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Hemophilic Arthropathy in Children: Pathophysiology, Diagnosis and Management

Çocukluk Çağında Hemofilik Artropati: Patofizyoloji, Tanı ve Tedavi

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ABSTRACT

Arthropathy is a serious and common problem in patients with hemophilia impairing the patient's quality of life seriously. The most commonly affected joints in hemophilic arthropathy are knees, ankles and elbows. Even a single bleeding could cause devastating effects to synovium, cartilage and also subchondral bones. Ultrasound and magnetic resonance imaging have been advocated for the studying of cartilage damage. Prophylaxis has been demonstrated as a standard choice of management to prevent hemophilic arthropathy development. Primary prophylaxis starting at early years of age is very important to prevent hemorrhages but secondary prophylaxis in adolescents has also significant success rates. As the duration of exposure to the blood increases in the joint cavity, degeneration of the cartilage matrix and resultant cartilage loss also increase, so the aspiration of the hematoma from joint plays an important role in prevention of the disease progression. Synovectomy may be required in cases where prophylaxis and aspiration does not prevent the recurrent hemorrhages. The purpose of synovectomy either with medical or surgical methods is to remove the problematic synovium to prevent the progression of hemophilic arthropathy. Medical synovectomy (synoviorthesis) has two basic types; radiosynovectomy and chemical synovectomy and the former one is appearing to be more effective with an about 85% success rates. If all of these measures fail to prevent the progressive cartilage damage, open or arthroscopic synovectomy, arthrodesis of the affected joint or even arthroplasty could be necessary. Here we tried to summarize the pathological mechanism, diagnosis and management of hemophilic arthropathy in children.
Keywords: Hemophilia; arthropathy; synovitis; diagnostic imaging.

ÖZ

Hemofili hastalarında yaygın olarak gözlenen artropati, yaşam kalitesini ciddi biçimde bozan bir problemdir. Hemofilik artropatide en sık etkilenen eklemler dizler, ayak bilekleri ve dirseklerdir. Eklem içinde gözlenen tek bir kanama bile sinovyum, kıkırdak ve subkondral kemikler dâhil eklemin tüm bileşenlerinde yıkıcı bir takım etkilere neden olabilir. Kıkırdak hasarı ve artropatinin erken dönem değişikliklerinin incelenmesi için daha çok ultrason ve manyetik rezonans görüntüleme kullanımı önerilmektedir. Profilaksi, hemofilik artropati gelişimini önlemek için standart bir tedavi seçeneği olarak gösterilmiştir. Erken yaşta başlanan primer profilaksi kanamaların önlenmesi açısından çok önemlidir ancak adölesan dönemde başlanan sekonder profilaksinin de tekrarlayan kanamaların önlenmesi ve kıkırdak hasarının gelişiminin geciktirilmesi konularında sağladığı ciddi faydaları bilinmektedir. Eklemin kanamaya maruz kalma süresi arttıkça, kıkırdak matriksinin dejenerasyonu ve buna bağlı kıkırdak kaybı da artacağı için eklem içindeki hematomun aspirasyonu hastalığın ilerlemesinin önlenmesinde önemli rol oynar. Profilaksi ve aspirasyonun tekrarlayan kanamaları engelleyemediği durumlarda ise hastalara sinovektomi uygulanması gerekebilir. Gerek medikal gerekse cerrahi yöntemlerle yapılabilen sinovektominin amacı sorunlu sinovyumu ortadan kaldırarak hemofilik artropatinin ilerlemesini önlemektir. Tıbbi sinovektomi (sinoviyortez) radyosinovektomi ve kimyasal sinovektomi olarak iki farklı başlık altında incelenebilir. Bunlardan radyosinovektominin yaklaşık %85 başarı oranı ile kimyasal sinovektomiye göre daha etkili olduğu bildirilmektedir. Tüm bu önlemlerin ilerleyici kıkırdak hasarını önlemede başarısız olduğu durumlarda, açık veya artroskopik sinovektomi, etkilenen eklemin artrodezi ve hatta artroplasti gibi uygulamalara ihtiyaç duyulabilmektedir. Burada, çocukluk çağında gözlenen hemofilik artropatinin patofizyolojisi, teşhis yöntemleri ve tedavisinin güncel literatür eşliğinde tartışılması amaçlanmıştır.
Anahtar kelimeler: Hemofili; artropati; sinovit; tanısal görüntüleme.

INTRODUCTION

Deficiency of specific coagulation factors like factor VIII (FVIII) and factor IX (FIX) causes hemophilia A and B respectively (1). The diseases are divided into three different degrees of severity according to the remaining defective factor activity level; severe, moderate and mild. Factor activity is less than 1% in severe, between 1 to 4% in moderate and between 5 to 40% in mild cases (2). The responsible genes to synthesize FVIII and FIX are located on the chromosome X. The prevalence of hemophilia is about 1 to 2 per 10.000 men and hemophilia A is 4 times more common than hemophilia B (3). Although they are well-known pathologies all over the world and there are very effective treatment modalities; if not managed properly since infancy, they could cause some lifelong disabilities (4). One of the most significant and disabling complications of hemophilia is hemophilic arthropathy (HA). In HA, repeated intra-articular bleedings that commonly seen in patients with severe hemophilia are responsible from the pathology and the knees, the elbows and the ankles are affected in most of the times from this complication (5). Different levels of dysfunction caused by HA can affect a person's quality of life severely. In this study it is aimed to elucidate pathophysiology, diagnosis, management modalities of HA in the light of the current literature.

PATHOPHYSIOLOGY

Recurrent bleeding is one of the major problems in patients with severe hemophilia eventually causing HA. Even a single bleeding could cause some devastating effects to all components of the joint (6). The elbows, the knees, hips and the ankles are the major joints affected by HA (7). Intensive mechanical forces and rich vascularization in these joints could be the cause of this high frequency. Blood clotting is already impaired even in a normal joint space and also local fibrinolysis is increased in hemophilic patients (8). The risk of recurrent bleedings increases with the formation of new and brittle vessels along with the synovial thickening after first hemorrhage. The pathophysiology of the joint disease in HA patients could be analyzed in three different sub-headings; synovium, cartilage and bone problems.

Synovium

The synovial tissue is the main cleaner of the joint cavity from the blood remnants especially erythrocyte derived iron. The synovium contains fibroblast like synoviocytes (FLSs). FLSs provide extracellular matrix and produce most of the constituents of the synovial fluid (9). In severe and recurrent cases of hemarthroses, the capacity of the synovium to carry the iron outside the joint is overwhelmed. The iron, accumulated in the synovial tissue in the form of hemosiderin, causes some pathological changes in this tissue like inflammation, hyperplasia and angiogenesis (10,11). The increased deposition of iron in the synovial tissues can also exaggerate the production of some cytokines like tumor necrosis factor (TNF)-α, interferon-c, interleukin (IL)-1 and IL-6 and can directly induce receptor activator of nuclear factor-κ B ligand (RANKL) to promote synovial hypertrophy (12). Synovial hypertrophy and thickening are the major pathological changes seen in HA patients and caused primarily by increased fibroblast proliferation and expression of some disrupted oncogenes. The oncogenes c-myc and mdm2 are highly present in the HA patients' synovium (13,14). The expression of oncogenes is thought to be related to the production of excessive amount of FLSs, monocyte infiltration and cell cycle arrest through the inhibition of direct apoptosis in patients with arthritis (15,16). These changes start a vicious cycle of repeated bleeding and chronic synovitis.

Cartilage

Cartilage degeneration mechanism in HA patients is complex and generally results from synovitis, bleeding into the joint and also mechanical stresses. The joint damage has both inflammatory and degenerative characters like in rheumatoid arthritis and osteoarthritis respectively (17). Production of some inflammatory cytokines like IL-1, IL-6 and TNF-α and proteases by synovial tissue causes degradation of cartilage (18). Also these mediators exacerbate synovial hypertrophy and neovascularization after recurrent bleeding into the joint (19). In addition to the cartilage damage caused by the synovitis via indirect ways, presence of the blood in the joint space itself causes direct damage to the cartilage. This explains the severity of the cartilage destruction in HA patients is more than in the inflammatory and degenerative arthritis patients. In an experimental study by Jansen et al. (20), synovial inflammation and apoptosis were shown even after only one bleed into a joint. Cartilage damage caused by synovial production of pro-inflammatory cytokines can be reversible after a single bleed because the chondrocytes could remain viable; but the blood causes extracellular matrix degradation and chondrocyte apoptosis by direct effect resulting irreversible damage (21). Destruction of the cartilage is directly related to the exposure time and blood concentration in the joint (22).

Bone

Patients with HA have some degree of systemic osteoporosis and local bone loss. Systemic osteoporosis is associated with short stature, lower body weight, decreased activity levels and some infectious diseases like hepatitis C and HIV (23). Local bone changes around the joints include cyst formation, subchondral sclerosis, osteophyte formation and epiphyseal enlargement (24). Cartilage degeneration could be the primary cause of bone loss in HA patients but the exact mechanism of bone changes are not well known. Even a single bleed might induce the bone loss and this loss can be exaggerated by inactivity, muscle weakness and recurrent bleeding (11). The RANKL/osteoprotegerin pathway plays an important role in regulation of bone turnover. This pathway induces osteoclast differentiation and maturation finally causing bone resorption. As in patients with osteoarthritis, HA patients also have a higher incidence of RANKL overexpression in the affected joints (25). In addition, pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α released in response to the synovitis, could increase the expression of the RANKL in HA patients (26). Subchondral bone cysts are the other prominent features of HA but the mechanism of its occurrence is not fully understood (27).

IMAGING

Plain Radiography

The plain radiography has been used successfully in evaluating the HA. It can show arthropathic changes like osteonecrosis, subchondral cysts, joint space narrowing

and angular problems (Figure 1). The Pettersson score for staging the HA using plain radiography is accepted in 1981 by the World Federation of Hemophilia (28). Each abnormality is graded from 0 to 2 in this scoring system and the highest score could be 13 (Table 1).

While the plain radiography is used in staging the disease, it cannot evaluate the early period of the joint involvement. For this reason, ultrasound (US) and magnetic resonance imaging (MRI) have been advocated for the studying of cartilage damage and early changes of HA.

Ultrasound

The joint damage and bleeding into the joint could be detected by US in patients with HA (29). It can show osteophytes, chondral defects and synovitis. With the help of Power Doppler US, hyperemia of the synovium caused by hypervascularity in asymptomatic patients might be diagnosed in early periods and this can help to prevent the progression of the joint damages (30). Also Doppler could differentiate the normal synovial flow and synovitis. But the Doppler imaging has some difficulties in detecting the severity of the bleeding in the joint, and accepted to be a key tool to predict the risk of hemorrhage rather than the active disease (31). Despite its limited ability for comprehensive evaluation of some details about cartilage defects, synovial changes and subchondral bone; it still maintains its value especially in children because it does not require sedation, can examine many joints in a single session and does not require special positioning.

Magnetic Resonance Imaging

Early detection of effusion in joints, synovitis, bony edema and cartilage involvement can be evaluated by MRI easily. MRI is also sensitive for the visualization of hemosiderin deposits in joints (32). In a prospective study to determine the relationship of MRI findings with joint functions, MRI revealed chondral and synovial abnormalities in 30% of Pettersson grade 0 patients (33). In this study, presence of hemosiderin was found to be related with the time elapsed between last bleeding and MRI evaluation; recent bled joints were showing significant hemosiderin deposits. But the clinical implications of finding abnormalities in MRI but not in Pettersson scoring system remain unclear. Although MRI is superior to US in evaluating the joint surfaces, the examination time is longer, only one joint can be evaluated at a session and sedation may be required with MRI. US should be the primary choice of screening method especially in patients with multi joint involvement and patients requiring repeated follow-up examinations (24).

Table 1. The Pettersson scoring system for hemophilic arthropathy grading by plain radiography

Radiographic finding	Score
Osteoporosis	
Absent	0
Present	1
Enlarged epiphysis	
Absent	0
Present	1
Irregular subchondral surface	
Absent	0
Partially involved	1
Totally involved	2
Narrowing joint space	
Absent	0
>1mm	1
<1mm	2
Subchondral cyst formation	
Absent	0
1 cyst	1
>1 cyst	2
Erosion of joint margins	
Absent	0
Present	1
Gross incongruence of articulating bone ends	
Absent	0
Slight	1
Pronounced	2
Joint deformity (angulation, displacement, or both)	
Absent	0
Slight	1
Pronounced	2

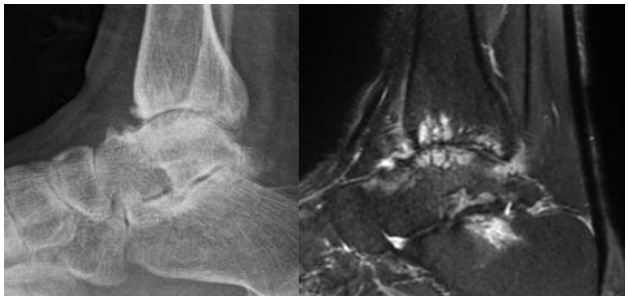

Figure 1. A 23-year-old female patient with an advanced ankle arthopathy secondary to hemophilia

MANAGEMENT

Prophylaxis

Prophylaxis has been demonstrated as a standard choice of management to prevent HA development. Primary prophylaxis starting at early years of age is very important to prevent hemorrhages but secondary prophylaxis in adolescents has also significant success rates (34). In a prospective large cohort study by Manco-Johnson et al. (35), the patients with severe hemophilia A taking regular infusions of recombinant factor VIII (prophylaxis) had les joint damage and decreased frequency of hemarthrosis than patients taking factor VIII at the time of bleeding periods (enhanced episodic treatment). Because the high cost of recombinant factor VIII is a significant barrier to be a standard care of prophylaxis worldwide, early detection and monitoring of the joint pathology after the bleeding and just before the joint damage had start is very important.

Joint Aspiration

As the duration of exposure to the blood increases, degeneration of the cartilage matrix and resultant cartilage loss also increase (36). If the blood in joint cavity is not evacuated at an early period of time, proteoglycan synthesis will be impaired and the chondrocyte apoptosis starts. Aspiration of the joints in patients with higher degree of arthropathy is not indicated because of the benefits thought to be taken by this method is very limited. Today, the mostly accepted modality of management for the acute hemarthrosis in hemophilic patients includes; early factor replacement if possible, joint aspiration, splinting of the extremity to reduce the recurrent bleedings,

ice applications and cyclooxygenase-2 inhibitors to relieve pain in selected cases (37,38).

Synovectomy

After the several trials of injections and other conservative measures fail to solve the bleeding episodes and progressing arthropathy in hemophilic patients, synovectomy should be considered (39). The purpose of synovectomy either with medical or surgical methods is to remove the problematic synovium to prevent the progression of HA. Medical synovectomy (synoviorthesis) has two basic types; radiosynovectomy and chemical synovectomy. Mostly intra-articular injections of rifampicin and oxytetracycline chloride are used for chemical synovectomy. Intra-articular injections of radionuclides 90Y (yttrium-90) (knees) and 186Rh (rhenium-186) (elbows and ankles) are used commonly for radiosynovectomy (40). Radiosynovectomy appears to be more effective than chemical synovectomy with an about 85% success rates. The safety of these radionuclide agents has been proven by more than 40 years of experience with no reported damaging effects (37). Synoviorthesis should be performed as early as possible to inhibit the chondral damage. Some patients do not need repetitive synovectomy and these patients can continue their lives without bleeding for years. However, in some cases, arthroscopic or open synovectomy should be considered in patients who do not benefit from medical synovectomy repeated 3 times consecutively (41). Arthroscopic synovectomy provides approximately 95% reduction in hemarthrosis attacks (42). But this procedure needs prolonged factor replacement and postoperative physiotherapy. Especially in children, if possible radiosynovectomy should be the primary choice of synovectomy methods.

Orthopaedic Procedures

Prophylaxis with appropriate factor replacement, joint aspiration with or without steroid injections and radiosynovectomy are the primary care of the children with HA. If all these measures fail to protect the joint from damaging effects of bleeding, arthroscopic synovectomy might be considered with handling its difficulties. More advanced orthopedic procedures should be taken into consideration after all strategies are insufficient in HA management (43). Arthrodesis can be applied to the patients with severe ankle and elbow arthropathies. In knee and hip joints, arthroplasty option can be considered as a last choice after the age of 18 because of its high complication rates (44). Care should be taken after blunt injury to the quadriceps or gluteal region. Hematoma may shift into compartment syndrome and finally contracture in the long term if it does not managed well. In young adult ages these patients need joint replacement and most common challenging part of the surgery is handling with contractures and deformity.

CONCLUSION

The mechanism of the joint degeneration in hemophilia patients is complex and not fully understood but the key factor triggering the pathological processes is bleeding into the joint. The joint enters a vicious circle of damage due to the hemorrhage and synovitis, this result in progression into severe arthritis, so the early diagnosis and treatment of this hemorrhage is crucial. US helps in early diagnosis and is a good choice of screening tool for multiple joint involvement and MRI plays an important role in detailed assessment of the joint. Prophylaxis with factor replacement, aspiration of the hematoma and synovectomy are the management options of HA. After the onset of the severe arthropathy, there are little amount of treatment modalities in children like arthrodesis or arthroplasty.

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Mortality Outcomes of Single-staged versus Multi-staged Complete Coronary Revascularization in Multivessel Non-ST Elevation Myocardial Infarction Patients

Çok Damarlı ST Yükselmesiz Miyokard İnfarktüsü Hastalarında Tek Aşamalı ve Çok Aşamalı Koroner Revaskülarizasyonun Mortalite Sonuçları

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ABSTRACT

Aim: The aim of this study was to compare the short-term and long-term mortality results of single-stage percutaneous coronary intervention (SS-PCI) and multi-stage percutaneous coronary intervention (MS-PCI) strategies in patients diagnosed with non-ST segment elevation myocardial infarction (NSTEMI) with multivessel disease.

Material and Methods: A total of 298 consecutive patients diagnosed with multivessel NSTEMI (71 (23.8%) patients in the SS-PCI group and 227 (76.2%) patients in the MS-PCI group) were included in this study. Data regarding mortality were obtained from the health information system of our institute and national health registry.

Results: Although in-hospital mortality rates were found to be significantly higher in univariate analysis in the SS-PCI group compared to the MS-PCI group (14.1% (n=10) vs 4.0% (n=9); p=0.005), it was not independently associated with total mortality in multiple model. Among the parameters predicted mortality determinants, low hemoglobin (odds ratio (OR)=0.485, 95% confidence interval (CI)=0.332-0.708; p=0.002), No-reflow occurrence (OR=6.194, 95% CI=1.310-29.300, p=0.021), not using post dilatation (OR=0.287, 95% CI=0.085-0.970, p=0.045) were independently associated with total mortality.

Conclusion: There was no statistical difference in overall mortality between the two study groups in multivessel NSTEMI patients who underwent complete coronary revascularization with the SS-PCI and MS-PCI strategy, while low hemoglobin, No-reflow phenomenon, and not using post-dilatation were found as independent predictors of mortality.

Keywords: Multivessel coronary artery disease; non-culprit lesion; non-ST segment elevation myocardial infarction.

ÖZ

Amaç: Bu çalışmanın amacı, çok damar hastalığı olan ST segment yükselmesiz miyokard enfarktüsü (non-ST segment elevation myocardial infarction, NSTEMI) tanısı aşan hastalarda tek aşamalı perkütan koroner girişim (single-stage percutaneous coronary intervention, SS-PCI) ile çok aşamalı perkütan koroner girişim (multi-stage percutaneous coronary intervention, MS-PCI) stratejilerinin kısa dönem ve uzun dönem mortalite sonuçlarının karşılaştırılmasıdır.

Gereç ve Yöntemler: Bu çalışmaya çok damar hastalığı olan NSTEMI tanısı alan (SS-PCI grubunda 71 (%23,8) hasta ve MS-PCI grubunda 227 (%76,2) hasta olmak üzere) ardışık toplam 298 hasta dahil edildi. Mortalite ile ilgili veriler, enstitümüzün sağlık bilgi sisteminden ve ulusal sağlık sicilinden alındı.

Bulgular: Hastane içi mortalite oranları tek değişkenli analizde SS-PCI grubunda MS-PCI grubuna göre anlamlı olarak daha yüksek saptamasına rağmen (%14,1 (n=10)'e karşı %4,0 (n=9); p=0,005), çoklu modelde bağımsız olarak genel mortalite ile ilişkili saptanmadı. Mortalite ile ilişkili olarak belirlenen parametreler içerisinde, hemoglobin düşüklüğü (odds raito (OR)=0,485; %95 güven aralığı (GA)=0,332-0,708; p=0,002), No-reflow gelişimi (OR=6,194; %95 GA=1,310-29,300; p=0,021) ,post dilatasyon kullanılmaması (OR=0,287; %95 GA=0,085-0,970; p=0,045) genel mortalitenin bağımsız ön gördürücüleri olarak saptandı.

Sonuç: SS-PCI ve MS-PCI stratejileri ile tam koroner revaskülarizasyon uygulanan çok damar hastalığı olan NSTEMI hastalarında total mortalite açısından iki grup arasında anlamlı bir fark saptanmamasına rağmen düşük hemoglobin, No-reflow gelişimi ve post-dilatasyon kullanılmaması mortalitenin bağımsız ön gördürücüleri olarak bulundu.

Anahtar kelimeler: Çok damarlı koroner arter hastalığı; sorumlu olmayan lezyon; ST segment yükselmesiz miyokard enfarktüsü.

INTRODUCTION

Atherosclerotic coronary plaques develop over years and may either lead to a clinically silent coronary artery obstruction or an acute coronary syndrome (ACS), mainly initiated by a plaque rupture or erosion along with overlying thrombosis. Even though the ratio of ST-segment elevation myocardial infarction (STEMI) has decreased significantly in the past decade, the ratio of non-ST elevation myocardial infarction (NSTEMI) has increased slightly in line with the rise of the elderly population and chronic diseases (1). Even though NSTEMI patients tend to have over short-term mortality rates than STEMI patients, long-term mortality rates at 1- or 2-year follow-up eventually become comparable (2). Angiographic features of the coronary arteries in patients with NSTEMI are diverse, ranging from normal or non-obstructive lesions in up to 20% to a severely and diffusely diseased coronary artery tree is up to 40-80% of the patients (3-5). Several studies have shown that NSTEMI patients with obstructive coronary artery disease (CAD) may have multiple coronary stenosis that up to 40% can meet the criteria for the guilty lesion (6-8). Therefore, the culprit lesion may be difficult to identify in this situation.

Percutaneous coronary intervention (PCI) remains the reasonable treatment option in NSTEMI patients. Uncertainty continues considering the optimal coronary revascularization strategy to be preferred in patients with NSTEMI and multivessel disease (9). As a matter of fact, the American College of Cardiology/American Heart Association (ACC/AHA) and European Society of Cardiology (ESC) guidelines are uncertain about which coronary revascularization strategy to recommend to these patients (10,11). Although complete coronary revascularization by routine PCI of the non-culprit obstructive lesions in patients with NSTEMI and multivessel disease tended to have a benefit in several clinical studies (8,12,13), not much data exist regarding the differences in clinical outcomes between single-staged and multi-staged complete coronary revascularization in this setting. The aim of our study was to compare the short and long-term mortality results of single-stage PCI (SS-PCI) and multi-stage PCI (MS-PCI) in patients diagnosed with NSTEMI with multivessel disease.

MATERIAL AND METHODS

Study Design and Patients

All patients who were diagnosed with NSTEMI and underwent PCI at our clinic between January 2014 and December 2014 were reviewed. After exclusion, a total of 298 patients who were diagnosed with NSTEMI and multivessel disease were included in this single-center, retrospective cohort study. Regarding the implemented strategy of complete coronary revascularization, the patients were divided into two groups; SS-PCI (n=71) and MS-PCI group (n=227).

Patients who fulfilled all of the following criteria were included: age ≥18 years, diagnosis of NSTEMI according to the current guidelines (14), and presence of multivessel disease at coronary angiography. Patients with previous coronary artery bypass graft surgery, Synergy Between Percutaneous Coronary Intervention With TAXUS and Cardiac Surgery (SYNTAX) score >32, severe congestive heart failure (ejection fraction <40%), severe valvular heart disease, history of cardiopulmonary resuscitation, malignancy, severe chronic kidney disease (estimated glomerular filtration rate <30 mL/min/1.73 m^2), chronic liver disease and who were a candidate for cardiac surgery were excluded. Patients who could not be followed up for 24 months were also excluded. This study was managed under the Declaration of Helsinki.

Considering the retrospective design of the study, the ethics committee confirmed the study design without requiring written informed assent. This study was approved by the Clinical Research Ethics Committee of Adana Numune Training and Research Hospital (Date: 26.04.2016, number: 77).

Multivessel disease was evaluated as more than 70% occlusion in at least one epicardial coronary artery accompanying more than 50% occlusion from the main coronary artery, or more than 70% occlusion in more than two epicardial coronary arteries. Due to the relatively small area of myocardial perfusion from these vessels, we did not regard lesions of the posterior descending artery, the second diagonal branch, or the third obtuse marginal. Throughout this study, a significant coronary lesion was defined as diameter stenosis of 50% or greater in the left main coronary artery or 70% or greater in one or more of the other epicardial vessels. The distributions of cardiac risk factors such as diabetes mellitus (DM), previous CAD, hypertension (HT), age, gender, hyperlipidemia (HPL), and smoking status were recorded through the patient files and hospital information system. Those who were found to be ≥140/90 at least twice in their office blood pressure measurement or who used medication due to hypertension in their history were evaluated as hypertensive (15). Those who had a fasting blood glucose of more than 126 mg/dL measured twice or more or had a history of antidiabetic oral and/or parenteral therapy due to diabetes were evaluated as diabetic (16). HPL was assessed if low-density lipoprotein (LDL) cholesterol was >100 mg/dL or total cholesterol >200 mg/dL, or if the patient had previously received lipid-lowering therapy following the "Adult Treatment Panel III" guidelines (17). Those who continued to smoke as of the time of application and had a history of quitting in the last month were registered as smokers. Data regarding mortality were obtained from the health information system of our institute and national health registry. No clinical follow-up and additional examinations specific to the study were performed.

Blood Samples and Laboratory Analysis

Venous blood samples of the forehead were included in the study at the time of admission to the hospital. Complete blood counts were measured with a Sysmex K-1000 auto-analyzer. Blood samples and lipid parameters tests were measured with a standard automatic analyzer device. Plasma levels of high-sensitivity C-reactive protein (Hs-CRP) were calculated with an Aero set auto-analyzer using a Spectrophotometric Analysis Kit (Scil Diagnostics GmbH, Viernheim, Germany). Serum levels of high-sensitivity cardiac troponin T (hs-cTnT) were measured with an Elecsys 2010 auto-analyzer using Elecsys immunoassay (Roche Diagnostics, Mannheim, Germany).

Coronary Angiography

Coronary angiography was organized by experienced interventional cardiologists in our cardiac catheterization

laboratory using Siemens and Toshiba devices. While the patients in the SS-PCI group underwent complete coronary revascularization during the index procedure, the patients in the MS-PCI group underwent only the culprit vessel revascularization during the index procedure, and the non-culprit vessels were gradually revascularized after 1 month. Femoral access was preferred for PCIs. All patients were treated according to current ESC guidelines (14). SYNTAX score was calculated by one cardiologist online from the website (https://syntaxscore2020.com). Thrombolysis in myocardial infarction (TIMI) flow grade was assessed at the angiographic laboratory by one cardiologist. Grade 0 - no antegrade flow to the distal of the occlusion point. Grade 1 - the contrast agent passes the coronary stenosis, but cannot fill the entire coronary bed distal to the stenosis during angiographic imaging. Grade 3 - antegrade full filling to the bed distal to the obstruction (18). The coronary flow of less than time 3 flows was evaluated as No-reflow (19).

Statistical Analysis

Statistical evaluation was applied through the SPSS v.20 statistical program. Whether continuous variables showed normal distribution was evaluated using the Kolmogorov-Smirnov test. The continuous variables were represented as mean±standard deviation or median (interquartile range) [min-max], whereas number and percentage were used when defining the categorical data. Comparisons of continuous variables between groups were made using the Student's t-test as a parametric test and the Mann-Whitney U-test as a nonparametric test. Chi-square test was used for the evaluation of categorical data, and Fisher's exact test was used in cases where its application criteria were not met. Bonferroni's method was used to determine differences between groups. After the univariate analysis for mortality markers, significant parameters were included in the multiple regression model and forward logistic regression method was applied to examine data that could be predictive for mortality. For each independent variable, the odds ratio (OR) and 95% confidence interval (CI) were determined. The significance level was determined as values less than 0.05 for the two-tailed p-value.

RESULTS

The basic features of the patients in the two groups we compared were similar according to strategy (Table 1). Angiographic and procedural features of the patients according to strategy are shown in Table 2. The prevalence of the culprit's vessel was significantly different between the two groups (p=0.001). The prevalence of the left main coronary artery (LMCA) as the culprit's vessel was higher in the SS-PCI group than in the MS-PCI group. SYNTAX score was significantly higher in the SS-PCI group than in the MS-PCI group (p=0.001). Initial TIMI flow (p<0.001), balloon pre-dilatation rate (p<0.001), tirofiban administration rate (p=0.005), stent type (p=0.006), cumulative stent length (p=0.037), final TIMI flow (p=0.016), and No-reflow phenomenon rate (p=0.015) were significantly different between the two groups.

Table 1. Basal features of the patients according to the strategy

Variable	SS-PCI (n=71)	MS-PCI (n=227)	p
Age (year), median (IQR) [min-max]	64 (19) [39-94]	61 (17) [35-91]	0.150
Gender (male), n (%)	46 (64.8)	154 (67.8)	0.633
CAD, n (%)	5 (7.0)	9 (4.0)	0.334
Smoking, n (%)	8 (11.3)	38 (16.7)	0.265
DM, n (%)	13 (18.3)	56 (24.7)	0.268
HPL, n (%)	17 (23.9)	72 (31.7)	0.212
HT, n (%)	39 (54.9)	138 (60.8)	0.380
ACE-I/ARB, n (%)	49 (69.0)	137 (60.4)	0.188
Statin, n (%)	47 (66.2)	139 (61.2)	0.451
Beta blocker, n (%)	58 (81.7)	172 (75.8)	0.300
Antiplatelet, n (%)			
Clopidogrel	57 (80.3)	176 (77.5)	
Prasugrel	6 (8.5)	27 (11.9)	0.720
Ticagrelor	8 (11.3)	24 (10.6)	
Hemoglobin (g/dL), mean±SD	13.0±2.0	13.3±1.8	0.279
HDL cholesterol (mg/dL), mean±SD	36.4±11.3	37.6±11.2	0.507
LDL cholesterol (mg/dL), mean±SD	123.1±56.2	122.4±45.9	0.930
Total cholesterol (mg/dL), mean±SD	184.3±62.9	185.7±49.7	0.864
WBC count (x10^3/uL), median (IQR) [min-max]	11.05 (5.6) [3.3-29.7]	11.0 (4.8) [4.9-21.1]	0.968
Platelet count (x10^3/uL), median (IQR) [min-max]	232 (102)[92-666]	251 (86) [103-474]	0.157
Creatinine (mg/dL), median (IQR) [min-max]	0.80 (0.33) [0.20-2.20]	0.87 (0.33) [0.36-2.34]	0.803
Triglyceride (mg/dL), median (IQR) [min-max]	127 (113) [46-459]	129 (128) [34-736]	0.469
Hs-CRP (mg/dL), median (IQR) [min-max]	0.6 (1.5) [0.0-9.6]	0.5 (1) [0.0-32.8]	0.641
Hs-cTnT (ng/mL), median (IQR) [min-max]	1.5 (19.7) [0.0-50.0]	1.7 (8.1) [0.0-1281.0]	0.318
LV ejection fraction (%), median (IQR) [min-max]	49 (18) [25-76]	56 (17) [23-77]	0.142

SS-PCI: single-stage percutaneous coronary intervention, MS-PCI: multi-stage percutaneous coronary intervention, IQR: interquartile range, SD: standard deviation, CAD: coronary artery disease, DM: diabetes mellitus, HPL: hyperlipidemia, HT: hypertension, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, HDL: high-density lipoprotein, LDL: low-density lipoprotein, WBC: white blood cell, Hs-CRP: high-sensitivity C-reactive protein, hs-cTnT: high-sensitivity cardiac troponin T, LV: left ventricle

Table 2. Angiographic and procedural features of the patients according to the strategy

Variable	SS-PCI (n=71)	MS-PCI (n=227)	p
SYNTAX score, median (IQR) [min-max]	16 (15) [5-41]	14 (9) [2-44]	**0.001**
Cumulative stent length (mm) median (IQR) [min-max]	32 (25) [102-108]	28 (22) [4-108]	**0.037**
Culprit vessel, n (%)			
LMCA[+]	5 (7.0)	1 (0.4)	**0.001**
LAD	38 (53.5)	98 (43.2)	
LCx	17 (23.9)	60 (26.4)	
RCA	11 (15.5)	68 (30.0)	
Initial TIMI flow, n (%)			
Grade 0[&]	21 (29.6)	10 (4.4)	**<0.001**
Grade 1	8 (11.3)	16 (7.0)	
Grade 2	13 (18.3)	56 (24.7)	
Grade 3	29 (40.8)	145 (63.9)	
Balloon pre-dilatation, n (%)	55 (77.5)	114 (50.2)	**<0.001**
Thrombus aspiration, n (%)	2 (2.8)	5 (2.2)	0.673
Tirofiban administration, n (%)	10 (14.1)	9 (4.0)	**0.005**
Stent type, n (%)			
Bare metal	14 (19.7)	85 (37.4)	**0.006**
Drug-eluting	57 (80.3)	142 (62.6)	
Post-dilatation with NCB, n (%)	16 (22.5)	37 (16.3)	0.230
Final TIMI flow, n (%)			
Grade 0	1 (1.4)	2 (0.9)	**0.016**
Grade 1	0 (0.0)	0 (0.0)	
Grade 2[†]	10 (14.1)	10 (4.4)	
Grade 3[†]	60 (84.5)	215 (94.7)	
No-reflow, n (%)	11 (15.5)	12 (5.3)	**0.005**

SS-PCI: single-stage percutaneous coronary intervention, MS-PCI: multi-stage percutaneous coronary intervention, IQR: interquartile range, SYNTAX: synergy between PCI with TAXUS™ and cardiac surgery, LMCA: left main coronary artery, LAD: left anterior descending artery, LCx: left circumflex artery, RCA: right coronary artery, TIMI: thrombolysis in myocardial infarction, NCB: non-compliant balloon, [+]: $p_{bonferroni}$=0.04 with Z test of 3.5 for LMCA, [&]: $p_{bonferroni}$<0.001 with Z test of 6.1 for Grade 0 initial TIMI flow, [†]: $p_{bonferroni}$=0.06 with Z test of 2.8 for Grade 2 final TIMI flow and Z test of -2.8 for Grade 3 final TIMI flow

Table 3. Basal features of the patients according to the mortality status

Variable	Non-survivor (n=19)	Survivor (n=279)	p
Age (year), median (IQR) [min-max]	71 (24) [44-94]	61 (17) [35-91]	**0.006**
Gender (male), n (%)	14 (73.7)	186 (66.7)	0.529
CAD, n (%)	3 (15.8)	11 (3.9)	0.051
Smoking, n (%)	6 (31.6)	40 (14.3)	0.054
DM, n (%)	7 (36.8)	62 (22.2)	0.161
HPL, n (%)	5 (26.3)	84 (30.1)	0.727
HT, n (%)	10 (52.6)	167 (59.9)	0.535
ACE-I/ARB, n (%)	16 (84.2)	170 (69.9)	**0.043**
Statin, n (%)	15 (78.9)	171 (61.3)	0.124
Beta blocker, n (%)	12 (63.2)	218 (78.1)	0.157
Antiplatelet, n (%)			
Clopidogrel	16 (84.2)	217 (77.8)	0.700
Prasugrel	1 (5.3)	32 (11.5)	
Ticagrelor	2 (10.5)	30 (10.8)	
Hemoglobin (g/dL), mean±SD	11.7±2.2	13.3±1.7	**<0.001**
HDL cholesterol (mg/dL), mean±SD	37.7±17.5	37.3±10.8	0.905
LDL cholesterol (mg/dL), mean±SD	118.1±45.9	122.8±48.5	0.930
Total cholesterol (mg/dL), mean±SD	181.3±45.5	185.6±53.4	0.784
WBC count (x10^3/uL), median (IQR) [min-max]	13.3 (7.8) [4.8-21.1]	11 (4.8) [3.3-29.7]	0.443
Platelet count (x10^3/uL), median (IQR) [min-max]	250 (106) [153-427]	244 (88) [92-666]	0.809
Creatinine (mg/dL), median (IQR) [min-max]	1 (0.5) [0.2-2.3]	0.84 (0.32) [0.3-2.2]	0.071
Triglyceride (mg/dL), median (IQR) [min-max]	126 (81) [57-277]	130 (126) [34-736]	0.478
Hs-CRP (mg/dL), median (IQR) [min-max]	2.6 (5.9) [0.0-32.8]	0.5 (1) [0.0-23.1]	0.065
Hs-cTnT (ng/mL), median (IQR) [min-max]	5.4 (21.5) [0.1-50]	1.56 (9.95) [0.0-1281]	0.250
LV ejection fraction (%), median (IQR) [min-max]	44 (7) [40-48]	56 (16) [23-77]	0.110

IQR: interquartile range, SD: standard deviation, CAD: coronary artery disease, DM: diabetes mellitus, HPL: hyperlipidemia, HT: hypertension, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, HDL: high-density lipoprotein, LDL: low-density lipoprotein, WBC: white blood cell, Hs-CRP: high-sensitivity C-reactive protein, hs-cTnT: high-sensitivity cardiac troponin T, LV: left ventricle

Table 4. Angiographic and procedural features of the patients according to the mortality status

Variable	Non-survivor (n=19)	Survivor (n=279)	p
SYNTAX score, median (IQR) [min-max]	26 (18) [7-44]	15 (10) [2-41]	**0.001**
Cumulative stent length (mm) median (IQR) [min-max]	33 (27) [12-72]	28 (24) [4-108]	0.402
Culprit vessel, n (%)			
LMCA	2 (10.5)	4 (1.4)	0.088
LAD	10 (52.6)	126 (45.2)	
LCx	4 (21.1)	73 (26.2)	
RCA	3 (15.8)	76 (27.2)	
Initial TIMI flow, n (%)			
Grade 0	1 (5.3)	30 (10.8)	**0.002**
Grade 1^{+}	5 (26.3)	19 (6.8)	
Grade 2	0 (0.0)	69 (24.7)	
Grade 3	13 (68.4)	161 (57.7)	
Balloon pre-dilatation, n (%)	19 (100)	150 (53.8)	**<0.001**
Thrombus aspiration, n (%)	0 (0.0)	7 (2.5)	0.999
Tirofiban administration, n (%)	4 (21.1)	15 (5.4)	**0.007**
Stent type, n (%)			
Bare metal	4 (21.1)	95 (34.1)	0.244
Drug-eluting	15 (78.9)	184 (65.9)	
Post-dilatation with NCB, n (%)	8 (42.1)	45 (16.1)	**0.009**
Strategy, n (%)			
SS-PCI	10 (52.6)	61 (21.9)	**0.005**
MS-PCI	9 (47.4)	218 (78.1)	
Final TIMI flow, n (%)			
Grade 0	1 (5.3)	2 (0.7)	**0.037**
Grade 1	0 (0.0)	0 (0.0)	
Grade 2	3 (15.8)	17 (6.1)	
Grade 3$^{\&}$	15 (78.9)	260 (93.2)	
No-reflow, n (%)	4 (21.1)	19 (6.8)	**0.048**

IQR: interquartile range, SYNTAX: synergy between PCI with TAXUS™ and cardiac surgery, LMCA: left main coronary artery, LAD: left anterior descending artery, LCx: left circumflex artery, RCA: right coronary artery, TIMI: thrombolysis in myocardial infarction, NCB: non-compliant balloon, SS-PCI: single-stage percutaneous coronary intervention, MS-PCI: multi-stage percutaneous coronary intervention, $^{+}$: $p_{bonferroni}$=0.16 with Z test of -3.0 for Grade 1 initial TIMI flow, $^{\&}$: $p_{bonferroni}$=0.21 with z test of -2.3 for Grade 3 final TIMI flow

Table 3 shows the basic characteristics of the patients according to their mortality status. Angiographic and procedural features of the patients according to mortality are shown in Table 4. The median age was statistically higher in the non-survivor group (p=0.006). The mean hemoglobin level was significantly lower in the non-survivor group (p<0.001). Renin-angiotensin system receptor blocker usage rates were higher in the non-survivor group (p=0.043). SYNTAX score (p=0.001), initial TIMI flow (p=0.002), balloon pre-dilatation rate (p<0.001), tirofiban administration rate (p=0.007), post-dilatation with non-compliant balloon (NCB, p=0.009), final TIMI flow (p=0.037), PCI strategy (p=0.005), and No-reflow phenomenon rate (p=0.048) were statistically different between the two groups.

Findings determined as the total mortality predictors of the patients are shown in Table 5. These findings were evaluated in our study as age, history of CAD, smoking, hemoglobin, creatinine, post-dilatation with NCB, SYNTAX score, Hs-CRP, No-reflow and PCI strategy. Among these parameters, low hemoglobin (OR=0.485, 95% CI=0.332-0.708, p=0.002), No-reflow phenomenon (OR=6.194, 95% CI=1.310-29.300, p=0.021), not using post-dilatation (OR=0.287, 95% CI=0.085-0.970, p=0.045) was found to be statistically significant.

Table 5. Results of the logistic regression analysis to predict mortality

Variable	OR (95% CI)	p
Post-dilatation with NCB	0.287 (0.085-0.970)	**0.045**
No-reflow	6.194 (1.310-29.300)	**0.021**
Hemoglobin	0.485 (0.332-0.708)	**0.002**

Nagelkerke R square=0.909, Omnibus tests of model coefficients p<0.001, OR: odds ratio, CI: confidence interval, NCB: non-compliant balloon

DISCUSSION

The main finding of our study, which accepted complete coronary revascularization as the strategy of choice in patients with multi-vessel NSTEMI, was that there was no difference in total mortality outcomes in those who underwent complete coronary revascularization with MS-PCI and SS-PCI.

The outcome from several large contemporary registries shows that performing complete multivessel percutaneous coronary intervention revascularization is associated with improved clinical outcomes in multivessel NSTEMI patients (8,20). In patients with cardiogenic shock presenting with acute myocardial infarction, complete lesion revascularization was associated with a lower risk of death from all causes than with culprit lesion revascularization alone (21). These findings highlight that, regardless of the timing of revascularization, complete coronary revascularization should be the recommended treatment strategy in patients with multi-vessel ACS.

Approximately 40-80% of patients presenting with NSTEMI have multivessel CAD (3,5,22,23). As mentioned previously, except the recently published data from the SMILE study, there are very few randomized controlled trials to the optimal time and strategy of

complete coronary revascularization in patients in the acute corner syndrome clinic with multi-vessel disease, where Sardella et al. (24) reported that the occurrence of a 1-year major adverse cardiovascular and cerebrovascular event(s), as well as target vessel revascularization rate, was significantly decreased in patients who underwent one-staged complete coronary revascularization than in patients who underwent multi-staged complete coronary revascularization. However, no significant differences were observed between the two study arms in overall death, cardiac death, myocardial infarction, stroke, and re-hospitalization. In line with our study, have no significant difference in terms of total mortality.

In multiple logistic regression analysis, low hemoglobin, high SYNTAX score, No-reflow phenomenon, not using post-dilatation were determinants as total mortality predicted. Studies examining the situation between anemia and MACE in patients with ACS are available in the literature. In the case of ACS, anemia is likely to worsen myocardial ischemia. Several studies investigated the impact of anemia on clinical outcomes in ACS. Sabatine et al. (25), studied the relationship between major adverse cardiovascular events and hemoglobin levels measured at the time of admission in approximately 40,000 patients. For reference hemoglobin 15 to 16 g/dL, when hemoglobin fell under 11 g/dL, there was a 1.5-fold increase in the probability of death, myocardial infarction, or recurrent ischemia for every 1 g/dL decrease in hemoglobin. In addition, Lorente et al. (26), revealed that anemia in patients diagnosed with ACS was independently associated with a significantly increased risk of total mortality.

Several studies investigated the effect of SYNTAX score, post-dilation, and No-reflow phenomenon on clinical outcomes in patients with ACS. Karjalainen et al. (27) revealed that balloon post-dilation improves clinical situations in patients with the acute coronary syndrome. Obeid et al. (28), demonstrated that the SYNTAX score is an independent predictor of all-cause deaths in ACS patients undergoing PCI. In addition, He et al. (29), revealed that clinical the SYNTAX score applied grouping patients by risk status for very long-term adverse clinical outcomes undergoing PCI and that predictive precision for 2-year all-cause mortality were improved using the clinical SYNTAX score. There are studies in the literature that demonstrated No-reflow was associated with increased all-cause mortality in patients with acute myocardial infarction (30,31). Our study reported comparable findings.

Our data showed different results, which might be associated with either patient-related or procedure-related factors in the SS-PCI group, including higher SYNTAX score, higher prevalence of LMCA involvement as culprit vessel, considerable reperfusion injury and inflammatory response, high rate of possible complications due to long procedures, including acute coronary syndrome, bleeding, stroke, nephropathy after exposure to a higher volume of contrast medium during the index procedure. Overall, these factors could have an essential impact on mortality at long-term follow-up in patients who underwent complete coronary revascularization with SS-PCI. In addition, in patients who underwent complete coronary revascularization with MS-PCI, overestimation of stenosis diameter due to infarction-related coronary vasospasm and, consequently, superfluous PCI was reduced to the minimum. This may have contributed to lower rates of mortality in this group. Conclusively, the decision of which complete coronary revascularization modality to prefer should be made in consideration of clinical presentation, co-morbidities, ventricular and renal functions, features of the coronary lesions of the patient, as well as the patient preference.

