kısa dönem sonuçlarını araştırmayı ve ICF prosedürünün güvenirliğini ve uygulanabilirliğini değerlendirmeyi amaçladık.

Hastalar ve Yöntemler: Çalışmamız 01.04.2022 ve 31.03.2024 tarihleri arasında kliniğimizde ICF uygulanan 50 POP hastasının verileri retrospektif olarak değerlendirilmiştir. Toplam 44 hasta çalışmada değerlendirme kriterlerine uygun bulundu. Hastaların demografik verileri ile preoperatif ve postoperatif 3. Aydaki klinik verileri değerlendirildi.

Bulgular: Çalışmamızın bulgularına göre, NSD ve CS hastalarında ortanca ameliyat süresi arasındaki fark istatistiksel olarak anlamlıdır ve NSD hastalarında ameliyat süresi daha uzundur (80 dakika (70-85) vs 60 dakika (50 -67,5), (p<0,05)). Sadece bir rekürrens, bir hematom ve enfeksiyon ve bir mesane yaralanması meydana geldi.

Sonuç: Çalışmamıza göre, ICF güvenli, dayanıklı ve diğer prosedürlerle birlikte yapılabilen cerrahi olarak uygulanabilir bir prosedürdür. Bu nedenle, SSLF' nin birçok ciddi komplikasyonunu önlediği için ideal yöntem bulunana kadar bu prosedürü şiddetle savunuyoruz.

Anahtar Kelimeler: pelvik organ prolapsusu, sakrospinöz ligament fiksasyonu, iliokoksigeal fiksasyon

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Introduction

Pelvic organ prolapse (POP), is one of the most frequently encountered diseases in the female population, especially in the elder age group [1]. It is reported that 11.1% of all women would experience a surgical procedure for this problem until the age of 80 [2]. Given the fact that we are living in an ever-growing older population, the graveness of the condition is self-evident. Since the usage of synthetic meshes is either banned or not advised after the announcements of the U.S. Food and Drug Administration, native tissue repair techniques are re-popularized to overcome this complex problem. The reconstruction of pelvic apical support remains as the most challenging obstacle in the field of urogynecology. Various surgical procedures are proposed to treat the condition such as sacrospinous ligament fixation (SSLF), sacrocolpopexy or hysteropexy, or other techniques such as lateral suspension. Nevertheless, the heritage of using paravesical space to anchor synthetic mesh kits to sacrospinous ligaments has proven the versatility and safety of utilizing an anterior vaginal approach to accomplish sacrospinous ligament fixation rather than using a posterior approach [3, 4].

Cespedes was the first to describe anterior SSLF and advocated the advantages of this approach when compared to classical unilateral SSLF (Richter's procedure) [3, 4]. However, neither SSLF nor abdominal sacrocolpopexy was free of complications such as bleeding or persistent buttock pain and, both also had considerable recurrence rates. When considering the pathophysiology of the apical prolapse, i.e. the loss of ligamentous support of Arcus Tendineus Fascia Pelvis, it seems more rational to mimic the original anatomy when treating this problem. Iliococcygeal fixation (ICF) was first described by Inmon and later advocated by Shull et al and Meeks et al. [5, 6]. Overall, the data shows that ICF has better or comparable anatomic results together with less serious complications. With this perspective, ICF has been utilized to correct apical compartment defects in our clinic since 01.04.2022 together with other procedures when needed or alone. In this study, we wanted to document its safety, feasibility (in terms of operation duration, bleeding amount), and short-term outcomes of this procedure.

Patients and Methods:

Our study is retrospectively done with the data of 50 POP patients aged between 40-70 years who underwent ICF in our clinic within the dates 01.04.2022 and 31.03.2024 and were followed up for a minimum of 3 months. Patients with gynecologic malignancy, pelvic mass, fertility desire, and younger than adolescent age were not included in the study. Six cases were excluded from the study due to incomplete data. Demographic data and pre- and postoperative clinical findings of a total of 44 patients were evaluated. Demographic data such as age, gravida, parity, history of D&C and prior surgeries, comorbidities and family history were noted. As clinical findings; preoperative ultrasound examination, magnetic resonance imaging (MRI) and PAP smear results were evaluated. Other surgical procedures done concomitantly with ICF, type of anesthesia, duration of the operation, postoperative complications, pre- and postoperative hemoglobin level, late postoperative complications, perioperative hemorrhage and the presence of infection were also noted.

Statistical Method

The data were analyzed with a package of software (SPSS 28). The normal distribution of numeric values was tested by the Shapiro-Wilk test. The normally distributed numeric values were expressed by average± SD, minimum, and maximum values. The values that are not normally distributed were expressed by Median (1st Quartile (Q1)- Third quartile (Q3)), minimum and maximum values. Categorical variables were summarized as numbers and percentages.

Mann-Whiney U test was utilized to compare two independent groups. Whereas, the Wilcoxon test was used for dependent groups. The statistical significance value (p) was taken as <0.05.

Results

A total of 50 cases were retrospectively reviewed. After the exclusion of six cases, because of missing data, a total of 44 women were found to be eligible for follow-up analysis. Demographic data, risk factors, and, some other general information

about the surgeries performed are summarized in Table 1.

Table 1 Demographic data, risk factors, and surgical characteristics of patients

	Total number of patients =44 (n)
Age	56,11±7,70 (41,00- 73,00)
Gravidity	3,00 (2,00-4,00)
Parity	3,00 (2,00-4,00)
Live birth	3,00 (2,00-3,75)
Type of Delivery	
-Nulliparous	3 (6.8%)
-Vaginal birth	31(70,5%)
-Cesarian	10(22,7%)
Ultrasonographic finding	
Pathology detected	29 (65,9%)
Pathology not detected	15 (34,1%)
History of chronic disease	25(56,8%)
Hypertension	17 (38,6%)
Diabetes	4 (9%)
History of prior operations	22(50%)
Preoperative hemoglobin	13,30 (12,20-14,08)
Postoperative hemoglobin	11,30±1,28 (8,70-13,90)
Duration of surgery	75,00 (60,00-85,00)

Of the 44 patients who underwent VH for POP, ICF and CAP were also performed on 26 (59.1%) patients whereas ICF alone was performed on 7 (15,9%) patients (Table 2). 7 (15,9%) patients were operated under general anesthesia and 37 (84,1%) patients were operated under spinal anesthesia. While no complication occurred in 41 (93,2%) of the patients, 3 patients had the complications as follows: relapse in one patient (2,3%), hematoma and infection in one patient (2,3%), and bladder injury in one patient (2,3%). 18 patients (40,9 %) had a decrease in hemoglobin of 1-2 g/dL whereas only 2 patients (4,5%) had a decrease of more than 3 g/dL. A combination of VH and ICF alone was observed to have less surgery duration than combining VH and ICF with other surgery types such as CAP (Table 3).

According to the findings of our study, the difference between the median duration of surgery in NSD and CS patients is statistically significant and the duration of surgery is longer in NSD patients (80 minutes (70-85) vs 60 minutes (50 -67,5), (p<0,05)). There was a negative, weak, and statistically significant relationship between surgical time and gravida, parity, abortion, and survival (p<0,005).

Table 2 Data on type of surgery and anesthesia and complications

	N	%
Surgery type		
VH+ ICF	7	15,9
VH+ ICF+ CAP	26	59,1
VH+ ICF+ CAP+ other	11	25,0
Anesthesia Type		
Spinal	37	84,1
General	7	15,9
Postoperative complication		
No	41	93,2
Yes	3	6,8
Complication type		
,00	41	93,2
Relapse	1	2,3
Hematoma+ infection	1	2,3
Bladder injury	1	2,3
Amount of decrease in hemoglobin		
None	2	4,5
0-1g/dL	9	20,5
1-2 g/dL	18	40,9
2-3 g/dL	13	29,5
3 g/dL and above	2	4,5
Infection		
No	43	97,7
Yes	1	2,3
Relapse		
No	43	97,7
Yes	1	2,3

VH: Vaginal hysterectomy, ICF: Iliococcygeal fixation, CAP: colporrhaphy

Tablo 3 Duration of surgery

	Type of Surgery		
	VH+ICF (n=7)	VH+ICF+CAP (n=26)	VH+ICF+CAP+ Other (n=11)
Duration of surgery (minutes)	70,00 (50,00-75,00)	75,00 (60,00-86,25)	80,00 (70,00-85,00)

VH: Vaginal hysterectomy, ICF: Iliococcygeal fixation, CAP: colporrhaphy

Discussion:

Our case series displays the surgical feasibility and, safety of ICF with comparable morbidity and anatomic success when treating pelvic organ prolapse (POP). POP is a disease with complex interactions with all interconnections within the pelvic floor and its resident organs. This nature of the pelvic floor is well depicted by the integral theory that considers the pelvic floor as a single unit. Hence any anatomic or functional derangement

that takes place in a certain compartment eventually affects the other compartments. De Lancey practically divided pelvic support into three compartments. Among these, apical support (Level III) might be considered as the hardest part to restore. To date, many surgical attempts have been made to correct the innate or acquired loss of support on this level. The major problem for restoring the apical support was to find a suitable anchoring site for a robust and durable repair. When synthetic mesh kits were introduced, this problem was seemingly solved by excellent anatomic outcomes reported by multiple studies utilizing these meshes. However, not much later a considerable number of complications regarding the usage of polypropylene mesh kits eventually led to their banning and removal from the market. This dichotomized the professional community regarding their usage. Some healthcare professionals expressed their frustration with their practices so that up-holding the utilization of mesh. They continued to believe that this concept should not be dismissed but ideal mesh search must continue. Others, on the other hand, adopted the legislative advice more rapidly and tried to minimize patient complaints such as pain, dyspareunia, erosion, or infection simply by avoiding the usage of synthetic meshes. Under the pressure of lawsuits, some reaching to millions of dollars of compensation fines, urogynecologists mostly started to convert back to native tissue repair techniques such as site-specific repair, SSLF, or SCP.

As a busy urogynecology unit, our clinic also adopted to utilize these conventional procedures while awaiting ideal mesh and conducted research studies with stem cells to overcome problems with polypropylene meshes.

The spinous process and sacrospinous ligament are believed to be one of the most convenient and strong anchoring sites to restore apical support. SSLF, therefore was found to be one of the most reliable procedures after the mesh kit debate. However, this procedure was hard to accomplish due to the proximity of the obturator neurovascular bundle with the ligament. Serious hemorrhage or neurologic injuries were reported inevitably by various studies [7]. Moreover, unilateral fixation resulted in significant deviation of the vaginal axis which was the preferred method by many surgeons. Interestingly Medina et al. reported that total vaginal length was longer in patients treated with ICF when compared to SSLF [8]. On the other hand, the anterior approach popularized by the mesh kit applications, and using the fascia of the iliococcygeus muscle for the anchoring site avoided these unwanted events successfully. In a review, done by Sze and Karram, there was 3% persistent gluteal pain after SSLF procedures while the was none after ICF [9]. Although it included only 7 patients operated with ICF procedure, the study of Biler et al compared the results and complication rates of common suspension techniques. In their study, there was no reported complication in ICF procedure whereas there were 9 and 4 complications in abdominal sacrocolpopexy and SSLF procedures respectively [10]. In a 10-year follow-up study, ICF was also reported to provide efficient apical support with acceptable complication and recurrence rates [11]. ICF may also provide a more anatomical repair since it follows the original Arcus Tendinous Fascia Pelvis attachment, which is believed to be the major apical and lateral support in the pelvic floor.

This study shows that it is a safe and efficient method evidenced by only one recurrence, one hematoma and infection, and one bladder injury. It should be noted that all the ICF procedures carried out were done concomitantly with other major pelvic surgeries such as vaginal hysterectomy and/or colporrhaphy or other procedures. Therefore, complication rates cannot be segregated from these concomitant procedures. Nevertheless, the complication rates and recurrence rates can be considered very low even though the procedures were undertaken with other major operations. Our study has limitations since it is retrospective in design with a limited number of patients. Another weakness can be considered that our clinic protocols did not include quality-of-life measurements and disease-specific questionnaires. Due to the lack of preoperative POP-Q measurements in every case, we did not include it in our study results. The reason for recurrence was further analyzed. It was concluded that the suture material failure was the reason since the recurrence was reported by the patient with a snap-like sound feeling in the first week postoperatively. In the examination, the left-

hand side suture was found to be broken and this led to the recurrence of prolapse. This case was further treated with abdominal sacrocolpopexy with a successful outcome after 3 months.

Limitations: Limitations of our study that may have influenced our results include the fact that our study was retrospective, our follow-up period was short, and our clinical experience with the ICF procedure was only 2.5 years. Nevertheless,, the outcomes of our study may contribute to national data and/or the systematic reviews and meta-analyzes [12] which will be at least together with other studies on the subject from our country at least together with other studies on the subject from our country. It may also contribute to a clinical guideline [13] based on the results of other national and/or international studies, systematic reviews and meta-analyses related to our research topic and its results.

Conclusion: We believe that ICF is a safe, durable, and surgically feasible procedure that can be done concomitantly with other procedures. We strongly advocate this procedure until the ideal mesh is found since it avoids many serious complications of SSLF.

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RESEARCH ARTICLE

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The role of oxidative stress, apoptosis and altered TRPM2 channel activation in doxorubicin-induced liver injury; the protective effect of selenium

Doksorubisin kaynaklı karaciğer hasarında oksidatif stres, apoptoz ve değişen TRPM2 kanalı aktivasyonunun rolü; selenyumun koruyucu etkisi

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ABSTRACT

Aim: Doxorubicin (DOXR) is frequently used alone or as combination therapy in the treatment of various types of cancer. Although dose-dependent side effects are known, its effects on liver health are not fully known. This study aimed to investigate the role of the transient receptor potential melastatin-2 (TRPM2) channel in DOXR-treated rats using the TRPM-2 channel blocker N-(p-amylcinamoyl) anthranilic acid (ACA) and to investigate the protective effects of selenium (Se).
Methods: Rats were allocated into six groups, each containing ten rats: control, DMSO, DOXR, DOXR + Se, DOXR + ACA, and DOXR + ACA + Se. Serum levels of AST, ALT, LDH, triglycerides, and total cholesterol were measured. Additionally, liver tissues were subjected to immunohistochemical tests for TRPM2 channel, 8-OHdG, and caspase-3 (Casp-3) expressions and also histopathological evaluation.
Results: Serum AST, ALT, LDH, triglyceride and total cholesterol levels, as well as liver 8-OHdG, TRPM2 channel and Casp-3 expressions in the DOXR group were significantly higher than in the DOXR + Se, DOXR + ACA and DOXR + ACA + Se groups ($p < 0.05$). However, these parameters were significantly reduced in the Se and ACA-treated groups compared to the DOXR group ($p < 0.05$).
Conclusions: The results suggest that simultaneous administration of Se or ACA with DOXR may provide an effective therapeutic approach to combat DOXR-induced hepatotoxicity.

Keywords: Apoptosis, Doxorubicin, Hepatotoxicity, Lipid Profile, TRPM2 Channel

ÖZ

Amaç: Doksorubisin (DOXR) çeşitli kanser türlerinin tedavisinde sıklıkla tek başına veya kombinasyon terapisi olarak kullanılmaktadır. Doza bağlı olarak değişen yan etkiler bilinmesine rağmen, karaciğer sağlığı üzerindeki etkileri tam olarak bilinmemektedir. Bu araştırma, DOXR ile tedavi edilen sıçanlarda geçici reseptör potansiyeli melastatin-2 (TRPM2) kanalının rolünü, TRPM-2 kanal blokörü N-(p-amilsinamoil) antranilik asit (ACA) kullanarak araştırmayı ve selenyum (Se)'un koruyucu etkilerini araştırmayı amaçladı.
Yöntemler: Sıçanlar altı gruba ayrıldı (n=10): kontrol, DMSO, DOXR, DOXR + Se, DOXR + ACA ve DOXR + ACA + Se. Serum AST, ALT, LDH, trigliserit ve total kolesterol seviyeleri ölçüldü. Ayrıca, karaciğer dokusunda TRPM2 kanalı, 8-OHdG ve kaspaz-3 (Casp-3) ekspresyonları için immünohistokimyasal testler ve ayrıca histopatolojik değerlendirme yapıldı.
Bulgular: Serum AST, ALT, LDH, trigliserid ve total kolesterol seviyeleri ve ayrıca karaciğer 8-OHdG, TRPM2 kanalı ve Casp-3 ekspresyonları DOXR grubunda DOXR + Se, DOXR + ACA ve DOXR + ACA + Se gruplarına göre anlamlı derecede yüksekti ($p <0.05$). Ancak, bu parametreler Se ve ACA ile tedavi edilen gruplarda DOXR grubuna kıyasla önemli ölçüde düşmüştü ($p <0.05$).
Sonuç: Sonuçlar, Se veya ACA'nın DOXR ile eşzamanlı uygulanmasının, DOXR kaynaklı hepatotoksisiteyle mücadelede etkili bir terapötik yaklaşım sağlayabileceğini göstermektedir.

Anahtar Kelimeler: Apoptoz, Doksorubisin, Hepatotoksisite, Lipid Profili, TRPM2 Kanalı

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Introduction

Doxorubicin (DOXR) is derived from the anthracycline class of chemotherapeutic agents [1]. Despite their higher toxicity compared to other medical treatments, the effectiveness of antineoplastic drugs against cancer, a leading and persistent cause of death, justifies their use [2]. DOXR mainly causes dose-dependent adverse effects on the heart, kidneys, and liver. Numerous studies have confirmed that DOXR primarily induces liver toxicity via the production of free radicals in non-target tissues. This process results in oxidative stress (OS), inflammation, the production of reactive oxygen species (ROS), and ultimately apoptosis [2].

