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ORIGINAL ARTICLE

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Sarcoidosis from the Perspective of Rheumatology: Three Years of Experience

Romatoloji Gözünden Sarkoidoz: Üç Yıllık Deneyim

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ABSTRACT

Aim: The aim of this study is to shed light on sarcoidosis from a rheumatology perspective and to provide a better understanding of sarcoidosis from the perspective of a rheumatologist.

Materials and Methods: The files of patients who applied to the rheumatology outpatient clinic with joint complaints between 2020 and 2023 and were diagnosed with sarcoidosis during follow-up or who were already diagnosed with sarcoidosis and had joint complaints during the course of the disease, were retrospectively examined.

Results: Joint involvement was observed in all patients (100%). When patients were grouped according to the joints involved, it was found that 17 patients had ankle involvement (60.7%), six patients had metacarpophalangeal joint involvement (21.4%), four (14.2%) patients had wrist involvement, and three (10.7%) patients had knee involvement. Shoulder involvement was detected in one (3.5%) patient.

Conclusion: Although sarcoidosis seems to primarily concern chest diseases, from the perspective of rheumatology, it is a very confusing and surprising disease with its heterogeneous nature and joint involvement. It is one of the diseases that should be kept in mind and further studied in rheumatology practice.

Keywords: Sarcoidosis, arthritis, joint involvement

ÖZ

Amaç: Bu çalışmanın amacı romatoloji gözüyle sarkoidoz hastalığına ışık tutmak ve romatolog gözüyle sarkoidozun daha iyi anlaşılmasını sağlamaktır.

Gereç ve Yöntem: Romatoloji polikliniğine 2020-2023 yılları arasında eklem şikayetleri ile başvurarak izlemde sarkoidoz tanısı almış veya halihazırda sarkoidoz tanısı mevcut olup hastalık seyrinde eklem şikayetleri mevcut olan hastaların dosyaları retrospektif olarak incelendi.

Bulgular: Hastaların hepsinde (%100) eklem tutulumu görüldü. Tutulan eklemlere göre hastalar gruplandırıldığında 17 hastada ayak bileği (%60,7) tutulumu, altı hastada metakarpofalangeal (%21,4) eklem tutulumu, dört (%14,2) hastada el bileği tutulumu, üç (%10,7) hastada diz tutulumu, bir (%3,5) hastada omuz tutulumu tespit edildi.

Sonuç: Sarkoidoz primer olarak göğüs hastalıklarını ilgilendiriyor gibi görünse de, romatoloji gözünden de heterojen doğası ve eklem tutulumlarıyla oldukça kafa karıştırıcı ve şaşırtıcı bir hastalıktır. Romatoloji pratiğinde akılda tutulması ve üzerinde daha fazla çalışma yapılması gereken hastalıklardan biridir.

Anahtar Kelimeler: Sarkoidoz, artrit, eklem tutulumu

INTRODUCTION

Sarcoidosis is a chronic, multisystemic, and inflammatory disease characterized by non-caseating granulomas, which can manifest throughout the body, with a predilection for the lungs and intrathoracic lymph nodes. Typically observed in individuals aged 20-40 years, it affects both men and women equally. However, a secondary peak has been observed in women over the age of 50 years. The development of sarcoidosis is believed to involve a combination of genetic predisposition and environmental factors[1]. While it has been demonstrated that infections and occupational factors play

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a role in the development of the disease, the exact cause of sarcoidosis remains unknown[2]. The incidence and prevalence of sarcoidosis exhibit significant variations among different racial groups. In our country, the annual incidence is reported as 4/100,000[3]. The clinical course of sarcoidosis and the affected organs can vary among societies. The first documented case of sarcoidosis in our country dates back to 1953, when Akkaynak reported it, and a comprehensive study by Demirkök et al.[4-6], involving 275 individuals, described the characteristics of sarcoidosis patients. However, a definitive profile of sarcoidosis patients in our country has not yet been established in the studies conducted thus far.

The organs most commonly affected by sarcoidosis are the lungs and intrathoracic lymph nodes, with an incidence of 90%[7]. Bilateral symmetric lymphadenopathy is frequently observed in the lungs. Following these organs, the skin ranks second in terms of involvement. Sarcoidal granulomas may manifest in the skin, accompanied by reactive, non-specific inflammation clinically presenting as erythema nodosum[8]. In addition to the lungs and skin, other organs such as the liver, spleen, nervous system, kidney, parathyroid glands, heart, and eyes may be affected during the course of the disease. Sarcoidosis can be identified through a routine chest X-ray, or it can involve multiple organs simultaneously. In this multisystemic disease, joints can also be affected, clinically appearing as arthritis. Although sarcoidosis is not commonly associated with acute arthritis, it is estimated that 15-20% of sarcoidosis cases are complicated by arthritis. Sarcoidosis should be considered in the differential diagnosis of ankle joint involvement, especially when respiratory symptoms are also present[9].

The most commonly reported symptoms in the course of sarcoidosis include cough, shortness of breath, and chest pain[10]. Diagnosis is established by the presence of one or more of the following criteria: radiological signs of sarcoidosis, evidence of systemic involvement, histopathologically proven non-caseating granulomas, demonstration of sarcoidosis findings in bronchoalveolar fluid, and exclusion of other granulomatous diseases. While there is no specific biomarker for the diagnosis of the disease, several markers aid in the diagnostic process, with angiotensin-converting enzyme (ACE) being the most commonly used. Granulomas developing during sarcoidosis can increase ACE activity and stimulate the synthesis of $1,25(OH)_2$ vitamin D_3, resulting in elevated plasma calcium levels. Due to the heterogeneous nature of the disease and variations in its course, diagnosis and treatment planning can pose challenges for clinicians. Despite being generally considered a benign disease with a favorable prognosis, mortality in sarcoidosis, which is observed in 1-5% of cases[11], is typically attributed to respiratory failure, cardiac involvement, and nervous system complications. The indications for treatment in this disease remain controversial.

Our study involves a retrospective analysis of patients who visited the rheumatology outpatient clinic between 2020 and 2023 and were diagnosed with sarcoidosis in that clinic. We also included patients diagnosed with sarcoidosis, who presented with the complaints of arthritis or arthralgia. The aim is to provide valuable insights for clinicians dealing with sarcoidosis in rheumatology practice.

MATERIALS AND METHODS

We conducted a retrospective examination of the medical records of patients who presented to the rheumatology outpatient clinic between 2020 and 2023. This included individuals diagnosed with sarcoidosis during follow-up or those who were already diagnosed with sarcoidosis and experienced joint complaints during the course of the disease. We collected and recorded demographic characteristics, comorbidities, organs involved, joint involvement at the time of diagnosis, medications used during follow-up along with their duration, patients' relapse status, and autoantibodies (rheumatoid factor, citrullinated protein antibodies, and antinuclear antibodies) from the hospital data system and patient files.

Statistical Analysis

The data were transferred to the Statistical Package for the Social Sciences 27.0 program for evaluation, where descriptive statistics such as frequency, percentage, and average were employed.

RESULTS

In our study, 28 patients were seen in the rheumatology outpatient clinic during the examined time period. Of these, 21 were female and 7 were male, resulting in a female-to-male ratio of 3. The average age of all patients was 52 years, ranging from a minimum of 39 years to a maximum of 68 years. The average age for female patients was 52.9 years, while it was 49.57 years for male patients. Examining accompanying diseases, 13 patients (46%) had no comorbidities. Two patients (7%) had concomitant hyperthyroidism, three (10%) had hypothyroidism, three (10%) had diabetes mellitus, and seven (25%) had hypertension. Two patients had ankylosing spondylitis and one of them developed sarcoidosis while using biological agents for ankylosing spondylitis. Additionally, two patients had heart failure, two had coronary artery disease, one had ulcerative colitis, and one had vitiligo. When assessing family histories, one of the 28 patients (3.5%) had a positive family history.

In terms of sarcoidosis involvement, joint involvement was observed in all patients (100%). The distribution of joint involvement included 17 patients with ankle involvement (60.7%), six patients with metacarpophalangeal joint involvement (21.4%), four patients with wrist involvement (14.2%), and three patients with knee involvement (10%). One patient (3.5%) had shoulder involvement (Table 1).

In our study focusing on organ involvement in sarcoidosis patients, the examination revealed lung involvement in 24 patients (85.7%), skin involvement in six patients (21.42%), liver involvement in three patients (10.7%), eye involvement in two patients (7.1%), and renal involvement in one patient (3.5%). Among the skin involvement cases, five were identified as erythema nodosum, and one patient exhibited skin involvement without further specification. Lymph node involvement outside the lung was observed in six patients (21.42%), with inguinal lymph node involvement in three of them and axillary lymph node involvement in the remaining three (Table 2).

When considering the years of diagnosis in our study, the longest duration of the disease was found to be 18 years. Two patients were diagnosed in 2023. Regarding diagnostic methods, mediastinoscopy and lymph node biopsy were performed in six patients (21.4%), endobronchial ultrasonography and lymph node biopsy in 14 patients (50%), axillary lymph node biopsy in one patient, inguinal lymph node biopsy in two patients, and skin biopsy in two patients. Additionally, the diagnosis was established through a biopsy in one patient, open lung sampling in one patient, kidney biopsy in one patient, and liver biopsy in one patient.

Table 1. Our patients according to joint involvements

	n=%
Ankle	60.7
MCF	21.4
Wrist	14.2
Knee	10.7
Shoulder	3.5

MCF: Metacarpophalangeal

Table 2. Extrapulmonary region involvement

Region	n=%
Joint	100
Lung	85.7
Extrapulmonary lymph node	21.4
Skin	21.4
Liver	10.7
Eye	7.1
Kidney	3.5

When examining patients based on their complaints at the time of diagnosis, it was noted that four patients (14.2%) were diagnosed with joint swelling and pain at the rheumatology outpatient clinic. Analyzing the complaints of the remaining twenty-four patients at the time of diagnosis revealed dyspnea in nine patients, erythema nodosum in five patients, left temporal lesion in one patient, anterior uveitis in two patients, and cough in seven patients (25%) (Table 3). Among the patients, 15 (53.5%) were identified as smokers at the time of diagnosis.

Regarding sarcoidosis treatment, 23 patients (82%) received systemic steroids, four were monitored without medication, and one was managed with local steroids. Six patients (21%) were prescribed methotrexate for arthritis accompanying sarcoidosis, and colchicine was used in four patients. In terms of treatment duration, the patient receiving systemic steroids intermittently for nine years experienced disease relapse upon steroid discontinuation.

In terms of laboratory parameters, hypercalcemia was observed in six patients (21.4%). Antinuclear antibody was positive in four patients (14.2%), and rheumatoid factor was positive in one patient (3.5%). The average serum ACE level was 57.96 U/L, with ten patients (35.7%) exceeding the laboratory ACE upper limit of 52 U/L.

DISCUSSION

In our study on sarcoidosis patients, 21 were female, and seven were male (F/M=3). The higher number of women aligns with findings from other studies conducted in our country. In a study by Karalezli et al.[12], the female-to-male ratio was 2.12, and in Aytemur et al.'s[13] study, this ratio was reported as 2.38. The average age of our patients was 52 years, consistent with literature findings. In a study by Fritscher-Ravens et al.[15], the average age of sarcoidosis patients was 60 years, while in a 2007 study by Miwa et al.[14], the average age was reported as 50 years. Examining our patients for chronic comorbid diseases, more than half were found to have chronic conditions. This observation may be attributed to the average age of the patients and the fact that they were evaluated in a multidisciplinary chest diseases and rheumatology outpatient clinic.

Table 3. Extrapulmonary region involvement

	n=28
Dyspnea	9 (32.1%)
Cough	7 (25%)
Erythema nodosum	5 (17.8%)
Arthritis, arthralgia	4 (14.2%)
Anterior uveitis	2 (7.1%)
Skin lesion	1 (3.57%)

Two of our patients had concurrent ankylosing spondylitis, a rare occurrence reported infrequently in the literature. In one study, three separate cases were examined, and the coexistence of spondyloarthritis and sarcoidosis was considered coincidental[16]. Remarkably, we observed sarcoidosis development in one patient undergoing biological agent treatment (infliximab) for ankylosing spondylitis. Upon the discontinuation of infliximab, we initiated adalimumab for ankylosing spondylitis in this patient and observed that the disease did not progress under adalimumab. Interestingly, the literature also mentions the development of sarcoid uveitis under biological agents, as reported in a multicentric retrospective study of 16 patients conducted by Sobolewska et al.[17] in 2022. Another study, published in June 2023, documented liver sarcoidosis in a patient receiving infliximab for inflammatory bowel disease[18]. Moreover, there is a growing body of research on the use of biological agents for the treatment of sarcoidosis. In our study, only one patient had familial sarcoidosis, aligning with findings from the study by Musellim et al.[3], where the rate of familial sarcoidosis was 1%. This rate appears consistent with our study and is in line with another study reporting a three percent rate. Considering this, it becomes crucial to inquire about family history in patient practice.

In daily clinical practice, arthritis and joint involvement are often overlooked in the context of sarcoidosis. Literature reports suggest that 15-25% of sarcoidosis patients may experience joint involvement[9]. However, in our study, joint involvement was observed in all patients, which can be attributed to the rheumatology perspective from which the study was conducted. The first joint affected in our study was the ankle, followed by the metacarpophalangeal joint. This is consistent with findings from a study by Sucharita Shanmugam in 2008, where sarcoidosis arthropathy was discussed, with a focus on metacarpophalangeal joint involvement. Similarly, a study by Kiely and Lloyd[19], published in June 2021, explored ankle arthritis, identifying sarcoidosis as one of the diseases associated with this condition.

The initial complaint in four patients in our study was joint involvement, and further investigation led to the diagnosis of sarcoidosis. Literature reports indicate that joint involvement can be the initial symptom of sarcoidosis, with some patients presenting under a specific subheading known as Löfgren's syndrome. However, in our patient group, only one of these four patients exhibited erythema nodosum during the clinical course, which is a characteristic feature of Löfgren's syndrome.

As a result, joint involvement in sarcoidosis can manifest both during the course of the disease and as its initial symptom. A multidisciplinary approach, involving specialties such as rheumatology, appears appropriate for the comprehensive follow-up and treatment of this condition, given its heterogeneous nature.

Sarcoidosis is a multisystem disease with the potential to affect various organs. Our study revealed that the most commonly involved organs were the lung and extrapulmonary lymph nodes, with the skin following closely. These findings align with existing literature. In a study conducted in Japan, approximately 20% of patients with systemic sarcoidosis exhibited skin involvement, with erythema nodosum being the most frequently described manifestation[20]. Consistently, our study found a similar rate of skin involvement, with erythema nodosum being the predominant presentation.

In our study, one patient received a diagnosis of sarcoidosis through kidney biopsy. Exploring the literature on the association between sarcoidosis and the kidneys, we found renal involvement to be relatively rare. A case report by Wang et al.[21] highlighted a patient diagnosed with sarcoidosis through a kidney biopsy conducted due to chronic kidney disease. A multicentric retrospective study conducted in Germany in 2023 defined the rate of renal involvement as 27.5% in sarcoidosis patients, emphasizing the significance of not overlooking renal complications in the course of sarcoidosis[22]. Consequently, clinicians should consider sarcoidosis as a potential cause in patients with unexplained chronic renal failure.

In our study, one patient received a diagnosis of sarcoidosis through liver biopsy, revealing liver involvement in 10% of the patients. In a sarcoidosis study conducted in France with 21 patients, liver involvement was observed in seven patients, representing a rate slightly higher than that observed in our study. Another study by Ibrahim et al.[23] suggested that liver involvement in sarcoidosis might vary between 5% and 30%. The rate of liver involvement in our study aligns with the range reported by Ibrahim. In conclusion, it emphasizes the importance of considering the liver as one of the organs that should be taken into account in the course of sarcoidosis.

Sarcoidosis is a systemic granulomatous inflammatory disease known for its frequent ocular involvement. A study in Tunisia reported ocular involvement, specifically in the form of uveitis, in two cases among a sarcoidosis group of 28 patients[24]. Similarly, in our study, two patients exhibited ocular involvement, with anterior uveitis identified upon examination. This underscores the importance of considering sarcoidosis in the etiology of uveitis.

Sarcoidosis may manifest extrapulmonary lymph node involvement during its course. Studies in the literature have reported cases of sarcoidosis exclusively involving cervical and inguinal lymph nodes without lung involvement. Isolated inguinal and axillary lymph node involvement has also been

documented. In our patient group, we observed axillary and inguinal lymph node involvement. Remarkably, 10% of patients in our study received their diagnosis through extrapulmonary lymph node biopsy, underscoring the significance of considering this aspect in the diagnostic process.

In our study, the most prevalent complaints among patients were dyspnea (32.1%) and cough (25%), in consistency with a sarcoidosis study conducted by Ertuğrul et al.[25] in 2008. Similarly, Karalezli et al.'s[12] sarcoidosis study highlighted cough and dyspnea as the most common complaints. These observations align with results from Sharma et al.'s[26] study on 156 sarcoidosis patients in the Indian population. Following dyspnea and cough, the most frequent presenting symptom was erythema nodosum, followed by arthritis. A study by Kıter et al.[27] in our country noted erythema nodosum in 17.1% of patients as a presenting symptom, and in our study, the incidence of erythema nodosum mirrored these findings.

In the sarcoidosis study by Musellim et al.[3], it was reported that 75% of the patient group consisted of non-smokers, suggesting a potential negative relationship between sarcoidosis and smoking. However, in our study, approximately half of the sarcoidosis patients were identified as smokers, indicating a higher smoking rate compared to other studies in the literature[28-30]. This variance might be attributed to the elevated prevalence of female smokers in our region.

82% of our patients received systemic steroid treatment after diagnosis, indicating a higher rate compared to Ertuğrul et al.'s[25] sarcoidosis study, where systemic steroids were indicated for 52.9% of patients. Literature suggests that spontaneous remissions may occur in about two-thirds of sarcoidosis patients. However, if organ damage develops during the course of the disease, the effectiveness of treatment diminishes[5,31]. Therefore, careful consideration is needed when determining which patients should receive systemic steroids at the time of diagnosis. Typically, systemic steroid treatment in the literature is reported to span around two years[32]. In our study, the duration of steroid administration varied based on the patient's clinical condition, with one patient experiencing relapses in the past and receiving intermittent systemic steroid treatment for nine years. The duration of systemic steroids used in the course of sarcoidosis should be assessed in accordance with the patient's relapses and overall clinical condition.

Hypercalcemia was detected in 21.4% of our patients. Granulomas in sarcoidosis, particularly those secreting cytokines such as interferon-γ, stimulate 1α hydroxylase, leading to the production of active vitamin D. This process increases calcium absorption through both intestinal absorption and bone resorption[33]. Comparatively, in a literature study with 1606 patients, the rate of sarcoidosis-related hypercalcemia was reported as 6%[34]. In an American sarcoidosis study with 196 patients, the frequency of hypercalcemia was found to be 18.3%, a rate resembling our study. Meanwhile, a study in Poland reported a 10% occurrence of sarcoidosis-related hypercalcemia. The varying rates across different countries highlight the diverse presentation of sarcoidosis-related hypercalcemia in the literature.

In our sarcoidosis patient group, the examination of ACE levels revealed that 35.7% of the patients had levels exceeding the laboratory upper limit. A study by Sejdić et al.[35], involving 101 patients, reported increased serum ACE levels in 48% of the patients, a rate similar to our findings. In a Chinese study, ACE demonstrated a specificity of 93% in diagnosing sarcoidosis, with reports suggesting higher ACE levels in patients with systemic involvement compared to those with simple lung involvement[36]. Notably, our study observed elevated ACE levels, particularly in patients with extrapulmonary organ involvement. In a sarcoidosis study in Tunisia involving 80 patients, serum ACE levels were found to be increased in all patients[37]. However, it is important to note that cut-off values for this enzyme vary in clinical practice across laboratories and countries. Ongoing research in some countries aims to update these cut-off values. In a Japanese study, it was emphasized that normal ACE levels in serum do not easily exclude sarcoidosis, and careful evaluation of this biomarker is necessary. While ACE levels may not be indicative in all cases, the literature underscores its significance, especially in certain sarcoidosis cases with involvement in unusual areas. Ongoing studies, particularly in phenotyping and the diagnostic use of ACE, contribute to the evolving understanding of sarcoidosis.

According to the laboratory results of our sarcoidosis patients, 14.2% showed ANA (antinuclear antibody) positivity, while 3.5% exhibited RF (rheumatoid factor) positivity. The association of these autoantibodies with sarcoidosis has been explored in the literature, although the nature of this relationship remains incompletely understood. A 2023 Japanese study suggested a correlation between autoantibody positivity and the severity of lung involvement[38]. A 2020 study in our country found that RF-positive sarcoidosis patients more frequently experienced hand joint involvement[39]. The literature has also investigated patients with sarcoidosis accompanied by Sjögren's syndrome, revealing ANA positivity in 23 and RF positivity in 12 out of 41 patients. It has been suggested that positive immunological parameters in sarcoidosis patients may indicate more multisystemic involvement[40]. In a study by Yildiz et al.[39] in our country, the relationship between anti-CCP and sarcoidosis was examined, indicating that anti-CCP positivity in sarcoidosis patients is similar to the healthy population, a result consistent with our study. None of our patients tested positive for anti-CCP. The study also found RF positivity in 16% of patients with sarcoidosis. While these findings provide insights, more studies are needed to comprehensively understand the relationship between sarcoidosis and autoantibodies.

Study Limitations

As our study focused on sarcoidosis from the rheumatology outpatient clinic perspective, all our patients exhibited arthritis during the course of the disease. Consequently, there is a limitation in terms of assessing the frequency of joint involvement. Additionally, given that sarcoidosis cases are typically directed to chest diseases outpatient clinics, our study's patient count is relatively small, and the sample size is limited.

CONCLUSION

In our study, we examined the demographic characteristics, comorbidities, organs involved, joint involvement at the time of diagnosis, medications used in follow-up, and autoantibodies of 28 sarcoidosis patients diagnosed in the rheumatology outpatient clinic or consulted to the rheumatology outpatient clinic. While sarcoidosis is commonly associated with chest diseases, it presents a perplexing and surprising aspect from the rheumatology perspective due to its heterogeneous nature and joint involvement. Thus, it underscores the importance of considering and further investigating sarcoidosis in rheumatology practice.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University of Local Ethics Committee (protocol no: 2023.176.10.05, date: 31.10.2023).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: R.M., Design: R.M., Data Collection or Processing: D.B.G., Analysis or Interpretation: D.B.G., Literature Search: D.B.G., Writing: D.B.G.

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

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ORIGINAL ARTICLE

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Effects of Nintedanib on the Lungs via NLRP3 in a Model of Lipopolysaccharide-induced Acute Lung Injury in Rats

Sıçanlarda Lipopolisakkarid Kaynaklı Akut Akciğer Hasarı Modelinde Nintedanibin NLRP3 Yoluyla Akciğerler Üzerindeki Etkileri

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ABSTRACT

Aim: To demonstrate the possible protective efficacy of nintedanib, a tyrosine kinase inhibitor with demonstrated antifibrotic and antitumor activity, in a model of acute lung injury (ALI), a severe lung disease, through NLR family pyrin domain containing 3 (NLRP3) and nuclear factor kappa B (NF-κB) pathways.

Materials and Methods: In this study, 40 male Wistar albino rats were used. These rats were divided into 5 groups of equal sizes. Before the experiment began, nintedanib was administered orally to selected groups at doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg. At 24 hours, 12 hours, or 1 hour after nintedanib administration, rats selected for the lung injury model were administered intratracheal LPS. Interleukin (IL)-1β and tumor necrosis factor-α (TNF-α) amounts were measured by ELISA method and NLRP3, caspase-1, IL-1β and NF-kB gene expressions were measured by reverse-transcriptase polymerase chain reaction.

Results: It was observed that administration of nintedanib lowered the elevated NLRP3, caspase-1, IL-1β, and NF-κB expressions and the IL-1β and TNF-α cytokine levels in the tissues of rats with LPS-induced ALI. The findings obtained for the rats included in the lung injury group that received 50 mg/kg nintedanib were most similar to those of the healthy control group.

Conclusion: In rats modeled with ALI, nintedanib was shown to modulate the NLRP3/NF-κB signaling pathway and reduce the effects of ALI.

Keywords: ALI, NF-κB, nintedanib, NLRP3, tyrosine kinase inhibitor

ÖZ

Amaç: Antifibrotik ve antitümör etkinliği gösterilmiş bir tirozin kinaz inhibitörü olan nintedanibin ciddi bir akciğer hastalığı olan akut akciğer hasarı (ALI) modelinde etkileri ve bu hastalığa karşı olası koruyucu etkinliğinin ortaya konulması ve NLRP3 / nükleer faktör kappa B (NF-κB) yolağı üzerine olası etkisinin incelenmesidir.

Gereç ve Yöntem: Bu çalışmada 40 adet erkek Wistar albino sıçan kullanıldı. Bu sıçanlar eşit büyüklükte 5 gruba ayrıldı. Deney başlamadan önce, nintedanib seçilen gruplara 25 mg/kg, 50 mg/kg ve 100 mg/kg dozlarında oral olarak uygulandı. Nintedanib uygulamasından 24 saat, 12 saat veya 1 saat sonra, ALI için seçilen sıçanlara intratrakeal LPS uygulandı. İnterlökin (IL)-1β ve tümör nekroz faktörü-α (TNF-α) miktarları ELISA yöntemiyle, NLRP3, kaspaz-1, IL-1β ve NF-kB gen ekspresyonları ise revers-transkriptaz polimeraz zincir reaksiyonu yöntemiyle ölçüldü.

Bulgular: Nintedanib uygulamasının, LPS ile indüklenen ALI'li sıçanların dokularında yüksek NLRP3, kaspaz-1, IL-1β ve NF-κB ekspresyonlarını ve IL-1β ve TNF-α sitokin düzeylerini düşürdüğü gözlendi. 50 mg/kg nintedanib alan akciğer hasarı grubunda yer alan sıçanlardan elde edilen bulgular, sağlıklı kontrol grubuna en çok benzeyen bulgulardı.

Sonuç: ALI ile modellenen sıçanlarda nintedanibin NLRP3/NF-κB sinyal yolunu modüle ettiği ve ALI'nin etkilerini azalttığı gösterilmiştir.

Anahtar Kelimeler: ALI, NF-κ, B, nintedanib, NLRP3, tirozin kinaz inhibitörü

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INTRODUCTION

Acute lung injury (ALI) is a type of lung inflammation with high mortality rates and it is characterized by the disruption of the alveolar capillary barrier and gas exchange dysfunction[1,2]. It is also characterized by decreased lung compliance, persistent hypoxemia, and respiratory failure. Cases of ALI are commonly observed in intensive care units. Various factors may cause ALI, but the most common causes are sepsis, pneumonia, disseminated intravascular coagulation, burns, acute pancreatitis, massive blood transfusions, aspiration of gastric contents, shock, emergency blood transfusions, and trauma[3]. The pathogenesis of this acute and severe disease is unclear. The current consensus suggests that ALI occurs as a result of damage caused by uncontrolled cytokine production due to an inflammatory process[4]. ALI has no specific treatment. Hence, new strategies, biomarkers, and treatments are needed to reduce ALI-related morbidity and mortality rates[5].

Prior studies have examined the use of different mechanical ventilation techniques and different pharmacological agents in the treatment of ALI[6-8]. However, despite the large number of studies, satisfactory results have not yet been achieved. Tyrosine kinases and their related pathways were more recently discovered, and it has been shown that tyrosine kinases and their related pathways may be involved in the physiopathology of many diseases. Accordingly, they have become new targets for therapeutic approaches.

Tyrosine kinase is an enzyme that plays an important role in intracellular signal transduction and catalyzes protein phosphorylation. Tyrosine kinases modulate numerous signaling pathways for survival and other various cellular functions via the phosphorylation of amino acid residues. On the other hand, tyrosine kinase inhibitors also interfere with the binding of ATP and its substrate, preventing phosphorylation[9,10].

Various tyrosine kinases and inhibitors are associated with these effects. Moreover, new tyrosine kinases and inhibitors continue to be discovered as time goes on. The different types of tyrosine kinase inhibitors are characterized by the receptors they inhibit and their mechanisms of action on different pathways. Inhibitors of the HER family (EGFR-1 and EGFR-2) and first-generation and second-generation multi-target tyrosine kinase inhibitors (VEGFR and PDGFR) are some examples of these compounds[11].

Among these inhibitors, nintedanib, which had recently begun being investigated experimentally in cases of inflammation and related diseases and which has been licensed for the treatment of pulmonary hypertension, is the subject of the present study. It was previously found that nintedanib mitigates the expression of mesenchymal markers, inhibits phosphorylation, and improves hemodynamics[12].

Nintedanib is a molecule designed as a multi-target first-generation tyrosine kinase inhibitor that inhibits both receptor (FGFR-1, VEGFR-2, and PDGFR) and non-receptor (Src, Lyn, and Lck) tyrosine kinases[13]. There are ongoing studies on the efficacy of nintedanib against various types of cancer, including ovarian cancer, colorectal cancer, and non-small-cell lung cancer. When prior studies are examined, it is seen that nintedanib has been used in the treatment of idiopathic pulmonary fibrosis due to its effects via PDGFR[14]. It has also been shown that this drug inhibits VEGF receptor expression and angiogenesis in prostate cancer cells *in vitro*, and it decreases microvessel density[15]. VEGF-C/VEGFR-3 signaling was shown to ameliorate the effects of experimental lung injury in macrophages, which suggests that this mechanism could also play a role in the treatment of ALI and acute respiratory distress syndrome (ARDS)[16].

NLRP3 is an inflammasome belonging to the NLR family. Inflammasomes are important functional members of the innate immune system and the most characteristic inflammasome is NLRP3. It is known as the main inflammasome associated with inflammation and it plays a pivotal role in cases of ALI[17].

Prior studies have explored the relationship between nintedanib and NLRP3. In the literature, it has been shown that nintedanib treatment reduces the number of inflammatory cells, effectively alleviating the lung damage caused by polyhexamethylene guanidine (PHMG). It was also determined that nintedanib significantly reduced the expression of inflammatory cytokines and fibrotic factors and the activation NLRP3 in lung tissues. These results suggest that nintedanib may reduce the inflammatory response and improve pulmonary fibrosis in the lungs of mice administered PHMG[18].

ALI-induced mortality is known to be associated with the loss of epithelial barrier function[19]. NLRP3 was shown to have a regulatory role in TGF-β signaling in tubular epithelial cells and it was also observed that, in addition to affecting IL-1β and IL-18 maturation and pyroptosis induction, NLRP3 affects immunity and tissue damage[20].

In light of these previous findings, it is possible that nintedanib could alleviate ALI by modulating the NLRP3/NF-κB signaling pathway. Thus, the aim of the present study is to examine the effects of nintedanib, a tyrosine kinase inhibitor shown to have antifibrotic and antitumor efficacy, in a model of ALI and to demonstrate its possible protective efficacy against this disease and the connection of its possible efficacy with the NLRP3 and NF-κB pathways.

MATERIALS AND METHODS

1. Experimental Animals

In this study, 40 Wistar albino male rats weighing 240-280 g were used. All rats were obtained from the Experimental

Animal Laboratory of the Medical Experimental Application and Research Center of Atatürk University. All treatments and procedures were carried out according to the national guidelines accepted by the local ethics committee. The study was approved by the Local Ethics Council of Animal Experiments of Atatürk University (date: 26.04.2022, no: E-42190979-000-2200127868). Rats were housed in standard polypropylene cages in an environment with controlled temperature (22±1 °C) and humidity (50-60%) and a 12-hour light/dark photoperiod. They were provided standard food and tap water ad libitum.

2. Chemicals

Nintedanib (Ofev, Boehringer Ingelheim, Germany), LPS (*E. coli* 055:B5, Sigma-Aldrich Chemie GmbH, Germany), ketamine (Ketalar 500 mg/10 mL, Pfizer, Turkey), and xylazine (Basilazine 2%, Biotek, Turkey) were used in this study.

3. Experimental Groups

The rats were randomly divided into 5 groups with 8 rats in each group as follows:

Group 1: Healthy control group,

Group 2: ALI group (5 mg/kg LPS),

Group 3: ALI (5 mg/kg LPS) + nintedanib (NDN) at 25 mg/kg,

Group 4: ALI (5 mg/kg LPS) + NDN at 50 mg/kg,

Group 5: ALI (5 mg/kg LPS) + NDN at 100 mg/kg.

4. Experimental Design and Preparation of the Utilized Drugs

The 40 male Wistar albino rats used in this study were divided randomly into 5 groups of equal size. Nintedanib was administered via oral gavage to selected groups at doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg. At 24 hours, 12 hours, or 1 hour after nintedanib administration, selected rats were administered LPS. The half-life of nintedanib was considered while selecting these 3 dosages[21,22].