Our study has limitations that to be taken into consideration. At the onset, the most important problem was its single-center study and limited patient inclusion. Second, the patients included in the study were in the low-risk group, for SYNTAX scores were not very high. Third, cerebrovascular and renal events, as well as bleeding events were not evaluated. Finally, structural (intravascular ultrasound) or functional assessment to analyze the non-culprit lesions were not used, although they have been suggested by recent studies (32).

CONCLUSION

Our study has clinical importance by showing that patients diagnosed with NSTEMI and multivessel disease, who underwent complete coronary revascularization with SS-PCI and MS-PCI have no statistical difference in overall mortality rates. Among the parameters predicted as total mortality determinants, low hemoglobin, No-reflow phenomenon, not using post-dilatation were found to be statistically significant.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Adana Numune Training and Research Hospital (26.04.2016, 77).

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Dynamic Thiol/Disulfide Balance and Ischemia Modified Albumin Levels in Patients with Polycythemia Vera

Polisitemi Veralı Hastalarda Dinamik Thiol/Disulfit Dengesi ve İskemi Modifiye Albumin Düzeyleri

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ABSTRACT

Aim: Polycythemia vera is a chronic myeloproliferative disease characterized by increased red cell mass and JAK2 mutation positivity. Transformation to myelofibrosis and acute leukemia is possible in patients with polycythemia vera. Oxidative stress causes DNA damage and might be a reason for malignant transformation. Thiol molecules can prevent the harmful effects of oxidative stress. Therefore, in this study, we aimed to analyze the state of thiol homeostasis in patients with polycythemia vera.
Material and Methods: Thirty-one patients with polycythemia vera and 80 healthy volunteers were included in this study. Serum samples of the cases were stored until the end of the study. Native thiol, total thiol, disulfide, and ischemia modified albumin levels were determined.
Results: The mean ischemia modified albumin (1.09±0.21 vs 0.67±0.08; p<0.001, mean disulfide (23.5±6.1 vs 10.7±2.6; p<0.001), the mean disulfide/native thiol ratio (5.6±1.1 vs 3.1±1.2; p<0.001), the mean disulfide/total thiol ratio (5.0±0.9 vs 2.9±1.0; p<0.001), the mean native thiol (418.9±80.6 vs 371.4±103.7; p=0.024), the mean total thiol (466.0±89.8 vs 393.0±105.5; p=0.001) and the mean disulfide/total thiol ratio (89.8±1.8 vs 94.1±2.0; p<0.001) were found higher in polycythemia vera patients. Ischemia modified albumin levels were also higher in high-risk polycythemia vera patients. Patients on ruxolitinib therapy had higher native thiol, total thiol and disulfide levels, and higher disulfide/native thiol and disulfide/total thiol ratios.
Conclusion: Oxidative stress markers are still high in patients with polycythemia vera who were under treatment. Besides, ruxolitinib may be helpful to decrease oxidative stress in these patients.
Keywords: Disulfide; polycythemia vera; oxidative stress; thiol.

ÖZ

Amaç: Polisitemi vera, eritrosit artışı ve JAK2 mutasyonu pozitifliği ile karakterize olan kronik bir miyeloproliferatif hastalıktır. Polisitemi veralı hastalarda miyelofibroz ve akut lösemiye dönüşüm görülebilir. Oksidatif stres, DNA hasarına neden olur ve malign dönüşüme yol açabilir. Tiyol molekülleri, oksidatif stresin zararlı etkilerini önleyebilir. Bu nedenle, bu çalışmada polisitemi veralı hastalarda tiyol homeostazı durumunun analiz edilmesi amaçlandı.
Gereç ve Yöntemler: Bu çalışmaya polisitemi veralı 31 hasta ve 80 sağlıklı gönüllü dahil edildi. Vakaların serum örnekleri toplanarak çalışma sonuna kadar saklandı. Doğal tiyol, toplam tiyol, disülfit ve iskemi modifiye albümin seviyeleri belirlendi.
Bulgular: Ortalama iskemi modifiye albümin (1,09±0,21'e karşı 0,67±0,08; p<0,001), ortalama disülfit (23,5±6,1'e karşı 10,7±2,6; p<0,001), ortalama disülfit/doğal tiyol oranı (5,6±1,1'e karşı 3,1±1,2; p<0,001), ortalama disülfit/toplam tiyol oranı (5,0±0,9'a karşı 2,9±1,0; p<0,001), ortalama doğal tiyol (418,9±80,6'ya karşı 371,4±103,7; p=0,024), ortalama toplam tiyol (466,0±89,8 393,0±105,5; p=0,001) ve ortalama disülfit/toplam tiyol oranı (89,8±1,8'e karşı 94,1±2,0; p<0,001) polisitemi vera hastalarında daha yüksek idi. İskemi modifiye albümin düzeyleri ayrıca yüksek riskli polisitemi vera hastalarında daha yüksek idi. Ruksolitinib tedavisi alan hastalar daha yüksek doğal tiyol, toplam tiyol ve disülfit seviyeleri ile daha yüksek disülfit/doğal tiyol ve disülfit/toplam tiyol oranlarına sahipti.
Sonuç: Tedavi altında olan polisitemi veralı hastalarda oksidatif stres belirteçleri hala yüksektir. Ayrıca, ruxolitinib bu hastalarda oksidatif stresi azaltmak için faydalı olabilir.
Anahtar kelimeler: Disülfit; polisitemi vera; oksidatif stress; tiyol.

INTRODUCTION

Polycythemia vera (PV) is one of the chronic myeloproliferative neoplasms (MPNs), which presents with increased hemoglobin levels. The clonal proliferation of myeloid cells in bone marrow is the leading cause of this disease. The most important feature that distinguishes PV from other chronic MPNs is increased red blood cell mass. In almost all cases, the JAK2 V617F mutation is present. Due to the increased proliferation of progenitor cells, PV has the potential to transform into myelofibrosis and acute leukemia. Therapeutic phlebotomy, hydroxyurea, interferon, and ruxolitinib are the treatment options in patients with PV. Ruxolitinib is an inhibitor of janus-associated kinases and commonly used in hydroxyurea-resistant or hydroxyurea-intolerant patients. Although the consequences of tyrosine kinase activation caused by JAK2 mutation are well known, the exact mechanism of uncontrolled cell proliferation in PV is still incompletely understood (1).

Oxidative stress is an imbalance between reactive oxygen species (ROS) and antioxidants (2). ROS contributes to DNA damage with toxic substances. There are several mechanisms to protect the organism against the unfavorable effects of ROS. Thiol compounds that contain sulfhydryl groups are among these mechanisms. ROS oxidizes the thiol groups and forms reversible disulfide bonds. Antioxidants degrade the disulfide bonds to thiol groups again, and thus thiol/disulfide homeostasis is maintained (3,4). This homeostasis has an essential role in regulating apoptotic pathways, signal transmission, and transcription. Abnormal dynamic thiol/disulfide hemostasis has been shown in several diseases such as solid malignancies, cardiovascular diseases, rheumatoid arthritis, myelodysplastic syndrome, Alzheimer's disease, and diabetes mellitus (5-10).

In ischemia conditions, free oxygen radicals modify the N-terminal of the albumin, and this new form is referred to as ischemia modified albumin (IMA) (11). Several studies suggested that IMA could be used as a marker for oxidative stress in diseases such as acute coronary syndrome, diabetes mellitus, preeclampsia, and acute pancreatitis (12-14).

We could not find any study about thiol compounds and IMA levels in patients with PV. Therefore, we aimed to analyze the thiol/disulfide homeostasis and IMA levels in patients with PV.

MATERIAL AND METHODS

Thirty-one patients who were being followed at Mersin University, Department of Hematology, and 80 healthy volunteers, were included in this study. The inclusion criteria for patient group were as follows: being over 18 years old, being diagnosed with PV and signing the informed consent form. PV diagnosis was confirmed by bone marrow samples, JAK2 V617F and JAK2 exon12 mutation status according to the 2016 World Health Organization diagnostic criteria. Patients who did not sign the informed consent form, and those who are currently using antioxidant drugs (statin, etc.) were excluded from the study. Between November 2020 and January 2021, blood samples were collected from patients who came to our clinic for control.

Management of patients with PV is based on a risk-adopted approach. Patients who are ≤60 years old with no history of thrombosis are classified as low risk; all others are considered high-risk (15). Thiol, disulfide, and IMA changes according to risk stratification were evaluated. In addition, patients were grouped according to the four different types of treatment (therapeutic phlebotomy, hydroxyurea, ruxolitinib, and pegylated interferon) they used.

This study was conducted in accordance with the Declaration of Helsinki. It was approved by the Mersin University Ethics Committee (decision date: 14.10.2020 no: 2020/708). Written informed consent of all patients and controls were obtained.

Biochemical Evaluation

Blood samples were taken following overnight fasting. The blood samples were centrifuged at 4000 rpm for 10 minutes, and the serum samples were separated. Serum samples were stored at -80 °C until all blood samples were collected.

IMA Measurement

IMA measurements were made by the ELISA method, and the results were given in ng/mL.

Dynamic Thiol/Disulfide Homeostasis

The thiol/disulfide homeostasis tests were studied with a new method previously described by Erel et al. (16). To mention briefly, reducible disulfide bonds were reduced to form free functional thiol groups using sodium borohydride. Formaldehyde was used to remove residual sodium borohydride and 5,5'-disulfanediylbis (2-nitrobenzoic acid) products. Later, both reduced and native natural thiol groups were determined. The amount of dynamic disulfide was acquired by dividing the variance between total and natural thiol. Disulfide/total thiol, disulfide/native thiol, and native thiol/total thiol levels were calculated as percentages.

Disulfide levels, disulfide/native thiol, and disulfide/total thiol ratios, native thiol/total thiol levels were compared in PV patients and the control group. The clinical relationship between disulfide, disulfide/native thiol, and disulfide/total thiol levels and PV risk stratification was investigated. Also, levels of thiol compounds and IMA levels were evaluated according to treatment modality in PV patients.

Statistical Analysis

Shapiro-Wilk test was used to control the normal distribution of data. Mean and standard deviation were given for parameters suitable for normal distribution, median and interquartile range values were given for the data that were not normally distributed. Number and percentage values were presented as descriptive statistics for variables in a categorical structure. The Student's t-test was used to compare two groups with a normal distribution. For groups that were not normally distributed, Mann-Whitney U test was used. The Pearson correlation coefficient was used to compare the relationships between variables. In assessing the data, SPSS v.22 statistical package program has been used. Statistical significance was taken as $p<0.05$.

RESULTS

Some demographic and clinical characteristics of the patients were presented in Table 1. The mean IMA (1.09±0.21 vs 0.67±0.08; $p<0.001$, mean disulfide (23.5±6.1 vs 10.7±2.6; p,0.001), the mean disulfide/native

thiol ratio (5.6±1.1 vs 3.1±1.2; p<0.001), the mean disulfide/total thiol ratio (5.0±0.9 vs 2.9±1.0; p<0.001), the mean native thiol (418.9±80.6 vs 371.4±103.7; p=0.024), the mean total thiol (466.0±89.8 vs 393.0±105.5; p=0.001) and the mean disulfide/total thiol ratio (89.8±1.8 vs 94.1±2.0; p<0.001) were found higher in PV patients. The mean WBC, neutrophile, and platelet counts were higher, while the mean lymphocyte count and hemoglobin level were lower in the PV group (Table 2).
Three (9.7%) of the patients were on phlebotomy treatment when needed, 22 (70.9%) of the patients were under hydroxyurea treatment, 5 (16.1%) of the patients had started on ruxolitinib, and 1 (3.2%) of the patients was under pegylated interferon therapy (Table 1).
Patients who were on ruxolitinib had higher native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, IMA, and lower native thiol/total thiol (Figure 1). The difference in terms of IMA levels was statistically significant (p=0.009).
Twenty (38.7%) of the patients had low-risk disease, while 19 (61.3%) had high-risk disease. Mean thiol parameters, according to PV risk stratification, were revealed in Table 3. There was no statistically significant difference between low-risk and high-risk patients in terms of native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol. On the other hand, mean IMA levels (0.99±0.16 vs. 1.15±0.21; p=0.045) were higher in high-risk patients.
The mean spleen size of the patients was 135.2±27.3 mm. Spleen size was negatively correlated with native thiol (r=0.396, p=0.027) and total thiol levels (r=0.399, p=0.026). Oher thiol parameters and IMA levels were not significantly associated with spleen size.

Table 1. Demographic and clinical characteristics of the patients

	PV (n=31)	Control (n=80)
Age (year), mean±SD	60.2±14.7	54.5±17.4
Gender, n (%)		
Female	12 (38.7%)	23 (28.8%)
Male	19 (61.3%)	57 (71.2%)
Current Treatment, n (%)		
Therapeutic Phlebotomy	3 (9.7%)	
Hydroxyurea	22 (70.9%)	
Ruxolitinib	5 (16.1%)	-
Pegylated-Interferon	1 (3.2%)	

PV: Polycythemia vera, SD: standard deviation

Table 2. Laboratory parameters of the patients

	PV (n=31)	Control (n=80)	p
IMA (ng/mL)	1.09±0.21	0.67±0.08	**<0.001**
Native thiol (mmol/L)	418.9±80.6	371.4±103.7	**0.024**
Total thiol (mmol/L)	466.0±89.8	393.0±105.5	**0.001**
Disulfide (mmol/L)	23.5±6.1	10.7±2.6	**<0.001**
SH/TT (%)	89.8±1.8	94.1±2.0	**<0.001**
SS/TT (%)	5.0±0.9	2.9±1.0	**<0.001**
SS/SH (%)	5.6±1.1	3.1±1.2	**<0.001**

PV: polycythemia vera, IMA: ischemia modified albumin, SH/TT: native thiol/total thiol, SS/TT: disulfide/total thiol, SS/SH: disulfide/native thiol

Table 3. Laboratory parameters according to risk stratification in PV patients

	Low risk (n=12)	High Risk (n=19)	p
IMA (ng/mL)	0.99±0.16	1.15±0.21	**0.045**
Native thiol (mmol/L)	435.45±75.4	408.46±84.1	0.373
Total thiol (mmol/L)	481.59±83.3	456.31±94.5	0.455
Disulfide (mmol/L)	23.06±5.27	23.92±6.75	0.712
SH/TT (%)	90.43±2.40	89.52±2.08	0.194
SS/TT (%)	4.78±0.70	5.23±1.04	0.194
SS/SH (%)	5.30±0.85	5.88±1.29	0.181

PV: polycythemia vera, IMA: ischemia modified albumin, SH/TT: native thiol/total thiol, SS/TT: disulfide/total thiol, SS/SH: disulfide/native thiol

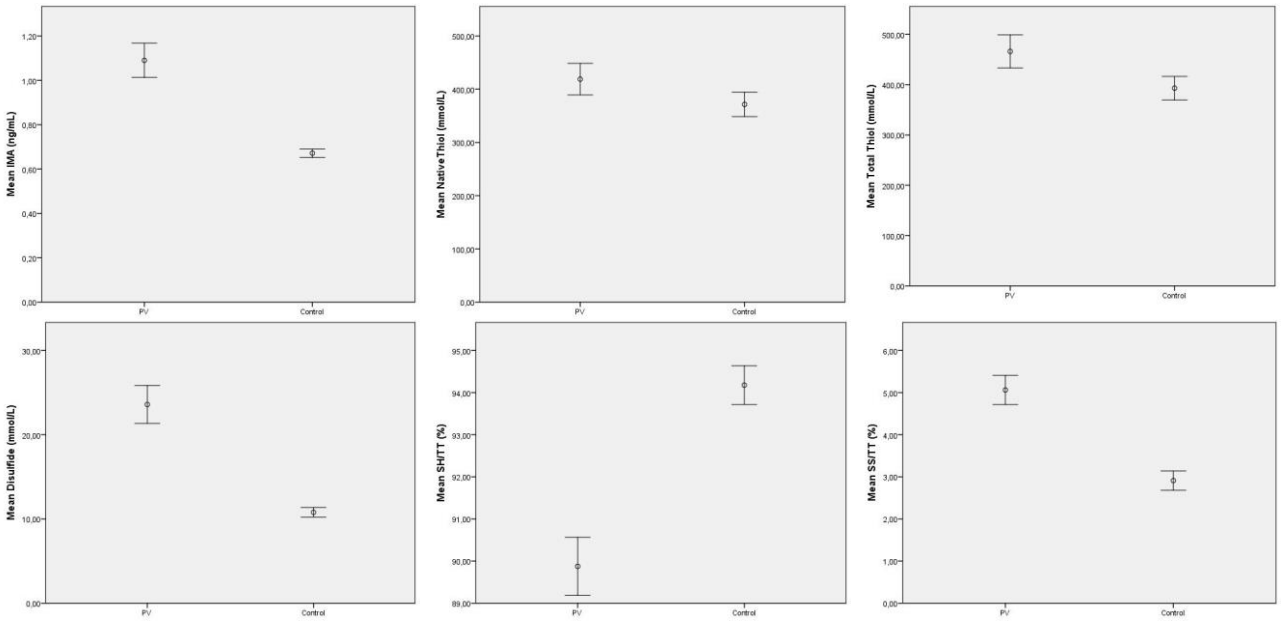

Figure 1. Error bars showing differences between patients with polycythemia vera and control group in terms of laboratory parameters, IMA: ischemia modified albumin, SH/TT: native thiol/total thiol, SS/TT: disulfide/total thiol

DISCUSSION

The results of this study have shown that IMA, native and total thiol, disulfide levels, disulfide/native thiol, and disulfide/total thiol ratios are elevated in PV patients. These results indicate that thiol/disulfide hemostasis shifts towards proliferation.

Several studies report that oxidative stress could play a role in myeloproliferative neoplasms' etiopathogenesis (17-20). However, we could not find any studies about the dynamic thiol/disulfide homeostasis in PV patients. We believe that this is the first study about this issue.

Oxidative stress and some genetic alterations in myeloid progenitor cells are related to myeloproliferative diseases. Some previous studies showed that oxidative stress markers other than thiol markers increase in patients with polycythemia vera (21,22). Cancer cells try to elevate their antioxidant capacities to adapt to oxidative conditions (23). The sulfhydryl groups of thiol compounds form a resistance mechanism against free oxygen radicals. The increase of free oxygen radicals due to the decrease in thiol levels causes obstacles in cellular pathways (24,25).

Erel et al. (16) showed that plasma disulfide levels are higher in patients with inflammatory and malignant diseases. In our study, it was observed that plasma disulfide levels were higher in the patient group. It was also found that native and total thiol levels were significantly lower in the patient group. Previous studies showed that native and total thiol levels are markedly higher in malignant hematological diseases such as myelodysplastic syndrome and multiple myeloma (4,26). From this point of view, it is seen that the results in our study are similar to the literature.

Ruxolitinib is an advanced treatment in patients with PV who are unresponsive to first-line therapies such as Therapeutic phlebotomy, hydroxyurea, and interferon. Although patients who were on ruxolitinib are resistant to other treatments, they had better oxidative stress results. This situation may prove that ruxolutinib is also effective on oxidative stress. Besides, higher IMA levels in these patients may be related to the fact that they had a worse prognosis than others. Since only five patients on ruxolitinib were included in this study, studies involving more patients are needed.

One of the most significant limitations of our study was that patients were not newly diagnosed. All of them had under a treatment modality. Moreover, the number of patients was small. New prospective studies should be designed to show thiol changes before and after treatment.

CONCLUSION

This study is critical because it supports that oxidative stress is still worse in patients with PV, even if they are under treatment. Additionally, ruxolitinib may be essential to decrease oxidative stress in these patients. With multicenter prospective and randomized controlled studies in the future, we believe that the importance of thiol/disulfide hemostasis will be better understood.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Mersin University (14.10.2020, 708).

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Histoultramicroscopic Investigation of the Rats' Thymus (Experimental Data)

Sıçanların Timusunun Histoultramikroskopik İncelenmesi (Deneysel Veri)

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ABSTRACT

Aim: The research paper presents the characteristic of cytoarchitectonics of the thymus of intact white mature male laboratory rats. Topicality of the study is due to the need to clarify the data on the contribution of each type of thymus cells in the formation of its structure. The aim of the research was to determine the specifics of localization and ultramicroscopic structure of thymus cells in male mature Wistar laboratory rats.
Material and Methods: The study was conducted using histological and ultramicroscopic methods on 10 mature male laboratory rats, weighing 130-150 g. Semi-thin (0.5-1 μm) and ultrathin (0.05-0.2 μm) sections were made on a microtome UMTP-4 (Ukraine), which were stained with 1% methylene blue solution with the addition of 1% sodium tetraborate solution. Histological analysis and photographic recording were performed using Olympus light microscope (Japan) and DSM 510 camcorder with magnification in 1000 times.
Results: With a detailed study of the semi-thin and ultrathin sections in the thymus lobules the specifics of localization and ultramicroscopic structure of thymus cells were clearly identified. The features of localization and ultramicroscopic structure of epithelial, mesenchymal, vascular and hematopoietic thymus cells were determined from the point of view of their functional loads and interactions.
Conclusion: The described structural peculiarities of the components of the thymus and their relative location in different zones reflect significant organ polymorphism, which must be taken into account in order to achieve the required level of objectivity in the result evaluation of simulated biomedical experiments.
Keywords: Thymus gland; rats; histology; ultrastructure; experiment.

ÖZ

Amaç: Bu araştırma makalesi, sağlam beyaz olgun erkek laboratuvar sıçanlarının timusunun sitoarkitektonik özellikleri hakkında bilgi sunmaktadır. Çalışmanın güncelliği, her bir timus hücresi tipinin timüs yapısının oluşumuna olan katkısı hakkındaki verilerin net bir şekilde ortaya çıkarılmasına olan ihtiyaçtan kaynaklanmaktadır. Bu araştırmanın amacı, erkek olgun Wistar laboratuvar sıçanlarında timus hücrelerinin lokalizasyon ve ultramikroskopik yapısının özelliklerini belirlemektir.
Gereç ve Yöntemler: Bu çalışma, 130-150 g ağırlığındaki 10 olgun erkek laboratuvar sıçanı üzerinde histolojik ve ultramikroskopik yöntemler kullanılarak yapıldı. Bir UMTP-4 (Ukrayna) mikrotomu ile yarı ince (0.5-1 μm) kesitler ve ultra ince (0.05-0.2 μm) kesitler yapıldı, yapılan bu kesitler %1 sodyum tetraborat solüsyonu ilave edilerek %1 metilen mavisi solüsyonu ile boyandı. Histolojik analiz ve fotoğraf kaydı Olympus ışık mikroskobu (Japonya) ve DSM 510 video kamera kullanılarak 1000 kez büyütme ile yapıldı.
Bulgular: Timus lobüllerindeki yarı-ince kesitler ve ultra-ince kesitlerin detaylı bir şekilde incelenmesi ile timus hücrelerinin lokalizasyon ve ultramikroskobik yapısının özellikleri net bir şekilde ortaya çıkarıldı. Epitelyal, mezenkimal, vasküler ve hematopoetik timus hücrelerinin lokalizasyon ve ultramikroskopik yapısının özellikleri, bu hücrelerin fonksiyonel yükleri ve etkileşimleri açısından dikkate alınarak belirlendi.
Sonuç: Timusun bileşenlerinin tanımlanmış yapısal özellikleri ve farklı bölgelerdeki göreceli konumları, simüle edilmiş biyomedikal deneylerin sonuçlarının değerlendirilmesinde gerekli olan ve istenen nesnellik düzeyinin elde edilebilmesi için dikkate alınması gereken önemli organ polimorfizmini yansıtır.
Anahtar kelimeler: Timus bezi; sıçanlar; histoloji; ultrastrüktür; deney.

INTRODUCTION

The immune system, as one of the central systems of homeostasis control, provides the physiological mechanisms for its regulation and takes direct part in the development of compensatory and adaptive reactions (1,2). Thymus is a central organ of lymphopoiesis, hematopoiesis and immune protection of the organism in all vertebrates, and is often studied in experimental biomedical research projects (3-8). The uniqueness of the thymus morphology lies in the significant heterogeneity of its cellular composition, which forms epithelial, mesenchymal, vascular, hematopoietic cells: T- and B-lymphocytes, macrophages, dendritic cells, etc. (9-11). The development of T-lymphocytes and the formation of recirculating pool of these cells is the main function of the thymus (12,13).

Numerous studies have shown that morphological changes at the ultrastructural level form the initial link of any pathogenetic mechanism. Therefore, there is no damaging factor that would not lead to structural changes in the cell. This attaches particular importance to further in-depth study of the submicroscopic organization of thymus cells in the normal conditions in connection with the search for key morphological equivalents of its normal function and the development of common criteria that can be used as benchmarking comparisons in conducting experimental medical and biological research.

A deep understanding of the normal cytoarchitectonics of the thymus and the morphology of all parts of the functional system: "Bone marrow - thymus - spleen - lymph nodes" – is extremely necessary for understanding the formation of the ultimate adaptive effect – immune protection of the organism. Topicality of the study is due to the need to clarify the data on the contribution of each type of thymus cells in the formation of its structure.

The aim of the research was to determine the specifics of localization and ultramicroscopic structure of thymus cells in male mature Wistar laboratory rats.

MATERIAL AND METHODS

Experimental research was carried out on the basis of the laboratory center of morphological research of Sumy State University (Sumy, Ukraine). The institutional and national guide for the care and use of laboratory animals was followed (Ethics committee of Sumy State University, date of approval 02.03.2020, Protocol №2/2).

Experimental Design

The experiment was conducted on 10 mature male laboratory rats, weighing 130-150 g. The maintenance and manipulation of animals was carried out in compliance with the requirements of bioethics and the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001), the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), the principles of the Helsinki Declaration on the Human Approach to Animals. All rodents were kept in conventional environment of vivarium with the usual food and water intake.

Electron Microscopy

After animals' decapitation and dissection of the chest wall under thiopental anesthesia thymus was extracted. Samples of the investigated organ tissue were first fixed in glutaral aldehyde (by Karnovsky), and then in 1% solution of tetraoxide osmium (by Palade). Subsequently, the samples were made through ethanol solutions of increasing strength and filled into a mixture of epoxy resins followed by polymerization. Semi-thin (0.5-1 μm) and ultrathin sections (0.05-0.2 μm) were made on a microtome UMTP-4 (Ukraine), which were stained with 1% methylene blue solution with the addition of 1% sodium tetraborate solution. Histological analysis and photographic recording were performed using Olympus light microscope (Japan) and DSM 510 camcorder with magnification in 1000 times. Ultra-thin sections were enhanced with uranyl acetate and lead citrate and investigated by electron microscope PEM-125 K (Ukraine).

RESULTS

With a detailed study of the semi-thin sections in the thymus lobules, according to the international classification of histological terms (5), morphologically distinct zones that had the specifics of the cellular structure organization were clearly identified: the cortical substance in the sections was more intensively stained, thymocytes were densely placed, the medulla was stained less intensively since contained fewer thymocytes (Figure 1b, Figure 2). The zones formed a lobe covered with a capsule (Figure 1a). The thin capsule of the connective tissue extended into small trabeculae, which partially divided the thymus into adjacent lobules of different sizes, extending from the periphery to the center of the lobes. In these partitions there were blood vessels and nerves. Between the capsule and trabeculae was a carrier carcass consisting of a network of epithelioreticular cells, which together with thymocytes are one of the major cellular components of the thymus. Between the cortex and medulla, an unclear boundary was determined, characterized by the presence of blood vessels (preferably arterioles) with a small amount of perivascular connective tissue, mature and immature thymocytes (Figure 3).

Important functional zones of thymus include intralobular perivascular spaces (14,15). These spaces were located close to the intercellular trabeculae and were limited from one side by a powerful, thickened basal membrane of blood vessels, from another side by a basal membrane of cortical and medullar epithelioreticular cellular nets. Intralobular perivascular cortical areas, together with the endothelium of the blood capillaries, are considered to be the structural basis of the blood-thymus barrier. The latter is defined as a functional and selective barrier between thymocytes and blood, into which, in addition to epithelial reticular cells, also include macrophages and perivascular thymocytes. In the medullar substance, this structure was not determined in its entirety.

It is generally recognized that in the cortical zone, the following cells are morphologically differentiated: epithelioreticulocytes cells (forming a cortical cellular network), macrophages, nursing cells, precursor cells (prolymphocytes, lymphoblasts) (5).

In the cortical zone of the intact rats' thymus were found epithelioreticular cells of the polygonal form (Figure 1a) with cytoplasm sprouts and a light euchromatic nucleus of irregular shape, with small lumps of heterochromatin, often

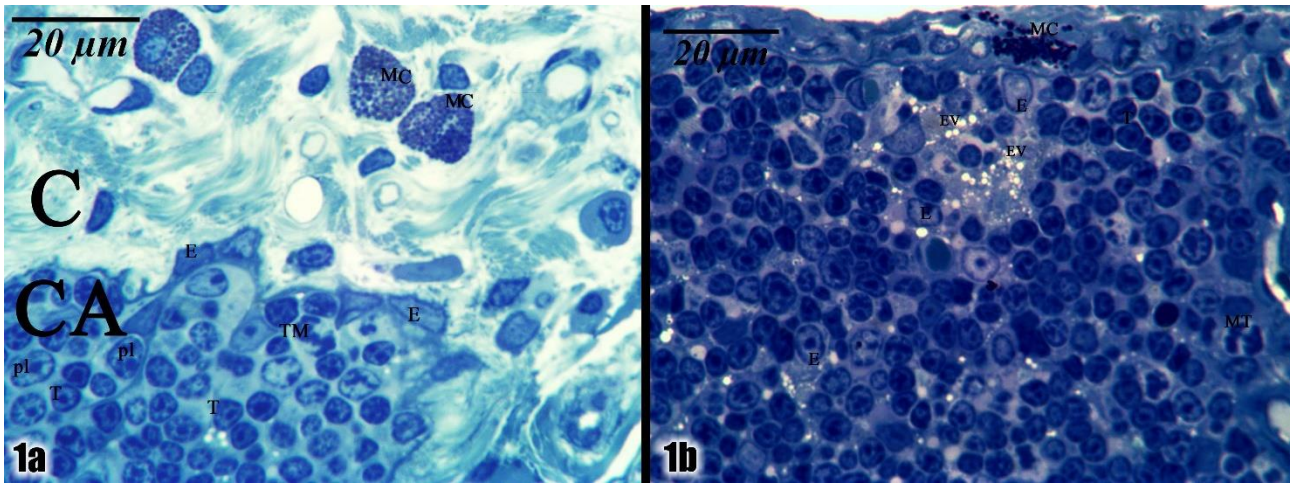

Figure 1a. Capsule (C) and cortex (CA) of the rat's thymus. Thymocytes (T), epitheliореticulocyte (E), mastocytes (MC), prolymphocytes (pl); **1b.** Capsule of the rat's thymus. Thymocytes (T), epitheliореticulocytes (E), epitheliореticulocytes with vacuoles (EV), mastocyte (MC), cell in the stage of mitosis (MT). Semi-thin slice. Methylene blue stain

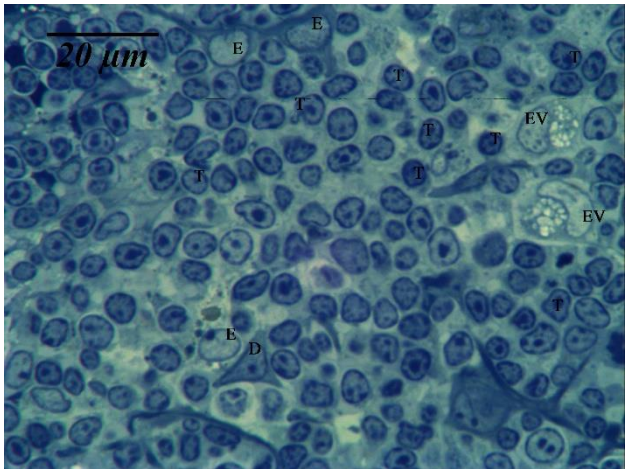

Figure 2. Medulla of the rat's thymus. Epitheliореticulocytes with vacuoles (EV), Thymocytes (T), epitheliореticulocytes (E), dendritic epitheliореticulocyte (D). Semi-thin slice. Methylene blue stain

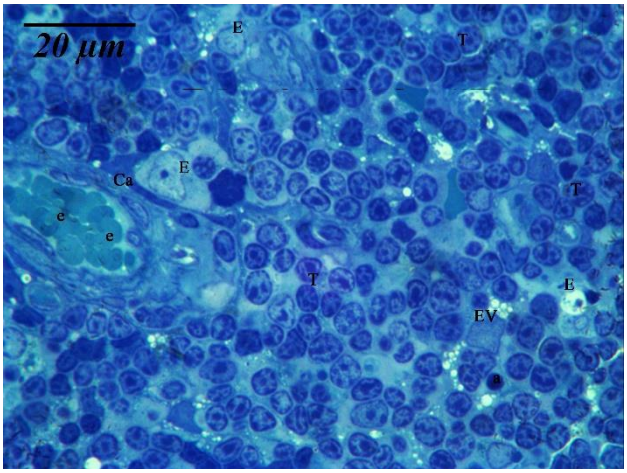

Figure 3. Cortico-medullar zone of the rat's thymus. Hemocapillar (Ca), Thymocytes (T), epitheliореticulocytes (E), epitheliореticulocyte with vacuoles (EV), erythrocyte in a hemocapillar lumen (e). Semi-thin slice. Methylene blue stain

with a developed nucleolus. The cytoplasm revealed mitochondria, the Golgi apparatus, and ribosomes. There were epitheliореticular cells with a lighter nucleus and cytoplasm without vacuoles. Alongside the nuclei, thin bundles of keratin tonofilaments were determined (Figure 4a, 4b). We also observed epitheliореticular irregular cells with a dark nucleus and a vacuolated cytoplasm. Vacuoles were often filled with fine-grained content (Figure 4c, 4d). Cells were located singly in both the cortical and medullar areas (Figure 1b).

In medullary zone the following cells are morphologically differentiated: epitheliореticulocytes (forming a cortical cellular network), macrophages, dendritic cells, precursor cells (prolymphocytes, lymphoblasts), as well as Hassall's corpuscles (5).

In the corticomedular zone, there were epithelioreticular cells with a rounded nucleus that contained heterochromatin lumps and the nucleolus. The cytoplasm was depleted by organelles, there were isolated ribosomes, mitochondria, Golgi apparatus, small clusters of tonofilaments (Figure 4e).

Epitheliореticulocytes of the thymus medulla were more numerous. More often occurred large cells sizes with bulky, not clearly expressed cytoplasmic processes. The nuclei of these cells had a crescent or irregular shape, with a predominance of euchromatin, contained nucleolus. The ultrastructural organization of these cells has shown high metabolic and secretory activity, as in their cytoplasm, generally, there were numerous transport vesicles, expanded rough endoplasmatic reticulum profiles Golgi apparatus, vacuoles located in a separate section of the cytoplasm near the nucleus (Figure 4f).

All thymic epithelioreticular cells were characterized by the presence of desmosomes and tonofilaments.

In the thymic medullary zone of the intact mature rats occasionally encountered Hassall's corpuscles (clusters of concentrically located flattened epithelial cells), which are considered one of the morphological markers of this zone.

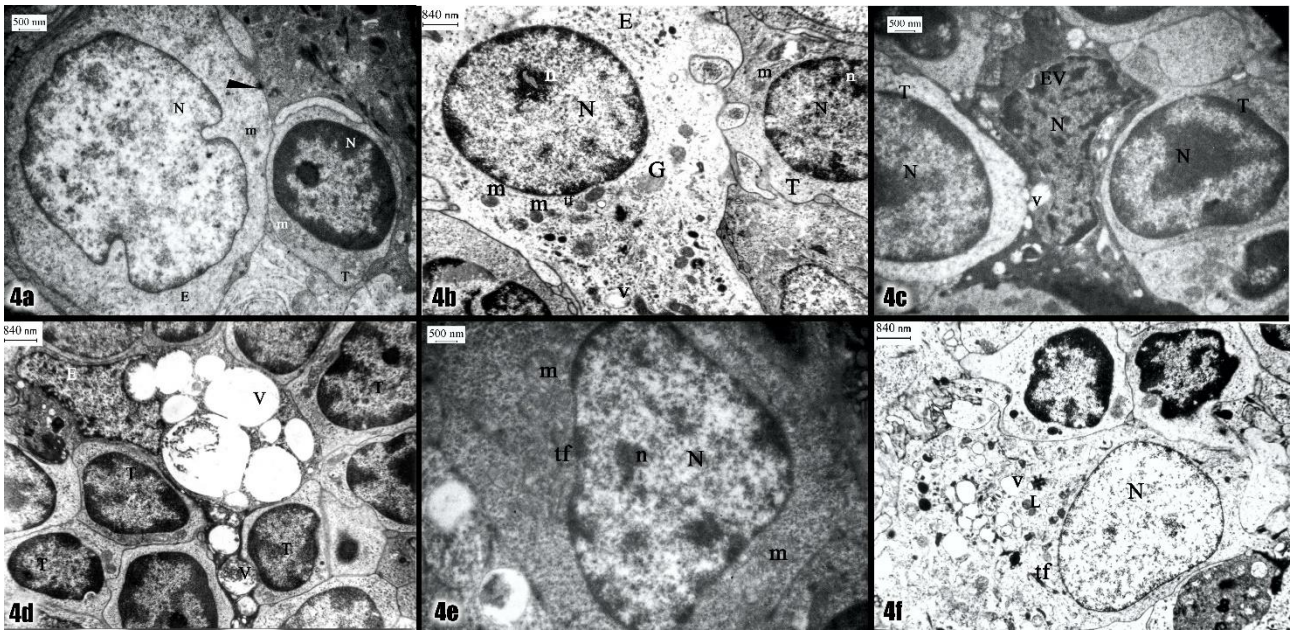

Figure 4(a,b,c,d,e,f). Polymorphism of epitheliореticulocytes of thymus in rats. Thymocyte (T), epitheliореticulocyte (E), epitheliореticulocyte with vacuoles (EV), nucleus (N), nucleolus (n), vacuoles (V), mitochondria (m), keratin tonofilaments (tf), lysosomes (L), black triangular arrow – desmosome (D), Golgi apparatus (G). Electronograph

Superficial layer of the corpuscles had irregular contours, was formed by flattened light structures that resembled epithelial cells without nuclei, but with fragments of ER, keratin tonofilaments, and single lysosomes. The structures of the superficial layer of the corpuscles had a small number of pronounced cytoplasmic processes. All visualized Hassall's corpuscles did not have a keratin nucleus and contained 1-2 epithelial cells in the center, which allows them to be identified as progressive; according to the classification of the stages of the development of Hassall's corpuscles, proposed by Beloveshkin AG (14). Centrally located epithelial cells had more often oval shape with uneven contours of light or dark enough broad cytoplasm, which contained isolated autophagosomes, oval or rounded form mitochondria, small lysosomes, keratin tonofilaments. Cell nuclei were often euchromatin with compactification of chromatin near the nuclear membrane, had an elongated shape, unequal contours with pronounced invaginations, which frequently split the nucleus into several blades. In the immediate proximity to Hassall's corpuscles, thymocytes were predominantly identified that did not form dense contacts with the corpuscles (Figure 5a, 5b).

During the investigations of semi-thin slices and electronographs were found only single epithelial cells which morphologically reminded nurse cells and contained 1-2 thymocyte in their invaginations.

In capsule were determined tissue basophils (mast cells). They formed groups of 3-6 cells in an intermittent chain or located apart (Figure 1a, 1b).

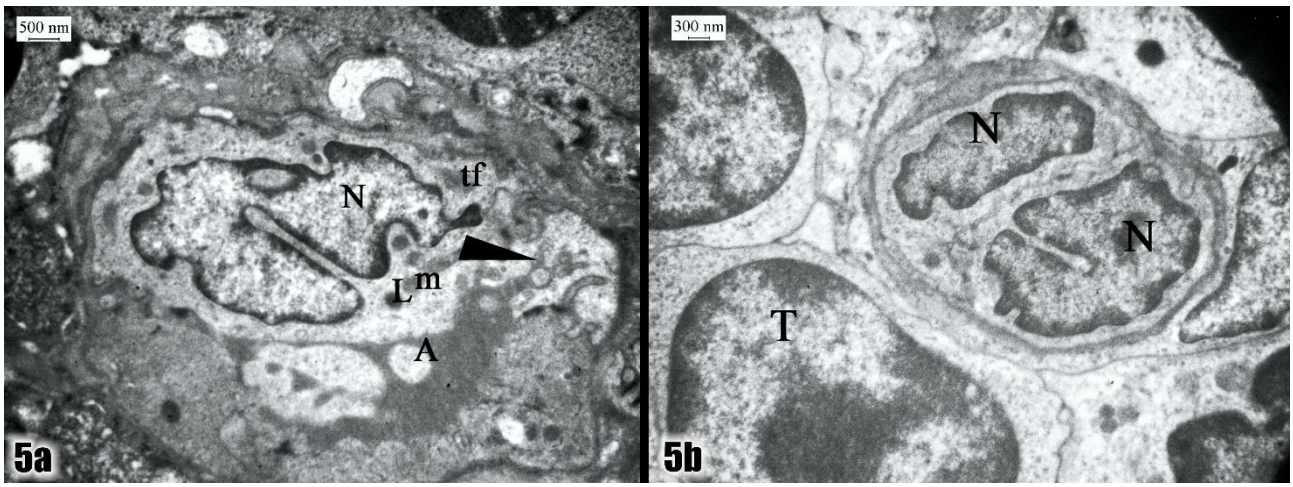

Figure 5a. Hassall's corpuscle of the rat's thymic medulla. The nucleus (N) of the epithelial cell with deep invaginations, mitochondria (m), autophagosome (A), black arrow – cytoplasmic processes, keratin tonofilament – (tf), (L) – lisosome; **5b.** Progressive body of the Hassall's corpuscle of the rat's thymic medulla. Thymocyte (T), nucleus (N) of the epithelial cell with deep invaginations. Electronograph

Underneath the capsule between single epitheliореticulocytes there were densely located, less differentiated lymphoid cells (prolymphocytes and lymphoblasts) that are precursors of T-lymphocytes (Figure 1a). The cells were predominantly round or oval shape with a rounded or oval nucleus, with one or two nucleoli. Chromatin was homogeneous but occurred denser areas of heterochromatin under the nuclear membrane. Narrow cytoplasmic rim surrounded the nucleus, oval mitochondria, single or multiple ribosomes, Golgi apparatus and refined ER were often visualized (Figure 6a). Perinuclear zone had less electron density. They were characterized by high mitotic activity. Segregate prethymocytes were at different stages of mitosis (Figure 6b).

During the study of semithin and ultrathin slices in the cortex and medulla of thymus, there were segregate apoptotic thymocytes with peculiar changes in the nucleus and cytoplasm. The consolidation of the nucleus with a change in the structure of its boundaries and the waviness of its contours, the condensation, and shrinkage of the cytoplasm were distinguished. An aggregation of nuclear chromatin in the form of various sizes of debris was observed. In some apoptotic lymphocytes, the condensed chromatin was located in a crescent form, sometimes filling the whole nucleus (Figure 7).

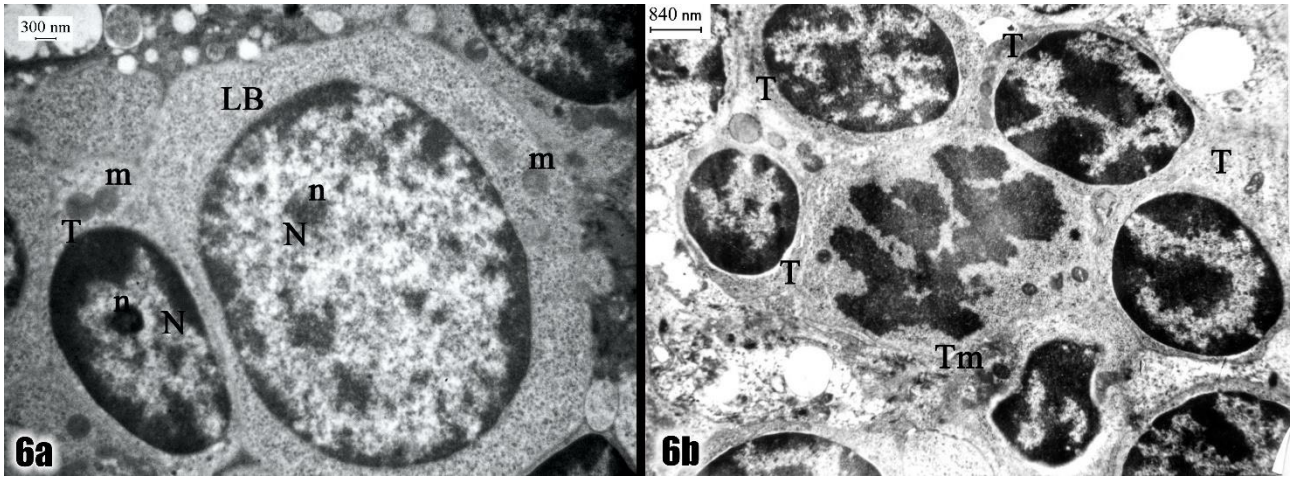

Figure 6a. Thymocyte (T) and lymphoblast (LB) of the rat's thymic cortical zone. Mitochondria (m), the nucleus (N), the nucleolus (n); **6b.** Thymocytes (T) of the rat's thymic cortical zone. In the center located the cell in the mitosis stage (Tm). Electronograph

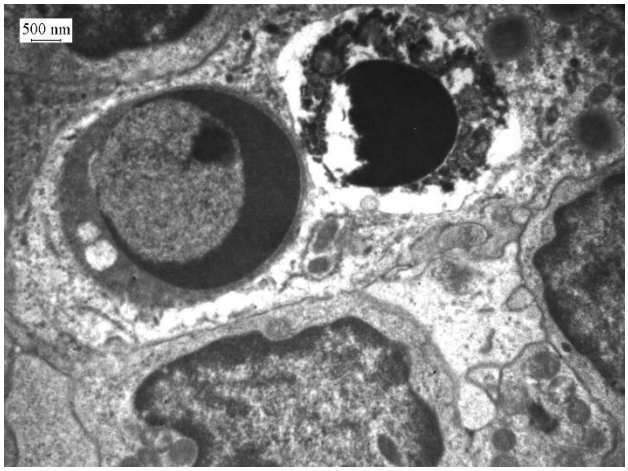

Figure 7. Apoptosis of thymocytes in the cortico-medullary zone of the rat's thymus. Electronograph.