Selenium (Se) plays a critical role in the body's antioxidant defence mechanisms and is vital for numerous biological functions as a component of the GSH-Px enzyme [3]. Malyar et al. showed that selenium supplementation can mitigate OS, inflammation, apoptosis, and fibrosis in thermally damaged liver tissues in mice [4]. Further studies have linked the roles of selenium and selenoproteins in various physiological processes, including neurotransmission, inflammation modulation, ion channel regulation (including TRP channels), protein phosphorylation, calcium balance, and cholesterol metabolism in the brain to their antioxidant properties [5].

The transient receptor potential melastatin-2 (TRPM2) channel, a member of the TRP channel family, is a voltage-independent, non-selective cation channel activated by oxidative molecules. Expressed in the liver, TRPM2 contributes to inflammation and apoptosis by increasing cellular calcium, thus functioning as an OS sensor [6]. This activation underscores TRPM2's critical role in cellular damage mechanisms [7, 8]. Recent studies have suggested TRPM2 as a potential therapeutic target for liver-related oxidative stress diseases. N-(p-amylcinnamoyl) anthranilic acid (ACA), initially used to inhibit leukotriene-mediated bronchoconstriction, has been found to inhibit phospholipase-A2 effectively [9, 10]. Thus, this research has chosen to investigate the blockade of the TRPM2 channel using ACA. To date, the role of the TRPM2 channel and the protective effects of selenium against DOXR-induced liver toxicity have not been simultaneously examined. This study explores the functionality of TRPM2 and the protective effect of Se against DOXR-induced hepatotoxicity.

Materials and Methods

Chemicals

Doxorubicin (DOXR) (T1020) and N-(p-amylcinnamoyl) anthranilic acid (ACA) (T5454) were supplied by TargetMol (Target Molecule Corp., USA), while sodium selenite (214485) was provided by Sigma Aldrich (St. Louis, MO, USA).

Animals and Experimental Design

The study utilized 60 Albino Wistar rats, aged 2-3 months. The rats were housed in plastic cages under a 12-hour light/dark photoperiod at 24°C. Total weight averages of the groups were optimized by measuring the weights of the rats at the beginning of the study. DMSO was used as a solvent for DOXR and ACA. Sodium selenite was dissolved in saline. DOXR, Se, and ACA dosages and duration were administered according to previously documented protocols [11-13]. The rats were divided into the following six groups:

1-Control: received daily intraperitoneal (i.p.) injections of isotonic solution (100 µL) for 14 days.

2- DMSO: received alternate day i.p. injections of DMSO (100 µL) for 14 days.

3- DOXR: received alternate day i.p. injections of DOXR (2.5 mg/kg) for 14 days [11].

4- DOXR+ACA: received alternate day i.p. injections of DOXR (2.5 mg/kg) and daily i.p. injections of ACA (25 mg/kg) for 14 days [12].

5- DOXR+Se: received alternate day i.p. injections of DOXR (2.5 mg/kg) and daily i.p. injections of Se (0.5 mg/kg) for 14 days [13].

6- DOXR+Se+ACA: received alternate day i.p. injections of DOXR (2.5 mg/kg) along with daily i.p. injections of Se (0.5 mg/kg) and ACA (25 mg/kg) for 14 days.

After the study, the rats were anesthetized with ketamine (50 mg/kg) + xylazine (20 mg/kg), and their abdominal cavities were surgically opened. Intracardiac blood samples were collected

and transferred to biochemistry tubes. After, centrifuged at 3500 xg for 10 min [14]. The liver was extracted and stored in the freezer for biochemical analysis, while liver tissues were preserved for histopathological and immunohistochemical evaluations.

Biochemical Evaluation

Serum AST and LDH levels were measured using spectroscopic methods with an Abbott Architect c16000 biochemical autoanalyzer (USA). Additionally, total cholesterol, HDL, LDL, and triglyceride levels were assessed through colorimetric analysis.

Histopathological Evaluation

Liver samples were preserved in 10% buffered formalin and subjected to dehydration with graded alcohols. After dehydration, samples were cleared in xylene and embedded in paraffin. Liver tissue sections, five micrometers thick, were sliced with a microtome and stained with Hematoxylin and Eosin, then examined under an Olympus BX53 microscope (Japan). Photographic records were created using Cellsens Imaging Software (Olympus, Japan). A semi-quantitative assessment was also performed, categorised as normal (-), mild (+), moderate (++), and severe (+++).

Immunohistochemical Evaluation

Paraffin blocks of 5 µm thick sections were deparaffinised and dehydrated. To reduce non-specific staining, sections were treated with 3% H_2O_2 for 10 min. Antigen retrieval was enhanced through microwave heating in citrate buffer (pH 6.1) for 5 min in two sessions. Non-specific staining was blocked at room temperature for 10 min using Ultra V Block. Primary antibodies against 8-OHdG (Santa Cruz Biotechnology, sc-20067, dilution: 1/50), TRPM2 (Bioss Inc., bs-2888R, dilution: 1/200), and Caspase-3 (Santa Cruz Biotechnology, sc-7272, dilution: 1/50) were applied and incubated overnight at 4°C in a humid chamber. Subsequently, the image sections were evaluated with an H-score using an Olympus BX53 microscope (Japan).

Statistical Methods: Statistical analysis was performed using SPSS version 21. The normal distribution of biochemical data was confirmed with the Kolmogorov-Smirnov test. Biochemical data with a normal distribution were analyzed using One-way ANOVA followed by Tukey's HSD test, with a significance level set at $p \leq 0.05$. Immunohistochemical and immunohistopathological data were analyzed using the Kruskal-Wallis test and the Bonferroni Adjusted Mann-Whitney U test to determine differences between groups. The intensity of positive staining was assessed over five randomly selected fields using ZEISS Zen Imaging Software. Data were considered significant at p values ≤ 0.05.

Results

Analysis of Research Findings

In the DOXR-induced liver injury scenario, liver function tests showed that serum AST, ALT, LDL, total cholesterol and triglyceride levels were increased, while HDL levels were lower in the DOXR group compared with the other groups ($p < 0.05$). No significant differences in these parameter levels were observed between the control and DMSO groups in these levels. Notably, the DOXR+ACA and DOXR+Se groups showed reductions in AST, ALT, LDL, total cholesterol, and triglyceride levels and increased HDL levels compared to the DOXR group ($p < 0.05$). The ACA+Se combination therapy displayed the lowest levels of AST, ALT, LDL, and total cholesterol and lower triglyceride levels compared to other treatments ($p < 0.05$, and Figure 1).

Histopathological and Immunohistochemical Observations

Normal liver histology was observed in the control and DMSO groups, as indicated in Table 1. DOXR treatment caused karyolysis, sinusoidal dilation, and disorganization of hepatic cords. Treatments with Se and ACA significantly ameliorated the hepatotoxic effects of DOXR (Figure 2).

Immunohistochemical examinations

Expressions of 8-OHdG (Figure 3A), TRPM2 channel (Figure 3B) and Casp-3 (Figure 3C) in liver tissue were evaluated by immunohistochemical method ($p < 0.05$). DOXR administration significantly increased the expression of 8-OHdG,

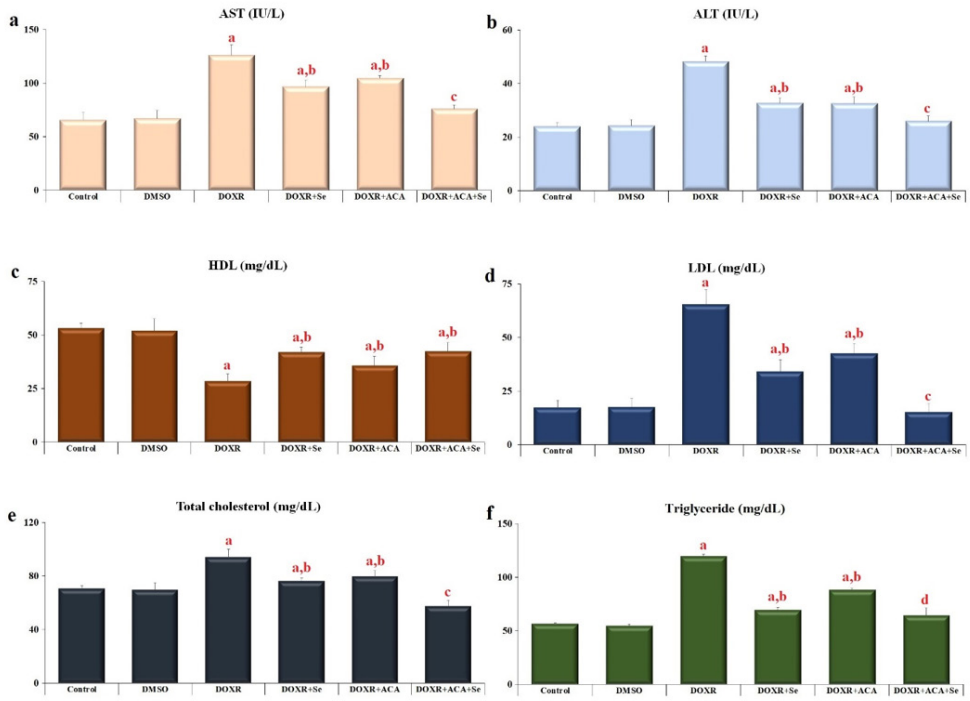

Figure 1. Effect of Se and ACA on serum parameters in DOXR-induced hepatotoxicity. a: AST (IU/L); b: ALT (IU/L); c: HDL (mg/dL); d: LDL (mg/dL); e: Total cholesterol (mg/dL); f: Triglyceride (mg/dL). (Values were given as mean ± SD; n=10). (ap < 0.05 compared with the control and DMSO groups; bp < 0.05 compared with DOXR group; cp < 0.05 compared with DOXR, DOXR+ACA and DOXR+Se groups; dp < 0.05 compared with DOXR and DOXR+ACA groups).

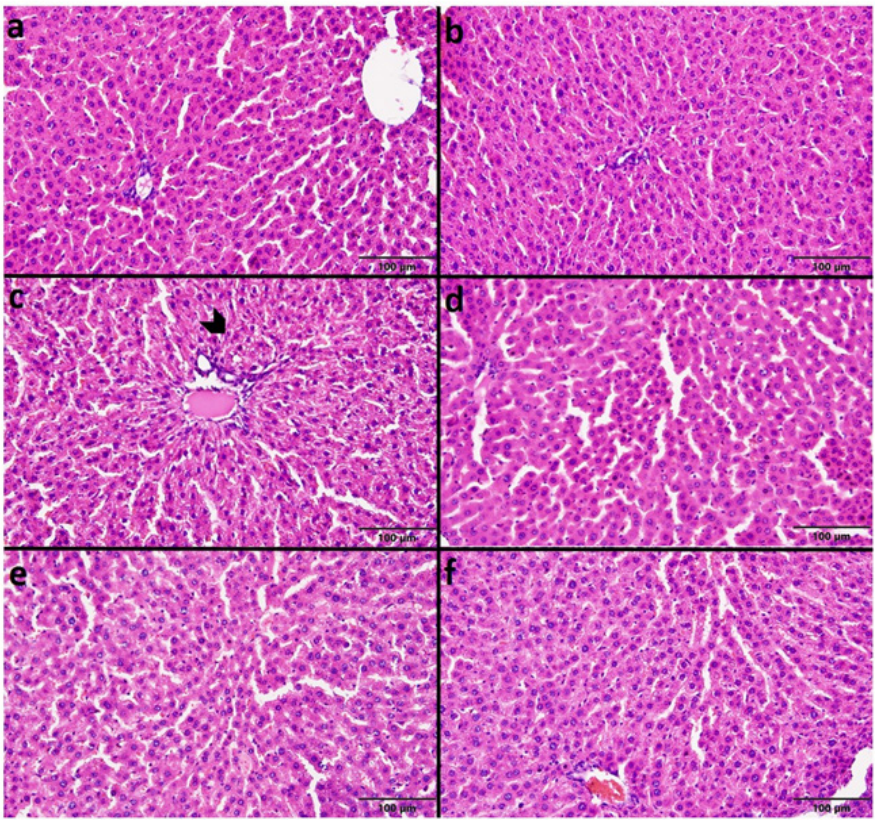

Figure 2. Histopathological Photomicrographs (H&E staining) rassociated with the effect of Se and ACA on DOXR-induced hepatotoxicity. Arrowhead: karyolytic nucleus. control group (a); DMSO group (b); DOXR group (c); DOXR+Se group (d); DOXR+ACA group (e); DOXR+ACA+Se group (f). (Bar; 100 µm).

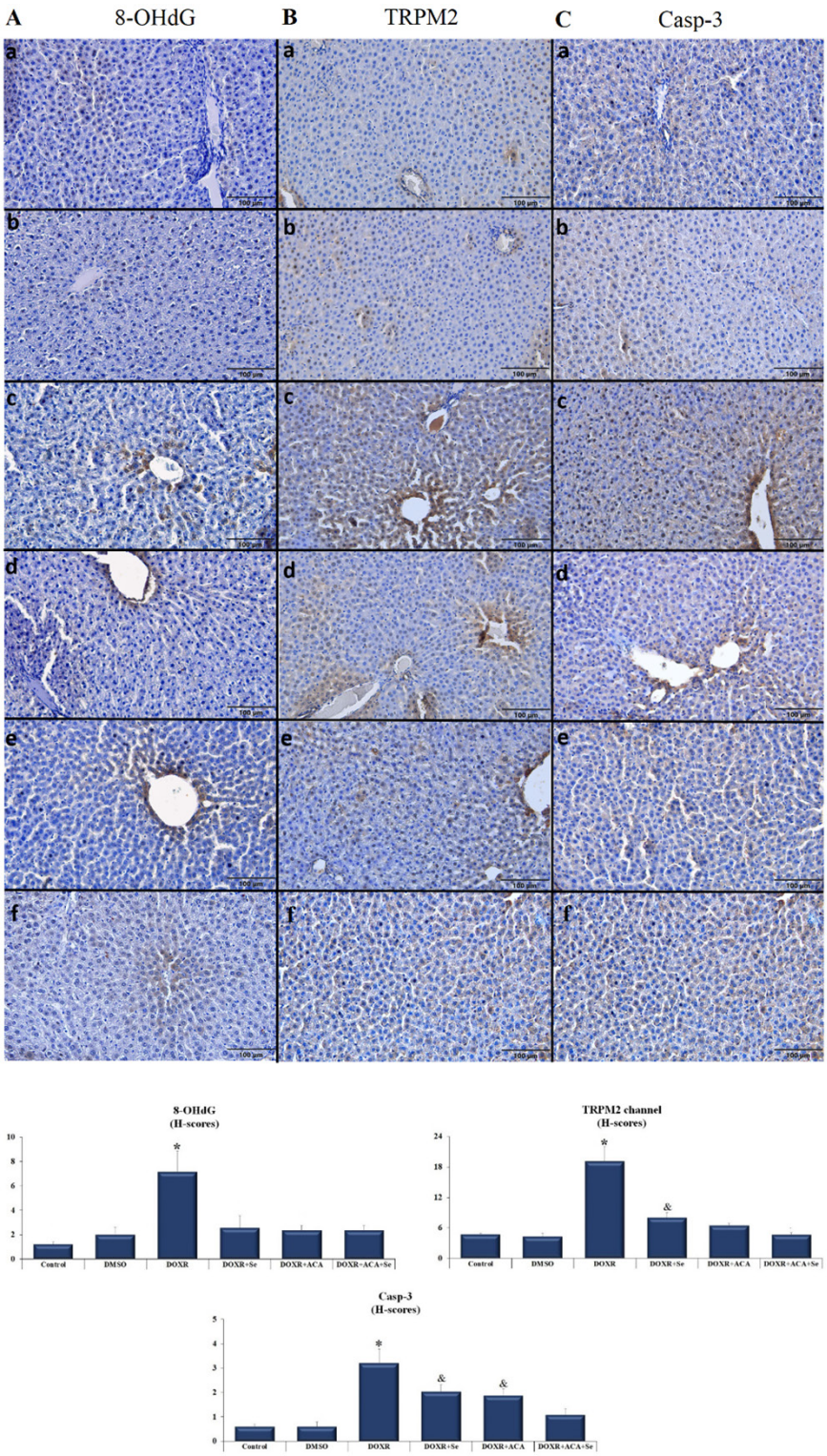

Figure 3. Immunohistochemical Photomicrographs and bar graphs associated with the effect of Se and ACA on 8-OHdG (A), TRPM2 channel (B), and Cas-3 (C) expression in DOXR-induced hepatotoxicity. The expressions were detected by immunohistochemical staining. Control group (a); DMSO group (b); DOXR group (c); DOXR+Se group (d); DOXR+ACA group (e); DOXR+ACA+Se group (f). (Bar; 100 µm). (*p < 0.05 versus DOXR groups; &p < 0.05 versus DOXR+Se and DOXR+ACA groups).