The rats were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) 1 hour after the last dose was given[23]. After sterilization, a midline neck incision was made using a surgical scalpel. After the incision was made, 5 mg/kg LPS[24] (Sigma-Aldrich) was dissolved in 0.2 mL of 0.09% NaCl and administered intratracheally to all groups except the healthy control group. In order to ensure homogeneous infiltration of the lungs, the rats were rotated repeatedly on 3 axes. The incision was then surgically sutured and sodium fusidate (2%) was applied topically[25]. Rats were euthanized with thiopental at a high dose of 50 mg/kg after 24 hours and then blood and lung tissue samples were collected.

5. Molecular Assays

This study was conducted *in vivo*. Within the scope of the study, gene expressions were examined molecularly. Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to examine lung NLRP3, caspase-1, NF-κB, and IL-1β mRNA expressions (Table 1). For this purpose, lung tissue samples were homogenized, their RNAs were isolated, their cDNAs were synthesized, and mRNA expressions were quantitatively examined.

6. RT-PCR

6.1. Extraction of RNA from Lung Tissues

Lung samples were weighed individually and stored at 4 °C for up to 4 weeks with RNAlater RNA Stabilization Reagent (QIAGEN, Germany). Tissues were homogenized with TissueLyser II (QIAGEN) and RNA extraction was performed in a QIAcube RNA isolation device (QIAGEN). Tissue samples were weighed individually and total RNA isolation was carried out using the QIAcube in combination with the RNeasy Mini Kit (QIAGEN) following the instructions provided by the manufacturer. Total mRNA was measured at 260 nm by nanodrop spectrophotometry (EPOCH, Biotek).

Table 1. RT-PCR gene sequencing

GAPDH	NM_017008.4	F: GCA AGT TCA ACG GCA CAG R: CTC AAC AGT ATA AAG AGC
NF-κB	NM_001276711.1	F: GAG ATT GTG CCA AGA GTG AC R: CTT GTC TTC CAT GGT GGA TG
NLRP3	NM_001191642.1	F: GTG GAG ATC CTA GGT TTC TCT G R: CAG GAT CTC ATT CTC TTG GAT C
IL-1β	NM_031512.2	F: TGC TGT CTG ACC CAT GTG AG R: GTC GTT GCT TGT CTC TCC TTG
Caspase-1	NM_012762.3	F: GAG CTG ATG TTG ACC TCA GAG R: CTG TCA GAA GTC TTG TGC TCT G

RT-PCR: Reverse-transcriptase polymerase chain reaction, NF-κB: Nuclear factor kappa B

6.2. Obtaining cDNA from RNA

cDNA synthesis was performed from total RNA using a cDNA reverse transcription kit. Each reaction was performed with the amount of RNA specified in the kit's instructions. The amount of cDNA obtained was measured by nanodrop spectrophotometry (EPOCH Plate, Biotek) and the cDNA was stored at -20 °C.

6.3. Quantitative Examination of mRNA Expressions by Real-time PCR

Lung mRNA expressions were examined using the SYBR GREEN Gene Expression Master Mix Kit. GAPDH was used as the reference gene. The recommended amount of cDNA was pipetted for the amplification and quantification processes, which were carried out in the recommended cycles.

6.4. Enzyme-Linked Immunosorbent Assay (ELISA)

Lung samples stored at -80 °C were subjected to physical homogenization in liquid nitrogen using the TissueLyser II device (QIAGEN). Each sample was weighed to 100 mg. The weighed tissues were then homogenized in 1 mL of PBS homogenate buffer in an Eppendorf tube using the TissueLyser II device. Subsequently, the tissues were centrifuged for ELISA as recommended by the manufacturer of the kits. The interleukin (IL)-1β and tumor necrosis factor-α (TNF-α) cytokine levels of the obtained supernatants were measured. For IL-1β measurements, a rat IL-1β ELISA kit (Cat. No. E0119RA-96T, BTLAB, UK) was used, while a rat TNF-α ELISA kit (Cat. No. E0764RA-96T) was used for TNF-α measurements. Protein amounts were measured using the Lowry method and the obtained data were normalized according to cell protein concentrations.

7. Histopathological Method

Lung tissues obtained via necropsy were placed in 3.7% neutral formalin solution. Routine alcohol-xylol follow-up procedures were performed for the tissues and then the tissues were embedded in paraffin blocks. Sections of 5 µm in thickness were transferred to poly-L-lysine slides and stained with Masson's trichrome. The histopathological findings observed in the lung tissues, including perivascular edema, submucosal edema, alveolar septal thickening, and inflammation, were scored semi-quantitatively as being absent (0), mild (1), moderate (2), or severe (3) compared to the healthy control group.

Statistical Analysis

Ct values were automatically converted to ΔCt values with the device and the obtained results were statistically evaluated with IBM Statistical Package for the Social Sciences statistics 25.0 (IBM Corp., USA). Data with non-homogeneous variance were subjected to one-way ANOVA and the Games-Howell test as a post-hoc multiple comparison test. Data with homogeneous variance were subjected to one-way ANOVA and the Tukey test as a post-hoc multiple comparison test. Values of $p<0.05$ were considered significant.

RESULTS

1. Molecular Findings

1.1. Caspase-1 Expression in Lung Tissues

When the caspase-1 expressions of the groups were examined, it was determined that the healthy control group had the lowest levels of caspase-1 expression. It was also observed that the ALI group had very significantly higher caspase-1 expression levels compared to the healthy control group ($p<0.001$). When the caspase-1 expressions of the ALI + NDN groups were examined, it was found that the groups that received all 3 dosages of nintedanib had very significantly lower caspase-1 expressions compared to the ALI group, the lowest being the caspase-1 expression of the ALI + 50 NDN group ($p<0.001$).

1.2. IL-1β Expression in Lung Tissues

When the IL-1β expressions of the groups were evaluated, it was determined that the healthy control group had the lowest levels of IL-1β expression. It was also observed that the ALI group had very significantly higher IL-1β expression levels compared to the healthy control group ($p<0.001$). When the IL-1β expressions of the ALI + NDN groups were examined, it was found that the groups that received all 3 dosages of nintedanib had very significantly lower IL-1β expressions compared to the ALI group, the lowest being the IL-1β expression of the ALI + 50 NDN group ($p<0.001$) (Figure 1).

1.3. NF-κB Expression in Lung Tissues

When the NF-κB expressions of the groups were examined, it was determined that the healthy control group had the lowest NF-κB expression. It was also observed that the ALI group had very significantly higher NF-κB expression levels compared to the healthy control group ($p<0.001$). When the NF-κB expressions of the ALI + NDN groups were evaluated, it was found that the groups that received all 3 dosages of nintedanib had very significantly lower NF-κB expressions compared to the ALI group, the lowest being the NF-κB expression of the ALI + 50 NDN group ($p<0.001$) (Figure 1).

1.4. NLRP3 Expression in Lung Tissues

When the NLRP3 expressions of the groups were examined, it was determined that the healthy control group had the lowest levels of NLRP3 expression. It was also observed that the ALI group had very significantly higher NLRP3 expression compared to the healthy control group ($p<0.001$). When the

NLRP3 expressions of the ALI + NDN groups were examined, it was found that the groups that received all 3 dosages of nintedanib had very significantly lower NLRP3 expressions compared to the ALI group, the lowest being the NLRP3 expression of the ALI + 50 NDN group (p<0.001) (Figure 1).

2. ELISA Findings

2.1. Effect of Nintedanib on TNF-α Cytokine Levels in Lung Tissues

When the TNF-α levels of the groups were examined, it was determined that the healthy control group had the lowest TNF-α levels. It was also observed that the ALI group had very significantly higher TNF-α levels compared to the healthy control group (p<0.001). The ALI + NDN groups had very significantly lower TNF-α levels compared to the ALI group. Among the ALI + NDN groups, the ALI + 50 NDN group was found to have TNF-α levels most significantly similar to those of the healthy control group (Figure 2).

2.2. Effect of Nintedanib on IL-1β Cytokine Levels in Lung Tissues

Considering IL-1β levels of the groups, it was revealed that the healthy control group had the lowest IL-1β levels. It was also observed that the ALI group had very significantly higher IL-1β levels compared to the healthy control group (p<0.001). When the IL-1β levels of the ALI + NDN groups were examined, it was found that the groups that received all 3 dosages of nintedanib had very significantly lower IL-1β levels compared to the ALI group, the lowest being the IL-1β levels of the ALI + 25 NDN and ALI + 50 NDN groups (p<0.001). Among the ALI + NDN groups, the ALI + 50 NDN group had IL-1β levels most significantly similar to those of the healthy control group (Figure 3).

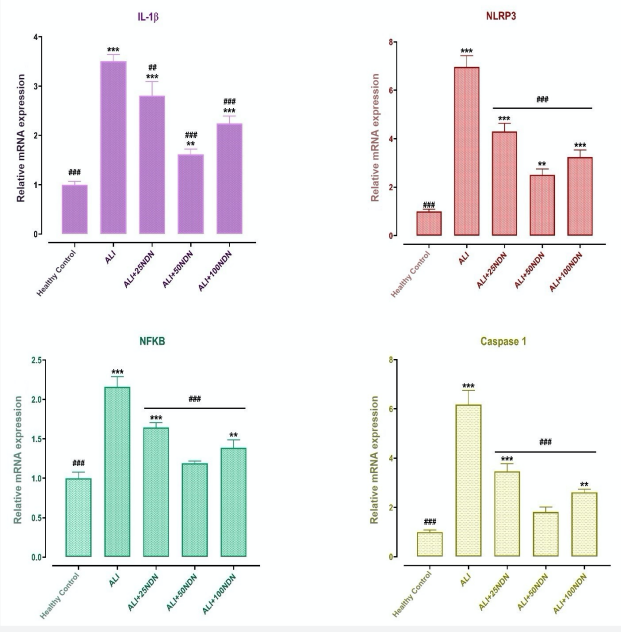

Figure 1. IL-1β, NLRP3, NF-κB, and caspase-1 expressions in lung tissues

****Signifies p<0.001 compared to the healthy group. ###Signifies p<0.001 compared to the ALI group*

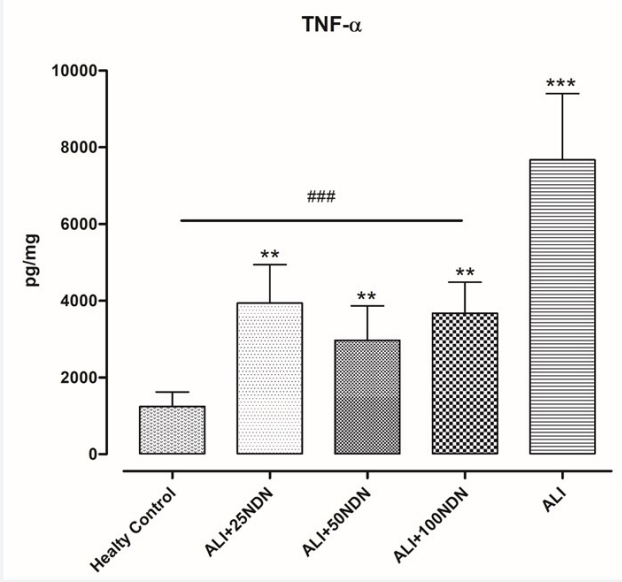

Figure 2. Lung tissue TNF-α ELISA results

****Signifies p<0.001 compared to the healthy group. ###Signifies p<0.001 compared to the ALI group*

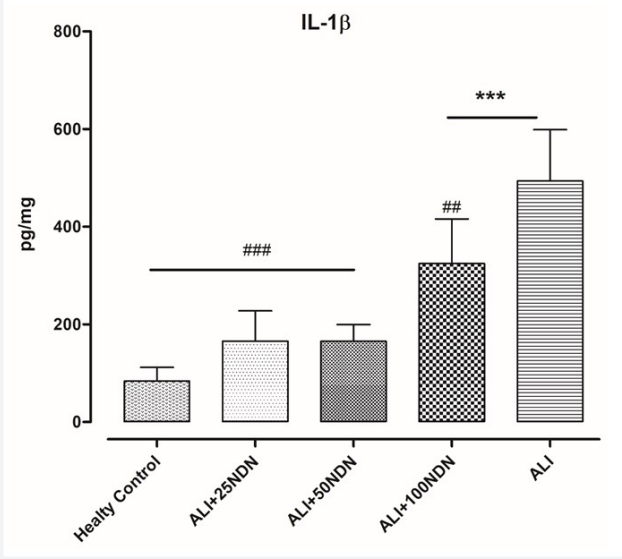

Figure 3. Lung tissue IL-1β ELISA results

****Signifies p<0.001 compared to the healthy group. ###Signifies p<0.001 compared to the ALI group*

3. Histopathological Findings

In the histopathological examination of the healthy control group, the bronchi, terminal bronchioles, respiratory bronchioles, alveolar sacs, alveolar walls, arteries, veins, and capillary vessel structures in the lung tissues were examined in detail. No pathological findings were found (Figures 4A, 4B).

Areas of advanced edema in the vascular adventitia and a large number of inflammatory cells (circles in images) were found in the ALI group. Alveolar septal thickening (stars) due to advanced edema was observed in the alveolar walls of the alveolar tree, encompassing the respiratory parenchyma of the lungs. Erythrocyte clusters infiltrating the alveoli from place to place in this tissue was another important finding (Figure 4C).

Similar to the ALI group, submucosal thickening due to edema and inflammatory cells were also observed in the ALI + 25 NDN group. However, the observed findings were not as severe in the ALI + 25 NDN group compared to the ALI group (Figure 4D). The alveolar septal thickening observed in the ALI + 25 NDN group was also not as severe compared to the ALI group (Figure 4D).

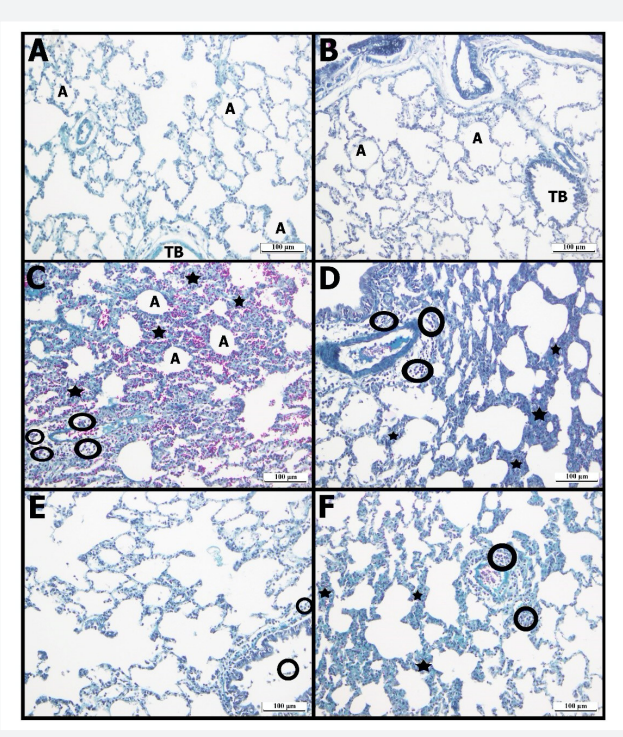

Figure 4. Lung histopathology findings with Masson's trichrome staining. A, B: Healthy control group, C: ALI group, D: ALI + NDN 25 group, E: ALI + NDN 50 group, F: ALI + NDN 100 group (A: alveoli, TB: terminal bronchiole, AD: alveolar wall, Circle: alveolar wall thickening and perivascular or submucosal edema, Star: alveolar septal thickening)

It was observed that the edematous areas around the submucosa surrounding the vessels and around the terminal bronchioles were improved significantly in the ALI + 50 NDN group. The alveolar septal thickening in the ALI + 50 NDN group was insignificant enough to be considered healthy. Inflammatory cells were also rarely observed in these tissues. In general, this group was observed to have a histological appearance similar to that of the healthy control group (Figure 4E).

Submucosal edematous areas around the vessels were also observed in the ALI + 100 NDN group. However, the observed findings were not as severe in this group as in the ALI group. In addition, mild alveolar septal thickening was observed in this group (Figure 4F).

DISCUSSION

In this study, the effects of nintedanib on LPS-induced ALI via NLRP3/NF-κB were examined. It was observed that the elevated expressions of IL-1β, caspase-1, NLRP3, and NF-κB and the elevated levels of IL-1β and TNF-α cytokines due to LPS administration decreased with the administration of all 3 selected dosages of nintedanib. The administration of nintedanib at 50 mg particularly improved inflammation and pulmonary edema and ultimately alleviated ALI, nearly reaching the same status as that seen in the healthy control tissues.

LPS is a glycolipid, and the LPS found on the outer membranes of Gram-negative bacteria binds to a specific lipopolysaccharide-binding protein (LBP), especially in serum[26]. LPS binding to LBP triggers the production of inflammatory mediators by activating the CD14/TLR-4 receptor complex in immune cells. LPS, which is an important mediator of Gram-negative bacterial sepsis, constitutes a very useful tool for studying the effects of Gram-negative bacterial infections in humans and animals. This approach allows for the examination of the effects of inflammatory responses that occur in cases of bacterial infections[27]. LPS is also one of the most suitable models for studying ALI caused by immune responses[28]. The lung consists of two fundamental anatomical structures, namely the vascular and airway structures. The lung epithelium protects the lung, especially from harmful toxins. Epithelial tissues that cover both airway and vascular structures play major roles in ALI[29]. Activation of the NF-κB pathway and the inflammation of the airway epithelium are the most important local and systemic responses to lung injury[30]. Mitogen-activated protein kinase (MAPK) regulates LPS-induced NF-κB activation[31], and NF-κB stimulation activates a large number of intracellular signaling pathways[32,33]. NF-κB also activates NLRP3, which is known as the main regulator of inflammation. NF-κB regulates the inflammatory response by directly converting pro-IL-

1β to active IL-1β, as well as activating NLRP3 to convert procaspase-1 to active caspase-1[34]. Inhibition of the PDGFRβ/Akt/NF-κB/NLRP3 pathway and modulation of NF-κB activity have been shown to be effective mechanisms for alleviating ALI[35,36]. NLRP3 is mainly activated in two ways. The first type of activation, as noted above, occurs through NF-κB and IL-1β. The second type of activation occurs via important signaling receptors in innate immune cells that can be activated by various pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs)[37]. NLRP3 is the primary protein that causes inflammation by forming a complex with pro-caspase-1 via ASC. The activation of the NLRP3 inflammasome leads to the transformation of procaspase-1 to caspase-1. This transformation also triggers the production and secretion of mature IL-1β and IL-18[38]. IL-1β, IL-18, and TNF-α are important proinflammatory cytokines produced by macrophages in response to PRR activation. TNF production is mainly regulated at the transcription level. It has also been shown that NLRP3 plays pivotal roles in the physiopathology of infectious agents such as *Staphylococcus aureus*[39], *Pseudomonas aeruginosa*[40], *Mycobacterium tuberculosis*[41], and influenza A virus[42], which cause ALI.

In a prior study, it was found that reduction of elevated NF-κB and NLRP3 transcription due to LPS via various treatment methods was effective in alleviating lung damage by reducing the amounts of proinflammatory TNF-α and IL-1β cytokines[43].

Previous studies also showed that NLRP3 played important roles in ALI and that experimental treatment with NLRP3 antagonists provided very significant benefits against ALI[17,44].

Similar to those studies, the findings of the present study revealed that the NLRP3/NF-κB signaling pathway was activated due to LPS administration. It was found that elevated NLRP3, NF-κB, caspase-1, and IL-1β gene expressions and TNF-α and IL-1β cytokine levels were alleviated with nintedanib treatment. These findings suggest that the inflammatory cascade is induced due to increased receptor activation following LPS administration, and lung damage occurs due to increased cytokine release activated as a result of NLRP3 activating the NF-κB signaling pathway[45]. Moreover, it was observed that this inflammatory cascade was blocked quite effectively in the groups that received nintedanib. In prior clinical studies, it was found that high levels of IL-1 and IL-18 were produced by the alveolar macrophages of patients with ALI, and it was shown that this increase in IL-1 and IL-18 production was directly associated with mortality and morbidity[46,47]. It is known that IL-1 and IL-18 cytokines are directly associated with the NLRP3 pathway. In this context, prior experimental studies of ALI achieved very positive results by antagonizing IL-1 and IL-18 cytokines[48,49].

Nintedanib is a first-generation multi-target tyrosine kinase inhibitor. It is used in the treatment of idiopathic pulmonary fibrosis because it reduces lung function disruption and inhibits the proliferation of vascular cells[50]. In addition to idiopathic pulmonary fibrosis, it is approved for use in cases of fibrosing interstitial lung diseases. Nintedanib can thus be applied against common health problems that occur around the world and its use has become a focus of attention. It has also been shown that nintedanib can be used in the treatment of pulmonary arterial hypertension since it blocks the phosphorylation of PDGF and FGF receptors, improving neointimal lesions and medial wall thickening in pulmonary arteries[12]. Nintedanib inhibits both receptor and non-receptor tyrosine kinases. Prior studies showed that the inhibition of Src tyrosine kinase alleviates ALI[51]. PDGF, a growth factor whose receptors are targeted by tyrosine kinase inhibitors, plays a key role in the pathogenesis of lung diseases such as pulmonary fibrosis, ALI, and ARDS. It is a chemotactic factor for monocytes and granulocytes during inflammation, and overexpression of PDGF can induce inflammatory damage[52]. In addition to PDGFR, the receptors of VEGF, which play important roles in many organs by directly regulating vascular permeability to water and proteins, are also targeted by nintedanib. VEGF increases mRNA expression, activates inflammation, and causes capillary leakage and pulmonary edema in cases of ALI[53]. Moreover, it was shown that VEGF inhibition reduced pulmonary edema due to ALI[54]. A prior study revealed that nintedanib inhibited angiogenesis by reducing the increased VEGF receptors in prostate cancer cells. In the same study, it was also observed that microvascular density decreased as a result of nintedanib administration[15]. When the results of the present study are considered, it can be said that nintedanib suppresses inflammation and alleviates ALI both through growth factors and directly via the innate immune system.

The possible effects of nintedanib observed in the present study can also be explained by its direct effects on NLRP3. Nintedanib, which is mainly used to treat idiopathic pulmonary fibrosis, has been the focus of many studies in the literature due to its effects on various signaling pathways. To date, however, only one study has examined the relationship between nintedanib and NLRP3. In that study, it was shown that nintedanib could alleviate lung fibrosis by reducing the activation of the NLRP3 inflammatory response in the lungs and improving pulmonary fibrosis, and it was furthermore shown that nintedanib reduced IL-1 and TNF-α levels[18]. In another study that examined the anti-inflammatory effects of nintedanib, it was found to reduce IL-1 levels[55]. In the present study, the effects of nintedanib on NLRP3 in ALI were elucidated for the first time in the literature.

This study also showed the improvement in lung tissues histopathologically. When the histopathologic findings of

the ALI group were examined, it was observed that there was diffuse perivascular and submucosal edema. At the same time, alveolar septal thickening and severe inflammation were detected. It was observed that the severity of these findings decreased and ALI was attenuated in the groups given all three doses of nintedanib. In the ALI + 50 NDN group, these findings were found to be very mild and close to the healthy group.

Study Limitations

The limitation of our study is that Western blot analysis, which is used to identify specific proteins, could not be performed in lung tissue.

CONCLUSION

Through the ALI model designed for this study, it was shown for the first time that nintedanib reduced LPS-induced ALI via the NLRP3/NF-kB signaling pathway. It was molecularly, biochemically, and histopathologically confirmed that nintedanib reduced the gene expressions of NLRP3, IL-1β, caspase-1, and NF-κB and the levels of IL-1β and TNF-α cytokines in cases of LPS-induced ALI. These results support the possibility of nintedanib providing beneficial results by protecting against ALI and suggest that the use of this drug may be effective.

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Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Council of Animal Experiments of Atatürk University (date: 26.04.2022, no: E-42190979-000-2200127868).

Informed Consent: Animal experiment.

Authorship Contributions

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

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Effect of Hypothalamic Adrenal Axis and Thyroid Function Alterations on Prognosis of Critically Ill COVID-19 Patients

Hipotalamik Adrenal Eksen ve Tiroid Fonksiyon Değişikliklerinin Kritik COVID-19 Hastalarının Prognozuna Etkisi

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ABSTRACT

Aim: The aim of this study was to evaluate the effect of changes in adrenal, and thyroid functions on the prognosis of Coronavirus disease-2019 (COVID-19) patients admitted to the intensive care unit (ICU).

Materials and Methods: This was a retrospective evaluation that included COVID-19 patients requiring ICU admission. Serum cortisol, adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH), free thyroxine (fT4) and triiodothyronine (fT3) levels were measured on admission and two more times during the hospitalization. Routine biochemistry, hemogram, C-reactive protein, procalcitonin, fibrinogen and D-dimer levels were also measured, along with hormones. All-cause mortality during ICU stay, inotropic drug and mechanical ventilation needs, and duration of hospitalization were recorded for each patient. Euthyroid sick syndrome (ESS) and hypocortisolism rates were determined. Deceased and surviving patients were compared in terms of hormone values, and logistic regression to determine independent associates of mortality was performed.

Results: Overall, 124 patients (58% male, mean age 70.7±11.3 years) were included. During the ICU stay, both fT3 and fT4, but not TSH, showed a statistically significant decrease compared to admission values. Serum cortisol and ACTH values increased compared to admission values, but this increase was not significant. ESS was present in 89.5% of the patients. Two-thirds of the patients died in ICU. Serum fT3 values were significantly lower among decedents compared to survivors. Hypocortisolism was detected in 20.1% of the patients. Only the fT3 level could independently and significantly predict all-cause mortality.

Conclusion: ESS was almost universal among critically ill COVID-19 patients. Serum fT3, but not other thyroid or adrenal hormones, could significantly predict all-cause mortality.

Keywords: Adrenal function, euthyroid sick syndrome, hypocortisolism, prognosis, thyroid function

ÖZ

Amaç: Bu çalışmanın amacı, yoğun bakım ünitesine (YBÜ) yatırılan Koronavirüs hastalığı-2019 (COVID-19) hastalarının adrenal ve tiroid fonksiyonlarındaki değişikliklerin prognoz üzerine etkisini değerlendirmektir.

Gereç ve Yöntem: Bu çalışma YBÜ'ye kabul edilen COVID-19 hastalarını içeren retrospektif bir değerlendirmeydi. Serum kortizol, adrenokortikotropik hormon (ACTH), tiroid uyarıcı hormon (TSH), serbest tiroksin (sT4) ve triiyodotironin (sT3) düzeyleri ilk yatışta ve yatış sırasında iki kez daha ölçüldü. Hormonların yanı sıra rutin biyokimya, hemogram, C-reaktif protein, prokalsitonin, fibrinojen ve D-dimer seviyeleri de ölçüldü. Her hasta için YBÜ'de kalış süresi boyunca tüm nedenlere bağlı ölümler, inotropik ilaç ve mekanik ventilasyon ihtiyaçları ve hastanede kalış süreleri kaydedildi. Ötiroid hasta sendromu (ESS) ve hipokortizolizm oranlar belirlendi. Ölen ve yaşayan hastalar hormon değerleri açısından karşılaştırıldı ve mortalitenin bağımsız birlikteliklerini belirlemek için lojistik regresyon yapıldı.

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Bulgular: Genel olarak 124 hasta (%58 erkek, ortalama yaş 70,7±11,3 yıl) dahil edildi. YBÜ'de kalış süresi boyunca, hem fT3 hem de fT4, ancak TSH, yatış değerlerine göre istatistiksel olarak anlamlı bir düşüş gösterdi. Serum kortizol ve ACTH değerleri yatış değerlerine göre arttı, bu artış anlamlı değildi. Hastaların %89,5'inde ESS mevcuttu. Hastaların üçte ikisi YBÜ'de öldü. Serum fT3 değerleri, hayatta kalanlara kıyasla merhumlarda anlamlı derecede düşüktü. Hastaların %20,1'inde hipokortizolizm saptandı. Yalnızca fT3 düzeyi tüm nedenlere bağlı ölümleri bağımsız ve anlamlı bir şekilde öngörebilir.

Sonuç: ESS, kritik durumdaki COVID-19 hastaları arasında neredeyse evrenseldi. Serum fT3 tüm nedenlere bağlı ölümleri önemli ölçüde tahmin edebilir fakat diğer tiroid veya adrenal hormonları tahmin edemez.

Anahtar Kelimeler: Adrenal fonksiyon, ötiroid hasta sendromu, hipokortizolizm, prognoz, tiroid fonksiyonu

INTRODUCTION

Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2), the etiologic agent of Coronavirus disease-2019 (COVID-19), might cause endocrine dysfunction on a wide range, including pituitary, thyroid, adrenal, gonadal, and pancreatic abnormalities[1]. Among these, perhaps thyroid hormone dysfunction, in the form of euthyroid sick syndrome (ESS), is the most common abnormality.

It has been reported that COVID-19 might cause thyroid dysfunction due to direct or indirect effects of the SARS-CoV-2 virus on the thyroid gland. This might be in the form of the ESS owing to severe systemic disease or may take the form of acute inflammation due to autoimmune reaction[2]. Several case reports demonstrated the development of subacute thyroiditis after COVID-19[3-5]. COVID-19 was also speculated as a trigger for autoimmunity and resultant Graves' disease in some patients, as well[6]. Several studies evaluated ESS and its severity on the prognosis of patients with COVID-19. These studies showed an increased prevalence of impaired thyroid function and an association between ESS and COVID-19 severity and level of inflammatory parameters[7-9].

Critical illness places great physical stress on the organism, and systemic availability of cortisol is a coping strategy with this stress. Several randomized studies produced conflicting results with respect to the impact of so-called relative adrenal failure-later renamed critical illness-related corticosteroid insufficiency-on clinical outcomes in patients with sepsis[10]. Subsequent research revealed the cause of increased systemic availability of cortisol, which was not due to increased adrenal secretion but rather owing to decreased degradation in the liver and kidney and less binding to cortisol-binding proteins in plasma[11]. Because of increased hospital and intensive care unit (ICU) admission rates of COVID-19 patients and dire outcomes for some, several investigators also evaluated the role of baseline levels of adrenocorticotropic hormone (ACTH) and cortisol on clinical outcomes and mortality in COVID-19 patients. Kanczkowski et al.[12], in their autopsy study, found that though not causing overt adrenal insufficiency, adrenal glands are a frequent target of SARS-CoV-2 and ensuing inflammation in deceased COVID-19 patients. A few case reports described cases of new-onset primary adrenal insufficiency following COVID-19[13-15]. Moreover, Mao et al.[16] found the level of serum cortisol significantly lower in ICU patients with COVID-19 compared to ICU patients without COVID-19. A very recent meta-analysis investigated the effect of serum cortisol levels on the prognosis of COVID-19 patients. The authors found significantly higher levels of serum cortisol in patients with severe COVID-19 compared to patients with moderate and mild COVID-19 patients[17]. Several treatment options were tried in COVID-19 patients with the hope of reducing mortality. Corticosteroids were one of the most promising among these potential treatment candidates. A meta-analysis by Boppana et al.[18] included 6 randomized controlled trials on 7 thousand patients with COVID-19 who were treated with corticosteroids and sought to answer whether corticosteroids were beneficial in reducing mortality. The analysis revealed that the use of systemic corticosteroids was associated with a reduction in all-cause mortality in patients with COVID-19 who required oxygen therapy or mechanical ventilation.

To the best of our knowledge, no study in the literature evaluated the combined power of thyroid and adrenal axes in the prediction of adverse clinical outcomes in patients with COVID-19. Thus, we aimed to evaluate the predictive ability of thyroid function along with serum cortisol and ACTH levels on clinical outcomes in COVID-19 patients who required ICU admission.

MATERIALS AND METHODS

Patients and Setting

This is a retrospective study that aims to evaluate the prognostic effects of admission serum cortisol level and thyroid function tests on the prognosis of patients admitted to a COVID-19 intensive care unit. The study was carried out in a teaching and research hospital in Karaman, Turkey. All patients who were admitted to the ICU due to COVID-19 were screened for eligibility for the study between March 11, 2020, and December 31, 2020. All patients had either a positive SARS-CoV-2 polymerase chain reaction (PCR) test from nasal swabs and/or a compatible chest computed tomography (CT) along with COVID-19 contact history. Exclusion criteria included the use of thyroid hormone therapy, the presence of

hypothalamic-pituitary gland dysfunction history, having the history of radiotherapy or chemotherapy in the last 6 months, the presence of thyroid surgery history and incomplete evaluation of thyroid gland function examination at admission (İndividuals with a history of thyroid disease were included in the study only if they lacked a history of active medication use or surgical intervention). Of the 95 patients who were excluded, the following reasons were ascertained: Being transfer from another service (n=5), having hypothyroidism (n=25), hyperthyroidism (n=7), change of thyroid gland revealed by chest CT scan (n=10), and nodular goiter (n=9), undergoing thyroidectomy (n=6) and radioiodine therapy (n=2), having multihormonal pituitary insufficiency (n=1), and lack of thyroid function test (n=30).