Typical plasmocytes were present among thymocytes in electronograms, as evidenced by the large eccentrically placed oval nucleus of the cell, where euchromatin dominated, a nucleus with a ribosomal aureole located. The cells had a well-developed Golgi apparatus, polysomes, and large, well-preserved membrane components of the mitochondria, which adhered tightly to the short tubules of a well-developed rough ER.

In the structure of different thymic zones, macrophages were presented. In the subcapsular zone of intact adult rats, single macrophages were observed, which represented large cells with a small amount in the cytoplasm of lysosomes and small vesicles that had electron-dense content. (Figure 8a).

The functional purpose of the subcapsular zone is to provide proliferation and initial stages of maturation of pre-T-lymphocytes (after their migration from the bone marrow) under the influence of appropriate signals from macrophages through the formation of contacts (16-18).

Compared with other thymic areas of intact adult rats, the cortical zone was characterized by the highest density distribution of macrophages that were morphologically similar to the macrophages of the subcapsular zone and localized in the perivascular spaces. Quite often occurred macrophages of elongated shape, as well as cells with small sprouts, which resembled pseudopodias (Figure 8b).

In the cortex of the thymus, contacts between macrophages and thymocytes, which in some cases were characterized by a large area of contacting cell membranes, were often determined (Figure 8c).

The macrophages of the cortico-medullary zone of thymus morphologically resembled the most resident monocytes: they were mostly round-shaped with a small amount of weakly expressed spherical invaginations of the cytoplasm, often a bean-shape form of the nucleus.

In the medullary zone of the intact adult rats' thymus, a few macrophages that located freely between thymocytes without contact formation between them were observed. This arrangement is likely to indicate their role as cells, which, together with epithelial cells and dendritic cells, provide microenvironment for antigen-dependent maturation of thymocytes in this zone of thymus (19,20).

In general, intact mature rats' macrophages of the thymus different zones were characterized by the following ultrastructural features. The cells had both round and elongated shapes with a rough surface and small protrusions of the cytoplasm, which resembled pseudopodias. On the cells' surface, there were invaginations of different sizes and in different quantity. The nuclear-cytoplasmic ratio was close to 1.0. Often were observed well-expressed nucleoli. Macrophages were characterized by developed intracellular membrane structures. Rough ER had the form of branched channels. The Golgi apparatus was formed by cisternaes, which were combined with small-sized vesicles. In the cytoplasm of all cells were determined, mostly small, sometimes larger granules, which resembled monocytes granules. Also, in individual macrophages, the elements of the cytoskeleton were identified: fibrillar and tubular structures. The mitochondria in the vast majority of cells were small and had close to round shape. In the cytoplasm of all macrophages, more or less pronounced primary and secondary lysosomes were detected in different amount, as well as vacuoles of various sizes were often found. Separate macrophages contained in the cytoplasm apoptotic bodies of thymocytes (Figure 8d).

The cytoarchitectonics of the thymus of the investigated rats group was characterized by the presence of dendritic cells (DC). DC were often found in medullary and cortico-medullary zones. Few DC were observed in the cortical and subcapsular areas, as well as in perivascular spaces, and segregate DCs were found in connective tissue trabeculaes. Occasionally, DCs were located separately, more often they formed clusters with thymocytes. In such clusters, cells are tightly contacted with membranes, but without the formation of desmosomes and tonofilaments.

DC – volumetric cells with electron light cytoplasm, which formed processes (predominantly from 3 to 6), which tended to penetrate more or less deeply between thymocytes. The nucleus was characterized by polymorphism (invagination), the presence of euchromatin with a thin layer of heterochromatin, located along the nuclear membrane. Most often the nucleus was located

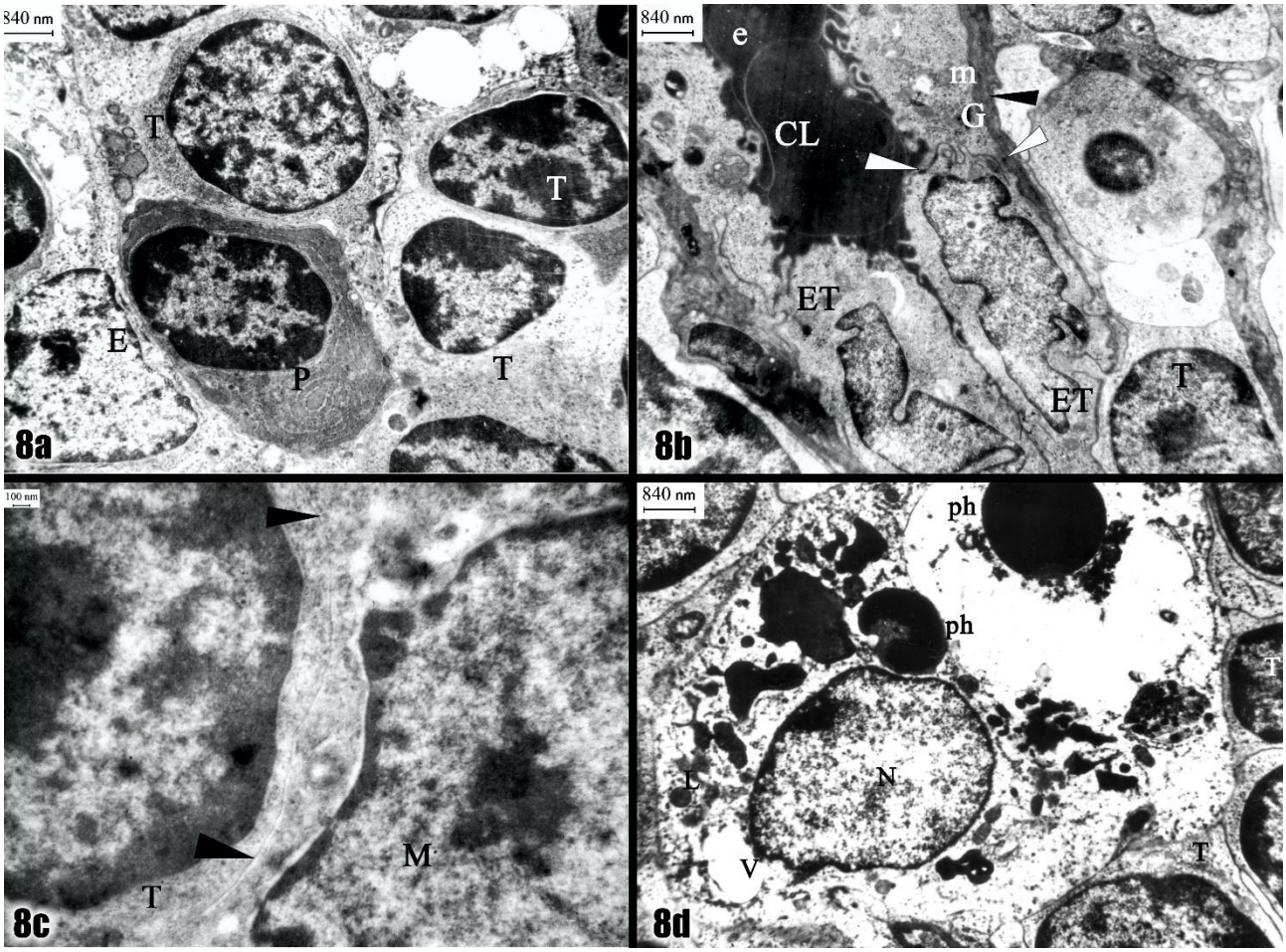

Figure 8a. Cells of the rats' thymic cortex. thymocyte (T), epitheliореticulocyte (E), plasmocyte (PT). **8b.** Blood capillary of the rat's thymic cortex. Capillary lumen (CL), erythrocytes (e), thymocyte (T), endotheliocyte (ET), black arrow – basal membrane, white arrow – desmosome, Golgi apparatus (G). **8c.** Contact of macrophage cell membranes (M) and thymocyte (T) of the rat's thymic cortex – arrows. **8d.** Cells of the rat's thymic cortex: epitheliореticulocyte (E), thymocytes (T), macrophage: phagosome (ph), nucleus (N), lisosome (L), endoplasmatic reticulum (EPR). Electronograph.

eccentrically and contained a nucleolus. In the cytoplasm of the DC near the perinuclear zone, there were organelles: a developed Golgi apparatus with small-scale vesicles, ER in the form of located peripherally narrow elongated cisternaes, sometimes large enough mitochondria. Also, in the DC cytoplasm, as a rule, were represented lysosomes in a small amount. In the cytoplasm of individual DC, there were vesicular structures that contained picnotial nuclei of lymphocytes (presumably phagolysosomes). Occasionally, in the cytoplasm of the DC, a few spindle-shaped formations similar to Birbeck granules located in the central part of the cell were observed (Figure 9).

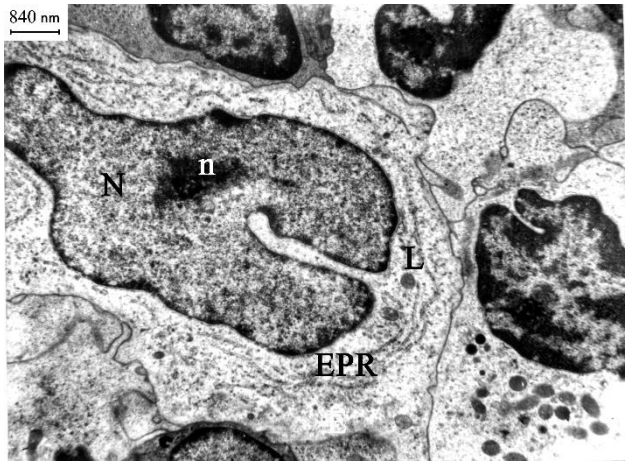

Figure 9. Dendritic cell (D): nucleus (N), nucleolus (n), lisosome (L), endoplasmatic reticulum (EPR). Electronograph

DISCUSSION

In the scientific literature, there are several opinions concerning the classification of the structural and functional zones of the thymus lobules. Some authors distinguish within the thymic lobule four zones: the outer subcapsular, internal cortical, medullar and perivascular spaces (21). Other authors in their works describe three zones: cortical, cortico-medullar and medullar (11,22,23). In studies of the last 10 years presumably the authors describe five zones in the thymus lobe, three of which are localized in the cortical substance; subcapsular, central cortical zone and marginal cortical zone, and two - in the medulla; marginal medullar zone and central medullar zone (24-26).

In our opinion, in the description of the thymic ultrastructural structure, the most appropriate is to use histological classification, but in order of further zones detailing, structures and cells location, additional definitions of different compartments can be used.

An important morphological element of the thymus lobe, which, in our view, is subject to mandatory ultrastructural evaluation, is a blood-thymus barrier. The latter is defined as a functional and selective barrier between thymocytes and blood, which, in addition to epithelioreticulocytes, also includes macrophages and perivascular thymocytes (5,25,27,28). The blood-thymus barrier protects thymocytes from circulating macromolecules (antigens). According to Pearse G (23) in this area, along with small blood capillaries, postcapillary venules with high endothelium are detected. There are also dendritic cells, thymocytes, B-lymphocytes, plasma cells. A detailed study of the blood-thymus barrier under the normal conditions allows us to get a proper idea of the cohesion between the thymus tissue and the structures of the microcirculatory bed.

By ultramicroscopic features, 6 types of epithelial cells are distinguished. Cells of 1 type limit the capsule, partitions, and surround perivascular spaces of the cortical substance. 2 and 3 type cells are localized in the cortical substance. Type 4 cells are located in the corticomedullar zone; cells of 5 and 6 types are stromal cells of the thymic medulla and thymic bodies. Based on the results of immunohistochemical studies, four main types of epithelial cells are identified: subcapsular (paraseptal, perivascular), cortical, medullary, and Hassall's corpuscles (16). Takahama et al. (11) suggest to distinguishing between 7 types of epithelial cells in the thymus. According to our ultrastructural data polymorphism of the mature rats' thymus is forming presumably in account of known existed 6 types of epithelioreticular cells.

Hassall's corpuscles are not numerous but compulsory component of the microenvironment of the medulla of rats' thymus. A thorough analysis and synthesis of literature on origin, morphological and functional features of Hassall's corpuscles were made by Beloveskin AG (14) in the monograph "The System Organization of Hassall's Corpuscles" (2014). The author proposed the classification of the stages development of corpuscles, according to which distinguished: progressive, mature and regressive bodies. Also, the author, according to our vision, is quite successfully, substantiated and detailed the concept of the Hassall's corpuscles systematic organization. At the heart of this concept lies the close interaction of all structural elements of the corpuscle, which is characterized by a large variety of information exchange, necessary for the harmonious work of individual parts of the body's immune protection system (for example, the removal of auto-reactive thymocytes, etc.). At the same time, the system-forming factor, according to the author, is the process of synthesis, mobilization, and transfer of tissue-specific autoantigens to dendritic cells that are in direct contact with the Hassall's corpuscles (14).

Concerning the age dynamics of the number and age-related morphological changes in Hassall's corpuscles, in humans, in particular, some authors note that the number of corpuscles increases until the period of puberty, after which it non-linearly decreases to an average of 70 years when again there is a slight increase. As the age-old involution of the thymus also changes the morphology of the Hassall's corpuscles, which can be gigantic in size and undergo cystic degenerative changes. All these changes are a reflection of age-related rearrangements in the cellular microenvironment of the thymus (21).

An important element of the thymic cytoarchitectonics is the nursing cells. According to some authors, nursing cells are located in the subcapsular zone and in the cortical substance, have deep invaginations, in which there are lymphocytes. Nursing cells are thymic nourishing cells and their cytoplasm penetrating between lymphocytes, can obtain the appearance of very thin and elongated bands. Typically, such cells contain 10-20 or more lymphocytes. Nursing cells are capable of producing thymosin.

Thymocytes are forming the most numerous and diverse by morphology and size of the thymus cell population. It is believed that thymocytes of the cortical substance migrate to the bloodstream, without passing the medulla. These lymphocytes differ in the composition of receptors from T-lymphocytes of the medulla. With blood flow they enter the peripheral organs of lymphocytopoiesis –lymph nodes and spleen, where they ripen, forming subpopulations. The thymocytes of the medulla are recirculating pool and can enter the bloodstream and proceed from the blood flow through the postcapillary veins (15).

Thymus macrophages originate from precursors of the myeloid line of the bone marrow and together with monocytes and dendritic cells form a mononuclear phagocytic system. Monocyte is considered as a circulating version of phagocytic cells, and the macrophage –as a tissue (resident) version of phagocytic cells (14).

Described by us DC form a unique, genetically and functionally heterogeneous component of the mononuclear phagocytic system and is considered as the main cells that recognize the pathogenic patterns (so-called "DC-detectors") and the presentation of antigens (the so-called "DC-presenters"). DC play a key role in the integration of responses of congenital and adaptive immunity and determine the central and peripheral tolerance of the immune system of the organism. At the heart of the mechanism of central tolerance, formation is the active participation of the thymus DC in the negative selection of T cells and their subsequent elimination. The origin, ultrastructure, localization, pathways of migration in the body, phenotypic features, and functions of individual subpopulations of DC continue to be studied actively (27).

In the majority of the research papers devoted to the question of the origin of DC, published over the past 10 years, the authors have expressed the common opinion that DCs originate from hematopoietic stem cells through a series of stages of the precursor cells within the two histogenetic series: myeloid and lymphoid. Guiliams et al proposed a classification of DC based on their origin, localization, functions and features of the phenotype. According to this classification, the DC have a common predecessor, which in turn comes from the stem cell of the bone marrow. From the common predecessor, two lines of DC develop: the precursors of the classical (myeloid) DC (cDK) and precursors of plasmacytoid DC (pDC). From predecessors of the pDC under the control of the E2-2 transcription factor develop mature pDC. From the precursors of the cDC, two phenotypes develop: under the control of BAFT3 (Basic leucine zipper transcriptional factor ATP-likes 3) - cDK1, and under the control of IRF4 (Interferon-regulatory factor 4) - cDC2 (22).

CONCLUSION

To sum up, it should be noted that the features of localization and ultrastructure of rat thymus cells described by us reflect the significant morphological heterogeneity of this organ. The study of certain issues of normal morphology of lymphoid and non-lymphoid thymus cells, their interrelationships, including with vascular structures, can not be described as complete nowadays. Knowledge of normal thymus morphology is a prerequisite for the objective evaluation of data obtained during model experiments.

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Short-Term Effects of Cell Phone Radiation on Fertility and Testosterone Hormone in Male Rats

Cep Telefonu Radyasyonunun Erkek Sıçanlarda İnfertilite ve Testosteron Hormonu Üzerine Kısa Dönem Etkileri

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ABSTRACT

Aim: Given the increasing usage of cell phones (6.9 billion subscriptions globally) and heterogeneous reports, this study aimed to determine the cell phone effect as non-ionizing radiation on the level of testosterone hormone and sperm parameters in male rats.
Material and Methods: Twenty-five matured male Wistar rats were randomly allocated to five groups with the same body weights. Radiofrequency radiation for the exposed groups was 1 h/day call, 2 h/day call, and 50 missed calls/day in 30 days. The other two groups were control (out of any radiation) and positive control (exposed to γ-radiation) groups. Sperm parameters (motility, morphology, viability, counting), histopathology, and serum level of testosterone were measured and analyzed.
Results: According to the results, the sperm viability significantly decreased compared to the control group ($p<0.001$). Also, the findings revealed that the sperm motility in all groups except missed call group ($p=0.475$). For sperm count and morphology only in Group C (2 h/day call) and Group D (positive control), there were significant reductions compared to the control group ($p<0.001$). The level of testosterone was not statistically significantly different between the groups ($p=0.451$).
Conclusion: This study suggests that cell phone hazard to infertility was mild to moderate, and cell phone usage might have long-term effects on infertility. However, the cell phone cannot significantly affect the serum testosterone level.
Keywords: Cell phone; infertility; semen; testosterone; histomorphometry.

ÖZ

Amaç: Cep telefonlarının artan kullanımı (küresel olarak 6,9 milyar abonelik) ve heterojen raporlar göz önüne alındığında, bu çalışmada, iyonlaştırıcı olmayan radyasyon olarak cep telefonu erkek sıçanlarda testosteron hormonu düzeyi ve sperm parametreleri üzerine etkisinin belirlenmesi amaçlanmıştır.
Gereç ve Yöntemler: Yirmi beş olgunlaşmış erkek Wistar sıçanı, aynı vücut ağırlıklarına sahip olacak şekilde rastgele olarak beş gruba ayrıldı. Maruz kalan gruplar için radyofrekans radyasyonu 30 gün içinde günde 1 saat görüşme, günde 2 saat görüşme ve günde 50 cevapsız çağrı şeklindeydi. Diğer iki grup ise kontrol (hiçbir radyasyon uygulanmayan) ve pozitif kontrol (γ-radyasyona maruz kalan) grupları idi. Sperm parametreleri (hareketlilik, morfoloji, canlılık, sayım), histopatoloji ve serum testosteron düzeyi ölçüldü ve analiz edildi.
Bulgular: Sonuçlara göre sperm canlılığı kontrol grubuna göre anlamlı olarak azaldı ($p<0,001$). Ayrıca bulgular cevapsız çağrı grubu dışındaki tüm gruplarda sperm hareketliliğinin olduğunu ortaya koydu ($p=0,475$). Sadece Grup C'de (günde 2 saat görüşme) ve Grup D'de (pozitif kontrol) sperm sayısı ve morfolojisi açısından kontrol grubuna kıyasla anlamlı şekilde azalma vardı ($p<0,001$). Testosteron düzeyi ise gruplar arasında istatistiksel olarak anlamlı şekilde farklı değildi ($p=0,451$).
Sonuç: Bu çalışma, cep telefonunun infertilite tehlikesinin hafif ila orta düzeyde olduğunu ve cep telefonu kullanımının infertilite üzerinde uzun vadeli etkileri olabileceğini düşündürmektedir. Bununla birlikte, cep telefonu serum testosteron seviyesini ise önemli ölçüde etkileyememektedir.
Anahtar kelimeler: Cep telefonu; infertilite; semen; testosteron; histomorfometri.

INTRODUCTION

Radiofrequency electromagnetic waves (RF-EMW) in optical waves in a vacuum or matter are released (1). These waves include electric and magnetic fields divided into the frequency range of radiofrequency (RF), microwave (MW), infrared, visible light, X-rays, and gamma rays (2). Cell phones are emitting RF-EMW, which between antennas and base stations, is transmitted (3). The frequency of these devices lies within the range of 450 to 3,800 megahertz (MHz), (4,5) that is part of non-ionizing radiation (NIR) (6).

According to statistics, 20-35% of males suffer infertility, a global problem (7). Reproduction in vertebrates requires coordination between the glands of the hypothalamus, pituitary, and gonads (8,9). Among the hormones that establish reproductive coordination, the luteinizing hormones (LH) with follicular stimulatory hormones (FSH) are realized by the pituitary gland (10).

FSH stimulates Sertoli cells in the spermatozoa to produce mature sperm (11). On the other hand, LH induces the synthesis of testosterone in testicular lining cells (12). Secondary marital characteristics, anabolism, and libido by testosterone are both generated, which also causes the hypothalamus-pituitary to regulate LH secretion (13).

Identification of the biological effects of cell phones due to NIR doesn't have enough energy to dislodge electrons, complex and controversial (14). Indeed, it may produce different biological effects in irradiated molecules in terms of intensity and frequency of radiation (15,16).

The increased usage of cell phones (6.9 billion subscriptions globally) and heterogeneous reports (17), which in recent years have been devastating about the damaging effects of these waves on different growth processes, have raised concerns about the harmful cell phone effects radiations on human health. This study aimed to determine the cell phone effect as NIR on the level of testosterone hormone and sperm parameters (motility, morphology, viability, counting) in male rats.

MATERIAL AND METHODS

Study Design

According to the Animal Research Reporting of in vivo Experiments (ARRIVE) guidelines checklist (18), the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (AJUMS) approved the protocol of this study under the number of IR.AJUMS.REC.1396.255. The duration of the study was 30 days.

Animals

Twenty-five mature male Wistar rats, with the same body weights, were prepared from the animal house center at AJUMS. The rats were housed in steel cages and maintained in a ventilated room at 25±3 °C, exposed to 12 hours light and 12 hours darkness. They were given free access to water and fed a commercial diet. Once acclimatized for 2 weeks, the animals were simply randomization based on a single sequence allocated to Group A (control; n=5), Group B (1 h/day call, n=5), Group C (2 h/day call, n=5), Group D (positive control, n=5), and Group E (50 missed calls/day, n=5).

The National Institutes of Health Guide conducted the investigation. The Institutional Review Board of AJUMS approved it, and every effort to minimize both the number of animals used and their suffering was made.

Sample Size

According to the animals randomized for each experimental group 'National Centre for the Replacement, Refinement, and Reduction of Animals in Research' (NC3RS)', the sample size was calculated with the formula 'Resource Equation' N=(E+T)/T (where 10≤E≤20, N: the number of animals per treated group, E: represents the degrees of freedom of the ANOVA) was chosen 5 rats for each group (19).

Exposure System

A cell phone simulation Mobile Telecom GSM signal (Bionic Mobin mobile frequency simulator, Iran) 900 MHz RF-EMW generating was used. The electric field density was set at 0.1 W/kg of the whole-body mean specific absorption rate (SAR). The single antenna of the simulation was confined and exposed to 900 MHz RF exposure emitted (Figure 1). RF radiation for exposed groups was 1 h, 2 h, and 50 missed calls in 30 days (seven days a week). The distance from each antenna to the head of the rats was 1 cm (20). 1-mm aluminum metals entirely covered the room walls for protection from possible outside telemetry exposure.

Other Groups

The control group was placed in a different room with the same temperature and condition (25±3 °C) but out of any radiation. Meanwhile, the positive control group was exposed to 6 Gy γ-radiation with a 1.9 Gy/min dose rate (Cobalt source, Elekta, England) to induce oxidative stress in the testis and cause permanent infertility in the rats (21).

Sperm Parameters

Animals were anesthetized with ketamine xylazine at the end of the experiment, also took blood directly from the heart. According to the World Health Organization (WHO) guideline (22), the cauda of the left epididymis was separated, and sperm was analyzed. Sperm motility was divided into four categories (1. fast progressive, 2. slow progressive, 3. non-progressive, 4. non-motile) in ten microscopic fields and were shown as the motility percentage in every sample. For analyzing sperm morphology used the Papanicolaou staining method. The sperm percentage with normal morphology was then determined. For identifying sperm viability, drop sperm mixed with a small eosin drop B (0.5% in saline) was set on a slide and analyzed at ×400 magnification. Live sperm does not absorb color, with the head of a dead sperm absorbing eosin and becoming red. In each fall, 100 sperms

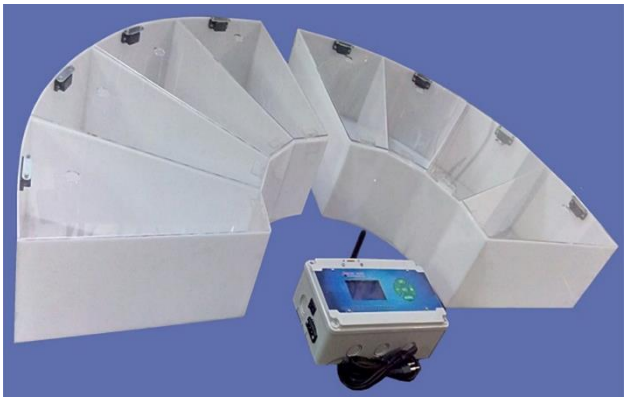

Figure 1. The single antenna of the simulation is confined in a Plexiglas carousel and exposed to 900 MHz RF

were counted, and the viable sperm percentage reported. To count sperms, used a Neubauer hemocytometer. The sperm count was calculated in one ml.

Testosterone Test

ELISA kits (monobind, USA, California) were used to measure the concentrations of serum testosterone. Intra-assay precision (precision within an assay) was used with the percent coefficient of variation (CV%) <15% for Testosterone; Inter-assay accuracy (accuracy between assays): CV% <15% for Testosterone. Finally, all homogenates were centrifuged (5,000 × g, 5 min, 4 °C), and the supernatants were stored at -80°C until measurement of Testosterone (23).

Histopathologic Analysis of the Testis

For pathologic examination, the rats' right testis was placed in 10% Bouin solution for 24 h. Following fixation, the pieces were subjected to standard histologic tissue preparation, dehydration, and paraffin embedding. With a microtome, paraffin blocks were cut to a thickness of 5 m, and the slices were stained with Hematoxylin and Eosin (H&E). They were then examined under a light microscope by the groups. The obtained images were measured in all groups equally according to the Modified Johnson scoring system (from 1 to 10), and the results were analyzed (24).

Statistical Analysis

The data showed descriptive statistics, including mean, standard deviation (SD) when a parametric test is used, and as median (interquartile range) [min-max] when using a non-parametric test. Shapiro-Wilk test used to examine normality and Levene test for homogeneity of variances. One-way ANOVA with the Tukey post hoc test or equivalent to the Kruskal-Wallis with Dunn post hoc test were used. Statistical analysis were done by Statistical Package for the Social Sciences (SPSS) v.26 software and $p<0.05$ was evaluated as statistically significant.

RESULTS

Epididymal Sperm Characteristics

Characteristics of epididymal sperm and the effects of cell phone radiation (900 MHz) on epididymal sperm were given in Table 1. According to the results, the sperm viability with 2 h/day call significantly decreased compared to the control group ($p<0.001$). However, there was no significant difference in sperm motility when compared between groups ($p=0.475$). The decrease in sperm count and morphology reductions were significant in Group C (2 h/day call) compared with the control group ($p<0.001$). Also, the weight of the left testis and left epididymis in Group B (1 h/day call) was considerably lesser than the control group ($p<0.001$).

Testosterone Levels

The testosterone levels were as follows and there was no significant difference between groups ($p=0.451$): Control group: 1.74±1.02 (2.38) [0.39-2.95], 1 h/day group: 2.33±1.41 (2.03) [0.71-4.61], 2 h/day group: 1.65±0.8 (1.68) [0.88-2.39], positive control group: 1.26±0.98 (1.64) [0.16-2.51], and 50 missed calls/day group: 1.09±0.92 (0.86) [0.23-2.44] ng/mL (Figure 2).

Histopathologic Results

Histopathological study of testes in Group A (control) showed a typical structure where seminiferous tubules were well preserved (Figure 3A). Microscopic examination of testes in Groups B (1 h/day call) and E (50 missed calls/day) revealed mild lesions (Figure 3B and 3E) while there were severe lesions in Groups D (positive control) and C (2 h/day call). They were as follows, arrest of spermatogenesis in some seminiferous tubules, interstitial edema, and undulation of basement membranes. Interstitial edema was characterized by free spaces or soft eosinophilic materials between seminiferous tubules (Figure 3D and 3C). Also, tubules were reduced and had a wavy basement membrane (Figure 3D).

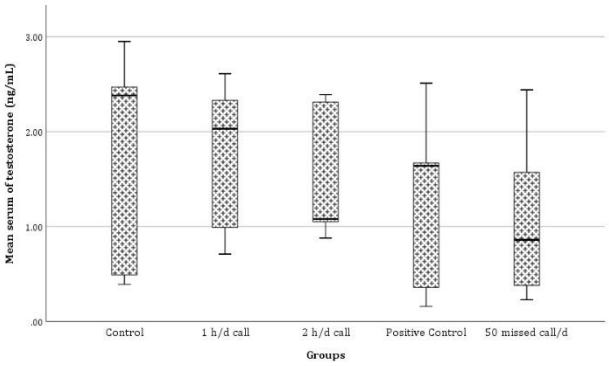

Figure 2. Serum level of testosterone in groups

Table 1. Comparison of sperm parameters between groups

	Control	1 h/day call	2 h/day call	Positive Control (γ-radiation)	50 missed calls/day	p
Sperm Viability (%)	90.52±1.56 (90) [89-93]	81.84±1.75 (82.3) [79-83.4]	69.32±3.17 (68.5) [66-74.5]	35.94±3.07 (34.5) [32.4-39.6]	82.02±1.51 (82) [80.1-83.6]	**<0.001**[*] **<0.001**[∫]
Sperm Motility (%)	52.30±3.55 (51.43) [48-56.78]	42.08±3.77 (41.5) [37.8-47.9]	37.76±4.35 (39.8) [30.23-40.98]	0.13±0.10 (0.1) [0.01-0.32]	48.14±5.63 (46.9) [42.7-57]	0.475[*] 0.812[∫]
Sperm Count ($\times 10^6$)	60.82±2.34 (61.7) [57-62.9]	58.19±6.42 (57) [50.1-68]	42.82±2.9 (43.2) [38.9-46]	0.56±0.07 (0.51) [0.43-0.67]	58.38±4.7 (59.1) [51-64]	**<0.001**[*] **<0.001**[∫]
Normal Morphology (%)	86.26±2.42 (86) [84-90]	85.91±3.17 (85) [81.3-90]	71.68±1.90 (71) [70.1-75]	23.6±3.36 (23) [20-28]	84.8±2.77 (84) [82-89]	**<0.001*** **<0.001**[∫]
Left Testis (g)	1.48±0.20 (1.45) [1.38-1.51]	1.42±0.04 (1.39) [1.35-1.46]	1.45±0.37 (1.46) [1.41-1.48]	0.92±0.07 (0.91) [0.89-0.94]	1.51±0.13 (1.52) [1.48-1.53]	**<0.001**[¥] **<0.001**[Θ]
Left Epididymis (g)	0.51±0.09 (0.52) [0.50-0.53]	0.49±0.12 (0.48) [0.45-0.50]	0.50±0.25 (0.51) [0.49-0.55]	0.36±0.01 (0.38) [0.34-0.40]	0.51±0.28 (0.53) [0.50-0.55]	**<0.001**[¥] **<0.001**[Θ]

[*]: Kruskal-Wallis test, [∫]: Dunn test, [¥]: One-Way ANOVA, [Θ]: Tukey test, descriptive statistics were given as mean±standard deviation (median) [minimum-maximum]

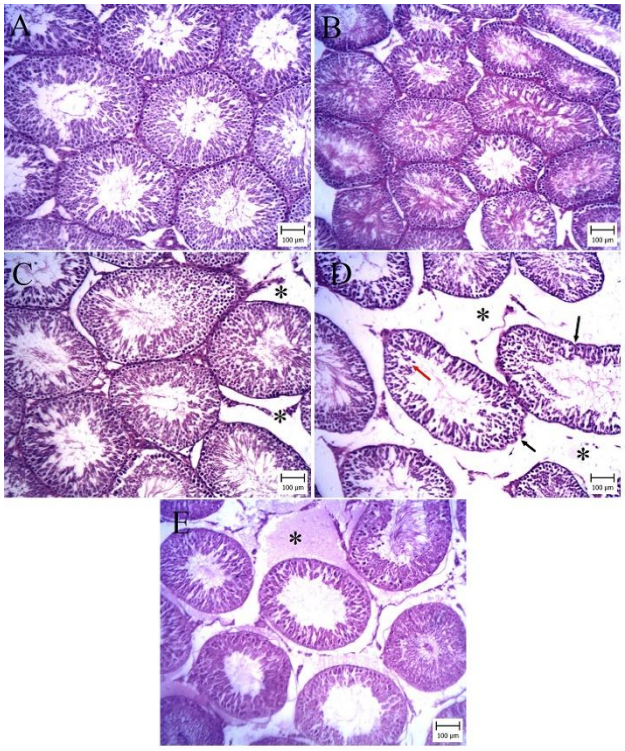

Figure 3. Photomicrograph of rat testes stained with H&E. **A)** Control, **B)** 1 h/day call, **C)** 2 h/day call, **D)** positive control, **E)** 50 missed calls/day. Note the interstitial edema (Asterisks) in (C), (D), and (E). Also, undulated basement membrane (Black arrows) and arrest of spermatogenesis (Red arrow) are seen in (D)

DISCUSSION

NIR does not have enough energy to move electrons (14). Radiation to forecast any biological effect of NIR, free radicals should be proved, oxidative stress, and DNA damage pathway. Our study indicated that cell phone radiation in the short term and sparse usage could not affect sperm motility. However, the sperm count and the average sperm viability were significantly decreased in Group C (2 h/day call) compared with the control group. Histometric indications of testicular reduced considerably than the control group, including the height of the epithelial cells of the spermatozoa, as well as the number and diameter of the Leydig cell nucleus and some of the anatomical parameters, including the size of the medium and the testicular weight in the group C (2 h/day call), and Group D (positive control).

Regarding humans studies, cell phone radiation's effects were harmful to sperm parameters (25-29). Cell phone use negatively affects sperm quality in men by reducing the semen volume (26,28), sperm count (25,27), motility, viability (25), regular morphology (25,29), and sperm DNA fragmentation could represent the only parameter significantly (26). Similarly, evidence shows that cell phone radiation can change sperm parameters (19,20,26-30). According to these studies, cell phone radiation negatively affects morphologic and histological changes (31,32). RF-EMF increased oxidative stress due to the heat and other stress-related (33), decreased gonadotropic hormonal (27), increase in apoptosis, reductions weight of the testes, negative impact on testicular architecture and enzymatic activity (34), and could negatively affect male fertility (19,20,29) by reducing sperm viability and motility (32). Consequently, our results follow other studies that considerably decreased the sperm count, the weight of the left testis and left epididymis, and the average sperm viability compared with the control group. However, Lewis RC et al. (35) and Nakatani-Enomoto S et al. (36) suggested that there was no evidence of a connection between cell phone usage and the quality of normal human spermatozoa or sperm.

Nonetheless, some studies showed no evidence for connecting cell phone use and abnormalities (37-42). Based on these studies, short-time exposure does not offer a significant risk factor for rat reproductive functions and the number of sperm in the testis and epididymis.

At the same time, evidence suggests that cell phone radiation influences infertility in men (33,43-48). These in vitro studies reported that cell phone transpiration has a harmful sperm acrosin activity, enhances mitochondrial reactive oxygen species generation (49), leads to oxidative stress (50), decreases sperm motility (33,38,40,51) and vitality (52). However, few in vitro studies show cell phones may increase safety exposure in their sample (44,45). Nevertheless, the current study showed a significant decrease in the sperm count and the average sperm viability; the mean percentage of motility was not significantly compared between groups.

Our study showed that cell phone radiation does not affect the testosterone hormone. Also, Jin YB et al. (51) suggested that RF did not affect serum levels in testosterone. However, some studies reported a decrease in the serum testosterone level with increasing the period of exposure (53-55). The most important argument for this is reducing the number and diameter of the Leydig cell nucleus and decreasing the interstitial testicular tissue as the site of synthesis and secretion of testosterone in the Leydig cell cytoplasm in the testicular tissue.

CONCLUSION

The results of this study suggest that cell phone hazard to infertility was mild to moderate, and usage of the cell phone might cause long-term effects on infertility. Also, the cell phone cannot significantly influence the serum testosterone level.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (16.05.2017, IR.AJUMS.REC.1396.255).

Conflict of Interest: None declared by the authors.

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Cotinine Effects on Peripheral Nerve Injuries: An Experimental Study

Periferik Sinir Yaralanmalarında Kotinin Etkileri: Deneysel Bir Çalışma

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ABSTRACT

Aim: Cigarette smoking is a common addictive manner and one of the greatest threats to health. Nicotine is one of the main components of cigarette. The aim of this study was to reveal the effect of cotinine which is an active metabolite of nicotine, on peripheral nerve injury in rats.

Material and Methods: We studied 42 male adult albino-Wistar rats that were divided into three groups with simple randomization method. Group 1 were given Cotinine® (C-5923-sigma) intraperitoneally, at a dose of 0.3 mg/kg/day for 21 days. Group 2 were given ethyl alcohol, the solvent of Cotinine in the same way, dose and period. Group 3 were subjected to sciatic nerve compression injury by a clip, which has a closing pressure of 50 gr/cm^2. Group 1 and 2 were subjected to the same type of injury at the end of 21 days. Four weeks later after trauma, both three groups were sacrificed and injured sciatic nerve sections are taken for histopathological analysis.

Results: It was observed that cotinine aggravated the traumatic degeneration and as privileged caused to fibrosis. In the Schwann cells of thick-myelinated fibers exhibited higher grades of degeneration and mitochondrial augmentation. According to the multiple comparison results, the number of Wallerian degenerations in the trauma group was significantly lower than in both the drug-control ($p=0.016$) and drug ($p<0.001$) groups. This situation was estimated as a response to oxidative stress.

Conclusion: This study reveals that peripheral nerve regeneration after traumatic injury may be affected negatively in smokers.

Keywords: Cigarette; nicotine; cotinine; peripheral nerve; trauma.

ÖZ

Amaç: Sigara içmek yaygın bir bağımlılık biçimidir ve sağlık için en büyük tehditlerden biridir. Nikotin, sigaranın ana bileşenlerinden biridir. Bu çalışmanın amacı, nikotinin aktif bir metaboliti olan kotininin sıçanlarda periferik sinir hasarına etkisini ortaya koymaktır.

Gereç ve Yöntemler: Basit randomizasyon metoduyla üç gruba ayrılan 42 erkek yetişkin albino-Wistar sıçan üzerinde çalıştık. Grup 1'e 21 gün boyunca 0,3 mg/kg/gün dozunda intraperitoneal olarak Cotinine® (C-5923-sigma) verildi. Grup 2'ye aynı şekilde Cotinine çözücüsü olan etil alkol, aynı doz ve sürede verildi. Grup 3, kapanma basıncı 50 gr/cm^2 olan klips ile siyatik sinir kompresyonu yaralanmasına maruz bırakıldı. Grup 1 ve 2 ise 21 gün sonunda aynı tipte yaralanmaya maruz bırakıldı. Travmadan dört hafta sonra bütün gruplar sakrifiye edildi ve hasarlı siyatik sinir kesitleri histopatolojik analiz için alındı.

Bulgular: Kotininin travmatik dejenerasyonu şiddetlendirdiği gözlenmiştir ve ayrıca kotininin özellikle fibrozun artışına neden olduğu görülmüştür. Kalın miyelinli fiberlerdeki Schwann hücrelerinde daha yüksek derecelerde dejenerasyona sebep olduğu ve ek olarak, bu hücrelerde mitokondriyal artış olduğu görüldü. Çoklu karşılaştırma sonuçlarına göre travma grubunda Wallerian dejenerasyon sayısı hem ilaç kontrol ($p=0,016$) grubu hem de ilaç grubuna ($p<0,001$) göre anlamlı derecede daha düşüktü. Bu durumun oksidatif strese bir yanıt olarak geliştiği tahmin edilmektedir.

Sonuç: Bu çalışma, sigara içenlerde travmatik yaralanma sonrası periferik sinir yenilenmesinin olumsuz şekilde etkilenebileceğini ortaya koymaktadır.

Anahtar kelimeler: Sigara; nikotin; kotinin; periferik sinir; travma.

INTRODUCTION

Precise estimation of smoking status is important for detection of smoking-attributable diseases and the effect of smoking on diseases. Also, it is crucial to authenticating the dimensions of the tobacco use, anticipating population risk, smoking-related disease burden, and interpreting the improvement of smoking cessation programs all along the world (1,2). The most commonly used tool is a standardized questionnaire. It is used to determine smoking prevalence and exposure to passive smoke. However, a standardized questionnaire might not always indicate a subject's real smoking status because of limitations. These limitations are a problem in recall the details of past smoking and having a desire to hide smoking because of social embarrassment. The questionnaire bias may increase over time. While social norms are altering, smoking has become less admissible. Moreover, a questionnaire may not be completely evaluate the passive smoke exposure of nonsmokers. The nonsmoker people who are exposed to smoke do not realize how much they are exposed to smoke. In this case, it is not possible to determine the level of exposure by a questionnaire. Also, this situation is valid for determination of smoking status of recent former smokers (3). For all these reasons, biological markers of cigarette smoking and passive exposure have been used to determinate smoking status, to monitor and track population exposure to cigarette smoke with respect to people, place, and time (4). Particularly, it has been preferred in prevalence surveys and epidemiologic studies for many years (5,6). Although the concentrations of tobacco-related components and their metabolites are used as biomarker mainly, the interaction between smoke-related chemical products and target molecules in biological materials also can be used (7). One of the most important biomarkers is nicotine and its metabolites (8,9). In humans, more than 80% of nicotine is metabolized to cotinine (10,11). Generally, nicotine is absorbed as particle-bounded or vapor phase by the body. First, nicotine is mostly metabolized by cytochrome P450 2A5 (CYP2A5) and cytochrome P450 2A6 (CYP2A6) enzymes to cotinine (10,12). Nicotine's half-time in the body is approximately 2-3 hours (13). Second, cotinine is mostly metabolized to its major metabolites, trans-3'-hydroxycotinine and its glucuronide by the liver (10,14). Only 10-15% of the total cotinine is excreted in the urine. Half-time of cotinine in the body is approximately 12-20 hours (15). This physiologically active form of cotinine tends to accumulate in the body as a result of smoking and exposure to tobacco smoke. The metabolic rate of cotinine synthesis is determined by a person's genetic background. Therefore, the rate of cotinine synthesis and accumulation in the body varies according to ethnicity (16). The studies show that body clearance of cotinine has a higher average for Caucasians than African Americans (15,16). Food consumption is also important for nicotine metabolization besides genetic factors (16). For example, grapefruit juice has been shown to prevent the production of cotinine by inhibiting the activity of CYP2A6 (17). De Leon et al. (18) showed that cotinine, the major metabolite of nicotine, is an appropriate determinant in revealing the effects of tobacco products due to its long half-life and metabolic changes.

Tissue damage by applying local compression or incision on the peripheral nerve results in increased regenerative capacity in the peripheral nervous system, which continues with the Wallerian degeneration process, in contrast to insufficient axonal regeneration in the central nervous system (Table 1). These findings have been demonstrated by the experiments conducted by Cajal (19). Similarly, this process can be detected by electron microscopic analysis. Parameters evaluated in neural tissue after trauma in electron microscope; intracytoplasmic edema, nucleus changes, mitochondrion formation, axonal changes and myelin sheath changes (19).

According to these findings, smoking habit has a negative effect on oxidative stress in addition to many harms it causes in the body. Evaluation of local oxidative stress after peripheral nerve injury is necessary to understand and improve the healing process of the nerve. In this study, we aimed to show the effect of smoking habit on peripheral nerve damage via cotinine, a metabolite of nicotine.