TRPM2 channel, and Casp-3 in the liver ($p < 0.05$). However, 8-OHdG, TRPM2 channel, and Casp-3 expression significantly decreased in DOXR+ACA and DOXR+Se+ACA groups compared to the DOXR group ($p < 0.05$). There was no significant difference in Casp-3 expression between the application of Se and ACA for combined or individual therapy ($p > 0.05$).

Table 1. Effect of Se and ACA on liver histology following DOXR-induced liver damage.

		Histological change		
		Karyolysis	Sinusoidal dilatation	Disarrangement of the hepatic cord structure
Groups	Control	-	-	-
	DMSO	-	-	-
	DOXR	++	++	+
	DOXR+Se	+	+	-
	DOXR+ACA	+	+	+
	DOXR+ ACA+ Se	+	-	-

normal (-); mild (+); moderate (++); and severe (+++), DMSO: Dimethyl sulfoxide, DOXR: Doxorubicin, ACA: N-(p-amylcinnamoyl) anthranilic acid, Se: Selenium.

Discussion

Doxorubicin (DOXR) is widely recognised as an effective anticancer agent for treating various cancer types [15]. The literature highlights significant concerns regarding DOXR's cardiotoxic and hepatotoxic effects. The liver, due to its numerous vital functions, is particularly susceptible to the adverse effects of drugs, and this feature underscores the necessity of strategies to mitigate these side effects. Selenium (Se), a critical trace element with robust antioxidant properties, plays a role in various biological functions [3]. It's antioxidant feature are fundamental to various physiological processes such as fertility, endocrine and immune functions, carcinogenesis, cardiovascular health, and gender-specific muscle development [16]. Studies by Cengiz et al. have emphasised that specific doses of Se could mitigate DOXR-induced liver damage by reducing pro-inflammatory cytokine levels [12].

The TRPM subfamily, especially TRPM2, has drawn significant interest due to its role in physiological and pathological processes such as cellular proliferation, temperature regulation, vascular development, neurological disorders, cancer progression, and endothelial dysfunction. The presence of TRPM2 in various cell types, including nerve, liver, endothelial, and kidney cells, further underscores its biological significance [7, 8]. Kheradpezhou et al. have shown that acetaminophen-induced liver toxicity is associated with the activation of the TRPM2 channel in hepatocytes, and blocking the channel with ACA and clotrimazole could reduce the severity of the damage [17]. Therefore, we used ACA to block the TRPM2 channel in this study.

The mechanisms of hepatotoxicity induced by doxorubicin (DOXR) are multifaceted. Recent studies have identified oxidative stress as one of the primary mechanisms driving oxidation-induced hepatotoxicity. Singla et al. have reported significant alterations in oxidative damage and hepatotoxicity parameters in the livers of rats subjected to DOXR-induced liver toxicity [18]. Wali et al. observed substantial increases in ALT, AST, ALP, and LDH serum levels in association with DOXR. Kuzu et al. found a significant rise in AST and ALT levels in liver function tests of rats administered DOXR [19]. Our study observed higher levels of serum AST, ALT, LDL, total cholesterol, and triglycerides in the DOXR group compared to other groups, aligning with previous findings. In addition, HDL levels were notably lower in the DOXR group compared to other groups. Additionally, we observed significant decreases in AST, ALT, LDL, total cholesterol levels, and triglycerides in the DOXR+ACA and DOXR+Se groups compared to the DOXR group. The lowest AST, ALT, LDL, and total cholesterol levels were seen in DOXR groups treated with a combination of ACA and Se. We also noted that triglyceride levels were significantly lower in the group treated with DOXR compared to the DOXR and DOXR+ACA groups (Figure 1).

Wali et al. reported that DOXR administration led to infiltration in liver cells, sinusoidal dilation, hepatocyte degeneration, periportal fibrosis, focal necrosis, and steatosis, thus resulting in tissue damage/atrophy [20]. Kuzu et al. observed that the liver tissues of the group not receiving DOXR maintained a normal histological appearance. Furthermore, severe coagulation necrosis, hydropic degeneration, serosal thickening, mononuclear cell infiltration in necrotic areas, severe hyperemia, and bleeding in interstitial vessels in liver tissues were seen in the group treated with DOXR [19]. Bilgiç et al. observed granular and vacuolar degeneration, hemorrhagic areas, macro and parenchymal mononuclear cell infiltration, picnotic nuclei in hepatocytes, sinusoidal dilation, and vascular congestion and dilation in the histopathological examination of liver

damage induced by DOXR [21]. We determined that the control and DMSO groups possessed normal liver histology in the histopathological examination of liver samples. However, DOXR administration caused karyolysis (apoptosis), sinusoidal dilation, and disorganization in the hepatic cord structure of hepatocytes. Furthermore, we found that treatments with Se and ACA significantly inhibited DOXR-induced hepatotoxicity (Table 1 and Figure 2). In a study investigating the function of the TRPM2 channel in liver ischemia/reperfusion (I/R) injury, TRPM2 expression was significantly up-regulated in liver tissue after I/R in the mouse liver I/R model. In contrast, histological damage, ALT, and AST levels in TRPM2 knockout mice were significantly lower compared to wild-type mice [22]. An in vitro study targeting the TRPM2 ion channel in non-alcoholic fatty liver injury-induced hepatocytes was showed that a new therapeutic strategy could be developed for oxidative stress-induced liver injury in non-alcoholic fatty liver injury. A study in hepatocytes isolated from rats showed that TRPM2 was mainly localized in intracellular organelles in rat hepatocytes, and oxidative stress-mediated damage to the cell results in increased expression of TRPM2 channels on the cell surface, most likely due to lysosomal trafficking. In addition, it has been emphasized that TRPM2 provides positive feedback that promotes further migration and cell death. Furthermore, the literature has reported that an increase in oxidative stress causes the expression of TRPM2 channels in many different cell types. Also, it has been reported that TRPM2 expression and increased channel activation trigger cell apoptosis mechanisms and promote cell death [7, 8, 12]. A different study evaluated the occurrence of DOXR-induced apoptosis/necrosis, genomic damage, oxidative stress and liver pathologies in rat liver tissue. In addition, in the histopathological examination of the liver tissue, it was determined that the use of DOXR caused remarkable histological changes in hepatocytes, including central vein occlusion, parenchymal inflammation around the main vein, periportal inflammation, and sinusoidal dilatation [23].

8-OHdG is an essential fundamental biomarker to measure endogenous oxidative DNA damage. Khan et al. examined the multiple side effects of DOXR in mice. They showed that the DOXR significantly increased 8-OHdG levels compared with the treatment groups. In addition, they noted that green synthesized selenium nanoparticles used for therapeutic purposes showed a protective effect against DOXR-induced damage [24]. Furthermore, it has been emphasised that oxidative stress, which also increases TRPM2 channel activation, triggers 8-OHdG levels in DOXR-induced liver damage [25]. Bilgic et al. showed that the Casp-3 expression in the DOXR-induced liver damage was increased compared to the control group [21]. In our study, was revealed that DOXR administration significantly increased the expression of 8-OHdG (Figure 3A), TRPM2 channel (Figure 3B) and Casp-3 (Figure 3C) in the liver. However, the expressions of 8-OHdG, TRPM2 channel and Casp-3 in the liver tissues were significantly decreased in the groups using Se and ACA together with DOXR compared to the DOXR group.

Limitations:

The limitations of the study are that more subjects could not be used in the study due to animal rights stated in the Declaration of Helsinki, which was revised in 2000, and that the studied parameters could not be examined molecularly due to the low project budget.

Conclusion: This study demonstrated that DOXR causes elevation of AST, LDH, HDL, LDL, and total cholesterol levels in serum samples and increases the expression of 8-OHdG, TRPM2 channel, and Casp-3 in liver tissue. However, the co-administration of Se and ACA with DOXR mitigated these effects and restored the lipid profile, and reduced the expression of 8-OHdG, TRPM2 channel, and Casp-3. These findings suggest that using Se and ACA as TRPM2 channel antagonists following DOXR administration may confer hepatic protection. Furthermore, further investigations at the molecular level are warranted to elucidate the underlying mechanisms of damage fully.

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Ethics Committee Approval: The Van Yuzuncu

Yil University Animal Experiments Local Ethics Committee approved this study (decision number: 2022/12-03, approval date: 01.12.2022).

ORCID and Author contribution: K.Y. (0000-0002-6585-4010), Z.H. (0000-0002-7623-1492) F.A. (0000-0002-7085-623X) M.H.B. (0000-0001-5821-4560). KY and ZH designed the study, performed the experiments, and analyzed the data. FA and MHB performed histology and IHC analyses. KY and ZH performed biochemical analyses and drafted the manuscript. All authors read and approved the final manuscript.

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RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

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The Relationship of Serum Uric Acid, Serum Uric Acid Creatinine Ratios With Disease Severity and Metabolic Syndrome in Schizophrenia Patients

Şizofreni Hastalarında Serum Ürik Asit, Serum Ürik Asit Kreatinin Oranlarının Hastalık Şiddeti Ve Metabolik Sendrom İle İlişkisi

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ABSTRACT

Aim: The objective of this research was to examine the association between serum uric acid (SUA) and serum uric acid/creatinine ratio (SUA/Cre), disease severity and metabolic syndrome in schizophrenia with a multifaceted etiopathogenesis.
Methods: The study comprised 240 participants in total, 120 of whom were healthy controls and 120 of whom were schizophrenia patients. Sociodemographic, clinical and laboratory data was collected and metabolic syndrome was assessed according to the established criteria. SUA and creatinine levels were measured and the SUA/Cre ratio was calculated. The severity of the disease was evaluated utilizing the Positive and Negative Syndrome Scale (PANSS). Statistical analyses were conducted to ascertain correlations and associations.
Results: SUA levels and SUA/Cre ratio higher in schizophrenia patients than controls (p=0.14, p=0.010, respectively). SUA/Cre ratio was positively correlated with PANSS negative score (r=0.266, p=0.03). SUA levels were elevated in individuals diagnosed with schizophrenia who also had metabolic syndrome, in comparison to those who did not have metabolic syndrome (p=0.009). Linear regression analyses showed that the association between SUA levels and SUA/Cre ratio and schizophrenia persisted when the effects of gender, age, metabolic syndrome, BMI and smoking were fixed.
Conclusions: This study highlights the association of SUA and SUA/Cre ratio with disease severity and metabolic syndrome among individuals diagnosed with schizophrenia.

Keywords: Schizophrenia, Uric acid, Uric acid creatinine ratio, Metabolic syndrome, Disease severity

ÖZ

Amaç: Bu araştırmanın amacı çok yönlü etiyopatogeneze sahip şizofrenide serum ürik asit (SUA) ve serum ürik asit/kreatinin oranı (SUA/Cre) ile hastalık şiddeti ve metabolik sendrom arasındaki ilişkiyi incelemektir.
Yöntem: Çalışmaya 120'si sağlıklı kontrol ve 120'si şizofreni hastası olmak üzere toplam 240 katılımcı dahil edilmiştir. Sosyodemografik, klinik ve laboratuvar verileri toplandı, kriterlere göre metabolik sendrom durumu değerlendirildi. SUA ve kreatinin düzeyleri ölçülmüş ve SUA/Cre oranı hesaplanmıştır. Hastalığın şiddeti Pozitif ve Negatif Sendrom Ölçeği (PANSS) kullanılarak değerlendirilmiştir. Korelasyon ve ilişkileri tespit etmek için istatistiksel analizler yapılmıştır.
Bulgular: SUA düzeyleri ve SUA/Cre oranı şizofreni hastalarında kontrol grubuna göre daha yüksekti (sırasıyla p=0.14, p=0.010). SUA/Cre oranı PANSS negatif skoru ile pozitif korelasyon gösterdi (r=0.266,p=0.03). SUA düzeyleri, metabolik sendromu da olan şizofreni tanılı bireylerde, metabolik sendromu olmayanlara kıyasla daha yüksekti (p=0.009). Doğrusal regresyon analizleri, SUA düzeyleri ve SUA/Cre oranı ile şizofreni arasındaki ilişkinin cinsiyet, yaş, metabolik sendrom, BMI ve sigara içmenin etkileri sabitlendiğinde de devam ettiğini göstermiştir.
Sonuç: Bu çalışma, şizofreni tanısı almış bireylerde SUA ve SUA/Cre oranının hastalık şiddeti ve metabolik sendrom ile ilişkisini vurgulamaktadır.

Anahtar Kelimeler: Şizofreni, Ürik asit, Ürik asit kreatinin oranı, Metabolik sendrom, Hastalık şiddeti

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Introduction

Schizophrenia is a psychiatric picture of chronic deterioration with a rate of approximately 1% in the population. In this psychiatric context, there are many psychiatric findings including hallucinations, delusions, disorganised speech and behavior. Although etiopathogenesis is still unclear, it has been reported that a number of factors may be responsible [1]. Although the role of dopaminergic, serotonergic and glutamatergic pathways in the etiology is emphasized, the immune system, inflammation, oxidative stress and related pathways are considered to play a role. It is thought that these pathways may be important in the evaluation of parameters not only in the etiology, but also the clinical course and response to treatment [2].

Uric acid is formed in the body from a compound called purine. Purines are involved in the purinergic cycle, a cycle that cells use to produce energy. In this cycle, they react with oxygen and are converted into uric acid, which in turn acts as an antioxidant in the body and fights free radicals. Free radicals are molecules that damage cells and lead to a condition called oxidative stress [3]. The formation and evolution of mental diseases are both significantly influenced by oxidative stress, which is a fundamental component. Thus, serum uric acid levels (SUA) have been associated with psychiatric disorders. In schizophrenia and some psychiatric disorders, SUA levels are lower than normal [4, 5]. This may indicate disruption of the purinergic cycle or decreased antioxidant activity [5]. However, some studies have also reported that SUA levels are higher than normal in patients with schizophrenia [6, 7]. This may suggest that the body produces uric acid as a defence mechanism against oxidative stress or when uric acid excretion is reduced [6]. The fact that the level of SUA gives different results in various neurological and psychiatric diseases suggests that its effect on diseases cannot be explained only by its antioxidant properties.

The presence of abdominal obesity, insulin resistance, dyslipidemia and hypertension are the defining characteristics of the metabolic syndrome, which is the principal condition. Individuals who receive a diagnosis of metabolic syndrome and satisfy the specified criteria are exposed to various risks including coronary diseases, type 2 diabetes and premature death [8]. The association between schizophrenia and metabolic syndrome predates the introduction of antipsychotic drugs in the 1950s [9]. Individuals diagnosed with schizophrenia or schizoaffective disorder, who have not previously received antipsychotic medication, have demonstrated hepatic insulin resistance in comparison to a control group. This implies that there may exist a direct relationship between schizophrenia and insulin resistance, regardless of the administration of antipsychotic medication [10].

The serum uric acid creatinine (SUA/Cre) ratio is a parameter that can be used to assess SUA levels independently of renal function. Since uric acid is metabolised in the kidney and excreted in the urine, serum uric acid concentration may vary according to age and gender. Therefore, both SUA levels and SUA/Cre ratios were compared in the studies. The SUA/Cre ratio was developed and used to reduce the interference on SUA caused by gender and renal function [11].

To the best of our knowledge, the relationship between SUA/Cre ratios and schizophrenia has not been investigated in the literature. The objective of our research was to examine the association between SUA, SUA/Cre ratios with disease severity and metabolic syndrome in schizophrenia patients.

Material methods

Study setting

The investigation was conducted at the psychiatry outpatient clinic and ward of Kütahya Evliya Çelebi Training and Research Hospital, involving patients diagnosed with schizophrenia according to DSM-V (Diagnostic Statistical Manual-V) criteria. The study employed a semi-structured interview method. The research received approval from the Ethics Committee of Kütahya Health Science University (2023/01-24). Each participant provided written informed permission to participate in this research.

Participant selection

A total of 240 participants were recruited,

comprising 120 individuals with schizophrenia (study group) and 120 healthy individuals (control group). Patients voluntarily agreed to participate and informed consent was obtained from either the participants themselves or their legal guardians. The control group included healthy people who were matched with the patient group based on age, gender and BMI. Participants in the control group sought medical attention for reasons unrelated to psychiatric disorders and willingly participated in the study.

Exclusion criteria

Participants with comorbid neurological diseases, chronic renal failure, gout, vegetarianism, chronic kidney disease, anemia, heart disease, obstructive pulmonary disease, chronic liver disease, chronic inflammatory bowel disease, thyroid dysfunction, tumors, cancer, active infections, excessive exercise habits, a history of steroid, colchicine, allopurinol, ascorbic acid, L-Dopa, alpha-methyldopa, isoniazid, isotretinoin, furosemide, indapamide, thiazide diuretics, antifungals, chemotherapeutics, excessive alcohol consumption or substance use, were all excluded.

Data collection

All participants completed a sociodemographic data form designed by the researcher, which included inquiries about age, gender and smoking habits. In the patient group, additional information was gathered, including age at onset of the disease, age at first treatment, age at first hospitalization, duration of illness (in years), time until treatment, number of hospitalizations, family history, suicide attempts and regular drug use. PANSS was employed to evaluate the intensity of pathological symptoms in the group diagnosed with schizophrenia. The publication was authored by Stanley Kay, Lewis Opler, and Abraham Fiszbein in the year 1987 [12]. Kostakoğlu et al. developed the Turkish version of the scale in 1999 [13].

Physical measurements

After completing the sociodemographic data form, height, weight, waist circumference and blood pressure were measured and recorded for each participant.