Approval was granted by the Ethics Committee of Karamanoğlu Mehmetbey University (date: 07/12/2020, no: 02-2020/04). This study was performed in line with the principles of the Declaration of Helsinki.

Data Collection

Age, sex, and comorbid medical conditions were recorded for each patient. Laboratory evaluations at ICU admission included complete blood count and differential, serum urea, creatinine, uric acid, lactate dehydrogenase, C-reactive protein (CRP), procalcitonin, and ferritin (daily COVID-19 sampling as part of hospital protocol). Serum levels of cortisol, ACTH, free triiodothyronine (fT3), free thyroxine (fT4), and thyroid-stimulating hormone (TSH) levels were measured both on admission and thereafter two times during hospitalization (weekly COVID-19 sampling as part of hospital protocol). Initial laboratory examinations were taken before patients received any treatment. Serum TSH and thyroid hormones, ACTH, and cortisol measurements were performed with the chemiluminescence method via an autoanalyzer (Advia Centaur XP, Siemens). All study laboratory measurements were performed again during the course of the hospitalization (second and third measurements in addition to admission (first measurement) values).

The ESS was described as having a decreased serum fT3 with normal or low TSH levels[7]. The normal reference values for fT3 at our laboratory were 2.7-4.3 pg/mL. Hypocortisolism was defined as having a serum morning cortisol value below 10 µg/dL[19].

The patient outcomes (discharge or exitus) were obtained from patient charts. The length of ICU stay was determined for each study participant. Patients who were administered pulse prednisone as a part of the COVID-19 treatment regimen and who were receiving thyroid hormone or drugs containing steroids were also recorded. Pulse steroid was given to 46.0% of the patients as mentioned in the national COVID-19 treatment guidelines. All patients received the same glucocorticoid agent. The patients were treated with pulse steroid treatment with an intermediate-acting glucocorticoid in the form of 250 mg methylprednisolone for the first 3 days and 1 mg/kg methylprednisolone for the next 7 days. Data regarding intubation and mechanical ventilation and positive inotropic use were also gathered.

Statistical Analysis

The Statistical Package for the Social Sciences 25.0 software package (IBM, Armonk, NY, USA) was used to analyze the data of the study. A probability value of $p<0.05$ was considered statistically significant, and two-tailed p-values were used for all statistics.

To determine whether the continuous variables were normally distributed, the Shapiro-Wilks test, histogram, and Q-Q plot were used for continuous variables. Continuous variables were expressed as mean±standard deviation or median (minimum-maximum) depending on the distribution of the variable. Categorical variables were reported as numbers and percentages. To compare the categorical variables between the groups, the chi-square test and Fisher's exact test were used. The Mann-Whitney U test and the independent t-test were employed to compare two-group comparisons for numeric variables. The correlation was displayed with the Spearman correlation and the point biserial correlation test. Repeated measured ANOVA or the Friedman test with post-hoc Durbin-Conover test were used to analyze repeated measurements of the variables.

We performed univariate and multivariate logistic regression analyses to determine the independent associates of mortality. According to the univariate logistic regression, among those with a p value less than 0.050, only the variables that had clinical significance were included in the multivariate logistic regression analysis test. We did not include CRP in the regression model because its confidence intervals crossed 1.000.

RESULTS

Baseline Clinicodemographic Features and Laboratory Values

In total, 124 patients (58.1% males) with COVID-19 who required ICU admission were included in the study. The mean age was 70.7±11.3 years. The most prevalent chronic medical comorbidity was hypertension (63.7%), followed by type 2 diabetes mellitus. The majority of the patients (75%) had a positive PCR test for SARS-CoV-2. During the course of the hospitalization, 74.2% of the patients required tracheal intubation and mechanical ventilatory support. Approximately half of all patients required the administration of positive

inotropic agents to support blood pressure. Pulse steroid was given to 46.0% of the patients as part of the national COVID-19 treatment guidelines. Table 1 summarizes the baseline clinicodemographic features of all study patients. Admission and repeat laboratory values for hemogram, routine biochemistry, free thyroxine, free triiodothyronine, TSH, ACTH, and cortisol values are shown in Table 2. All controls were created one week apart. Controls were performed on 124 patients upon admission to intensive care (1st), 103 patients after 1 week (2nd), and 57 patients after 2 weeks (3rd).

Sixty-seven patients (54%) were not given pulse steroids during their hospitalization. In relation to patients who were administered pulse steroid treatment, clinical outcomes (length of ICU stay, rates of mechanical ventilation and inotropic drug use, and mortality) were comparable in both groups.

Thyroid and Adrenal Function

During the course of the ICU stay, both fT3 and fT4 showed a gradual decrease from their corresponding admission values. And these trends were statistically significant. Serum TSH did not significantly change during ICU stay. Although repeat values of serum cortisol and ACTH values increased in all patients compared to admission values, these increases were not statistically significant (Table 2).

Table 1. Demographic and clinical features of the whole study cohort

	Patients (n=124)
Age (years)	70.7±11.3
Sex	
Male	72 (58.1%)
Female	52 (41.9%)
Comorbidities	
Hypertension	79 (63.7%)
Diabetes mellitus	40 (32.3%)
Coronary artery disease	25 (20.2%)
Pulmonary disease	21 (16.9%)
Cerebrovascular disease	19 (15.3%)
Valvular heart disease	15 (12.1%)
Thyroid disease	15 (12.1%)
Chronic kidney disease	10 (8.1%)
Dyslipidemia	6 (4.8%)
Malignancy	6 (4.8%)
Pulse steroid administration	57 (46.0%)
Inotropic drug administration	68 (54.8%)
PCR test positivity	93 (75.0%)
Need for mechanical ventilation	92 (74.2%)
Length of hospital stay (days)	8 (1-60)
Outcome	
Survivors	42 (33.9%)
Decedents	82 (66.1%)

PCR: Polymerase chain reaction

When admission laboratory values were evaluated in the whole cohort of patients, 89.5% of the patients had ESS. This rate was maintained during the course of the hospitalization [ESS (2nd)=93.2%, ESS (3rd)=93.0%] (Table 3).

We also assessed ESS frequency among deceased and survivors who were not given pulse steroid treatment. The ESS frequency was similar at all measurement points during the hospitalization between survivors and deceased patients (Table 3).

Although admission values were comparable, repeat values of serum TSH were significantly lower in patients who were administered pulse steroid compared to patients who were not. On the other hand, fT3 and fT4 measurements were not different. All ACTH measurements, including admission value, were consistently and significantly lower in patients who were administered pulse steroids compared to patients who were not. Similarly, baseline and repeat serum cortisol values were significantly lower in patients who were treated with pulse steroids (Table 4).

Hypocortisolism (serum cortisol value below 10 µg/dL) was present in 25 (20.1%) patients. Inotropic medications were given to 14 (56%) patients with hypocortisolism. The coexistence of ESS and hypocortisolism was evident in 24 (19.4%) patients. The frequency of hypocortisolism in survivors and decedents was 21.4% and 19.5%, respectively. Mortality rate was not different between patients with and without hypocortisolism. In addition, serum fT3 levels were also comparable between patients with and without hypocortisolism. In a cohort of 25 patients with hypocortisolism, 22 received pulse steroid therapy, while 3 did not. Among the 22 patients who received pulse steroid therapy, 15 (68.2%) experienced an adverse outcome (exitus). Of the 3 patients who did not receive pulse steroid therapy, 2 (66.7%) had an adverse outcome. Statistical analysis using the Fisher's Exact test indicated no significant difference in mortality between these two groups (p=0.704).

Patient Survival and Associated Factors

Two-third of all patients died during ICU stay. When compared, survivors and decedents had comparable age and comorbid disease distribution. As expectedly, mechanical ventilation requirement and inotropic drug administration were significantly more frequent among the deceased compared to survivors. The mean admission serum fT3 and T4 levels were comparable between the survivor and deceased groups. However, repeat values taken during the course of the

Table 2. Comparison of laboratory parameters (on admission, second, and third) of the whole study cohort

	On admission (1st) (n=124)	Second (2nd) (n=103)	Third (3rd) (n=57)	p value
WBC count (K/uL)	12.76±6.01	13.31±7.59	13.15±6.00	0.516
Hemoglobin (g/dL)	12.2±2.43	11.48±2.12	11.17±2.20	**0.001***
Hematocrit (%)	36.91±6.65	35.61±6.53	34.22±7.61	**0.001****
Lymphocyte count (K/µL)	0.84±0.48	0.88±0.48	1.05±0.93	0.077
Platelet count (K/µL)	230.5 (25.0-597)	252.7±121.7	251.3±119	0.266
Ferritin (ng/mL)	420 (13-2000)	489 (53-2000)	525 (65-2000)	**0.002†**
D-dimer (mg/L)	2528 (401-9999)	2398 (306-9999)	2257 (931-9999)	0.824
LDH (U/L)	562.9±371.5	459.3±221.1	469.6±372.1	0.325
Fibrinogen (g/L)	4.77±1.46	4.80±1.37	5.10±1.39	0.154
Free triiodothyronine (pg/mL)	1.44 (0.52-6.0)	1.33±0.37	1.27±0.43	**0.003††**
Free thyroxine (ng/dL)	1.13±0.36	1.05±0.36	0.96±0.37	**0.003≠**
TSH (mU/L)	0.64 (0.01-39.8)	0.44 (0.01-20.13)	0.76 (0.02-33.14)	0.166
ACTH (pg/mL)	9.77 (1.0-166.5)	10.58 (1.58-74.34)	11.17 (1.0-69.0)	0.374
Cortisol (µg/dL)	22.57±17.78	18.10±13.08	18.81±12.19	0.123
Triglyceride (mg/dL)	161.6±82.3	162.4±84.4	147.7±67.5	0.577
Uric acid (mg/dL)	6.35±2.94	4.81±2.62	4.54±2.39	**<0.001≠≠**
CRP (mg/L)	164.1±96.2	124.3±79.2	149.7±83.5	**0.004ϕ**
Procalcitonin (µg/L)	0.57 (0.03-100)	0.40 (0.01-49.05)	0.52 (0.01-75.0)	0.390
Urea (mg/dL)	64.5 (10.6-301)	77.0 (23.0-315)	114.6±77.2	**0.011ΦΦ**
Creatinine (mg/dL)	1.19 (0.58-7.70)	1.03 (0.52-8.60)	1.09 (0.52-6.84)	0.197

All controls were created one week apart.

*Post-hoc significant difference: Hemoglobin (1st) vs. hemoglobin (2nd), hemoglobin (1st) vs. hemoglobin (3rd).

**Post-hoc significant difference: Hematocrit (1st) vs. hematocrit (2nd), hematocrit (1st) vs. hematocrit (3rd).

†Post-hoc significant difference: ferritin (1st) vs. ferritin (3rd).

††Post-hoc significant difference: triiodothyronine (1st) vs. triiodothyronine (2nd), triiodothyronine (1st) vs. triiodothyronine (3rd).

≠Post-hoc significant difference: thyroxine (1st) vs. thyroxine (2nd), thyroxine (1st) vs. thyroxine (3rd).

≠≠Post-hoc significant difference: uric acid (1st) vs. uric acid (2nd), uric acid (1st) vs. uric acid (3rd).

ϕPost-hoc significant difference: CRP (1st) vs. CRP (2nd).

ΦΦPost-hoc significant difference: Urea (1st) vs. Urea (3rd).

ACTH: Adrenocorticotropic hormone, CRP: C-reactive protein, LDH: Lactate dehydrogenase, TSH: Thyroid-stimulating hormone, WBC: White blood cell

hospitalization were significantly lower in deceased patients compared to survivors (Table 5). On the other hand, serum TSH values at all time points were lower among deceased compared to survivors. However, only the second repeat TSH value difference reached statistical significance. There was no significant difference in serum basal cortisol and ACTH values between decedents and survivors except for second repeat serum cortisol values. Figure 1 shows changes of serum fT3 level throughout ICU stay in survivor and decedent groups.

In addition, deceased patients had significantly higher serum CRP and procalcitonin values compared to survivors.

We performed univariate and multivariate logistic regression analyses to evaluate independent predictors of mortality. In multivariate analysis, only serum fT3 level appeared as a significant predictor of all-cause mortality (Table 6). Age, comorbid conditions, serum cortisol, ACTH values, and inflammatory markers were not independent associates of mortality.

DISCUSSION

The salient findings of the present study were as follows: (1) ESS was very common among COVID-19 patients admitted to ICU (2). Serum fT3 level was comparable on admission; however, it remained significantly lower during ICU stay in decedents compared to survivors (3). Serum fT3 was the sole predictor of all-cause mortality among several factors, including inflammatory markers, kidney function, and comorbid conditions (4). One-five of our patients had hypocortisolism. However, hypocortisolism rates, serum cortisol, and ACTH levels were comparable between the decedents and survivors (5). Serum ACTH and cortisol levels were significantly different throughout the ICU stay between patients who were given and not given pulse steroids. Values

Table 3. Frequency of ESS in the whole cohort and patients who did not receive pulse steroid throughout ICU stay in the survivor and decedent groups

	Euthyroid sick syndrome	TFT not showing a specific pattern	p value
Whole cohort			
On admission (1st) (n=124)			
Survivors	36 (85.7%)	6 (14.3%)	0.361
Decedents	75 (91.5%)	7 (8.5%)	
Second (2nd) (n=103)			
Survivors	36 (92.3%)	3 (7.7%)	>0.999
Decedents	60 (93.8%)	4 (6.2%)	
Third (3rd) (n=57)			
Survivors	20 (95.2%)	1 (4.8%)	>0.999
Decedents	33 (91.7%)	3 (8.3%)	
Patients who did not receive pulse steroid			
On admission (1st) (n=67)			
Survivors	22 (84.6%)	4 (15.4%)	0.727
Decedents	36 (87.8%)	5 (12.2%)	
Second (2nd) (n=53)			
Survivors	21 (87.5%)	3 (12.5%)	0.649
Decedents	27 (93.1%)	2 (6.9%)	
Third (3rd) (n=29)			
Survivors	11 (100%)	0	0.512
Decedents	16 (88.9%)	2 (11.1%)	

P values belong to comparisons in which the frequency of ESS was compared between decedents and survivors.

ESS: Euthyroid sick syndrome, ICU: Intensive care unit

apart from admission might reflect the effects of pulse steroid administration on the hypothalamic adrenal axis (6). Coexistence of ESS and hypocortisolism was evident in 24 (19.4%) patients. However, this cooccurrence did not pose a mortality disadvantage.

A study by Świstek et al.[20] found that ESS was present in 38.1% of COVID-19 patients, with higher levels of inflammatory markers and mechanical ventilation need. The study found that ESS increased the risk of death by 3.1 times. ESS was also found to be a prognostic factor even in mild cases. Sparano et al.[21] found that the frequency of ESS was 57% among hospitalized COVID-19 patients. Serum fT3 level was found to be inversely related to inflammatory markers and an independent associate of mortality.

Although it might be associated with *de novo* development of Graves' disease and subacute thyroiditis, ESS remains the most common thyroid pathology in COVID-19 patients with serum fT3 levels significantly lower than non-severe patients[22]. ESS is an independent predictor of all-cause mortality in these

Table 4. Comparison of clinical features and admission laboratory data between groups stratified according to pulse steroid administration

	Pulse steroid administration		p value
	Not given (n=67)	Given (n=57)	
Age (years)	71.9±11.4	69.4±11.2	0.218
Sex			
Male	38 (56.7%)	34 (59.6%)	0.883
Female	29 (43.3%)	23 (40.4%)	
Comorbidities			
Diabetes mellitus	24 (35.8%)	16 (28.1%)	0.467
Thyroid disease	9 (13.4%)	6 (10.5%)	0.827
Hypertension	44 (65.7%)	35 (61.4%)	0.760
Dyslipidemia	4 (6.0%)	2 (3.5%)	0.686
Valvular heart disease	11 (16.4%)	4 (7.0%)	0.186
Coronary artery disease	14 (20.9%)	11 (19.3%)	>0.999
Malignancy	5 (7.5%)	1 (1.8%)	0.217
Cerebrovascular disease	13 (19.4%)	6 (10.5%)	0.264
Pulmonary disease	9 (13.4%)	12 (21.1%)	0.375
Chronic kidney disease	6 (9.0%)	4 (7.0%)	0.752
Inotropic drug administration	36 (53.7%)	32 (56.1%)	0.930
Need of mechanical ventilation	46 (68.7%)	46 (80.75)	0.186
Length of hospital stay (day)	10.8±9.3	13.4±13.8	0.219
Outcome			
Survivors	26 (38.8%)	16 (28.1%)	0.285
Decedents	41 (61.2%)	41 (71.9%)	
TSH (1st)	0.66 (0.01-39.8)	0.54 (0.05-3.39)	0.287
TSH (2nd)	0.56 (0.01-20.13)	0.33 (0.04-2.8)	**0.035**
TSH (3rd)	1.28 (0.03-33.14)	0.70 (0.02-1.96)	**0.022**
ACTH (1st)	13.74 (1.61-77.8)	5.85 (1.00-166.5)	**<0.001**
ACTH (2nd)	14.41 (2.05-68.0)	6.52 (1.58-74.34)	**0.002**
ACTH (3rd)	14.56 (3.07-69.0)	8.43 (1.0-31.46)	**0.008**
Cortisol (1st)	26.33±12.90	18.14±21.48	**0.014**
Cortisol (2nd)	20.96±11.74	15.06±13.85	**0.022**
Cortisol (3rd)	22.01±12.36	15.48±11.27	**0.042**

ACTH: Adrenocorticotropic hormone, TSH: Thyroid-stimulating hormone

patients[23]. Apparently, as the severity of COVID-19 increases, the frequency of ESS also increases[24]. In our cohort of patients, the frequency of ESS on admission was 89.5%. Different from the studies published to date, we also evaluated thyroid function during the course of the ICU stay. In our patients, the rate of ESS never descended below 90% during ICU stay. However, the frequency of ESS on admission was not statistically significant between the decedents and survivors. The patients who died in ICU had significantly lower fT3 levels compared to survivors. Serum fT4 values were significantly lower in decedents during hospitalization compared to survivors, and serum fT3 was found to be an independent and significant predictor of all-cause mortality.

During the critical illness, cortisol secretion normally increases as a stress response[24]. However, in some critically ill patients, this increase is not sufficient to meet the needs of the body.

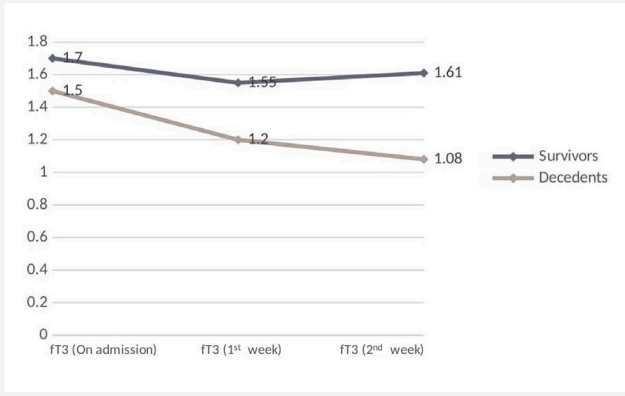

Figure 1. Changes of serum fT3 level throughout ICU stay in the survivor and decedent groups

ICU: Intensive care unit

Table 5. Comparison of clinical features and on admission laboratory data between groups stratified according to in-hospital mortality

	Survivors (n=42)	Decedents (n=82)	p value
Age (years)	68.4±11.8	71.9±11.0	0.114
Sex			0.669
Male	26 (61.9%)	46 (56.1%)	
Female	16 (38.1%)	36 (43.9%)	
Comorbidities			
Diabetes mellitus	15 (35.7%)	25 (30.5%)	0.699
Thyroid disease	4 (9.5%)	11 (13.4%)	0.735
Hypertension	27 (64.3%)	52 (63.4%)	>9.999
Dyslipidemia	4 (9.5%)	2 (2.4%)	0.178
Valvular heart disease	5 (11.9%)	10 (12.2%)	>9.999
Coronary artery disease	11 (26.2%)	14 (17.1%)	0.336
Malignancy	1 (2.45%)	5 (6.1%)	0.663
Cerebrovascular disease	8 (19.0%)	11 (13.4%)	0.575
Pulmonary disease	7 (16.7%)	14 (17.1%)	>9.999
Chronic kidney disease	3 (7.1%)	7 (8.5%)	>9.999
Pulse steroid administration	16 (38.1%)	41 (50.0%)	0.285
Inotropic drug administration	6 (14.3%)	62 (75.6%)	**<0.001**
Need of mechanical ventilation	10 (23.8%)	82 (100.0%)	**<0.001**
Length of hospital stay (day)	9 (2-60)	7.5 (1-55)	0.239
Free triiodothyronine (1st)	1.48 (0.68-6.0)	1.40 (0.52-3.46)	0.077
Free triiodothyronine (2nd)	1.55±0.37	1.20±0.31	**<0.001**
Free triiodothyronine (3rd)	1.61±0.34	1.08±0.36	**<0.001**
Free thyroxine (1st)	1.20±0.37	1.10±0.34	0.129
Free thyroxine (2nd)	1.16±0.33	0.99±0.37	**0.013**
Free thyroxine (3rd)	1.16±0.19	0.84±0.40	**<0.001**
TSH (1st)	0.84 (0.01-5.72)	0.54 (0.06-39.80)	0.214
TSH (2nd)	0.70 (0.05-15.74)	0.34 (0.01-20.13)	**0.017**

Table 5. Continued

	Survivors (n=42)	Decedents (n=82)	p value
TSH (3rd)	1.14 (0.05-3.02)	0.47 (0.02-33.14)	0.103
ACTH (1st)	9.79 (1.61-79.10)	9.76 (1.00-166.50)	0.782
ACTH (2nd)	14.23 (2.05-40.69)	9.93 (1.58-74.34)	0.446
ACTH (3rd)	11.48 (3.66-54.40)	10.93 (1.00-69.00)	0.418
Cortisol (1st)	19.27±10.67	24.26±20.35	0.076
Cortisol (2nd)	15.09±6.44	19.93±15.59	**0.031**
Cortisol (3rd)	18.63±9.02	18.91±13.82	0.925
CRP (1st)	138.3±83.5	178.4±99.9	**0.024**
CRP (2nd)	93.7±70.1	143.0±79.1	**0.001**
CRP (3rd)	122.2±77.9	165.8±83.4	0.053
Procalcitonin (1st)	0.30 (0.04-100.0)	0.84 (0.03-100.0)	**0.007**
Procalcitonin (2nd)	0.21 (0.01-6.10)	0.74 (0.08-49.05)	**<0.001**
Procalcitonin (3rd)	0.26 (0.01-75.0)	0.70 (0.06-44.25)	0.082

ACTH: Adrenocorticotropic hormone, CRP: C-reactive protein, LDH: Lactate dehydrogenase, TSH: Thyroid stimulating hormone, WBC: White blood cell

Table 6. Univariate and multivariate logistic regression analyses to determine independent predictors of in-hospital mortality in the whole cohort

	Univariate			Multivariate		
Parameters	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Age	1.028	0.994-1.063	0.107	-	-	-
Dyslipidemia	4.211	0.738-24.007	0.106	-	-	-
Ferritin	1.001	1.000-1.003	**0.033**	1.000	0.999-1.002	0.720
D-dimer	1.000	1.000-1.001	0.054	-	-	-
Free triiodothyronine	0.017	0.002-0.148	**<0.001**	0.959	0.929-0.989	**0.007**
Free thyroxine	0.057	0.008-0.412	**0.005**	0.994	0.965-1.024	0.708
Cortisol	1.035	0.996-1.074	0.077	--		-
Uric acid	1.194	0.928-1.535	0.167	-	-	-
CRP	1.005	1.000-1.009	**0.035**		--	-
Procalcitonin	1.631	1.087-2.446	**0.018**	0.152	0.742-6.827	2.251
Creatinine	2.010	1.102-3.665	**0.023**	0.747	0.423-1.853	0.886

Hosmer and Lemeshow test p=0.844 (for multivariate regression).
OR: Odds ratio, CI: Confidence interval

When a disorder of the hypothalamic-pituitary-adrenal (HPA) axis is present and not appropriately treated with high doses of hydrocortisone, acute stress may induce a life-threatening adrenal crisis. In the absence of adrenal insufficiency, stress doses of hydrocortisone are also used to lessen the requirement for vasopressors in patients suffering from septic shock. Research on the HPA axis during critical illness have led to the realization that neither of these disorders can be classified as "critical illness-induced corticosteroid insufficiency" or CIRCI. Instead, these results advised adopting the term CIRCI for a syndrome that may develop in individuals who are critically sick for an extended period of time. Patients who rely on organ support for weeks may develop central adrenal insufficiency due to increased systemic glucocorticoid availability, suppressed circulating cortisol-binding proteins, and suppressed hepatic/renal cortisol metabolism. This negative feedback inhibition at the hypothalamus/pituitary is exacerbated by high levels of other glucocorticoid receptor ligands and drugs like opioids. The adrenal cortex may become physically and functionally damaged, leading to inadequate cortisol production and potentially contributing to persistent vasopressor demand and encephalopathy, impeding recovery[25]. Patients with adrenal crisis usually present with nonspecific symptoms and signs, including severe fatigue, weakness, myalgia, postural dizziness, nausea, vomiting, abdominal pain, and fever[26]. These symptoms are also common in an acute COVID-19 infection, and it might be difficult to differentiate between the features of acute adrenal crisis and acute COVID-19 infection. Since

adrenal insufficiency clinic and COVID-19 clinical findings can be confused with each other, we created a treatment plan for relative adrenal insufficiency by combining the cortisol cut-off value with clinical findings. Given the dynamic nature of the pituitary-adrenal axis, single measurements of serum cortisol cannot fully explain the adrenal reserve. Some studies accepted a serum cortisol level below 10 µg/dL as a hypocortisolism response to critical illness[19]. Das et al.[24] found that among patients with severe and mild COVID-19, hypocortisolism rates were 38.5% and 6.8%, respectively. In our study, the frequency of hypocortisolism was 20.1%, which was lower than the findings of Das et al.[24], because Das et al.[24] used 15 µg/dL as the cut-off value in their study, unlike the 10 µg/dL we used in our study. Patients with severe disease had significantly lower serum ACTH values compared to patients with mild COVID-19[24]. Another case-control study involving noncritical patients hospitalized for COVID-19 revealed that, compared to normal subjects, serum levels of ACTH were significantly lower, and cortisol was higher in COVID-19 patients[27]. A significant number of our patients received pulse steroid treatment as part of COVID-19 treatment guidelines. However, admission values reflected the steroid-naive status before pulse steroid administration. Hypocortisolism frequencies were comparable between the survivors and decedents. Moreover, neither serum cortisol nor serum ACTH values were independent predictors of all-cause mortality. There was no correlation between serum fT3 and cortisol values, either.

Study Limitations

Some limitations of the current study are worthy of mention. First, our sample size was not large, and this might have led to a failure to unravel subtle differences in thyroid hormone and adrenal hormone levels. Moreover, ESS was almost universal among our patients, and this precludes us from meaningfully comparing patients with and without ESS in terms of study outcomes. Second, pulse steroid administration in a significant portion of patients might affect adrenal axis evaluation during the hospitalization period. It might also have impacted the thyroid axis to some extent. However, several strengths of this study make our results contributive to the current literature. For the first time, we evaluated the combined prognostic impact of thyroid and adrenal axes on the prognosis of critically ill COVID-19 patients. Second, we also evaluated hormone values throughout the hospitalization but not at a single time point, as was the case in the previous studies in the literature.

CONCLUSION

In conclusion, ESS was prevalent in critically ill COVID-19 patients. One-five of all patients also had hypocortisolism. Although the frequencies of ESS and hypocortisolism were similar between survivors and decedents, patients who died during ICU stay had significantly lower levels of serum fT3. In addition, fT3, but not serum cortisol and ACTH levels, was an independent predictor of all-cause mortality.

Ethics

Ethics Committee Approval: Approval was granted by the Ethics Committee of Karamanoğlu Mehmetbey University (date: 07/12/2020, no: 02-2020/04).

Informed Consent: Retrospective study.

Authorship Contributions

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

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Investigation of Antibacterial Activity of Curcumin and Synergistic Effect with Gentamicin Sulfate

Kurkuminin Antibakteriyel Aktivitesinin ve Gentamisin Sülfat ile Sinerjistik Etkisinin Araştırılması

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ABSTRACT

Aim: In this study; it was aimed to examine the antibacterial activities and synergistic effects of curcumin, a phytotherapeutic agent, and gentamicin sulfate on bacteria.

Materials and Methods: Antibacterial activity of different concentrations of curcumin and gentamicin sulfate on *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 3851, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 25923 minimal inhibitory concentration (MIC) and tested by disc diffusion methods. The synergistic effects of combinations of curcumin and gentamicin sulfate were examined by checkerboard test.

Results: It was found that antibacterial activity was seen in all bacteria and the lowest MIC was 7.81 µg/mL in *E. faecalis* for curcumin and 0.08 µg/mL in *K. pneumoniae* for gentamicin sulfate. As a result of the disk diffusion test, inhibition zone diameters were detected at concentrations of 32 and 16 mg/mL in all bacteria tested. As a result of the checkerboard test, an additive effect was observed in four of the bacteria (*P. vulgaris*, *B. cereus*, *L. monocytogenes*, *S. aureus*) and a indifferent effect was observed in three of them (*P. aeruginosa*, *K. pneumoniae*, *E. faecalis*). The finding of the lowest fractional inhibitor concentration index (FICI=0.75) in *B. cereus*, one of the gram-positive bacteria, was interpreted as the combination of curcumin and gentamicin sulfate having a partial synergistic effect.

Conclusion: This study is the first to evaluate the synergistic effect of curcumin and gentamicin sulfate. Antibacterial activity results suggest that curcumin can be used as an alternative agent in the treatment of bacterial infections. However, in order to clearly determine the effect of both the antibacterial activity of curcumin and its synergy with gentamicin sulfate during the treatment process, these results need to be supported by large-scale *in vitro* and *in vivo* studies that will include clinical isolates.

Keywords: Curcumin, gentamicin sulfate, antibacterial activity, synergistic effect, checkerboard test

ÖZ

Amaç: Bu çalışmada; fitoterapötik bir ajan olan kurkuminin ve gentamisin sülfatın bakteriler üzerine antibakteriyel aktiviteleri ile sinerjistik etkilerinin incelenmesi amaçlandı.

Gereç ve Yöntem: Kurkumin ve gentamisin sülfatın farklı konsantrasyonlarının *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 3851, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644 ve *Staphylococcus aureus* ATCC 25923 üzerine antibakteriyel aktivitesi minimal inhibitör konsantrasyon (MİK) ve disk difüzyon metodları ile test edildi. Kurkumin ile gentamisin sülfatın kombinasyonlarının sinerjistik etkileri dama tahtası testi ile incelendi.

Bulgular: Antibakteriyel aktivitenin tüm bakterilerde görüldüğü ve en düşük MİK'in kurkumin için *E. faecalis*'te 7,81 µg/mL, gentamisin sülfat için ise *K. pneumoniae*'da 0,08 µg/mL olduğu bulundu. Disk difüzyon testi sonucu test edilen tüm bakterilerde 32 ve 16 mg/mL konsantrasyonlarda inhibisyon zon çapı tespit edildi. Dama tahtası testi sonucu bakterilerin dördünde (*P. vulgaris*, *B. cereus*, *L. monocytogenes*, *S. aureus*) additif,

Note: This study constitutes the master's thesis of the primary author entitled 'Investigation of Antibacterial Activity of Curcumin and Synergistic Effect with Gentamicin Sulfate' within the Medical Microbiology Program at Tekirdağ Namık Kemal Institute University of Health Sciences.

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üçünde (*P. aeruginosa*, *K. pneumoniae*, *E. faecalis*) indiferans etki görüldü. Gram-pozitif bakterilerden *B. cereus*'ta en düşük fraksiyonel inhibitör konsantrasyon indeksinin (FİKİ=0,75) bulunması kurkumin ile gentamisin sülfat kombinasyonunun kısmi sinerjistik etkili olduğu şeklinde yorumlandı.