MATERIAL AND METHODS

Animal experiment protocol and guidelines of study was ratified by Ethical Committee of Ankara Training and Research Hospital (Decision no: 0291/2155). Animal Laboratory of the same hospital was used for all experimental procedure. 42 male adult albino-Wistar rats with body weight 180-220 gr were used. All rats had been 4-6 months old. Standardized conditions were prepared for rats. They were put in a standard laboratory cage, sufficient food and water, at 18-21 ºC. The light and dark cycle was divided equally 12 hours of light and dark (20).

Anesthesia and Surgical Procedure

The rats were divided into 3 groups as drug, drug-control and trauma with simple randomization method. It was arranged as 14 rats in each group. Cotinine® (C5923-Sigma) was administered intraperitoneally to the first group at a dose of 0.3 mg/kg/day for 21 days. Ethyl alcohol, which is the solvent of Cotinine®, was applied to the second group. Ethyl alcohol was administered intraperitoneally for 21 days in a volume equal to the amount of Cotinine given to the first group. The third group is the trauma group and only compression damage was done with the clip that was closed with a pressure of 50 gr/cm^2. Similarly, compression injury was applied to the first and second groups at the end of 21 days.

Four weeks later after trauma, both three groups were sacrificed and injured sciatic nerve sections are taken for histopathological examination. Subjects were fasted 12 hours before the surgical procedure. All subjects were weighed before the procedure. Surgical procedures were performed under general anesthesia. For this purpose, Ketamine Hydrochloride (Ketalar®, 5% solution, with Parke Davis License and Eczacıbaşı İlaç Sanayi) 50 mg/kg and Xylocaine (Rhompun®, 2% solution Bayer) 10 mg/kg were added. The mixture was given intraperitoneally (20,21). Under general anesthesia, the skin was shaved in the prone position, including the sacrum and both lower extremities, and superficial sterilization was achieved with polyvinylpyrolidone iodine (Batticon®, 10% solution, Genesis İlaç Sanayi). A longitudinal skin incision was made at the trochanter level in the proximal right lower extremity, and the skin and subcutaneous tissue were

passed through. The gluteus maximus muscle was incised vertically at the trochanter level with its fascia and dissected, and the sciatic nerve just below it was explored. The sciatic nerve was carefully dissected from the surrounding tissues, preserving it with its epineurium.

Nerve damage was created using a clip (Yaşargil FE 693 temporary aneurysm clip - Aesculap) with a compression force of 50 g/cm^2 (mean compression time 1 minute) in each rat sciatic nerve (21,22). The incision was closed anatomically after the sciatic nerve injury was created. The incision was reopened by giving general anesthesia to the rats 4 weeks after the trauma. The damaged sciatic nerve segment was exposed and samples were taken including the proximal and distal parts for histopathological examination. At the end of the experiment, the rats were sacrificed with pentobarbital.

Examination by Light and Electron Microscope

Tissue samples were divided into 1 mm^3 pieces and the tissue samples were fixed in 0.1 M phosphate buffered 2.5% glutaraldehyde (pH 7.4) for 2 hours. At the end of the fixation period, the tissues were washed 3 times with a tampon for 1 hour. And after washing, post-fixation was performed by 1% tic osmium tetraoxide active. At the end of the period, the tissues passed through graded alcohol series for dehydration. Finally, tissues that were activated by propylene oxide were blocked with the embedding material prepared with Araldite CY212 kit. Semi-thin sections were taken from the blocks polymerized for 48 hours in a 56 °C oven and dyed with toluidine blue and examined under a light microscope. Thin sections taken from the marked areas were stained with uranyl acetate-lead citrate, evaluated by Carl Zeiss EVO LS 10+ ED transmission electron microscope (TEM) and illustrated at appropriate magnifications reflecting the findings.

For assessment of histological scoring and changes of myelinated fibers, specimens were examined by light microscopy (Table 1). More than 12 microscopic fields were selected randomly from injured nerve specimens of each rat. Then, a protocol used for counting degenerated axons. The protocol consisted of starting from the first right corner of the rectangular field to the last left corner. All samples evaluation were performed by two independent histopathologists blind to the present study.

Statistical Analysis

Statistical analyses were performed by the SPSS v.26 statistical package. Normality assumption was examined using Shapiro-Wilk test. Kruskall-Wallis test was used to compare groups and Mann-Whitney U test with Bonferroni adjustment was performed for post hoc comparisons. Descriptive statistics were presented as median (interquartile range) [min-max], and a p value of 0.05 was considered statistically significant.

RESULTS

Semi Thin Section Analysis Results

In the sciatic nerve semi-thin sections that belong to the drug-control group, the epineurium and perineurium structures were observed to be normal. Schwann's cells and axonem were normal in myelinated and unmyelinated nerve fibers. While sporadic separations in the myelin sheath were determined in thick myelinated fibers, this finding was evaluated due to the physical compression that occurred during dissection. Thin fibers had normal texture (Figure 1).

In the semi-thin section evaluation of the trauma group, the epineurium and perineurium were observed with normal structure. Unlike the other groups, separation of myelin sheaths and myelin sheath duplication were noted. In addition to axon withdrawal and local thickening of myelin

Table 1. Histological grading score of nerve injury

Histology Score	Grade
0	Normal
1	Duplication of the myelin sheath
2	Undulation of the myelin sheath
3	Axonal degenerations (axonal withdrawal)
4	Nerve fibers with severe degeneration

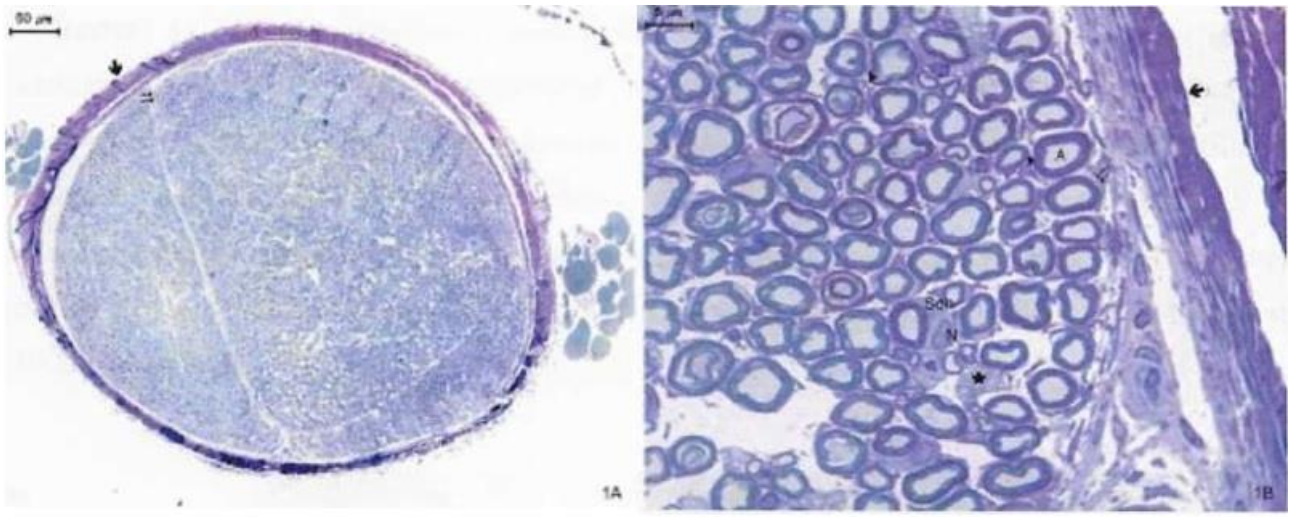

Figure 1. The epineurium (^) and perineurium (=>) structures were observed normally in the sciatic nerve semi-thin sections belonging to the drug-control group (**1A**, x100). Myelinated nerve fibers and unmyelinated fibers, Schwann cells and axonem were normal. Partial separations (>) due to physical compression of the myelin sheath were detected in some thick myelinated fibers (**1B**, x1000, Toluidine Blue).

sheath, also Schwann cell hypertrophy were prominent in the trauma group. Another striking degenerative change was Schwann cells' pyknotic nucleus structure. It was noted that Schwann cells with pyknotic nuclei were commonly observed at unmyelinated fibers. This finding was accepted as an indicator of the cells' apoptosis process. Endoneural edema was another distinguishing feature in this group.

In the drug-control group which applied trauma + ethyl alcohol (solvent of cotinine), the findings were generally similar to the trauma group. In the drug-control group, mast cell infiltration was observed in the connective tissue of the nerve. These findings were interpreted as the subjects' response to alcohol.

In the drug group which cotinine was administered, intense degenerative changes were observed in the sciatic nerve. While detachment and duplication in the myelin sheath were clearly observed, the most typical finding of this group was myelin sheath hypertrophy. It was observed that the axonemic structure disappeared in some nerve fibers. It was noteworthy that the myelin sheath ondulations and the pyknotic nucleus appearance in Schwann cells became common in this group. Nerve loss was also observed in some areas due to intense degeneration in the myelin sheath. Fibrosis and edema in endoneurium was another distinguishing feature of this group. Mast cell infiltration was evident in the nerve sheaths in this group as well (Figure 2). Distribution of Wallerian degenerations measurement in all groups was illustrated at Table 2. There was a statistically significant difference between the groups in terms of axon numbers with Wallerian degeneration ($p<0.001$). According to the multiple comparison results, the number of Wallerian degenerations in the trauma group was significantly lower than in both the drug-control ($p=0.016$) and drug ($p<0.001$) groups. Also, the difference between the drug-control and drug group was also significant ($p=0.005$). Comparison of the number of axons with Wallerian degeneration between the trauma, drug-control and drug groups was shown in Figure 3a and 3b.

Electron Microscopic Examination Results

In the electron microscopic examination of the trauma group, although small myelinated nerve fibers were observed in normal structure, various degenerative changes were observed in large fibers. Local dilatations were observed in the myelin sheaths in a group of fibers. In a group of nerve fibers, it was observed that dilatations caused myelin duplication. Myelin ondulation was detected in large fibers in patches. It was remarkable that

Table 2. Wallerian degenerations measurement in all groups

Groups	Number of Axons with Wallerian Degenerations
Trauma	21 (2.5) [18-23]
Drug-Control	25 (2.3) [22-29]
Drug (0.3 mg/kg/day)	32 (2.0) [30-35]

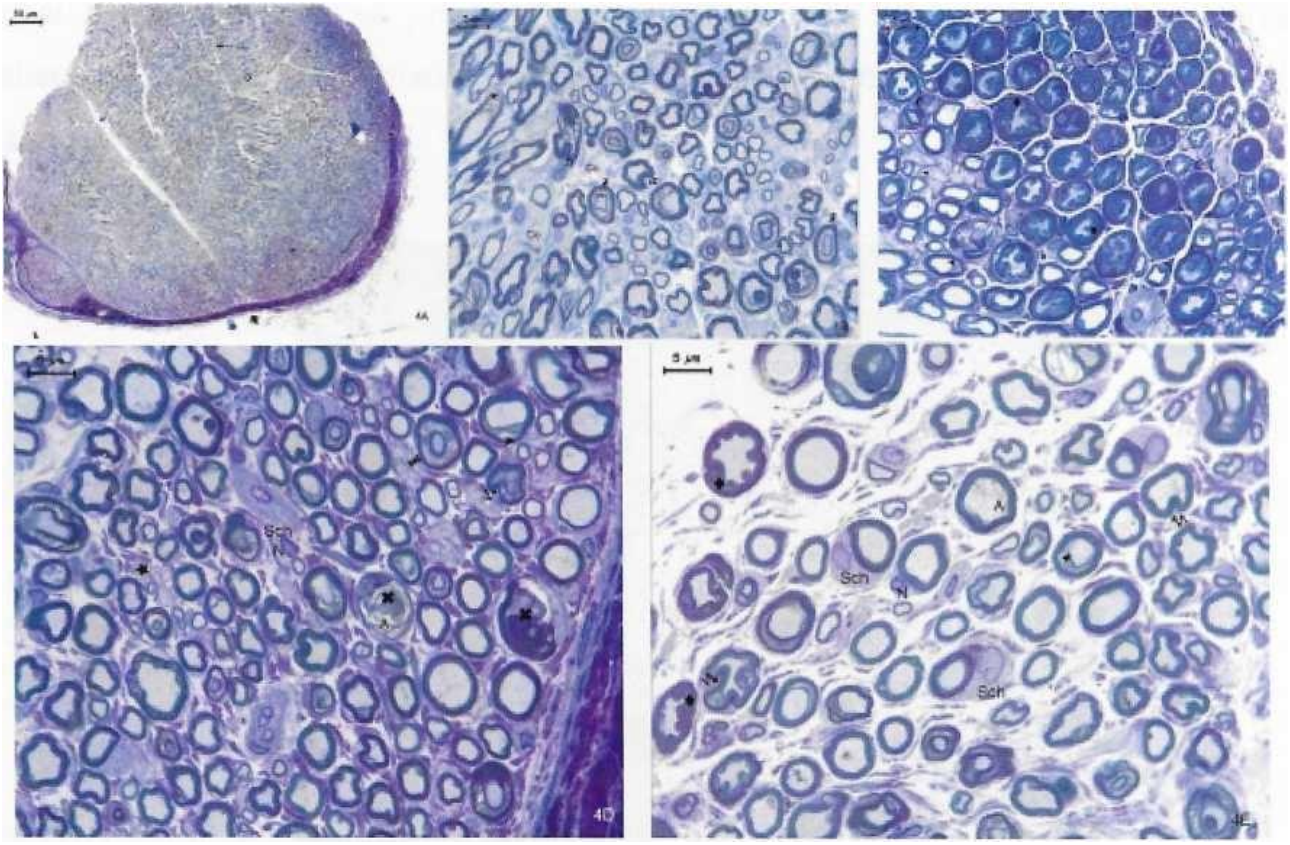

Figure 2. In the group in which cotinine was applied with trauma, epineurium (■ ♦) and perineurium (concealed, **2A** x100) in the semi-thin section evaluation with small magnification of the sciatic nerve. It is observed that the axonem (0) structure disappears in some fibers (**2B**, x1000) with thickening (*) to form corrugations in places (**2C**, x1000). (A) (**2D**, x1000), hypertrophic Schwann cells (Sch), Schwann cells with locally picnotic nucleus (N) (**2E**, x1000, Toluidine Blue).

the Schwann cells surrounding these fibers had an increase in mitochondria and their matrix was electron-dense. Nuclear degradation was observed in Schwann cells surrounding the unmyelinated fibers.

The general appearance of the sciatic nerve was observed to be normal in the electron microscopic examination of the drug-control group. It was determined that the findings were generally identical with the trauma group. Degenerative changes at different levels in the myelin sheath, mitochondrial density in Schwann cells of myelinated fibers where ondulation was observed. Also, hypertrophy in most Schwann cells were similar to the trauma group (Figure 4).

In the electron microscopic examination of the cotinine administered drug group, irregularities in the course of myelin were evident, especially in large fibers. The most prominent change in this group was axon recession, especially observed in myelinated nerve fibers. In addition, fibrosis caused by the increase in collagen fiber in the endoneurium was another distinctive finding (Figure 5).

As a result, it was observed that cotinine administration increased the degeneration caused by trauma. Also, it was shown that cotinine administration caused fibrosis exclusively. The myelinated fibers were the most affected fiber type in the sciatic nerve by trauma. The mitochondrial increase in Schwann cells observed with trauma was evaluated as the response of the cells to oxidative stress. Moreover, it was concluded that myelin sheath degeneration was the result of oxidative stress.

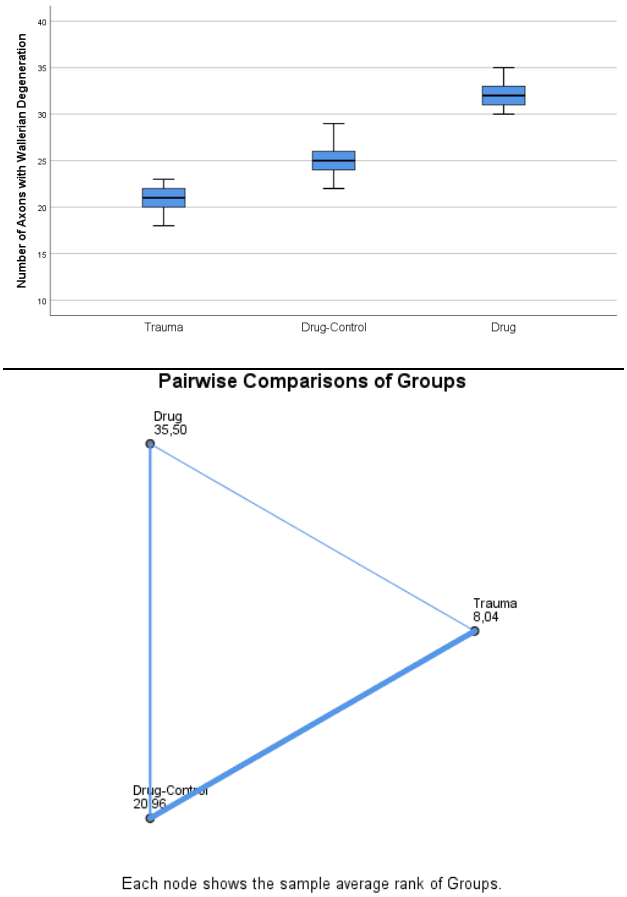

Figure 3. Number of axons with Wallerian degeneration in groups (**3A**) and multiple comparison of groups (**3B**)

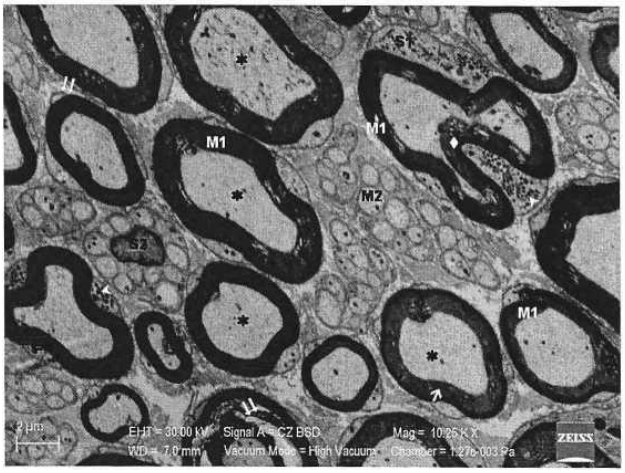

Figure 4. Drug-control group electron microscopic picture. M1: myelinated nerve fiber, M2: Unmyelinated nerve fiber, S1: Hypertrophic Schwann cell, *: axonem, ith: Duplicate separations in the myelin sheath of myelinated nerve fibers, 'f: Decrease in electron density in the inner part of myelinated nerve fibers before separation, : Undulation in myelin sheath, >: Electron-dense matrix mitochondria, Top: endoneurium where edema is observed locally (Uranyl acetate - lead citrate).

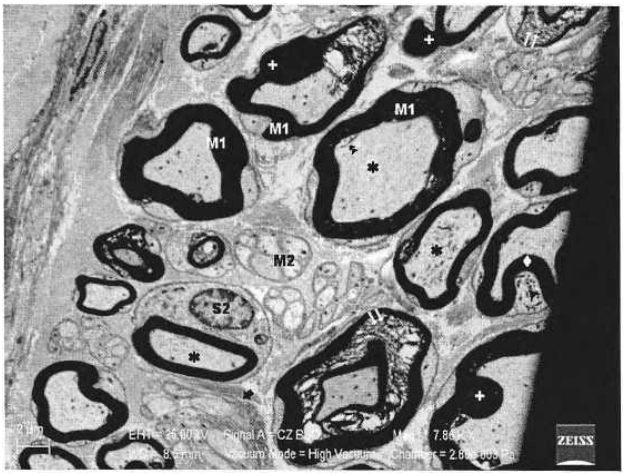

Figure 5. Electron microscopic picture of the Cotinine administered drug group. M1: myelinated nerve fiber, M2: Unmyelinated nerve fiber, S2: Schwann cell belonging to the unmyelinated nerve, *: axonem, ÎT: Separations in the myelin sheath of myelinated nerve fibers, +: Irregular course in myelin sheath, ♦: Ondulation in myelin sheath, >: Electron dense matrix mitochondria, ►►: Axon withdrawal, ♦: Fibrosis in the endonorium (Uranyl acetate - lead citrate).

DISCUSSION

The effects of nicotine on oxidative stress and neural protection differ in terms of applied dose and mechanism. This two-way effect is thought to be due to the nicotine dose exposed and the mechanism of action. Nicotine at very high doses (1/10 microM) increases lipid peroxidation, while at very low doses (10 pM) it acts as an antioxidant. It probably inhibits the Fenton reaction by binding excess iron in the environment at low doses. On the other hand, it may also reduce the formation of superoxide anions by binding NADH and disrupting the mitochondrial electrotransfer chain (22,23).

The effect of cotinine, the main metabolite of nicotine, on human metabolism has not been studied sufficiently. In humans, about 70-80% of nicotine is converted into cotinine. The half-life of cotinine is longer than nicotine and its residence time in the bloodstream is 48-96 hours. Since blood cotinine level is proportional to exposure to cigarette, cotinine levels are evaluated as an indicator of nicotine exposure (10,23).

In studies conducted to investigate the adverse effects of smoking, it has been reported that pyridine and pyrazine content cause toxic effects by inhibiting angiogenesis (24,25). Therefore, although there is information that smoking has a teratological effect by causing developmental malfunctions of various organs by inhibiting angiogenesis, no statistical information has been revealed (26,27). However, it has been reported that it affects the vascular endothelial structure in adults and so causes coronary artery disease and cerebrovascular strokes (28). On the other hand, in studies on nicotine intake during pregnancy has been reported that causes damage to brain neuron cells during the neurulation phase of embryogenesis (29).

Peripheral nerve damage is begun with compression pressure and capillary pressure's encounter. When the compression pressure on peripheral nerves is exceeded capillary perfusion pressure limits, ischemia occurs. Ischemia and injury to peripheral nerves cause microvascular damage. This vascular damage and impairment of perfusion can cause to endoneural edema and promote the increase of endoneural fluid pressure. Moreover, oxidative stress-induced cell impairment follows the ischemia (20). After the ischemia, if reperfusion is achieved, blood flow provide oxygen, but also increases the formation of free oxygen radicals and lipid peroxidation, and this process is called reperfusion injury (20,30). The greatest effects of free oxygen radicals on ischemia-reperfusion injury are on lipid peroxidation. Lipid peroxidation is a very toxic chain reaction. It is important because it is a self-progressing and destructive (30,31). Far worse than, the central and peripheral nervous systems are rich in myelin, a substance rich in lipids. This situation makes the nervous system susceptible to lipid peroxidation.

In this study, sciatic nerve trauma was performed after the administration of cotinine (0.3 mg/kg/day) at a dose equivalent to a mild smoker's blood level of cotinine for 21 days. As a result of histopathological examination, it was observed that the most affected fiber type of sciatic nerve was myelinated fibers. The mitochondrial increase in Schwann cells observed after trauma. This increase was evaluated as the response of the cells to oxidative stress. Myelin sheath degeneration concluded that as a result of the oxidative stress on these cells. It was observed that the administration of cotinine increased the degeneration caused by the trauma.

In our study, the histopathological degenerative changes caused by cotinine has been shown, in the rat peripheral nerve experimental trauma model. While small myelinated nerve fibers were observed in normal structure, degenerative changes were observed in large fibers. The number of Wallerian degenerations in the trauma group was significantly lower than in both the drug-control and drug groups. Also, the difference between the drug-control and drug group was also significant. It showed that cotinine administration increased the degeneration caused by trauma, and it was also found to cause fibrosis.

CONCLUSION

According to these results, regular daily intake of cotinine increases secondary damage related to peripheral nerve injury. Nonetheless it might induce or reduce degenerative process particularly which necessitates dose dependent further investigations. Despite the absence of concrete correlation of histological recovery and neurological findings, the present study demonstrated that cotinine might have additive effects on neurodegeneration and potentially neurodestructive which might be a critical guide to future studies in this field.

Ethics Committee Approval: The study was approved by the Ethics Committee of Ankara Training and Research Hospital (Decision no: 0291/2155).

Conflict of Interest: None declared by the authors.

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Author Contributions: Idea/Concept: RA, AD; Design: RA, AD; Data Collection/Processing: RA, AD, GT; Analysis/Interpretation: RA, AD, GT; Literature Review: RA, AD, US; Drafting/Writing: RA, AD, GT, US, EÇ; Critical Review: AD.

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Reconstruction of Vulva and Perineal Defects After Gynecological Oncological Surgery and Effectiveness of Local Flaps

Jinekolojik Onkolojik Cerrahi Sonrası Oluşan Vulva ve Perine Defektlerinin Rekonstrüksiyonu ve Lokal Fleplerin Etkinliği

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ABSTRACT

Aim: Although most defects can close by primary suturing after radical surgery of gynecological malignancies, different reconstruction options are available when large defects that require reconstruction occur. In this study, we present the treatment strategy and results for patients who underwent reconstruction after resection for gynecological cancer in the vulva and perineum.
Material and Methods: A total of 18 patients who underwent reconstruction between May 2018 and July 2020 were included in this retrospective study. Demographics and clinical data, the resection operation, characteristics of the defect, and the reconstruction methods applied were evaluated. Postoperative treatment strategy and complication rates were evaluated.
Results: The mean age was 62.3±13.2 (42-83) years. 88.9% of the patients had additional diseases. Pelvic exentration was performed in 5 (27.8%) patients, anterior resection in 2 (11.1%) patients and vulvectomy in 11 (61.1%) patients. The most common malignancy was squamous cell carcinoma, and mean defect size was 106±97 (12-476) cm^2. Reconstruction was performed with a local fasciocutaneous flap in 16 (88.9%) patients, pedicled rectus myocutaneous flap in one (5.6%) patient, and skin graft in one (5.6%) patient. Wound complications occurred in 5 (27.8%) patients, partial flap necrosis in one (5.6%) patient, and recurrence in one (5.6%) patient in the long term.
Conclusion: It is possible to reconstruction most of the vulva and perineal defects with local flaps after oncological resections, Considering the characteristics of the area and patient comorbidities, it should be kept in mind that prolonged wound problems may be seen, especially in vulvectomy patients.
Keywords: Vulvectomy; pelvic exenteration; reconstructive; neoplasm; morbidity; wounds.

ÖZ

Amaç: Jinekolojik malignitelerin radikal cerrahisi sonrası çoğu defekt primer sütüre edilerek kapatılabilse de rekonstrüksiyon gerektiren geniş defektler oluştuğunda farklı rekonstrüksiyon seçenekleri de mevcuttur. Bu çalışmada, vulva ve perine bölgesinde jinekolojik kanser nedeniyle rezeksiyon sonrası rekonstrüksiyon uygulanan hastalarda uyguladığımız tedavi strateji ve sonuçları sunulmaktadır.
Gereç ve Yöntemler: Bu geriye dönük çalışmaya Mayıs 2017 ve Temmuz 2020 arasında rekonstrüksiyon uygulanan toplam 18 hasta dahil edildi. Hastaların demografik ve klinik bilgileri rezeksiyon operasyonu, defekt özellikleri, uygulanan rekonstrüksiyon yöntemleri değerlendirildi. Operasyon sonrası tedavi stratejisi ve komplikasyon oranları değerlendirildi.
Bulgular: Hastaların ortalama yaşı 62,3±13,2 (42-83) yıl olarak saptandı. Hastaların %88,9'unda ek hastalık bulunmaktaydı. Beş (%27,8) hastada pelvik egzantasyon, 2 (%11,1) hastada anterior rezeksiyon ve 11 (%61,1) hastada vulvektomi uygulanmıştı. En sık görülen malignite skuamöz hücreli karsinom ve ortalama defekt genişliği 106±97 (12-476) cm^2 olarak saptandı. On altı (%88,9) hastada lokal fasyokutan flep, bir (%5,6) hastada pediküllü rektus myokutan flebi ve bir (%5,6) hastada deri grefti ile onarım yapıldı. Beş (%27,8) hastada yara yeri komplikasyonları, bir (%5,6) hastada parsiyel flep nekrozu ve uzun vadede bir (%5,6) hastada nüks gelişti.
Sonuç: Jinekolojik onkolojik rezeksiyonlar sonrası vulva ve perine defektlerinin birçoğunun lokal flepler ile başarılı bir şekilde kapatılması mümkündür. Özellikle vulvektomi hastalarında bölgenin özellikleri ve hasta komorbiditeleri dikkate alındığında uzamış yara yeri problemleri görülebileceği akılda bulundurulmalıdır.
Anahtar kelimeler: Vulvektomi; pelvik egzantrasyon; rekonstrüktif; neoplazm; morbidite; yara.

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INTRODUCTION

Vulva and perineum defects most commonly occur due to curative surgical resections. Less commonly, it can also be caused by trauma and necrotic soft tissue infections (1). The most common histological type in vulvar cancers is squamous cell carcinoma associated with the human papilloma virus (HPV). Although older patients are the predominant population in cancer patients, there has been an increase in the diagnosis of HPV-associated in-situ carcinomas and different histological types in younger women. Other histological tumor types affecting the vulvar region are extra-mammary Paget's disease, melanoma, sarcoma, basal cell carcinoma, adenocarcinoma, and verrucous carcinoma (2). Ablative surgery of vulvar cancers often causes extensive soft tissue defects, and rapid postoperative recovery is required to allow adjuvant therapies (3-7).

For an aesthetically and functionally successful reconstruction, adequate skin cover and well-vascularized tissue should be transferred to the defect area in such a way that no dead space is left, sufficient vaginal and urethral opening with minimal urine and stool contamination (5-9). In the literature, some algorithms for the reconstruction of these defects have been published in the past, but in practice, the management of these defects depends not only on the size and location of the defect, but also on the patient's comorbidities, adjuvant treatments (radiotherapy and chemotherapy), the stage of the disease, and the experience of the reconstructive surgeon (5-7). This study, it was aimed to present the conclusions we have obtained by examining the reconstruction methods applied based on the key points mentioned above in vulvar and perineal defects that occur after gynecological cancer surgery in our clinic.

MATERIALS AND METHODS

In the study, which was approved by the clinical research ethics committee (Istanbul Medeniyet University, 2020/0643) and planned retrospectively, the resections which is applied to the perineum of the vulva due to pelvic-vulvar gynecological malignancy by Istanbul Medeniyet University Göztepe Prof. Dr. Süleyman Yalçın City Hospital Gynecology and Obstetrics Clinic between May 2018 and July 2020 were addressed. The reconstructions performed by Plastic, Reconstructive and Aesthetic Surgery Clinic of the patients whose primary repair of the defect after resection was not possible were scanned. Patients' demographic information, clinical characteristics, comorbidities (diabetes, hypertension, smoking, etc.), previous surgeries in this region, resection surgery, defect characteristics, reconstruction methods, duration of mobilization, post-operative treatment protocol, and length of stay in hospital were evaluated. Histopathological diagnosis, recurrence rates, complications were scanned and the results were evaluated.

All patients were operated under general anesthesia and in the lithotomy position. Resection of the tumor in all patients was performed by the same gynecologist and all reconstructions were performed by the same plastic surgeon. Considering the characteristics of the defect that occurred after resection and the patient, reconstruction was performed with a local flap, distant flap, or skin graft (Figure 1. A, B).

In cases where total pelvic exenteration and anterior resection were performed, a part of the transferred flap, up to one-third of it as needed, was de-epithelized and tightly sutured to each other, and it was used to support the cavity formed in the pelvic floor due to resection and support the floor by filling the space formed in the pelvic floor due to resection (Figure 2. A-E).

After subcutaneous was closed with absorbable sutures in such a way that skin tension is minimal, vulva perineum area and mucosal junction surfaces were closed with absorbable (polyglactin) sutures. Flap donor areas in the thigh area were closed with non-absorbable (polypropylene) sutures.

One or two hemovac drains were used in all patients who underwent with flap surgery. In addition, permanent colostomy was performed by the general surgery specialist and permanent urostomy by the urology specialist in all patients who underwent exenteration. In patients who underwent anterior resection, only urostomy was performed.

In the postoperative period, immobilization was applied for 1-3 days. During the immobilization period, prophylactic anticoagulant (Fraksiparin 6000 U/day-subcutaneous) treatment was given. The first dose

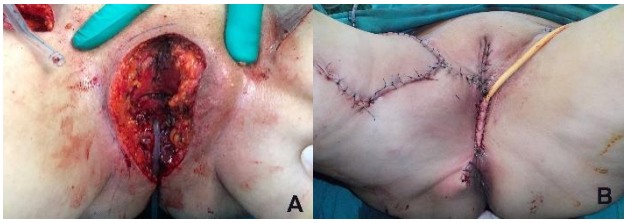

Figure 1. The patient underwent radical vulvectomy due to squamous cell carcinoma. The posterior-based fastocutaneous rotation flaps were elevated from the inner side of the right thigh to close the more defect (**A**) on the right side, and the defect was closed by detaching the existing skin on the left (**B**).

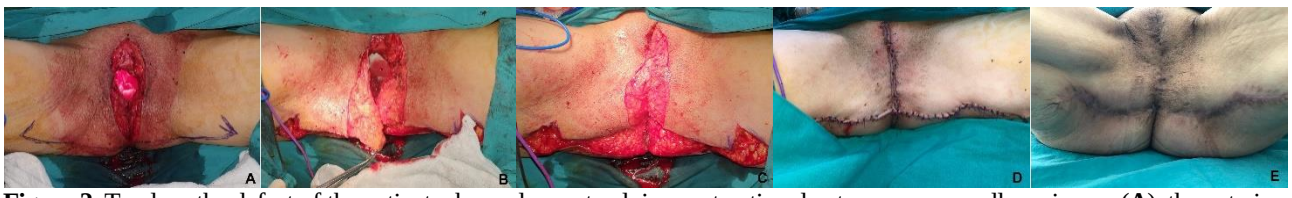

Figure 2. To close the defect of the patient who underwent pelvic exenteration due to squamous cell carcinoma (**A**), the anterior-based fastocutaneous rotation flaps were elevated from the inner thigh to close the defect, the ends were de-epithelized (**B**), the ends were used to fill the space in the pelvis (**C**), and the skin defect was closed (**D**). Postoperative 5 months view of the patient (**E**).

of cefazolin 1000 mg (intravenous) was administered peroperatively and 3 doses were administered per day for 5-7 days. Foley catheter was used for at least 5 days in all patients who did not undergo urostomy, in the post-operative period. In patients who did not undergo colostomy, a liquid diet (regimen-1) was given for 3 days and 250 mg of Lyophilized *Saccharomyces boulardii* was administered orally 3 times a day to reduce the frequency of defecation. The dressing was changed every day during the hospitalization period. In patients who underwent vulvectomy, daily vaginal tampons were used for 3 days to prevent wetting of the suture area with secretions. When the 24-hour amount of material coming from the drains fell below 25 milliliters, the drains were withdrawn. Patients were advised not to sit in a sitting position for more than a few minutes for two weeks. Non-absorbable sutures were removed after 14-21 days. Absorbable sutures were left until they dissolve spontaneously.

In the long term, recurrence rates and functional results were evaluated, and all results were visually examined. Six months after the reconstruction, all patients were asked about their satisfaction with the reconstruction.

Statistical Analysis

Statistical analysis of the data was performed by using the SPSS v.22 (IBM SPSS Statistics v.22.0) package program. Descriptive statistics were calculated as mean and standard deviation for numerical variables, and as number and percentage for categorical variables. The Student's t-test was used to compare two independent groups. A p value of less than 0.05 was considered significant.

RESULTS

In the study in which a total of 18 patients were included, the results are summarized in Table 1. The mean age of the patients was 62.3±13.2 (42-83) years. Average body mass index was observed as 29.3±4.5 (24.1-39.0) kg/m^2. Although 16 (88.9%) of the patients had comorbid diseases, 10 (55.6%) patients had more than one comorbidity. The most common accompanying disease was hypertension. Three (16.7%) patients previously had a history of malignancy in a different region. One (5.6%) patient was previously operated for vulvar malignancy in an external center and subsequently relapsed. Seven (38.9%) of the patients received neoadjuvant chemotherapy and radiotherapy, and one (5.6%) patient received only neoadjuvant chemotherapy.

Total pelvic exentration was performed in 5 (27.8%) patients, anterior resection in 2 (11.1%) patients, radical vulvectomy in 7 (38.9%) patients, partial vulvectomy in 3 (16.7%) patients and superficial vulvectomy in one (5.6%) patient. The smallest defect was 4x3 cm (12 cm^2) and the largest was 30x14 cm (420 cm^2).

For the reconstruction of the defects, fasciocutaneous rotation, transposition or V-Y advancement flaps prepared from the bilateral medial thigh in 13 (72.2%) patients, unilateral medial thigh in 3 (16.7%) patients and vertical skin island rectus abdominis myocutaneous flap in one (5.6%) patient, and partial thickness skin graft in one (5.6%) patient were applied. Bilateral superficial inguinal lymph node dissection was performed in 7 (38.9%) of the patients. The mean length of hospital stay was 21.1±5.4 (8-120) days, and the mean follow-up period was 12.3±7.3 (2-30) months.

As complications, wound infection and partial suture separation were observed in 5 (27.8%) patients. All patients with this condition were those who underwent vulvectomy and were repaired with flap. Three of them were re-operated and revised. Secondary healing was seen in other patients with wound care. In one patient who underwent vulvectomy, due to partial flap necrosis, repeated debridements and restorations were performed; however, wound healing was achieved after 4 months. While the mean body mass index of patients with wound complications was 34.8±3.5 (32.2-39.0) kg/m^2, it was observed as 26.5±1.7 (24.1-30.1) kg/m^2 in patients without complications. This difference was found to be statistically significant (p=0.001). *Enterococcus faecalis* were grown in 4 and fungal growth in 1 of the culture samples taken from the wound of patients with wound infection. According to the culture results, sensitive antibiotics were administered for 7-10 days. In one patient who underwent vulvectomy and bilateral fasciocutaneous flap was applied, local recurrence developed in the 10th month postoperatively and wide resection was performed again; the defect was reconstructed with a unilateral fasciocutaneous local flap. There was no significant difference in the complication rates of patients who received chemotherapy and/or radiotherapy and those who underwent lymph node dissection (p=0.650).

Table 1. Patient characteristics and treatment summary

Age (years)	62.3±13.2 (42-83)
Body mass index (kg/m^2)	29.3±4.5 (24.1-39.0)
Comorbidity (total 25 for 16 patients)	
Hypertension	8 (44.4%)
Hypothyroidism	4 (22.2%)
Diabetes	2 (11.1%)
Coronary artery disease	3 (16.7%)
Arrhythmias	2 (11.1%)
Smoking	3 (16.7%)
Other	3 (16.7%)
Preoperative neo-adjuvant therapy	
Radiotherapy	7 (38.9%)
Chemotherapy	6 (33.3%)
Previous cancer history (another system)	
Breast cancer	1 (5.6%)
Hodgkin lymphoma	2 (11.1%)
Resection operation performed	
Vulvectomy	11 (61.1%)
Anterior Resection	2 (11.1%)
Pelvic exentration	5 (27.8%)
Defect size (cm^2)	106±97 (12-476)
Inguinal lymph node dissection (bilateral)	7 (38.9%)
Reconstruction operation performed	
Bilateral fasciocutaneous flap	13 (72.2%)
Unilateral fasciocutaneous flap	3 (16.7%)
Rectus abdominis myocutaneous flap	1 (5.6%)
Skin grafting	1 (5.6%)
Complications	
Wound infection-detachment	5 (27.8%)
Partial flap necrosis	1 (5.6%)
Relapse	1 (5.6%)
Pathology	
Squamous cell carcinoma	11 (61.1%)
High-grade squamous intraepithelial lesion	4 (22.2%)
Paget's disease	2 (11.1%)
Malignant melanoma	1 (5.6%)

In the long term, complete wound closure was achieved within one month at the latest, except for prolonged wound closure (4 months) in one patient. No patient developed chronic urinary tract infection. Vaginal osteal stenosis did not develop in any patient. In three patients, the increased vaginal discharge regressed within 3 months and became normal. Sensory return was observed at the flap area for one year in all patients. Among the patients who underwent vulvectomy, 4 patients (under 55 years of age) who had an active sexual life stated that they returned to their sexual activities. Other patients stated that they were not sexually active. Total patient satisfaction was determined as 88.9%.

Histopathological examinations revealed that the most common malignancy was squamous cell carcinoma in 11 (61.1%) patients. High-grade squamous intraepithelial lesion (HGSIL) in 4 (22.2%) patients, vulvar Paget's disease in 2 (11.1%) patients, and malignant melanoma in 1 (5.7%) patient were observed.

Looking at the long-term complications, prolonged wound closure (4 months) was observed in one patient. In other patients, complete wound closure was achieved within one month at the latest. No patient developed chronic urinary tract infection. Vaginal osteal stenosis did not develop in any patient. Increased vaginal secretion was detected in three patients; it regressed within 3 months and reached the normal level.

DISCUSSION

Since the vulvar region has a flexible skin, many defects can be primarily closed after large resections (1,10). The number of cases requiring reconstruction in vulvar oncological resections constitutes approximately 3% of all cases (10). However, the selection of the reconstruction technique of the defect that occurs after primary non-repairable vulvar and perineal resections may vary according to the defect and many factors related to the patient. Local fasciocutaneous flaps are usually the first choice of many surgeons in the absence of extensive tissue deficiency (2,7). Successful results have even been reported by leaving primary non-closable defects to secondary healing (11). The blood circulation of the perineal area, gluteal region and medial part of the thigh is provided by a rich perforator vascular system. Due to the pudental arteries, branches of the deep femoral artery and branches of the inferor gluteal arteries, the blood supply of this area is quite well (12,13). Based on this blood flow pattern, many options have been described in perineal region defects including rotation, advancement and perforator flaps. Bilateral flaps can be performed in cases where unilateral flap is insufficient or to provide symmetry. Oily and loose donor areas allow primary closure (2,14). Considering these advantages of local flaps in our cases, we preferred the use of local flaps primarily and often.

Defects that occur after resection of malignancies resulting from irregular and uncontrolled growth in external genital structures such as vulvar cancers are also irregular. Not specific subunits; It can also affect the structures in the vulva neighborhood (7). Therefore, we think that most defects should be planned within an algorithm and not be limited to a few reconstruction options. Considering the basic principles of plastic surgery and the characteristics of adjacent tissues, we anticipate that most defects can be repaired with local flaps. In all vulvectomy patients included in the study; all defects except one patient could be closed with local flaps, and in a large case (30x14 cm) that could not be closed with local flaps, they were repaired with a partial thickness skin graft. Repair with local flaps also provides shorter operation time and better tissue compatibility (4,7,15).

In defects that occur after pelvic excentration or anterior resection, a defect that connects the abdominal cavity and the external environment must be safely closed and the dead space formed in place of the resected organs must be filled (7). In such cases, although most references suggest distant and voluminous flaps such as the epigastric region, rectus abdominis, or vastus lateralis, we used anterior-based large fasciocutaneous rotation flaps elevated from the bilateral inner thigh in 6 of these patients (7). We used the medial parts of the flaps by disepithelializing according to the need and to the extent that tension allowed, in order to close the dead space in the pelvic floor. However, since one patient had a large skin defect (20x12 cm) with a pelvic dead space, we performed the defect repair using the vertical skin island rectus abdominis myocutaneous flap, through which the pedicle passes through the abdominal area.

Malignancies are more common in older ages and accompanying diseases are also more common in older ages (16). Therefore, patients with gynecological malignancies often have accompanying diseases. This situation also increases the frequency of complications during and after surgery. 88.9% of our patients also had comorbid diseases. Although anesthesia-related complications were not observed in our patient group, wound-related complications were observed in 33.3% of all patients. Also, resections that require reconstruction are more prone to complications than primary repairable defects. Martin et al. (10) in his study; complication rates were examined in 2098 patients who were operated and reconstructed for vulvar cancer, and they found that all major and minor complication rates were 45%. In the same study, the rate of patients who do not require reconstruction was stated as 10%.

Obesity in the reconstruction of vulva and perineum defects; emerges as another disadvantage. Increased body mass index and obesity are important negative factors in the development of wound complications (11,17). In our study, body mass index was 34.8±3.5 (32.2-39.0) kg/m^2 in patients with wound complications; determination of body mass index as 26.5±1.7 (24.1-30.1) kg/m^2 in patients without wound complications supports these findings.

As is known, having received chemotherapy and/or radiotherapy before the operation slows down wound healing (11,18). However, in our patients, as expected, there was no significant difference between the patients who developed and did not develop complications, between the rates of radiotherapy and/or chemotherapy. Similarly, although it was reported that lymph node dissection increased wound-related complications, in our study, there was no significant difference in the complication rate between patients who underwent and did not undergo lymph node dissection (18).

In our opinion, the exposure of the moist labium minor or vaginal mucosa after resections involving labium majus

and the suturing of this area with the skin tissue is another factor that makes wound healing difficult. Vaginal secretions slow down wound healing and increase the rate of infection by causing maceration in the suture line (2,19). Therefore, even if we use a vaginal tampon for 3 days after surgery, it is not possible to eliminate this effect. For this reason, we think that there is inevitably prolonged wound healing in patients undergoing vulvectomy.

The limitations of our study may be that the number of patients was limited to 18 and that many different reconstruction options that were defined and reported as successful were not implemented. However, obtaining a large and single-center series will take a very difficult and long process due to the proportional low number of cases requiring reconstruction in oncological resections in the vulva region (10). We think that our local flap strategy, which we primarily apply in such cases we encounter, provides successful and high patient satisfaction.