Metabolic Syndrome assessment

Each participant underwent evaluation for metabolic syndrome (MetS) using the criteria established by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III). MetS diagnosis required the presence of three or more of the following factors: abdominal obesity (waist circumference: M>102 cm, F>88 cm), hypertriglyceridemia (>150 mg/dL), low HDL (M<40 mg/dL, F<50 mg/dL), hypertension (BP>130/85 mmHg), and hyperglycemia (fasting blood glucose>110 mg/dL) [14].

Biochemical analysis

Biochemical blood parameters, including fasting blood glucose, triglyceride, HDL, uric acid, and creatinine levels, were determined from venous blood samples obtained after an 8-hour fasting period.

Statistical analysis

The Statistical Package for the Social Sciences (Version 22, SPSS Inc., Chicago, IL, USA) was used to analyse the data. The Kolmogorov-Smirnov test, the Shapiro-Wilk test and some histograms were used to evaluate the normality of distributions. Normally distributed data was expressed as mean + SD, non-normally distributed data was expressed as median (min-max) and categorical data was expressed as n (%). An independent sample test was used for normally distributed parameters and the Mann Whitney U test was used for non-normally distributed parameters. The Chi-square and Fisher Exact tests were used to compare categorical data. The Pearson correlation test was used for correlation analyses. Linear regression analysis was used to evaluate the parameters that may affect serum uric acid and SUA/Cre ratios. p significance value was considered significant as two-way and <0.05.

Results

Table 1 compares the demographic, clinical and laboratory characteristics of schizophrenia patients and healthy control group. It shows clinical characteristics of schizophrenia patients such as age at onset of illness, age at first treatment, age at first hospitalization, duration of illness, time to treatment, number of hospitalizations,

family history, suicide attempt, regular medication use, regular follow-up visits and PANNS scores. Schizophrenia patients and controls are similar in terms of age, body mass index (BMI), gender, systolic and diastolic blood pressure, fasting blood glucose, creatinine and metabolic syndrome. However, patients with schizophrenia had significantly higher smoking rates, triglycerides, waist circumference, SUA, and SUA/Cre ratio compared to the control group (p=0.001, p=0.38, p=0.001, p=0.14, p=0.010, respectively). In addition, HDL levels of patients with schizophrenia were significantly lower compared to the control group (p=0.001).

Table 1. Demographic, clinical and laboratory characteristics of the patient and control groups

Parameters		Patient (n=120)	Control (n=120)	p value
Age		45.3 ± 10.4	46.2 ± 10.3	0.516*
BMI (kg/m2)		28.3 ± 5.9	29.2 ± 4.6	0.199*
Gender	Female	53(44.2%)	65(54.2%)	0.121**
	Male	67(55.8%)	55(45.8%)	
Smoking		70(58.3%)	41(34.2%)	0.001**
Systolic blood pressure (mmHg)		124.3 ± 10.5	126.2 ± 8.4	0.120*
Diastolic blood pressure (mmHg)		78.8 ± 7.9	80.4 ± 6.9	0.084*
Fasting blood glucose (mg/dL)		113.3 ± 33.5	118.7 ± 23.3	0.145*
Triglyceride (mg/dL)		192.4 ± 139.0	163.3 ± 62.8	0.038*
HDL (mg/dL)		41.1 ± 9.9	45.7 ± 10.5	0.001*
Waist circumference(cm)		92.5 ± 13.1	86.7 ± 9.1	0.001*
Metabolic syndrome		57(47.5%)	55(45.8%)	0.796**
SUA levels (mg/dL)		5.7 ± 1.3	5.2 ± 1.6	0.014*
Creatinine (mg/dL)		1.0 ± 0.1	1.0 ± 0.2	0.713*
SUA/Cre ratio		6.0 ± 1.5	5.5 ± 1.6	0.010*
Age at disease onset		21.8 ± 5.3		
Age at first treatment		24.3 ± 6.3		
Age at first hospitalisation		25.3 ± 6.2		
Disease duration(years)		23.5 ± 9.7		
Time until treatment		2 (0 - 16)		
Number of hospitalizations		3 (0 - 20)		
Family history		39 (32.5%)		
Suicide attempt		30 (25.0%)		
Regular use of medication		27 (22.5%)		
Regular check-ups		103 (85.8%)		
PANNS Negative		25.2 ± 10.0		
PANNS Positive		18.1 ± 7.8		
PANNS General		44.5 ± 15.8		
PANNS Total		87.9 ± 30.3		

Data were given as mean± SD. * independent t test, ** Chi Square test(n%) , BMI: Body Mass Index, HDL: High Density Lipoprotein, SUA: Serum Uric Acid, SUA/Cre: Serum Uric Acid/ Creatinine, PANSS: Positive and Negative Syndrome Scale, p <0.05 was considered significant.

Table 2 compares the demographic, clinical and laboratory characteristics of schizophrenia patients according to the presence of metabolic syndrome. Patients with schizophrenia who were positive for metabolic syndrome had significantly higher BMI, systolic and diastolic blood pressure, fasting blood glucose, triglycerides, waist circumference and uric acid levels than patients with negative metabolic syndrome (p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.009, respectively). In addition, HDL levels of schizophrenia patients with positive metabolic syndrome were significantly lower than those with negative metabolic syndrome (p=0.001). In addition, the PANNS general scores and the number of hospitalizations of schizophrenia patients with positive metabolic syndrome were significantly higher than those with negative metabolic syndrome (p=0.039, p=0.005, respectively).

The PANNS Negative Score has a weak positive correlation with the SUA/Cre ratios (r = 0.266, p = 0.03) and is not significantly correlated with SUA levels(p> 0.05) (Figure 1).

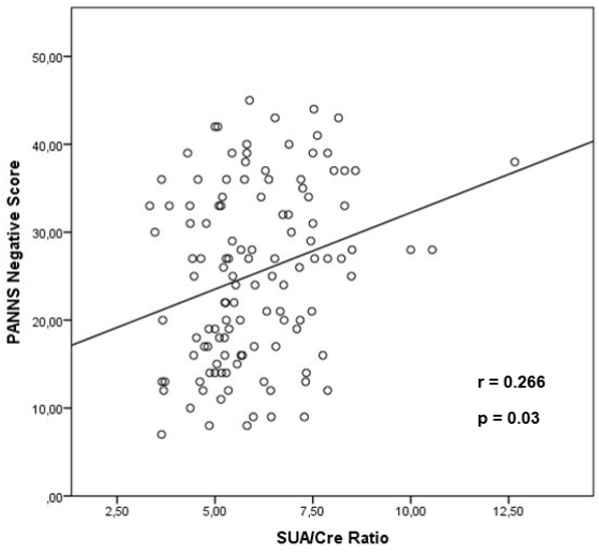

Figure 1. The Correlation between PANSS Negative Score and SUA/Cre Ratio

Table 3 analyses the factors affecting SUA level and SUA/Cre ratio by multiple linear regression analysis. There is a negative relationship between SUA level and age. This indicates that SUA level

Table 2. Comparison of the groups based on the presence or absence of metabolic syndrome within the patient group

Parameters	Metabolic syndrome negative (n=63)	Metabolic syndrome positive (n=57)	p value
Age	44.8 ± 9.8	45.8 ± 10.9	0.588*
BMI (kg/m2)	24.6 ± 4.0	32.3 ± 5.0	0.001**
Systolic blood pressure (mmHg)	120.8 ± 7.8	128.2 ± 11.6	0.001*
Diastolic blood pressure (mmHg)	75.4 ± 6.4	82.4 ± 7.9	0.001*
Fasting blood glucose (mg/dL)	96.8 ± 20.4	131.5 ± 35.7	0.001**
Triglyceride (mg/dL)	149.8 ± 88.6	239.3 ± 167.6	0.001**
HDL (mg/dL)	43.8 ± 11.0	38.0 ± 7.2	0.001*
Waist circumference(cm)	84.7 ± 10.4	101.1 ± 10.1	0.001**
SUA (mg/dL)	5.4 ± 1.2	6.0 ± 1.4	0.009*
Creatinine (mg/dL)	0.9 ± 0.1	1.0 ± 0.2	0.267*
SUA/Cre ratio	5.8 ± 1.5	6.3 ± 1.6	0.067*
PANNS Negative Score	24.2 ± 9.6	26.5 ± 10.5	0.208*
PANNS Positive Score	17.1 ± 7.5	19.4 ± 8.1	0.107*
PANNS General Score	41.7 ± 15.9	47.6 ± 15.2	0.039*
PANNS Total	82.9 ± 30.1	93.5 ± 29.9	0.056*
Age at disease onset	22.1 ± 5.6	21.5 ± 5.2	0.566*
Age at first treatment	24.7 ± 6.8	23.8 ± 6.0	0.424*
Age at first hospitalization	25.9 ± 6.7	24.8 ± 5.8	0.378*
Disease duration(years)	22.8 ± 9.0	24.3 ± 10.5	0.381*
Time until treatment	2 (0 – 15)	1 (0 – 16)	0.388*
Number of hospitalizations	3 (0 – 12)	5 (0 – 20)	0.005*

Data were given as mean± SD. * independent t test, **Mann Whitney U test

BMI: Body Mass Index, HDL: High Density Lipoprotein, SUA: Serum Uric Acid, SUA/Cre: Serum Uric Acid/ Creatinine, PANSS: Positive and Negative Syndrome Scale, p <0.05 was considered significant.

Table 3. Regression analysis for SUA and SUA/Cre ratio between age, sex, metabolic syndrome, BMI, smoking and groups

Dependent variable: SUA	B	SE		95%Cl (LL/UL) for B	p-value
Age	-.032	.009	-.228	-.050 / -.015	0.001
Gender	.204	.188	.070	-.167 / .575	0.279
Metabolic syndrome	.142	.243	.048	-.338 / .621	0.561
BMI	.023	.023	.085	-.022 / .069	0.308
Smoking	-.223	.193	-.076	-.604 / .159	0.251
Group	.382	.189	.130	.010 / .754	0.044
Dependent variable: SUA/Cre ratio	B	SE		95%Cl (LL/UL) for B	p-value
Age	-.043	.010	-.276	-.062 / -.024	0.001
Gender	-.073	.203	-.023	-.473 / .327	0.719
Metabolic syndrome	-.158	.262	-.049	-.675 / .359	0.547
BMI	.050	.025	.164	.001 / .098	0.046
Smoking	-.050	.209	-.016	-.461 / .361	0.811
Group	.536	.204	.167	.135 / .937	0.009

For SUA: R square; 0,10, Adjusted R Square; 0,79

For SUA/Cre ratio: R square; 0,125, Adjusted R Square; 0,102

BMI: Body Mass Index, SUA: Serum Uric Acid, SUA/Cre: Serum Uric Acid/ Creatinine

decreases with increasing age. No significant relationship was found between SUA level and gender, metabolic syndrome, BMI and smoking. There was a positive correlation between SUA level and group. This shows that SUA levels in schizophrenia patients are significantly higher than the healthy control group. There was a negative relationship between SUA/Cre ratio and age,

which indicates that the SUA/Cre ratio decreases as age increases. No significant relationship was found between the SUA/Cre ratio and gender, metabolic syndrome and smoking. There was however a positive relationship between SUA/Cre ratio and BMI, which shows that as BMI increases, the SUA/Cre ratio also increases. There was also a positive relationship between the SUA/Cre ratio and the group, which in turn indicates that the SUA/Cre ratio of schizophrenia patients is significantly higher than the healthy control group.

Discussion

In our study, SUA levels and SUA/Cre ratios were found to be high in patients with schizophrenia. As far as the available literature indicated, our study is the first study showing the relationship between schizophrenia patients and SUA/Cre ratio.

SUA levels have been investigated in psychiatric and many other diseases due to their activity via antioxidant mechanisms. There is a possible connection between SUA and mental problems, according to the findings of several studies [5, 7]. A study conducted in Egypt examined the levels of SUA as a biomarker in patients diagnosed with schizophrenia, major depressive disorder, and bipolar affective disorder. The study revealed that SUA levels were elevated specifically in patients with schizophrenia [15]. Similar to our study, when fifty-five schizophrenia patients and a healthy control group were analysed in a 5-year follow-up study, high SUA levels were found in the stable stage of schizophrenia compared to healthy controls when standardized according to age, gender and smoking habits [6]. Yao et al. found that the levels of SUA were notably reduced in patients with schizophrenia when compared to healthy individuals, which contradicts our own results [4]. Similarly, in a meta-analysis conducted in 2020, no significant difference was found in SUA levels between schizophrenia patients and healthy controls [16].

In the literature, there are also studies investigating SUA levels according to the clinical phases of schizophrenia patients [16-18] Reddy et al. found that patients with first-episode schizophrenia had significantly lower SUA levels than healthy controls [17]. In contrast to this study, Malewska-Kasprzak et al. found no difference in SUA concentration between schizophrenia patients in the acute and remission phases [18]. When the studies conducted to date are evaluated, it remains unclear whether SUA levels are elevated at the onset of schizophrenia or because of the progression of the disease. These studies show that SUA levels may be associated with schizophrenia, but this relationship is not related to the etiology. Studies have also shown that elevated SUA levels are associated with higher oxidative stress and more inflammation in patients with schizophrenia. Nevertheless, the exact relationship between schizophrenia, oxidative stress and endogenous antioxidant levels remains unclear and requires further studies [6].

At the same time, it should not be forgotten that SUA levels may be affected by antipsychotics [19]. It was not possible to explain the clear effects of antipsychotic drugs on SUA for the reason that the patient population in our study consisted of patients with chronic schizophrenia, multiple drug use, duration of drug use and dose changes during the treatment process.

In our study, the SUA/Cre ratio was found to be high in patients with schizophrenia. In the literature, the SUA/Cre ratio has been investigated in many clinical conditions such as hypertension, diabetes, kidney diseases and heart diseases in recent years [11, 20]. Some studies even show that the SUA/Cre ratio may be a better prognostic marker than the SUA level in the diagnosis, prognosis and treatment of diseases. Similarly, the SUA/Cre ratio was found to be a better marker than the SUA in our study. This was associated with the fact that SUA/Cre ratio better reflects endogenous uric acid production [20].

In our study, the SUA/Cre ratio was also found to be associated with the negative score of PANNS, which is an indicator of clinical severity. However, this relationship was not found with the SUA level. There are no studies in the literature examining the SUA/Cre ratio and clinical severity; however, there are studies examining SUA and clinical severity [7, 15, 18, 21]. Like our study, no correlation was observed between SUA levels and PANSS scores in these studies [7, 15, 18]. However, Borovcanin et al. found a relationship between SUA level and clinical severity in their

study [21]. Negative symptoms are one of the indicators of poor prognosis in schizophrenia that may be resistant to treatment. It includes important symptoms affecting functioning such as social withdrawal, decreased emotional expression, lack of motivation, anhedonia, and avolition. Many studies emphasize the role of inflammation and oxidative stress in contributing to the severity of negative symptoms in schizophrenia [22]. It suggests that patients with worse clinical findings have higher oxidative stress and more inflammation.

It should be borne in mind that uric acid disorders may not only be a consequence of the disease but also of metabolic abnormalities such as smoking, weight gain, abdominal obesity, dyslipidemia, hypertension or insulin resistance [11, 23]. There is significant evidence in the literature supporting the relationship between metabolic syndrome and SUA levels [24]. Various research has examined the association between SUA levels and metabolic syndrome, highlighting the possible influence of uric acid on metabolic syndrome [24, 25]. In our study, when schizophrenia patients were examined according to the association of metabolic syndrome, SUA level was found to be significantly higher in schizophrenia patients with metabolic syndrome, while there was no difference between the two groups in terms of SUA/Cre ratio. In a study investigating the relationship of SUA concentration with metabolic syndrome in schizophrenia and schizoaffective disorder, it was found that metabolic syndrome was more common in patients with high SUA levels [25].

Our study had some limitations. The medications and doses of the patients for schizophrenia treatment were different from each other. It was challenging to assess the impact of antipsychotic medications since the research was carried out with a group of patients with varying levels of therapy. Eating habits and life activities such as exercise may differ for the patient and control groups. Larger sample groups and longitudinal studies are needed.

Conclusion

SUA and SUA/Cre ratios were found to be higher in schizophrenia patients. In addition, the SUA/Cre ratio was found to be higher in schizophrenia patients with negative symptoms. Therefore, we think that SUA concentrations and SUA/Cre ratio may be a biomarker that can be used in diagnosis or treatment follow-up.

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RESEARCH ARTICLE

ARAŞTIRMA MAKALESI

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Evaluation of nutritional status (Nutritional Risk Screening-2002) of hospitalized inpatients and comparison with various variables

Hastanede Yatan Hastaların Beslenme Durumunun (Nütrisyonel Risk Taraması-2002) Değerlendirilmesi ve Çeşitli Değişkenlerle Karşılaştırılması

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ABSTRACT

Aim: This study aimed to evaluate the nutritional status of hospitalized patients according to Nutritional Risk Screening-2002 (NRS-2002) and to examine the effects of various variables on nutritional status.

Methods: The sample of the cross-sectional study consisted of 469 inpatients reached by simple random sampling method. Data was collected from hospitalized patients with a survey form using face-to-face interview method between January and March 2024. Personal information and hospital stay characteristics form, NRS-2002 form was used in the survey used to collect data.