Sonuç: Bu çalışma kurkumin ile gentamisin sülfatın sinerjistik etkisinin değerlendirildiği ilk çalışmadır. Antibakteriyel aktivite sonuçları, kurkuminin bakteriyel enfeksiyonların tedavisinde alternatif bir ajan olarak kullanılabileceğini düşündürmektedir. Ancak hem kurkuminin antibakteriyel etkinliğinin hem de gentamisin sülfat ile sinerjisinin tedavi sürecindeki etkisini net olarak belirlemek adına bu sonuçların klinik izolatların dahil edileceği geniş ölçekli *in vitro* ve *in vivo* çalışmalarla desteklenmesi gerekmektedir.

Anahtar Kelimeler: Kurkumin, gentamisin sülfat, antibakteriyel aktivite, sinerjistik etki, dama tahtası testi

INTRODUCTION

Bacterial infections are one of the major causes of chronic infections and mortality. Antibiotics used in the treatment of these infections are preferred due to their potent effects. However, it is also known that the widespread use of antibiotics leads to the emergence of multidrug resistant (MDR) bacterial strains[1]. In recent years, the increase in infections caused by resistant strains has attracted attention. MDR bacteria show resistance to three or more classes of antibiotics. High morbidity and mortality rates are observed in diseases caused by these bacteria[2].

In order to prevent the increase in antibiotic resistance, there is a need for antimicrobial compounds that can be used as an alternative to conventional antibiotic therapy. This has led to the discovery of new natural or synthetic antimicrobial compounds[3]. The side effects of synthetic drugs have led to a growing interest in natural plant-derived antimicrobial agents and a growing interest in treating infections naturally[4]. Natural products are also being investigated in combination therapies to manage antibiotic resistance. Synergistic studies are expected to be important in the future to overcome antimicrobial resistance[5].

Curcumin is a food spice that is a natural component of *Curcuma longa* (turmeric, turmeric) rhizomes. It has been widely used as a medicine in the treatment of various diseases in Asian and Middle Eastern countries for years[6]. Curcumin, also known as turmeric, has been shown to have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and anticancer activities[7-9]. In the society, it is known to be used for therapeutic purposes against various malignant diseases, diabetes, arthritis, gastritis, urinary tract infections, skin diseases and other chronic diseases[10]. Studies have shown that combinations of curcumin with different agents, including various antibiotics, have synergistic effects against bacteria[11-13].

The aim of this study was to determine the antibacterial activities of curcumin and gentamicin sulfate, an antimicrobial drug, against various Gram-positive and Gram-negative bacteria. It was also planned to investigate the synergistic effects of gentamicin sulfate, which is used in clinical applications, when combined with a phytotherapeutic agent such as curcumin as well as its effects alone.

MATERIALS AND METHODS

This study was reviewed and approved by Tekirdağ Namık Kemal University Non-interventional Clinical Research Ethics Committee (approval no: 2022.68.04.18, date: 26.07.2022).

Bacteria Strains

Gram-negative bacteria of *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 3851 and Gram-positive bacteria of *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923 strains were used in the study. Before each experiment, bacterial strains were inoculated on 5% sheep blood agar (BESLAB, Turkey) and incubated at 37 °C for 18-20 hours.

Preparation of Curcumin and Gentamicin Sulfate Stock Solutions

Curcumin (Sigma-Aldrich, USA) was weighed 0.00154 g at a concentration of 1000 µg/mL, dissolved in 200 µL 0.5 M sodium hydroxide and 800 µL phosphate buffered saline, filtered and diluted with Mueller Hinton Broth (Himedia, India). Gentamicin sulfate (Biological Industries, Israel) was diluted 1:5000 at a stock concentration of 10 µg/mL and dilutions were prepared.

Determination of Antibacterial Activity

Minimal Inhibitory Concentration Test

Minimal inhibitory concentration (MIC) values for each bacterial strain were performed by liquid microdilution method according to EUCAST recommendations[14]. Serial dilutions of curcumin (1000-1.95 µg/mL) and gentamicin sulfate (10-0.019 µg/mL) were performed in 96-well U-bottom microplates. Bacterial suspensions were diluted to $5x10^5$ CFU/mL and 10 µL each was added to the wells. Positive (growth) and negative (sterility) control wells were also prepared in microplates. Microplates were incubated at 35 ± 1 °C for 18-20 hours. The results were evaluated visually and by spectrophotometer reading. The lowest concentration without growth was considered as the MIC value. Each test was repeated three times.

Minimum Bactericidal Concentration Test

Bacterial concentrations where no growth was observed at the end of incubation were evaluated as minimum bactericidal concentration (MBC) value. In the minimum bactericidal concentration test study, firstly, the wells for which the MIC test was completed and no growth was observed were identified. 2 µL of Mueller Hinton Agar (Merck, Germany) was inoculated from the non-growing wells. The medium plates were incubated at 35±1 °C for 18-20 hours. Bacterial concentrations at the end of incubation were considered as MBC values.

Disc Diffusion Test

Antibacterial activities of curcumin and gentamicin sulfate were determined using disk diffusion test[15]. 0.5 McFarland bacterial suspensions were inoculated on Mueller Hinton Agar. Dilutions of curcumin at concentration ranges of 32-2 mg/mL and gentamicin sulfate at different concentrations selected according to the results of the MIC test were impregnated on 6 mm blank disks (Bioanalyse, Turkey) in 100 µL. Gentamicin (10 µg/mL, Bioanalyse, Turkey) disk was used as a positive control. Petri dishes were then incubated at 35±1 °C for 18-20 hours. Inhibition zone diameters (mm) were measured at the end of incubation. Each test was repeated three times.

Checkerboard Test

The synergistic effect between curcumin and gentamicin sulfate was demonstrated with the checkerboard test[16]. Concentrations were determined at four times above (x16 MIC) and three times below (1/8 MIC) the MIC value. Serial dilutions were prepared with curcumin on the vertical axis and gentamicin sulfate on the horizontal axis. In 96-well U-bottom microplates, 100 µL combinations of curcumin (50 µL) and gentamicin sulfate (50 µL) were prepared in each well. The prepared 0.5 McFarland bacterial suspensions were diluted 1:10 and 5 µL was added to each well to ensure a final bacterial concentration of $5x10^5$ CFU/mL. Wells for positive (growth) and negative (sterility) controls were also prepared in microplates. Microplates were incubated at 35±1 °C for 18-20 hours. The results were evaluated visually and spectrophotometrically at 490-630 nm.

The synergy relationship between curcumin and gentamicin sulfate was calculated as fractional inhibitor concentrations (FIC) sums and fractional inhibitor concentration index (FICI) as follows.

$$FIC_{CURCUMIN} = \frac{\text{MIC of curcumin in combination}}{\text{MIC of curcumin alone}}$$

$$FIC_{GENTAMICIN\ SULFATE} = \frac{\text{MIC of gentamicin sulfate in combination}}{\text{MIC of gentamicin sulfate alone}}$$

$$FICI = \frac{\text{MIC of curcumin in combination}}{\text{MIC of curcumin alone}} + \frac{\text{MIC of gentamicin sulfate in combination}}{\text{MIC of gentamicin sulfate alone}}$$

Synergy between curcumin and gentamicin sulfate was evaluated by calculating the FICI. If FICI ≤0.5, it was interpreted as synergistic effect; if 0.5 < FICI ≤1, it was interpreted as additive effect; if 1 < FICI ≤4, it was interpreted as differential effect; and if FICI >4, it was interpreted as antagonistic effect[16].

Statistical Analysis

Statistical analysis of the data obtained for the disk diffusion test applied in the study was performed with the GraphPad Prism 8 program. Mean±standard deviation values of inhibition zone diameters were calculated in these experiments performed in triplicate.

RESULTS

Antibacterial Activity Results

In the MIC test, the antibacterial activities of curcumin (1000-1.95 µg/mL) and gentamicin sulfate (10-0.019 µg/mL) were tested on standard reference strains. The results of the MIC and MBC tests are shown in Table 1. It was determined that curcumin was effective in all bacteria tested and generally showed activity at a concentration of 62.5 µg/mL. The antibacterial activity of curcumin was highest in the Gram-positive bacteria

Table 1. MIC and MBC values of curcumin and gentamicin sulfate

Bacteria strains	Curcumin		Gentamicin sulfate	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
P. aeruginosa	62.5	125	0.16	0.31
K. pneumoniae	62.5	125	0.08	0.08
P. vulgaris	62.5	62.5	0.16	0.16
B. cereus	62.5	125	0.31	0.31
E. faecalis	7.81	31.25	2.5	5
L. monocytogenes	62.5	62.5	0.31	0.31
S. aureus	62.5	62.5	0.31	0.31

MIC: Minimal inhibitory concentration, MBC: Minimum bactericidal concentration

E. faecalis (7.81 µg/mL). For gentamicin sulfate, antibacterial activity was found at different concentrations in the tested bacteria. MIC and MBC results were obtained at a concentration of 0.31 µg/mL in three bacteria (*B. cereus*, *L. monocytogenes*, *S. aureus*). The most sensitive bacteria to gentamicin sulfate was *K. pneumoniae*, while the most resistant bacteria was *E. faecalis*. At the same time, it was determined that the MIC and MBC results of gentamicin sulfate were compatible (except *E. faecalis*).

Disk diffusion test showed that curcumin showed antibacterial activity at concentrations of 32 and 16 mg/mL. Similar zone diameters were obtained at both concentrations. The most sensitive bacteria to curcumin were *K. pneumoniae*, *E. faecalis* and *L. monocytogenes*, which formed inhibition zone diameter at 4 mg/mL concentration (Table 2).

As a result of the disk diffusion test, the most sensitive bacteria to gentamicin sulfate were *P. aeruginosa*, *B. cereus*, *L. monocytogenes* and *S. aureus*. The lowest concentration effective against these bacteria was 0.63 µg/mL. The most resistant bacteria to gentamicin sulfate was *E. faecalis* (Table 3).

Checkerboard Test Results

Checkerboard test was used to evaluate the synergistic effect between curcumin and gentamicin sulfate. The MIC values of gentamicin sulfate alone and in combination with curcumin are given in Table 4. It was observed that the combination of curcumin and gentamicin sulfate decreased the MIC values in *P. vulgaris*, *B. cereus*, *L. monocytogenes* and *S. aureus* bacteria (Table 4).

The FIC and FICI values of the bacterial strains tested in the study are given in Table 5. Among the Gram-negative bacteria tested, 1 showed additive effect and 2 showed differential effect, while 3 gram-positive bacteria showed additive effect and 1 showed differential effect. The lowest FICI value (0.75)

Table 2. Inhibition zone diameters formed at curcumin concentrations (mm)

Bacteria strains	Inhibition zones (mm) (mean±standard deviation)				
	Curcumin				Gentamicin
	32 mg/mL	16 mg/mL	8 mg/mL	4 mg/mL	10 µg/mL
P. aeruginosa	7.17±0.08	7.11±0.08	7.02±0.08	NZ	21.17±0.07
K. pneumoniae	8.15±0.05	7.13±0.05	7.06±0.06	7.02±0.01	23.02±0.11
P. vulgaris	9.83±0.10	8.84±0.04	8.65±0.05	NZ	23.02±0.13
B. cereus	8.11±0.06	8.04±0.08	NZ	NZ	22.02±0.09
E. faecalis	9.13±0.03	8.14±0.06	8.11±0.04	7.07±0.04	20.06±0.14
L. monocytogenes	9.12±0.12	8.07±0.09	8.03±0.03	7.05±0.03	21.12±0.12
S. aureus	8.16±0.07	8.02±0.06	NZ	NZ	23.12±0.11

NZ: No zone

Table 3. Inhibition zone diameters formed at gentamicin sulfate concentrations (mm)

Bacteria strains	Inhibition zones (mm) (mean±standard deviation)				
	Gentamicin sulfate concentrations				Gentamicin
P. aeruginosa	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**0.16 µg/mL**	**10 µg/mL**
	10.04±0.12	7.25±0.14	NZ	NZ	23.01±0.11
K. pneumoniae	**0.63 µg/mL**	**0.31 µg/mL**	**0.16 µg/mL**	**0.08 µg/mL**	**10 µg/mL**
	7.14±0.14	NZ	NZ	NZ	22.04±0.10
P. vulgaris	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**0.16 µg/mL**	**10 µg/mL**
	9.12±0.12	NZ	NZ	NZ	22.16±0.17
B. cereus	**2.5 µg/mL**	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**10 µg/mL**
	14.11±0.10	11.04±0.14	8.16±0.15	NZ	22.03±0.13
E. faecalis	**20 µg/mL**	**10 µg/mL**	**5 µg/mL**	**2.5 µg/mL**	**10 µg/mL**
	17.12±0.12	13.21±0.19	12.01±0.13	NZ	20.12±0.11
L. monocytogenes	**2.5 µg/mL**	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**10 µg/mL**
	14.02±0.12	11.14±0.14	7.28±0.12	NZ	22.91±0.10
S. aureus	**2.5 µg/mL**	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**10 µg/mL**
	15.01±0.11	12.53±0.13	8.21±0.10	NZ	23.17±0.06

NZ: No zone

Table 4. MIC values of gentamicin sulfate alone and in combination with curcumin

Bacteria strains	Gentamicin sulfate	
	MIC value alone (µg/mL)	MIC value in combination (µg/mL)
P. aeruginosa	0.16	0.16
K. pneumoniae	0.08	0.16
P. vulgaris	0.16	0.08
B. cereus	0.31	0.08
E. faecalis	2.50	5
L. monocytogenes	0.31	0.16
S. aureus	0.31	0.16

MIC: Minimal inhibitory concentration

Table 5. FIC and FICI values as a result of checkerboard test

Bacteria strains	$FIC_{CURCUMIN}$	$FIC_{GENTAMICIN\ SULFATE}$	FICI	Interaction
P. aeruginosa	0.02	1	1.02	Indifference effect
K. pneumoniae	0.13	2	2.13	Indifference effect
P. vulgaris	0.50	0.50	1	Additive effect
B. cereus	0.50	0.25	0.75	Additive effect
E. faecalis	0.03	2	2.03	Indifference effect
L. monocytogenes	0.50	0.50	1	Additive effect
S. aureus	0.50	0.50	1	Additive effect

FIC: Fractional inhibitor concentrations, FICI: Fractional inhibitor concentration index

in *B. cereus*, a Gram-positive bacterium, suggests that the combination of curcumin and gentamicin sulfate has a partial synergistic effect on this bacterium.

DISCUSSION

The most common treatment for bacterial infections is the use of antibiotics[17]. However, due to the increase in antibiotic resistance in recent years, there is a need to discover new therapeutic agents with strong antibacterial activity[11]. Curcumin is a natural agent whose therapeutic effects have been investigated due to its various biological and medicinal properties[18]. In this study investigating the antibacterial activity of curcumin on some standard strains, it was found that curcumin showed antibacterial activity at a concentration of 62.5 µg/mL against all tested bacteria except *E. faecalis* (7.81 µg/mL). The results of this study and similar studies also emphasize the antibacterial activity of curcumin[19,20]. In a study by Ungphaiboon et al. (2005)[19], in which they investigated the antibacterial activity of curcumin extracts obtained from *Curcuma longa* L. rhizomes on various microorganisms, they found the MIC values of curcumin as 16 and 128 µg/mL for *Bacillus subtilis* NCTC 10073 and *S. aureus* ATCC 25923, respectively. On the other hand, they could not detect any activity against *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Anbari et al. (2021)[20] examined the antibacterial effects of curcumin nanoparticles and pure curcumin on S. *aureus* PTCC 1764. They found that the MIC values of curcumin nanoparticles were in the range of 0.48-0.34 mg/mL, while pure curcumin was 0.56 mg/mL. Compared to pure curcumin, they found that the MIC values of curcumin nanoparticles were lower than pure curcumin due to their smaller size and easier penetration into bacteria. In the studies, it is seen that the MIC values differ in each study due to the preparation of curcumin by different methods, the use of different methods for the determination of antibacterial activity and testing against different microorganism species.

It is known that gentamicin sulfate, an aminoglycoside group, is an agent that shows broad antibacterial activity with low MIC values against Gram-positive and Gram-negative bacteria[21]. In this study, although the MIC values of gentamicin sulfate varied in the bacteria studied, the most resistant bacteria was *E. faecalis* (2.5 µg/mL) and the most sensitive bacteria was *K. pneumoniae* (0.08 µg/mL). At the same time, the MBC results were similar to the MIC results (except for *E. faecalis*). In a similar study, Arisoy et al. (2013)[22] found that the highest gentamicin sulfate MIC values were found in ESBL-producing *Escherichia coli* (1024 µg/mL) and *K. pneumoniae* (256 µg/mL). Dorati et al.[23] (2018) examined the antibacterial activity of gentamicin sulfate and gentamicin sulfate-loaded nanoparticles in clinical isolates. In the study,

they determined the MIC and MBC test results of gentamicin sulfate as 2/4 µg/mL for *Escherichia coli*, 1/2 µg/mL for *P. aeruginosa*, 4/8 µg/mL for *Proteus mirabilis*, 1/1 µg/mL and 8/16 µg/mL for two S. *aureus* isolates. For the reference strain *Escherichia coli* ATCC 25922, the MIC/MBK values were 0.5/0.5 µg/mL. As a result of the study, they concluded that there was no difference between the MIC/MBK test results of gentamicin sulfate and gentamicin sulfate loaded nanoparticles.

Disk diffusion test is another method used to investigate antibacterial activity. In the literature, there are limited number of studies investigating the antibacterial activity of curcumin by disk diffusion method[6,24]. In this study, disk diffusion test was applied in addition to MIC test. The first two highest concentrations of curcumin, 32 and 16 mg/mL, were found to produce zone diameters of inhibition in all bacteria tested, while large zone diameters were observed at low concentrations of gentamicin sulfate. The limited mobility of bacteria in the disk diffusion test compared to liquid culture in the MIC test may cause the results of these two tests to be different[25]. Khan et al. (2021)[6] investigated the antimicrobial activities of curcumin nanoparticles and its aqueous extract (5, 10, 20 mg/mL) by disk diffusion method and used *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC BAA-1705, *P. aeruginosa* ATCC 27853, *S. aureus A*TCC 23235 and *Aspergillus niger* FCBP-PTF0198 strains. They found that the most effective concentration of curcumin nanoparticles and aqueous extract was 20 mg/mL and at this concentration, nanoparticle inhibition zone diameters were higher than aqueous curcumin.

It is thought that synergistic studies will play an important role in the future of antibiotic therapies to overcome antibacterial resistance and prevent new resistance mechanisms[5]. In recent years, the combined use of natural products and antibiotics in the treatment of infectious diseases has come to the fore in order to expand the pool of therapeutic agents and manage emerging resistance. It has been reported that the combination of curcumin, a natural product, with antibiotics increases the membrane permeability of the bacterial cell and facilitates the entry of antibiotics into the cell[18]. In this study, the combination of curcumin with gentamicin sulfate decreased the MIC values in *P. vulgaris, B. cereus, L. monocytogenes* and *S. aureus* bacteria. In addition, the lowest FICI result (FICI=0.75) in *B. cereus* was interpreted as a partial synergistic effect of curcumin and gentamicin sulfate. In similar studies, it has been shown that combinations of curcumin with different antibiotics are antibacterial and these combinations can be used for therapeutic purposes[13,26]. Bahari et al. (2017)[13] investigated the synergistic activity of curcumin with azithromycin and gentamicin against *P. aeruginosa* (PAO1) by checkerboard test and found that the MIC values of the antibiotics alone were higher than the MIC values of the combination with curcumin. As a result of the study, they found that there was a synergistic effect between curcumin and azithromycin (FICI=0.25) and gentamicin (FICI=0.37). Mun et al. (2013)[26] showed that the combination of curcumin with oxacillin, ampicillin, ciprofloxacin and norfloxacin against eight clinical and two reference MRSA strains reduced the MIC values of these antibiotics and showed synergy/partial synergy between them and curcumin. In studies with curcumin, it is seen that the synergistic effect results vary according to the strain and antimicrobial agent used.

This study shows that curcumin alone or in combination with gentamicin sulfate has antimicrobial activity. However, these results need to be supported by large-scale *in vitro* and *in vivo* studies including clinical isolates to clearly determine the effect of curcumin and gentamicin sulfate synergy in the treatment process of bacterial infections.

Study Limitations

The limitation of this study is that the synergistic effects of curcumin and gentamicin sulfate on bacteria isolated from clinical samples were not investigated.

CONCLUSION

In conclusion, the use of different natural therapeutic agents in combination with antibiotics in the empirical treatment of bacterial infections seems promising in terms of reducing the toxicity of antibiotics and increasing their antimicrobial efficacy.

Ethics

Ethics Committee Approval: This study was reviewed and approved by Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (approval no: 2022.68.04.18, date: 26.07.2022).

Informed Consent: It is a laboratory study that does not require patient consent.

Authorship Contributions:

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

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Prognostic Value of NTCP p.Ser267Phe Variant in Patients with Chronic Hepatitis B

Kronik Hepatit B'li Hastalarda NTCP p.Ser267Phe Varyasyonun Prognostik Değeri

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ABSTRACT

Aim: In this study, the aim is to detect polymorphisms in the gene encoding the sodium taurocholate cotransporting polypeptide (NTCP), the functional receptor for hepatitis B virus (HBV) and hepatitis D virus.

Materials and Methods: The study included a total of 293 patients, with 150 diagnosed with chronic HBV (CHB) and 143 undergoing liver parenchyma biopsy procedures due to different clinical indications. Total DNA was isolated from liver biopsy samples. The TaqMan SNP genotyping method was used to determine the rs2296651 polymorphism in the *SLC10A1* gene, which leads to the NTCP S267F variation.

Results: In patients with CHB and the control group, the NTCP-interacting domain was highly conserved, and no variation of the SNP rs2296651 in the *SLC10A1* gene leading to the NTCP S267F variation was detected in any of the patients.

Conclusion: It was thought that in patients with CHB, the impact of the NTCP S267F variation on the progression of HBV-associated diseases and its influence on the therapeutic efficacy of anti-viral agents targeting NTCP blockade may be limited.

Keywords: Hepatitis B virus, chronic hepatitis B, single nucleotide polymorphism, sodium taurocholate co-transporting polypeptide

ÖZ

Amaç: Bu çalışmada hepatit B virüsün (HBV) ve hepatit D virüs fonksiyonel reseptörü sodyum taurokolat kotransporter polipeptitini (NTCP) kodlayan gendeki polimorfizmlerin tespiti amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmaya 150'si kronik HBV (KHB) ve 143'ü farklı klinik endikasyonlar nedeniyle karaciğer parankim biyopsisi işlemi gerçekleştirilen toplam 293 hasta dahil edildi. Karaciğer biyopsi örneklerinden total DNA izole edildi. NTCP S267F varyasyonuna sebep olan *SLC10A1* genindeki rs2296651 polimorfizminin belirlenmesinde TaqMan SNP genotiplendirme yöntemi kullanıldı.

Bulgular: KHB hastaları ve kontrol grubunda NTCP-etkileşim domaini oldukça iyi korunmakta olup, hiçbir hastada NTCP S267F varyasyona sebep olan *SLC10A1* genindeki SNP rs2296651 varyasyonuna rastlanmadı.

Sonuç: KHB'li hastalarda NTCP S267F varyasyonunun HBV ilişkili hastalıkların progresyonu ile NTCP blokajını hedef alan anti-viral terapötiklerin tedavi etkinliğini üzerindeki etkisinin sınırlı olabileceği düşünüldü.

Anahtar Kelimeler: Hepatit B virüs, kronik hepatit B, tek nükleotid polimorfizmi, sodyum taurokolat kotransporter polipeptiti

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INTRODUCTION

Although the development of cirrhosis and liver cancer, which may be related to hepatitis B virus (HBV), can be prevented with current treatments, it remains an important public health problem because there is no treatment that will ensure complete viral clearance and it may cause approximately one million people to lose their lives worldwide every year[1]. Chronic HBV (CHB) infections are a dynamic process shaped by the interaction of viral factors belonging to the virus, host factors belonging to the infected individual and environmental factors. Therefore, viral factors such as genotype and mutations, genetic and immunologic factors of the infected individual and environmental factors are critical determinants of the natural course of chronic viral hepatitis, the development and prognosis of end-stage liver diseases such as cirrhosis and hepatocellular carcinoma (HCC), and treatment responses[2,3].

Sodium taurocholate cotransporter polypeptide (NTCP) is a functional receptor for human HBV and its satellite virus hepatitis D virus (HDV)[4]. NTCP is a cell surface glycoprotein localized on chromosome 14, encoded by the *SLC10A1* gene, the number one member of the solute carrier family 10 (sodium/bile acid cotransporter family, SLC10) and involved in the enterohepatic circulation of bile salts expressed by hepatocytes[5]. In this respect, the entry step involving the interaction of the viral surface protein (surface) PreS1 with the PreS1-specific receptor NTCP on the hepatocyte surface and subsequent transfer into the cell via endocytosis is of critical importance for HBV infections. Therefore, NTCP constitutes one of the new therapeutic targets for preventing the binding and entry of the virus into hepatocytes for anti-HBV treatment[6-8].

On the other hand, it has been reported that the p.Ser267Phe (S267F) polymorphism (single nucleotide polymorphism; SNP) detected on the *NTCP* gene, especially p.Ser267Phe (S267F) polymorphism, may cause changes in the physiological function of NTCP, leading to a decrease in HBV entry into the cell and infection load. Therefore, it is hypothesized that these genetic variants of NTCP may be associated with resistance to HBV infection and the risk of developing HBV infection-related liver cirrhosis and HCC. It is also suggested that sequence differences in the NTCP interaction domain (HBV PreS1) may negatively affect treatment responses to HBV entry inhibitors designed for anti-HBV therapy by causing changes in NTCP binding affinity[9-15].

Therefore, the aim of this study was to identify polymorphisms in the gene encoding the HBV and HDV functional receptor NTCP and to evaluate the effects of variation of the NTCP S267 polymorphism on the clinical outcomes of patients and the prognostic impact of new therapeutics targeting NTCP in predicting treatment efficacy.

MATERIALS AND METHODS

This study was supported by the Scientific and Technical Research Council of Turkey (TÜBİTAK) project number 218S769. Ethical approval of the study was granted by the Ethics Committee of İstanbul University, İstanbul Faculty of Medicine (no: 2018/1251, date: 14.09.2018).

Selection and Identification of the Cases

Patient Study Group

In this study, 150 treatment-naive patients with CHB who were diagnosed with CHB infection due to HBsAg seropositivity for more than 6 months and who were followed up by the Gastroenterohepatology Clinic of İstanbul University, İstanbul Medical Faculty, and in whom liver parenchymal biopsy was planned for histologic and clinical evaluation were included (Table 1).

Control Group

This study included 63 patients diagnosed with chronic hepatitis C infection and 80 patients who underwent liver parenchymal biopsy for different kinetic indications other than viral hepatitis agents (non-viral) by the Gastroenterohepatology Clinic of İstanbul University, İstanbul Medical Faculty (Table 1).

Clinical Material

Liver biopsy samples were obtained from the patients. Liver biopsy procedures were performed in the Gastroenterohepatology Clinic and interventional radiology units of İstanbul Medical Faculty. Total DNA samples obtained from liver biopsy specimens using a commercial kit (QIAamp DNA Mini kit, Qiagen GmbH, Hilden, Germany) were stored at -20 °C for SNP genotyping and sequence analysis to detect viral mutations.

SNP Genotyping (SLC10A1/rs2296651)

Genotype Analysis

Diagnosis and analysis of SNPs from human DNA samples isolated intrahepatically from patients' liver tissue samples were performed using a real-time PCR (RT-PCR) device and method provided by the manufacturer (Applied Biosystems, Foster City, CA, USA). TaqMan SNP genotyping method was used for genotyping the NTCP S267F variant (c.800G>A, SLC10A1/rs2296651). For SNP analyses, StepOne Software programs were used for amplification curves obtained from TaqMan SNP genotyping assay and allelic discrimination.

Demographic and clinical laboratory data: Demographic, other clinical laboratory and pathologic data were obtained from patient files and/or the electronic data recording system of the hospital.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) (16.0 software, SPSS Inc., Chicago, IL). Chi-square test and/or Fisher's tests were used to compare categorical variables between data, and Mann-Whitney U and one-way ANOVA tests were used to compare non-categorical data. Results were expressed as mean and standard deviation. P<0.05 values were accepted as statistically significant differences.

RESULTS

Characteristics of Patients

Demographic, clinical and histological data of the patients included in the study are summarized in Table 1. In summary, 150 CHB patients, 78 males and 72 females, with a mean age of 41.63±13.17 years, were included in this study. The mean necroinflammatory activity (grade) level was 4.96±2.8 and the mean fibrosis stage (stage) level was 2.04±1.30 according to the histologic activity index obtained from the histologic evaluation of liver biopsy specimens. These data indicate that the patients included in the study group generally had limited inflammatory activity and fibrosis consistent with active chronic hepatitis.

Demographic, clinical, clinical laboratory, virologic and histopathologic data of 143 control group patients, including 63 patients with chronic hepatitis C infection and 80 patients with non-viral liver disease, 58 males and 85 females, are summarized in Table 1. The histologic grade of inflammation was 6.00±2.10 and the stage of fibrosis was 2.59±1.13 in patients with chronic hepatitis C. Of the patients who underwent liver biopsy for clinical indications other than viral hepatitis, 32 patients (40%) had fatty liver disease.

SLC10A1/rs2296651 Polymorphism (S267F, NTCP Variant)

In this study, S267F (G/A) genotype and polymorphism in *SLC10A1/rs2296651* gene were investigated in total human DNA obtained from the liver tissue of 150 CHB patients and 143 patients in the control group. No S267F (G/A) polymorphism was found in any of the patients in both CHB and control groups. In the molecular analysis of all samples in the patient and control groups, the allele in the codon encoding the 267th amino acid (AA) of the *SLC10A1/rs2296651* gene was homozygous as G/G. G/A heterozygous or A/A homozygous alleles were not detected in any patient (Table 2).

Table 1. Characteristic features of patients

		Control group		
	CHB, n=150	**CHC, n=63**	**Non-viral liver disease, n=80**	**p**
Age mean±SD (years)	41.63±13.17	56.08±16.38	47.48±13.86	N.S.
Gender (M/F)	78/72	21/42	37/43	N.S.
Cynical laboratory, mean±SD				
AST (U/L)	51.68±54.08	52.08±42.13	96.68±148.84	**<0.05**
ALT (U/L	68.38±89.46	69.03±99.97	114.22±141.29	**<0.05**
ALP (U/L)	74.62±26.85	80.30±27.46	171.40±207.59	**<0.05**
GGT (U/L)	31.26±34.44	56.84±58.14	174.50±246.13	**<0.05**
Viral factors				
Log HBV DNA (IU/mL)	7.29±7.69	NA	NA	
Log HCV RNA (IU/mL)	NA	8.08±8.03	NA	
Histology, mean±SD				
Inflammation (grade)	4.96±2.84	6.00±2.10	NA	**<0.05**
Fibrosis (stage)	2.04±1.30	2.59±1.13	NA	**<0.05**
NASH	NA	NA	21	
NAFLD	NA	NA	11	
Non-specific change*	NA	NA	26	
Cholestatic liver disease**	NA	NA	12	
Cirrhosis	NA	NA	5	
Portal hypertension/venopathy	NA	NA	5	

*Non-structural changes, absence of fibrosis, minimal portal and lobular inflammatory infiltrates.

**Primary biliary cholangitis, primary sclerosing cholangitis.

AST: Aspartate aminotransferase, ALT: Alanin aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, NASH: Non-alcoholic steatohepatitis, NAFLD: Non-alcoholic fatty liver disease, NA: Not applicable, N.S.: Not significant, HBV: Hepatitis B virus, HCV: Hepatitis C virus, SD: Standard deviation, M/F: Male/female

Table 2. SLC10A1/rs2296651 polymorphism (S267F, NTCP variant) results				
	rs2296651 Allel			
Patients (n)	**G/G**	**G/A**	**A/A**	**p**
Chronic hepatitis B (150)	150	0	0	-
Control (143)	143	0	0	-

DISCUSSION

In recent years, detection of genetic variations in the *SLC10A1* gene encoding NTCP and investigation of their effects on the HBV-associated receptor function of NTCP is a current approach. The most common NTCP-associated genetic variant is reported to be the S267F polymorphism in scientific studies conducted predominantly in Asian populations to detect genetic variations in the *SLC10A1* gene. In some of the scientific studies conducted for this purpose, it has been reported that the S267F polymorphism detected on the *SLC10A1* gene may cause a change in the physiological function of NTCP, leading to a decrease in HBV entry into the cell and infection load. Therefore, it is hypothesized that NTCP S267F genetic variant may be associated with resistance to HBV infection and the risk of development of liver cirrhosis and HCC associated with HBV infection[10-13,16,17].

It has also been suggested that the NTCP S267F (c.800G>A, rs2296651) variant, which is located in the 4th exon of the *NTCP* gene and causes missense mutation, causes a decrease in the receptor function of HBV, which may result in loss of HBV binding to the receptor, intracellular entry and decreased replication capacity[9].