CONCLUSIONS

After radical resection of gynecological malignancies, large defects may occur in the vulva and perineum. Reconstruction of these defects with minimal complications should be aimed; reconstruction should be planned according to the characteristics of each patient and defect. Defects in this area, local tissues are often sufficient to close the defects. Keeping the recovery period short by providing minimal morbidity should be considered, especially in advanced stage patients, as adjuvant treatments may be required. It should be kept in mind that the wound healing process may be prolonged, especially in vulvectomy cases with a high body mass index, where the vaginal mucosa and skin are sutured together, because the region is prone to maceration and infection, and the suturing of tissues with different histological features to each other.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Istanbul Medeniyet University (18.11.2020, 643).

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Clinical Course of Pregnant Women with Maternal Hydronephrosis: Retrospective Clinical Study

Maternal Hidronefrozlu Gebe Kadınların Klinik Seyri: Retrospektif Klinik Çalışma

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ABSTRACT

Aim: The study aims to investigate the clinical course of symptomatic physiological hydronephrosis in pregnant women and the results of treatment in patients required treatment.
Material and Methods: A hundred and two consecutive pregnant women who presented with clinical signs and symptoms related to the upper urinary system were included in the study. Renal ultrasonography, urinalysis, serum creatinine levels, white blood cell count, and urine culture were done in all patients at the first visit and repeated at least once a month until 1 month after delivery. In patients with acute pyelonephritis, urinalysis was repeated every 3 days until white blood cell count, erythrocyte sedimentation rate and C-reactive protein levels normalized; urine culture and kidney ultrasonography were performed monthly until 1 month after delivery. Conservative measures (positioning, analgesia, antibiotics) were used in all patients with symptomatic physiological hydronephrosis. If the patient's condition was resistant to medical treatment and the degree of hydronephrosis was increased, drainage was performed by inserting a double J stent into the ureter.
Results: Conservative treatments were successful in 98 (96.1%) of 102 patients, but 4 (3.9%) had signs and symptoms of acute pyelonephritis progressing to urosepsis. Antibiotics were continued in patients who developed pyelonephritis, symptoms regressed rapidly, signs of kidney infection returned to normal. Follow-up pregnancies ended with normal vaginal delivery.
Conclusion: Symptomatic hydronephrosis in pregnancy can be treated conservatively, should be treated carefully and patients should be followed up. However, ureteral double-J stenting is an effective and safe treatment method in patients with resistant symptoms.
Keywords: Hydronephrosis; pregnancy; renal ultrasonography.

ÖZ

Amaç: Bu çalışmanın amacı gebe kadınlarda semptomatik fizyolojik hidronefrozun klinik seyrinin ve tedavi gereken hastalarda tedavi sonuçlarının araştırılmasıdır.
Gereç ve Yöntemler: Üst üriner sistem ile ilgili klinik belirti ve semptomlarla başvuran ardışık 102 gebe kadın çalışmaya dahil edilmiştir. Tüm hastalarda ilk vizitte renal ultrasonografi, idrar tahlili, serum kreatinin düzeyi, beyaz kan hücresi sayımı ve idrar kültürü yapıldı, doğumdan 1 ay sonrasına kadar ayda en az bir kez tekrarlandı. Akut piyelonefritli hastalarda idrar tahlili, beyaz kan hücresi sayısı, eritrosit sedimantasyon hızı ve C-reaktif protein seviyeleri normalleşene kadar her 3 günde bir tekrarlandı; idrar kültürü ve böbrek ultrasonografisi doğumdan 1 ay sonrasına kadar ayda bir kez yapıldı. Semptomatik fizyolojik hidronefrozlu tüm hastalarda konservatif önlemler (pozisyon verme, analjezi, antibiyotikler) uygulandı. Hastanın durumu tıbbi tedaviye dirençli ve hidronefroz derecesinde artış var ise, üretere double J stent takılarak drenaj yapıldı.
Bulgular: Konservatif tedaviler 102 hastanın 98'inde (%96,1) başarılıydı, ancak 4'ünde (%3,9) ürosepsiye ilerleyen akut piyelonefrit belirtileri ve semptomları vardı. Pyelonefrit gelişen hastalarda antibiyotiklere devam edildi ve semptomları hızlı bir şekilde geriledi, böbrek enfeksiyonunun bulguları normale döndü. Takip edilen gebelikler normal vajinal doğum ile sonlandı.
Sonuç: Gebelikte semptomatik hidronefroz konservatif olarak tedavi edilebilir, dikkatli bir şekilde tedavi edilmeli ve hastalar takip edilmelidir. Bununla birlikte dirençli semptomları olan hastalarda ureteral double-J stent takılması etkili ve güvenli bir tedavi yöntemdir.
Anahtar kelimeler: Hidronefroz; gebelik; renal ultrasonografi.

INTRODUCTION

During pregnancy, dilatation of ureters and pelvicalyceal systems due to hormonal and mechanical reasons are commonly seen. Maternal hydronephrosis is a condition seen at rates of up to 90% in pregnancy. However, acute symptomatic dilatation of the collecting system is seen in 0.2-3% of these pregnancies (1,2). Increased urine volume, growing uterus pressure, and progesterone's myorelaxant effect on smooth muscle are the main reasons for maternal hydronephrosis during pregnancy. Maternal hydronephrosis mostly affects the right side and is seen during the second half of pregnancy and also mostly is seen in primipara patients (1,3). Gravid uterus and dilated ovarian veins, as well as hormonal factors, maybe the underlying mechanism of ureteral compression in the pregnant woman. As a result of this, enlargement of the urinary tract is often expected in pregnant women with a large uterus, such as twin pregnancies or cases of polyhydramnios (4). Most cases are asymptomatic even if some complaint of acute severe pain. In these patients, recurrent urinary tract infections and renal function deterioration may also occur. Most patients are a medical response to treatment, about 6% of the patients may be required urinary drainage such as ureteral catheterization procedures (5). It is still a controversial issue how to treat symptomatic hydronephrosis in pregnancy (6). In this study, it was aimed to evaluate retrospectively the clinical course of pregnant women with maternal hydronephrosis who were followed in our clinic.

MATERIAL AND METHODS

This retrospective study was carried out at Mersin City Training and Research Hospital. This study was approved by the Mersin University Clinical Research Ethics Committee (17.03.2021/239). 120 pregnant women with maternal hydronephrosis aged between 21 and 48 who consult our clinic between July 2017 and February 2020 were included. History of recurrent bladder infection was not considered exclusion criteria, while newly diagnosed kidney or ureteral stones and a history of kidney disease were considered exclusion criteria. For each woman, renal ultrasonography (Siemens Sonoline Adara, Erlangen, Germany) with a 3.5-MHz probe was performed. Renal ultrasonography was performed at least once until 1 month after delivery. 18 of our patients had urinary calculi, 17 were in the third trimester, and one in the second trimester. They were all excluded owing to kidney stones. In all patients with symptomatic hydronephrosis and acute pyelonephritis symptoms, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cell (WBC) count and urinalysis were tested every 3 days until their normal. Also, urine culture as well as renal ultrasonography was performed monthly until 1 month after delivery. Hydronephrosis was graded according to the maximum calyceal diameter as Zwergel et al. (7). 5-10, 10-15, and >15 mm were determined mild, moderate, and severe hydronephrosis, respectively. Urinalysis, urine culture, serum creatinine levels, WBC counts were checked. All patients were treated conservatively with analgesics and intravenous fluids, and if there were signs of infection such as fever and leukocytosis, intravenous cefuroxime and gentamicin were added to the treatment. Surgical drainage was performed in cases where conservative treatment was failed, such as signs of infection that did not improve after 48 hours, impaired renal function (i.e. increase in serum creatinine or blood urea nitrogen (BUN) values), absence of 'jet mark' on renal ultrasonography that indicating ureteral flow showing ureter obstruction, or presence of persistent pain. A 4,8 F 26 cm double-J stent (polyurethane ureteric stent; Arılar, Istanbul, Turkey) was passed under cystoscopy with the help of intravenous sedation. With this procedure, internal drainage was provided from the ureteropelvic junction to the bladder.

Statistical Analysis

SPSS v.21.0 package program was used for statistical evaluation and $p < 0.05$ was considered to be statistically significant. Descriptive statistics for continuous variables were done, and also given as mean±standard deviation, while number and percentage values were calculated for categorical variables.

RESULTS

All patients were in the second half of pregnancy and the mean gestational week was 19±3 (13-24). Sixty five (63.7%) women were in their first pregnancy and 37 (36.3%) were in their second. Ninety five (93.1%), of them have pain in one kidney and 7 (6.9%) in both kidneys while all unilateral ones were in the right kidney. Bacteriuria was found in 38 (37.3%), pyuria in 70 (68.6%) patients, while an acute urinary infection was detected in 20 (19.6%) patients in the first visit. Bacteria were seen in the urine culture, including *Escherichia coli* (%70.2), *Klebsiella pneumonia* (%13.4), *Enterococcus faecalis* (%10.4), mixed cultures (%3.4) and *Streptococcus agalctiae* (%2.6) detected. Patients' demographic and clinical data were shown in Table 1. At the same time, it was shown with renal ultrasonography that 60 (58.8%) of the 102 patients were grade I, while 34 (33.3%) of them were grade II, and 8 (7.8%) were grade III upper urinary tract dilatation (Table 1 and 2).

Conservative treatments provided pain relief in 90 (88.2%) of the patients at the end of the 5th day. After 1 month, bacteriuria was observed in 31 (30.4%) and pyuria in 22 (21.6%) of the patients' urine. In 19 (18.6%) of the patients, only recurrent flank pain was observed without any complications until delivery. In these patients, no increase in the degree of hydronephrosis was observed in renal ultrasonography until birth, and those with bacteriuria were followed up with low-dose prophylactic antimicrobial therapy. Four (3.9%) of the patients did not respond to conservative treatment, degrees of hydronephrosis increased on ultrasonography (Table 2) and showed signs and symptoms of acute pyelonephritis (fever, high WBC count, flank pain, high CRP). Calyceal kidney diameter was above 15 mm. ESR, CRP, WBC count and serum BUN (mmol/L) levels were higher than the conservative treatment group (Table 1). Although the treatments were arranged according to the urine culture results, patients did not respond to the treatment and urosepsis developed.

Later, a ureteral double-J stent was placed in these patients under local anesthesia. During the endoscopic procedure, urine drainage was observed after the double-J stent was placed. Four days after ureteral stenting, WBC count and

body temperature were normal, and in all patients, the pain was resolved. Six days later, in all 4 patients the urine was also sterile. Symptoms developed in patients after double-J stent insertion are shown in Table 3. The pregnancies of the patients progressed smoothly and 4 patients delivered vaginally at an average of 39 weeks. One month after delivery the ureteric stent was removed without any complication. At the same time renal ultrasonography was normal in all patients and renal function remained within the normal limits as measured by serum creatinine.

Table 1. Patients' demographic and clinical data

Parameter	Conservative Treatment	Double-J stent insertion cases			
		Case 1	Case 2	Case 3	Case 4
Age (years)	26±4	29	20	30	30
GA (weeks)	19±3	28	26	28	26
GA at delivery weeks	39.2±1.4	39	39	38	34
Primipara	65 (63.7%)	Primipara	Primipara	Primipara	Primipara
Hydronephrosis site		Right	Right	Right	Right
Right	89 (87.3%)				
Left	13 (12.7%)				
Hydronephrosis grade		3	2	3	3
1	60 (58.8%)				
2	34 (33.3%)				
3	8 (7.8%)				
Serum BUN (mmol/L)	2.1±0.8$^{\beta}$	9.3	2.5	10.7	3.6
Serum creatinine (mmol/dL)	0.6±0.1$^{\beta}$	1.2	0.7	1.3	0.8
WBC (x10^3/µL)	12.1±0.4	22	9	8.5	11.7
CRP level (mg/l)	4.2±2.2$^{\beta}$	23.2	14.1	11.9	16.2
ESR (mm/hr)	21.3±9.4$^{\beta}$	60	42	51	44
Hospitalization (days)	5.3±1.6	8	10	16	11

GA: gestational age, BUN: blood urea nitrogen, WBC: white blood cell count, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, $^{\beta}$: at admission

Table 2. Renal ultrasonography in 102 pregnant women with symptomatic physiologic hydronephrosis

Grade	Calyceal Kidney Diameter	At the first visit		After therapy	
		Right	Left	Right	Left
I	**5-10 mm**	52	8	22	2
II	**10-15 mm**	28	6	10	2
III	**>15 mm**	7	1	4	0

Table 3. Symptoms noticed in four pregnant women with urosepsis caused by symptomatic physiologic hydronephrosis during for a maximum of 6 days following ureteral stenting

Symptom	n*
Frequency <3 h	4
Stranguria	3
Hematuria	1
Loin pain	1

*: Four patients had more than one symptom

DISCUSSION

Physiological hydronephrosis in pregnant women may become symptomatic. Hydronephrosis that develops during pregnancy may be responsible for the progression of asymptomatic bacteriuria to symptomatic infection. The infection can progress if left untreated and may threaten the life of either the mother or the baby (8). It has been reported that hydronephrosis can give signs in the form of acute pain, as well as cause treatment-resistant urosepsis and renal failure (4). As previously reported, our study showed that urinary tract dilatation was much more common in primigravida (7). Ultrasonography with the assistance of a doppler is suitable for the evaluation of hydronephrosis grade and ureteric function. Intravenous pyelography was unnecessary in all cases. In most patients (96%), symptoms declined significantly with 3 to 5 days of medical treatment (hydration, antibiotics and analgesia), this agrees with the incidence reported in previous studies. Although Zwergel et al. (7) offered β1-adrenoreceptor blockers for acute hydronephrosis in pregnancy, we did not try on using them because of concern for the safety of the pregnant woman and her fetus. According to the literature, approximately 70-80% of pregnant women with symptomatic hydronephrosis can be treated with a conservative approach (7,8). In our study, we can attribute this rate to a high value of 96.1%, close follow-up, regular antibiotic use and patient compliance.

In patients with symptoms and signs of urosepsis, a ureteral double-J stent was placed immediately. Although this procedure has the risk of many complications such as vesicoureteral reflux, catheter migration, development of pyelonephritis and stone formation, no such complication was seen in our cases (9,10). Patients with a double-J stent had only complaints of discomfort and pain in the suprapubic region. Notably, none of the patients needed previously practiced more aggressive modalities, such as percutaneous nephrostomy (11,12). There is limited

information on the risk of premature birth in those with renal colic without urolithiasis. Ercil et al. (1) reported that preterm labor was observed in 10 of 211 patients (4.7%) with symptomatic physiological hydronephrosis. Additionally, the preterm birth rate was statistically higher in the surgical treatment group.

Although symptomatic hydronephrosis is seen very rarely in pregnant women, extreme care should be taken in these patients. Conservative measures should be taken for all patients with flank pain, bacteriuria or acute urinary infection in pregnant women. Urinary ultrasonography should be performed on these patients and they should be followed up.

CONCLUSION

Most patients with acute symptomatic hydronephrosis during pregnancy respond well to conservative treatment. Although our study included a small number of patients, its results are similar to the literature. Pregnancy hydronephrosis should be cared for and these patients should be followed closely.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of the Mersin University (17.03.2021, 239).

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Soluble ST2 in Predicting Adverse Outcome after Revascularization with Percutaneous Coronary Intervention in Patients with ST-Elevation Myocardial Infarction

ST-Yükselmeli Miyokard Enfarktüslü Hastalarda Perkütan Koroner Girişim ile Revaskülarizasyon Sonrası Advers Sonucu Öngörmede Çözünür ST2

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ABSTRACT

Aim: The aim of the study was to investigate the relationship between the soluble suppression of tumorigenicity 2 (sST2) level and the degree of epicardial blood flow recovery in patients with myocardial infarction with ST-segment elevation (STEMI) after percutaneous coronary intervention.

Material and Methods: The study involved 61 patients (83.6% males), with a mean age of 59.85±10.01 years. sST2 level was measured by enzyme immunoassay. Patients were divided into two groups. The first group (n=12) included patients with thrombolysis in myocardial infarction (TIMI) ≤II flow grade, the second group (n=49) with TIMI III flow grade.

Results: The sST2 level was significantly higher in the first hours of the disease in the group with decreased epicardial blood flow (TIMI ≤II) after percutaneous coronary intervention (p=0.003). Receiver operating characteristics curve analysis showed that sST2 levels over 34.2 ng/ml, detected on admission, was an independent predictor of adverse revascularization (TIMI ≤II) in patients with STEMI with a sensitivity of 92.3% and a specificity of 62.5%; the area under curve was 0.811 (95% CI: 0.651 - 0.873; p=0.001). Both the univariate (OR: 1.020, 95% CI: 1.001-1.041, p=0.028) and multivariate (OR: 1.030; 95% CI: 1.002-1.057; p=0.033) analyzes showed that sST2 was a significant predictor of the unfavorable outcome of epicardial vascular revascularization (TIMI ≤II).

Conclusion: sST2 is highly associated with the degree of blood flow recovery after percutaneous coronary intervention in patients with STEMI and is of great clinical importance as a prognostic marker.

Keywords: Acute myocardial infarction; sST2; no-reflow phenomenon; percutaneous coronary intervention.

ÖZ

Amaç: Bu çalışmanın amacı, ST-segment yükselmesi (STEMI) olan miyokard enfarktüslü hastalarda perkütan koroner girişim sonrası tümörijenisitenin çözünür baskılanması 2 (soluble suppression of tumorigenicity 2, sST2) seviyesi ile epikardiyal kan akımının düzelme derecesi arasındaki ilişkinin araştırılmasıdır.

Gereç ve Yöntemler: Çalışmaya ortalama yaşı 59.85±10.01 yıl olan 61 hasta (%83,6 erkek) dahil edildi. sST2 seviyesi enzim immunoassay ile ölçüldü. Hastalar iki gruba ayrıldı. Birinci grup (n=12) miyokard enfarktüsünde tromboliz (thrombolysis in myocardial infarction, TIMI) ≤II akım dereceli hastaları, ikinci grup (n=49) ise TIMI III akım dereceli hastaları içeriyordu.

Bulgular: Epikardiyal kan akımı azalmış (TIMI≤II) olan grupta perkütan koroner girişim sonrası hastalığın ilk saatlerinde sST2 düzeyi anlamlı olarak daha yüksekti (p=0.003). Alıcı işlem karakteristiği eğrisi analizi, başvuru sırasında saptanan 34,2 ng/ml'nin üzerindeki sST2 düzeylerinin, STEMI hastalarında %92,3 duyarlılık ve %62,5 özgüllük ile advers revaskülarizasyonun (TIMI ≤II) bağımsız bir ön gördürücüsü olduğunu göstermiştir; eğri altında kalan alan 0,811 (%95 GA: 0,651 - 0,873; p=0,001) idi. Hem tek değişkenli (OR: 1,020; %95 GA: 1,001-1,041; p=0.028) ve hem de çok değişkenli (OR: 1,030; %95 GA: 1,002-1,057; p=0.033) analizler sST2'nin epikardiyal vasküler revaskülarizasyonun olumsuz sonucunun (TIMI≤II) anlamlı bir ön gördürücüsü olduğunu gösterdi.

Sonuç: sST2, STEMI olan hastalarda perkütan koroner girişim sonrası kan akımındaki iyileşme derecesi ile yüksek oranda ilişkilidir ve prognostik bir belirteç olarak büyük klinik öneme sahiptir.

Anahtar kelimeler: Akut miyokard enfarktüsü; sST2; no-reflow fenomeni; perkütan koroner girişim.

INTRODUCTION

Acute coronary syndrome (ACS) continues to be among the most significant causes of death worldwide, which is clearly seen in developed countries. Despite the introduction of high-tech interventions, the use of new highly effective drugs and an increase in public awareness of the need to seek early medical care, mortality remains high. Percutaneous coronary intervention (PCI) restores blood flow in infarction-dependent artery in patients with acute myocardial infarction (AMI), making a significant contribution to the regression of disease symptoms and improving a prognosis. However, the effectiveness of stenting of subepicardial arteries is not 100% always. It is considered that the no-reflow phenomenon is among the reasons for this, which reduces the beneficial effects of PCI. The no-reflow phenomenon is defined as insufficient myocardial perfusion that persists despite a mechanically open infarction-dependent artery. The frequency of the no-reflow phenomenon varies and reaches 50%, depending on the assessment methods, especially in patients with ACS (1). There are studies that show that this phenomenon is associated with a poor prognosis, such as increased hospital and long-term mortality, and progression of heart failure (HF) (2,3) The thrombolysis in myocardial infarction (TIMI) scale is used to assess the degree of blood flow restoration in the infarction-related artery. It is of great importance to identify new sustainable markers that can predict the unfavorable course of revascularization.

Soluble suppression of tumorigenicity 2 (sST2) can be considered as the newest marker, and it is a member of the IL-1 family. It plays an important role in myocardial remodeling and inflammation processes (4). It has been shown that sST2 levels are increased in cardiovascular stress and myocardial fibrosis (5,6). sST2 competitively binds to IL-33 and blocks its positive effect on the myocardium, causing fibrosis and myocardial hypertrophy (7). There are some studies that show sST2 as an independent predictor of mortality in patients with ACS, both in the short and in the long term (8,9). The relationship between high sST2 levels and adverse cardiovascular events in patients with AMI has been established (10). Recent data suggest sST2 as a promising prognostic marker in heart failure (11). There are findings showing that sST2 in contrast to the rest of heart biomarkers is linked to cardiac remodeling irrespective of renal function (12).

Herewith, the prognostic value of sST2 in the prognosis of an unfavorable course of revascularization in patients with AMI with ST-segment elevation has not yet been sufficiently studied. In our study the relationship between sST2 levels and unfavorable outcomes of revascularization in patients with ST-elevation myocardial infarction (STEMI) after PCI was analyzed.

The purpose of the study was to find out the relationship between the level of sST2 and the degree of epicardial blood flow restoration in patients with ST-elevation myocardial infarction after PCI.

MATERIAL AND METHODS

The study involved 61 patients with AMI with ST segment elevation, among them 51 (83.6%) male and 10 (16.4%) female, with a mean age of 59.85±10.01 years. The patients were hospitalized and examined during the first hours of the disease. The adverse event was considered as coronary flow TIMI≤II after PCI.

The study was conducted at the Department of Prevention and Treatment of Emergency Conditions in Government Institution "L.T. Malaya Therapy National Institute of the National Academy of Medical Sciences of Ukraine", Kharkiv, between 2016 and 2017.

The diagnosis of AMI was established on the basis of data from clinical, electrocardiographic and biochemical studies in accordance with the European guidelines for the diagnosis and treatment of AMI with ST segment elevation, 2017 (13).

The studies were carried out in accordance with the provisions of the Declaration of Helsinki. The study protocol was approved by the Ethics and Deontology Commission (№ 12 from 21.10.2015). Each patient signed an informed consent to participate in the study.

Clinical and biochemical parameters were determined in all patients on the first day of the disease. Additionally, the level of sST2 was determined by the enzyme immunoassay using the Presage ST2 Assay reagent kit, Critical Diagnostics (USA) and NT-proBNP using the NTproBNP-ELISA-BEST kit (Russia). Immunoassay studies (sST2, NT-proBNP, troponin I) were carried out on an Immunochem-2100 enzyme immunoassay analyzer (USA), head. No. 501322057FSE. Echocardiographic examination was carried out using the Medison SonoAceX6 apparatus (Korea), end-systolic volume (ESV), end-diastolic volume (EDV) of the left ventricle (LV), LV diastolic dysfunction were assessed - the maximum early diastolic filling velocity E (m/s), maximum speed of atrial diastolic filling A (m/s), their ratio E/A and LV ejection fraction (EF) according to Simpson method.

Patients received therapy in accordance with the current recommendations of the European Society of Cardiology during the entire period of treatment (13).

Inclusion criteria in the study were: patients with AMI with ST-segment elevation, hospitalization within 24 hours after the onset of the disease, age ≥50≤75 years, signed informed consent to participate in the study.

Exclusion criteria were refused informed consent, infectious and inflammatory diseases in the acute stage, glomerular filtration rate (GFR) <60 ml/min/1.73m^2, acute liver failure, type 1 diabetes mellitus, hypertrophic cardiomyopathy, life-threatening arrhythmias, thyroid disease, severe obesity (body mass index, BMI >35 kg/m^2), arrhythmias, inability to follow the study protocol, and patient's discontinue at any stage of the study.

Sample size were calculated with help of online sample calculator http://www.raosoft.com/samplesize.html, with margin of error accepted 10%, confidence level of 95%, the size of all performed PCI in the center of 200, the recommended sample size was 66 patients.

Statistical Analysis

Statistical processing of the obtained data was carried out using the statistical software Statistica 10.0 (StatSoft Inc, USA), Microsoft Office Excel 2013. Shapiro-Wilk test was used to test normality of distribution. Indicators were presented as median (interquartile range, IQR) [min-max] in descriptive statistics, taking into account the different from the normal distribution of variables. Categorical

variables were given as percentages. Intergroup differences in qualitative characteristics were assessed using a non-parametric test, Mann-Whitney U test. The Kruskal-Wallis test was used to compare more than two independent groups. Two-sided p-value of Fisher's exact test was used for categorical variables comparison. The determination of optimal cut-off value of sST2 to distinguish the degree of blood flow restoration in the infarction-related artery was performed by the receiver operating characteristics (ROC) curve analysis. In order to find independent predictors of degree of blood flow restoration in the infarction-related artery (TIMI score) logistic regression analysis was used. Possible confounding factors were analyzed in univariate regression analysis and confounders with a p value of <0.1 were tested in multivariate logistic regression analysis. A p value of <0.05 was accepted as statistically significant.

RESULTS

The patients were divided into two groups depending on the degree of blood flow restoration in the infarction-dependent artery (TIMI): the first group (n=12) included patients with TIMI 0, I, II, the second group (n=49) with TIMI III. Table 1 presents the baseline clinical characteristics of patients with STEMI who participated in the study. As it comes from Table 1, the both groups divided by blood flow recovering were matched by all baseline parameters; sex, age, presence of arterial hypertension, diabetes mellitus type 2 and hypercholesterolemia, smoking state, BMI, kidney function and STEMI localization. Examined initial levels of biomarkers troponin I peak and NT-pro BNP were also statistically similar in the groups, in contrast to this the sST2 values differed significantly (81.39; 95% CI: 38.53-136.19 ng/mL vs 29.26; 95% CI: 22.77-52.78 ng/mL; p=0.003). Herewith, there were significant differences in percentage of patients with infarction-dependent artery: patients with TIMI III were prevalent with left main coronary artery (0% vs 49%, p=0.002), but those with TIMI ≤2 with left anterior descending artery (47.1% vs 6.1%, p=0.001). Right main coronary artery and first diagonal (D1) artery of the left anterior descending artery were involved without statistical differences in proportions, p=1.000and p=0.357, respectively.

The data presented in Table 2 evidence statistically similar of majority hemodynamic and ultrasound values in both groups. It should be marked that there was a significant difference between TIMI groups just in systolic blood pressure (SBP, p=0.029) and LVEF (p=0.041). Aside from that, the rest investigated parameters: heart rate, diastolic blood pressure (DBP), left atrium (LA), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), the ratio (E/A) of mitral peak velocity of early filling (the E wave) to mitral peak velocity of late filling (the A wave), left ventricle mass (LVM) did not demonstrated significant differences.

There were also analyzed the differences in sST2 and NT-pro BNP levels in dependence of occlusion localizations with the aim to find out the possible association between biomarker values and occlusion localizations - right main

Table 1. Baseline characteristics of patients with STEMI

	Patients with TIMI 0-II (n=12)	Patients with TIMI III (n=49)	p
Demographic parameters and risk factors			
Age (years)	62.7 (17.5) [52 - 74]	57.2 (14.0) [50 - 75]	0.104
Males, n (%)	10 (83.3%)	41 (83.7%)	1.000
Arterial hypertension, n (%)	11 (91.7%)	34 (69.4%)	0.156
Diabetes mellitus type 2, n (%)	1 (8.3%)	12 (24.5%)	0.432
Smoking, n (%)	8 (66.7%)	19 (38.8%)	0.109
Hypercholesterolemia, n (%)	8 (66.7%)	34 (69.4%)	1.000
Body mass index (kg/m^2)	29.57 (4.45) [26.30 - 34.51]	30.07 (5.91) [21.97 - 35.00]	0.863
GFR CKD-EPI (ml/min/1.73m^2)	79.13 (23.07) [61.45 - 119.82]	78.03 (31.26) [60.06 - 135.42]	0.498
STEMI localization, n (%)			
Anterior MI	7 (58.3%)	23 (46.9%)	0.534
Posterior MI	5 (41.7%)	22 (44.9%)	1.000
Other	0 (0.0%)	3 (6.1%)	1.000
Number of affected vessels, n (%)			
1 vessel	2 (16.7%)	10 (20.4%)	1.000
2 vessels	4 (33.3%)	11 (22.4%)	0.467
3 vessels	0 (0.0%)	16 (32.7%)	**0.028**
Infarction-dependent artery, n (%)			
Left main coronary artery	0 (0.0%)	24 (49.0%)	**0.002**
Left anterior descending artery	6 (50.0%)	3 (6.1%)	**0.001**
Right main coronary artery	5 (41.7%)	21 (42.9%)	1.000
D1 Left anterior descending artery	1 (8.3%)	1 (2.0%)	0.357
Biomarkers' level			
Troponin I peak (ng/ml)	32.4 (32.6) [8.8 - 51.0]	22.4 (17.8) [9.2 - 49.8]	0.446
sST2 (ng/ml)	81.39 (98.19) [20.94 - 319.12]	29.26 (30.01) [13.05 - 155.70]	**0.003**
NT-proBNP (pg/ml)	232.87 (275.51) [62.67 - 415.83]	247.54 (268.86) [55.99 - 431.67]	0.668

STEMI: ST-elevation myocardial infarction, TIMI: thrombolysis in myocardial infarction, GFR: glomerular filtration rate; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration, MI: myocardial infarction, sST2: soluble suppression of tumorigenicity 2, descriptive statistics were presented as median (interquartile range) [min-max].

Table 2. Hemodynamic and ultrasound parameters in patients with STEMI

Indices	Patients with TIMI 0-II (n=12)	Patients with TIMI III (n=49)	p
Heart rate (per min)	70 (27.5) [38 - 100]	76 (20.0) [44 - 115]	0.617
SBP (mm Hg)	145.5 (30.5) [110.0 - 175.0]	130.0 (17.5) [100.5 - 155.0]	**0.029**
DBP (mm Hg)	85.0 (15.2) [55.0 - 100.5]	83.5 (22.5) [60.0 - 110.0]	0.325
LA (cm)	4.2 (0.8) [3.0 - 5.5]	3.9 (0.8) [3.1 - 5.3]	0.322
LVEDV (ml)	136.5 (62.5) [89.0 - 196.0]	126.0 (39.0) [90.5 - 180.0]	0.480
LVESV (ml)	69.8 (35.2) [40.0 - 102.5]	65.1 (29.5) [35.0 - 104.5]	0.328
LVEF (%)	48.7 (12.0) [45.0 - 59.0]	53.5 (11.7) [45.0 - 73.5]	**0.041**
E/A	1.08 (0.70) [0.59 - 1.75]	1.22 (0.39) [0.67 - 2.39]	0.163
LVM (gr)	263 (64) [150 - 354]	239 (79) [105 - 390]	0.414

STEMI: ST-elevation myocardial infarction, TIMI: thrombolysis in myocardial infarction, SBP: systolic blood pressure, DBP: diastolic blood pressure, LA: left atrium, LVEDV: left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, LVEF: left ventricle ejection fraction, E/A: the ratio of mitral peak velocity of early filling (the E wave) to mitral peak velocity of late filling (the A wave), LVM: left ventricle mass, descriptive statistics were presented as median (interquartile range) [min-max].

coronary artery, left anterior descending artery, D1 artery of the left anterior descending artery, left main coronary artery. The Kruskal-Wallis test indicated no significant difference in the levels of the both studied biomarkers sST2 and NT-proBNP in dependence of the infarct-dependent coronary artery (p=0.687). When analyzing the level of sST2 in the studied patients, it was found that its level was significantly higher in the first hours of the disease in the group with unrecovered or decreased epicardial blood flow (TIMI≤II) after PCI (p=0.003).

The receiver operating characteristics (ROC) curve analysis was used for determination of optimal cut-off value of sST2 for the prediction revascularization degree (TIMI 0-II). During the ROC analysis, it was found that the sST2 level above 34.20 ng/ml, determined in the first hours of the disease, is suggested to be a possible biomarker for distinguishing TIMI ≤II from TIMI >II in patients with STEMI with a sensitivity of 92.3% and a specificity of 62.5%; the area under the AUC curve was 0.811 (95% CI: 0.651 - 0.873; p=0.001).

Univariate and multivariate logistic regression analyses were performed to study independent predictors of the adverse outcome of revascularization indicated as TIMI level degree. When conducting univariate regression analysis it was found that sST2 level (OR: 1.020, 95% CI: 1.001 - 1.041, p=0.028); SBP (OR: 1.083, 95% CI: 1.036 - 1.151, p=0.011); LVEDV (OR: 0.776, 95% CI: 0.511 - 0.983, p=0.039); LVESV (OR: 0.675, 95% CI: 0.351 - 0.997, p=0.042); and EF (OR: 1.011, 95% CI: 1.003 - 1.263, p=0.017) were independent confounding factors for unfavorable outcome of epicardial revascularization (TIMI 0-II). The multivariate logistic regression analysis which included confounders with a p value of <0.1 obtained from univariate logistic regression analysis indicated that sST2 remained as significant predictor of TIMI 0-II by PCI in patients with STEMI (OR: 1.030; 95% CI: 1.002 - 1.057; p=0.033) as is shown in Table 3.

Table 3. Univariate and multivariate logistic regression analysis of the influence of the studied factors on the TIMI score (≤II) in patients with STEMI after PCI

Indices	OR (95% CI)	p
Univariate logistic regression		
sST2, ng/ml	1.020 (1.001 - 1.041)	**0.028**
SBP (mm Hg)	1.083 (1.036 - 1.151)	**0.011**
DBP (mm Hg)	1.029 (0.889 - 1.076)	0.343
Gender (M/F)	2.155 (0.755 - 1.939)	0.477
Age (years)	0.989 (0.961 - 1.023)	0.949
BMI (kg/m^2)	1.058 (0.919 - 1.139)	0.280
Smoking	3.659 (0.541 - 29.187)	0.653
TC (mmol/L)	0.861 (0.519 - 1.314)	0.119
LVEDV (ml)	0.776 (0.511 - 0.983)	**0.039**
LVESV (ml)	0.675 (0.351 - 0.997)	**0.042**
LVEF (%)	1.011 (1.003 - 1.263)	**0.017**
Multivariate logistic regression		
sST2, ng/ml	1.030 (1.002 - 1.057)	**0.033**
SBP (mm Hg)	1.097 (0.991 - 1.189)	0.057
LVEDV (ml)	0.990 (0.931 - 1.062)	0.871
LVESV (ml)	0.903 (0.802 - 1.199)	0.576
LVEF (%)	1.845 (0.948 - 1.291)	0.633

STEMI: ST-elevation myocardial infarction, TIMI: thrombolysis in myocardial infarction, PCI: percutaneous coronary intervention, OR: odds ratio, CI: confidence interval, sST2: soluble suppression of tumorigenicity 2, SBP: systolic blood pressure, DBP: diastolic blood pressure, M: male, F: female, BMI: body mass index, TC: total cholesterol, LVEDV: left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, LVEF: left ventricle ejection fraction.

DISCUSSION

It can be highlighted three main findings of our study. First, the values of sST2 in STEMI patients with TIMI ≤II were significantly higher than that of TIMI >II patients. Second, sST2 is independent predictor for the development of adverse revascularization in patients with STEMI after PCI treatment. At last, high sST2 levels on admission evidence on increased risk for no-reflow development compared with initial low sST2 levels. Our study is the first attempt to identify association between sST2 and no-reflow phenomenon in PCI-administered STEMI patients. The presented results prove that sST2 level on admission is significantly associated with no-reflow phenomenon and can be used to predict it development.

Our study possibly gave additional proof that the essential chain in the process of no-reflow is inflammation. The phenomenon of no-reflow, which is defined as incomplete reperfusion at the microvascular level, despite the complete opening of the infarction-dependent artery, which may be due to tissue edema, the presence of microthrombi, accumulation of neutrophils and the formation of free radicals, which are believed to be associated with

inflammation after coronary perfusion (14). Potential risk factors for no-reflow are thought to be associated with inflammatory activity after AMI. Most likely, the no-reflow phenomenon prediction becoming rather difficult due to the complexity of its occurrence mechanism.

sST2 is one of the molecules that cardiomyocytes release when the myocardium is damaged. Recently, this biomarker has been included in the clinical guidelines for HF and is widely used and studied in patients with HF (15-17). The search for an association of sST2 with genetic polymorphisms in HF continues (18). Herewith its pathophysiological features in AMI have not been adequately studied, especially in terms of the no-reflow phenomenon. The existing data on the role of sST2 in AMI are contradictory (19,20). In the study on mice, it was shown that the IL-33/sST2 pathway induces IL-6 and IL-8 and causes systemic inflammation (21).

In his study Tuegel et al. (22) found that in patients with renal disease with higher circulating sST2 levels have increased mortality, possibly through inflammatory, fibrotic changes and heart remodelling. Thus, this result indirectly is aligned with our preposition that higher sST2 levels give negative prognosis in patients. On the other hand, Tuegel et al. (22) did not find sST2 interconnection with heart failure or atherosclerotic CVD in multivariable models.

As we found a prognostic role of admission sST2 level in low TIMI score after PCI in STEMI patients, another investigation proved this biomarker is a possible predictor of contrast-induced nephropathy (CIN) in STEMI patients (23). Now, it can be seen that sST2 became great clinical prognostic parameter for several end-points.

Findings from European multicenter randomized placebo-controlled study (PROTECT) investigating 44 novel heart biomarkers in heart failure. It verified sST2 as a strong independent predictor all-cause mortality and cardiovascular or kidney rehospitalization (24). Therefore, we consider that our results are in correspondence with these conclusions from mentioned European study, because no-reflow can be essential reason for poor prognosis (25).

The results from our study are important due to appearance of a new predictor of no-reflow phenomenon in addition to previously described ones in Fajar et al. (26) review.

Balta et al. (27) devoted their investigation to find another new predictor of no-reflow in STEMI patients, and proved that monocyte to high density lipoprotein ratio (MHR) is an independent predictor of no reflow after pPCI. The ROC analysis from that study showed that the MHR level cut-off point was 22.5 ng/ml, with a sensitivity and specificity of 70.2% and 73.3%, respectively (AUC=0.768, 95% CI: 0.725-0.811), but we showed, that sST2 with a higher sensitivity level and 11% less specificity and the closely equal AUC. Our consideration is that the both parameters can be used for no-reflow predicting, and further researches obligatory to find optimal marker or to use them simultaneously.

The purpose of our study was close to Zhao et al. (28) study. In that study they evaluated if admission fibrinogen-to-albumin ratio (FAR) values is associated with no-reflow in 510 STEMI patients. It was similar to our study, because it also specifying TIMI ≤II flow grade influence on “no-reflow”. Zhao et al. (28) showed that admission FAR can be considered as an independent predictor of no-reflow. ROC analysis elucidated the FAR cut-off value can predict no-reflow with a sensitivity of 79.59% and a specificity of 69.42%, that was shade less than our ROC results. Zhao et al. (28) showed admission FAR value is positively associated with all-cause mortality, as well as sST2 (22,24).

sST2 plays a role in the release of proinflammatory cytokines from macrophages and induces inflammation and free radical release in the acute phase of myocardial infarction and, it seems that it may be the cause of the no-reflow phenomenon in AMI patients. This is consistent from our data according to which the level of sST2 in patients with blood flow according to the TIMI ≤II scale is significantly higher than in patients with TIMI III blood flow. There is a study showing the relationship of IL-6 with the phenomenon of slow coronary flow. Thus that results also impliedly accord idea that the high levels of sST2 might lead to an increase in the frequency of the phenomenon of no-reflow (29).

The study by Sabatine et al. (30) determined sST2 in patients with STEMI who underwent coronary angiography. The follow-up period was 30 days. The sST2 value was associated with a significantly greater risk of cardiovascular death or heart failure. In our study, when conducting a logistic regression analysis, sST2 turned out to be a significant predictor of an unfavorable outcome of revascularization in patients with AMI with ST segment elevation.

The obtained results confirm the data of studies in which the role of sST2 in predicting the unfavorable course of AMI was studied. Shimpo et al. (31), investigated sST2 in patients with STEMI in the TIMI 14 and TIMI 23 studies. The baseline sST2 level was significantly higher in deceased patients and in patients with heart failure developed by 30 follow-up days. When analyzing adverse events by day 30 of follow-up, both death and the combined death/HF endpoint showed a significant association with sST2 levels. Also, in-hospital mortality and death/HF were associated with higher sST2 levels. The data we obtained correspond to the available literature data - determination of sST2 over 34.2 ng/ml makes it possible to predict an unfavorable outcome of revascularization in patients with AMI with TIMI score ≤II.

This study presents several limitations. First, the limitation of our study was the small sample size to achieve definitive conclusions. Another was that only one clinical center was involved. Also, it should be indicated that males were predominantly presented in the study. Additionally, it was a prevalence of the patients with TIMI III compared to those ones with TIMI ≤II, hence it could intend to some statistical discordances, on the other hand, we used appropriate (non-parametric) statistical methods doing our best to avoid this. We also measured just TIMI score as outcome, without considering other possible endpoints.

Further studies are required on the role of sST2 in the pathogenesis of the no-reflow phenomenon, and the search for effective methods to prevent it. The promising for further study is the investigation of the no-reflow phenomenon with the assessment of the possible relationship of myocardial perfusion on the myocardial blush grade (MBG) scale with the studied marker.

CONCLUSION

No-reflow is incomplete reperfusion after percutaneous intervention in STEMI patients resulting in adverse endpoints. We demonstrated that the sST2 level at admission is independently associated with the no-reflow phenomenon in STEMI patients after PCI. The sST2 level estimation is suggested to be important to prevent the no-reflow phenomenon development in STEMI patients. Further research is needed to understand the sST2 influence on decreased blood flow development and to prognosticate patients at high risk for poor outcomes.

Ethics Committee Approval: The study was approved by the Ethics Committee of Government Institution "L.T. Malaya Therapy National Institute of the National Academy of Medical Sciences of Ukraine" (21.10.2015, 12).

Conflict of Interest: This manuscript was produced based on the PhD thesis of Yaroslava Hilova, and all authors declare that there is no conflict of interest.

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Inverted 'Ɔ' Technique without Loss of Skin Tissue Compared to Classical Open Surgery Technique in Pediatric Pilonidal Sinus Disease

Pediyatrik Pilonidal Sinüs Hastalığında Klasik Açık Cerrahi Tekniğine Göre Deri Dokusunda Kayıp Olmaksızın Ters 'Ɔ' Tekniği

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ABSTRACT

Aim: Although the pilonidal sinus disease is commonly treated with surgical methods, search for optimal surgical procedures and minimally invasive methods continues. The aim of this study was to evaluate the inverted 'Ɔ' technique which provides a tension-free closure with minimal tissue loss, by comparing with the classical open surgery technique.
Material and Methods: Sixty-five patients, 37 (56.9%) male and 28 (43.1%) female, with a mean age of 15.85±1.82 years, admitted between January 2014 and October 2020 and underwent inverted 'Ɔ' surgery and classical midline open surgery were retrospectively evaluated.
Results: As an early postoperative complication, bleeding was observed in 2 (7.4%) patients in inverted 'Ɔ' group and 3 (11.1%) patients had wound infection. The same complications were 4 (10.5%) and 4 (10.5%) in classical group, respectively. The time to return to full activities was statistically different between two groups (10.36±0.43 versus 11.30±0.93 days, p<0.001). During the follow-up period, 1 (3.7%) recurrence in the inverted 'Ɔ' group and 9 (23.7%) recurrences in the classical group were statistically significant (p=0.037).
Conclusion: The present technique aims to remove the pathological tissue that causes sacrococcygeal pilonidal disease by the subcutaneous surgical work area under the flap created through the 'Ɔ' incision and to close it without tension, away from the midline without tissue loss. We believe that the operation involving the 'Ɔ' incision and primary suture method should be considered as the first choice in the pediatric patient group because of being a simple and effective surgical technique for sacrococcygeal pilonidal disease treatment.
Keywords: Sacrococcygeal sinus disease; children; surgery.

ÖZ

Amaç: Pilonidal sinus hastalığı genellikle cerrahi yöntemlerle tedavi edilmesine rağmen, optimal cerrahi prosedürler ve minimal invaziv yöntemler arayışı devam etmektedir. Bu çalışmanın amacı, minimal doku kaybı ile gerilimsiz bir kapatma sağlayan ters 'Ɔ' tekniğini klasik açık cerrahi tekniği ile karşılaştırarak değerlendirmektir.
Gereç ve Yöntemler: Ocak 2014 ve Ekim 2020 tarihleri arasında başvuran, ters 'Ɔ' cerrahi ve klasik orta hat açık cerrahi uygulanan, yaş ortalaması 15,85±1,82 olan 37'si (%56,9) erkek ve 28'i (%43,1) kadın 65 hasta geriye dönük olarak değerlendirildi.
Bulgular: Erken postoperatif komplikasyon olarak ters 'Ɔ' grubunda 2 (%7,4) hastada kanama görüldü ve 3 (%11,1) hastada yara enfeksiyonu vardı. Aynı komplikasyonlar klasik grupta sırasıyla 4 (%10,5) ve 4 (%10,5) idi. Tamamen normal yaşam aktivitelerine dönme süresi iki grup arasında istatistiksel olarak farklıydı (10,36±0,43'e karşı 11,30±0,93 gün, p<0.001). Takip süresi boyunca hastalarda ters 'Ɔ' grubunda 1 (%3,7) nüks, klasik grupta 9 (%23,7) nüks gelişmesi istatistiksel olarak anlamlı idi (p=0,037).
Sonuç: Mevcut teknik, sakrokoksiks pilonidal hastalığa neden olan patolojik dokuyu 'Ɔ' kesi ile oluşturulan flebin altındaki deri altı cerrahi çalışma alanı ile ortadan kaldırmayı ve orta hattan doku kaybı olmadan gerilimsiz kapatmayı amaçlamaktadır. Sakrokoksiks pilonidal hastalığın tedavisi için basit ve etkili bir cerrahi teknik olması nedeniyle pediatrik hasta grubunda 'Ɔ' insizyonu ve primer sütür yöntemini içeren operasyonun ilk seçenek olarak değerlendirilmesi gerektiğine inanıyoruz.
Anahtar kelimeler: Sakrokoksigeal sinus hastalığı; çocuk; cerrahi.