Results: The average age of the patients included in the study was found to be 59.8±18.1 years. Of the patients, 51.4% were male and 48.6% were female. According to the total NRS-2002 score result, 410 (87.3%) of the patients were classified as no risk of malnutrition and 59 (12.6%) were classified as risk of malnutrition. No statistically significant difference was found between patients' malnutrition risk and gender, education level, hospital diets, hospital clinics ($p>0.05$). However, a statistically significant difference was found between the malnutrition risk of the patients and their age, body mass index (BMI) and length of hospital stay ($p<0.05$).

Conclusion: Nutrition screening tools should be applied more frequently to these patients, especially since the risk of malnutrition is higher in patients who are elderly, have a long hospital stay, and have a low BMI.

Keywords: Malnutrition, Nutritional status, Nutritional Risk Screening

ÖZ

Amaç: Bu çalışmada, hastanede yatan hastaların beslenme durumlarının Nütrisyonel Risk Taraması-2002 (NRS-2002)'ye göre değerlendirilmesi ve çeşitli değişkenlerin beslenme durumu üzerindeki etkilerinin incelenmesi amaçlandı.

Yöntem: Kesitsel tipte yapılan araştırmanın örneklemini basit tesadüfi örnekleme yöntemiyle ulaşılan 469 yatan hasta oluşturmuştur. Veriler, Ocak-Mart 2024 tarihleri arasında hastanede yatan hastalardan yüz yüze görüşme yöntemi kullanılarak anket ile toplandı. Verilerin toplanmasında kullanılan ankette kişisel bilgilervehastanede kalış özellikleri formu, NRS-2002 formu kullanıldı.

Bulgular: Çalışmaya alınan hastaların yaş ortalaması 59,8±18,1 yıl olarak bulundu. Hastaların %51,4'ü erkek, %48,6'sı kadındı. NRS-2002 skoru sonucuna göre hastaların 410'u (%87,3) malnütrisyon riski yok, 59'u (%12,6) malnütrisyon riskli olarak sınıflandırıldı. Hastaların malnütrisyon riski ile cinsiyet, eğitim düzeyi, hastane diyeti ve hastane klinikleri arasında istatistiksel olarak anlamlı bir farklılık bulunamadı ($p>0,05$). Ancak hastaların malnütrisyon riski ile yaş, beden kütle indeksi (BKİ) ve hastanede kalış süresi arasında istatistiksel olarak anlamlı bir farklılık bulundu ($p<0,05$).

Sonuç: Özellikle yaşlı, hastanede yatış süresi uzun ve BKİ düşük olan hastaların malnütrisyon riskinin daha yüksek olması nedeniyle bu hastalara beslenme tarama araçlarının daha sık uygulanması gerekmektedir.

Anahtar Sözcükler: Malnütrisyon, Beslenme durumu, Nütrisyonel Risk Taraması

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Introduction

One of the main problems for hospitalized patients is malnutrition. Developed countries try to minimize the risk of malnutrition in hospitalized patients. Malnutrition causes an increase in hospital stay, morbidity and mortality [1-3]. It also reduces the patient's quality of life and leads to significant increases in healthcare expenses. Hospitalization causes patients to become malnourished. Micro and macronutrient deficiencies caused by malnutrition affect the immune system of patients and increase the risk of infection. Therefore, early detection of malnutrition in hospitalized patients is important [3]. The American Society for Parenteral and Enteral Nutrition (ASPEN) recommends that all hospitalized patients undergo nutritional screening at the beginning of hospitalization [4].

In the hospital setting, one of the most important screening tools used to identify patients at risk of malnutrition is the NRS-2002 [1]. Additionally, European Society for Clinical Nutrition and Metabolism (ESPEN) recommends the use of NRS-2002 in nutritional assessment [5]. Implementation of the NRS-2002 screening tool does not require high training for healthcare professionals, is rapid and easy to administer [6]. In NRS 2002, patients are evaluated and scored in terms of nutritional deficiency and disease severity. Patients with a total score ≥ 3 are considered to be at nutritional risk [5]. It has been determined that there is a significant relationship between the increase in the NRS score and the increase in hospital stay, morbidity, mortality and hospital costs [7-10].

It is important to evaluate the relationship between the malnutrition risk of hospitalized patients and information about their personal characteristics or hospital stay. In addition, upon examining previous literature on the subject, it is notable that there is no data detecting hospital malnutrition in Alanya, an important tourism district in our country. This makes the contribution of our study to the literature very significant. This study aimed to evaluate the nutritional status of hospitalized patients according to NRS-2002 and to examine the effects of various variables on nutritional status.

Materials and Method

Sample

The sample of the study consists of inpatients at Alanya Alaaddin Keykubat University Training and Research Hospital. The sample calculation was made according to the malnutrition prevalence (%15-50) in other studies conducted in hospitals [11, 12]. Known universe size it was calculated according to the sampling formula (α=0.05, p=0.5, d=0.05) and the sample size to represent the population was determined as 393 inpatients. The sample of the study consisted of 469 inpatients reached by simple random sampling method. Those included in the study were voluntary inpatients who were 18 years of age and over and had no speech problems and were hospitalized for at least 2 days. Pregnant and breastfeeding women, unconscious individuals and patients in pediatric, psychiatric and intensive care clinics were not included in the study.

Ethical Regulations

"Ethics Committee Approval" dated 09.01.2024 and numbered 01/11 (10/2024) was received for the research from the " Alanya Alaaddin Keykubat University non-invasive clinical research ethics committee decision". In addition, before starting the study, written permission was obtained from the hospital chief physician to conduct the study. Data were collected by face-to-face interviews and survey method from hospitalized patients between January and March 2024. Individuals participating in the research were provided with information about the purpose of the study and "voluntary participation consent" was obtained. This research was conducted in accordance with the "Principles of the Declaration of Helsinki" and "Research and Publication Ethics".

Data Collection Tools

The data was collected using personal information and hospital stay characteristics form, Nutritional Risk Screening-2002.

Personal information and hospital stay characteristics form

The data form created by the researchers includes personal information such as age, gender, height,

body weight, education level, as well as data on the characteristics of the hospital stay such as the hospital clinics, hospital diets, length of hospital stay (LOHS). Anthropometric data measured by the researchers were evaluated by calculating body mass index (BMI) as kg/m² using the formula body weight (kg)/height (m²) according to the World Health Organization (WHO) classification. According to the WHO, BMI classification was as: BMI <18.5 kg/m2 as underweight; 18.5-24.9 kg/m2 as normal; 25-29.9 kg/m2 as preobese; ≥30 kg/m2 as obese [13].

Nutritional Risk Screening-2002 (NRS-2002)

NRS-2002, a nutritional screening tool, was developed in 2002 by Kondrup and colleagues with the contributions of the Danish Parenteral and Enteral Nutrition community [5]. The Turkish validity and reliability of the scale was conducted by Bolayır et al (2019). This screening tool aims to determine individuals' malnutrition levels and malnutrition risk rates. NRS-2002 is scored based on weight loss, food intake and BMI (1-3 points), disease severity score (1-3 points) and age correction (+1 point) in individuals over 70 years of age. Patients are classified as having no risk of malnutrition (<3 points) and having a risk of malnutrition (≥3 points) [14].

Data Assessment

For statistical analyses of the data obtained, SPSS 25.0 for Windows software (SPSS, Chicago, Il, USA) was used. Frequencies, percentages (%), mean, standard deviation (± SD), minimum(min) and maximum(max) values were used in descriptive statistics. Normal distribution of the data was assessed using the Kolmogorov-Smirnov test. In determining the differences between groups, the Chi-Square test was used to evaluate categorical variables, while the t test was used to evaluate continuous variables. A value of $p<0.05$ was considered significant.

Results

A total of 469 adult patients with an average age of 59.8±18.1 years were included in the study. Of the patients, 51.4% were male and 48.6% were female. When the education level of the patients was examined, it was found that more than half (55%) were primary school graduates. The most common hospital diets taken by patients in the hospital were normal diet, diabetic diet and salt-free diet (26.3%, 22.0% and 14.9%, respectively). The average hospital stay of the patients was 10.7±5.2 days (Table 1).

Table 1. Personal information and hospital stay characteristics of inpatients (n=469)

Variables		Results
Gender, n (%)	Female	228 (48,6)
	Male	241 (51,4)
Age, years (mean±SD) / (min-max)		59,8±18,1 / (18-97)
Education level, n (%)	Literate	74 (15,8)
	Primary school	258 (55,0)
	High school	91 (19,4)
	University	46 (9,8)
BMI, kg/m2 (mean±SD) / (min-max)		26,7±5,5 / (14,6-47,9)
Hospital diets, n (%)	Clear liquid diet (regimen1)	45 (9,6)
	Full liquid diet (regimen 2)	59 (12,6)
	Normal diet (regimen 3)	123 (26,3)
	Salt-free diet	70 (14,9)
	Diabetic diet	103 (22,0)
	Other diets	69 (14,8)
Hospital clinics, n (%)	General surgery	80 (17,0)
	Orthopedy	53 (11,3)
	Internal medicine	77 (16,4)
	Cardiology	57 (12,1)
	Gynecology	58 (12,4)
	Neurology	70 (15,0)
	Palliative	74 (15,8)
LOHS, days (mean±SD) / (min-max)		10,7±5,2 / (2-82)

In our study, the nutritional status of the patients was evaluated according to NRS 2002. According to the total NRS-2002 score result, 410 (87.3%) of the patients were classified as no risk of malnutrition and 59 (12.6%) were classified as risk of malnutrition (Table 2).

In this study, no significant difference was found between patients' nutritional risk and gender, education level, hospital diets, hospital clinics ($p>0.05$). However, a significant difference was found between the patients' nutritional risk and age, BMI and LOHS ($p<0.05$). According to the results of our study, the hospital stay of patients at risk of malnutrition was longer than that of patients no risk of malnutrition, and the difference was

found to be statistically significant (6.21±10.84 vs 9.49±10.10; p=0.029). In determining the risk of malnutrition according to body mass index, the BMI of patients at risk of malnutrition was lower than patients no risk of malnutrition and the difference was found to be statistically significant (27.32±5.31 vs 22.46±5.36; p<0.001). In determining the risk of malnutrition according to age, the average age of patients at risk of malnutrition was older than patients no risk of malnutrition and the difference was found to be statistically significant (57.48±17.79 vs 72.42±13.79; p<0.001) (Table 3).

Table 2. NRS-2002 scores of inpatients and nutritional status according to NRS-2002 score.

NRS-2002 Score	n	%
0	319	68,0
1	36	7,7
2	55	11,7
3	38	8,1
4	15	3,2
5	6	1,3
6	-	-
Total	469	100
Total NRS-2002 Score		
No risk of malnutrition(NRS score<3 points)	410	87,4
Risk of malnutrition (NRS score≥3 points)	59	12,6
Total	469	100

Discussion

This study was conducted to evaluate the nutritional status of hospitalized patients and to examine the variables affecting their nutritional status. In our study, the risk of malnutrition was found in 12.6% of hospitalized patients. In a comprehensive study conducted in 13 hospitals in Germany, the rate of malnutrition in inpatients was determined to be 27.4% [15]. In a study conducted by the Clinical Enteral and Parenteral Nutrition Association (KEPAN) in Turkey, where 29139 patients in 34 hospitals from 19 provinces were evaluated, it was determined that 15% of the patients were at risk of malnutrition at the time of hospitalization [16]. In a study in which 407 inpatients at Kırıkkale University Faculty of Medicine Hospital were evaluated with NRS-2002, malnutrition was detected in 13.6% of the patients, and this rate is very close to the malnutrition rate in our study [17]. In studies evaluating the risk of malnutrition in hospitalized patients with NRS 2002, it is seen that the malnutrition rate spreads over a wide range [12, 16, 17]. The reason for this wide range may be differences in the distribution of the services where patients are hospitalized. The rate of malnutrition also increases in studies with a higher proportion of intensive care patients. Since intensive care patients were not included in our study, the malnutrition rate may have been found to be lower than other studies. Other reasons for the differences in malnutrition rates in the literature may be the size of the provinces and hospitals where the studies were conducted, the types of diseases and differences in the methodology used.

Table 3. Relationship between nutritional status of various variables (n = 469).

		No risk of malnutrition (n=410)	Risk of malnutrition (n=59)	p-value
Gender, n (%)	Female	201 (88,2)	27 (11,8)	0,639*
	Male	209 (86,7)	32 (13,3)	
Age (years), mean±SD		57,48 ±17,79	72,42 ±13,79	<0,001**
Education level, n (%)	Literate	61 (82,4)	13 (17,6)	0,066*
	Primary education	221 (85,7)	37 (14,3)	
	High school	86 (94,5)	5 (5,5)	
	University	42 (91,3)	4 (8,7)	
BMI, (kg/m2)mean±SD		27,32±5,31	22,46±5,36	<0,001**
Hospital diets, n (%)	Clear liquid diet (regimen1)	38(84,4)	7(15,6)	0,171*
	Full liquid diet (regimen 2)	50(84,7)	9(15,3)	
	Normal diet (regimen 3)	113 (91,9)	10 (8,1)	
	Salt-free diet	59(84,3)	11(15,7)	
	Diabetic diet	88(85,4)	15(14,6)	
	Other diets	62(89,9)	7(10,1)	
Hospital clinics, n (%)	General surgery	72 (90,0)	8 (10,0)	0,412*
	Orthopedy	47 (87,0)	6 (13,0)	
	Internal medicine	66 (85,7)	11 (14,3)	
	Cardiology	49 (86,0)	8 (14,0)	
	Gynecology	52 (89,0)	6 (11,0)	
	Neurology	61 (87,1)	9 (12,9)	
	Palliative	63 (85,1)	11 (14,9)	
LOHS (days), mean±SD		6,21±10,84	9,49±10,10	0,029**

p<0,05, *Chi-Square test, **t test

In this study, the malnutrition rate was found

to be 11.8% in women and 13.3% in men, and there was no significant difference in the risk of malnutrition according to gender (p>0.05). In the study conducted by Güngör et al. in 2022, it was found that the risk of malnutrition was higher in men and the difference was significant [18]. There are studies showing that the rate of malnutrition in women is significantly higher than in men [17, 19]. There appears to be no consensus in the literature regarding the relationship between malnutrition and gender.

In this study, there was no significant relationship between the type of diet the patients took and the clinic they stayed in and the risk of malnutrition (p>0.05). Güngör et al. found that the risk of malnutrition was higher in patients hospitalized in oncology, general surgery and cardiac surgery services [18]. The reason why no difference was found in the risk of malnutrition depending on the ward where the patients were hospitalized may be due to the lack of oncology and intensive care services in our study. In our study, although the risk of malnutrition was found to be higher in individuals with low education levels, no significant relationship was found (p>0.05). In a study conducted in Kırıkkale in 2023, they found an inverse relationship between education level and malnutrition risk [17].

In our study, the average age of patients at risk of malnutrition was older than patients without risk of malnutrition, and the difference was found to be statistically significant (p<0.05). In another study conducted with 762 female and 662 male patients, it was reported that the risk of malnutrition was associated with increasing age, similar to our study [20]. Other studies have also found that increasing age increases the risk of malnutrition statistically significantly [21, 22].

It is expected that nutritional status screening tools and some anthropometric measurements will be correlated with each other. An inverse correlation is also expected between BMI and NRS-2002. Our study also meets this expectation and the BMI of patients at risk of malnutrition was lower than patients no risk of malnutrition and the difference was found to be statistically significant (p<0.05). In the study conducted by Kroc et al. in 2021, a negative relationship was found between both BMI and waist circumference and the NRS-2002 score [21]. In a comprehensive meta-analysis study, it was stated that NRS-2002 had a significant negative correlation with BMI [23].

This study, the hospital stay of patients at risk of malnutrition was longer than that of patients no risk of malnutrition, and the difference was found to be statistically significant (p<0.05). There are many studies in the literature showing a significant relationship between an increase in the NRS-2002 score and an increase in the length of hospital stay [7-10]. According to these results, we can think that a long hospital stay is an important criterion that increases the risk of malnutrition.

Limitation: The main limitation of this study is that the patient group is heterogeneous and the reasons for hospitalization are different. Another limitation is that patients in intensive care units were not included in the study.

Conclusion: Nutritional problems of hospitalized patients due to their current illness and complications and the resulting risk of malnutrition are common in hospitals. Preventing and treating malnutrition also contributes significantly to the treatment of the patient's current disease and accelerates recovery. NRS-2002 is a reliable screening tool used to detect malnutrition status of patients all over the World. Malnutrition risk screening tools should be applied to hospitalized patients at the time of hospitalization and at frequent intervals thereafter. In particular, nutritional screening tools should be applied more frequently, as patients of older ages, longer hospital stays and low BMI have a higher risk of malnutrition. Establishing nutrition support teams in hospitals and/or supporting their work can minimize the risk of malnutrition in patients.

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0002-9277-100X) and **A.E.B. (0000-0003-2254-2348)** All authors contributed to the manuscript conception, design, literature research, writing, critical review and final approval.

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RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

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Investigation of Mean Platelet Volume/Platelet, Neutrophil/ Leucocyte Ratio And Troponin Values in Geriatric Patients Admitted to Hospital with Akut Ischaemic Stroke

Hastaneye Akut İskemik inme ile Başvuran Geriatrik Hastalarda Ortalama Trombosit Hacmi/ Trombosit, Nötrofil/Lökosit Oranı Ve Troponin Değerlerinin Araştırılması

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ABSTRACT

Aim: The aim of this study was to investigate the mean MPV/PLT ratio, NLR and troponin I levels in geriatric patients admitted to hospital with acute ischaemic stroke (AIS) and to evaluate the prognostic value of these parameters. In this way, a better clinical decision can be provided in the evaluation and treatment processes of AIS patients.