In a study conducted to examine the association of the NTCP S267F variant with HBV clearance, HBV-associated cirrhosis, HCC and resistance to HBV infection, the S267F (A allele) variant was detected at a higher rate in the healthy group than in HBV-infected patients. Therefore, it was reported that the S267F variant may be associated with a decreased risk of developing HBV-related cirrhosis and HCC and disease progression, but may not be associated with spontaneous HBV clearance. It has been reported that the S267F variant is generally protective against HBV infection and related diseases; however, this variant does not prevent the progression of cirrhosis towards HCC[10].

In a study involving an Asian (Chinese) population of approximately 2000 patients and 2000 healthy controls investigating the effect of NTCP variations on resistance to CHB infection and clinical outcomes, it was reported that the NTCP Ser267Phe variant was associated with resistance to CHB and a decrease in the incidence of HBV-related liver diseases[12].

Today, Bulevirtide, a synthetic lipoprotein based on the first 47 aa of HBV Pre-S1, has been synthesized and pre-clinical and clinical studies are ongoing to test its therapeutic efficacy[19-21]. Therefore, sequence differences in the NTCP interaction domain (HBV Pres1) and identification of single nucleotide polymorphisms (SNP) in NTCP may be useful in predicting the therapeutic efficacy of HBV cell entry inhibitors planned for clinical use in the near future for the treatment of HBV infections.

In a large-scale study in Taiwan, in which the association of the NTCP Ser267Phe variant with the serostatus of CHB infection and the risk of HBV-associated cirrhosis and HCC development was examined in 3801 people with CHB and 3801 HBsAg-negative people as the control group, the S267F variant was found in 18.5% of the control group, 17.2% in cirrhotic cases and 13.2% in non-cirrhotic HCC patients. It was reported that the risk of developing HCC was 25 times higher in individuals with GG genotype compared to GA and AA genotypes, and AA genotype was statistically significantly associated with HBsAg seronegativity. In conclusion, it was reported that the S267F variant was associated with resistance to CHB infection, HBV-related cirrhosis and decreased risk of development of HCC, and it was also predicted that analyzing the detection of this variant in patients together with HBV DNA levels may be useful in identifying patients with low risk of HBV-related HCC[22].

On the other hand, the data of some scientific studies investigating the effects of these genetic variants on the HBV-related receptor function of NTCP do not confirm the data in this study[23-25]. In a study examining the relationship between HBV infection and S267F (rs2296651) variation, it was reported that rs2296651 variant was not detected in either the infected or control groups and as a result, this SNP may be specific for the Asian population[26]. Therefore, the role of these variants on resistance to HBV infection and development of CHB-related cirrhosis, HCC and anti-HBV therapies is still controversial. Moreover, the prevalence of NTCP SNP rs229665 may vary according to ethnicity, geography and HBV endemicity.

In our study, the SNP rs2296651, which causes the S267F variation in the *SLC10A1* gene encoding the functional receptor NTCP of HBV, was not detected in any of the patients. Therefore, the effects of S267F variation on the clinical and histologic features of the patients could not be evaluated in this study. Therefore, the data obtained in this study suggest that the utility of SLC10A variants as a novel biomarker for identifying patients with CHB with a low risk of CHB-

associated cirrhosis and HCC is limited, and that their role in reducing the efficacy of anti-viral therapeutics targeting NTCP blockade may be quite limited. Therefore, the prognostic value of the SNP rs2296651, which causes the S267F variation in the *SLC10A1* gene, remains unclear.

Study Limitations

The limitations of this study were the small number of patients and samples included in this study and the detection of the S267F variation in the *SLC10A1* gene at the mRNA level.

CONCLUSION

In conclusion, the data obtained from this study suggest that the NTCP interaction domain is highly conserved in patients with CHB, and therefore the potential negative impact of S267F variations in the *SLC10A1* gene encoding NTCP on the progression of HBV-associated liver damage and antiviral therapeutics targeting NTCP may be quite limited.

Ethics

Ethics Committee Approval: Ethical approval of the study was granted by the Ethics Committee of İstanbul University, İstanbul Faculty of Medicine (no: 2018/1251, date: 14.09.2018).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: B.Ç., A.A., B.Ç., M.B., F.A., Concept: B.Ç., F.A., Design: B.Ç., F.A., Data Collection or Processing: B.Ç., A.A., M.P., B.Çav., M.B., M.G., F.A., Analysis or Interpretation: B.Ç., M.P., M.G., F.A., Literature Search: B.Ç., F.A., Writing: B.Ç., F.A.

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Management of Hemorrhoid Rubber Band Ligation Complications: Massive Rectal Bleeding

Hemoroid Lastik Band Ligasyonu Komplikasyonlarının Yönetimi: Masif Rektal Kanama

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ABSTRACT

Aim: The aim of this study is to present rubber band ligation (RBL), which is one of the non-surgical treatment options in hemorrhoidal disease, its complications and management of massive rectal bleeding, which is an important complication due to its morbidity.

Materials and Methods: Between 2018-2022, five hundred and sixty-four RBL was performed for stage 1-2 and 3 internal hemorrhoidal disease. Seventy two patients with previous anorectal surgery, pregnancy, chronic liver disease were excluded. Four hundred and ninety two patients were included in the study. All patients underwent detailed anorectal examination, in patients over 50 years were evaluated by colonoscopy. The demographic characteristics of the patients, the number of applied band ligation and complications (minor-major) were evaluated.

Results: The mean age of the patients was 33.4±11 (18-65) years, 385 were male (78.3%) and 107 were female (21.7%). Thirty-nine patients (8%) had single band ligation, 448 (91%) patients had double band ligation and 5 patients had triple band ligation. After RBL minor complications (anal pain, vasovagal symptoms, minor rectal bleeding, urinary retention) developed in twenty patients (4%) and massive rectal bleeding developed in 4 (0.8%) patients as a major complication.

Conclusion: Hemorrhoidal banding is a safe and effective method for treatment of hemorrhoidal disease. This study highlights a rare, life-threatening complication of RBL.

Keywords: Hemorrhoids, hemorrhage, ligation

ÖZ

Amaç: Çalışmanın amacı, hemoroidal hastalıkta cerrahi dışı tedavi seçeneklerinden biri olan lastik band ligasyonunu (RBL) ve buna bağlı gelişen komplikasyonları irdelemektir. Morbiditesi nedeniyle önem arz eden masif rektal kanama komplikasyonunun yönetimini sunmaktır.

Gereç ve Yöntem: Evre 1-2 ve 3 internal hemoroidal hastalık nedeniyle 2018-2022 yılları arasında kliniğimizde RBL yapılan 564 hasta retrospektif olarak irdelenmiştir. Gebelik durumu, geçirilmiş anorektal cerrahi, kronik karaciğer hastalığı ve antikoagülan kullanımı nedeniyle 72 hasta çalışma dışında bırakılmıştır. Hastaların 492'si çalışmaya dahil edilmiştir. Tüm hastalara proktoloji ünitesinde detaylı anorektal muayene, 50 yaş üstündekilere ise kolonoskopik değerlendirme yapılmıştır. Hastaların demografik özellikleri, gelişen komplikasyonlar (minör/majör) ve uygulanan band ligasyon sayısı standardize edilmiş formlara kayıt edildi.

Bulgular: Hastaların ortalama yaşı 33,4±11 (18-65) yıl olup, 385'i (%78,3) erkek, 107'si (%21,7) kadındı. Hastaların 39'una (%8) tek kadran, 448 hastaya (%91) iki kadran ve 5 hastaya üç kadran RBL uygulandı. RBL sonrası minör komplikasyonlar (anal ağrı, vazovagal semptomlar, minör rektal kanama, üriner retansiyon) yirmi hastada (%4) gelişirken, hastaların 4'ünde (%0,8) masif rektal kanama meydana gelmiştir. Masif rektal kanama gelişen hastaların hepsi acil şartlarda hospitalize edildi ve operasyona alındı. Bu hastaların birine 3 ünite, üç hastaya ise 4 ünite eritrosit transfüzyonu yapıldı.

Sonuç: Hemoroidal band ligasyonu, hemoroidal hastalık tedavisinde güvenli ve etkili bir yöntemdir. Ancak hayatı tehdit edecek ciddi kanamalara yol açabileceği göz önünde bulundurulmalıdır.

Anahtar Kelimeler: Hemoroid, kanama, ligasyon

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INTRODUCTION

Hemorrhoidal disease is a very common anorectal disease encountered in colorectal clinics[1]. Blood entering the hemorrhoidal pads via the hemorrhoidal arteries at rest easily returns to the venous system from the anal canal. As a result of the slowing of venous return due to forced and prolonged straining during defecation and the adverse effect on lymphatic circulation, anal edema may develop following defecation. In addition to these vascular changes, hemorrhoidal disease is also caused by degeneration of muscle and connective tissue. The sliding anal cushion theory developed by Thomson[2-4] is currently the most widely accepted theory of hemorrhoidal disease formation.

Non-surgical day-to-day procedures, surgical methods and advanced technological instruments are used in the treatment of hemorrhoidal disease. Popular interventions include sclerotherapy, infrared coagulation, radiofrequency ablation (RF), rubber band ligation (RBL), laser ablation, Milligan Morgan hemorrhoidectomy (open), Ferguson hemorrhoidectomy (closed), LigaSure (LigaSure™, Valleylab, Covidien) hemorrhoidectomy, ultrasonic scalpel (US hemorrhoidectomy), stapled hemorrhoidectomy (PPH), and Doppler guided hemorrhoidal artery ligation.

The application of RBL in hemorrhoidal disease was described by Blaisdell[5] in the 1950s and has survived to the present day after being modified by Barron[6]. Since then, this technique, with hemorrhoidal band ligation, has gained importance as an effective and cost-effective method in the treatment of internal hemorrhoidal disease by causing fixation, retraction and fibrosis of hemorrhoidal pads[7]. Recently, extensive clinical studies and data emphasize that RBL is an effective outpatient alternative to surgery in the treatment of stage 2 and 3 internal hemorrhoidal disease with minimal complication rate, no need for general anesthesia and no need for hospitalization[8-10].

In this study, we discuss the approach and treatment methods for a rare complication of massive rectal bleeding, related to RBL, which is an effective and safe method.

MATERIALS AND METHODS

Five hundred-sixty four patients who were admitted to our clinic as outpatients between 2018 and 2022 and underwent RBL for stage 1-2 and 3 internal hemorrhoidal disease were retrospectively analyzed. Four hundred ninety-two patients were included in the study. Seventy-two patients were excluded from the study because of anticoagulant use outside the physician's control after the procedure, history of previous anorectal surgery, pregnancy status, and chronic liver disease. Ethics committee approval with number 17 was obtained from Atılım University Faculty of Medicine, Medicana International Ankara Hospital on 14.07.2023 for this study.

All patients underwent a detailed anorectal examination in the proctology unit, and colonoscopy was performed in addition to the examination in patients over 50 years of age. All band ligations were performed in the endoscopy unit preferably under intravenous sedation in the left lateral position. One-, two-, and three-quadrant RBL was performed according to the current hemorrhoidal disease status of the patients (Table 1). The procedure was performed with a disposable anoscope and Mc Gown band ligator (Figure 1). RBL was applied to diseased hemorrhoidal veins approximately 1 cm proximal to the dentate line. All band ligations were performed at the root of the aspirated hemorrhoidal vein after aspiration with the vacuum chamber pressure of the band ligator between 400-600 mmHg. Patients were discharged as outpatients after recovery in the recovery room. Demographic characteristics of the patients, complications (minor/major) and the number of band ligations performed were recorded on standardized forms.

All patients who developed massive rectal bleeding underwent urgent anal exploration after stabilization of vital signs and fluid replacement, and the abundant blood accumulated in

Table 1. Demographic characteristics of the patients and the number of bands applied to the patients

Demographic characteristics	n (%)
Age	33.4±11
Gender	
Female	107 (21.7)
Male	385 (78.3)
Number of bands	
Single quadrant	39 (8)
Two quadrants	448 (91)
Three quadrants	5 (1)

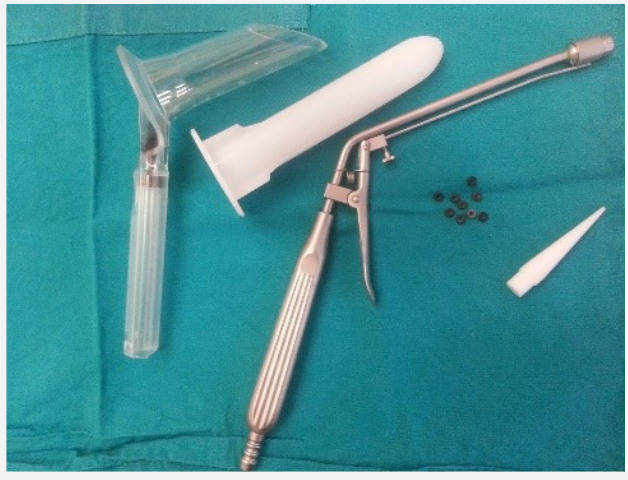

Figure 1. Anoscope, Mc Gown ligator, rubber bands

the rectum was aspirated. On anal exploration, foci of mucosal necrosis and active arterial bleeding were observed in the band ligation quadrant. Primary hemostasis of this area was performed with 3/0 vicryl suture. After stopping the bleeding focus, it was checked whether there was any other bleeding area. Patients who were hemodynamically stable and had defecation without rectal bleeding on post-operative day 2 were discharged.

Statistical Analysis

All data were computerized and Statistical Package for the Social Sciences (SPSS) 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data were expressed as median (range) and categorical data were expressed as percentages.

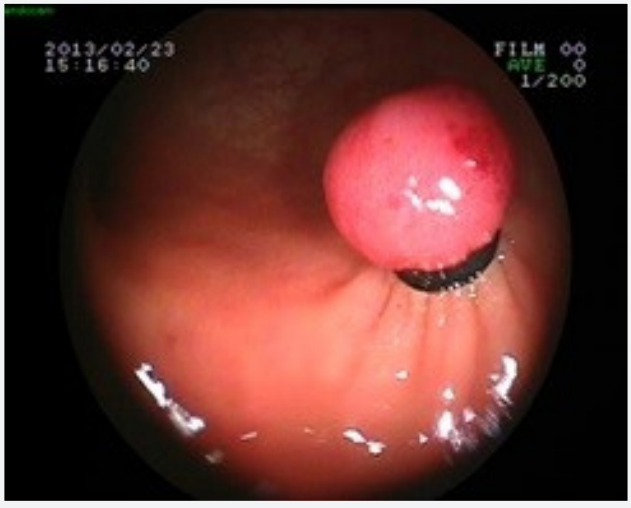

Figure 2. Endoscopic view of single (1) quadrant rubber band ligation

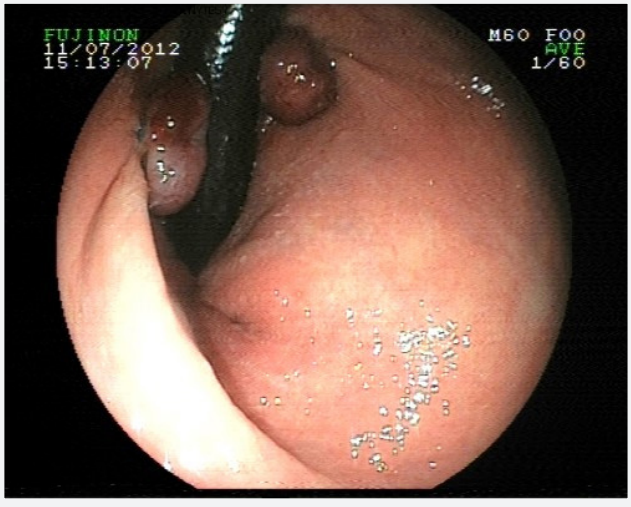

Figure 3. Endoscopic view of two (2) quadrant rubber band ligation

RESULTS

The mean age of the patients was 33.4±11 (18-65) years, 385 were male (78.3%) and 107 were female (21.7%). Single quadrant RBL was performed in 39 (8%), two quadrants in 448 (91%) and three (3) quadrants in 5 patients (Table 1 and Figures 2, 3, 4). Twenty patients (4%) developed minor complications (anal pain, vasovagal symptoms, minor rectal bleeding, urinary retention) after RBL, while massive rectal bleeding occurred in 4 patients (0.8%) (Table 2).

Minor complications included anal pain in 1 patient, vasovagal symptoms secondary to possible anal pain in 1 patient, urinary retention in 1 patient, and minor rectal bleeding occurring between days 7 and 10 of the procedure in 17 patients. While anal pain in one patient resolved with the use of non-steroidal anti-inflammatory drugs, the other patient was re-examined on the same day due to severe anal pain and it was observed

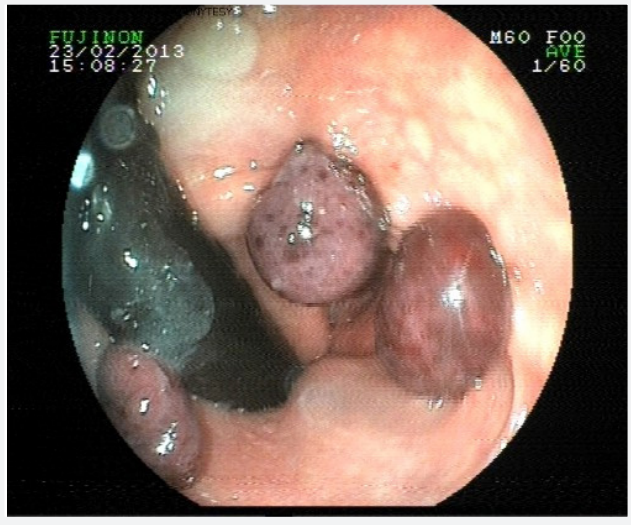

Figure 4. Endoscopic view of three (3) quadrant rubber band ligation

Table 2. Complications developing due to rubber band ligation application to patients

Complications	n (%)
Major complications	
Massive lower gastrointestinal bleeding	4 (0.8%)
Pelvic sepsis	0
Minor complications	20 (4%)
Anal pain	1
Minor rectal bleeding	17
Vasovagal symptoms	1
Urinary retention	1

that the band was very close to the dentate line. In the same session, the band was removed and band ligation was performed again more proximal to the dentate line.

All patients with massive rectal bleeding required hospitalization and operation, one patient received 3 units of erythrocyte transfusion and three patients received 4 units. All patients with massive rectal bleeding, which was a major complication, were male. Massive bleeding developed between 10-14 days in the late postoperative period. Patients with massive rectal bleeding presented to the emergency department with a dramatic rectal bleeding picture. They had clinical picture of hypovolemia. Patients were admitted to the clinic, their vital signs were stabilized and they were operated under emergency conditions. Two patients underwent anal exploration under general anesthesia and 2 patients under spinal anesthesia in jack-knife position.

DISCUSSION

Many surgical and non-surgical methods such as laser ablation, RBL, infrared coagulation, and sclerotherapy have been described in the treatment of hemorrhoidal disease. RBL is a widely used treatment method for hemorrhoidal disease with a lower complication rate compared to conventional surgery[9-11]. Although band ligation is considered as a non-surgical intervention, some complications after the procedure have been described. These complications include pain, rectal bleeding, vasovagal symptoms, anal fissure, thrombosis of the external piles, urinary retention (glob vesica) and infection (pelvic sepsis, Fournier's gangrene, etc.)[12].

In a study by Iyer et al.[13], the complication rates of 2114 rubber band applications for hemorrhoidal disease were as follows: pain 8.7%, bleeding 2.8%, external vein thrombosis 1.5%, pelvic sepsis 0.09%. Bat et al.[14] found a major complication rate of 2.5% requiring hospitalization in their study. El Nakeeb et al.[15] compared patients operated for grade 2 and 3 hemorrhoidal disease who underwent 2122 RBL procedures and found no statistically significant difference in the treatment of the disease. The complication rates after RBL were 4.1% pain, 4.1% minor bleeding, 1.3% vasovagal symptoms, 0.13% infection, 0.13% perianal fistula, and 0.4% perianal fissure. They observed that rectal bleeding developed between days 7-14 of the procedure. Similarly, in our study, the rate of minor complications was 4%, while the rate of major complications requiring hospitalization was 0.8%.

Bleeding is an important complication of RBL. Although most bleeding is self-limiting and does not require hospitalization or transfusion, massive lower gastrointestinal bleeding is rare and can be life-threatening. Bleeding rates have been reported to be 2-4% in various publications[13-15]. In our study, the rate of minor rectal bleeding that was self-limiting and did not require hospitalization was found to be 3.5%. Bleeding was observed in the late post-procedural period, usually on the 10th-14th day, suggesting that it was due to necrosis caused by the band[12]. Clopidogrel, aspirin and non-steroidal anti-inflammatory drugs have been shown in the literature to increase the risk of massive rectal bleeding after RBL[16,17].

Massive lower gastrointestinal bleeding has been reported in the literature in large series as well as case reports[12,15-18]. Although most of the massive lower gastrointestinal bleeding reported in these studies was emphasized to be related to the use of aspirin, non-steroidal anti-inflammatory drugs and anticoagulants, there was no drug use in our series. Similarly, bleeding after RBL was observed between 7-14 days and massive rectal bleeding was observed after the 10th day in all patients.

Study Limitations

In our study, complications after RBL in hemorrhoidal disease and the management of these complications were examined. No comparison was made with other methods used in the treatment of hemorrhoidal disease.

CONCLUSION

Although hemorrhoid RBL is considered an economical, effective and safe method, it should be kept in mind that it has life-threatening complications. Patients should be advised that bleeding may occur within 7-14 days after the procedure and patients should be advised to come for follow-up especially during this period. During the band ligation procedure, excessive suction should be avoided and the negative pressure should be 400-600 mmHg. Patients should be carefully questioned about any bleeding disorders, aspirin, non-steroidal anti-inflammatory drugs and anticoagulant use before the band ligation procedure. Although it was not observed in our series, it is important to follow the patient in terms of perianal infection/pelvic sepsis, which is another major complication other than bleeding reported in the literature.

Ethics

Ethics Committee Approval: Ethics committee approval with number 17 was obtained from Atılım University Faculty of Medicine, Medicana International Ankara Hospital on 14.07.2023 for this study.

Informed Consent: It is a retrospective study.

Author Contributions

Surgical and Medical Practice: B.E., M.G. Design: B.E., A.C.E., Data Collection or Processing: M.G., Interpretation: B.E., M.G.,

Literature Search: A.C.E., M.G., Writing: M.G., A.C.E.

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Can the Neutrophil/Lymphocyte*Platelet Ratio Predict Acute Appendicitis? An Analytical Study

Nötrofil/Lenfosit*Trombosit Oranı Akut Apandisiti Öngörebilir mi? Bir Analitik Çalışma

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ABSTRACT

Aim: Our primary aim is to determine whether the neutrophil/lymphocyte*platelet ratio (NLPR) has the ability to predict acute appendicitis. Our secondary aim is to show whether the NLPR is effective in differentiating complicated and non-complicated acute appendicitis.

Materials and Methods: Our study was planned retrospectively, and patients over 18 years of age who were diagnosed with acute appendicitis and admitted to the Health Sciences University Turkey, Ümraniye Training and Research Hospital between 01.04.2022 and 01.04.2023, were included in the study.

Results: A total of 492 patients were included in our study, and 242 (49.18%) of them were diagnosed with acute appendicitis. Neutrophil lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR) and NLPR were statistically significantly higher in patients with acute appendicitis compared to the control group ($p<0.001$, $p<0.001$, $p<0.001$, respectively) (the area under the curve was 0.96, 0.70, 0.94, respectively).

Conclusion: NLR, PLR and NLPR can be used as predictors for the diagnosis of acute appendicitis, but according to our study, NLR, PLR and NLPR should not be used as prognostic indicators of acute appendicitis.

Keywords: Acute appendicitis, NLR, PLR, NLPR

ÖZ

Amaç: Birincil amacımız nötrofil/lenfosit*trombosit oranının (NLPR) akut apandisiti öngörebilme yeteneğinin olup olmadığının belirlenmesidir. İkincil amacımız ise NLPR'nin komplike ve non-komplike akut apandisit ayrımında etkin olup olamadığını göstermektir.

Gereç ve Yöntem: Çalışmamız retrospektif olarak planlandı ve 01.04.2022-01.04.2023 tarihleri arasında Sağlık Bilimleri Üniversitesi, Ümraniye Eğitim ve Araştırma Hastanesi'ne başvuran 18 yaş üstü akut apandisit tanısı alan hastalar dahil edildi.

Bulgular: Çalışmamıza 492 hasta dahil edildi. Hastalarımızın 242'si (49,18%) akut apandisit tanısı alan hastalardı. Akut apandisit tanısı konulan hastaların yaş ortancası 33 (25-46) yıldı ve kontrol ve hasta grubu arasında anlamlı bir fark yoktu (p=0,078). Nötrofil lenfosit oranı (NLR), platelet lenfosit oranı (PLR) ve NLPR akut apandisit tanılı hastalarda, kontrol grubuna göre anlamlı derecede yüksekti (sırası ile $p<0,001$, $p<0,001$, $p<0,001$) (eğrinin altındaki alan sırasıyla 0,96, 0,70, 0,94 idi).

Sonuç: NLR, PLR ve NLPR akut apandisit tanısını koymada prediktör olarak kullanılabilir, fakat çalışmamıza göre akut apandisit prognoz göstergesi olarak NLR, PLR ve NLPR kullanılmamalıdır.

Anahtar Kelimeler: Akut apandisit, NLR, PLR, NLPR

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INTRODUCTION

Acute appendicitis is the most common cause of acute abdomen. While the rate of incidence with perforation is 20%, its prevalence is approximately 7%[1]. Diagnosis is made with clinical and radiological imaging. Laboratory tests are also helpful in diagnosing[2]. About half of adults have obvious symptoms. Periumbilical abdominal pain, anorexia, vomiting, and fever are the common symptoms[3]. C-reactive protein (CRP) and white blood cell (WBC) are often preferred for diagnosis, but there is no specific laboratory test for the diagnosis of appendicitis[4]. Although it is mostly considered as an inflammatory disease, it is known that immune dysfunctions will increase the susceptibility to infection[5].

Neutrophils, lymphocytes, and platelets that play a role in the coagulation cascade play an important role in immune system dysfunction and systemic inflammation[6]. Neutrophil lymphocyte ratio (NLR) was accepted as an easy-to-calculate, low-cost indicator of systemic inflammation[7,8]. Neutrophils are the most abundant cell of leukocytes, and they regulate other cell functions such as neutrophils, mast cells and macrophages and are effective in inflammation. Like NLR, platelet lymphocyte ratio (PLR) has been used as an early marker of infections in various diseases[9]. Since appendicitis is an inflammatory disease associated with immune system dysfunction, it was the subject of NLR and PLR studies[2,10-13]. Another parameter used as an early marker of inflammatory diseases was the neutrophil/lymphocyte*platelet ratio (NLPR). Whether NLPR is a prognostic marker for sepsis[14], Coronavirus disease-2019 (COVID-19)[15] and post-surgery[16] were discussed in the literature.

To the best of our knowledge, there is no study evaluating the effect of NLPR on diagnosis and prognosis in adult patients with acute appendicitis.

Our primary aim is to determine whether the NLPR has the ability to predict acute appendicitis. Our secondary aim is to show whether the NLPR is effective in differentiating complicated and non-complicated acute appendicitis.

MATERIALS AND METHODS

Ethics

The instant study was carried out with the permission of the Ümraniye Training and Research Hospital Local Ethics Committee (date: 24/04/2023, decision no: B.10.1.TKH.4.34.H.GP.0.01/131).

Study Design

Our study was retrospectively planned and more than three hundred thousand patients admitted to the emergency department of Ümraniye Training and Research Hospital between 01.04.2022 and 01.04.2023, who were diagnosed with acute appendicitis through computer tomography or ultrasound and examination findings and who were surgically over the age of 18 years were included.

Study Population

Patients whose hemogram parameters were measured and registered in the emergency department were included in the study, but patients with a history of trauma, younger than 18 years of age, and with incomplete data were excluded from the study. The control group was selected from patients without suspected acute appendicitis. Gangrenous appendicitis, intra-abdominal abscess, perforation, plastron formation, and generalized peritonitis were considered in the differentiation of complicated and non-complicated. Patients who could not differentiate between complicated and non-complicated were also excluded from the study.

Data Collection

Demographic characteristics, symptoms and examination findings of the patients were recorded using the hospital data system. Age (year), gender, localized pain, nausea, vomiting, anorexia, right lower quadrant tenderness, right lower quadrant rebound, high fever, leukocytosis, left shift in WBC and complicated appendicitis status were recorded by the investigators from the patient electronic file. The values of WBC, neutrophil count, lymphocyte count, hemoglobin, and hematocrit values, mean corpuscular volume (MCV), red cell distribution width, platelet count, mean platelet volume, and NLPR obtained from hematology laboratory were recorded. Alanine aminotransferase, aspartate aminotransferase, albumin, glucose, calcium, blood urea nitrogen, creatinine, sodium, potassium, and CRP levels were also recorded. The examinations and data of patients who attended the emergency department were used. The patients were classified both as acute appendicitis and control group, and also as complicated and uncomplicated acute appendicitis. Hematological values were compared between the patient group with acute appendicitis and the control group. Then, a comparison was made between patients with a diagnosis of complicated and uncomplicated acute appendicitis.

NLPR=Neutrophil count (10^9/L) * 100: Lymphocyte count (10^9/L) * Platelet count (10^9/L).

Statistical Analysis

Quantitative variables were presented as median and interquartile range (IQR) (IQR, 25^{th}-75^{th} percentile) values, and the Mann-Whitney U test was used in analyzing the paired groups. The categorical data were done using the Fisher's Exact test and chi-square test. Statistical Package for Social

Sciences (SPSS) (SPSS Inc., version 20.0; Chicago, IL) was used for statistical analyses. The Spearman's correlation analysis test was employed for correlation analysis. Statistical significance was accepted as p<0.05.

RESULTS

A total of 492 patients were included in our study, and 242 (49.18%) of our patients were diagnosed with acute appendicitis. Twenty hundred and fifty (50.82%) patients constituted the control group. The most common symptom in patients diagnosed with acute appendicitis was right lower quadrant pain (96%). The most common finding was leukocytosis with a rate of 48%. The frequency of symptoms and signs of patients diagnosed with acute appendicitis, and the median (IQR) values of laboratory tests are shown in Table 1.

The mean age of the patients diagnosed with acute appendicitis was 33 years (25-46 years) (p=0.078); Likewise, 58.7% of the patients diagnosed with acute appendicitis were male (p<0.001). WBC, neutrophil, and hemoglobin values were higher in patients with acute appendicitis than in the control group (p<0.001, p<0.001, p=0.001 respectively). Lymphocyte and MCV values were lower in patients with acute appendicitis than in the control group (p<0.001, p=0.001 respectively). NLR, PLR and NLPR values were statistically significantly higher in patients with acute appendicitis (p<0.001, p<0.001, p<0.001 respectively). The comparison of hemogram parameters of the control group and patients diagnosed with acute appendicitis is shown in Table 2.