INTRODUCTION

Mayo first described sacrococcygeal pilonidal disease (SPD) in 1883, but despite the passage of time and being a common disease, the search continues for the gold standard in treatment (1). The incidence of the disease gradually increases from the age of onset to adolescence and peaks in young adulthood. Although the incidence in the pediatric patient group was written to be 1.2-2 /10000, the number of studies about the treatment in this age group is quite low (2,3).

Although its etiology is not fully understood, recently it is believed to be an acquired disease. Being overweight, excessive hairy phenotype, long sitting periods and poor hygiene conditions are the main factors. Knowing these is important for post-treatment disease management (4).

Although the disease is commonly treated with surgical methods, the search for optimal surgical procedures and minimally invasive methods continues. Despite the success of surgical methods in recurrence, the main problems are wound opening due to the location of the pathological area, delay in healing and long-term effects on normal life activities. For this reason, asymmetric incisions (5) and various flap methods (6) have been developed to remove closure incisions from the midline. However, the main problem of these incisions is tissue loss, which further increases the tension in the process area (7).

We think that the inverted 'ɑ' technique, which is a surgical technique that prevents skin tissue loss, provides a tension-free closure with minimal tissue loss. Especially in this age group, when the speed of wound healing is combined with this procedure, we aimed to evaluate this technique for the first time in the pediatric patient series in order to investigate the health of better tissue healing, less hospital stay and faster return to normal life in a less invasive manner.

MATERIAL AND METHODS

With the approval of the 2021/272 institutional review board, pediatric patients who applied to Düzce University Pediatric Surgery Clinic between January 2014 and October 2020 and underwent inverted 'ɑ' surgery (group 1) and classical midline open surgery (group 2) were retrospectively evaluated.

After the surgical procedures were explained to the children and their parents in detail, the consent of the patients and their parents was obtained in writing, including the information that hospital data could be used. Twenty-seven pediatric patients included in our series were defined as type I-V according to the classification made by Tezel (8) according to the navicular area in SPD. Before deciding on the surgical technique, ultrasound imaging was performed to determine the location, size and content of the pilonidal sinus under the skin. All patients' age, gender, medical history of the family, body mass index (BMI), duration of symptoms, preoperative treatments, complaints at the time of application, number and location of sinus pits, type of anesthesia, duration of surgery, analgesia need, return to activity, complications, and recurrence were learned from the records. In all cases included in the series, sinus excisions with the 'ɑ' incision technique were accomplished by a single surgeon. The choice of direction in the 'ɑ' incision method for SPD depends on the location of the surgeon according to the patient. When the surgeon is placed on the right side of the table, dissection can be easily performed under the flap formed by the 'ɑ' incision made on the left side of the patient. Using the visual analog scale (VAS), we evaluated the pain perceived by the children from the day of operation to the 7th day. While the mean VAS score was 1.3 on the day of operation and increased to 3.0 on the 5th postoperative day in open surgery group, the mean vas score was 1.5 on the day of operation and decreased regularly in inverted 'ɑ' group postoperatively. Also, the patients in inverted 'ɑ' group stated that they did not feel any pain in the postoperative 5th day and after. We also recorded the number of postoperative laser treatments added to the treatment to prevent recurrence of the disease.

Ultrasound Imaging

Before the surgical procedure was decided in all patients, an ultrasound image was taken to determine the size and location of the sinus in the subdermal region. Right before the operation, the sinus position was drawn on the skin under ultrasound guidance (Figure 1).

Surgical Technique

Although spinal anesthesia was recommended for pediatric patients, the choice was left to them. After the child was laid prone under anesthesia, the intergluteal fold was removed from the midline with adhesive tapes. The borders were clarified by introducing methylene blue with mild pressure into the sinus. While the classical open surgical method is used with a midline incision as the first option in pediatric patients, we have given priority to the inverted 'ɑ' technique in recent years. In this technique, taking care not to perforate the cyst, the orifis/es of sinus was cut off with a scalpel number 11 and separated from the skin. The flap, which will create a surgical work area under it, was lifted and laid in the opposite direction with a 'ɑ' incision of an average width of 4 cm and a length of 7-8 cm, far from the midline (Figure 2). The orifice was moved from the flap into the surgical area with the help of a clamp. Using electrocautery, the cyst and surrounding dirty tissue were freed from the healthy tissue. After hemostasis, local field cleaning was performed with H_2O_2 and povidone-iodine, and a negative pressure hemovac drain is placed under the skin after the operating team changed their gloves with new ones. Approximation of the subcutaneous tissue was performed using 3/0 polyglactin suture. Primary closure was performed with Polypropylene using the Allgöwer-Donati suture technique. On the second postoperative day, the drain was withdrawn and the patient was sent home. Skin stitches were removed postoperatively on the 10th day if they were clean, but on the 15th day if they were dirty.

Follow-Up

Later, the patients were discharged to come for control on the 7th postoperative day and for stitch removal on the 14th day. Later, appointments were made by the surgeon as first month, sixth month and annual controls. They were called for a final check just before this research paper was designed. Patients included in the study were followed up and controlled for at least two years by the authors themselves.

Statistical Analysis

Shapiro-Wilk test was used to examine normality assumption, and Independent samples t test was used to analyze numerical variables with normal distribution while

Mann-Whitney U test was used for numerical variables showing not normal distribution. Categorical variables were analyzed with Pearson chi-square or Fisher's exact test. Descriptive statistics were given as mean±standard deviation or median, interquartile range, minimum, maximum values according to the distribution, and categorical variables were summarized with frequency and percentage. Statistical analyses were done by SPSS v.22 statistical package and 0.05 level was considered as statistical significance level.

RESULTS

Sixty-five patients, 37 (56.9%) males and 28 (43.1%) females, with a mean age of 15.85±1.82 years, were included in the study and 15 (23.1%) of them had first-degree relatives suffering from the same disease. The patients were divided into two groups according to the surgical procedure: group 1 inverted 'ᗡ' (27 patients) and group 2 classical open surgery (38 patients) group. Disease classification was made according to the navicular area concept (8). Primary symptoms were swelling in 17 (26.2%) patients, continuous purulent drainage in 15 (23.1%) patients, and intermittent discharge in 25 (38.5%) patients for all patients, and there was no significant difference between the groups (Table 1). There was a significant difference between the two groups in terms of symptom duration (p=0.002). Five (7.7%) of the operated patients were asymptomatic and presented with the complaint of a hole in the sacral region, and all of them were in the inverted 'ᗡ' group. There was no significant difference between the groups in terms of age (p=0.124), gender (p=0.851) and BMI (p=0.442).

All SPD removal procedures were made through a 'ᗡ' incision. Thus, wound closure was performed primarily with a suture line away from the midline (Figure 3). Despite careful dissection in 2 patients with type 3 SPD, the sinus cavity was entered incidentally. In the other group, the skin tissue in the form of a fish mouth in the middle line, including the sinus orifice, was completely removed with the cyst and closed primarily in the other group.

While there was no need for analgesia in patients operated on with spinal anesthesia, 24-hour postoperative analgesia was applied to those who were given general anesthesia. As an early postoperative complication, in the first group, 2 (7.4%) patients had minimal bleeding after the drain was removed, and 3 (11.1%) patients had wound infection that resolved with antibiotic treatment. The same complications were 4 (10.5%) and 4 (10.5%) in the second group, respectively. While the mean length of stay in the hospital was 2.24±0.34 days in the first group, it took 4.03±0.34 days to return to normal activities. The same periods were 2.41±0.44 days of hospitalization and 4.16±0.39 days of returning to normal activities in the second group (Table 2). The time for our patients to return to full activities was statistically different between the two groups: 10.36±0.43 versus 11.30±0.93 days (p<0.001). During the follow-up period, 1 (3.7%) recurrence in the inverted 'ᗡ' group and 9 (23.7%) recurrences in the classical group developed in our patients (p=0.037).

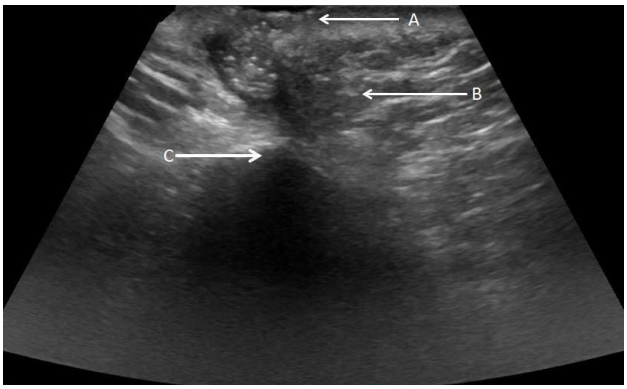

Figure 1. Preoperative ultrasonographic image of sacrococcygeal pilonidal disease

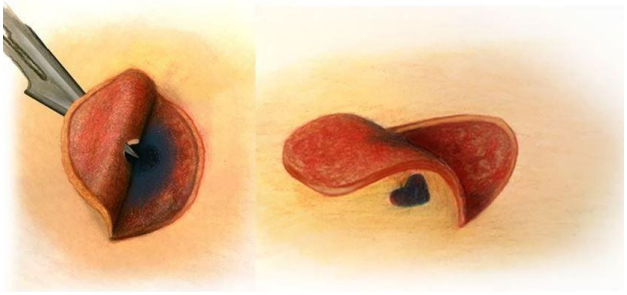

Figure 2. Schematic images of inverted 'ᗡ' flap; **A)** freeing the sinus offices with a No.11 scalpel and view of the surgical work area under the flap; **B)** top view of the flap

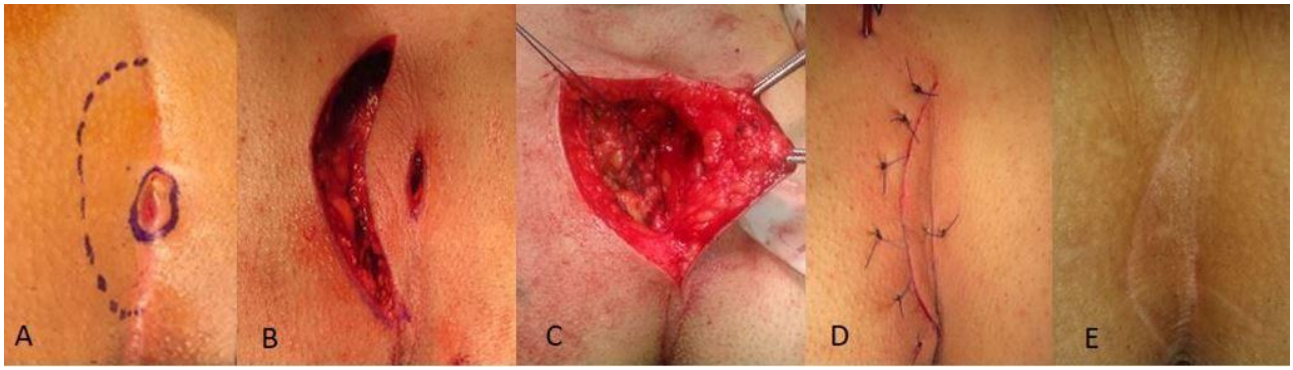

Figure 3. Surgery pictures of sacrococcygeal pilonidal disease; **A)** marking the incision; **B)** deepening the incision towards the presacral area; **C)** Surgical workspace under the flap; **D)** Primary closure without tissue loss; **E)** healed view of the incision

Table 1. Demographic information and SPD types of patients

	Inverted 'ɑ' (n=27)	Open Surgery (n=38)	p
Gender, n (%)			
Female	12 (44.4)	16 (42.1)	0.851
Male	15 (55.6)	22 (57.9)	
Age (years), mean±SD (min-max)	16.26±1.51 (13-18)	15.55±1.98 (11-18)	0.124
Body mass index, mean±SD (min-max)	28.41±3.11 (22.9-35.5)	29.02±3.10 (22.6-35.5)	0.442
Family history of SPD, n (%)	6 (22.2)	9 (23.7)	0.890
Duration of symptoms (month), median (IQR) (min-max)	5 (3) [2-24]	14.5 (14) [2-36]	**0.002**
Tezel classification, n (%)			
I	5 (18.5)	10 (26.3)	0.948
II	12 (44.4)	16 (42.1)	
III	7 (25.9)	8 (21.1)	
IV	0 (0.0)	0 (0.0)	
V	3 (11.1)	4 (10.5)	

SPD: sacrococcygeal pilonidal disease, SD: standard deviation, IQR: interquartile range

Table 2. Postoperative data in patients undergoing inverted 'ɑ' technique and long-term follow-up

	Inverted 'ɑ' (n=27)	Open Surgery (n=38)	p
Type of anesthesia, n (%)			
Spinal block	16 (59.3%)	21 (55.3)	0.749
General	11 (40.7%)	17 (44.7)	
Operation time (minute), mean±SD, (min-max)	36.81±3.82 (32-45)	37.05±4.05 (30-45)	0.812
Need for analgesia (hour), mean±SD (min-max)	23.89±3.15 (18-36)	24.87±3.78 (18-36)	0.275
Hospital stay (day), mean±SD (min-max)	2.24±0.34 (1.8-2.8)	2.41±0.44 (1.8-3.8)	0.086
Time to return to normal activity (day), mean±SD (min-max)	4.03±0.34 (3.6-5.0)	4.16±0.39 (3.6-5.0)	0.171
Time to return to full activity (day), mean±SD (min-max)	10.36±0.43 (9.6-11.0)	11.30±0.93 (9.8-12.8)	**<0.001**
Follow-up time (year), mean±SD (min-max)	2.66±0.49 (2.0-3.6)	2.96±0.32 (2.2-3.6)	**0.004**
Complications, n (%)			
Wound infection	3 (11.1%)	4 (10.5)	0.912
Bleeding	2 (7.4%)	4 (10.5)	
Recurrence, n (%)	1 (3.7%)	9 (23.7%)	**0.037**

SD: standard deviation

DISCUSSION

Although it may appear in other regions of the body, the pilonidal sinus is most commonly seen in the intergluteal sulcus in the midline sacrococcygeal zone. SPD mostly affects young adults after puberty and occurs predominantly in males of all age groups (9,10). In Turkey, in parallel with social security regulations, pediatric surgeons are more likely to encounter SPD, which peaks at the age of 16-25 but starts to appear at the age of 14-15, due to the fact that the pediatric age group is raised to 18 years of age. While it was known that pediatric surgeons rarely operated on pilonidal sinus disease cases in the previous years due to the low number of complicated cases in adolescence and early adolescence, in recent years, due to the interest of pediatric surgeons in this age group, cases operated in Turkey have been increasingly reported (11).

Although a lot of medical and surgical treatment procedures have been defined to heal pilonidal sinus, none of them could eliminate the risk of recurrence (10). These treatments may cause restrictions in the postoperative period, to a greater extent than the problems caused by the disease itself (12). It is generally accepted that the treatment of choice for SPD is less invasive and should provide rapid wound healing, easy postoperative care, short hospitalization, short return to normal life and a low recurrence ratio, but the gold standard has not yet been decided (13,14).

Although non-surgical methods are tried to be introduced to the literature, the most preferred treatment for SPD is surgery (15). Although complex and difficult flap techniques are used in complicated cases, less invasive techniques should be preferred instead of these methods in the treatment of primary disease: Excision and laying open of the sinus tract, narrow or wide and deep excision to the sacrum with primary closure, incision and marsupialization, asymmetrical incisions. In techniques of open surgery, the excision is left open with the expectation of secondary recovery, which means long-term treatment and late return to normal life. To overcome this disadvantage of leaving the wound open, various methods of primary closure in SPD surgical treatment have been reported (16,17).

The most difficult complication after primary closure surgery for SPD is a midline wound that does not heal permanently after excision or opens due to tension. With the previously developed asymmetric incisions, it was aimed to remove the primary closure away from the

midline and thus increase the healing rate and reduce the recurrence rate, but the skin loss in the incision could not bring the success rate to the desired level and caused complaints such as incision opening due to tension or discomfort in normal life.

A D-shape incision was developed to reduce these complications of primary closure. After the cyst borders are determined with methylene blue, the sinus holes are freed from the skin with an 11 scalpel and an asymmetrical excision is made with an average 8 cm long and 4 cm wide incision down to the presacral fascia with a 'ᗡ' shaped incision. The pilonidal sinus and surrounding tissue are totally removed by excision made lateral to the gluteal fascia and periosteum. After the surgical area is washed with hydrogen peroxide and povidone iodine, it is appropriate to place the aspiration drain into the cavity.

In the early period, no complications were encountered except minimal bleeding after removal of the hemovac drain in 2 patients and wound infection treated with antibiotics in 3 patients. While the complication rate was similar in the classical group, there was a significant difference in the number of recurrences. Despite more than 2 years of follow-up, just one of our inverted 'ᗡ' cases recurred. Compared to the literature, these rates are close to perfect (18). We think that the reason for the low recurrence rate is that it provides the opportunity to work comfortably in the large surgical area that occurs after flap removal and thus, the sinus and the surrounding dirty tissues can be removed completely. While providing this, we would like to add that the methylene blue we use in marking and moving away from the midline in the incision closure has an effect. Our recommendations for reducing the risk of recurrence for all of our patients have, of course, been; such as frequent showering, antiperspirant underwear, shorter sitting intervals and epilation.

The primary purpose of the inverted 'ᗡ' technique, which is the subject of the article, is to ensure the surgical wound is closed first without causing tension in the suture line with minimal tissue loss in the flap after sinus excision. The second goal was to place this closing line away from the midline. A surgical defect that can be closed with minimal strain after excision or other procedures is definitely the target of all surgeons as it will facilitate wound healing. In SPD, it is recommended to move this closure suture line away from the midline to prevent both wound healing and recurrence (19). The location of the surgeon relative to the inverted 'ᗡ' incision facilitates tissue dissection in the area under the flap. In our study, while removing the cysts, only the pit(s) were freed from the skin causing no tissue loss in the flap of the 'ᗡ' incision, which the surgical working area was located under. This enabled us to primary closure of the surgical wound with the least possible tension. In a population of pediatric patients undergoing a similar study, a trephine device was used to perform extensive resection with minimally invasive surgery and the wound was left open without sutures (20). Nevertheless, although this surgery was minimally invasive, it had high rates of reoperation (28%), and the cost of the authors to widen the excision to reduce the reoperation rate (9%) was both long 0 days (14 days) and a long transition time to full activity (45 days). In our series, we found significantly lower bleeding and infection rates, shorter recovery, and transition to full activity.

Karydakis (21) demonstrated his own method to improve wound healing and reduce the recurrence rate and found a reoperation rate of less than 1 percent but the authors who followed him could not achieve the same rate, researchers such as Bascom (22) and Brusciano et al. (19) tried to develop the method. The common point of these authors was that they aimed to remove the closure wound from the midline, but in order to completely remove the pathological tissue that caused the disease, it always removed together with the skin in the upper navicular area, causing a tense closure. Likewise, in all flap methods developed for the treatment of the disease, there was a loss in the skin tissue in the surgical area, which caused tensile closure of the wound. Doğan and his colleagues (23) developed a method in which the cyst can be removed completely without any skin loss and published it with a high success rate (recurrence rate 1.3%).

In our series, which is the first publication in the literature regarding the D-shaped incision in a series of pediatric patients, we think that the reason for the close-ratio of boys and girls to each other, contrary to the literature, is the earlier onset of the SPD process with the early start of puberty in girls.

We included patients who were followed for at least 2 years to evaluate the complication rates clearly. While initially following the asymptomatic cases in Group 1 with recommendations in accordance with the literature (24), we started to recommend surgery as the first option in this patient group as all these cases evolved into Groups 2 and 3 in the process.

CONCLUSION

In conclusion, the present technique aims to remove the pathological tissue that causes SPD by the subcutaneous surgical work area under the flap created through the 'ᗡ' incision and to close it without tension, away from the midline without tissue loss. Off the midline and tension-free primary wound closure provides better cosmetic recovery, less suffering, and an earlier return to the routine quality of life. The recurrence rate after subcutaneous sinus excision is almost perfect in pediatric patients with proper evaluation and compliance with surgical rules. We believe that the operation involving the 'ᗡ' incision and primary suture method should be considered as the first choice in the pediatric patient group because of being a simple and effective surgical technique for SPD treatment.

Ethics Committee Approval: The study was approved by the Ethics Committee of Düzce University Faculty of Medicine (04.01.2021, 272).

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Induction of Autophagic Cell Death by Thymoquinone in Docetaxel Resistant Prostate Cancer Cells

Dosetaksel Dirençli Prostat Kanseri Hücrelerinde Timokinon Tarafından Otofajik Hücre Ölümünün İndüklenmesi

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ABSTRACT

Aim: Acquired docetaxel (DOC) resistance of prostate cancer (PCa) is still a clinical problem. In addition to failure in chemotherapy treatment, it causes tumor recurrence. Therefore, novel and more effective compounds are needed in DOC-resistant PCa treatment. This study aimed to investigate the possible cytotoxic and cell death-inducing activities of thymoquinone (TQ), one of the main active components of *Nigella sativa* L., on DOC-resistant prostate cancer cells.
Material and Methods: DOC-resistant PC3 cells (DOC-R/PC3) were developed by the continuous culture with increment concentrations of DOC (1-10 nM) until they improved their growth and division abilities. The cell viability was determined by MTT assay. The Muse™ Annexin V & Dead Cell kit was performed to detect apoptotic cell death. Autophagic vacuoles were observed by staining autophagic vacuoles. The levels of LC3I, LC3II and Beclin-1 proteins were investigated via western blot analysis.
Results: TQ inhibited the viability of DOC-R/PC3 cells in a dose- and time-dependent manner ($p=0.014$). The IC50 value of TQ for DOC-R/PC3 cells was calculated as 60 µM at 72 h. Treatment of TQ did not induce apoptotic cell death in DOC-resistant prostate cancer cells but induced the formation of autophagic vacuoles. Moreover, Beclin-1 and LC3-II protein levels were increased in TQ-treated DOC-R/PC3 cells, however, LC3-I levels were decreased in DOC-R/PC3 cells.
Conclusion: All these results show that TQ may become a new therapeutic target for DOC-resistant prostate cancer in the future.
Keywords: Autophagy; Beclin-1; LC3; prostate cancer; resistance; thymoquinone.

ÖZ

Amaç: Prostat kanserinde (prostate cancer, PCa) edinilen dosetaksel (docetaxel, DOC) direnci hala klinik bir sorundur. Kemoterapi tedavisinde başarısızlığa ek olarak tümör nüksüne neden olmaktadır. Bu nedenle, DOC'a dirençli PCa tedavisinde yeni ve daha etkili bileşiklere ihtiyaç duyulmaktadır. Bu çalışmanın amacı, *Nigella sativa* L. bitkisinin etken bileşenlerinden biri olan timokinon (thymoquinone, TQ)'un, DOC dirençli prostat kanseri hücreleri üzerindeki olası sitotoksik ve hücre ölümünü tetikleyici aktivitelerinin araştırılmasıdır.
Gereç ve Yöntemler: DOC dirençli PC3 hücreleri (DOC-R/PC3), büyüme ve bölünme yeteneklerini geliştirene kadar artan DOC (1-10 nM) konsantrasyonlarında devamlı kültürle çoğaltıldı. Hücre canlılığı, MTT yöntemi kullanılarak belirlendi. Muse™ Annexin V & Dead Cell kiti, apoptotik hücre ölümünün tespiti için kullanıldı. Otofajik vakuoller spesifik boya kullanılarak gösterildi. TQ mualemesi sonucu LC3-I, LC3-II ve Beclin-1 protein düzeylerindeki değişiklikler western blot analizi ile araştırıldı.
Bulgular: TQ muamelesi, DOC-R/PC3 hücrelerinin canlılığını doza ve zamana bağlı olarak inhibe etti ($p=0.014$). DOC-R/PC3 için TQ'nun IC50 değeri 72. saatte 60 µM olarak hesaplandı. TQ uygulaması, DOC dirençli prostat kanseri hücrelerinde apoptotik hücre ölümünü indüklemedi, ancak otofajik vakuol oluşumunu indükledi. Ayrıca TQ ile muamele edilen DOC-R/PC3 hücrelerinde Beclin-1 ve LC3-II protein seviyelerinin arttığı, ancak DOC-R/PC3 hücrelerinde LC3-I seviyelerinin azaldığı tespit edildi.
Sonuç: Tüm bu sonuçlar, TQ'nun gelecekte DOC dirençli prostat kanseri için yeni bir terapötik ajan olabileceğini göstermektedir.
Anahtar kelimeler: Otofaji; Beclin-1; LC3; prostat kanseri; direnç, timokinon.

INTRODUCTION

Castrate-resistant prostate cancer (PCa) is one of the most prevalent cancers in men worldwide, after lung cancer (1). In most cases, PCa is detected as advanced, depending on the diagnosed stage and prostate-specific antigen levels. Although PCa is diagnosed as much as possible in the early stages, metastases may develop in patients after various local treatments (2).

In recent years, drugs and drug combinations used in the treatment of PCa have been an important development for PCa patients. Among them, docetaxel (DOC), which averts the depolarization of microtubules and mitotic division, is a standard chemotherapeutic treatment method for PCa patients (3,4) Although DOC is an effective treatment in PCA, it can cause various undesirable side effects such as fatigue, pneumonia, and infusion reactions. Moreover, DOC resistance resulting from an exaggeration of ABC transport proteins, which confines the cellular amounts of the drug, is an important problem in PCa treatment. Therefore, novel and more effective compounds are needed in DOC-resistant PCa treatment (5).

Thymoquinone (TQ), known as black cumin, is one of the main active components of *Nigella sativa* L. essential oil (6). Black seed is utilized both as a spice and in the treatment of diseases in many societies, especially in Arab countries. Many studies have been conducted to identify the cytotoxic effects of TQ on prostate, colon, breast, liver, lung, colon, brain cancer cells *in vitro* (7). It has been discovered that many cancer-related mechanisms such as angiogenesis, invasion, metastasis, cell death, and tumor growth are impaired by TQ (8,9). Besides its anticancer properties, TQ fortifies the immune system, protects normal cells from the effects of oxidative stress, and averts various side effects (10). TQ can make cancer cells sensitive to traditional treatments such as chemotherapy and radiotherapy by regulating resistance mechanisms (11,12), therefore it is recommended that TQ be used as an adjuvant in combination with radiotherapy and chemotherapy (7).

The two primary types of programmed cell death (PCD) are apoptosis and autophagy, which can be recognized by their morphological characteristics. Apoptosis, or type I PCD is characterized by cell shrinkage, nuclear condensation and fragmentation, dynamic membrane blebbing, and phosphatidylserine externalization (13). Autophagy or type II PCD is a catabolic process characterized by the formation of autophagosomes, double membrane-bound structures surrounding cytoplasmic macromolecules and organelles (14). Beclin-1 and microtubule-associated light chain 3 (LC3) are two autophagy-related (Atg) genes/proteins that play key functions and are commonly considered as potential markers of autophagy. Beclin-1 is involved in the nucleation phase (early stages) of autophagosome formation and is an important molecule for the initiation of autophagy. LC3 is a key autophagy-related protein that exists in two forms, LC3-I and LC3-II. When autophagy is triggered, the cytosolic form of LC3 (LC3-I) is transformed to LC3-II, a lipid molecule known as phosphatidyl ethanolamine (PE), which is then incorporated into the membrane of autophagosomes (15). It suggests that reduced autophagy contributes to the progression of prostate cancer (16).

This study aimed to investigate the possible cytotoxic and cell death-inducing activities of TQ on DOC-resistant prostate cancer cells.

MATERIAL AND METHODS

Cell Culture Conditions and Chemicals

DOC and TQ were provided from Sigma (USA). The DOC (10 mM) and TQ (10 mM) stock solutions were formulated in dimethyl sulphoxide (DMSO) and stored at -20 °C. We arranged new stock solutions before each experiment and formulated the dilutions just before application.

Human PC3 prostate cancer cell lines were purchased from Interlab Cell Line Collection (ICLC). The cells were maintained in RPMI 1640 with heat-inactivated fetal bovine serum (10%), penicillin-streptomycin (1%), and L-glutamine (1%) supplementation. Cells were cultivated at 37 °C and 5% CO_2- containing incubators. The growth and morphology of the cells were monitored daily under an inverted light microscope. All cell culture procurations were purchased from Sigma.

DOC-resistant derivatives of PC3 (DOC-R/PC3) were produced by the continuous culture at increasing doses of DOC until they improved their growth and division abilities. PC3 cells were cultured with the increasing doses of DOC (1-10 nM) for 6 months before they were able to survive and differentiate in the presence of 10 nM DOC (a >10-fold rise in the IC_{50} value for DOC). MDR1 protein levels were measured using western blot analysis at each stage. When the PC3 cells began to divide in 10 nM of DOC medium and increased MDR1 activity, they were named DOC-R/PC3 and these cells were used for the next experimental steps. Sigma provided all of the other chemicals not listed above.

MTT Viability Assay

The MTT assay was employed to evaluate the cytotoxic effect of TQ on DOC-R/PC3 cells. In 100 µL of culture media, DOC-R/PC3 cells were seeded at 10^4 cells per well in a 96-well plate. TQ formulated by dilution in increasing concentrations (25, 50, 75, 100, 150 µM) was applied to the cells in well-plate for 24, 48, 72 hours. A control without TQ treatment was also incorporated. After incubation term, TQ treated and untreated cells were exposed to 20 µL MTT solution and held at 37 °C for 4 h. Then, all the media was abolished and DMSO was applied to the cells to dissolve formazan crystals. Then, the optic densities of the wells were measured using a spectrophotometer (Tecan Infinite 200 PRO) at 570 nm wavelength. For DOC-R/PC3 cells, the IC_{50} value, indicating the TQ concentration displaying 50% cell viability, was determined using Biosoft CalcuSyn version 2.0 software (USA).

Apoptosis Assay

The Muse™ Annexin V & Dead Cell kit (Millipore) was used to detect the presence of apoptosis in DOC-R/PC3 cells in response to treatment with TQ. The DOC-R/PC3 cells were plated at $4x10^5$ cells and were subjected to pre-determined IC_{50} values of TQ (60µM) or TQ free media (control). The cells were then held at 37 °C for 72 h in a CO_2 incubator. Centrifugation (1000 rpm, 10 min) was used to extract full cells, which were then washed in PBS. The cells were resuspended in RPMI-medium combined with Muse™ Annexin V & Dead Cell reagent and

maintained for 20 min at RT. Finally, apoptosis analysis was assessed with the Muse™ Cell Analyzer (Merck Millipore, Billerica, MA, USA) (17).

Detection of the Autophagic Activity of TQ

In DOC-R/PC3, the Autophagy Assay Kit (ab139484) was used to determine which type of cell death triggered in response to TQ therapy. The cells were subjected to TQ (60 μM) for 72h. Cells were harvested and washed in PBS after being treated. Finally, cells were stained and maintained for 30 minutes at 37 °C in a dark environment concerning the manufacturer's guide. Wide-field fluorescence microscopy was used to monitor stained cells.

Western Blot

First, cells were exposed to 60 μM TQ for 72 h. Cell pellets were prewashed in phosphate-buffered saline (PBS) and then M-PER Mammalian Protein Extraction Reagent (Thermo Scientific) was utilized to make cell lysates. After centrifugation at 14 000 g for 15 min, total protein contents were evaluated by using the Bradford method. Polyacrylamide gel electrophoresis was used to isolate equal quantities of protein loaded onto SDS polyacrylamide. For PAGE, the gels were run at 120 V for 2 hours. Proteins were separated and then moved to nitrocellulose membranes (Bio-Rad Laboratories) under 115 V for 75 minutes. The membranes were blocked for 1 h with a blocking buffer with 5% nonfat dry milk prepared in TBS with 0.1 percent Tween 20. After blocking the membranes, they were incubated with primary antibodies against LC3 (1:750; Cell Signaling) and Beclin-l (1:2000; abcam). After overnight incubation with the primary antibodies, membranes then treated with secondary antibodies (1:2000) for 1 h. Membranes were washed three times with TBS containing Tween 20 for 10 min. The Kodak Gel Logic 1500 Imaging System was utilized to monitor protein bands. Abcam provided all the antibodies (Cambridge, UK). β-Actin was the loading control, and an orbital shaker was used throughout the incubation process (18). Western blot bands were analyzed via Image J software.

Statistical Analysis

Statistical analysis was conducted via one-way analysis of variance (ANOVA) followed by a Dunnett's t-test for multiple comparisons (Normality analysis was done via Shapiro-Wilk test). Statistical analysis and graphs were done via Graph Pad Prism 5 (Graphpad Software). Statistical significance was attributed to values with a $p<0.05$.

RESULTS

Cytotoxic Effect of TQ on the DOC-R/PC3 Cells

The effect of TQ on the viability of DOC-R/PC3 was evaluated by the MTT assay. The increasing concentrations of TQ (25-150 μM) were applied to DOC-R/PC3 cells for 24, 48 and 72 h. MTT assay results showed that cell viability decreased depending on time and increasing TQ concentration. There were 12%, 15%, 25%, 38%, 57% reductions in the cell viability of DOC-R/PC3 cells exposed to 25, 50, 75, 100, 150 μM of TQ, respectively, at 24 h ($p=0.028$). There were 12%, 15%, 33%, 58%, 76% reductions in the cell viability of DOC-R/PC3 cells exposed to 25, 50, 75, 100, 150 μM of TQ, respectively, at 48 h ($p=0.020$). Cell viability after 72 hours of TQ exposure was significantly reduced in TQ-exposed cells relative to non-exposed cells (control). There were 20%, 38%, 60%, 75%, 82% reductions in the cell viability of DOC-R/PC3 cells exposed to 25, 50, 75, 100, 150 μM of TQ, respectively, at 72 h ($p=0.014$). The IC_{50} of TQ for DOC-R/PC3 was measured as 60 μM at 72 hours, when the highest cytotoxic effect was observed.

Evaluation of Apoptosis in TQ Treated DOC-R/PC3 Cells

To determine the proportion of apoptotic cells after TQ treatment, AV/PI staining was conducted, and flow cytometric analysis was done. Since AV-FITCH has a good affinity for phosphatidylserine, AV/PI staining cells indicates early apoptotic cells. Furthermore, while AV-/PI- cells indicate viable cells, AV+/PI+ cells indicate late apoptotic cells. Based on a comparison of TQ-exposed and non-exposed cells, dot plots of flow cytometric apoptosis analysis revealed the proportion of early apoptosis and late apoptosis (Figure 1A, 1B). There was no statistically significant difference in the viability between TQ-treated (%8.2 apoptotic cells) and TQ-untreated cells (%0.1 apoptotic cells) ($p=0.088$), as shown in Figures 1A and 1B. This showed that TQ had no apoptotic effect on human prostate cancer cells resistant to docetaxel (DOC-R/PC3).

Induction of Autophagy in TQ Treated DOC-R/PC3 Cells

The induction of autophagy was examined in DOC-R/PC3 via Autophagy Assay Kit (ab139484) coupled with fluorescence microscopy. Fluorescent microscopy analysis showed the autophagic vacuoles in DOC-R/PC3 cells exposed to TQ for 72 h compared to untreated DOC-R/PC3 cells (Figure 1C, 1D).

LC3-I, LC3-II, and Beclin-1 Protein Expression Levels in DOC-R/PC3 Cells

Western blot analysis was used to determine the levels of LC3-I, LC3-II, and Beclin-1 protein expression in DOC-R/PC3 cells as markers of autophagy. Levels of Beclin-1 protein were induced by 3.2±0.2 fold in TQ-treated DOC-R/PC3 cells (Figure 2). Levels of another autophagy related protein LC3-II were increased by 2.8±0.4 fold in TQ-treated DOC-R/PC3 cells, but LC3-I levels were decreased by 2.4±0.6 fold in DOC-R/PC3 cells compared to untreated control cells, suggesting enhanced autophagic activity in DOC-R/PC3 cells (Figure 2).

DISCUSSION

In patients with metastatic prostate cancer, DOC-based therapy is the preferred first-line treatment. Previous research has suggested that when used as a high-dose monotherapy for prostate cancer, DOC, has major adverse effects and results in resistance. The current study aimed to identify a novel drug with low or no cytotoxicity that could inhibit viability and induce cell death in DOC-resistant human PCa cells. By exposing PC3 cells to gradually the concentrations of DOC, DOC-R/PC3 were created. The resistance to DOC of the subclones was validated by evaluating cell viability and MDR1 activity.

In the literature, it was proven that TQ inhibits cell viability of PCa cells in a dose- and time-dependently (19,20). However, there is no study investigating the possible cytotoxic effect of TQ on DOC-resistant PCa cells. The findings of this study showed that treating the DOC-resistant PCa cell line DOC-R/PC3 with TQ, a new and more powerful drug, resulted in substantial cytotoxic activity.

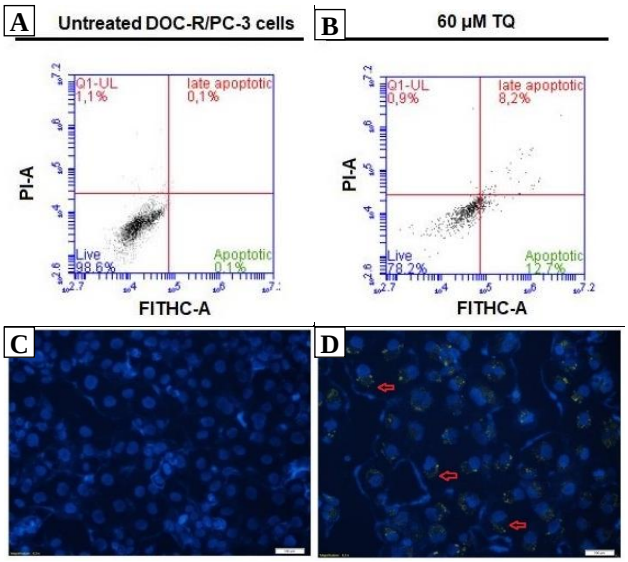

Figure 1. Flow cytometric analysis of **(A)** untreated DOC-R/PC3 cells and **(B)** 60 µM TQ treated DOC-R/PC3 cells via the AV/PI staining. Fluorescent microscopy analysis of **(C)** untreated and **(D)** 60 µM TQ treated DOC-R/PC3 cells

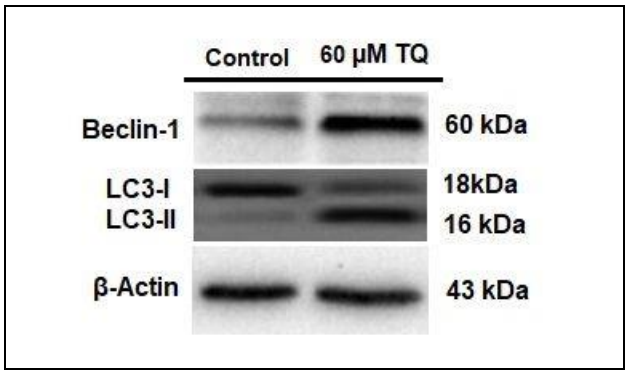

Figure 2. Western blot analysis of LC3-I, LC3-II and Beclin-1 protein levels after 60 µM TQ treatment in DOC-R/PC3 cells

This effect was observed depending on time and concentration manner. It was also reported in previous studies that TQ triggers apoptosis in various human cancer cells including prostate cancer cells (5,21-24). Thus, to investigate the possible apoptotic feature of TQ on DOC-R/PC3 cells, Annexin V-FITC and propidium iodide (PI) levels were evaluated. Annexin V-FITC detects phosphatidylserine externalization in apoptotic cells, while PI attaches to DNA and recognizes necrotic cells. Apoptosis was not induced in TQ-treated DOC-R/PC3 cells, according to flow cytometry.

Another main mechanism underlying effective anti-cancer chemotherapy treatments is autophagy. To enlighten the underlying cytotoxic mechanism of TQ on DOC-R/PC3 cells, the autophagic effect was investigated via staining autophagic vacuoles. The most prominent morphological change in autophagy is the vesicles formed in the cytoplasm surrounded by a membrane of two or more layers, containing parts of the cytoplasm and/or organelles. Lysosomal enzymes break down the cargo of these vesicles as they fuse with lysosomes (25).

To verify the autophagy-inducing effect of TQ in DOC-R/PC3 cells, LC3-I, LC3-II and Beclin-1 protein levels were evaluated. Autophagy is activated in response to similar stress stimuli, coordinated through some regulatory proteins. Various enzyme-substrate relationships (for example, caspase8 both cutting autophagy proteins and being the target of autophagy), protein-protein relationships (such as Atg5-FADD), and protein-protein competition (between autophagy proteins and pro-apoptotic proteins race) play a role. All these data show that there may be several molecular mechanisms underlying TQ inducing autophagy in DOC-R/PC3 cells. After the cellular stress and cell death-related kinases, DAPk or JNK1/2 phosphorylate Beclin1 and Bcl-2, respectively, Beclin1 can escape from the suppressive effect of Bcl-XL protein and activate autophagy (26,27). Moreover, a ubiquitin-like mechanism converts LC3-I into LC3B-II (28).

CONCLUSION

Our data showed that TQ has a significant cell death-inducing effect on PCa cells that have developed resistance to docetaxel, a traditional chemotherapeutic agent. Although studies are showing that TQ is a powerful anticancer agent, our findings also confirm this, however, more thorough research is required to determine the molecular mechanism of action. As a result, this report shed light on autophagy and TQ-induced cytotoxicity in human PCa cells. Based on these findings, TQ can be a new therapeutic option for DOC-resistant prostate cancer in the future.

Ethics Committee Approval: Since our study was not an experimental study including human or animal subject, ethics committee approval was not required.

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Clinical Outcomes after En Bloc Resection of Periosteal Chondroma: A Retrospective Clinical Study

Periosteal Kondromanın en blok Rezeksiyonu Sonrası Klinik Sonuçları: Geriye Dönük Klinik Çalışma

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ABSTRACT

Aim: Periosteal chondroma is a rare chondroma that is difficult to differentiate. Its localization is similar to other surface periosteal lesions. These lesions have a wide distribution of age. Curettage, marginal excision, or en bloc resection are applied in the surgical treatment. En bloc resection is preferred to reduce recurrence. In this study, we aimed to share the experience of two orthopedic oncology centers in the differential diagnosis and treatment of periosteal chondroma.

Material and Methods: Data from two clinics were analyzed retrospectively. Data were collected on demographic data (age, gender), clinical findings (pain, swelling, pressure-related symptom, duration of follow-up), radiological findings (size, bony invasion), pathology results (biopsy, excision), and postoperative complications (recurrence).

Results: Fourteen patients were included in the study. En bloc resection was performed in all cases. The mean age of the patients was 31.5±16.5 (range, 8-58) years. 10 (71.4%) patients were male. The mean duration of symptoms was 6.6±4.8 (range, 0-18) months, and the mean follow-up was 46.7±39.6 (range, 6-132) months. Nine (64.3%) patients had pain. Six (42.9%) patients had swelling. One patient (7.1%) had a palpable mass. There was no complaint in 1 (7.1%) patient. One (7.1%) patient underwent biopsy. During the follow-up, no recurrence or complication was observed after en bloc resection.

Conclusion: Imaging and histopathological findings of benign and malignant periosteal chondroid tumors may overlap, and accurate differential diagnosis is crucial in the treatment of these lesions. En bloc resection prevents recurrence during follow-up.

Keywords: En bloc resection; periosteal chondroma; chondrosarcoma; recurrence.

ÖZ

Amaç: Periosteal kondroma oldukça nadir gözlenen ve ayırıcı tanısı zor bir kondroid tümördür. Yerleşimi diğer yüzeyel periosteal lezyonlar ile benzerlik gösterir. Bu lezyonlar farklı yaş gruplarında görülmektedir. Cerrahi tedavisinde küretaj, marjinal eksizyon veya en blok rezeksiyon uygulanmaktadır. Nüksü azaltmak amacıyla en blok rezeksiyon tercih edilir. Bu çalışmada, periosteal kondromanın ayırıcı tanı ve tedavisinde iki ortopedik onkoloji merkezinin tecrübesinin aktarılması amaçlanmıştır.

Gereç ve Yöntemler: İki kliniğe ait veriler geriye dönük olarak incelendi. Demografik veriler (yaş, cinsiyet), klinik bulgular (ağrı, şişlik, basıya bağlı semptom, takip süresi), radyolojik bulgular (kitle büyüklüğü, kemik invazyonu), patoloji sonuçları (biyopsi, eksizyon) ve ameliyat sonrası komplikasyonlar (nüks) hakkında veri toplandı.