Methods: Using a retrospective design, the data of geriatric patients admitted to hospital with a diagnosis of AIS were analyzed. Demographic characteristics, clinical findings, laboratory results and radiological findings were obtained from electronic medical records. Platelet volume, platelet count, neutrophil count, leukocyte count and troponin I levels were recorded as primary data and other demographic, clinical and laboratory parameters were recorded as secondary data.

Results: MPV/PLT ratio was significantly higher in AIS patients (0.04±0.02) compared to non-ischemic stroke patients (0.03±0.02) (p<0.001). Similarly, NLR (5.29±5.09) was significantly higher in AIS patients compared to the other group (1.93±0.87) (p<0.001). In addition, Troponin I level was significantly higher in AIS patients (10.48±7.88 ng/mL) than the other group (2.18±1.11 ng/mL) (p<0.001).

Conclusion: This study demonstrates the predictive value of mean MPV/PLT ratio, NLR, and troponin I levels in geriatric patients admitted to hospital with AIS. Using these parameters in clinical evaluations may be helpful in the follow-up of AIS patients and in determining treatment strategies.

Keywords: Ischaemic stroke, Mean Platelet Volume/Platelet ratio, Neutrophil/ Leukocyte ratio, Troponin I

ÖZ

Amaç: Bu çalışmanın amacı, akut iskemik inme ile hastaneye başvuran geriatrik hastalarda ortalama MPV/PLT oranı, NLR ve troponin I düzeylerini araştırmak ve bu parametrelerin prognostik değerlerini değerlendirmektir. Bu şekilde, akut iskemik inme hastalarının değerlendirme ve tedavi süreçlerinde daha iyi bir klinik karar verme sağlanabilir.

Yöntem: Geriye dönük bir tasarım kullanılarak, akut iskemik inme tanısıyla hastaneye başvuran geriatrik hastaların verileri retrospektif olarak incelendi. Hastaların demografik özellikleri, klinik bulguları, laboratuvar sonuçları ve radyolojik bulguları elektronik tıbbi kayıtlardan elde edildi. Trombosit hacmi, platelet sayısı, nötrofil sayısı, lökosit sayısı ve troponin I düzeyleri primer veriler, diğer demografik, klinik ve laboratuvar parametreleri de ikincil veriler olarak kaydedildi.

Bulgular: MPV/PLT oranı, akut iskemik inme hastalarında (0,04±0,02) akut iskemik inme olmayan hastalara (0,03±0,02) göre anlamlı oranda daha yüksek olarak gözlemlendi (p<0,001). Benzer olarak akut iskemik inme hastalarında NLR (5,29±5,09), diğer gruba oranla (1,93±0,87) anlamlı oranda daha yüksek idi (p<0,001). Buna ek olarak Troponin I düzeyi, akut iskemik inme hastalarında (10,48±7,88 ng/mL) diğer gruptan (2,18±1,11 ng/mL) bariz olarak yüksek idi (p<0,001).

Sonuç: Bu çalışma, akut iskemik inme ile hastaneye başvuran geriatrik hastalarda ortalama MPV/PLT oranı, NLR ve troponin I düzeylerinin prognostik değerlerini ortaya koymaktadır. Bu parametrelerin klinik değerlendirmelerde kullanılması, akut iskemik inme hastalarının takibinde ve tedavi stratejilerinin belirlenmesinde yardımcı olabilir.

Anahtar kelimeler: İskemik inme, Ortalama Trombosit Hacmi /Platelet oranı, Nötrofil/ Lökosit oranı, Troponin I

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Introduction

Acute ischaemic stroke (AIS) has become an important health problem among older adults. The aging process increases the risk of AIS with the development of vascular diseases [1, 2]. The frequency and severity of AIS are growing in the geriatric population; therefore, an effective prognostic evaluation and determination of treatment strategies are of great importance [3].

In recent years, the role of platelets and neutrophils in the mechanisms of AIS has become important to determine the prognostic factors. Platelets are cells that play a critical role in hemostasis and platelet activation is a key factor in the development of ischaemic events such as plaque formation and arterial occlusion [4]. Neutrophils play an important role in inflammation processes and may secrete proinflammatory molecules that may increase brain damage during AIS [5].

In this context, the examination of some hematological parameters to evaluate platelet and neutrophil activation in geriatric patients admitted to hospitals with ischaemic stroke has become an important research area. The Mean Platelet volume (MPV)/Platelet (PLT) ratio draws attention as a parameter that can reflect platelet activation. Furthermore, the Neutrophil/Leukocyte ratio (NLR) is a marker used in inflammation and infection and may be a potential prognostic indicator for AIS patients [6].

Troponin I is a protein recognized as an indicator of heart damage and is closely associated with cardiovascular incidents such as acute coronary syndrome and myocardial infarction. It has been suggested that troponin I levels may be increased in AIS patients, and therefore, may be used in the management of the patients [7].

The objective of this research was to investigate the mean MPV/PLT ratio, NLR, and troponin I levels in geriatric patients admitted to hospital with AIS and to evaluate the predictive value of these parameters. In this way, better clinical decision-making can be provided in the evaluation and treatment processes of AIS patients.

Patients and Method

Study Design

This study was carried out taking into account the rules of the Declaration of Helsinki and was approved by the scientific research ethics committee of Mardin Artuklu University (Date: 10.07.2023, decision no 2023/7-12). The confidentiality of all patients' files was maintained. Using a retrospective design, the data of geriatric patients admitted to the hospital with a diagnosis of AIS were retrospectively analyzed. This study was carried out in compliance with ethical guidelines and principles of research conduct.

Participants

Geriatric patients admitted to our hospital between January 2020 and June 2023 and diagnosed with AIS were included in the study. Participants had to be aged 65 years and older. Uncontrolled diabetes and hypertension patients, cancer patients, steroid drug users, smokers, and alcohol users were not included in the study. These were enrolled as group I. People with similar demographic characteristics who applied to our hospital with no significant clinical problems were included as control group. In the AIS patient group, those who were discharged after hospitalization were recorded as survivors and those who died within 30 days of hospitalization were recorded separately. Comparisons were also made between alive and dead patients with AIS.

Data Collection

Demographic characteristics, clinical findings, laboratory results, and radiological findings were obtained from electronic medical records. Platelet volume, platelet count, neutrophil count, leukocyte count, and troponin I levels were recorded as primary data, and other demographic, clinical, and laboratory parameters were recorded as secondary data. The diagnosis of the patients was based on computed tomography, diffusion magnetic resonance imaging results, and anamnesis reports.

Statistical Analysis

The data were analyzed using SPSS software (ver. 26.0; SPSS Inc., Chicago, IL). The normality of the data distribution was determined by the Shapiro-Wilk test. Continuous variables are presented as mean ± standard deviation and

categorical variables are presented as frequencies and percentages. Continuous variables were calculated using a student t-test. A p-value <0.05 was set to indicate statistical significance.

Results

The included individuals were 108 AIS patients [52 (48.1%) females and 56 (51.9%) males] and 52 [33 (63.5%) females and 19 (36.5%) males]. Table 1 summarizes the distribution of laboratory data and mean age. Although there was a difference between the gender distributions, the difference was not meaningful (p=0.09). The mean age of patients with a history of AIS was 72.84±7.07 years and 70.42±5.85 years for those without AIS. The mean age was higher in AIS patients compared to the other group (p=0.045). MPV/PLT ratio was significantly higher in AIS patients (0.04±0.02) compared to non-AIS patients (0.03±0.02) (p<0.001).

Table 1. Distribution of laboratory data and mean age of AIS patients and controls

Parameters	AIS patients Mean±SD	Non-AIS patients Mean±SD	P
Age(years)	72.84±7.07	70.42±5.85	0.045
Glucose (mg/dL)	152.09±83.051	129.09±39.40	0.104
Urea (mg/dL)	46.62±21.21	37.59±11.12	0.014
Creatinine (mg/dL)	0.98±0.52	0.93±0.56	0.599
Albumin (g/dL)	4.71±5.34	4.39±0.39	0.863
Cholesterol (mg/dL)	192.76±56.48	203.2660±32.06	0.502
WBC (10*3/uL)	9.98±4.41	8.02±2.46	0.003
HCT (%)	40.94±6.75	42.69±5.07	0.099
MCV (fL)	88.79±8.09	88.54±7.15	0.844
MCH (pg)	28.55±2.77	28.07±2.62	0.301
MPV/PLT	0.043±0.021	0.03±0.016	<0.001
NLR	5.29±5.09	1.93±0.87	<0.001
Troponin I (ng/mL)	10.48±7.88	2.18±1.11	<0.001

WBC: White blood cell, HCT: Haematocrit, MCV: Mean Corpuscular Volume, PLT: Platelet, MPV: Mean Platelet Volume, PLT: Platelet NLR: Neutrophil to lymphocyte ratio MPV/PLT: The mean platelet volume/ platelet count ratio

Similarly, NLR (5.29±5.09) was significantly higher in AIS patients compared to the other group (1.93±0.87) (p<0.001). In addition, Troponin I level was significantly higher in AIS patients (10.48±7.88 ng/mL) than in the other group (2.18±1.11 ng/mL) (p<0.001).

Table 2 shows the mean distribution of WBC, MPV/PLT, NLR, and Troponin I values between alive and dead AIS patients within 30 days. NLR was significantly higher in the dead patients (13.41±6.21) than in alive patients (3.77±3.01) (p<0.001). On the other hand, although the mean MPV/PLT ratio and Troponin I values were higher in dead patients, the differences were not statistically significant (p=0.448 and p=0.125, respectively).

Table 2. Distribution of WBC, MPV/PLT, NLR and Troponin I values of discharged and dead patients with AIS

Parameters	Survivor patients Mean±SD	Died patients Mean±SD	P
MPV/PLT	0.042±0.021	0.047±0.020	0.448
NLR	3.77±3.01	13.41±6.21	<0.001
Troponin I (ng/mL)	9.68±7.27	14.68±10.16	0.125

PLT: Platelet, MPV: Mean Platelet Volume, NLR: Neutrophil to lymphocyte ratio

In patients with AIS, the MPV/PLT ratio showed a strong positive correlation with neutrophils (R=0.348, p<0.001), while the NLR showed a strong positive correlation with albumin (R=0.511, p<0.001), WBC (R=0.560, p<0.001), and neutrophils (R=0.754, p<0.001). In contrast, both the MPV/PLT ratio and NLR showed no significant correlation with Troponin I (Table 3).

Table 3. Correlation of MPV/PLT and NLR with other data

Parameters	MPV/PLT	NLR
Albumin (mg/dL)	(R=-0.093,P=0.524)	(R=0.511,P<0.001)
WBC (10*3/uL)	(R=-0.165,P=0.089)	(R=0.560, P<0.001)
NEU (10*3/uL)	(R=0.348** , P<0.001	(R=0.754, P<0.001)
Troponin I (ng/mL)	(R=0.200,P=0.194)	(R=0.130,P=0.400)

PLT: Platelet, MPV: Mean Platelet Volume WBC: White blood cell, NEU: Neutrophil, NLR: Neutrophil to lymphocyte ratio R: Pearson Correlation rank, P: Correlation is significant at the 0.01 level (2-tailed).

Discussion

In this study, it was found that the mean MPV/PLT ratio, NLR, and troponin I levels were significantly higher in geriatric patients with AIS. These parameters were also observed to be elevated in deceased AIS patients in comparison to survivors. These findings are essential for assessing the prognosis and treatment of AIS patients.

Platelets start the formation of blood clots by sticking together and accumulating. This process continues until inflammation occurs, leading to

the development of more organized blood clots and reduced blood flow [4, 8]. The levels of MPV and PLT have been studied in various diseases including cerebral ischemia, cardiovascular diseases, and cancer. Studies have shown that high MPV and MPV/PLT ratios are associated with poor outcomes in these conditions [9-11]. Ho Lim et al. showed that MPV/PLT was parallel with clinical severity in patients with AIS and that the values at the time of presentation could be used as a good tool to predict prognosis [6]. Our study findings are similar to the outcomes of these studies. According to this data, we can say that there is a significant relationship between the mean MPV/PLT ratio and AIS. Higher MPV/PLT ratios suggest increased platelet activation and thus increased risk of ischaemic events such as plaque formation and arterial occlusion. These findings underline the importance of a more detailed examination of platelet function in AIS patients.

Furthermore, NLR should also be considered as a prognostic marker in AIS patients. High NLR suggests increased inflammation processes and increased release of proinflammatory molecules that may increase brain damage. This finding emphasizes the importance of evaluating the inflammatory response in AIS patients [12, 13]. Li et al. showed that NLR values at admission were significantly negatively correlated with improvement in clinical outcomes in patients with AIS in the first 90 days after stroke [14]. In their study, Gong et al. also suggested that NLR is an independent factor for early neurological deterioration after thrombolysis [15]. Similarly, NLR was significantly higher in AIS patients in our study. This parameter was found to be increased in dead AIS patients compared to survivor patients. The results obtained provide important findings in the evaluation of the prognosis and treatment processes of AIS patients.

Troponin I level is also an important parameter that should be evaluated in AIS patients. Elevated troponin I levels are considered a marker of cardiac damage and are associated with cardiovascular events including acute coronary syndrome and myocardial infarction. Elevated troponin levels have been reported in many different pathological conditions including AIS [16, 17]. In their study, Schietz et al. reported that troponin I levels were elevated in one of seven patients with AIS, and these levels were independently associated with short-term mortality [18]. Troponin I levels were significantly increased in AIS patients in our study. Moreover, troponin I levels were significantly higher in patients who died due to AIS compared to those who were discharged. The findings of this study suggest that troponin levels may be increased in AIS patients and these levels may be used in the evaluation of prognosis.

Limitations: Limitations of this study should also be considered. Firstly, due to the retrospective design of the study, causal relationships cannot be determined and only associations are revealed. Secondly, the number of patients included in the study may be limited, which may affect the results. The results of this study should be confirmed by prospective studies involving larger sample groups.

Conclusion: In conclusion, this study demonstrates the predictive value of mean MPV/PLT ratio, NLR, and troponin I levels in geriatric patients admitted to hospital with AIS. Using these parameters in clinical evaluations may help in the follow-up of AIS patients and in determining treatment strategies. Future studies are necessary to further confirm these findings and to better understand the efficacy of these parameters in clinical practice.

Conflict of Interest: The authors declare no conflict of interest related to this article.

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RESEARCH ARTICLE

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Investigation of the effects of Protocatechuic acid on apoptosis, oxidant and antioxidant status in Caco-2 colorectal cancer cells

Protokatekuik asidin Caco-2 kolorektal kanser hücrelerinde apoptoz, oksidan ve antioksidan durum üzerine etkilerinin araştırılması

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ABSTRACT

Aim: Protocatechuic acid (PCA) is one of the common phenolic acids found in many foods and plants and it has multiple biological activities. Although PCA has been investigated for its antioxidant, anti-inflammatory, and anticancer effects in various cell lines, its effects on molecules involved in the apoptotic pathway, especially in human colon cancer (Caco-2) cells, have not been fully elucidated. This study aimed to investigate the effects of PCA on possible oxidant, antioxidant, and apoptosis mechanisms in Caco-2 cells, depending on dose and time.
Methods: In the experimental study, 4 groups were created: control (K), PCA (250-500-1000 µM). Total oxidant capacity (TOC), total antioxidant capacity (TAC), Oxidative stress index (OSI), Bax, Bad, Bcl-2, Bcl-xl, and Caspase 9 protein levels were determined by the ELISA method in the cell lysates obtained from the groups.
Results: The results showed that PCA treatment had apoptotic effects on Caco-2 cells at 24 and 48 h. PCA also decreased OSI levels by increasing TAC levels and decreasing TOC levels in a dose-dependent manner.
Conclusion: As a result, it was determined that PCA has an apoptotic effect on the Caco-2 cell line and can be useful in the prevention and/or treatment of colon cancer.

Keywords: Colon cancer, Caco-2, Protocatechuic acid, Apoptosis, Oxidative stress

ÖZ

Amaç: Protokatekuik asit (PCA), birçok biyolojik aktiviteye sahip birçok gıda ve bitkide bulunan yaygın fenolik asitlerden biridir. PCA'nın çeşitli hücre hatlarında antiinflamatuvar, antioksidan, antikanser etkileri araştırılmasına rağmen insan kolon kanseri (Caco-2) hücrelerinde özellikle de apoptotik yolaklakta yer alan moleküller üzerindeki etkileri tam olarak aydınlatılamamıştır. Bu çalışmanın amacı PCA'nın Caco-2 hücrelerinde doza ve zamana bağlı olarak olası oksidan, antioksidan ve apoptoz mekanizmaları üzerindeki etkilerini araştırmaktır.
Yöntem: Deneysel çalışmada kontrol (K), PCA (250-500-1000 µM) olmak üzere 4 grup oluşturulmuştur. Gruplardan elde edilen hücre lizatlarında toplam oksidan kapasite (TOC), toplam antioksidan kapasite (TAC), Oksidatif stres indeksi (OSI), Bax, Bad, Bcl-2, Bcl-xl, Kaspaz 9 protein seviyeleri ELISA yöntemiyle belirlenmiştir.
Bulgular: Sonuçlar PCA tedavisinin Caco-2 hücrelerinde 24 ve 48 saatte apoptotik etkilere sahip olduğunu göstermiştir. Ayrıca PCA doza bağlı bir şekilde TAC seviyelerini artırırken TOC seviyelerini azaltarak OSI seviyelerini de azaltmıştır.
Sonuç: Sonuç olarak PCA'nın Caco-2 hücre hattında apoptotik etkiye sahip olduğu ve kolon kanserinin önlenmesinde ve/veya tedavisinde yararlı olabileceği belirlenmiştir.