Table 1. Symptoms of patients diagnosed with acute appendicitis and laboratory findings other than hematological parameters

Characteristic (n, %) (median IQR)	N=242
Localized pain (n, %)	26 (11%)
Anorexia (n, %)	21 (8.7%)
Vomiting (n, %)	74 (31%)
Sensitivity in right lower quadrant (n, %)	232 (96%)
Rebound (n, %)	114 (47%)
High fever (n, %)	3 (1.2%)
Leukocytosis (n, %)	200 (83%)
WBC left shift (n, %)	117 (48%)
Complicated (n, %)	70 (29%)
ALT (IU/L)	20 (13-31)
Albumin (g/dL)	44.6 (41.0-47.2)
AST (IU/L)	21 (17-25)
Glucose (mmol/L)	105 (93-122)
Calcium (mg/dL)	9.13 (8.85-9.40)
BUN (mg/dL)	24 (20-30)
Creatinine (mg/dL)	0.79 (0.66-0.89)
Potassium (mEq/L)	4.30 (4.07-4.63)
Sodium (mEq/L)	136.70 (135.00-137.20)
CRP (mg/mL)	40 (9-110)

WBC: White blood cell count, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, CRP: C-reactive protein, IQR: Interquartile range

Table 2. Evaluation of hematological parameters of acute appendicitis patients and control group

(n, %), (median IQR)	Control group	Acute appendicitis group	Total	p value
Gender (n, %)				
Male	67 (26.8%)	142 (58.7%)	209 (42.5%)	<0.001
Female	183 (73.2%)	100 (41.3%)	283 (57.5%)	
Age, median (IQR)	34.0 (30.0-42.0)	33.0 (25.0-46.0)	34.0 (28.0-43.0)	0.078
WBC (10^3 µ/L)	7.0 (6.1-8.1)	14.8 (11.9-16.9)	9.4 (6.9-14.7)	<0.001
Neutrophil (10^3 µ/L)	4.0 (3.4-4.9)	12.1 (9.5-14.1)	6.2 (3.9-11.9)	<0.001
Lymphocyte (10^3 µ/L)	2.2 (1.9-2.8)	1.8 (1.2-2.3)	2.0 (1.6-2.6)	<0.001
Hemoglobin (g/dL)	13.3 (12.2-14.5)	14.0 (12.4-15.1)	13.6 (12.3-14.9)	0.001
Hematocrit (%)	40.5 (37.8-43.6)	41.7 (37.8-45.1)	40.8 (37.8-44.2)	0.059
MCV (fl)	88.3 (84.8-92.1)	86.7 (83.9-90.0)	87.5 (84.4-90.9)	0.001
RDW (fl)	13.1 (12.7-13.8)	13.2 (12.8-13.8)	13.1 (12.7-13.8)	0.472
Platelet (10^3 µ/L)	257.0 (223.0-297.0)	254.5 (215.2-311.5)	256.0 (218.0-302.0)	0.634
MPV (fl)	9.6 (9.0-10.3)	9.8 (8.9-10.5)	9.7 (8.9-10.5)	0.563
NLR	1.8 (1.4-2.2)	6.5 (4.5-10.2)	2.8 (1.8-6.4)	<0.001
PLR	112.6 (93.5-139.3)	150.9 (112.3-217.9)	125.7 (100.9-166.9)	<0.001
NLPR	0.7 (0.5-0.9)	2.7 (1.6-4.0)	1.1 (0.7-2.6)	<0.001

WBC: White blood cell count, IQR: Interquartile range, MCV: Mean corpuscular volume, RDW: Red cell distribution width, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, NLPR: Neutrophil-to-lymphocyte*platelet ratio

Of the 242 patients diagnosed with acute appendicitis, 70 (28.92%) were those diagnosed with complicated acute appendicitis. 58.6% of patients with complicated acute appendicitis were male (p=1.00). While WBC was high in patients with complicated acute appendicitis, neutrophils and hemoglobin levels were lower than in patients with uncomplicated acute appendicitis. However, WBC, neutrophil and hemoglobin values were not statistically significant in the diagnosis of complicated and uncomplicated acute appendicitis (p=0.902, p=0.952, p=0.836 respectively). While lymphocyte is higher in patients with complicated acute appendicitis, MCV was lower. Lymphocyte and MCV values were not statistically significant in the diagnosis of complicated and uncomplicated acute appendicitis (p=0.427, p=0.337, respectively). NLR, PLR and NLPR values were not statistically significant in the differentiation of complicated and non-complicated acute appendicitis (p=0.561, p=0.973, p=0.280, respectively). Hemogram and NLPR values of patients with complicated and uncomplicated acute appendicitis are shown in Table 3.

Table 4 presents the cut-off, sensitivity, specificity, 95% confidence interval and area under the curve (AUC) values.

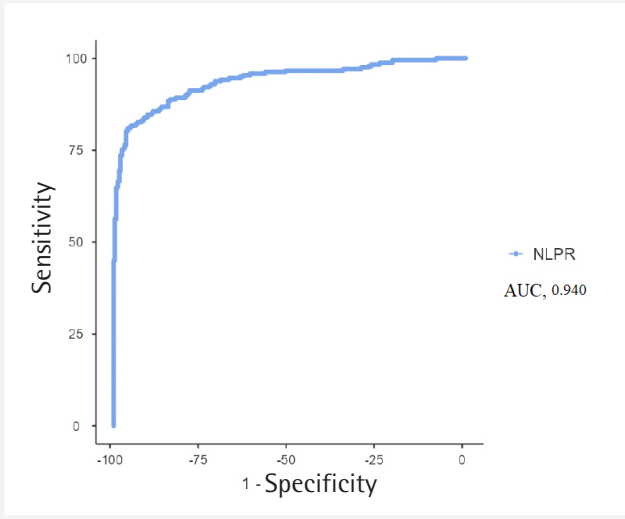

Figure 1. AUC of NLPR

*AUC: Area under the curve, NLPR: Neutrophil-to-lymphocyte*platelet ratio*

Table 3. Evaluation of hematological parameters of patients with non-complicated and complicated acute appendicitis

	Non-complicated	Complicated	Total	p value
Gender n (%)				0.999
Male	101 (58.7)	41 (58.6)	142 (58.7)	
Female	71 (41.3)	29 (41.4)	100 (41.3)	
Age, median (IQR)	31 (24-41)	43 (29-56)	33 (25-46)	<0.001
WBC (10^3 µ/L)	14.7 (11.9-16.9)	14.9 (11.8-16.7)	14.8 (11.9-16.9)	0.902
Neutrophil (10^3 µ/L)	12.1 (9.3-14.2)	12.0 (9.6-14.0)	12.1 (9.5-14.1)	0.952
Lymphocyte (10^3 µ/L)	1.7 (1.2-2.3)	1.9 (1.2-2.5)	1.8 (1.2-2.3)	0.427
Hemoglobin (g/dL)	14.0 (12.4-15.1)	13.9 (12.5-15.1)	14.0 (12.4-15.1)	0.836
Hematocrit (%)	41.8 (37.9-45.0)	41.0 (37.7-45.1)	41.7 (37.8-45.1)	0.821
MCV (fl)	86.8 (84.1-90.0)	86.2 (83.5-89.7)	86.7 (83.9-90.0)	0.337
RDW (fl)	13.1 (12.7-13.8)	13.2 (12.8-14.0)	13.2 (12.8-13.8)	0.324
Platelet (10^3 µ/L)	253.5 (214.8-304.2)	258.0 (221.8-331.5)	254.5 (215.2-311.5)	0.278
MPV (fl)	9.8 (8.9-10.5)	9.7 (8.8-10.5)	9.8 (8.9-10.5)	0.538
NLR	6.5 (4.6-10.5)	6.4 (4.1-9.8)	6.5 (4.5-10.2)	0.561
PLR	151.7 (113.7-213.9)	150.9 (107.5-236.7)	150.9 (112.3-217.9)	0.973
NLPR	2.7 (1.7-4.3)	2.4 (1.4-3.8)	2.7 (1.6-4.0)	0.280

WBC: White blood cell count, IQR: Interquartile range, MCV: Mean corpuscular volume, RDW: Red cell distribution width, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, NLPR: Neutrophil-to-lymphocyte*platelet ratio

Table 4. Receiver operating characteristic analysis of NLPR, NLR, and PLR for the prediction of diagnosis

	Cut point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index	AUC	Metric Score
NLPR	1,42	80.58%	96%	95.12%	83.62%	0.77	0.94	1.77
NLR	3,27	89.26%	95.60%	95.15%	90.19%	0.85	0.96	1.85
PLR	148	52.48%	81.20%	72.99%	63.84%	0.34	0.7	1.34

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, NLPR: Neutrophil-to-lymphocyte*platelet ratio, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio

The cut-off values of NLR, PLR and NLPR were statistically significant in the diagnosis of acute appendicitis, and the AUC values were at a good level. According to the diagnostic test performance analysis report, the NLR, PLR, and NLPR were statistically significant predictors of the diagnosis of acute appendicitis with AUC values being calculated as 0.96 (0.919-0.95) for NLR at a cut-off value of 3.27, 0.70 (0.63-0.72) for PLR at a cut-off value of 148, and 0.94 (0.83-0.95) for NLPR at a cut-off value of 1.42. AUC of NLPR is presented in Figure 1.

DISCUSSION

In our study, it was determined that NLR, PLR and NLPR were effective in diagnosing acute appendicitis, and NLR, PLR and NLPR were not effective in distinguishing complicated and non-complicated acute appendicitis. In the ROC analysis of NLR, PLR and NLPR for the diagnosis of acute appendicitis, it was determined that NLR had the highest AUC value. NLPR, on the other hand, was found to be a strong predictor in diagnosing acute appendicitis with a value of 0.94 AUC. In addition, we have concluded that it cannot be an indicator of prognosis. As far as we could detect, there was no study showing the relationship between acute appendicitis and NLPR in terms of diagnosis and prognosis in adult patients over the age of 18 years. However, studies evaluating the relationship of acute appendicitis with hematological parameters were available in the literature[10-13].

In a study conducted in pregnant patients diagnosed with appendicitis, the lymphocyte value was statistically significantly lower in patients compared to the control group, similar to our study, and no statistically significant difference was observed in platelet values. NLR and PLR were found to be significantly higher than those in the control group[10]. In a meta-analysis, it was reported that NLR was effective in both the clinical course and diagnosis of acute appendicitis[11]. In a study conducted in pediatric acute appendicitis patients, NLR and NLPR were found to be statistically high in patients, and it was stated that these rates could be used in diagnosis[12]. In the literature, different results were obtained from our study in differentiating complicated and non-complicated acute appendicitis[2,13]. In a study comparing NLR and PLR in complicated and uncomplicated acute appendicitis cases, unlike our study, NLR and PLR were found to be statistically significantly higher in complicated acute appendicitis cases[2]. In a study by Ribeiro et al.[13] in which 841 patients with acute appendicitis were included, leukocyte value and NLR were statistically significantly higher in cases of complicated acute appendicitis than in cases of uncomplicated acute appendicitis. In a study by Uludağ et al.[17], including 702 acute appendicitis patients, it was found that perforation developed in 10% of the patients, and NLR and PLR were statistically significantly higher in the perforated patient group compared to the non-perforated acute appendicitis patients.

Apart from acute appendicitis, sepsis[14], COVID-19[15], kidney damage after major surgery[16], pneumonia[18], inflammatory diseases such as spondylarthritis[19], decompensated heart failure[20], contrast nephropathy[21], minor surgery and even psychiatric diseases[22] were evaluated together with hematological parameters. In a study conducted in 173 patients diagnosed with sepsis, in which mortality was found to be close to 38%, a statistically significant correlation was found between the 5th day neutrophil and platelet values and mortality. In the same study, NLR and NLPR calculated on the 5th day were also found to have a statistically significant relationship with mortality[14]. In a study in which hematological parameters were examined in patients with a diagnosis of COVID-19 and 500 patients were included, there was no statistically significant relationship between neutrophil and platelet values and disease severity, although lymphocyte was statistically significantly lower in the severe COVID-19 patient group compared to mild COVID-19 patients. NLR and NLPR were statistically significantly higher in severe COVID-19 patients[15]. In a study examining the development of acute kidney injury after major abdominal surgery, no statistically significant correlation was found between postoperative neutrophil and platelet values and the development of acute kidney injury. However, there was a statistically significant correlation between low lymphocyte count and high NLPR and the development of acute kidney injury[16]. In an examination of pneumonia patients in inpatient and outpatient group and control group, it was determined that CRP, NLR and PLR were statistically significantly higher in inpatients and outpatients compared to the control group. In the same study, there was no statistically significant difference between inpatients and outpatients in terms of CRP, NLR, and PLR[18]. In a study examining spondylarthritis, it was reported that NLR and PLR could be considered as independent predictors in patients with severe sacroiliitis[19]. In a study evaluating cardiac deaths in patients with decompensated heart failure, NLR and PLR were found to be statistically significantly higher in cardiac deaths[20]. In a study examining the relationship between contrast nephropathy and hematological parameters and including patients with acute coronary syndrome, it was found that neutrophil lymphocyte and platelet values were not associated with contrast development. NLR and NLPR were also not associated with the development of contrast nephropathy[23]. In a prospective study examining patients who underwent septorhinoplasty, with a high postoperative periorbital ecchymosis score, while lymphocytes were statistically significantly lower, neutrophil and platelet values were not statistically significant. No statistically significant correlation was found between NLR and high periorbital ecchymosis score. PLR, on the other hand,

was statistically significantly higher[22]. In a study in which patients with a diagnosis of schizophrenia were compared with a healthy control group, neutrophils and lymphocytes were statistically significantly higher in patients with schizophrenia. No statistically significant correlation was found between platelet and NLR and schizophrenic patients. PLR was found to be statistically significantly lower in male patients compared to the control group[22]. In a study conducted in Turkey that evaluated the ability of hematological parameters to predict short-term mortality in patients with acute cholecystitis, there was a significant difference between survivors and non-survivors in terms of NLR, but not in terms of PLR. Additionally, among the combined hematological parameters evaluated, the parameter with the highest AUC value was NLR, which was 0.708[24]. In our study, while high levels of NLR, PLR and NLPR, as well as high neutrophil and low lymphocyte levels, were statistically significant in diagnosing acute appendicitis, it was determined that the platelet level was not statistically significant in diagnosing. It was determined that neutrophil, lymphocyte, and platelet levels were not statistically significant in the differentiation of complicated and non-complicated acute appendicitis.

Study Limitations

Since our study was planned retrospectively, the clinical progress of the patients was obtained from the records. Although the included patients were over the age of 18 years, they consisted of young patients and the number of patients diagnosed with complicated acute appendicitis was small.

CONCLUSION

NLR, PLR and NLPR can be used as predictors for the diagnosis of acute appendicitis, but according to our study, NLR, PLR and NLPR should not be used as prognostic indicators of acute appendicitis.

Ethics

Ethics Committee Approval: The instant study was carried out with the permission of the Ümraniye Training and Research Hospital Local Ethics Committee (date: 24/04/2023, decision no: B.10.1.TKH.4.34.H.GP.0.01/131).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices - Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: A.Ö., S.Ö., H.Ş.A., M.T.A.

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

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Factors Effecting the Duration of Breast Feeding and the Time of Weaning

Anne Sütü Alım Süresi ve Kesilme Zamanını Etkileyen Faktörler

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ABSTRACT

Aim: Breast milk is the ideal type of nutrition for infants. Breastfeeding has many benefits for the mother, baby and society. In this study, we aimed to determine the sociodemographic factors that affect the duration of breastfeeding and the time of weaning in the children of mothers who have children aged 5 and under, to detect the problems that will occur during the breastfeeding process in advance and to contribute to the precautions that need to be taken.

Materials and Methods: This is a cross-sectional study in which a survey was conducted with mothers or caregivers of children aged 5 and under. Our study was conducted by the researcher using the face-to-face survey method. In the survey; the child's gender, age, type of birth, week of birth, birth weight, when he first breastfed, whether he used formula or not, if so, the reason, when he started the first complementary food, how much breast milk he received only and in total, the number of siblings, if any, for how long. Breastfeeding, household's total income, mother's prenatal breastfeeding education status, mother and father's sociodemographic characteristics, occupations, working and health status, and mother's emotional state were questioned.

Results: The cesarean delivery rate in the group with breastfeeding for less than 1 year was significantly ($p<0.05$) higher than the group with breastfeeding for more than 1 year. The rate of the mother receiving prenatal breastfeeding education in the group with breastfeeding for more than 1 year was significantly ($p<0.05$) higher than the group with breastfeeding for less than 1 year. The rate of breastfeeding within the first hour in the group with breast milk intake over 1 year was significantly higher than in the group with breast milk intake under 1 year ($p<0.05$). In the group whose breastfeeding is over 1 year, the proportion of those who think that their child should be breastfed until the age of 2 is; Breast milk intake was significantly higher than the group under 1 year. Maternal education level was significantly ($p<0.05$) higher in the group with breastfeeding for more than 1 year than in the group with breastfeeding for less than 1 year. The maternal employment rate in the group with breastfeeding for more than 1 year was significantly ($p<0.05$) higher than the group with breastfeeding for less than 1 year.

Conclusion: Breastfeeding is very important for a baby's healthy development and protection from diseases in the first two years of life. It is every baby's natural right to be fed with breast milk. Determining the timing of breastfeeding and the factors that lead to its cessation in our country and region will contribute to taking precautions in this regard and increasing the duration of breastfeeding. If possible, breastfeeding education starting before birth, supporting the mother after birth, and providing appropriate opportunities for breastfeeding for working mothers will increase the duration of breast milk intake.

Keywords: Human milk, breastfeeding, duration of time

ÖZ

Amaç: Anne sütü, bebekler için en ideal besindir. Anne sütü ile beslenmenin; anneye, bebeğe ve topluma çok sayıda yararı bulunmaktadır. Biz de bu çalışmamızda, 5 yaş ve altı çocuk sahibi olan annelerin çocuklarındaki anne sütü alım süresi ve kesilme zamanında etkili olan sosyodemografik faktörlerin belirlenmesini, anne sütü ile beslenme sürecinde yaşanacak sorunların önceden tespit edilmesini ve alınması gereken önlemlere katkıda bulunmayı amaçladık.

Gereç ve Yöntem: Araştırmamız 5 yaş ve altı çocukların anneleri veya bakım verenleriyle anket uygulanan kesitsel tipte bir çalışmadır. Çalışmamız araştırıcı tarafından yüz yüze anket yöntemi ile yapıldı. Ankette; çocuğun cinsiyeti, yaşı, doğum şekli, doğum haftası, doğum kilosu, ilk ne zaman

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emdiği, formül mama kullanıp kullanmadığı, kullandıysa nedeni, ilk ek gıdaya başlama zamanı, sadece ve toplam ne kadar anne sütü aldığı, kardeş sayısı, varsa kardeşin ne kadar süre anne sütü aldığı, hane halkının toplam geliri, annenin doğum öncesi emzirme eğitimi alma durumu, anne ve babanın sosyodemografik özellikleri, meslekleri, çalışma ve sağlık durumları ile annenin duygudurumu sorgulanmıştır.

Bulgular: Anne sütü alımı 1 yıl altı olan grupta sezaryen doğum oranı, anne sütü alımı 1 yıl üstü olan gruptan anlamlı (p<0,05) olarak daha yüksekti. Anne sütü alımı 1 yıl üstü olan grupta annenin doğum öncesi emzirme eğitimi alma oranı, anne sütü alımı 1 yıl altı olan gruptan anlamlı (p<0,05) olarak daha yüksekti. Anne sütü alımı 1 yıl üstü olan grupta ilk 1 saat içinde emzirme oranı, anne sütü alımı 1 yıl altı olan gruptan anlamlı olarak daha yüksekti (p<0,05). Anne sütü alımı 1 yıl üstü olan grupta, çocuğunun 2 yaşına kadar anne sütü alması gerektiğini düşünenlerin oranı; anne sütü alımı 1 yıl altı olan gruptan anlamlı olarak yüksekti. Anne sütü alımı 1 yıl üstü olan grupta anne eğitim durumu, anne sütü alımı 1 yıl altı olan gruptan anlamlı (p<0,05) olarak daha yüksekti. Anne sütü alımı 1 yıl üstü olan grupta anne çalışma oranı, anne sütü alımı 1 yıl altı olan gruptan anlamlı (p<0,05) olarak daha yüksekti.

Sonuç: Anne sütü ile beslenme hayatın ilk iki yılında bir bebeğin sağlıklı gelişimi ve hastalıklardan korunması için çok önemlidir. Her bebeğin anne sütü ile beslenmek en doğal hakkıdır. Ülkemizde ve bölgemizde anne sütü alım zamanı ve kesilmesine yol açan faktörlerin belirlenmesi, bu konuda önlemler alınmasına ve anne sütü alım süresinin arttırılmasına katkı sağlayacaktır. Mümkünse doğum öncesi başlanan emzirme eğitimi, annenin doğum sonrası desteklenmesi, çalışan anneler için emzirme için uygun olanakların sağlanması anne sütü alım süresini arttıracaktır.

Anahtar Kelimeler: Anne sütü, alım süresi, emzirme

INTRODUCTION

Breast milk is the ideal food for babies. Breastfeeding has many benefits for the mother, the infant and society. Exclusive breastfeeding (EB), especially for the first six months, is very important for the functional, neuromotor and cognitive development of infants. The World Health Organization (WHO) and the European Committee for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommend that all infants should be fed with EB for the first 6 months, and that supplementary foods with high nutritional value should be started in addition to breast milk from the 6th month onwards, and breastfeeding should be continued until at least 2 years of age[1,2].

Breastfeeding reduces infant mortality and morbidity rates, provides optimal growth and development, and establishes a close bond between mother and child. In addition, breast milk protects infants against diseases through antibodies transmitted from the mother, prevents nutritional deficiencies and prevents foodborne infections[3].

Breastfeeding has benefits for the mother as well as the baby. Breastfeeding prevents complications that may occur in the breast due to milk accumulation. Postpartum bleeding, breast, ovarian and uterine cancers, urinary tract infections and osteoporosis are less common in breastfeeding women compared to non-breastfeeding women[4,5].

The WHO and the United Nations Children's Fund (UNICEF) have been working hard to promote breastfeeding. For this reason, in 1992, the WHO initiated the "Baby Friendly Hospital" practice on an international platform and established practical practices, especially in developing countries, to increase breastfeeding. This approach of WHO has led to visible changes in hospital practices in most countries to increase breastfeeding rates[6,7].

In this study, we aimed to determine the sociodemographic factors affecting the duration of breast milk intake and discontinuation of breast milk in the children of mothers with children aged 5 years and residing in Tekirdağ province, to determine the problems that would be experienced during the breastfeeding process in advance and to contribute to the measures to be taken.

MATERIALS AND METHODS

Type, Sample, Place and Time of the Study

Our study is a cross-sectional study in which mothers or caregivers of children aged 5 years and under were surveyed. This study was conducted on 400 children aged 5 years and younger between October 2021 and December 2021 at Tekirdağ Namık Kemal University Hospital, Department of Pediatrics, Pediatrics Outpatient Clinics.

Data Collectıon Method

Our study was conducted by the researcher with face-to-face questionnaire method. The questionnaire takes approximately 10 minutes to complete. In the questionnaire, the child's gender, age, mode of delivery, birth week, birth weight, when he/she first breastfed, whether he/she used formula milk or not, if so, why, the time of starting the first supplementary food, how much breast milk he/she received exclusively and in total, the number of siblings, how long the sibling received breast milk, if any, the total income of the household, the mother's prenatal breastfeeding education, the sociodemographic characteristics, occupations, employment and health status of the mother and father, and the mother's emotional status were questioned.

While categorizing the answers to the question on the total income of the household in the survey, based on the May 2021 data of the TÜRK-İŞ Confederation, the hunger limit of 2850 TL

and below was determined as the lowest limit and the poverty limit of 9300 TL and above was determined as the upper limit.

Statistical Analysis

Mean, standard deviation, median minimum, maximum, frequency and ratio values were used in descriptive statistics of the data. The distribution of variables was measured with the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to analyze quantitative independent data. The chi-square test was employed in the analysis of qualitative independent data, and the Fisher's test was used when chi-square test conditions were not met. Statistical Package for the Social Sciences 28.0 program was used in the analysis.

Research Ethics

The families and other caregivers of the children admitted to the Tekirdağ Namık Kemal University, Pediatrics Outpatient Clinics of the Department of Pediatrics were informed and the children who agreed to participate in the study and signed the Informed Consent Form by their families or caregivers were included in the study.

Approval decision with protocol number 2021.213.07.21 was obtained from the Tekirdağ Namık Kemal University Medical Faculty Non-Interventional Clinical Research Ethics Committee (date: 27.07.2021).

RESULTS

Children aged 5 years and younger who were admitted to the Tekirdağ Namık Kemal University Hospital, Pediatrics Outpatient Clinics of the Department of Pediatrics between October 2021 and December 2021 were included in the study (Table 1, 2, 3).

The mean age was significantly ($p<0.05$) higher in the group with breast milk intake of more than 1 year than in the group with breast milk intake of less than 1 year. Gender distribution did not differ significantly ($p>0.05$) between the groups with breast milk intake less than 1 year and above 1 year. The rate of cesarean delivery was significantly ($p<0.05$) higher in the group with breast milk intake less than 1 year than in the group with breast milk intake more than 1 year. The preterm birth rate was significantly ($p<0.05$) lower in the group with breast milk intake above 1 year than in the group with breast milk intake below 1 year. Low birth weight was significantly ($p<0.05$) lower in the group with breast milk intake above 1 year than in the group with breast milk intake below 1 year.

The number of siblings and duration of breastfeeding did not differ significantly ($p>0.05$) between the groups with breastfeeding intake of less than and above 1 year.

The rate of prenatal breastfeeding education was significantly higher in the group with breastmilk intake of more than 1 year than in the group with breastmilk intake of less than 1 year ($p<0.05$). The rate of breastfeeding within the first 1 hour

Table 1. Distribution of information on mothers' breastfeeding education, time of first breastfeeding, duration of breast milk use, time of first supplementary food initiation and duration of breast milk intake

		Minimum-maximum	Median	Mean±SD	n	%
Did the mother receive prenatal breastfeeding education?	Yes				183	45.8%
	No				217	54.3%
When did she first breastfeed?	None				14	3.5%
	First 1 hour				256	64%
	1-4 hours				85	21.3%
	After 4 hours				55	13.8%
Duration of breast milk use	None	0.0-36	17.0	15.0±9.3	14	3.5%
	0-6 months				80	20%
	6-12 months				62	14.5%
	12-18 months				74	18.5%
	18-24 months				117	29.3%
	24-30 months				45	11.3%
	30-36 months				8	2.0%
When is the first supplementary food?		0.0-16.0	6.0	5.8±1.7		
Has she/he started supplementary food?	Not started				19	4.8%
	Started				381	95.3%
How long should a child be breastfed?	First 6 months				27	6.8%
	Until one year of age				50	12.5%
	Until two years of age				323	80.8%

SD: Standard deviation

was significantly higher in the group with breastmilk intake of more than 1 year than in the group with breastmilk intake of less than 1 year ($p<0.05$). The proportion of those who thought that their child should receive breast milk until the age of 2 years was significantly higher in the group with breast milk intake of more than 1 year than in the group with breast milk intake of less than 1 year ($p<0.05$) (Table 4).

Maternal age did not differ significantly between the groups with breast milk intake of less than 1 year and more than 1 year ($p>0.05$). Maternal education level was significantly higher in the group with breast milk intake over 1 year than in the group with breast milk intake less than 1 year ($p<0.05$). Maternal employment rate was significantly higher in the group with breastmilk intake above 1 year than in the group with breastmilk intake below 1 year ($p<0.05$). In the group

Table 2. Distribution of maternal sociodemographic characteristics, number of siblings and duration of breastfeeding

		Minimum-Maximum	Median	Mean±SD	n	%
Age of mother		3.0-46.0	31.0	31.7±5.3		
Mother's education level	Primary school				38	9.5%
	Middle school				46	11.5%
	High school				79	19.8%
	University				215	53.8%
	Master's degree				22	5.5%
Does the mother work?	Yes				206	51.5%
	No				194	48.5%
If the mother works, what is her occupation?	Teacher				57	27.7%
	Private sector employee				31	15%
	Civil servant				30	14.6%
	Nurse				16	7.8%
	Doctor				13	6.3%
	Engineer				12	5.8%
	Worker				8	3.9%
	Health officer				7	3.4%
	Security				6	2.9%
	Self-employment				5	2.4%
	Emergency medical technician				5	2.4%
	Tradesmen				4	1.9%
	Academician				3	1.5%
	Lawyer				2	1%
	Dentist				2	1%
	Bank employee				2	1%
	Physiotherapist				2	1%
	Police officer				1	0.5%
Did the mother start working after the birth?	Not working				194	48.5%
	Not started				19	4.8%
	Started				187	46.8%
When did the mother start working after the birth?		1.0-120.0	7.0	11.6±14.4		
How long did she breastfeed the siblings?		0.0-40.0	1.0	8.3±10.5		
Number of siblings	1				189	47.3%
	2				176	44%
	3				26	6.3%
	4				7	1.8%
	5				2	0.5%

SD: Standard deviation

Table 3. Distribution of the child's formula intake status and reason, and total duration of breastfeeding

		n	%
Did she/he take formula?	Yes	199	49.8%
	No	201	50.2%
If so, when did she/he first start formula?	First 1 week	83	41.7%
	1. month	11	5.5%
	2. month	37	18.6%
	3. month	18	9%
	4. month	15	7.5%
	5. month	8	4%
	6. month	14	7%
	7. month	5	2.5%
	8. month	4	2%
	9. month	2	1%
	12. month	2	1%
Why did she/he start formula?	Because breast milk was insufficient	147	73.9%
	Because the doctor recommended it	30	15.1%
	Because the mother thought the baby was not full	22	11.1%
Who recommended formula?	Doctor	152	76.4%
	Nurse	7	3.5%
	Relative	2	1%
	Herself	38	19.1%
Breast milk intake	Less than 1 year	156	39%
	More than 1 year	244	61%

Table 4. Distribution of information about the mother's breastfeeding education, time of first breastfeeding and duration of breastfeeding in the groups with breast milk intake of less than or more than 1 year

		Breast milk intake				
		Less than 1 year		More than 1 year		p
		Mean±SD		Mean±SD		
		n	%	n	%	
Did the mother receive prenatal breastfeeding training?	Yes	58	37.2%	125	51.2%	**0.006** x^2
	No	98	62.8%	119	48.8%	
When did she first breastfeed	First 1 hour	76	48.7%	170	69.7%	**0.000** x^2
	1-4 hours	34	21.8%	51	20.9%	
	After 4 hours	32	20.5%	23	9.4%	
How long did the child receive breast milk?	First 6 months	24	15.4%	3	1.2%	**0.000** x^2
	Up to 1 year old	38	24.4%	13	5.3%	
	Up to 2 years old	94	60.3%	228	93.4%	

x^2: Chi-square test, SD: Standard deviation

with breast milk intake of more than 1 year, the rate of maternal employment after birth was significantly higher than the group with breast milk intake of less than 1 year ($p<0.05$). There was no significant difference between the groups with breast milk intake of less than 1 year and more than 1 year in the mother's time of starting work after delivery ($p>0.05$). The rate of maternal smoking did not differ significantly between the groups with breast milk intake below and above 1 year ($p>0.05$). The rate of maternal alcohol use was significantly higher in the group with breast milk intake over 1 year than in the group with breast milk intake less than 1 year ($p<0.05$). The rate of known maternal illness was significantly lower in the group with breast milk intake over 1 year than in the group with breast milk intake less than 1 year ($p<0.05$). The rate of maternal postpartum mood disorder did not differ significantly between the groups with breast milk intake of less than or more than 1 year ($p>0.05$) (Table 5).

Table 5. Distribution of mothers' age, education level, employment and health status, and postnatal mood in the groups with breast milk intake of less than or more than 1 year

		Breast milk intake				
		Less than 1 year		More than 1 year		p
		n	%	n	%	
Mother's education level	Primary school	22	14.1%	16	6.6%	**0.000 x^2**
	Middle school	28	17.9%	18	7.4%	
	High school	34	21.8%	45	18.4%	
	University	64	41%	151	61.9%	
	Master's degree	8	5.1%	14	5.7%	
Is the mother working?	Yes	62	39.7%	144	59%	**0.000 x^2**
	No	94	60.3%	100	41%	
Did the mother start working after the birth?	Not working	94	60.3%	100	41%	**0.000 x^2**
	Not started	13	8.3%	6	2.5%	
	Started	49	31.4%	138	56.6%	
Does the mother smoke?	Yes	34	21.8%	42	17.2%	0.255 x^2
	No	122	78.2%	202	82.8%	
Does the mother drink alcohol?	Yes	0	0%	12	4.9%	**0.005 x^2**
	No	156	100%	232	95.1%	
Does the mother have a known disease?	Yes	27	17.3%	25	10.2%	**0.041 x^2**
	No	129	82.7%	219	89.8%	
Postpartum mood disorder in the mother	Yes	32	20.5%	54	22.1%	0.701 x^2
	No	124	79.5%	190	77.9%	

x^2: Chi-square test

Paternal age did not differ significantly between the groups with breast milk intake less than 1 year and above 1 year (p>0.05). Paternal education level was significantly higher in the group with breast milk intake over 1 year than in the group with breast milk intake less than 1 year (p<0.05). Paternal employment rate did not differ significantly between the groups with breast milk intake of less than and above 1 year (p>0.05). The rate of paternal smoking was significantly lower in the group with breast milk intake over 1 year than in the group with breast milk intake less than 1 year (p<0.05). There was no significant difference between the groups with breast milk intake of less than or more than 1 year (p>0.05). There was no significant difference in the rate of known disease in the father between the groups with breast milk intake of less than 1 year and more than 1 year (p>0.05). Total household income was significantly higher in the group with breastfeeding intake above 1 year than in the group with breastfeeding intake below 1 year (p<0.05).

The rate of formula feeding was significantly lower in the group with breast milk intake over 1 year than in the group with breast milk intake less than 1 year (p<0.05). In the group with a breast milk intake of more than 1 year, the rate of starting formula at 3 months and later was significantly lower than the group with a breast milk intake of less than 1 year (p<0.05). The reason for the initiation of formula feeding did not differ significantly between the groups with breast milk intake of less than 1 year and more than 1 year (p>0.05). The person who recommended formula did not differ significantly between the groups with breast milk intake of less than or more than 1 year (p>0.05).