Bulgular: Çalışmaya 14 hasta dahil edildi. Tüm vakalarda en blok rezeksiyon uygulandı. Hastaların ortalama yaşı 31,5±16,5 (aralık, 8-58) yıl idi. 10 (%71,4) hasta erkek cinsiyetti. Ortalama şikayet süresi 6,6±4,8 (aralık, 0-18) ay, ortalama takip süresi ise 46,7±39,6 (aralık, 6-132) ay idi. Dokuz (%64,3) hastada ağrı şikayeti mevcuttu. Altı (%42,9) hastada şişlik şikayeti mevcuttu. Bir (%7,1) hastada palpe edilebilen bir kitle mevcuttu. Bir (%7,1) hastada şikayet bulunmuyordu. Bir (%7,1) hastaya biyopsi yapıldı. Takip süresince nüks veya en blok rezeksiyon sonrasında herhangi bir komplikasyon görülmedi.

Sonuç: Benign ve malign periosteal kondroid tümörlerin görüntüleme ve histopatolojik bulguları çakışabilir ve bu lezyonların tedavisinde, ayırıcı tanının doğru yapılması oldukça önem arz eder. En blok rezeksiyon takip sırasında nüksü önlemektedir.

Anahtar kelimeler: En blok rezeksiyon; periosteal kondroma; kondrosarkom; nüks.

INTRODUCTION

Periosteal chondroma is a rare benign cartilaginous lesion. It is very rare and is not usually involved in the differential diagnosis of chondromatous lesions (1). It was first defined by Liechtenstein (2) and then by Jaffe (3).

These tumors originate from the periosteal surface. The differential diagnosis is challenging as many other benign and malignant chondroid lesions can be mistaken, located at the periosteal surface. These entities comprise periosteal chondrosarcoma, periosteal osteosarcoma, and surface high-grade osteosarcoma, osteosarcoma, cortical desmoid, non -epiphyseal chondroblastoma. The most common frequent sites are metaphyses, metadiaphyses of long bones (femur, tibia, and humerus). Atypical lesions can also be encountered at the spine and the rib (4).

These lesions are generally 3 cm in size (5). In lesions bigger than 7 cm, malignancy should be suspected. Periosteal chondroma and malignant chondroid lesions can spread to extracompartmantal areas (6). Palpable painful mass is the most common symptom. However, patients can present with asymptomatic lesions. Radiographic images usually have well-defined borders with marginated erosions and endosteal scalloping. The imaging feature may resemble both benign and malignant lesions. So, biopsy does not help in making a definite diagnosis. Pathologic analysis revealed nuclear pleomorphism and binucleation. These findings may sometimes lead to misdiagnosis as chondrosarcomas. Hyaline cartilage demarcated by the periosteum is a typical finding. There is a narrow transition zone between the soft tissue and the periosteal chondroma. There is no invasion of the underlying bone with no atypical mitotic figures. En bloc resection is safe in terms of prevention of recurrence and improvement in symptoms.

In this study, we aimed to share the clinical experience of two centers in the surgical treatment of periosteal chondromas.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of İstanbul Medeniyet University (2021/285). A retrospective review was performed in two clinics between 2015 and 2021. A total of 14 cases were identified. A prior diagnosis was made based on clinical and radiological findings. Final histopathologic evaluation by two pathology experts in musculoskeletal oncology verified the diagnosis. Inclusion criteria included patients with complete data at least one year of follow-up, lesions of appendicular skeleton involving upper and lower extremities. Exclusion criteria included incomplete patient data, lesions of axial skeleton, final pathological diagnosis of malignancy. In all lesions, surgical excision was performed using direct approach over the lesion under general anesthesia.

Statistical Analysis

Descriptive statistics were given as mean, standard deviation and range values. Categorical variables were summarized as numbers and percentages.

RESULTS

The demographic and clinical findings of patients were presented in Table 1. 14 patients were included in this study. All cases underwent en bloc resection. The mean age of the patients was 31.5±16.5 (range, 8-58) years, and 10 (71.4%) patients were male. The mean duration of symptoms was 6.6±4.8 (range, 0-18) months. The mean follow-up was 46.7±39.6 (range, 6-132) months. Symptoms included pain in 9 (64.3%) patients and swelling in 6 (42.9%) patients. One (%7.1) patient had a palpable mass. One (%7.1) patient was asymptomatic. Lesions were located at distal femur in 4 (28.6%) patients, hand in 4 (28.6%) patients, metatarsal in 3 (21.4%) patients, tibia in 1 (%7.1) patient, calcaneus in 1 (%7.1) patient, and humerus in 1 (%7.1) patient. The size of the tumor was more than 3 cm in all lesions. Preoperative biopsy was made in only 1 (%7.1) patient with a lesion more than 7 cm in size. There was no recurrence and no other complication after en bloc resection. Radiologic MRI features were well-defined lesions with a sharp sclerotic margin, scalloping of the cortex, and multilobular mass without bone marrow invasion. The lesions predominantly show high signal intensity on T2-weighted images and low signal intensity on T1-weighted images (Figure 1, 2). Pathologic specimens demonstrated double-nucleated cells with moderate myxoid changes in the matrix consistent with periosteal chondroma (Figure 3).

Table 1. Demographic and clinical findings of all cases

Case	Age	Gender	Localization	Symptom	Duration of symptom	Follow-up
1	58	Male	left third metatars	(-)	(-)	36
2	28	Male	left second finger	swelling	6	36
3	55	Male	left fourth finger	firm immobile sensitive mass	7	48
4	26	Female	right second metatars	pain	3	24
5	22	Male	left posterior knee	pain and sensitivity	12	12
6	27	Male	right proximal humerus	night pain	1	24
7	13	Female	left distal tibia	pain	18	24
8	21	Male	left fifth finger	swelling	5	120
9	20	Male	right fourth finger	swelling	3	132
10	48	Female	left proximal humerus	pain	7	36
11	55	Male	left calcaneus	pain and sensitivity	12	96
12	21	Male	distal femur	pain and swelling	5	37
13	8	Male	distal femur	pain and swelling	8	24
14	39	Female	left distal femur	pain and swelling	6	6

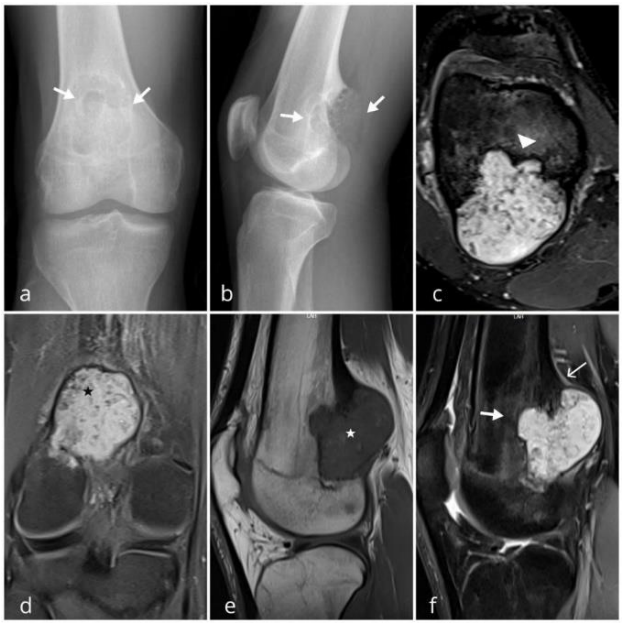

Figure 1. Radiologic features of periosteal chondroma located at distal femur (Case 12). **a, b)** Anteroposterior and lateral radiograph of right femur shows periosteal-based lesion with extrinsic scalloping of posterolateral cortex of distal femoral metaphysis (arrows) There is internal chondroid calcification. **c, d, f)** Axial, coronal and sagittal fat-supressed T2-weighted MR images show multilobular, predominantly high signal intensity mass (asteriks and arrow) along posterior cortex of distal femur without bone marrow invasion (arrow head). No adjacent soft tissue edema is seen. **e)** Sagittal T1-weighted MR image shows low signal intensity soft tissue mass causing cortical scalloping (arrow)

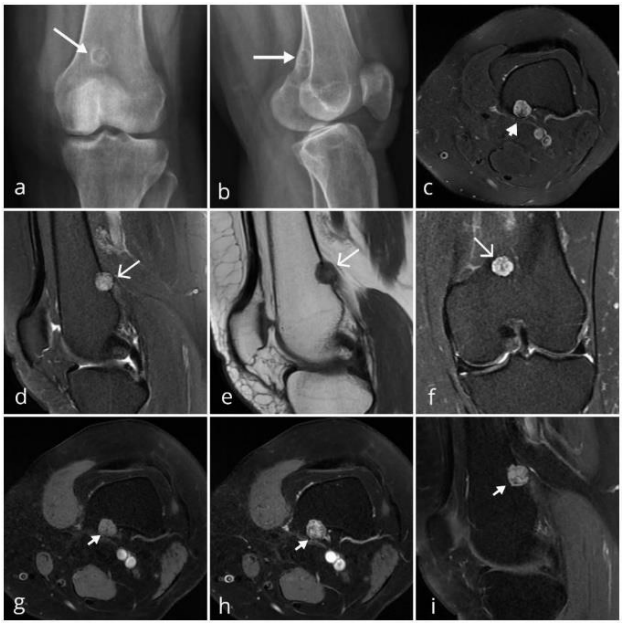

Figure 2. Radiologic features of periosteal chondroma located at distal femur (Case 14). **a, b)** Anteroposterior and lateral radiograph of left femur shows jukstracortical well-defined lesion with sharp sclerotic margin and extrinsic scalloping of posterolateral cortex of distal femoral metaphysis(arrows). **c, d, f)** Axial, coronal and sagittal fat-supressed T2-weighted MR images show high signal intensity mass (arrows) along posterior cortex of distal femur without bone marrow invasion. No adjacent soft tissue edema is seen. **e)** Sagittal T1-weighted MR image shows low signal intensity soft tissue mass causing cortical scalloping (arrow) **g)** Precontrast axial fat-supressed T1-weighted image show mildly intens signal in lesion. Contrast enhanced fat-suppressed T1-weighted image demonstrates internal patchy enhancement (arrows)

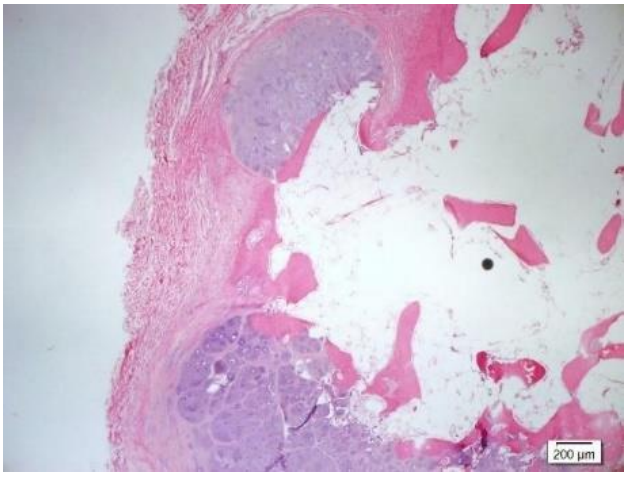

Figure 3. Radiologic features of periosteal chondroma located at distal femur (Case 13). Lobes of hyaline cartilage surrounded by fibrous connective tissue or cortical bone form the periosteal chondroma. This tumor is well-circumscribed. It does not penetrate into the bone and permeate the surrounding soft tissues. The cartilage is normocellular and the nuclei are plump and hyperchromatic. Double-nucleated cells are common, and moderate myxoid changes may be seen in the matrix (H&E, x40)

DISCUSSION

The diagnosis of periosteal chondroma is usually problematic and need clinical, radiological, and pathological correlation. In this study, we performed en bloc resection in 14 cases. At a mean of 4 years of follow-up, we were unable to observe any recurrence.

The surgical treatment in periosteal chondroma is usually marginal excision or en bloc resection with wide margins. However, intralesional curettage has the risk of local recurrence. Studies regarding outcomes after surgical treatment are usually limited to case reports and case series as it is a rare entity.

Previous studies demonstrated satisfactory clinical outcomes after excision. Boriani et al. (7) performed marginal or wide resection in 20 cases. Most lesions were located at the proximal metaphysis of long bones. Five lesions were located in the hand, which was also common in our study. Wide excision was done in three cases. He found that marginal excision is efficient in most cases. In atypical lesions except for the metaphysis of long bones, excision is a preferred treatment. Motififard et al. (8) reported a pelvic periosteal chondroma in a 39 year-old male with paresthesia, gluteal muscle atrophy, and claudication. He performed marginal excision. At six months after surgery, the patient reported clinical improvement. Samaddar et al. (9) performed second rib wide resection in a 12-year-old female child due to periosteal chondroma. The follow-up was not given, but the patient was reported to be good. Kang et al. (10) performed wide excision and left T5/6 hemilaminectomy in a 41-year-old male due to periosteal chondroma in the thoracic spinal canal. At 18 months of follow-up, there was no recurrence. Pandey et al. (11) observed no recurrence in radial diaphyseal lesion at two years after marginal excision of periosteal chondroma. Nishio et al. (12) performed excision in a 25-year-old female with a distal tibial lesion. At four months of follow-up, the patient was satisfied with no signs of recurrence. Debbarma et al. (13)

performed a subtotal scapulectomy in a 24-year-old male due to periosteal chondroma at the right scapula. At 1-year follow-up, the patient had an excellent outcome.

Rolvien et al. (14) performed en bloc resection in a periosteal chondroma of cuboid. At 9 months after surgery, the patient had no symptoms with good functional outcomes. Zheng et al. (15) made en bloc resection of distal femur periosteal chondroma in a 14-year-old female. At 6 months after resection, the patient was well and the fibular graft was well incorporated with complete healing of the defect. These studies demonstrated that en bloc resection provides satisfactory outcomes without recurrence.

Intralesional curettage is another option in the management of periosteal chondromas. Imura et al. (5) performed intralesional curettage, bone grafting, and plate fixation in the distal femoral lesion in a 17-year-old boy. There was no recurrence at 12 months after resection. However, intralesional curettage can present with recurrence in one year after initial surgery. In their case series involving 24 hand periosteal chondromas, Rabarin et al. (16) reported recurrence in a 10-year-old child, 10 months after curettage.

After resection or curettage of the lesion, cement or bone graft has been used to fill the defect in previous studies. Imura et al. (5) used bone graft after curettage of chondroma of the distal femur. At 6 months after surgery, the graft was consolidated. Rolvien et al. (14) used cement after en bloc resection of cuboidal periosteal chondroma. In our study, we did not use any bone void filler without concern about biomechanical stability, and the defect was healed uneventfully.

In terms of clinical complaints, localized swelling and pain are common findings in periosteal chondroma, and the clinical course facilitates the diagnosis of periosteal chondroma. Swelling followed by moderate pain is characteristic (7). In our series, 12 (%85.7) patients had a history of pain and swelling for 3-6 months which was comparable to previous studies (7,8). However, there are also cases who had symptoms for two years as reported by Pandey et al. (11)

Age at diagnosis is variable in previous studies. In our study, the mean age at diagnosis was 31.5 years. In series of Boriani et al. (7), the mean age was 22.15 years with a mean duration of symptoms of 15 months. In other studies, the age at diagnosis ranged between 12-41 years (5,8-12). Similarly, our study also confirmed that periosteal chondromas can be seen in patients with a wide range of age.

Imaging can help in the identification of periosteal chondroma and differentiation from other chondroid lesions. Lesion size with more than 3 cm increases the likelihood of chondrosarcoma. Although the size of lesions was more than 3 cm in some of our cases, a radiological appearance with sharp sclerotic margin, chondroid calcification, and extrinsic scalloping of cortex without adjacent soft tissue edema supports the diagnosis of periosteal chondroma. One recent study (17) indicated that PET-CT could aid in distinguishing chondromas from chondrosarcomas with a cut-off SUVmax value of 2.0. This is yet to be evaluated in other studies. Pathological clues depend on whether the lesion's microscopy demonstrated osteoid (osteosarcoma) or chondroid matrix (periosteal chondroma). In our cases, there was no peripheral ossification present which is possible in periosteal osteosarcomas.

Observation can be preferred in a painless periosteal chondroma. We did not encounter any morbidity at the affected extremity. However, if the size of the lesion exceeds more than 3 cm, it is reasonable to consider that periosteal chondroma should be approached like a malign tumor and en bloc resection without cementation or grafting can be preferred.

CONCLUSION

This study confirms the importance of differential diagnosis and wide excision in periosteal chondromas. These lesions have a wide distribution of age. Its localization is similar to other surface periosteal lesions, which is why the identification from malignant chondroid lesions is challenging.

Ethics Committee Approval: The study was approved by the Ethics Committee of İstanbul Medeniyet University Göztepe Training and Research Hospital (26.05.2021, 285).

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Comparison of the Mental Status of COVID-19 Intensive Care Unit and General Intensive Care Unit Staff

COVID-19 Yoğun Bakım Ünitesi ve Genel Yoğun Bakım Ünitesi Personelinin Ruhsal Durumunun Karşılaştırılması

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ABSTRACT

Aim: The coronavirus disease 2019 (COVID-19) pandemic may predispose front-line intensive care staff to experience mental health problems. The aim of this study was to compare the COVID-19 fear experienced by COVID-19 intensive care unit staff and general intensive care unit staff, and the effects of this fear on mental health.
Material and Methods: In this cross-sectional study, a total of 156 participants, 90 (57.7%) participants from the COVID-19 intensive care unit and 66 (42.3%) from the general intensive care unit, were included. A printed questionnaire consisting of the Fear of COVID-19 Scale (FCV-19S), Depression Anxiety Stress Scale (DASS-21), and demographic data were used.
Results: Female gender, being a nurse, and working in COVID-19 intensive care unit, were associated with higher depression, anxiety, and stress scores. A significant relationship was found between fear of COVID-19 and depression (ρ=0.399, p=0.044), anxiety (ρ=0.456, p=0.019), and stress (ρ=0.418, p=0.033). Furthermore, as compared to general intensive care unit staff, COVID-19 intensive care unit staff who may have high-risk contact were approximately twice times more likely to experience anxiety and fear of COVID-19 and 3.5 times more likely to suffer from depression and stress.
Conclusion: The COVID-19 pandemic has adversely affected the mental health of intensive care staff. Attention should be paid to the mental health of females and nurses working in the COVID-19 intensive care unit. The mental health of intensive care workers should be supported to protect the health workforce.
Keywords: Anxiety; COVID-19; depression; fear; mental health; intensive care.

ÖZ

Amaç: Koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) pandemisi, ön saflardaki yoğun bakım personelini ruhsal sağlık sorunları yaşamaya yatkın hale getirebilir. Bu çalışmanın amacı, COVID-19 yoğun bakım ünitesi personeli ile genel yoğun bakım ünitesi personelinin yaşadığı COVID-19 korkusunu ve bu korkunun ruh sağlıkları üzerindeki etkilerini karşılaştırmaktır.
Gereç ve Yöntemler: Bu kesitsel çalışmaya COVID-19 yoğun bakım ünitesinden 90 (%57,7) katılımcı ve genel yoğun bakım ünitesinden 66 (%42,3) katılımcı olmak üzere toplam 156 katılımcı dahil edildi. COVID-19 Korku Ölçeği (Fear of COVID-19 Scale, FCV-19S), Depresyon Anksiyete Stres Ölçeği (Depression Anxiety Stress Scale, DASS-21) ve demografik verilerden oluşan basılı bir anket kullanıldı.
Bulgular: Kadın cinsiyet, hemşire olmak ve COVID-19 yoğun bakım ünitesinde çalışmak daha yüksek depresyon, anksiyete ve stres puanları ile ilişkili idi. COVID-19 korkusu ile depresyon (ρ=0,399; p=0,044), kaygı (ρ=0,456; p=0,019) ve stres (ρ=0,418; p=0,033) arasında anlamlı bir ilişki bulundu. Ayrıca, genel yoğun bakım ünitesi personeline kıyasla, yüksek temas riski olan COVID-19 yoğun bakım ünitesi personelinin, anksiyete ve COVID-19 korkusu yaşama olasılığı yaklaşık iki kat, depresyon ve stresten muzdarip olma olasılığı 3,5 kat daha fazlaydı.
Sonuç: COVID-19 pandemisi, yoğun bakım personelinin ruh sağlığını olumsuz etkilemiştir. COVID-19 yoğun bakım ünitesinde çalışan kadın ve hemşirelerin ruh sağlığına dikkat edilmelidir. Yoğun bakım çalışanlarının ruh sağlığı, sağlık iş gücünün korunması için desteklenmelidir.
Anahtar kelimeler: Anksiyete; COVID-19; depresyon; korku; ruh sağlığı; yoğun bakım.

INTRODUCTION

The World Health Organization (WHO) defined the novel coronavirus disease 2019 (COVID-19) as a pandemic in March 2020. WHO has determined protecting the mental well-being of healthcare workers who care for people with COVID-19 as a necessity to sustain the health workforce in the long term (1).

During past pandemics, healthcare workers experienced anxiety, stress, depression, and fear for various reasons such as intense work stress, loneliness, social isolation, fear of getting infected, and fear of infecting others. It has even been reported that these adverse psychological effects may continue for months or years (2-5).

Global health systems have struggled to fight as a result of the rapid spread of the COVID-19 pandemic (6). During the COVID-19 pandemic, patients' intensive care support needs also increased (7). Due to the nature of their profession, healthcare professionals are always at high risk of getting infected with infectious diseases such as COVID-19. While working in an intensive care setting, continuous close contact, and repeated contact with patients is inevitable (8). Intensive care workers are exposed to heavy workload, long-term fatigue, and the threat of infection for their family and their safety (3,6,7). They also face uncertainty about the duration of a pandemic, capacities of proven therapies, personal protective equipment, and health resources. Healthcare professionals may experience psychological distress due to providing direct care to COVID-19 patients, having a relative or someone familiar who is sick or critically ill, and being quarantined (9). These conditions can create high levels of stress, fear, and anxiety in employees and may expose them to permanent adverse psychological effects. Therefore, healthcare workers are at risk of subclinical mental health disorder symptoms and long-term burnout (7).

Despite meticulous global combat efforts, COVID-19 continues to rise around the world. It is substantial to evaluate the mental status of intensive care workers to take the necessary measures and make improvements to protect the health workforce in the fight against COVID-19.

In this study, we aimed to evaluate the fear of COVID-19 situation of COVID-19 intensive care unit (COVID-19 ICU) and general intensive care unit (ICU) staff and to compare the effect of this fear on their mental health.

MATERIAL AND METHODS

Participants and Procedures

This cross-sectional study was conducted after the approval of the Kırşehir Ahi Evran University Ethics Committee (24.06.2020, 09-63). We planned our research according to current Helsinki guidelines. The hospital has both the 3rd line COVID-19 ICU, where the treatment and care of COVID-19 patients are provided and the ICU, where patients who are not infected with COVID-19 are treated. The study was conducted on 156 personnel working in these two intensive care units. Doctor, nurse, and assistant health personnel working in intensive care units were asked to fill out printed questionnaires between 1 July and 10 July 2020. Within the scope of COVID-19 contact isolation rules and protection measures, the responsible researcher informed the participants about the research. Signed, enlightened consents of the participants were obtained. Volunteers diagnosed with psychological and cognitive disorders and currently receiving medical support were excluded from the study.

Screening Questionnaire

The questionnaire form used in this study consists of 3 parts: socio-demographic data, Fear of COVID-19 Scale (FCV-19S), and Depression Anxiety Stress Scales (DASS-21).

Socio-demographic Data: These data include age, gender, profession (doctor, nurse, assistant healthcare personnel), working year (<5, 6-10, 11-15, >15 years), and marital status (single, married, married-with children).

Fear of COVID-19 Scale (FCV-19S): Ahorsu et al. (10) have developed the FCV-19S scale. It is a one-dimensional scale with a 5-point Likert-type rating system consisting of seven items (1: Strongly disagree to 5: Strongly agree). A minimum of 1 point and a maximum of 5 points are given for each question. The total score is calculated by adding the scores of 7 items (between 7 and 35 points). In our study, the Turkish scale adaptation of FCV-19S by Satici et al. (11) was used.

Depression Anxiety Stress Scales (DASS-21): This scale has been developed from the DASS-42 with 42-item (12). DASS-21 consists of three sub-dimensions (depression, anxiety, and stress) and 21 items. There are 7 items in each sub-dimension. The scale has a 4-point Likert-type rating system (0: Never to 3: Always). Each sub-dimensions score is calculated by adding each item score. Since the DASS-21 scale is developed as a short form of DASS-42, it is calculated by multiplying each sub-dimension score by two. DASS-21 scores are interpreted as follows: Depression: normal (0-9), mild (10-13), moderate (14-20), severe (21-27), and extremely severe (28-42); Anxiety: normal (0-7), mild (8-9), moderate (10-14), severe (15-19), and extremely severe (20-42); Stress: Normal (0-14), mild (15-18), medium (19-25), severe (26-33), and extremely severe (34-42). In this study, Turkish scale adaptation by Yılmaz et al. (13) was used.

Statistical Analysis

Statistical analyses were performed using SPSS v.21.0 software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). The normality assumption was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. Since the study is a questionnaire study, kurtosis and skewness coefficients were also taken into account in the test of the normality assumption. Descriptive statistics of variables that provide normality assumption were given as mean±standard deviation (min-max); median (25^{th} - 75^{th} percentile) were given for variables not provide normality assumption. Categorical variables were summarized as frequency and percentage. Group comparisons based on the total scores were made by using the Mann-Whitney U and Kruskal-Wallis tests. When a significant difference was found between the groups by the Kruskal-Wallis test, the Mann-Whitney U test was used as the post hoc test. Chi-square and Fisher-Freeman-Halton tests were used to compare categorical variables. The relationship between fear of COVID-19 and anxiety, depression, and stress were tested with Spearman correlation analysis. Multivariate analyzes for fear of COVID-19, depression, anxiety, and stress were performed using the ordinal logistic regression model. The reliability of the scale was conducted using Cronbach's alpha coefficient.

RESULTS

Reliability of the DASS-21 and FCV-19S

Cronbach's alpha values of the depression, anxiety, and stress sub-dimensions of the DASS-21 scale were determined as 0.810, 0.800, and 0.755, respectively, in the study of Yılmaz et al (13). In our study, Cronbach's alpha values of DASS-21 sub-dimensions were found to be 0.889, 0.848, and 0.827, respectively. Cronbach's alpha value of the FCV-19S scale was 0.840 in the study by Satici et al. (11). The Cronbach's alpha value of FCV-19S in our study was found as 0.916.

Participants' Characteristics

One hundred fifty-six employees voluntarily participated in the study among 162 employees working in our hospital's intensive care units. There were 90 (57.7%) participants from the COVID-19 ICU and 66 (42.3%) from the ICU. Descriptive statistics and group comparisons of the demographic characteristics of COVID-19 ICU and ICU personnel are given in Table 1.

Comparison of COVID-19 Fears

The results for FCV-19S are shown in Table 2. The effect of gender on fear of COVID-19 was found to be significant (p=0.001). FCV-19S value of male staff was found higher than female staff (23 (16.0-27.0) vs. 18.5 (13.75-22.0)). Although the FCV-19S scores of the COVID-19 ICU staff group were slightly higher in both male and female compared to the ICU group, this difference was not significant (p=0.381, p=0.104, respectively).

Analysis of Psychological Features: DASS-21

The statistically comparisons of DASS-21 depression, anxiety, and stress scores are shown in Table 3. When the scores in our and similar studies are examined, it will be more appropriate to interpret both statistically and clinically. Although a statistically significant difference was found in some comparisons (married-with children and nurse anxiety, etc.) of COVID-19 ICU and ICU; when evaluated according to subgroups as depression, anxiety,

Table 1. Comparison of demographic data of COVID-19 ICU and ICU staff

	Total (n=156)	COVID-19 ICU (n=90)	ICU (n=66)	p
Age (years), mean±SD (min-max)	32.61±8.78 (18-57)	32.98±8.87 (20-57)	32.10±8.70 (18-52)	0.537
Gender, n (%)				
Male	66 (42.3%)	39 (59.1%)	27 (40.9%)	0.762
Female	90 (57.7%)	51 (56.7%)	39 (43.3%)	
Marital Status, n (%)				
Single	64 (41.0%)	38 (59.4%)	26 (40.6%)	
Married (no children)	15 (9.6%)	9 (60.0%)	6 (40.0%)	0.898
Married (with children)	77 (49.4%)	43 (55.8%)	34 (44.2%)	
Profession, n (%)				
Doctor	27 (17.3%)	15 (55.6%)	12 (44.4%)	
Nurse	96 (61.5%)	56 (58.3%)	40 (41.7%)	0.967
Assistant healthcare personnel	33 (21.2%)	19 (57.6%)	14 (42.4%)	
Working year, n (%)				
0-5	87 (39.2%)	48 (55.2%)	39 (44.8%)	
6-10	57 (25.7%)	33 (57.9%)	24 (42.1%)	0.874
11-15	41 (18.5%)	25 (61.0%)	16 (39.0%)	
>15	37 (16.7%)	23 (62.2%)	14 (37.8%)	

COVID-19 ICU: COVID-19 intensive care unit, ICU: general intensive care unit, SD: standard deviation

Table 2. Comparison of COVID-19 ICU and ICU staff fears based on FCV-19S scores

	Total (n=156)	COVID-19 ICU (n=90)	ICU (n=66)	p
Gender				
Male	23 (16.0-27.0)	23 (19.0-27.0)	22 (12.0-27.0)	0.381
Female	18.5 (13.75-22.0)	19 (14.0-23.0)	17 (11.0-21.0)	0.104
p	**0.001**	**0.009**	**0.035**	
Marital Status				
Single	20 (14.0-24.0)	20.5 (15.75-24.0)	18.5 (10.75-24.25)	0.503
Married (no children)	20 (17.0-26.0)	22 (17.0-25.5)	19 (14.5-29.0)	0.776
Married (with children)	21 (14.5-26.0)	22 (19.0-27.0)	19.5 (12.0-24.25)	0.073
p	0.407	0.198	0.867	
Profession				
Nurse	22 (15.0-26.0)	22 (17.0-26.0)	19 (12.0-25.0)	0.092
Assistant healthcare personnel	20 (14.0-26.0)	18 (12.0-24.0)	21 (13.0-29.25)	0.553
Doctor	19 (14.0-23.0)	21 (19.0-24.0)	19 (11.75-20.75)	0.075
p	0.433	0.198	0.550	
Working year				
0-5	18 (12.0-23.0)	18.5 (15.75-23.0)	15 (10.0-23.75)	0.197
6-10	23 (19.0-27.0)	23.5 (20.25-26.75)	22 (17.0-31.0)	0.633
11-15	21.5 (14.0-26.0)	21 (14.5-26.0)	21.5 (13.5-25.75)	0.673
>15	20 (14.5-25.0)	21.25 (17.5-26.75)	19 (10.5-23.5)	
p	0.055	0.077	0.161	

COVID-19 ICU: COVID-19 intensive care unit, ICU: general intensive care unit, FCV-19S: fear of COVID-19 scale

Table 3. Comparison of DASS-21 scores of COVID-19 ICU and ICU staff

	DASS-21 Depression			DASS-21 Anxiety			DASS-21 Stress		
	COVID-19 ICU	**ICU**	**p**	**COVID-19 ICU**	**ICU**	**p**	**COVID-19 ICU**	**ICU**	**p**
Gender									
Female	12 (4.0-22.0)	4 (2.0-8.0)	**0.006**	10 (4.0-12.0)	4 (2.0-10.0)	**0.007**	16 (12.0-22.0)	8 (6.0-12.0)	**<0.001**
Male	6 (2.0-14.0)	4 (2.0-8.0)	0.405	2 (2.0-6.0)	2 (0.0-4.0)	0.134	12 (6.0-14.0)	8 (4.0-14.0)	0.241
p	**0.012**	0.509		**<0.001**	**0.028**		**0.002**	0.958	
Marital Status									
Single	9 (2.0-20.5)	6 (1.5-15.0)	0.360	4 (2.0-12.0)	2 (0.0-8.5)	0.070	14 (8.0-17.5)	8 (4.0-8.5)	**0.019**
Married (no children)	6 (0.0-16.0)	5 (4.0-12.5)	0.864	4 (2.0-10.0)	6 (1.5-18.5)	0.529	12 (9.0-18.0)	8 (7.5-14.0)	0.272
Married(with children)	8 (4.0-16.0)	3 (2.0-6.0)	**0.001**	4 (2.0-10.0)	2 (1.5-6.0)	**0.016**	14 (8.0-18.0)	8 (4.0-12.0)	**0.001**
p	0.634	0.144		0.540	0.355		0.919	0.863	
Profession									
Nurse	11 (4.5-18.0)	4 (2.0-7.5)	**<0.001**	7 (4.0-12.0)	2 (2.0-7.5)	**0.001**	16 (10.5-21.0)	8 (4.0-10.0)	**<0.001**
Assistant healthcare personnel	6 (2.0-16.0)	4 (1.5-14.5)	0.653	4 (0.0-10.0)	2 (0.0-7.0)	0.529	12 (4.0-20.0)	7 (4.0-13.5)	0.377
Doctor	2 (2.0-6.0)	7 (4.5-17.5)	0.059	2 (0.0-6.0)	2 (0.0-9.5)	0.867	10 (6.0-14.0)	12 (10.0-16.0)	0.167
p	**0.013**	0.149		**0.002**	0.660		**0.018**	**0.017**	
$p^{(N\text{-}AHP)}$	0.231	0.741		**0.024**	0.455		0.188	0.872	
$p^{(N\text{-}D)}$	**0.003**	0.059		**0.002**	0.494		**0.003**	**0.004**	
$p^{(AHP\text{-}D)}$	0.286	0.347		0.471	0.980		0.632	**0.003**	
Working year									
0-5	7 (2.0-22.0)	5 (2.0-8.0)	0.288	6 (2.0-12.0)	2 (0.5-9.0)	0.098	14 (7.5-20.5)	8 (4.0-11.5)	**0.018**
6-10	4 (1.0-9.0)	6 (2.0-8.0)	0.192	6 (4.0-12.0)	2 (2.0-8.0)	**0.033**	15 (12.0-21.0)	8 (6.0-12.0)	**0.001**
11-15	7 (2.5-16.0)	2 (2.0-7.0)	0.156	4 (2.0-9.5)	2 (0.0-7.0)	0.237	12 (6.5-17.5)	9 (5.0-12.0)	0.151
15+	6 (4.0-14.0)	4 (1.0-5.0)	0.251	4 (2.0-12.5)	2 (2.0-8.0)	0.239	12 (6.0-20.5)	10 (6.0-14.0)	0.257
p	0.931	0.529		0.433	0.768		0.336	0.726	

DASS-21: depression anxiety stress scale, COVID-19 ICU: COVID-19 intensive care unit, ICU: general intensive care unit, N: nurse, AHP: assistant healthcare personnel, D: doctor

and stress clinical levels (normal, mild, moderate, severe, extremely severe), no clinically significant difference was found between the groups. For example, in the comparison of depression scores of participants married-with children although a significant difference was found between the groups (8 (4.0-16.0) vs. 3 (2.0-6.0), p=0.001); both group scores were <9, that is, in the normal group. Similarly, in comparison of anxiety scores (4 (2.0-10.0) vs. 2 (1.5-6.0), p=0.016), score of both groups were <7, and they were in the normal group. Again, in comparison of nurse anxiety (7 (4.0-12.0) vs. 2 (2.0-7.5), p=0.001) both groups score were <7, and they were in the normal group.

COVID-19 ICU female staff were found to have higher depression values than male (p=0.012). While the effect of working in COVID-19 ICU or ICU on depression values was insignificant in male workers (p=0.405), depression levels of female working in COVID-19 ICU were higher than female healthcare workers working in ICU (p=0.006). Among the personnel of COVID-19 ICU, depression levels of nurses were found to be the highest, while doctors' levels were found to be the lowest (p=0.013). According to the classification of DASS-21 depression severity (normal, mild, moderate, severe, extremely severe), working in the COVID-19 ICU had a significant effect on the depression levels of the staff (p=0.012, Table 4). It was determined that both COVID-19 ICU and ICU female staff had higher anxiety values than males (p<0.001, p=0.028, respectively). COVID-19 ICU female staff had higher anxiety levels than ICU female staff (p=0.007). On the other hand, the effect of working in COVID-19 ICU or ICU on anxiety levels of male staff was insignificant (p=0.134). While the effect of occupational titles on the anxiety values of COVID-19 ICU staff was significant (p=0.002), the effect on the anxiety values of ICU staff was found to be insignificant (p=0.660). Among the COVID-19 ICU staff, nurses had the highest anxiety score (Table 3). The relationship between the distribution of the DASS-21 anxiety severity classification and the type of intensive care unit (COVID-19 ICU or ICU) was not statistically significant (p=0.302, Table 4).

Female staff in COVID-19 ICU had higher stress levels than males (p=0.002). There was no difference between males and females working in ICU in terms of stress level (p=0.958). Female staff in COVID-19 ICU had higher stress levels than ICU female staff (p<0.001). The effect of the type of intensive care unit on the stress levels of male staff was insignificant (p=0.241). Nurses had the highest stress levels amongst COVID-19 ICU staff (p=0.018), on the other hand, doctors were the most stressful among ICU staff (p=0.017). COVID-19 ICU nurses had higher stress levels than ICU nurses (p<0.001). The stress levels of COVID-19 ICU personnel with 0-5 years of working experience, and 6-10 years of working experience were significantly higher than the ICU personnel with the same working years (p=0.018, p=0.001, respectively, Table 3). According to the DASS-21 classification of depression severity, working in the COVID-19 ICU had a significant effect on the stress levels of the staff (p=0.008, Table 4).

Fear of COVID-19, Depression, Anxiety and Stress

The scores of FCV-19S and DASS-21 scales and group comparisons are given in Table 5. The FCV-19S score of the COVID-19 ICU staff group was significantly higher than the ICU staff group (p=0.022). DASS-21 depression, anxiety, and stress levels were significantly higher in the COVID-19 ICU staff group than in the ICU group (p=0.011, p=0.002, p<0.000, respectively).

Relationship between Fear of COVID-19 and Depression, Anxiety and Stress Reactions

Spearman's rho coefficients showing the relationship between fear of COVID-19 and DASS-21 depression, anxiety, and stress values were summarized in Table 6. Increasing fear of COVID-19 caused an increase in DASS-21 depression, anxiety, and stress values of ICU workers. The relationship between fear of COVID-19 and depression (ρ=0.399, p=0.044), anxiety (ρ=0.456, p=0.019), stress (ρ=0.418, p=0.033) is positive and significant.

COVID-19 ICU staff who may have high-risk contact were 3.26 times more likely to experience depression (OR=3.26, 95% CI=1.595-6.692, p=0.001), 1.98 times anxiety (OR=1.98, 95% CI=1.010-3.892, p=0.047) and 3.61 times stress (OR=3.61, 95% CI=1.688-7.750, p=0.001) than ICU staff (Table 7).

DISCUSSION

In the current study, we aimed to compare the COVID-19 fear experienced by COVID-19 ICU staff and ICU staff and the effects of this fear on mental health. The study has three main contributions to the literature. First of all, our study; revealed that COVID-19 ICU staff working at the forefront of care and treatment of COVID-19 patients had higher levels of COVID-19 fear, depression, anxiety, and stress than ICU staff. We found a significant positive correlation between fear of COVID-19 and participants' depression, anxiety, and stress levels. Second, female gender, being a nurse, and working in COVID-19 ICU were associated with higher depression, anxiety, and stress scores. Finally, it revealed that as compared to ICU staff, COVID-19 ICU staff were approximate twice times more likely to experience anxiety and fear of COVID-19 and 3.5 times more likely to suffer from depression and stress.

The first COVID-19 case in Turkey was detected on March 9, 2020. The first death associated with COVID-19 was seen on March 17, 2020 (14). The Ministry of Health announced the daily status report of COVID-19 as of July 1, 2020. Turkey reported the daily number of new cases as 1 198, the number of newly hospitalized patients as 688, and the number of newly intubated patients as 52 (15). The Government of the Republic of Turkey took some national measures to prevent the spread of the disease: They gradually suspended flights to risky countries. They banned foreign nationals from entering the country. For cases with suspected infection, 14 days of isolation and symptom follow-up were enforced. Administrative leave was granted for staff with chronic illness. They restricted face-to-face education in schools. Temporarily stopped the work of recreation and entertainment venues. They reorganized the way institutions such as dormitories and nursing homes work. They took measures for public transportation and intercity buses. A weekend curfew was imposed in the country. Non-urgent surgical interventions were postponed. Besides scientific studies have been started on subjects such as virus isolation, vaccine, and drug therapy (14). In Turkey, COVID-19 vaccine applications started on January 14, 2021, with the approval of the Ministry of Health, primarily for healthcare workers. As of July 2020, when we conducted the research, vaccination of healthcare workers had not yet started (16). Restrictions affecting the flow of daily life and being unvaccinated may have negatively affected the fears and mental health of intensive care personnel.

When the current literature is examined, there are clinical studies conducted in different countries evaluating the fear, anxiety, depression, and stress levels of healthcare workers during the COVID-19 pandemic and related factors (6,7,10,11,17-20). Ahorsu et al. (10) evaluated the effects of fear of COVID-19 on the mental health of the Iranian population. They showed that there was a significant positive correlation between fear of COVID-19 and depression and anxiety. COVID-19 fear levels of

Table 4. DASS-21 score distribution according to COVID-19 ICU and ICU

	COVID-19 ICU	ICU	p
Depression, n (%)			
Normal	48 (53.3%)	53 (80.3%)	
Mild	6 (6.7%)	2 (3.0%)	
Moderate	20 (22.2%)	3 (4.6%)	**0.012**
Severe	8 (8.9%)	7 (10.6%)	
Extremely severe	8 (8.9%)	1 (1.5%)	
Anxiety, n (%)			
Normal	52 (57.8%)	49 (74.2%)	
Mild	16 (17.8%)	6 (9.1%)	
Moderate	12 (13.3%)	6 (9.1%)	0.302
Severe	3 (3.3%)	2 (3.0%)	
Extremely severe	7 (7.8%)	3 (4.6%)	
Stress, n (%)			
Normal	52 (57.8%)	55 (83.3%)	
Mild	17 (18.9%)	6 (9.1%)	
Moderate	11 (12.2%)	2 (3.0%)	**0.008**
Severe	10 (11.1%)	3 (4.6%)	
Extremely severe	0 (0.0%)	0 (0.0%)	

DASS-21: depression anxiety stress scale, COVID-19 ICU: COVID-19 intensive care unit, ICU: general intensive care unit

Table 5. Comparison the COVID-19 fear, depression, anxiety and stress between COVID-19 ICU and ICU staff

	COVID-19 ICU	ICU	p
FCV-19S	21 (17-25)	19 (12-23)	**0.022**
DASS-21			
Depression	6 (2-16)	4 (2-8)	**0.011**
Anxiety	4 (2-10)	2 (0-6)	**0.002**
Stress	12 (7-17)	8 (4-12)	**<0.001**

COVID-19 ICU: COVID-19 intensive care unit, ICU: general intensive care unit, FCV-19S: fear of COVID-19 scale, DASS-21: depression anxiety stress scale

Table 6. Correlation coefficients between COVID-19 fear and DASS-21 depression, anxiety, and stress scores

	FCV-19S	p
DASS-21		
Depression	0.399	**0.044**
Anxiety	0.456	**0.019**
Stress	0.418	**0.033**

DASS-21: depression anxiety stress scale, FCV-19S: fear of COVID-19 scale

Table 7. Multivariate analysis of COVID-19 fear, depression, anxiety and stress between COVID-19 ICU and ICU

Variables	OR (95% CI)	p
FCV-19S		
ICU	1	
COVID-19 ICU	1.65 (0.986-2.452)	0.077
DASS-21 Depression		
ICU	1	
COVID-19 ICU	3.26 (1.595-6.692)	**0.001**
DASS-21 Anxiety		
ICU	1	
COVID-19 ICU	1.98 (1.010-3.892)	**0.047**
DASS-21 Stress		
ICU	1	
COVID-19 ICU	3.61 (1.688-7.750)	**0.001**

COVID-19 ICU: COVID-19 intensive care unit, ICU: general intensive care unit, OR: odds ratio, CI: confidence interval, FCV-19S: fear of COVID-19 scale, DASS-21: depression anxiety stress scale

university students and graduates in Russia and Belarus Ahorsu et al. (10) slightly less than his work (19). In the study by García-Reyna et al. (20) where COVID-19 fear levels of Mexican medical staff were investigated, the FCV-19S median score was 19. In this study, they found that females had higher FCV-19S scores than males. In our study, we found a significant positive correlation between fear of COVID-19 and the depression, anxiety, and stress levels of intensive care workers. COVID-19 ICU staff had increased levels of COVID-19 fear compared to ICU staff. Similar to previous studies, we found that female staff members had higher COVID-19 fear levels than males. Previous studies had analyzed COVID-19 fear levels of the general population or healthcare professionals in a low-risk area. Interestingly, although our study included an intensive care unit with high-risk contact, similar to previous studies, we detected a moderate level of fear. The cause for this can be due to the relatively low incidence of COVID-19 cases in our region during the time we conducted the research. Also, it can be attributed to the effective execution of the COVID-19 pandemic preparation phase in our hospital and our country from March to July, when the first cases were recorded (21).