Anahtar kelimeler: Kolon kanseri, Caco-2, Protokatekuik asit, Apoptoz, Oksidatif stres

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Introduction

Colon cancer is the third most common type of cancer in the world, starting in the large intestine and spreading to the lower digestive system [1, 2]. Various surgical methods and chemotherapy drugs are used in the treatment of the disease. However, the side effects of these drugs are high, and the cancer cells become resistant to the drugs used over time, which negatively affects the treatment process [3]. Therefore, there is a need to develop new drug candidates that will minimize the side effects. Phenolic compounds with antioxidant activity especially stand out in controlling this disease [4-6]. Flavonoids and phenolic compounds have a strong cytotoxic effect against colon cancer cells with lower risk and fewer side effects [7]. In addition, metabolites such as flavonoids and phenolic compounds can reduce tumor cell proliferation through various mechanisms, such as activating caspases and promoting apoptosis [2]. Protocatechuic acid (3,4-dihydroxybenzoic acid, PCA), among the phenolic compounds, may be a useful agent with its remarkable antioxidant activity [8,9]. Various studies have reported that PCA has antioxidant, antibacterial, antimutagenic, antiviral, anti-inflammatory, antiulcer, antidiabetic, neuroprotective, and anticancer properties [10,11,12]. Although there are many studies on PCA in various types of cancer, studies on colon cancer cell lines are limited.

It is well known that apoptosis is one of the main pathways of tumor cell death. According to literature reviews, PCA is considered a potential chemopreventive compound for clinical applications in the prevention of neoplastic diseases and a highly promising compound in the treatment of various diseases. However, the mechanism of action of PCA on apoptotic pathways, where it disrupts specific pathways leading to cell death, has not yet been elucidated. Therefore, further research is needed in this area. The mechanism of apoptosis is complex and involves many signaling pathways. Apoptosis activation is initiated by two main pathways: intrinsic (mitochondria-dependent) extrinsic. Both pathways combine to activate caspases and ultimately cause morphological and biochemical cellular changes, leading to the onset of apoptosis [13,14]. The intrinsic pathway is regulated by Bcl-2 (B cell lymphoma-2) family members [(anti-apoptotic (Bcl-2, Bcl-xl)- pro-apoptotic (Bad, Bax)]. Pro-apoptotic proteins such as Bcl-2 Bax (Bcl-2-associated X) and Bad (Bcl-2-associated death) activate caspases, releasing the mitochondrial intermembrane space and promoting cell death [15]. In addition, members such as Bcl-2 inhibit cell death by suppressing cytochrome c release. Therefore, the balance between pro- and antiapoptotic proteins is important in determining apoptosis. In summary, apoptosis is a promising target in tumor therapy.

In light of this information, this study investigated the effects of PCA on possible oxidant, antioxidant, and apoptosis mechanisms in Caco-2 cells, depending on dose and time. I believe that in the future, PCA will contribute to developing new candidate anticancer agents by elucidating the signaling pathways associated with apoptosis mechanisms.

Materials and Methods

Cell Culture and Experimental Groups

PCA was purchased from Sigma Aldrich (Cat No. 99-50-3). The human colon cancer cell line (Caco-2) was used from cells available in our stock. Caco-2 cells were cultured in DMEM medium containing 10% FBS (fetal bovine serum), L-glutamine, 1% penicillin-streptomycin, and NaHCO3 at 37oC in an environment containing 5% CO2 and atmospheric humidity. Incubated at 37°C and passaged every 2-3 days. PCA was dissolved in ethyl alcohol, and the concentration of ethyl alcohol was less than 0.1% for all treatments. The PCA concentrations used in the study were determined based on the results of our previous study [16].

In this study, 4 main groups were created as follows.

Group I (Control): Cells in this group were not treated with any chemicals.

Group II (250 µM PCA): Cells were incubated with PCA (250 µM) for 24 and 48 h [16].

Group III (500 µM PCA): Cells were incubated with PCA (500 µM) for 24 and 48 h [16].

Group IV (1000 µM PCA): Cells were incubated

with PCA (1000 µM) for 24 and 48 h [16].

Detection of apoptosis

The apoptotic markers in the PCA-treated or untreated control groups were determined using the ELISA method. According to the manufacturer's instructions, the Bad, Bax, Bcl-2, and Caspase 9 protein levels in Caco-2 cell culture lysates were determined using commercial ELISA assay kits (BT-LAB ELISA kits, China). The results were obtained by taking OD values at a wavelength of 405 nm with an ELISA microplate reader (BIOTEK ELx808, USA).

Determination of TAC, TOC, and OSI

To evaluate antioxidant and oxidative stress levels, TOC, TAC, and levels were analyzed in line with the instructions of the manufacturer company using ELISA kits (RL0017 Rel Assay Total Antioxidant Status, RL0024 Rel Assay Total Oxidant Status). Studies were performed using an ELISA reader and an ELISA plate washer. TAC findings were expressed as mmol Trolox Equiv/L, while TOC findings were expressed as mmol H2O2 Equiv/L [17]. In addition, the OSI was calculated using TOC and TAC measurements [18].

Statistical analyses

All data were expressed as mean ± standard deviation (SD). The “SPSS 20.0 for Windows” package program and the “One Way ANOVA-Tukey” test were used to evaluate the data obtained in our study. Differences between the groups were tested by Tukey’s multiple comparison post hoc tests, and p<0.05 was determined to be statistically significant. Other statistical analyses between experimental groups were calculated using GraphPad Prism (Version 7.04 for Windows, USA) software.

Results:

Results of Apoptosis

The effect of PCA on molecules involved in the apoptotic pathway is shown in Figure 1. When the study results regarding Bad protein were evaluated, a statistically significant decrease was observed in the concentrations applied to PCA in Caco-2 cells at 250 µM and 1000 µM after 24 h compared to the control group (p <0.01). After 48 h, a significant increase was observed only in the group applied with 1000 µM PCA compared to the control group (p <0.05) (Figure 1A).

Bax/Bcl-2 ratio showed a significant increase in Caco-2 cells exposed to PCA at a concentration of 250 µM for 24 h compared to the control group (p<0.01), while this increase at other doses was not statistically significant. After 48 h of application, the Bax/Bcl-2 ratio showed a significant decrease only at the concentration of 1000 µM (p<0.05) (Figure 1B). Bax/Bcl-xl ratio showed a statistically significant decrease at all concentrations applied at the end of the 24 h compared to the control group (p<0.05, p<0.01). At the end of the 48 h, a significant decrease was observed only at the concentration of 1000 µM (p<0.01) compared to the control group (Figure 1C). Caco-2 cells treated with PCA for 24 h, showed a significant increase in caspase 9 levels at all concentrations applied compared to the control group (p<0.001). At the end of the 48 h, it was observed that the increase in caspase 9 protein level continued significantly in PCA-applied Caco-2 cells compared to the control group, depending on the increasing dose concentration (p<0.001, p<0.0001). (Figure 1D).

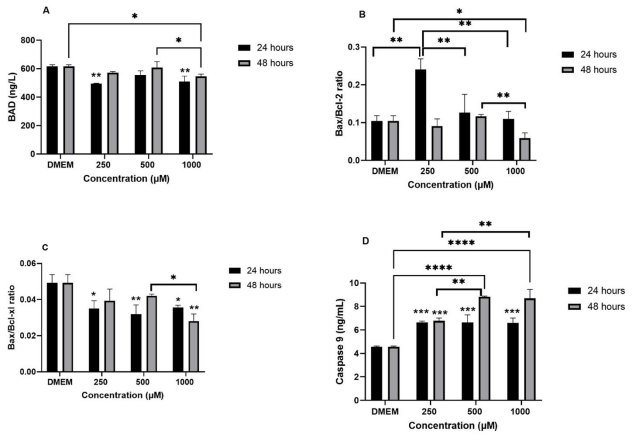

Figure 1. The effect of PCA treatment on Bad protein (A), Bax/Bcl-2 (B), Bax/Bcl-xl ratio (C), Caspase 9 protein (D) levels (mean ± SD). *P<0.05 **P<0.01, ***P<0.001, ****P<0.0001 when compared with control and other groups (n=3)

Results of TAC, TOC, and OSI

It was determined that TAC levels in Caco-2 cells applied with PCA at concentrations of 250, 500, and 1000 µM for 24 h increased significantly compared to the control group (p<0.0001). Similarly, it was

found that the increase in TAC levels continued in all treatment groups after 48 h compared to the control group ($p<0.0001$) (Figure 2A). In Caco-2 cells, a decrease in TOC levels was detected in the groups applied with PCA at concentrations of 250, 500, and 1000 µM for 24 h compared to the control group, but the decrease was significant only at the dose of 500 µM compared to the control group ($p<0.05$). In 48 h PCA applications, TOC levels decreased at doses of 250 and 500 µM compared to the control group, while TOC levels increased significantly at the dose of 1000 µM compared to the control group ($p<0.01$) (Figure 2B). When OSI values were examined at the end of the 24 and 48-h period, a significant decrease was detected in all PCA-applied groups compared to the control group ($p<0.0001$) (Figure 2C).

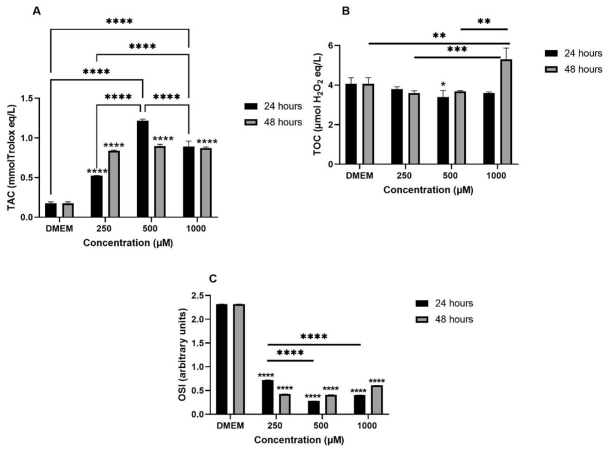

Figure 2. Mean ± standard error plot of the effects of PCA treatment on Caco-2 cells TAC (A), TOC (B), and OSI (C) **p<0.01, ***p<0.001, ****p<0.0001 when compared with control and other groups (n=3)

Discussion

Colon cancer death rates are increasing every year in the world. The apoptotic mechanism in colon cancer is a potential path that is being investigated to predict aggressive behavior. Many studies have reported the PCA anti-inflammatory, antioxidant, and anti-cancer activities in different cell lines [13]; however, very few research studies have revealed potential apoptosis mechanisms. Studies have shown that PCA protects against cell death forms such as apoptosis and pyroptosis in other types of cancer [19, 20]. Therefore, our study aimed to elucidate the effect of PCA on apoptotic pathways in the Caco-2 cell line. There are limited studies in the literature explaining the effect of PCA on Caco-2 cells through apoptosis mechanisms [16, 19].

To determine the apoptotic effect, Bcl-xl and Bcl-2 protein levels from the anti-apoptotic protein group were examined, and to determine the pro-apoptotic effect, Bax and Bad protein levels were examined. Apart from these, since caspases also carry out the apoptotic process, the caspase 9 protein level was examined.

In addition, the TAC and TOC activities of PCA were also evaluated. The suppressive effects of different doses of PCA on cell proliferation and IC50 dose after 24 and 48 h were shown in our previous study by the 3-(4,5-Dimethyltriazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) method [16]. Based on these results, the doses used in the study were decided. Today, apoptosis is the main goal of many treatment strategies and, therefore, plays an important role in the treatment of colon cancer. Bad protein is one of the pro-apoptotic proteins involved in apoptosis and triggers apoptosis via the internal pathway in the cell [21]. When the study results are considered, the concentrations applied to Caco-2 cells at 250 µM and 1000 µM PCA showed a significant decrease in 24 h compared to the control group. While there was a statistically insignificant decrease in 250 and 500 µM doses at 48 h, especially the 1000 µM dose significantly reduced Bad levels compared to the control group. These results made us think the applied doses could not have a pro-apoptotic effect.

The most studied ratio in the evaluation of apoptosis is the pro-apoptotic/anti-apoptotic (Bax/Bcl-2 or Bax/Bcl-xL) protein ratio. The imbalance between these two protein levels is considered an important indicator of apoptosis because it leads to apoptosis [22]. The Bax/Bcl-2 ratio of a cell responding to an apoptotic signal is high. This ratio was significantly increased in Caco-2 cells exposed to PCA at 250 µM concentrations for 24 h compared to the control group. The increase at 250 µM concentration in 24 h is particularly striking. After 48 h, the highest Bax/Bcl-2 ratio was observed at 500 µM concentration, but this increase was insignificant compared to the control group. These increases show us that PCA is induced in the intrinsic apoptosis pathway in Caco-

2 cells, especially at a concentration of 250 µM for 24 h, and increases sensitivity to apoptosis. When the effect of PCA on Caco-2 cells was evaluated after 48 h, it was determined that there was no statistically significant change in the Bax/Bcl-2 ratio. This is because the concentration of PCA and the incubation period may not be sufficient to increase the Bax/Bcl-2 ratio in Caco-2 cells. In this study, the Bax/Bcl-xl ratio significantly decreased in Caco-2 cells at all doses applied compared to the control group at the end of the 24 h, while it significantly decreased only at the dose of 1000 µM applied at 48 h compared to the control group. We can say that this dose has an anti-apoptotic effect. In other words, we can say that PCA reduces the Bax/Bcl-xl ratio in Caco-2 cells depending on time and dose, making the internal pathway more resistant to apoptosis. When we look at the existing studies, it has been determined that PCA has different effects on cell protection depending on the type of cancer, the time, and the concentration applied. While a study showed that high PCA concentrations induced apoptosis in Caco-2 cells [19], another study showed that it induced apoptosis at low concentrations [23].

Induction of cell death in mammalian cells in both intrinsic and extrinsic apoptotic pathways is associated with the activation of caspases. Therefore, caspase-9 protein levels were examined to determine which apoptotic pathway PCA was used in this study. Caspase-9, which is effective in the intrinsic pathway, showed a significant increase in Caco-2 cells at all concentrations applied at 24 and 48 h compared to the control group. Thus, it was determined that PCA caused caspase-9 activation in Caco-2 cells and used the intrinsic pathway. When the studies were examined, it was seen that PCA showed a significant ability to positively regulate the Bax and caspase-mediated death signaling cascade. Conversely, it created an environment that helped apoptosis induction in cancer cells by interfering with the activity of the Bcl-2 family [11]. Tsui-Hwa Tseng et al. reported that PCA showed a dose- and time-dependent inhibitory effect on human promyelocytic leukemia cell (HL-60) survival. This effect was achieved by increasing the expression of Bax, a key protein regulating apoptotic processes, while decreasing the expression of Bcl-2 [9]. In another study, it was shown that PCA, at concentrations of 1-10 µg/mL, depending on the dose used, activated the intrinsic apoptosis pathway by upregulating Bax and caspase-9, and the extrinsic apoptosis pathway by regulating caspase-8 [24]. Free radicals, oxidative stress, and antioxidants are widely studied topics today. In our study, it was determined that PCA caused an increase in TAC levels in Caco-2 cells at 24 and 48 h, depending on the increasing dose. The highest TAC values were observed at the applied concentration of 500 µM. TOC levels decreased in Caco-2 cells at 24 h compared to the control group only at the 500 µM applied concentration. While the decrease continued at 250 and 500 µM applied in Caco-2 cells at 48 h, the highest TOC values were seen in the group applied at 1000 µM. As a result, we can say that PCA significantly increased TAC levels in Caco-2 cells at 24 and 48 h compared to the control group. According to the obtained data, PCA suppressed oxidative stress by inducing antioxidant capacity in the Caco-2 cell line depending on the increasing dose concentration. In a study similar to our results, it was shown that PCA could oxidative stress, and improve the activity of antioxidant enzymes by reducing ROS levels, thus preventing oxidative damage in colon tissues [25].

Limitation: This study investigated the effect of PCA on apoptotic and antioxidant mechanisms on the Caco-2 colon cancer cell line. In addition, studies examining signaling pathways at the molecular level are needed to better understand how PCA affects the molecular mechanisms of Bax, Bcl-2, Bad genes.

Conclusion: According to our results, PCA exhibited apoptotic effects on the Caco-2 cell line depending on the dose and time. However, the mechanisms by which PCA triggers apoptosis in Caco-2 cells should be investigated in more detail at the advanced gene level with different signaling pathways and mechanisms.