The rate of prenatal breastfeeding education did not differ significantly between the groups who received formula for the first 4 months and those who did not (p>0.05). The duration of EB was significantly lower in the group receiving formula for the first 4 months than in the group not receiving formula for the first 4 months (p<0.05). The rate of breastfeeding within the first hour was significantly lower in the group receiving formula for the first 4 months than in the group not receiving formula for the first 4 months (p<0.05). The proportion of those getting breast milk for 1 year or less in the group who received formula for the first 4 months (57.9%) was significantly higher than the proportion of those who used breast milk for 1 year or less in the group who did not receive formula for the first 4 months (25.8%) (p<0.05). The rate of those who thought that their child should receive breast milk until the age of 2 years was significantly lower in the group who received formula for the first 4 months than in the group who did not receive formula for the first 4 months (p<0.05).

DISCUSSION

In this cross-sectional study conducted among parents with children aged 5 years and younger, who applied to the Pediatrics Outpatient Clinic of Tekirdağ Namık Kemal University Hospital, sociodemographic data affecting the duration of breast milk intake and weaning time, problems experienced in this process and solutions were investigated.

Total fertility rate refers to the average number of children a woman can give birth to in the age group of 15-49 years during her fertile period. According to Turkish Statistical Institute 2020 data, the total fertility rate is 1.76[8]. In our study, 47.3% of the families had one child, 44% had two children, 6.5% had three children, and 2.2% had more than three children.

In our study, the mode of delivery was questioned and 63.8% were delivered by cesarean section and the rate of cesarean section was 57% according to 2019 data in Turkey[8]. The rate of cesarean delivery is increasing in our country and the data of our study were found to be close to the average of Turkey. Considering that cesarean delivery affects breastfeeding, the high rates are striking. In a study conducted by Hobbs et al.[9] in 2016, when planned cesarean delivery and normal spontaneous vaginal delivery were compared, it was reported that women who gave birth by cesarean delivery had a higher rate of discontinuation of breastfeeding before 12 weeks postpartum and that cesarean delivery negatively affected breastfeeding initiation time and total breastfeeding duration. In our study, we found that cesarean delivery had a negative effect on EB and total breastfeeding duration. However, Pérez-Escamilla et al.[10] reported that cesarean delivery had no effect on total breastfeeding duration if mothers started breastfeeding from birth and continued breastfeeding for at least 4 weeks after birth. Similarly, in the study conducted by Watt et al.[11] no difference was found between the total breastfeeding duration of mothers who continued breastfeeding up to 6 weeks postpartum and mothers who delivered by cesarean section or normal spontaneous vaginal delivery.

Delays in the initiation of breastfeeding may lead to a decrease in the baby's sucking ability and sensitivity, as well as a decrease in the amount of milk[12-20]. In a study by Raghavan et al.[21], it was reported that cesarean delivery and male gender were the greatest risk factors for early initiation of breastfeeding. In our study, we found that babies who were breastfed within the first 1 hour after birth had a higher duration of EB intake and total breast milk intake for the first 6 months compared to babies who were not breastfed within the first 1 hour. These results are consistent with the WHO's 10 steps for successful breastfeeding, which encourage initiation of breastfeeding within the first 1 hour after birth and recommend that babies stay with their mothers for 24 hours[22-24].

Difficulties experienced in initiating and maintaining breastfeeding in the first child lead to the same problems in subsequent births[25,26]. In our study, we found that mothers who breastfed their first child had higher rates of feeding their children with EB for the first 6 months in other births, but this did not affect the total duration of breastfeeding.

Preterm babies have many problems, including irregular sucking ability, low birth weight and low alertness levels. These adversely affect initiation and maintenance of breastfeeding[27,28]. In our study, preterm birth and low birth weight negatively affected the duration of EB and total breast milk intake in the first 6 months.

Scime et al.[29] reported that there was no significant difference between the rates of EB in the first 6 months between mothers with and without a chronic disease, but the presence of a chronic disease in the mother may negatively affect the total breastfeeding time. In our study, the presence of a known chronic disease in the mother did not affect the duration of EB in the first 6 months but shortened the total duration of EB.

In their meta-analysis, Cohen et al.[30] found that higher educational level had a positive effect on both initiation and continuation of breastfeeding. It is thought that mothers with higher levels of education and working mothers may have more control over their home or working environment, which has a positive effect on the duration of breastfeeding[31]. In a study conducted by Laksono et al.[32] in 2021 using data from 53,528 children aged 5 years and younger, it was reported that as the mother's education level increased, the duration of EB intake increased, but the duration of EB intake was inversely associated with the mother's employment status. In our study, we concluded that a high level of maternal education and employment status of the mother had a positive effect on the duration of EB and total breast milk intake in the first 6 months.

In a study by Mathew et al.[33] including children aged 4 years and younger published in 2021, it was found that breastfeeding rates were higher in families with higher household income, and that this was inversely related to maternal age. In another study, sociodemographic characteristics such as low household income and low maternal age were associated with a decreased likelihood of initiation and continuation of breastfeeding[34]. In our study, high total household income had a positive effect on the duration of SAS and total breastfeeding in the first 6 months, whereas maternal age and infant gender were not found to be associated with these durations. In a study by Hacıan-Tilaki[35], it was reported that mothers with higher educational level breastfed their children for a longer period of time, whereas maternal age and infant gender had no effect on the duration of breastfeeding.

Mothers who received prenatal breastfeeding education are more likely to initiate and continue breastfeeding than mothers who did not receive prenatal breastfeeding education[36-40]. In our study, no significant difference was found between mothers who received prenatal breastfeeding education and mothers who did not receive prenatal breastfeeding education in the rates of feeding their babies with EB, and we concluded that mothers who received prenatal breastfeeding education fed their children with breast milk for longer periods.

In many studies, it has been found that smoking by breastfeeding mothers shortens the duration of breastfeeding[41-45]. In our study, no significant difference was found between the duration of feeding their babies with EB and total breastfeeding in smoking and nonsmoking mothers. This result made us think that mothers may have hesitated to answer the more personal questions in the questionnaire.

Study Limitations

Our study is limited to individual statements as it was conducted with the survey method. The fact that the survey was conducted face-to-face by a single interviewer helped to ensure that the questions were asked in the same way to each participant and the answers were clearer, but it was not always possible for the participants to perceive all the questions in the survey in the same way. In particular, the question "Have you received prenatal breastfeeding education?" was misunderstood and a high percentage (45.8%) answered AS yes. At the same time, the presence of the mother's relatives or other people in around during the survey may have prevented the mothers from always giving the clearest and most accurate answer.

Ethics

Ethics Committee Approval: Approval decision with protocol number 2021.213.07.21 was obtained from the Tekirdağ Namık Kemal University Medical Faculty Non-Interventional Clinical Research Ethics Committee (date: 27.07.2021).

Informed Consent: Cross-sectional study.

Authorship Contributions

Concept: B.N., Design: B.N., G.Y.Ç., Data Collection or Processing: B.N., G.Y.Ç., A.N., Analysis or Interpretation: B.N., A.N., Literature Search: B.N., G.Y.Ç., Writing: B.N., G.Y.Ç.

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Evaluation of Postoperative Complications in VASER®-assisted Liposuction: A Retrospective Study of 1,486 Cases

VASER® Destekli Liposuctionda Postoperatif Komplikasyonların Değerlendirilmesi: 1.486 Olgunun Retrospektif Çalışması

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ABSTRACT

Aim: Liposuction is a popular plastic surgery procedure with a growing number of cases. Despite advancements, complications remain a significant concern. Modifications like ultrasound-assisted techniques aim to improve safety and efficacy. The aim of this study was to assess the postoperative complications in patients who underwent a vibration amplification of sound energy at resonance (VASER®)-assisted liposuction (VAL) procedure.

Materials and Methods: A retrospective study of 1,486 VAL cases was conducted. Patient demographics, surgical procedures, postoperative care, and complications were recorded.

Results: Of 1,486 patients, 45 (3.02%) experienced minor complications including loss of sensitivity, tissue stiffness, seroma, hyperpigmentation, and prolonged edema. No major complications or fatalities were observed. There was no significant correlation between fat aspirate volume and complications.

Conclusion: VAL demonstrates safety and effectiveness, with a relatively low complication rate. Sensory loss and tissue stiffness were the most common complications. Hyperpigmentation was transient and resolved with postoperative care. Attention to patient selection, meticulous technique application, fluid management, and postoperative care is crucial to minimize complications in VAL procedures. Further studies are required to explore the specific impacts of ultrasound-assisted liposuction on patient outcomes.

Keywords: Liposuction, vibration amplification of sound energy at resonance (VASER®), complications, patient safety

ÖZ

Amaç: Liposuction, giderek artan sıklıkla gerçekleştirilen bir plastik cerrahi işlemidir. Ancak, bu alandaki güncel gelişmelere rağmen komplikasyonlar hala önemli bir endişe kaynağıdır. Ultrason destekli teknikler gibi modifikasyonlar güvenliği ve etkinliği artırmayı amaçlamaktadır. Bu çalışmanın amacı, rezonansta ses enerjisinin titreşim amplifikasyonu (VASER®) destekli liposuction (VAL) prosedürü uygulanan hastalarda postoperatif komplikasyonları değerlendirmektir.

Gereç ve Yöntem: 1.486 VAL olgusu retrospektif olarak incelendi. Hastaların demografik özellikleri, cerrahi prosedürler, postoperatif bakım ve komplikasyonlar kaydedildi.

Bulgular: 1.486 hastanın 45'inde (%3,02) hassasiyet kaybı, doku sertliği, seroma, hiperpigmentasyon ve uzun süreli ödem gibi minör komplikasyonlar görüldü. Majör bir komplikasyon veya ölüm gözlenmedi. Aspire edilen yağ hacmi ile komplikasyonlar arasında anlamlı bir ilişki bulunamadı.

Sonuç: VAL nispeten düşük komplikasyon oranıyla güvenli ve etkin bir yöntem olma özelliği göstermektedir. Serimizde, duyu kaybı ve doku sertliği en sık görülen komplikasyonlardı. Hiperpigmentasyon geçiciydi ve ameliyat sonrası bakımla çözüldü. VAL işlemlerinde komplikasyonları en aza indirmek için hasta seçimine, titiz teknik uygulamasına, sıvı yönetimine ve ameliyat sonrası bakıma dikkat edilmesi çok önemlidir. Ultrason destekli liposuctionın hasta sonuçları üzerindeki spesifik etkilerini araştırmak için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Liposuction, rezonansta ses enerjisinin titreşiminin yükseltilmesi (VASER®), komplikasyonlar, hasta güvenliği

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INTRODUCTION

As of 2021, liposuction stands out as the most prevalent plastic surgery procedure, for both women and men, with an increasing popularity, boasting a 25% increase, which corresponds to approximately two million cases in the United States alone[1]. Furthermore, the report also describes the liposuction as the second most common plastic surgery procedure, accounting for 59,696 cases, which is 12.9% of all cases.

The liposuction is briefly defined as a suction-assisted removal of fat tissue using various cannulas, and the procedure can be conducted under either general or local anesthesia. The amount of adipose tissue removed during liposuction can vary significantly, ranging from a few hundred milliliters to several liters.

However, irrespective of the surgeon's expertise and the utilized technique, the procedure causes significant complications due to several factors such as inappropriate selection of patients, disturbances in the perioperative and postoperative care, and several unpredictable issues[2].

In order to overcome these challenges and possible complications, several modifications, including ultrasound-assisted liposuction (UAL), the injection of a tumescent solution into the targeted area, a subdermal or superficial approach, and the utilization of a wide-range of cannulas, have been suggested. All these modifications aim to enhance the safety and efficacy of the liposuction procedure.

In the existing literature, the overall complication rate associated with liposuction has been documented within the range of 8.6-20%. The most prevalent complication is contour deformity, which has been reported in approximately 20% of cases. Other complications include seroma, hyperpigmentation, asymmetry, and hypertrophic scar, but these are less common[3,4].

Major or life-threatening complications, including skin necrosis, infection, necrotizing fasciitis, pulmonary embolism, and fatal outcomes, have been documented in approximately 0.02-0.25% of liposuction cases[5,6].

In this article, we briefly reviewed and discussed the postoperative complications in our patients who underwent a vibration amplification of sound energy at resonance (VASER®)-assisted liposuction (VAL) procedure.

MATERIALS AND METHODS

The study cohort comprised 45 patients in 1,486 VAL cases, who experienced complications following the procedures performed between January 2018 and February 2023. All patients provided informed consent, and the study was conducted in accordance with the Declaration of Helsinki. Ethical approval for the study was granted by the İstanbul Atlas University Local Ethics Committee (document no: 27247, date: 10.05.2023).

Patients with pre-existing chronic medical conditions such as diabetes mellitus, anemia, or disorders affecting the cardiovascular, renal, or hepatopancreaticobiliary systems were excluded for liposuction procedures.

The inclusion criteria encompassed patients who had undergone VAL procedures, did not exhibit the aforementioned exclusion criteria, and had developed postoperative complications. These patients were closely monitored until the resolution of their complications.

All operations were performed by the same board-certified plastic surgeon under general anesthesia. Prior to surgery, the patient's surgical site was prepared with a povidone-iodine solution, and the patient was draped in a sterile manner and given the prone position. A tumescent solution prepared with 1 mg adrenaline and 10% anti-arrhythmic (lidocaine hydrochloride) into each 1000 mg Ringer's lactate solution was infiltrated in the areas of liposuction and fat harvesting.

The liposuction procedures employed third-generation VASER® technology and encompassed multiple areas of aspiration, including the abdomen, gluteal region, arms, flanks, back, and thighs. During the procedure, VASER® cannulas with dimensions of 3.7, 2.9, and 2.2 mm were employed, operating in both continuous and pulse modes. The VASER® mode was configured at 100% energy (C) using a 3.7 mm 5-groove probe, with an infiltration rate of 100 mL per minute, allowing the fat emulsification.

The liposuction was performed using the conventional technique, employing 3-4, and 5 mm reverse triangular cannulas on specific regions and maintaining a flow rate of 24-26 mmHg per second.

Patients were given prophylactic antibiotherapy with a single dose of the first-generation cephalosporins prior to, and pain medication with non-steroidal anti-inflammatory drugs such as acetaminophen or ibuprofen after the procedure. Patients were advised to wear compression garments for a minimum of one-month post-surgery and to undergo regular lymph drainage massage for at least 15 days.

To ensure proper postoperative care, all patients were scheduled for follow-up visits, including check-ups, and photographs were taken of those residing abroad.

Statistical Analysis

Descriptive statistics were conducted using the Statistical Package for the Social Sciences (SPSS) 21.0 program (SPSS Inc., Chicago, IL, USA). To assess the relationships between variables, a correlation analysis was performed and Pearson correlation

coefficients were calculated. A statistical significance level of <0.05 was considered as indicative of a significant correlation between variables.

RESULTS

During the study period, a total of 1,486 patients underwent VAL procedures targeting various areas such as the flanks, hips, waist, abdomen, neck, upper arms, chest (in male patients), medial and lateral thighs, and knees. Among 1,486 cases, 45 (3.02%) patients developed minor complications including loss of sensitivity, tissue stiffness, seroma, hyperpigmentation, and prolonged edema.

The patient group had a mean age of 36.4±6.28 years, ranging from 23 to 48 years, with a male-to-female ratio of 5/40. The mean body mass index (BMI) was 27.16±1.89 kg/m², ranging from 19.88 to 31.33 kg/m². The average volume of the total aspirate was 7833±1821 mL, ranging from 5450 to 10280 mL. The total number of aspirated regions was 5±2.8 (range 3-9), while the duration of VASER® was 72.4±18.6 minutes (range 34-98) (Table 1). Figure 1 shows the total volume of the tumescent solution given, and the aspirated fat tissue.

Table 1. Demographics and intraoperative data of the study profile

	Patients with complications (n=45)	
Characteristics	**Mean±SD**	**Minimum-maximum**
Age (years)	36.4±6.28	23-48
Gender (male/female) (n; %)	(5/40; 11.11/88.89)	
BMI (kg/m²)	27.16±1.89	19.88-31.33
Volume of total aspirate (mL)	7833±1821	5450-10280
Volume of tumescent solution (mL)	11430±1836	8650-13920
Number of aspirated regions	5±2.8	3-9
Duration of VASER® (min)	72.4±18.6	34-98

VASER®: Vibration amplification of sound energy at resonance, SD: Standard deviation, BMI: Body mass index

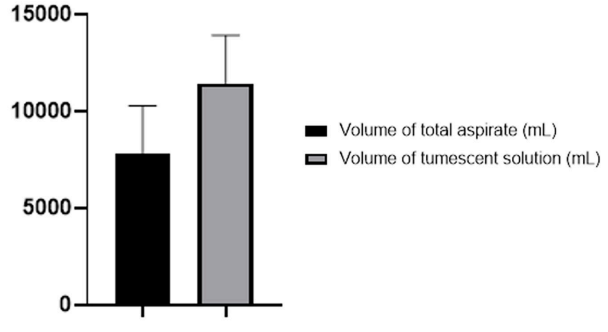

Figure 1. The total volume of the tumescent solution, and the aspirated fat tissue

Among the 41 patients, a total of 71 complications were recorded. The most common complication was the loss of sensitivity, observed in 34 cases (47.88%), followed by tissue stiffness in 26 cases (36.61%). Seroma occurred in 15 patients (21.12%), hyperpigmentation in 5 patients (7.04%), and prolonged edema in one patient (1.40%). Within the overall patient group consisting of all 1,486 cases, the most prevalent complication was loss of sensitivity (2.28% of cases), followed by tissue stiffness (1.74%), seroma (1.00%), hyperpigmentation (0.33%), and prolonged edema (0.06%) (Table 2).

Multiple complications were present for some patients. Specifically, 14 cases (31.11%) experienced both the loss of sensitivity and tissue stiffness, while 6 patients (13.33%) had seroma in addition to these complications. Four cases (8.88%) had both seroma and loss of sensitivity, and one patient (2.22%) exhibited tissue stiffness along with hyperpigmentation. In the overall patient group, complications included a combination of loss of sensitivity and tissue stiffness in 14 cases (0.94%), seroma along with loss of sensitivity and tissue stiffness in 6 cases (0.40%), seroma combined with loss of sensitivity in 4 cases (0.26%), and a combination of tissue stiffness and hyperpigmentation in 1 case (0.06%) (Table 3).

Notably, there was no significant correlation between the volume of aspirate and the number of complications per patient (r=0.12, p=0.67).

Table 2. Overall evaluation of the complications

Type of complication	Overall patient group (n=1,486) (n; %)	Complication group (n=45) (n; %)
Loss of sensitivity	34; 2.28	34; 47.88
Tissue stiffness	26; 1.74	26; 36.61
Seroma	15; 1.00	15; 21.12
Hyperpigmentation	5; 0.33	5; 7.04
Prolonged edema	1; 0.06	1; 1.40

Table 3. Patients with multiple complications

Type of complication	Overall patient group (n=1,486) (n; %)	Complication group (n=45) (n; %)
Loss of sensitivity + tissue stiffness	14; 0.94%	14; 31.11%
Seroma + loss of sensitivity + tissue stiffness	6; 0.40%	6; 13.33%
Seroma + loss of sensitivity	4; 0.26%	4; 8.88%
Tissue stiffness + hyperpigmentation	1; 0.06%	1; 2.22%

All of the complications were resolved within 3.2±2.8 months (range 2-8 months).

We did not observe other complications, including contour irregularities, chronic induration, infection, operation site burn or distant site burn, and skin necrosis in our patient group. Furthermore, there were no major life-threatening complications or fatalities.

Figure 2 shows the preoperative and postoperative pictures of a female patient.

DISCUSSION

Since the introduction of liposuction, the procedure has undergone several modifications over time to maximize patient safety and decrease the occurrence of complications. Complications stemming from a liposuction procedure liposuction can be broadly classified into three subcategories:

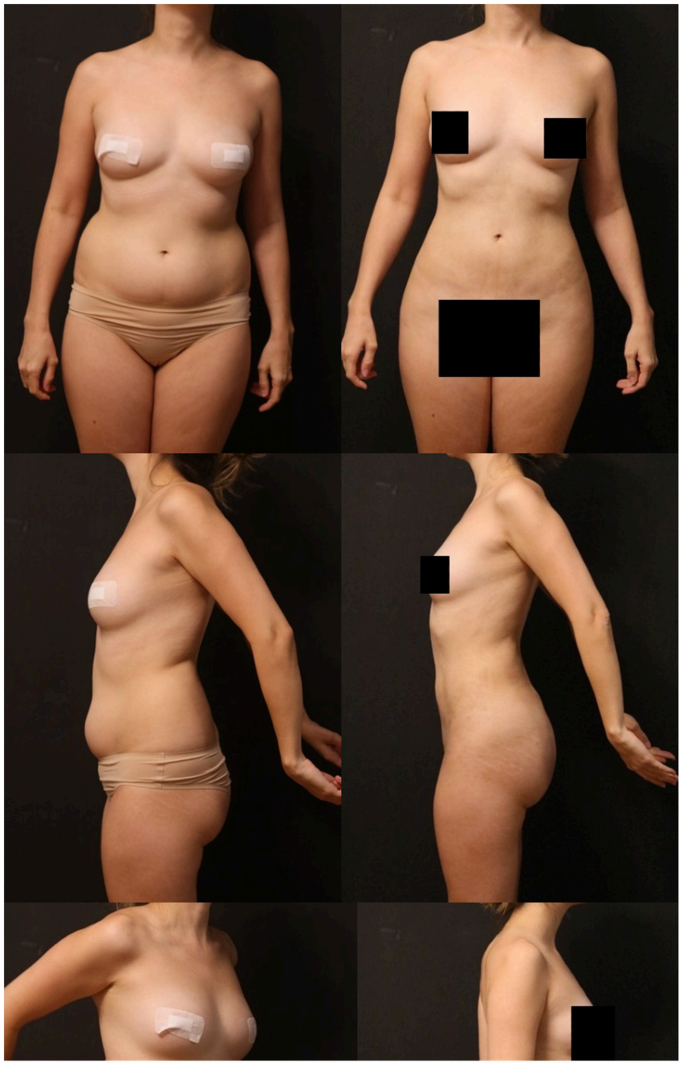

Figure 2. Pre- and post-operative photographs of a female patient who underwent VAL procedure

VAL: VASER®-assisted liposuction

local and systemic complications, alongside patient dissatisfaction[4].

Third-generation VAL emerges as a safe and effective technique for body contouring, enabling surgeons to more precisely target the superficial fat layer and promote skin tightening while maintaining a relatively low rate of complications and achieving higher levels of patient satisfaction[7,8].

In our study, we specifically address local complications, which encompassed the following issues: loss of sensitivity, tissue stiffness, seroma, hyperpigmentation, and prolonged edema. Among the 45 patients in our study group, 25 individuals (55.56%) experienced the presence of more than one complication, and six of them (13.33%) had the occurrence of three different types of postoperative liposuction-related complications.

The overall complication rate was 3.02% in our study group, while the complication rates for liposuction were reported to be in the range of 8.6-20%[3].

Chow et al.[9] conducted a study with 4,534 patients, revealing a 1.5 percent postoperative complication rate in liposuction, identifying liposuction volume and BMI as notable independent risk factors. However, their study had limitations, as it relied on data from the American Society of Plastic Surgeons (ASPS) member database and lacked information on the specific types of liposuction procedures, potentially leading to an underrepresentation of complications and resulting in a relatively lower reported complication rate.

In a 5-year study involving 551 consecutive patients, liposuction alone had a 4.2% complication rate, and they suggest a limited epigastric ultrasound time of less than 1 minute, and liposuction time of 2 minutes minimizes the risk of seroma formation[10].

In their recent case series of 261 patients who underwent UAL, Tran et al.[11] reported an overall complication rate of 4.6%, contour irregularity being the most common complication. However, they frequently avoided large-volume liposuction and the lipoaspirate volume in their cohort was an average of 2284 mL.

It is a well-known fact that traditional suction-assisted liposuction (SAL) is linked to significant complications, some of which can be life-threatening. Since its introduction by Zocchi[12] and Kloehn[13] in 1996 and 1998, respectively, the reports have revealed lesser complication major rates with the use of UAL[14]. However, UAL is associated with an increased risk of thermal injury and skin necrosis to subdermal tissues due to the exothermic energy caused by ultrasound. In our 1,486 cases operated within five years, we did not encounter such major complications.

Despite the studies suggesting a correlation between the volume of aspirated fat tissue and an increased risk of complications, our study did not find any significant association between the types and number of complications and the volume of aspirated fat tissue.

One notable finding from our study was that the incidence of seroma, a common complication following liposuction, which is collection of serous fluid originating from the fibrous tissue, was surpassed by the occurrences of loss of sensitivity and tissue stiffness. We speculate that this finding might be attributed to the use of an ultrasound-assisted approach, which is relatively safer compared to the traditional SAL, providing better control over the shredding and flow of adipose tissue cells.

It is worth mentioning that many liposuction procedures are performed in conjunction with abdominoplasty, where the use of electrodissection and the absence of Scarpa fascia preservation can lead to higher tissue damage and seroma formation[15]. None of the patients in our study underwent additional procedures, which could explain the lower rates of seroma in our group. Furthermore, the placement of drains in the abdominal and sacral regions, along with secondary healing after drain removal, might also contribute to the lower incidence of seroma. We also advocate the use of postoperative compression garments and frequent lymph drainage massage, which could be additional factors contributing to the lower incidence of seroma and absence of hematoma in our patient group.

On the other hand, we propose that the use of a tumescent solution containing lidocaine and epinephrine could be one of the underlying reasons for the relatively higher rate of complications such as loss of sensitivity and tissue stiffness in our patient group. Supporting this hypothesis, these complications resolved shortly after the liposuction procedure in most cases. While some reports have suggested an increased incidence of these complications with a larger volume of aspirates, our study did not find a significant correlation between the volume of aspirated fat and any type of complications[9]. A report by Francis et al.[16] indicates the acidic nature of the tumescent solution, which was also enriched by lactated Ringer's solution, can be overcome by the addition of sodium bicarbonate as a buffer, which also enhances adipose stem cell viability. In addition to the tumescent solution, other factors such as the mechanical effect of the liposuction cannula and the conversion of ultrasound energy into thermal energy might also contribute to temporary postoperative neuropraxia. However, these potential confounders cannot be confirmed with absolute certainty and require further investigation through randomized and controlled studies.

In our patient series that experienced complications after liposuction procedures, 5 out of 45 individuals (7.04%) developed increased skin pigmentation, primarily in the abdominal and buttocks areas. Post-liposuction hyperpigmentation is a multifactorial condition with multiple potential causes, including hemosiderin deposition, excessive pressure made by the compression bandages, friction between clothing and the treated skin areas, sun exposure, and exogenous drugs such as iron supplements, hormonal therapy, and minocycline[4]. While the patients were evaluated in an attempt to find the underlying etiology, two reported intake of contraceptive pills, and two were under oral iron supplement therapy. However, the hyperpigmentation resolved in all patients within the first year after the liposuction procedure with regular use of sunscreen and topical application of hydroquinone.

Large-volume liposuction in the context of liposuction procedures is typically defined as the removal of 5000 ml or more of total aspirate during a single procedure, according to the ASPS[17]. However, some studies have set a lower threshold, considering 3500 mL or more of total aspirate volume as significant[18,19]. This volume is often considered safe to remove and is roughly equivalent to 5-8% of the patient's body weight[2].

In large-volume liposuction procedures, postoperative anemia is a significant concern and one of the most important causes of morbidity, thus it is crucial to consider the patient's physiological condition while ensuring the desired aesthetic outcomes. This necessitates a closer monitoring of patients during the preoperative and postoperative periods. In our series, which evaluated blood loss in large-volume liposuction cases using third-generation internal UAL, we observed that the amount of aspirated supernatant was responsible for 44.4% of the change in hemoglobin and 30.9% of the change in hematocrit levels after the procedure[20]. Additionally, the presence of epinephrine in the tumescent solution can have an impact on the cardiac index, heart rate, and mean pulmonary arterial pressure, and a detailed patient screening before the surgery and appropriate patient selection are also critical factors. A recent survey by the ASPS has reported a mortality rate of 19.1/100,000, corresponding to 0.019% of all liposuction procedures, defining the major cause of death as pulmonary thromboembolism[5]. However, a study focusing on tumescent liposuction reported no death in a series of 66,000 cases[21]. Major risk factors associated with severe complications included poor sterility, infiltration of large volumes of wetting solution, early postoperative discharge, selection of medically unfit patients, and procedures performed by clinicians without accreditation in plastic surgery[22].

A histopathological comparison of abdominoplasty specimens in patients who underwent both abdominoplasty and liposuction, with UAL treatment on one side and standard liposuction on the other side, revealed that disrupted collagen

and elastin structures in the treated tissues were associated with longer application times[23]. Hence, we recommend that UAL is a safe and efficient technique when performed by experienced professionals. A close monitorization of application in terms of amplitude settings, as well as paying attention to signs such as the decreased resistance of tissue to probe movement and any alterations in the color of the aspirate in favor of different shades of pink and red. Additionally, it is crucial to avoid keeping the ultrasonic probes in one place for an extended period in order to prevent prolonged contact with the dermis.

Study Limitations

This study has several limitations worth noting. Firstly, its retrospective nature may introduce biases, and the findings are based on a single liposuction method. However, the primary focus of this study was to investigate potential factors associated with the use of an ultrasound-assisted approach and to report different complication trends compared to traditional liposuction methods.

CONCLUSION

In conclusion, the choice of liposuction technique should involve careful consideration of patient selection, meticulous technique application, appropriate fluid management, postoperative care, and the surgeon's expertise with the chosen method and handling and closely monitoring the ultrasound energy, and its effects on the operation sites. These factors are crucial for minimizing undertreated cases, reducing the need for re-operation, which can increase costs, prolong recovery times, and overall pose greater risks to patients.

Ethics

Ethics Committee Approval: The study was approved by the İstanbul Atlas University of Local Ethics Committee (document no: 27247, date: 10.05.2023).

Informed Consent: Retrospective study.

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Relationship Between Endometriosis, Borderline Adnexal Tumors and Malignant Tumors: A Retrospective Case Study

Endometriozis ile Adneksiyal Borderline ve Malign Tümörler Arasındaki İlişki: Retrospektif Olgu Serisi Çalışması

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ABSTRACT

Aim: The main purpose of this study is to investigate the relationship between ovarian endometriosis and borderline ovarian tumor (BOT) and malignant ovarian tumor.

Materials and Methods: Data were retrospectively collected from patients with BOT or malignant adnexal tumor and endometriosis in the surrounding tissue (in the same piece) in the pathology after gynecological surgery at the tertiary center Obstetrics and Gynecology Clinic, between 2017 and May 2023. Patient age, gravidity, body mass index, family history of cancer, clinical complaints, ultrasound and magnetic resonance imaging (MRI) features of the detected mass, preoperative tumor markers and pathological stages were recorded.

Results: A total of 49 BOTs were diagnosed in this 5.5-year period. 19 of them were serous, 22 of them were mucinous, 7 of them were seromucinous, and 1 of them was endometrioid BOT. In 9 BOT cases, there was pathologically confirmed endometriosis in the same ovary or pelvic tissue remaining in the surgical area. There were 37 malignant epithelial ovarian cancer diagnoses in 5.5 years. Of these, 11 had endometrioid ovarian cancer (1 had endometrioid and clear cell cancer), 25 had serous ovarian cancer and 1 had mucinous ovarian cancer. Endometriosis was associated with 4 cases of serous adenocarcinoma and 4 cases of endometrioid ovarian cancer. Among these patients, BOT patients with endometriosis were younger. Tumor markers were slightly elevated in 3 cases in the BOT group. In women having endometriosis with malignant pathology, preoperative tumor markers were slightly elevated in 3 patients, and tumor markers were normal in the other cases. Infertility was more common in the malignant patient group. MRI findings were more consistent with the pathological diagnosis in the malignant group. All but 1 patient in the malignant group had peroperative frozen pathology and complementary surgery in a single session. Frozen pathology was not performed in all patients in the BOT group, and some patients underwent cystectomy only.

Conclusion: Endometriosis can be associated with malignancies and borderline adnexal tumors with or without endometrioma. We believe that when an adnexal mass is seen in patients who have undergone surgery for the diagnosis of endometriosis, the possibility of malignancy should be considered and per-operative frozen pathology should be performed.

Keywords: Adnexal tumours, borderline ovarian tumour, endometriosis, endometrioma, ovarian cancer

ÖZ

Amaç: Bu çalışmanın temel amacı over endometriozisi ile borderline over tümörü (BOT) ve malign over tümörü arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntem: Veriler, 2017 ve Mayıs 2023 tarihleri arasında üçüncü basamak bir merkezin Kadın Hastalıkları ve Doğum Kliniği'nde jinekolojik cerrahi sonrası patolojide BOT veya malign adneksiyal tümör ve çevre dokuda (aynı piyeste) endometriozis olan hastalardan retrospektif olarak toplandı. Hasta yaşı, gravidite, vücut kitle indeksi, ailede kanser öyküsü, klinik şikayetler, tespit edilen kitlenin ultrason ve manyetik rezonans görüntüleme (MRG) özellikleri, ameliyat öncesi tümör belirteçleri ve patolojik evreler kaydedildi.

Bulgular: Bu 5,5 yıllık dönemde toplam 49 BOT tanısı kondu. Bunların 19'u seröz, 22'si müsinöz, 7'si seromüsinöz ve 1'i endometrioid BOT idi. Dokuz BOT olgusunda aynı overde veya cerrahi alanda kalan pelvik dokuda patolojik olarak doğrulanmış endometriozis vardı. 5,5 yıl içinde 37 malign

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epitelyal over kanseri tanısı konuldu. Bunların 11'inde endometrioid over kanseri (1'inde endometrioid ve berrak hücreli kanser), 25'inde seröz over kanseri ve 1'inde müsinöz over kanseri vardı. Endometriozis 4 seröz adenokarsinom ve 4 endometrioid over kanseri olgusu ile ilişkilendirildi. Bu hastalar arasında endometriozisli BOT hastaları daha gençti. Tümör belirteçleri, BOT grubundaki 3 olguda hafifçe yükselmişti. Malign patolojiye sahip endometriozisli kadınlarda, ameliyat öncesi tümör belirteçleri 3 hastada hafifçe yükselirken, diğer olgularda tümör belirteçleri normaldi. Malign hasta grubunda infertilite daha yaygındı. MRG bulguları malign grupta patolojik tanı ile daha tutarlıydı. Malign gruptaki 1 hasta hariç tüm hastalara peroperatif frozen patoloji ve tek seansta tamamlayıcı cerrahi uygulandı. BOT grubundaki tüm hastalara frozen patoloji yapılmadı ve bazı hastalara sadece kistektomi uygulandı.

Sonuç: Endometriozis, endometrioma olsun ya da olmasın maligniteler ve borderline adneksiyal tümörler ile ilişkili olabilir. Endometriozis tanısı ile cerrahi uygulanan hastalarda adneksiyal kitle görüldüğünde malignite olasılığının göz önünde bulundurulması ve peroperatif frozen patoloji yapılması gerektiğine inanıyoruz.

Anahtar Kelimeler: Adneksiyal tümörler, borderline over tümörleri, endometriozis, endometrioma, ovarian kanser

INTRODUCTION

Endometriosis is an inflammatory disease that affects 10% of women and carries a risk of malignant transformation[1].

Endometriosis is a non-neoplastic pathology with endogenous estrogen production and progesterone-resistant chronic inflammatory features such as tissue invasion, angiogenesis, and reduced apoptosis. These properties may predispose to cancer.

Endometriosis is associated with a 50% increased risk of epithelial ovarian cancer[2,3].

In endometriosis, the total ovarian cancer is increased, especially the endometrioid and clear cell cancer[4]. However, endometrioma excision is not recommended for ovarian cancer prophylaxis. The risk of malignant transformation of endometriosis has been estimated at 1% for premenopausal females and 1% to 2.5% for postmenopausal females[5,6]. Ovarian cancer associated with endometriosis is associated with better overall survival and early stage. Epidemiological studies investigating the relationship between endometriosis and cancer have shown that endometriosis increases the risk of cancer (1.3 to 1.9 times)[7].

Borderline ovarian tumors (BOTs) represent 10-20% of all ovarian epithelial tumors and they are characterized by nuclear atypia and up-regulated cellular proliferation without stromal invasion. They have a low incidence of 4.8/100,000 (Europe) and 1.5-2.5/100,000 (United States) new cases per year, primarily affecting women in their childbearing years. Based on their histologic features, BOTs are classified as serous, mucinous, endometrioid, clear cell, transitional cell, mixed epithelial cell, and Brenner tumors[8]. The same publication has noted that BOTs are more common in endometriosis than ovarian malignancies. In another study, the prevalence of concomitant endometriosis in borderline tumors was reported to be 12%[9].

Our aim in this study is to draw attention to the increase in ovarian malignancies and borderline tumors in women with endometriosis.

MATERIALS AND METHODS

The data of patients who underwent surgery in a tertiary state hospital between May 2017 and January 2023 and were diagnosed with borderline or malignant adnexal tumors in addition to endometriosis in their pathology were screened in accordance with the İstanbul Medipol University Non-invasive Clinical Research Ethics Committee date-decision numbered: 16.03.2023-271 and the Declaration of Helsinki. In the study, a total of 49 BOT and 37 malignant ovarian tumors were operated, and pathologically confirmed endometriosis was detected in nine BOT and eight malignant ovarian tumors. The data obtained from the patients' age, gravida, clinical complaints, ultrasonography and Doppler findings, tumor markers, magnetic resonance imaging (MRI) characteristics, the operation performed, frozen pathology if performed, final pathology, the final state of the patients as a result of hospital and telephone interviews were obtained.

Statistical Analysis

The study is a case series and statistical analysis was not performed.

RESULTS

We screened 49 patients with BOTs and 37 patients with malignant ovarian tumors between May 2017 and January 2023. Nine of the BOT patients had endometriosis, and the median age of these patients was 41 years (2080 years). There were eight endometriosis patients with malignant ovarian tumors, and the mean age of these patients was 52 years (45-76 years) (Tables 1, 2).

The tumour markers were normal in six out of nine patients with a BOT and endometriosis. Tumor markers were normal in five out of eight patients with ovarian malignancy and endometriosis, and did not increase more than twofold in three patients with high tumor markers.

Of 49 patients (19 serous, 22 mucinous, 7 seromucinous, 1 endometrioid borderline tumor) with a pathological diagnosis of BOT, three were serous BOTs (15% of all serous BOTs), and two mucinous BOTs (all mucinous BOTs 9% of patients), three

seromucinous BOTs (40% of all seromucinous BOTs) and one endometrioid BOT were associated with endometriosis.

Of the 37 patients diagnosed with malignant ovarian tumors, one was a mucinous adenocarcinoma, 25 were serous adenocarcinomas (nine lowgrade, 16 highgrade serous adenocarcinomas), 11 were endometrioid-type ovarian carcinomas and one was of mixed type with endometrioid + clear-cell carcinoma cells. Endometriosis accompanied malignant ovarian tumors in eight of the women, three of which were high-grade serous adenocarcinomas (12% of all malignant ovarian serous cancer cases), and five were endometrioid carcinomas (45% of all endometrioid ovarian cancer cases) (Tables 1, 2).

In one high grade serous cancer and one endometrioid cancer, the primary cancer focus was the fimbrial end of the tubal.

In the BOT group, every patient had abdominal pain, cyst detection, and known cyst enlargement. There were no menstrual irregularities and no family histories of cancer. Vaginal bleeding (postmenopausal bleeding) was the most common complaint in malignant cases. In these patients, a cystic mass with solid areas was diagnosed with ultrasound and ovarian cancer was diagnosed with MRI.

The body mass index of the patients in the BOT group was in the normal weight range. Two of the malignant patients were obese.

Four of the malignant patients had a family history of breast and stomach cancer. There was no family history of cancer in the BOT group.

The fertility potential of virgins and young patients in the BOT group was unknown; every patient in the malignant group was married and four were infertile.

In the BOT group, in addition to three patients with known endometrioma, who underwent surgery because the endometrioma grew rapidly during followup, three patients underwent surgery because cysts of unknown origin were diagnosed.

Endometriosis was not mentioned in the preoperative complaints and examinations of patients with malignancy and endometriosis, but preoperative cysts with dense contents were observed in almost all of them.

In both the BOT and malignant groups, a dense cyst, solid area, papillary structure, and a mural nodule were observed on ultrasound, and an intra-cyst enhancing papillary structure or mural nodule was observed on MRI (Tables 1, 2).

Table 1. Characteristics of borderline ovarian tumor cases with endometriosis

No.	Age	Gravida	USG/Doppler	MRI	Tm markers	Operation and time after surgery	Frozen pathology	Final diagnosis
1	20	0	Endometrioma with solid component on the left	Endometrioma with solid component on the left	n	Cystectomy (1 year ago)	Benign ovarian tumor	SMBT (left)
2	23	0	Endometrioma with solid component on the left	Endometrioma with solid component on the left	n	Cystectomy (2.5 years ago)	Borderline tumor	SMBT (left)
3	35	3	Right homogeneous cyst	T1 hypointense-T2 hyperintense cyst	n	1. Cystectomy 2. L/S USO (5 years ago)	None	MBT (right)
4	36	3	Endometrioma with solid component on the right	Endometrioma	n	1. Cystectomy 2. Debulking (1 year ago)	None	SMBT (right)
5	41	5	Homogeneous cyst with bilateral solid areas	Homogeneous cyst	CA 125:47 U/mL	L/S BSO (2021-March)	None	SBT (bilateral)
6	43	0	Endometrioma with solid component on the left-endometrioma on the right	Solid space	n	Debulking (3 years ago)	SBT	SBT (bilateral)
7	43	2	Endometrioma with solid component on the left	Papillary structure	CA 125:56 U/mL	Debulking (4.5 years ago)	Borderline tumor	EBT (left)
8	49	2	Endometrioma on the right	Cyst	CA 125:250 U/mL	TAH+BSO+ omentectomy (1.5 years ago)	Serous cystadenofibroma	MBT (right)
9	80	3	Endometrioma on the left	Endometrioma	n	1. uso 2. tah+uso (2 years ago)	BOT	SBT (left)

SMBT: Seromucinous borderline tumor, SBT: Serous borderline tumour, EBT: Endometrioid borderline tumor, MBT: Mucinous borderline tumor, L/S BSO: Laparoscopic bilateral salpingooopherectomy, L/S USO: Laparoscopic unilateral salpingooopherectomy, MRI: Magnetic resonance imaging, USG: Ultrasonography, Ca: Carcinoma, n: Normal

Table 2. Characteristics of malignant ovarian tumor cases with endometriosis

No.	Age	Gravida	USG/Doppler	Tumor stage	Tm markers	Operation and time after surgery	Frozen	Final diagnosis	CT/RT
1	39	1	Cyst with solid component on the	pT1c2N0Mx	CA 125:87	Debulking (1.5 years ago)	Malignant tumor	HGSC[a] (left tuba)	CT
2	45	1	Bleeding; cancer on the endometrial polyp	PT1a? (tuba) PT3a? (uterus)	CA19-9 2x	TAH+BSO+ right lymphadenectomy (2 years ago)	Endometrium cancer[a]	Endometrium ca+ right tuba endometiroid ca[b]	CT
3	45	1	Cyst with solid component on the left	p T1a N1b Mx (over) p T1b N1a Mx (uterus)	n	Type 3 hysterectomy (3 years ago)	Malignant tumor	Endometrioid over Ca (left side)+ endometrium Ca	CT+RT
4	45	0	Cyst with solid component on the right	pT1aN0Mx	CEA:4	Debulking (2 years ago)	Endometrioid cancer	HGSC[a] (right side)	CT
5	52	0	Concentrated cyst, endometrioma on the left	pT1c1NxMx	n	TAH+BSO (5.5 years ago)	None	Endometrioid (left side)	CT
7	56	4	Cyst with solid component	pT1c1N0Mx	n	Debulking (3 years ago)	Malignant tumor	Endometrioid Ca (right side)	CT
6	58	2	Malignant mass	PT1c2N0Mx (over) PT1aN0Mx (uterus)	n	Debulking (1.5 years ago)	Malignant tumor	Endometrioid over Ca (left side) + endometrium Ca	CT+RT
8	76	0	Cyst with solid component on the right	pT1aN0Mx	n	Debulking (3 years ago)	HGSC[a]	HGSC[a] (right side)	?

[a]Frozen pathology was done for endometrium.

[b]The cancer in the tube has emerged from the endometriosis focus.

HGSC: High grade serous ovarian carcinoma, Ca: Carsinoma, CT: Chemotherapy, RT: Radiotherapy, USG: Ultrasonography

Only cystectomies were performed on two virgin patients in the BOT group. Frozen pathology was performed on six patients and was reported as benign in two patients and BOT in four patients. Peroperative frozen pathology was not performed in three patients, and due to the presence of BOT in the postoperative pathology and keeping in mind their age and fertility expectations, complementary surgery was performed in the second operation, and close observation was performed in one patient. Frozen pathology was performed in seven patients whose final pathology was malignant ovarian tumor, and no frozen pathology was performed in one patient. Patients who were found to be malignant on frozen pathology underwent additional surgery in the same session, and one patient did not have frozen pathology and underwent postoperative chemotherapy (CT) and radiotherapy (RT). CT was given to five of eight malignant patients and CT+RT was given to two patients. Malignant patients with a postoperative period of 1.5 to 5.5 years were contacted by telephone, and every patient was found to be alive and experiencing no recurrence.

None of the patients in the BOT group had peritoneal or distant organ implants, gastrointestinal symptoms or ascites. There were also no recurrences.

DISCUSSION

Endometrioma is a benign condition and malignant transformation is rare (1% in premenopausal women and 1% to 2.5% in postmenopausal women). In the case of large endometriomas (9 cm) and advanced age (>45 years), malignancy should be considered. The mean age of the patients in our malignant patient group was 52 years.

Endometriosis is defined as the presence of endometrial-type mucosa outside the uterine cavity[2].

Endometriosis increases the frequency of clear cell ovarian cancer, endometrioid ovarian cancer and epithelial ovarian cancer[10-12]. Endometriosis is the most common precursor of endometrioid and clear cell carcinoma with a clonal relationship. There is believed to be a relationship between BOTs and endometriosis, explained by molecular and genetic aberrations[13].

Ovarian seromucinous borderline tumors, a rare pathology, are a different tumor group from ovarian epithelioid tumors seen in women of reproductive age. In 2014, the new classification defined these tumors separately, whereas they were previously classified as epithelial ovarian tumors[14]. Finally, the World Health Organization 2020 Classification of Female Genital Tumors recognized seromucinous carcinoma as a separate entity and reclassified it as endometrioid carcinoma with mucinous differentiation[15].

There is information in the literature that the type of endometriosis associated with malignancy is ovarian endometriosis, and that peritoneal or deep endometriosis does not increase the risk of cancer. About one-third of endometrioid and clear cell cancer cases have endometriosis. In fact, endometriosis has been defined as a risk factor or precursor lesion for these cancers. In a ten year follow up, an additional two cases of ovarian cancer are seen for every 1,000 women with ovarian endometriosis[4]. In our study, BOT and endometriosis were found in the pathological specimens of two patients. There was no endometrioma in these patients, and there were no clinical (pain, abnormal vaginal bleeding, etc.) or examination findings (nodule, urethral dilatation, hydrosalpinx, etc.) suggestive of endometriosis. In the group of patients with malignant tumors, an endometrioma was observed in one patient prior to surgery and endometriosis externa was found in the pathological examination of the other patients. As a result of this study, we believe that not only endometrioma but also endometriosis elsewhere should have an impact on the development of malignancy.

CA 125, the most commonly used tumor marker for malignancy in patients, is a tumor marker with low specificity and is already high in women with endometriosis However, it should be considered if the CA 125 test result is >200 U/mL. This is because CA 125 is elevated in 80% of epithelial ovarian tumors[16,17]. However, based on the literature and the data from our study, we found that tumor markers were not useful in the diagnosis of BOTs[18].

BOTs are the tumors with nuclear atypia and up-regulated cellular proliferation, but without stromal invasion. In this way, it is seen less aggressively and at an early age (40s) and the type of treatment should be determined by considering the patient's age and fertility expectation. In our study, the median age in the BOT group was 41 years, which is in line with the literature. Moreover, we performed cystectomy on the patients in the BOT group, who wanted to have a child, and followed them closely. There was no recurrence.

In our patients in whom BOT and ovarian cysts were seen together, we decided to operate because of the most common appearance of solid areas/mural nodules/papillary structures within the cyst. BOT cysts are the cysts with papillary structures in a unilateral cyst, and in an examination, it shows microscopically characteristic broad papillae lined by serous type epithelial cells with abundant eosinophilic cytoplasm admixed with a varying number of endocervical-like mucinous cells[19]. In patients with cysts with malignant pathology, intracyst hyperechogenicity (such as papillary structure or solid area) and Doppler flow in these areas were remarkable on ultrasound.

Seromucinous tumors were associated with endometriosis in 23.1% of the cases and they were bilateral in 30.8%. In the BOT group in our study, two patients had bilateral BOT, three had a lesion on the right, and four had a left lesion. In three of the malignant ovarian tumors detected together with endometriosis, there was ovarian cancer synchronized with endometrial cancer (two in the right ovary, one in the left ovary), two patients had a malignancy originating from the left adnexa and three patients had a malignancy originating from the right adnexa. There were no bilateral malignancies in the malignant patients. In a study examining ovarian cancer data spanning 16 years in 2018, it was reported that 7.3% of ovarian cancer cases were observed in conjunction with endometriosis. Among 35 cases with identified atypical endometriosis in their pathology, BOTs were detected in 11 cases (31%), and the average age of these patients was reported to be 44 years (range: 22-70 years)[9]. Despite the mandatory hiatus imposed by COVID-19 during the five years we scrutinized the data, we identified 8 malignant cases associated with endometriosis. The oldest patient among them was 76 years old, while the average age of the remaining patients was 48 years, aligning with the literature. It should be noted that epithelial ovarian cancer is more common in individuals aged 60 years and above[20].

In ovarian tumors, frozen pathology can diagnose suspicious masses at a high rate (65100%), but the diagnostic value is reduced in borderline tumors and large masses[21]. Peroperative frozen pathology was not used in one patient with malignant pathology in our clinic, and one patient was diagnosed with endometrial cancer synchronized with frozen pathology. The diagnosis of a 3 mm endometrioid type tubal cancer originating from the endometriosis focus at the fimbrial end of the tube in this patient could not be made. This patient was operated on for endometrial cancer and no adnexal mass was found on ultrasound, so the pathology was not alerted for adnexal malignancy.

Malignancy should be considered, especially in adnexal cysts in infertile women. The frequency of infertile patients in the group we studied is remarkable[22].

Endometriosis is not a precancerous disease, but it is not a completely benign disease, either. A malignancy that develops from an endometrioma has similar characteristics to other ovarian tumors. Especially in cases of atypical endometrioma with a solid area and papillary structure, malignancy should

be considered, and then the patient should be referred to MRI. Almost all of our cases with endometrioma in our study had atypical cyst features (solid areas, mural nodules, and increased blood flow in Doppler)[23].

In the presence of abnormal vaginal bleeding in women with endometriosis, we believe that preoperative endometrial sampling is necessary to screen for endometrial pathologies and other genital tumors, aiming to avoid overlooking or missing them (in our study, we had three patients with malignant adnexal tumors).

Study Limitations

In the study, we excluded the results of patients whose specimens were sent to the hospital pathology laboratory from outside centers and patients who underwent surgery in other surgical departments and were found to have additional malignancy/borderline tumors in addition to endometriosis.

CONCLUSION

Currently, surgical treatment is avoided for the treatment of endometriosis/endometrioma. However, based on our study results and the literature, we believe that ultrasound, Doppler and MRI should be used more freely in endometriosis patients. We also recommend that if there is clinical or radiologic suspicion of malignancy, the patient should be informed about the surgical treatment option and frozen pathology should be performed intraoperatively in those who undergo surgery.

Ethics

Ethics Committee Approval: The study was approved by the İstanbul Medipol University Non-invasive Clinical Research Ethics Committee date-decision numbered: 16.03.2023-271.

Informed Consent: Retrospective study.

Authorship Contributions

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

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

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CASE REPORT

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Metastatic Adenoid Cystic Carcinoma: Case Report

Baş Boyun Metastatik Adenoid Kistik Karsinomu Olgu Sunumu

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We regret to inform you that Ahmet YOLCU MD, has passed away.

ABSTRACT

Among head and neck cancers, adenoid cystic carcinoma (ACC) has a separate place compared to other histological types. Our case report is about the follow-up of a patient with ACC originating from the left parotid gland, who applied to our clinic for about 10 years, after different treatments were applied in different centers.

Keywords: Adenoid cystic carcinoma, metastatic, radiation oncology, oncologic surgery, chemotherapy, radiotherapy

ÖZ

Baş boyun kanserleri içinde adenoid kistik karsinom (AKK) diğer histolojik tiplere göre ayrı bir yere sahiptir. Bizim olgu sunumumuz yaklaşık 10 yıldır farklı merkezlerde farklı tedaviler uygulanmış, ardından kliniğimize başvuran sol parotis bezinden kaynaklanan bir AKK hastasının takibi hakkındadır.

Anahtar Kelimeler: Adenoid kistik karsinom, metastatik, radyasyon onkolojisi, onkolojik cerrahi, kemoterapi, radyoterapi

INTRODUCTION

Approximately 4% of head and neck cancers originate from the salivary gland. Adenoid cystic carcinoma (ACC) is a rare malignancy of the glands, accounting for approximately 1% of head and neck cancers and 10% of salivary gland cancers[1]. It is more common in the submandibular gland, minor salivary glands and mucinous glands in the oral cavity and oropharynx, but less common in the parotid gland. It can also occur in glands in other tissues where mucinous glands are present, such as the tracheobronchial tree, esophagus, breast, lungs, prostate, cervix uteri, lacrimal, Bartholin's glands and skin[2]. Salivary gland malignancies are pathologically heterogeneous tumor groups. According to the World Health Organization 2017 classifications, 32 subgroups characterized by morphological and genetic features as well as specific clinical behaviors have been classified[3]. Among salivary gland cancers, ACC has a distinct place compared to other histologic types due to its different clinical course including perineural spread, progression rate and late systemic metastasis. In our case report, we present the treatment and follow-up of a patient with ACC arising from the left parotid gland, who was treated in different centers and then admitted to our clinic.

CASE REPORT

A 49-year-old female patient was admitted to our outpatient clinic in April 2014 after recurrent ACC operation in the left parotid lobe.

The patient underwent mass excision and superficial parotidectomy operation in February 2013 due to a mass in the inferior left auricle and total parotidectomy + left radical neck dissection in March 2014 due to recurrence. The adjuvant radiotherapy schedule of the patient was performed in our

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clinic between May and June 2014 with field-in-field (FIF) technique using 6 MV photon energy in 33 fractions of 2 Gy/day with a total dose of 66 Gy (Figure 1).

Carboplatin (AUC 5.5) and paclitaxel (175 mg/m^2) were administered six times as three-hour infusions every three weeks by the Medical Oncology clinic.

Metastasis was detected in the 3rd lumbar vertebra approximately 4 years later. The patient's palliative radiotherapy program was performed in June 2018 in our clinic using the FIF technique for the lumbar 1-4 vertebrae using 6 MV photon energy in 10 fractions of 3 Gy/day, with a total dose of 30 Gy. As a precaution for medication-related osteonecrosis of the jaw, the patient was started on bisphosphonate treatment after oral and dental health examination. The local treatments applied to the patient are schematized in Figure 1.

Approximately 3 months later, lung metastasis was detected, etoposide treatment was started at an external center and pulmonary metastasectomy was performed in October 2019. In the molecular examination of the tissue biopsy taken from the pulmonary metastatic lesion, *AKT1, ALK, BRAF, CTNNB1, DDR2, EGFR, HER2 (ERBB2), ERBB3, ERBB4, ESR1, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, HRAS, KIT, KRAS, MAP2K1, MAP2K2, MET, NOTCH1, NRAS, PDGFRA, PIK3CA, RAF1, SMAD4, STK11* genes were studied. ALK, BRAF, EGFR, ERBB2, FGFR1, FGFR2, FLT3, KIT, KRAS, MAP2K1, MET, PIK3CA copy number changes were also investigated. No clinically significant mutation was found according to the available medical literature.

Progression was detected and paclitaxel was added to the treatment. After the completion of the chemotherapy program, the patient was followed up and etoposide was started again upon the progression of lung nodules. When the treatment response was evaluated as stable disease, treatment was continued with oral endoxan.

The patient complained of low back pain radiating to the right leg and underwent an operation for laminectomy and stabilization by the department of neurosurgery in February 2022.

The patient is being followed up as Eastern Cooperative Oncology Group 1 at 120 months after the first operation with pulmonary metastasis at the last follow-up (Figure 2).

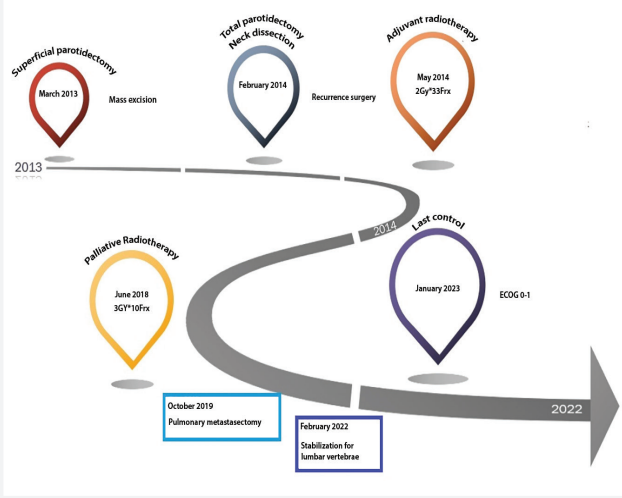

Figure 2. Local treatments during the course of the disease
ECOG: Eastern Cooperative Oncology Group

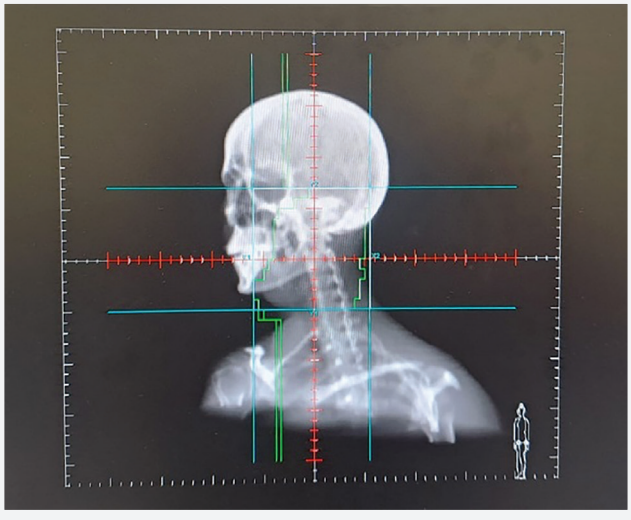

Figure 1. Digital reconstructed radiogram from adjuvant radiotherapy plan

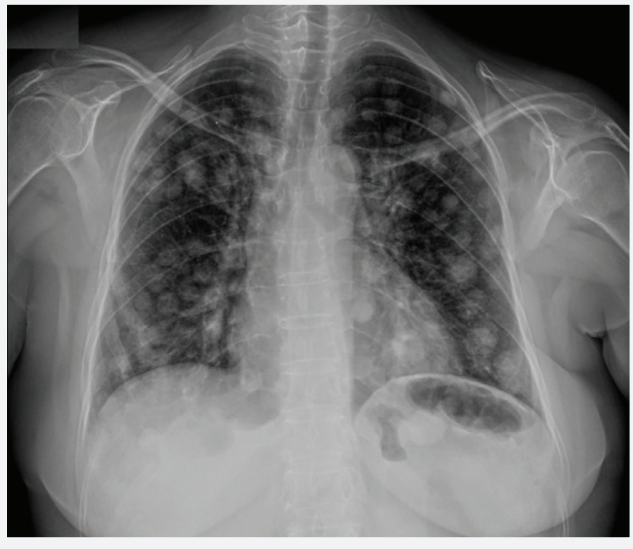

Figure 3. Posterior-anterior chest radiograph

DISCUSSION

A palpable mass is the most common finding on physical examination for superficial parotid gland tumors. Ultrasound examination with color Doppler imaging is an economical and reliable method widely used to detect and evaluate parotid gland masses. The disadvantage of ultrasonographic examination is its low sensitivity in differentiating malignant from benign masses[4].

Due to the rarity of the disease, it is difficult to prospectively examine head and neck ACC patients, but the French study (French National Network on Rare Head and Neck Cancers)[5], which started with 95 patients in 2012, was recently updated with 470 cases[6]. The female/male ratio in the study was reported to be approximately 3/2, in line with previous literature[7]. Minor salivary glands are more frequently involved than major salivary glands, similar to the sex ratio. In head and neck cancers, adjuvant radiotherapy is recommended in the presence of invasive margins and perineural invasion in low-grade T1 tumors, whereas it is recommended for ACC regardless of these after complete surgery. For ACC of the head and neck region, adjuvant radiotherapy at doses of 60 Gy and above after surgery is recommended as standard treatment[7].

In our case, systemic metastasis developed after local adjuvant radiotherapy after recurrence, although there was no recurrence at the primary tumor site. It is important to perform timely local adjuvant therapies by risk assessment of the patients. The symptomatic success of palliative radiotherapy to the metastasis sites has been of limited benefit despite persistent progression of the disease.

Metastatic ACC is a slowly progressive disease with limited response to treatment. Platinum-based standard chemotherapy regimens should be considered in metastatic patients who have no chance of local treatment due to differentiation of tumor cells. The combination of cisplatin, doxorubicin and cyclophosphamide is recommended for ACC, and the addition of 5-fluorouracil is not frequently preferred due to its side effects, although it affects treatment responses. Paclitaxel-carboplatin and cisplatin-vinorelbine regimens are often preferred[8,9]. Current studies have shifted to targeted therapies. Although the platelet-derived growth factor receptor and the tyrosine kinase receptor c-kit proto-oncogene, which is structurally similar to the colony stimulatory factor receptor, have been detected at a high rate in ACC, treatments with imitanib targeting the receptor have not yielded satisfactory responses (Table 1)[8,9].

Lenvatinib is a next-generation multi-kinase inhibitor against FGFR1-3, VEGFR2, cKIT, RET, and PDGFR alpha and beta and has been reported to have more promising results[11]. Following studies reporting median progression-free survival between 9.1 and 17.5 months, the combination of pembrolizumab in particular has found a place in current treatment guidelines[12].

Table 1. Current systemic agents and combinations in ACC[10]

Chemotherapy	Cisplatin
	Cyclophosphamide + doxorubicin + cisplatin
	Cisplatin + gemcitabine
	Paclitaxel
	Vinorelbine
	Vinorelbine + cisplatin
Targeted therapies	Imatinib
	Imatinib + cisplatin
	Dasatinib
	Cetuximab
	Gefitinib
	Lapatinib
	Dovtinib
	Sunitinib
	Regorafenib
	Nintedanib
	Lenvatinib
	Axitinib
	Sorafenib
Immunotherapy	Pembrolizumab
	Pembrolizumab + vorinostat
	Pembrolizumab + radiotherapy

ACC: Adenoid cystic carcinoma

CONCLUSION

Metastatic ACC patients have longer survival than patients with different histopathologic head and neck cancers in the metastatic stage. Planning studies with follow-up periods of more than 5-10 years seems to be more appropriate for slowly progressive and progressive disease. In addition to palliative treatments during the long treatment period, the appropriateness of the developments in the current literature to the patient should be checked periodically. Clinical studies and additional case reports in the literature on this subject should be taken into consideration in the treatment of patients in follow-up.

Ethics

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: A.Y., Concept: Y.B., Design: Ö.Ç., Data Collection or Processing: L.Ş., Analysis or Interpretation: A.Y., Literature Search: Ö.Ç., Writing: A.Y., L.Ş.

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

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

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RETRACTION

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Retracted: Evaluation of the Antidepressant Effect of Propolis in Chronic Unpredictable Mild Stress-induced Depression Model in Rats

The Editor-in-Chief of Namık Kemal Medical Journal has retracted the following article:

Evaluation of the Antidepressant Effect of Propolis in Chronic Unpredictable Mild Stress-induced Depression Model in Rats, authored by Ali Taşkıran, Fadime Canbolat, Sena Nur Yücelli, and Burcu Çevreli, published in Volume 11, Issue 2, pages 94-104, with DOI 10.4274/nkmj.galenos.2023.48303.

Upon further investigation, it has been determined that an error occurred in the method section of the manuscript. Specifically, it was found that the characteristics of the kit content used for the analysis of chromatographic quantity determination of Serotonin and its metabolite were mistakenly reported.

The manufacturer of the analysis kits demanded the corresponding author to take appropriate actions in accordance with scientific research and publication ethics; therefore, the full article has been removed. All authors have agreed on the retraction.