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CASE REPORT

OLGU SUNUMU

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A Variation of the Double Deep Fibular Nerve with Multiple Connections to the Superficial Fibular Nerve on the Dorsal Aspect of the Foot

Ayağın Dorsal Yüzünde Nervus Fibularis Superficialis ile Çoklu Bağlantılara Sahip Çift Nervus Fibularis Profundus Varyasyonu

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ABSTRACT

In this report, we aim to present our observation of a rare variation on the dorsum of the foot, including the double deep fibular (peroneal) nerve and its connections to superficial nerves. The cutaneous nerves of both feet ' dorsum were examined during a routine dissection on a formalin-fixed adult male cadaver. During the dissection of the dorsum of the right foot, it was observed that the deep fibular nerve (DFN) surfaced at two distinct points by piercing the investing fascia. Then, these two branches converged, and DFN had connections at three different points with the superficial fibular (peroneal) nerve (SFN). However, the superficial nerve anatomy of the dorsum of the left foot exhibited the anatomical structure commonly described in classical textbooks. To the best of our knowledge, the variation of double DFN with multiple connections to the SFN has not been previously described in the literature. Understanding the innervation of the dorsum of the foot may be clinically substantial in preventing nerve injuries during surgical interventions. The knowledge regarding rare superficial nerve variations may contribute to the success of anaesthesia applications, especially in the ankle and dorsum of the foot.

Keywords: Fibular nerve, peroneal nerve, cadaver, variation, cutaneous innervation

ÖZ

Bu vaka raporunda ayak sırtında çift nervus fibularis (peronealis) profundus (DFN) ve yüzeyel sinirlerle olan bağlantılarını içeren nadir bir varyasyona ilişkin gözlemimizi sunmayı amaçladık. Formalinle fikse edilmiş yetişkin bir erkek kadavra üzerinde rutin eğitim diseksiyonu sırasında her iki ayak sırtının kutanöz sinirleri incelendi. Sağ ayak sırtı diseksiyonu sırasında DFN'nin fascia investiens'i iki ayrı noktada delerek yüzeye çıktığı görüldü. Daha sonra bu iki dal birleşti ve DFN'nin nervus fibularis (peronealis) superficialis (SFN) ile üç farklı noktada bağlantısı oldu. Ancak sol ayak sırtının yüzeyel sinir anatomisi, klasik ders kitaplarında yaygın olarak tanımlanan anatomik yapıyı sergiliyordu. Bildiğimiz kadarıyla, çift DFN'nin SFN'ye çoklu bağlantılarla varyasyonu literatürde daha önce tanımlanmamıştır. Ayak sırtının innervasyonunun anlaşılması, cerrahi müdahaleler sırasında sinir yaralanmalarının önlenmesi açısından klinik açıdan önemli olabilir. Nadir görülen yüzeysel sinir varyasyonlarının bilinmesi, özellikle ayak bileği ve ayak sırtında anestezi uygulamalarının başarısına katkı sağlayabilir.

Anahtar Kelimeler: Nervus fibularis, nervus peroneus, kadavra, varyasyon, kutanöz inervasyon

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Introduction

The cutaneous innervation of the dorsum of the foot is supplied by the superficial fibular nerve (SFN), deep fibular nerve (DFN), and sural nerve (SN). During its course, SFN gives motor branches to the lateral compartment muscles. It becomes superficial at 1/3 of the distal part of the leg. It moves to the dorsum of the foot and is divided into two branches known as the medial branch of the medial dorsal cutaneous nerve of the foot (MDCNF) and the intermediate dorsal cutaneous nerve of the foot (IDCNF). The medial branch of MDCNF innervates the medial aspect of the great toe, while the lateral branch innervates the adjacent surfaces of the second and third toes. IDCNF provides sensory innervation to a substantial portion of the dorsum of the foot, the lateral aspect of the third toe, both sides of the fourth toe, and the medial surface of the fifth toe [1].

The anterior tibial artery accompanies DFN on the anterior surface of the interosseous membrane of the leg. After crossing, the inferior extensor retinaculum divides into medial and lateral terminal branches. The medial terminal branch of the DFN runs with the dorsalis pedis artery on the dorsum of the foot, is divided into two branches at the first intermetatarsal space, and becomes superficial. These branches innervate the adjacent sides of the first and second toes. The lateral terminal branch of the DFN travels deep into the ankle and penetrates the extensor digitorum brevis muscle, expanding like a pseudo-ganglion before innervating the muscle. Additionally, the SN passes behind the lateral malleolus and continues as the lateral dorsal cutaneous nerve of the foot (LDCNF), supplies the skin on the lateral side of the little toe [1].The branching pattern of the SFN should be considered in procedures such as flap surgery in the dorsum of the foot [2], it is essential to note that iatrogenic injuries of cutaneous nerves are common complications in surgical interventions of the foot and ankle region [3].

This case report describes a variation of the DFN/SFN. We aimed to present our observation regarding the communicating branches between DFN and branches of SFN, as well as the atypical course and branching pattern of DFN.,

Case Presentation

The study followed the ethical principles outlined in the 1964 Declaration of Helsinki and its later amendments. The dissection was performed on a cadaver obtained in compliance with national legal and ethical procedures and kept in the Anatomy Laboratory of the Medical Faculty. Informed consent was obtained during cadaver donation process. During a routine educational dissection performed on a formalin-fixed adult male cadaver in our laboratory, the cutaneous nerves of the right and left dorsal aspects of both feet were examined. The cadaver had no lower extremity deformity. On the right side, SFN was superficial at the 1/3 distal part of the leg proximal to the ankle. Just before crossing the ankle, SFN divided into two branches. These branches are described as the intermediate dorsal cutaneous nerve of the foot (IDCNF) and the medial dorsal cutaneous nerve of the foot (MDCNF). These branches were coursing towards the dorsum of the foot (Figure 1).

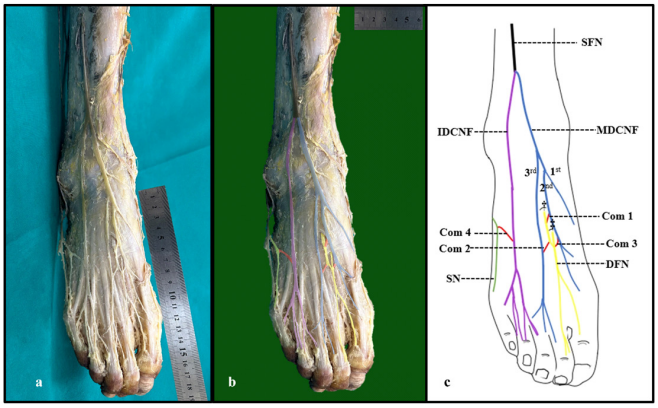

Figure 1. Cutaneous innervation on the dorsum of the right foot. a. Original photograph. b: coloured/illustrated photograph. c: schematic view. [Yellow, deep fibular nerve (DFN); Black, superficial fibular nerve (SFN; Blue, medial dorsal cutaneous nerve of the foot (MDCNF) (1st: first branch, 2nd: second branch, 3rd: third branch); Purple, intermediate dorsal cutaneous nerve of the foot (IDCNF); Red: communicating branches (Com1, Com2, Com3, Com4), Green, sural nerve (SN); †: deep fibular nerve 1 (DFN1), ‡: deep fibular nerve 2 (DFN2).]

At the first intermetatarsal space on the right foot, in the proximal-distal direction, two nerves were observed where DFN was expected to become superficial by piercing the fascia investiens. The distal nerve originated from the proximal nerve just beneath the investing fascia. These nerves were named DFN1 (located proximally) and DFN2

(located distally). DFN2 was running medially to DFN1. Then, DFN1 and DFN2 converged to a single DFN.MDCNF was divided into three distinct branches on the dorsum of the foot. The first branch extended to the medial aspect of the great toe. With an intermediate course, the second branch gave branches extending the dorsal aspect of the same toe. The third branch extended to the second toe's lateral aspect and the third toe's medial aspect.

MDCNF was communicating with the branches of the DFN at three points. These branches were named Com 1, Com 2, and Com 3. Com 1 established a connection between the DFN1 and the second branch of MDCNF, while Com 2 established a connection between the DFN1 and the third branch of MDCNF. Com 3 connected the nerve formed by the convergence of DFN1 and DFN2 and the second branch of MDCNF. IDCNF was divided into branches extending to the lateral aspect of the third toe, both sides of the fourth toe, and the medial aspect of the fifth toe. A communicating branch (Com 4) arose from the SN and joined this nerve on the dorsum of the left foot, the DFN was branching at the first intermetatarsal space and supplied the adjacent surfaces of the first and the second toe. The SFN and the SN innervated the rest of the dorsum of the foot. We observed that the cutaneous nerves on the dorsum of the left foot were consistent with the basic descriptions in classical anatomical textbooks [1].

Discussion

In our report, we observed that (I) DFN became superficial at two different distinct points by piercing the investing fascia at the first intermetatarsal space, resulting in the formation of two branches (DFN1 and DFN2), (II) then these two branches converged, and (III) DFN1 and DFN2 contact with MDCNF at three points with communicating branches on the dorsum of right foot. We observed that DFN1 and DFN2 provided superficial innervation to the adjacent sides of the first and second toes. On the dorsum of the left foot, a familiar anatomical pattern, frequently encountered in essential resources, was observed regarding the superficial nerves [1].

There are many studies in the literature regarding the innervation of the dorsum of foot [4-9]. The earliest document we could find and access was published in 1891 by the Collective Research Committee of the Great Britain and Ireland Anatomy Society. According to this report, 229 dorsa of feet were examined, and variations were classified into 12 types (Type A-L). The most common variation according to this classification is Type A (%55, classical type) [8]. Type A appears consistent with the most commonly observed anatomical pattern and is accepted as usual in classical textbooks. However, the terminology used by Thomson is no longer in use today [10]. In this source, the terms musculocutaneous nerve for SFN, internal branch for MDCNF, external branch for IDCNF, anterior tibial nerve for DFN, and external saphenous nerve for SN were preferred [8]. The classification of Thomson et al. was compiled by current terminology, and 12 types (Type K1-12) were defined as Kosinski's classification [6]. However, the variation observed on the right foot in our case was not included in these classifications.

Cheredath et al. reported 5 variations in their study about cutaneous nerve innervation of the dorsum of the foot in the Indian population. The vast majority of variations they identified were observed in the sural nerve. Among these types, there was a communicating branch between SFN and DFN, and DFN became superficial from a single point in variations 2 and 3 [4]. In another study conducted by Nayak et al., cutaneous nerve innervation of the dorsum of the foot was classified into four groups in the Indian cadavers. They identified the communicating branch between MDCNF and DFN in 10% of the cases. This variation was defined as Group 4 [7]. In our case, DFN formed communicating branches with MDCNF at three different points in the variational pattern observed on the dorsum of the right foot. Additionally, the presence of the superficialising of the DFN from two distinct points in the first metatarsal interspace was not concordant with any specific variation type.

In a fetal cadaver study in India, the innervation of the dorsum of the foot was classified into 4 main types. Main variation types were divided into subtypes by considering the locations of the communicating branches between the nerves that supply the dorsum Type 1a-g, Type 2a-d, Type

3, Type 4a-c) [5]. According to this classification, in 25% of the cases, at least one communicating branch was observed between the SFN and DFN. Type 1f was the most concordant variation with our case. In this type, there are three communicative connections ‘‘C1: communication between DFN and MDCNF, between MDCNF and IDCNF, and between IDCNF and SN). In our case, although DFN innervated the same region that is described in Type 1f, DFN reported in our report superficialised at two distinct points (DFN1 and DFN2), and DFN1 and DFN2 were communicating with MDCNF at three different points.

In the literature, to the best of our knowledge, the only study in which double DFN is defined in the dorsum of the foot is a fetus study in the Indian population. In this study, the cutaneous nerves of the dorsum of the foot were classified into six main types. DFN was defined as Type 4b by superficialising at two distinct points. Type 4b (1.7%) was scarce, and the innervation region of the DFN was the adjacent sides of the second and third toe in addition to the adjacent sides of the first and second toe [9]. In our report, DFN1 and DFN2, which became superficial from 2 different points, merged again and innervated only the first interphalangeal space (adjacent sides of the first and second toe). DFN1 and DFN2 also established multiple communicating branches with SFN (Com1: between DFN1 and NCMDP 1st branch, Com2: between DFN1 and MDCNF 2nd branch, Com3: between the union of DFN1 and DFN2 and MDCNF 2nd branch, Com4: between SN and IDCNF).

Clinical Importance

According to a systematic review examining complications of ankle arthroscopy procedures, neurological injuries are the most common among all complications, with 55.4% (180/325). SFN is affected in 32.7% of all neurological injuries. In addition, it has been reported that 20% of all neurological damage is permanent [3]. Injuries in the distal part of the DFN primarily lead to sensory deficits and the formation of painful neuromas [11].

In addition, different variations of these cutaneous nerves should be considered in nerve block anaesthesia of the foot and ankle region and during the evaluation of chronic traumatic or atraumatic pain syndromes of the leg [12].

Conclusions

In this case we presented a rare cutaneous variation of DFN on the dorsum of the foot. The knowledge of such as these variations can provide a deeper understanding of the clinical reflections of the neural network of the foot. Thus, we believe that it will be beneficial for surgeons and clinicians dealing with the region in terms of less iatrogenic injuries and better postoperative results.

Conflict of Interest: The authors declare no conflict of interest related to this article.

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Ethics Approval and/or Informed consent: The study followed the ethical principles outlined in the 1964 Declaration of Helsinki and its later amendments. The dissection was performed on a cadaver obtained in compliance with national legal and ethical procedures and kept in the Anatomy Laboratory of the Medical Faculty. Informed consent was obtained during cadaver donation process.

ORCID and Author contribution: All authors contributed to the study's conception and design. **M.K. (0000-0002-9267-1164):** Material preparation and dissection, writing. **H.K. (0000-0003-4804-3678):** Material preparation and dissection. **K.E.O. (0000-0002-9778-3325):** reviewed and edited the manuscript. All authors read and approved the final manuscript.

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LETTER TO EDITOR

EDİTÖRE MEKTUP

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The Impact of Prolotherapy and Steroid Injection on De Quervain's Tenosynovitis: A Retrospective Outcome Study

Proloterapi ve Steroid Enjeksiyonunun De Quervain Tenosinoviti Üzerindeki Etkisi: Retrospektif Sonuç Araştırması

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Dear Editor,

I read with interest the article by Zora and Bayrak [1] titled “Effect of Prolotherapy and Steroid Injection on De Quervain Tenosynovitis: Retrospective Outcome Study”. I appreciate the authors of this study comparing the effects of prolotherapy and steroid injection on short-term functional outcomes in patients with De Quervain stenosing tenosynovitis. Based on this study, I have an important statement and comments in order to develop future studies on De Quervain Tenosynovitis.

De Quervain's tenosynovitis is the stenosing tenosynovitis of the M. Abductor Pollicis Longus (APL) and M. Extensor Pollicis Brevis (EPB) tendons located in the 1st dorsal compartment of the wrist, under the dorsal carpal ligament and radial tunnel. De Quervain's tenosynovitis is usually recognized clinically, and the classic test used in diagnosis is the Finkelstein test. Although this test is considered pathognomonic for De Quervain, the patient may have difficulty in distinguishing the pain of tenosynovitis in the radial styloid from different pathologies such as thumb arthritis/arthrosis etc. The specificity of the Finkelstein test is good, but there are arguments against this method due to false-positive results and examination discomfort, as it can also cause pain in healthy individuals [2]. However, in clarifying the diagnosis, it would be better to use an objective imaging method such as ultrasonography, which is easily accessible, fast and effective, especially during injection treatment. Because, the detection of abnormalities in tendon sliding movement, narrowing in the fibro-osseous channel or thickening in the tendons can be recognized by ultrasonography [3].

In this study, VAS (visual analog scale) was used for wrist evaluation, QuickDASH (Quick Disability Assessment of Arm, Shoulder, and Hand Problems) and HAQ (Health Assessment Questionnaire) were used for hand and wrist function and problems. Since hand grip functions may be weakened, especially in De Quervain tenosynovitis, hand grip strength could be checked before and after treatment. Evaluation

of standard, palmar and lateral grips, which are the grip types most used in daily living activities and expected to be affected by De Quervain's tenosynovitis, in terms of position and strength. In De Quervain's tenosynovitis, it is observed that the functionality of the hand is affected and the intense pain caused by weakness in the APL and EPB muscles or increased tension in these muscles causes a decrease in endurance in hand functions. Therefore, using tests that more objectively evaluate the functionality of the hand, such as the Minnesota test, to evaluate the functionality and endurance of the hand would make the future studies on de Quervain's Tenosynovitis study more meaningful [4].

In addition, the isolated effect of prolotherapy and steroid injection could not be evaluated due to the lack of a placebo (non-treatment) control group in the study and the administration of cold application, massage and home exercise program to both groups. It would be more more valuable in evaluating effectiveness in future studies of De Quervain's tenosynovitis if patients were compared only with prolotherapy and steroid injection, independent of other treatments. Also, further research may be designed in the future to better evaluate other potential predictors of treatment success, such as conservative treatment options include thumb spica splinting or kinesio taping for De Quervain’s tenosynovitis.

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Editorial Note and Authors' Response: I appreciate this scientific letter on Zora and Bayrak's [1] article, “The impact of Prolotherapy and Steroid Injection on De Quervain's Tenosynovitis: A Retrospective Outcome Study." On the other side, the authors of the original article claimed that the assessments and insightful explanations about the article contributed to a better understanding of the subject and content.