Previous pandemics have had adverse effects on the mental health of healthcare workers. Mental distress of personnel working in high-risk units such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome-coronavirus (MERS-CoV) intensive care units have been associated with higher stress, anxiety, and fear than those working in low-risk hospital areas (2,17). In our study, working in a high-risk COVID-19 ICU unit resulted in a significant increase in the COVID-19 fear, depression, anxiety, and stress levels of employees compared to the ICU unit. Lai et al. (17) reported that during the initial period of the COVID-19 outbreak, healthcare workers in China felt depression, anxiety, and stress (50%, 44%, and 71%, respectively). During the COVID-19 pandemic Chew et al. (18) discovered moderate to very severe depression, anxiety, and stress (50%, 55%, and 42%, respectively) in healthcare workers in Singapore and India. In our study, we found that 40% of COVID-19 ICU staff suffered from moderate to very severe depression, and 24% from anxiety and stress. Mental distress levels in our study were lower than those of previously reported studies (17,18). We believe that there may be several reasons for this; increased mental preparedness of staff, the effectiveness of infection control measures, and reduced uncertainty at the onset of the pandemic. Greenberg et al. (22) investigated the mental status of COVID-19 intensive care staff in June-July 2020. They reported that 59% of the staff were in good mental health. Similarly, we found that more than half of the COVID-19 ICU staff had no mental health problems. However, they reported that 45% of the staff experienced at least one of post-traumatic stress disorder 40%, severe depression 6%, severe anxiety disorder 11%, and alcohol consumption problems 7%. The surveys we used were different from the ones they used. Nevertheless, our results showed that COVID-19 ICU staff suffered from higher severe to very severe depression, and our rates of stress and anxiety were similar (42% stress, severe to very severe depression, and anxiety disorder 17% and 11%, respectively).

In the SARS outbreak, Styra et al. (2) had found that the gender factor did not affect the mental health of healthcare professionals. In contrast, in the study conducted in the MERS-CoV outbreak, female nurses had significantly higher levels of anxiety and fear compared to their male counterparts (23). Another study conducted during the COVID-19 pandemic showed that gender affects mental health, especially nurses and females, experiencing more symptoms of depression, anxiety, insomnia, and distress (17). In our study; when both ICU staff were evaluated, it was found that the fear, depression, anxiety, and stress scores of females were higher than males. However female staff in COVID-19 ICU was more likely to experience mental distress than the ICU group. Previous studies have suggested that fluctuations in female's estrogen and progesterone levels may be the source of mood and anxiety disorders. Studies have revealed that females are more likely to experience psychological problems during periods of hormonal change (puberty, menopause, perimenstrual, and postpartum). Studies on males have shown that testosterone is a protective factor against anxiety and depression (24). It would be incomplete to look at this issue from a purely biological point of view. We think that these three structures will have an impact on mental health during the pandemic process since humans have biological, psychological, and social characteristics. Quarantine and social restrictions can trigger psychological distress (25). In addition, other studies have shown that females increased burden of unpaid labor and care in the household (daily housework, care of children, distance education process, care for family members during curfew, hygiene management, etc.) may be associated with poor mental health during the pandemic process (26).

In the previous SARS outbreak, Styra et al. (2) reported that nurses' risk of emotional distress was higher than other healthcare workers. Similarly, studies conducted during the COVID-19 pandemic have shown that nurses' psychological distress levels are higher than other healthcare professionals (22,27). They also reported that in addition to being a nurse, the female gender also significantly increased psychological distress (17,27). In our study, similar to previous studies, being a nurse, and female gender increased the depression, anxiety, and stress levels of COVID-19 ICU staff. The reason for the prevalence of mental distress among nurses may be that they play a front-line role in providing treatment and care for the patient. Nurses' working periods in the isolation department are longer than doctors and their direct contact with the patient can easily lead to fatigue and tension (27). Styra et al. (2) found that in the SARS epidemic, the work experience did not affect the mental status of healthcare workers. In our study, the anxiety and stress levels of the personnel with less than 10 years of working experience in COVID-19 ICU were slightly higher than the ICU group.

Pandemic is a long process. The incidence of the disease varies depending on the localization in different periods. Our study is a cross-sectional study conducted in the specified period and it coincides with the end of the first wave of the pandemic in our region. It should be taken into consideration that the scores we have determined may vary in different periods of the pandemic. Although there are differences between ICU staff characteristics and work experiences, it may be a recommendation.

The success of newly developed treatment strategies and vaccination studies will also have an impact on fear and

mental health. Therefore, we planned to repeat our research as the outbreak progressed. We intend to re-evaluate the results of this study, which contributes to the current literature, with the results of our next study.

CONCLUSION

This study provides insight into the adverse mental effects of the COVID-19 pandemic on frontline intensive care staff. Increasing fear of COVID-19 led to an increase in intensive care workers' depression, anxiety, and stress values. The mental well-being of females, and nurses, working in the COVID-19 ICU should be protected to sustain the health workforce efficiently for a long time. Psychological support should be provided for intensive care staff. We think that this study will contribute to the Ministry of Health and hospital administrators to gain awareness of the mental problems of intensive care staff and to take corrective measures.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of the Kırşehir Ahi Evran University (24.06.2020, 09-63).

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Investigation of the Usability of CT in Clinical Decision Making by Comparing COVID-19 Positive and Probable Patients Diagnosed According to CT Imaging Findings

BT Görüntüleme Bulgularına Göre Tanı Alan COVID-19 Pozitif ve Olası Hastaları Karşılaştırarak Klinik Karar Vermede BT'nin Kullanılabilirliğinin Araştırılması

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ABSTRACT

Aim: In this study, the usability of thoracic computed tomography (CT) in clinical decision making was investigated by comparing laboratory results of patients with probable and definite coronavirus disease 2019 (COVID-19) diagnosis according to CT imaging features.
Material and Methods: Within the scope of this single-center retrospective clinical study, data of possible and definite cases of COVID-19 were scanned from the hospital electronic database and patient files. Laboratory and CT imaging results of the patients were obtained. Patients were divided into two groups as positive and negative according to their CT imaging results, and compared.
Results: Of the 995 patients included in the study, 57% (n=567) were male, and the mean age was 45.7±20.2 years. It was found that 65.1% (n=648) of the patients had positive CT. Real-time polymerase chain reaction (RT-PCR) test result was found positive in 22.2% (n=144) of the CT positive patients, and 32.0% (n=111) of the CT negative patients, and it was statistically significant (p<0.001). In the logistic regression analysis, it was determined that C-reactive protein (CRP), lymphocyte count, ferritin, procalcitonin, D-dimer, lactate and RT-PCR were statistically significant with CT positivity.
Conclusion: In this study, COVID-19 positive and probable patients were compared according to thoracic CT findings and the usability of CT for clinical decision making was investigated. It has been determined that thorax CT can be used to initiate the treatment of COVID-19 in patients with negative RT-PCR test results but positive CT findings and high biochemical parameters such as CRP, D-dimer, ferritin and lactate.
Keywords: COVID-19; computed tomography; SARS-CoV-2; pneumonia; pandemic.

ÖZ

Amaç: Bu çalışmada bilgisayarlı tomografi (BT) görüntüleme özelliklerine göre olası ve kesin koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) tanısı alan hastaların laboratuvar sonuçları karşılaştırılarak, klinik karar verme sürecinde torasik BT'nin kullanılabilirliği araştırılmıştır.
Gereç ve Yöntemler: Tek merkezli retrospektif klinik çalışma kapsamında olası ve kesin COVID-19 vakalarının verileri hastanenin elektronik veri tabanından ve hasta dosyalarından taranmıştır. Hastaların laboratuvar ve BT görüntüleme sonuçları elde edilmiştir. Hastalar BT görüntüleme sonuçlarına göre pozitif ve negatif olmak üzerek iki gruba ayrılmış ve karşılaştırılmıştır.
Bulgular: Çalışmaya dahil edilen 995 hastanın %57'si (n=567) erkekti ve hastaların yaş ortalaması 45,7±20,2 yıl idi. Hastaların %65,1'inde (n=648) BT pozitifliği saptandı. Gerçek zamanlı polimeraz zincir reaksiyonu (real-time polymerase chain reaction, RT-PCR) testi sonucu BT pozitif hastaların %22,2'sinde (n=144) ve BT negatif hastaların %32'sinde (n=111) pozitif olarak bulundu ve istatistiksel olarak anlamlı idi (p<0,001). Lojistik regresyon analizinde C-reaktif protein (CRP), lenfosit sayısı, ferritin, prokalsitonin, D-dimer, laktat ve RT-PCR'nin BT pozitifliği ile istatistiksel olarak anlamlı olduğu tespit edildi.
Sonuç: Bu çalışmada, torasik BT bulgularına göre COVID-19 pozitif ve olası hastalar karşılaştırılmış ve BT'nin klinik karar verme amaçlı kullanılabilirliği araştırılmıştır. RT-PCR test sonucu negatif ancak BT bulguları pozitif olan ve CRP, D-dimer, ferritin ve laktat gibi yüksek biyokimyasal parametreleri olan hastalarda, COVID-19'un tedavisine başlanmasında toraks BT'nin kullanışlı olduğu tespit edilmiştir.
Anahtar kelimeler: COVID-19; bilgisayarlı tomografi; SARS-CoV2; pnömoni; pandemi.

INTRODUCTION

Coronaviruses are large, enveloped, positive single-stranded RNA viruses that are transmitted from animals to humans. Seven subtypes of these viruses cause serious illness and death in humans (1). Coronaviruses have led to two major pandemics in recent years; severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (1). Today, the novel agent was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as the causative of coronavirus disease 2019 (COVID-19) (2). COVID-19 was seen for the first time in Wuhan, China, in December 2019 and spread all over the world in a short time (3,4). The World Health Organization (WHO) declared this epidemic as a global health emergency and declared as a pandemic in March 2020 (5). In Turkey, the first COVID-19 positivity was seen on March 11th, 2020 (6).

The first case of COVID-19 associated with SARS-CoV-2 was identified as pneumonia. In subsequent follow-ups, it was determined that the disease appeared with many clinical symptoms and even in some people the disease could be asymptomatic. In symptomatic patients, clinical signs of the disease mostly, but not only consist of upper respiratory tract infection symptoms such as fever, cough, fatigue, common muscle and joint pain, and nasal congestion. Moreover, the patients can present with a wide spectrum of other clinical symptoms such as shortness of breath, headache, gastrointestinal symptoms and progressive respiratory failure (1,2). Pneumonia is usually seen 2-3 weeks after the symptoms appear. As in viral pneumonias, lymphopenia is common in COVID-19 and inflammatory markers such as C-reactive protein (CRP) and cytokines are elevated. In imaging of the disease, findings such as ground glass appearance, irregular consolidations, linear opacities, and pleural effusion are seen in computed tomography (CT) (7,8).

Real-time polymerase chain reaction (RT-PCR) analysis is routinely used to detect acute respiratory pathogens, and COVID-19 is diagnosed with this method (9). Despite the high specificity of this test, it has been observed that it frequently causes false negative results to be reported due to the incorrect and inappropriate swab samplings (7,10). To overcome this situation, the use of the thoracic CT imaging to detect the presence of pulmonary disease in patients with suspected COVID-19, may avoid delaying the chance of diagnosis and treatment in these patients, regardless of the RT-PCR test result. For this purpose, it was aimed to compare the sociodemographic, clinical and laboratory characteristics of the patients according to thoracic CT findings of possible/definite cases of COVID-19.

MATERIAL AND METHODS

Study Design and Setting

This study is a retrospective clinical study examining possible/definite patients with COVID-19. This study was conducted in a tertiary university hospital in Erzurum, Turkey. The study was carried out between 01.06.2020-01.10.2020. The required permission for the study was obtained from the Scientific Research Platform of the Ministry of Health, General Directorate of Health Services, and then the approval of the local ethics committee (28.05.2020, 06/15). Our study was conducted in accordance with the Declaration of Helsinki.

Patients

Clinical and epidemiological data of patients diagnosed with COVID-19 were obtained from the hospital electronic data system and file scanning. These patients consisted of patients who applied to the COVID-19 polyclinics established in our hospital during the pandemic period. The records of patients with symptoms (such as cough, fever, shortness of breath, headache, sore throat, muscle-joint pains, diarrhea and nausea-vomiting) who were pre-diagnosed or definitively diagnosed with COVID-19 by RT-PCR test were scanned according to ICD-10 codes. Patients with these symptoms who were not tested for COVID-19 by RT-PCR tests were excluded from the study. In addition, patients younger than 18 years of age and patients with insufficient medical data were excluded.

In our hospital, the swab samples from COVID-19 probable/definite patients were taken by nasopharyngeal swap sampling and the diagnosis was made by RT-PCR analysis. RT-PCR analyzes were performed in the reference laboratory of the Ministry of Health. Furthermore, in our hospital, patients with pulmonary symptoms and signs with possible/definite cases of COVID-19 were mostly scanned with thoracic CT. Thorax CTs were reported according to Radiological Society of North America expert consensus document on reporting chest CT findings related to COVID-19 (8).

The RT-PCR test results of the patients at the first admission to the hospital were included in the study and evaluated. According to the Ministry of Health COVID-19 guideline, patients with positive RT-PCR test are considered as definite cases, patients with negative RT-PCR tests as possible cases (11). For the study, thorax CT reports of the patients were scanned from the hospital data system, and if there were more than one, only first CTs of the patients were included in the study. The patients whose thorax CT report were compatible with COVID-19 disease, constituted the CT positive group, and the patients who were not compatible, constituted the CT negative group.

According to the inclusion and exclusion criteria of the study, 1261 patients were examined as probable/definite cases of COVID-19. Of these patients, 266 persons were excluded from the study because thorax CT was not performed. As a result, 995 patients who had RT-PCR results with nasopharyngeal swap were included in the study. Sociodemographic data such as age, gender, comorbidities, and laboratory test results, thorax CT reports and RT-PCR results of the patients were compared and analyzed.

Test Methods

Biochemical test levels of the patients included in the study were studied by chemical immunoassay method with Unicel DXI 600 Access Immunoassay System device (Beckman Coulter, Porterville, CA, USA). Swab samples were studied with the Bio-Rad CFX96 TouchTM Real-Time PCR device (Agilent Technologies, Inc. US) in the reference laboratory and the Bio-Speedy® SARS-CoV-2 + VOC202012 / 01 RT-qPCR kit (Bioeksen R&D Technologies, Inc. Turkey) in the reference laboratory.

Statistical Analysis

Statistical analyzes were performed by using SPSS v.25.0 program (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA).

Kolmogorov-Smirnov test was used to evaluate normal distribution. Categorical variables were defined as frequencies and percentages. Continuous variables were defined by using mean and standard deviation if normally distributed, and median and interquartile range (IQR) values if not normally distributed. For the comparison of continuous variables, Student's t-test was used if the data were normally distributed, and the Mann-Whitney U test was used if it was not normally distributed. Categorical variables were compared using the Pearson's Chi-squared test if the minimum expected number was greater than 25; Fisher's exact test if the minimum expected count is less than 5; or Continuity correction was used if the minimum expected count is between 5 and 25. Logistic regression analysis was used to determine independent predictors of thoracic CT positive patients. For whole study, $p<0.05$ was considered as statistically significant.

RESULTS

Of the 995 patients included in the study, the mean age of the patients was 45.7±20.2 years, and 57% (n=567) were male. The sociodemographic characteristics and clinical characteristics of the groups are shown in Table 1. It was found that 65.1% (n=648) of the patients had positive CT. The mean age was 46.59±20.52 years in CT positive group, and 44.02±19.38 years in CT negative group and it was not statistically significant (p=0.055). Similarly, when CT positivity was compared with the gender of the patients, no statistically significant difference was found in terms of gender (p=0.761). When the patients admitted to the hospital were examined and compared in terms of whether they were CT positive or not, it was seen that the patients with low oxygen saturation level (p=0.003), and with the comorbidities together with hypertension + diabetes + chronic renal failure (p=0.017) were found to be statistically significant, whereas other physical examination findings and presence of comorbidities were not statistically different between CT groups.

The comparison of the laboratory findings of the groups is given in Table 2. Accordingly, it was found that CRP, D-dimer, ferritin, lactate values were high in the CT positive group, and they were statistically significant (all $p<0.001$). Alanine aminotransferase (ALT) value was found to be low and statistically significant (p=0.038). In addition, the RT-PCR test result was found to be positive in 22.2% (n=144) of the CT positive patients and 32.0% (n=111) of the CT negative patients and it was found to be statistically significant ($p<0.001$).

The logistic regression analyses were performed by applying all independent and categorical factors, in terms of sociodemographic, clinical characteristics and laboratory findings and CT positivity of the patients included in the study. Logistic regression was performed using the Enter model. The independent predictors with significant difference obtained as a result of logistic regression analyses are presented in Table 3, but other insignificant data were discarded and not presented.

Accordingly, it was determined that CRP (Odds Ratio (OR)=1.201, 95% CI=1.024-1.432, $p<0.001$), lymphocyte count (OR=1.917, 95% CI=1.155-3.183, p=0.012), ferritin (OR=1.001, 95% CI=1.001-1.002, $p<0.001$), procalcitonin (OR=0.957, 95% CI=0.917-0.998, p=0.039), D-dimer (OR=1.001, 95% CI=1.001-1.001, p=0.019), lactate (OR=1.303, 95% CI=1.067-1.592, p=0.009) and RT-PCR (OR=1.646, 95% CI=1.229-2.204, p=0.001) were statistically significant with CT positivity.

Table 1. Comparison of demographic and clinical characteristics of patients with positive and negative CT findings

	CT Positive (n=648)	**CT Negative (n=347)**	**p**
Age (years), mean±SD	46.59±20.52	44.02±19.38	0.055[a]
Gender (male), n (%)	367 (56.6)	200 (57.6)	0.761[b]
Comorbidity, n (%)			
Absent	348 (53.7)	183 (52.7)	0.771[b]
COPD	35 (5.4)	12 (3.5)	0.169[b]
CAD	30 (4.6)	20 (5.8)	0.435[b]
HT	14 (2.2)	9 (2.6)	0.832[c]
DM	21 (3.2)	19 (5.5)	0.087[b]
Malignancy	26 (4.0)	10 (2.9)	0.464[c]
CRF	8 (1.2)	4 (1.2)	0.999[d]
Autoimmune diseases	1 (0.2)	2 (0.6)	0.280[d]
HT + DM	31 (4.8)	10 (2.9)	0.204[c]
COPD + HT	8 (1.2)	6 (1.7)	0.577[d]
HT + CAD	20 (3.1)	8 (2.3)	0.611[c]
CAD + DM	18 (2.8)	10 (2.9)	0.999[c]
COPD + CAD	7 (1.1)	7 (2.0)	0.263[d]
HT + DM + CAD	28 (4.3)	12 (3.5)	0.623[c]
HT + DM + CRF	14 (2.2)	18 (5.2)	**0.017**[c]
COPD + CAD + DM	8 (1.2)	6 (1.7)	0.577[d]
COPD + HT + DM + CRF	31 (4.8)	11 (3.2)	0.298[c]
Physical Examination on Arrival, median (IQR) [min-max]			
Systolic blood pressure (mmHg)	134 (12) [124-143]	135 (14) [123-150]	0.062[e]
Diastolic blood pressure (mmHg)	79 (6) [74-89]	81 (7) [77-90]	0.125[e]
Heart rate (per minute)	86 (11) [68-98]	90 (13) [70-101]	0.464[e]
Fever (°C)	36.4 (0.5) [36.2-36.8]	36.3 (0.6) [36.2-36.7]	0.143[e]
Respiratory rate (per minute)	18 (5) [14-25]	17 (4) [15-27]	0.245[e]
Oxygen saturation level (%)	81 (8) [72-97]	92 (6) [86-99]	**0.003**[e]

CT: computed tomography, SD: standard deviation, COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, HT: hypertension, DM: diabetes mellitus, CRF: chronic renal failure, IQR: interquartile range, [a]: Student's t-test, [b]: Pearson chi-square test, [c]: continuity correction test, [d]: Fisher's exact test, [e]: Mann-Whitney U test

Table 2. Comparison of the laboratory findings of patients with positive and negative CT findings

Median (IQR) [min-max]	CT Positive (n=648)	CT Negative (n=347)	p
WBC ($\times10^3/\mu L$)	7.5 (5.9) [2.5-35.7]	8.5 (6.1) [2.0- 26.5]	0.710[a]
Neutrophil count ($\times10^3/\mu L$)	5.0 (6.0) [0.3-30.0]	5.2 (6.3) [0.0-23.8]	0.817[a]
Lymphocyte count($\times10^3/\mu L$)	1.6 (1.6) [0.0-13.8]	1.6 (1.6) [0.0-10.9]	0.379[a]
C-reactive protein (mg/L)	84.3 (14.2) [5.3-212.5]	16.7 (8.4) [3.1-82.3]	**<0.001[a]**
Albumin (g/dl)	3.34 (0.7) [3.28-3.92]	3.35 (0.8) [3.32-4.05]	0.425[a]
GGT (U/L)	14.0 (15.5) [8.0-899.0]	14.0 (19.0) [8.0-859.0]	0.948[a]
CK (U/L)	87.0 (51.0) [10.0-9354.0]	73.0 (37.0) [14.0-1065.0]	0.394[a]
AST (U/L)	24.0 (14.0) [10.0-1111.0]	25.0 (21.0) [7.0-714.0]	0.240[a]
ALT (U/L)	18.0 (23.0) [9.0-976.0]	19.0 (34.0) [9.0-882.0]	**0.038[a]**
LDH (U/L)	260.0 (114.0) [147.0-4014.0]	263.0 (116.0) [150.0-1190.0]	0.211[a]
Total bilirubin (mg/dL)	0.51 (0.54) [0.12-27.25]	0.50 (0.57) [0.13-3.90]	0.400[a]
Direct bilirubin (mg/dL)	0.11 (0.12) [0.00-17.48]	0.13 (0.14) [0.02-1.86]	0.516[a]
Glucose (mg/dL)	99.0 (40.0) [69.0-591.0]	100.0 (36.0) [76.0-501.0]	0.223[a]
BUN (mg/dL)	13.6 (8.9) [6.1-166.4]	13.1 (5.6) [6.5-106.5]	0.261[a]
Creatinine (mg/dL)	0.88 (0.36) [0.25-8.06]	0.78 (0.39) [0.26-7.06]	0.099[a]
Na (mmol/L)	138.0 (6.0) [129.0-167.0]	138.0 (6.0) [123.0-158.0]	0.870[a]
K (mmol/L)	3.9 (0.7) [3.1-5.8]	3.9 (0.6) [3.2-5.4]	0.399[a]
Cl (mmol/L)	102.0 (3.0) [89.0-127.0]	102.0 (3.0) [88.0-116.0]	0.841[a]
Troponin (ng/L)	7.1 (11.1) [0.3-2208.7]	7.2 (7.0) [0.4-299.6]	0.155[a]
D-dimer (ng/mL)	821.0 (1442.5) [37.0-10435.0]	568.0 (662.0) [61.0-6360.0]	**<0.001[a]**
Ferritin (ng/mL)	1780.0 (2057.0) [1100.0-13185.0]	196.0 (533.0) [7.0-2858.0]	**<0.001[a]**
Procalcitonin (ng/mL)	(n=325) 0.1 (0.7) [0.0-84.6]	(n=192) 0.1 (0.7) [0.0-831.0]	0.118[a]
Lactate (mmol/L)	1.6 (1.1) [0.6-12.4]	1.2 (0.8) [0.6-6.1]	**<0.001[a]**
RT-PCR test positive, n (%)	144 (22.2)	111 (32.0)	**<0.001[b]**

CT: computed tomography, WBC: white blood cell count, GGT: gamma glutamyl transferase, CK: creatine kinase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, BUN: blood urea nitrogen, Na: Sodium, K: Potassium, Cl: Chlorine, IQR: interquartile range, [a]: Mann-Whitney U test, [b]: Pearson chi-square test

Table 3. Logistic regression analysis results for CT positive patients

	OR	95% Cl	p
C-reactive protein	1.201	1.024-1.432	**<0.001**
Lymphocyte count	1.917	1.155-3.183	**0.012**
Ferritin	1.001	1.001-1.002	**<0.001**
Procalcitonin	0.957	0.917-0.998	**0.039**
D-dimer	1.001	1.001-1.001	**0.019**
Lactate	1.303	1.067-1.592	**0.009**
RT-PCR	1.646	1.229-2.204	**0.001**

OR: odds ratio, CI: confidence interval, Hosmer and Lemeshow test $p<0.001$; $\chi^2=41.218$, $p<0.001$, percentage correct=80.6%, Nagelkerke R square: 0.412

DISCUSSION

In this study, sociodemographic, clinical and laboratory characteristics of probable and definite cases were compared according to the positive and negative thorax CT findings according to the COVID-19 guideline of the Ministry of Health in Turkey. The first thorax CT reports of all patients included in the study were evaluated and compared with the RT-PCR test results and laboratory test results of the patients. CRP, D-dimer, ferritin and lactate values were found to be higher in the CT positive group compared to the CT negative group. RT-PCR positivity was higher in the CT negative group.

Furthermore, low lymphocyte count and procalcitonin levels, increased CRP, ferritin, D-dimer, lactate levels and negative RT-PCR test results were found as independent predictors for CT positivity in the logistic regression model created with the CT positive group. However, other laboratory tests, demographic features and comorbidities of the patients were not found to be statistically significant. Among these results, the detection of PCR negativity and low procalcitonin levels as predictors for CT positivity were very interesting results. This reveals the importance of screening for CT positivity, especially in patients with negative PCR test results and negative procalcitonin levels.

The RT-PCR test results obtained from nasopharyngeal, oropharyngeal or swap samples from lower respiratory tract such as sputum, tracheal aspirate, or bronchoalveolar lavage are considered variable and potentially unstable (10,12). RT-PCR is the primary method for the diagnosis of COVID-19, but it can cause false negativity (7,13,14). In a study in which Li et al. (10) investigated the positivity of the RT-PCR test in patients diagnosed with COVID-19 clinically, they emphasized that there was a high rate of false negativity in the RT-PCR test. According to the results of the RT-PCR test, which is commonly used in the world, the isolation, discharge or transfer of COVID-19 patients are performed. The isolation of the patient with false negativity can be terminated or discharged. Therefore, even if the RT-PCR result is negative, clinical status and radiological imaging should guide clinical decision-making about these patients. In this study, the negative RT-PCR test results of the majority of patients with positive thoracic CT findings support the necessity of CT in decision-making.

Thorax CT is a useful method that detects changes in the lungs at an early stage and plays an important role in the evaluation and management of COVID-19 patients (15). Even thorax CT findings can be seen before RT-PCR positivity (8,16). In addition, thorax CT is used both for diagnosis and to determine the severity of the disease (17,18). In a meta-analysis on the thoracic CT findings of COVID-19, due to the variability of the RT-PCR test positivity, it is recommended that clinicians perform CT scans and combine with RT-PCR to detect high probability COVID-19 patients (19).

In the study of Song et al. (20) comparing the thoracic CT findings and clinical features of 211 COVID-19 patients, 163 patients were diagnosed with viral pneumonia by thoracic CT. It was determined that 66.3% (n=108) of these patients were RT-PCR positive, and 33.7% (n=55) were RT-PCR negative. They were also emphasized that thorax CT has a high sensitivity compared to the RT-PCR test in COVID-19. In our study, 65.1% (n=648) of the patients had signs of COVID-19 disease pneumonia in CT, and only 22.2% (n=144) of these patients were RT-PCR positive. In other words, although 225 of the patients had positive RT-PCR test, 648 patients had CT positivity. This means that although the RT-PCR test is widely used for diagnosis, the diagnosis of COVID-19 disease will increase with thoracic CT scan and these symptomatic patients will be caught, especially in RT-PCR negative patients.

In a study conducted by Alanli et al. (21) in 114 patients in Turkey, the compatibility of PCR with thoracic CT findings was investigated and they found similar imaging changes in both PCR negative and PCR positive groups. Since the study group was smaller than our study, also, they did not grade CT findings with radiological evaluations. In our study, CT findings of 995 patients were classified and graded according to the North American expert consensus document of the Society of Radiology. After the patients were categorized as CT positive and CT negative, RT-PCR test results were found to be statistically significant between the groups.

Luo et al. (7) investigated the relationship between the thorax CT findings and the clinical course of COVID-19 patients and found that the lymphocytes and CRP levels were higher; white blood cells, neutrophil and albumin levels were lower, and they were statistically significant. They emphasized that this is related to the clinical course of the patients. On the contrary, in our study, lymphocyte levels were lower and neutrophil levels were higher in the CT positive group.

In another study, CT findings were found to be correlated with the severity and duration of the symptoms of COVID-19, and similar to our study, lymphocyte count was found to be negatively correlated with lung involvement (22). Again, there are studies showing that laboratory findings, such as CRP, ferritin, D-dimer, and lactate are elevated in COVID-19 patients and correlate with the disease severity and mortality (4,23-25).

One of the important results of our study is that, by logistic regression analysis, it is determined that low lymphocyte count, CRP, D-dimer, ferritin and lactate elevations in patients can be used to predict the probability of CT positivity in COVID-19 patients. This situation shows us that it is possible to predict lung involvement and disease positivity in COVID-19 by combining these biochemical test results. This is particularly critical for early prediction of COVID-19 and early treatment of patients.

Limitations: Our study has some limitations. The first of these is that our study was single-center and designed retrospectively. Another point is that the second test results of the patients with the first negative RT-PCR test were not included in the study, even if they were performed. The fact that, the RT-PCR tests were mostly repeated after the first test, the subsequent results, even if different from the first, were ignored in our study. The relationship between the CT positivity at the time of first presentation was investigated in patients who were found to be positive for the subsequent tests, would be a further support for our study. As another limitation, the treatments, prognosis and the outcomes of the patients were not investigated and not included in our study. This study focused on the initial diagnosis of COVID-19 to catch the opportunity to start treatment earlier.

CONCLUSION

It is important to detect COVID-19 disease early to start treatment quickly. The possibility of a false negative result of the RT-PCR test result should be considered. For this reason, the diagnostic effectiveness of thoracic CT has been investigated in this study in terms of its usability in clinical decision making. According to the results we have achieved, it has been determined that CT can be used for decision-making in the immediate initiation of treatment due to the possibility of being positive for COVID-19 in patients with negative RT-PCR test results but positive CT evaluation and high biochemical parameters.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Atatürk University Faculty of Medicine (28.05.2020, 06/15).

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Evaluation of Alexithymia, Anger and Temperament Features in Insomnia Patients with Sexual Dysfunction

Cinsel İşlev Bozukluğu Olan İnsomni Hastalarında Aleksitimi, Öfke ve Mizaç Özelliklerinin Değerlendirilmesi

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ABSTRACT

Aim: One of the most common sleep disorders is insomnia, and it is also an independent risk factor related to sexual dysfunction (SD). The aim of the present study was to investigate the anger parameters, temperament parameters, and alexithymia in insomnia patients with SD.
Material and Methods: The study group consisted of 92 patients diagnosed with insomnia according to the third edition of the International Classification of Sleep Disorders. The sociodemographic data form, Temperament Evaluation of Memphis, Pisa, Paris and San Diego Auto-questionnaire (TEMPS-A), Insomnia Severity Index (ISI), Toronto Structured Interview for Alexithymia (TSIA), Arizona Sexual Experiences Scale (ASEX), Pittsburgh Sleep Quality Index (PSQI), State-Trait Anger Expression Inventory (STAXI) were applied to the patients.
Results: While 62 patients had SD, 30 patients had no SD. ISI, PSQI, anger in score were significantly higher in patients with SD (p=0.048, p=0.007, p=0.032, respectively). While depressive and anxious temperament was significantly higher in patients with SD (p=0.026, p=0.008, respectively), hyperthymic temperament was significantly higher in patients without SD (p=0.013). ISI score, depressive, and anxious temperament were significantly correlated with the ASEX score (r=0.214, p=0.041; r=0.261, p=0.012; r=0.286, p=0.007, respectively). Linear regression revealed that depressive, cyclothymic, and irritable temperaments were predictors of ISI (p=0.001). According to logistic regression, hyperthymic temperament was an independent predictor of SD (p=0.001).
Conclusion: Psychological factors should also be considered in studies conducted on the relationship between insomnia and SD. Further research is needed on temperament characteristics, alexithymia and anger issues. Thus, patients can be approached more comprehensively.
Keywords: Sexual dysfunction; insomnia; temperament.

ÖZ

Amaç: İnsomni en yaygın uyku bozukluklarından biridir ve cinsel işlev bozukluğu (CİB) ile ilişkili bağımsız bir risk faktörüdür. Bu çalışmanın amacı CİB olan insomni hastalarında öfke parametrelerini, mizaç parametrelerini ve aleksitimiyi araştırmaktır.
Gereç ve Yöntemler: Çalışmaya uluslararası uyku bozuklukları sınıflandırması üçüncü versiyonuna göre insomni tanısı alan 92 hasta dahil edildi. Hastalara sosyodemografik veri formu, Memphis, Pisa, Paris and San Diego mizaç değerlendirme ölçeği (Temperament Evaluation of Memphis, Pisa, Paris, San Diego Auto-questionnaire, TEMPS-A), insomni şiddet ölçeği (Insomnia Severity Index, ISI), Toronto aleksitimi ölçeği (Toronto Structured Interview for Alexithymia, TSIA), Pittsburgh uyku kalitesi ölçeği (Pittsburgh Sleep Quality Index, PSQI), Arizona cinsel yaşantılar ölçeği (Arizona Sexual Experiences Scale, ASEX) ve durumluk-sürekli öfke ifade ölçeği (State-Trait Anger Expression Inventory, STAXI) uygulandı.
Bulgular: 62 hastada CİB varken, 30 hastada CİB yoktu. CİB olan hastalarda ISI, PSQI ve öfke içeri skoru anlamlı olarak yüksekti (sırasıyla p=0,048, p=0,007, p=0,032). CİB olan hastalarda depresif ve anksiyöz mizaç anlamlı derecede yüksek iken (sırasıyla p=0,026, p=0,008), hipertimik mizaç CİB olmayan hastalarda anlamlı olarak daha yüksekti (p=0,013). ISI skoru, depresif ve anksiyöz mizaç ile ASEX skoru arasında anlamlı korelasyon saptandı (sırasıyla r=0,214, p=0,041; r=0,261, p=0,012; r=0,286, p=0,007). Lineer regresyon analizi depresif, siklotimik ve irritabl mizacın ISI'nın ön gördürücüleri olduğunu ortaya koymuştur (p=0,001). Lojistik regresyon analizine göre hipertimik mizaç, CİB'in bağımsız bir ön gördürücüsüdür (p=0,001).
Sonuç: İnsomni ve CİB ilişkisine dair yürütülen çalışmalarda psikolojik faktörler de göz önünde bulundurulmalıdır. Mizaç özellikleri, aleksitimi ve öfke konularının daha fazla araştırılması gerekmektedir. Böylece hastalara daha kapsamlı yaklaşılabilir.
Anahtar kelimeler: Cinsel işlev bozukluğu; insomni; mizaç.

INTRODUCTION

One of the most common sleep disorders is insomnia. Many factors such as age, gender, and socioeconomic status are associated with the prevalence of insomnia. It is seen in more than 50% of people over the age of 65. It is more common especially in individuals with low socioeconomic status, single or separated, female gender, and during the menopausal period (1). In terms of diagnosis, there should be one of the symptoms such as difficulty in initiating sleep, awaking in the night, waking up early in the morning, and inability to sleep again for at least three months at a frequency of at least three nights a week, and it should cause symptoms that continue throughout the day (2).

In the literature, studies are examining the relationship between insomnia and sexual dysfunction (SD). Sexual desire problems, difficulty in sexual arousal and orgasm, or experiencing rapid orgasm, and sexual pain are the symptoms of SD. The prevalence of low sexual function is about 40-45% for female and 20-30% for male (3). It is generally associated with personal and relationship distress, low self-esteem, and decreased quality of life.

Insomnia is an independent risk factor related to SD. Sleep loss can lead to hormonal changes that cause poorer sexual functioning. The effect of insufficient sleep time on sexual function is regulated by low androgen levels. In the literature, the effect of sleep time on sexual desire and the genital response was shown (4).

SD commonly occurs with emotional disorders like depressive and anxiety disorders, although it is independently associated with negative outcomes. It was shown that SD and emotional disorder comorbidity is associated with higher suicidality and increased severity of the disorder (5). Therefore, more studies are needed on the relationship between SD and psychopathology.

Temperament characteristics of patients with SD were investigated in some studies. The level of neuroticism has been found higher in patients with SD. In a study, the NEO-Five Factor Inventory was applied to patients, and frequency of negative sexual cognitions are predicted by neuroticism scores (6). Moreover, SD shares a high level of negative affect that characterizes internalizing disorders, which are a general dimension of sadness, anxiety, anger, and guilt. High levels of these emotions are mostly related to SD, especially to lower sexual arousal and sexual desire in both genders. By following this, patients with SD report higher levels of negative affect, especially during sexual activity. Wiegel et al. (7) reported that SD is generally associated with temporary or recurrent negative affect.

Alexithymia is defined as the difficulty in identifying feelings, distinguishing between bodily sensations of emotions and feelings. Alexithymia causes dysfunctions in cognitive processing and emotion regulation, so it can lead to lots of psychiatric and medical disorders. Recently, most studies propose the relation between alexithymia and sleep quality. In a previous study, Lindholm et al. (8) demonstrated that alexithymic features were associated with insomnia symptoms such as sleep latency time, and awakenings from sleep. Also, the relation between alexithymia and erectile dysfunction (ED) (9), hypoactive sexual desire (10), and premature ejaculation (11) were shown. The relationship of alexithymia with decreased sexual desire (12), more sexual dissatisfaction (13), and lesser vaginal intercourse frequency (14) were shown in studies with nonclinical female samples.

Anger is another psychological factor that has correlated with SD. The relation of anger with negative penile tumescence and sexual desire was found by Bozman et al. (15) in 1991. They suggested that anger can be one of the mechanisms that prevent desire and arousal in people with hypoactive sexual desire. Incidence of ED is associated with increased expression and suppression of anger.

In this study, we aimed to examine the relations of SD with various psychological factors in insomniac patients. We investigated the temperament characteristics, alexithymia, anger parameters in insomniac patients with SD and compared them with insomniac patients without SD.

MATERIAL AND METHODS

This study was performed at the Sleep Disorder outpatient clinic of the University Hospital. Following the Helsinki declaration, approval was obtained from the Ethics Committee with decision number 41 on 17.06.2019. The informed consent form was signed by all participants.

Patients

This observational study consisted of 92 consecutive patients who applied to the Sleep Disorder outpatient clinic of the University Hospital between July 2019 and January 2020 and diagnosed with insomnia according to the third edition of the International Classification of Sleep Disorders (ICSD-3). Inclusion criteria; i. patients without any other accompanying sleep disorder, ii. volunteering to participate in the study and signing the informed consent, iii. being literate, iv. being over the age of 18. Exclusion criteria; i. having active psychotic symptoms, ii. having active mood disorder, iii. having mental retardation that is understandable by interview, iv. presence of neurocognitive impairment as a result of clinical observation and medical history, v. previous history of any medical or urological diseases that may affect SD.

The sociodemographic data form, Temperament Evaluation of Memphis, Pisa, Paris and San Diego Auto-questionnaire (TEMPS-A), Insomnia Severity Index (ISI), Toronto Structured Interview for Alexithymia (TSIA), Arizona Sexual Experiences Scale (ASEX), Pittsburgh Sleep Quality Index (PSQI), State-Trait Anger Expression Inventory (STAXI) were applied. Cut off score was taken as 11 according to the ASEX, and patients with a score above 11 were considered as SD. Insomnia patients were divided into two groups as "with sexual dysfunction" and "without sexual dysfunction", and anger, alexithymia levels, and temperament characteristics were compared in both of the groups. SD, anger, and alexithymia scores were compared in all insomnia patients, also.

Questionnaires

Temperament Evaluation of Memphis, Pisa, Paris and San Diego Auto-questionnaire (TEMPS-A) is a self-report that consists of 35 questions. Questions 1-7 refer to the depressive temperament, 8-14 to the cyclothymic, 15-21 to the hyperthymic, 22-28 to the irritable, and 29-35 to the anxious temperament. It is responded in a five-point anchored Likert-type scale ranging from 1 to 5 (1-not all; 2-a little; 3-moderately; 4-much; 5-very much) based on the degree that better describes the way one feels during a

large part of the life. To evaluate the dominant temperament, the cut points are 13, 18, 20, 13, and 18 points respectively (16).

Insomnia Severity Index (ISI) is a seven-item questionnaire used to assess sleep quality and insomnia severity (17). The ISI scores range between 0 and 28. We evaluate the total ISI score.

Pittsburgh Sleep Quality Index (PSQI) measures subjective sleep quality. It consists of 19 self-rated questions and 5 questions rated by the bed partner. It is divided into seven components: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. These component scores are added to a total PSQI score with a range of 0 to 21. While PSQI scores of above 5 are taken as abnormal, higher scores indicate worse sleep quality. The Turkish adaptation of the scale was done by Agargun et al. (18).

Arizona Sexual Experiences Scale (ASEX) is a 5-item scale that measures sexual function in six different levels of answers. The questionnaire enquires about sex drive, erection, arousal, orgasm, and satisfaction. Patients who have ASEX levels above 11 points are regarded as SD.

Toronto Structured Interview for Alexithymia (TSIA) is a 20-item self-report scale. Items are rated on a 1-5 scale. Higher total scores indicate greater levels of alexithymia. It has also three subscales measuring difficulty in identifying feelings and distinguishing them from bodily sensations of emotion, difficulty expressing feelings, and externally oriented thinking. The Turkish translation of the TAS-20 has good reliability (α=0.76, 19).

State-Trait Anger Expression Inventory (STAXI) is a self-report inventory which is consisting of 44 items coded on a 4-point scale. State anger, trait anger, anger in, anger out, anger control, and anger expression are subscales. The first 10 items in the scale are the items of the Trait Anger subscale. With the addition of items 13, 15, 16, 20, 23, 26, 27, and 31, the score of the anger in sub-scale of the Anger Style scale; Anger Outward subscale score, with the addition of items 12, 17, 19, 22, 24, 29, 32 and 33; the scores of the Anger Control subscale are obtained by summing the items no 11, 14, 18, 21, 25, 28, 30 and 34. High scores from Trait Anger indicate that the level of anger is high; high scores on the Control Anger scale indicated that anger can be controlled; high scores on the Anger-Out Scale indicate that anger is easily expressed.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows (version 20.0; SPSS Inc, Chicago, Illinois). The Shapiro-Wilk test was used to determine whether the data were normally distributed. Continuous data were expressed as mean±standard deviation or median (interquartile range) while categorical data were presented as the number of patients and percentages. Student's t-test or Mann-Whitney-U test was used to compare parametric and non-parametric continuous variables, respectively. Pearson chi-square and Fisher's exact tests were used for statistical evaluation of the categorical variables. Correlation analysis was performed by Pearson or Spearman's correlation test. Linear regression analysis was performed to demonstrate the predictors of the ISI score. Parameters included depressive temperament, cyclothymic temperament, hyperthymic temperament, irritable temperament, anxious temperament, alexithymia, and ASEX based on the univariate analysis findings. Logistic regression analysis was performed to demonstrate the predictors of SD. Parameters included age, gender, ISI score, total PSQI score, anxious temperament, and depressive temperament based on the univariate analysis findings. Hosmer-Lemeshow goodness-of-fit statistic was used to assess model fit. A p-value <0.05 was considered statistically significant.

RESULTS

The mean age of patients was 54.9±8.7 years. Most of the patients (73.9%, n=68) were female. 19 (20.7%) of the patients had a cigarette and 20 (21.7%) had alcohol use. While the mean duration of the insomnia complaints of the patients was 11.7±6.4 years, the mean duration of the insomnia diagnosis was 3.3±2.8 years. The mean ISI score of the patients was 19.8±4.8. The mean total PSQI score of the patients was 16.8±3.7 (Table 1).

The cut-off score was taken as 11 according to the ASEX, and patients with a score above 11 are considered SD. Insomnia patients are divided into two groups as "patients with SD" and "patients without SD". While 62 patients had SD, 30 patients had no SD. The mean ASEX score of the patients with SD was 20.9±5.7. There was no significant difference between the groups in terms of age (p=0.187). While 52 (83.9%) of the patients with SD were female, there were 16 (53.3%) females in the group without SD. There was a significant difference between the groups in terms of gender (p=0.002). There was no significant difference between the groups in terms of marital status, employment status, smoking, and alcohol use (p=0.293, p=0.095, p=0.281, p=0.778, respectively). Duration of insomnia and diagnosis time were significantly higher in patients with SD (p=0.021, and p=0.003 respectively). ISI and PSQI scores were significantly higher in patients with SD (p=0.048, and p=0.007, respectively, Table 2).

Anger scores, TEMPS-A parameters, and alexithymia were compared between patients with and without SD in Table 3. When the anger scores were compared between the groups; anger in score was significantly higher in patients with SD (p=0.032). Trait anger and anger out scores were significantly higher in patients without SD (p=0.028, and p=0.029, respectively). Anger control scores was not different between groups (p=0.676). According to the examination of temperament characteristics, depressive and anxious temperament were

Table 1. Baseline characteristics and clinical data of the patients (n=92)

Age (years), mean±SD	54.9±8.7
Sex (female), n (%)	68 (73.9)
Smoking, n (%)	19 (20.7)
Alcohol, n (%)	20 (21.7)
Married, n (%)	62 (67.4)
Working, n (%)	21 (22.8)
Onset of symptoms (years), mean±SD	11.7±6.4
Diagnosis of insomnia (years), mean±SD	3.3±2.8
Insomnia Severity Index, mean±SD	19.8±4.8
Total Pittsburgh Sleep Quality Index, mean±SD	16.8±3.7

SD: standard deviation

Table 2. Comparison of baseline characteristics and clinical data between groups

	Patients without sexual dysfunction (n=30)	Patients with sexual dysfunction (n=62)	p
Age (years), mean±SD	53.1±7.5	55.7±9.2	0.187
Gender (female), n (%)	16 (53.3)	52 (83.9)	**0.002**
Married, n (%)	18 (60.0)	44 (71.0)	0.293
Working, n (%)	10 (33.3)	11 (17.7)	0.095
Smoking, n (%)	4 (13.3)	15 (24.2)	0.281
Alcohol, n (%)	6 (20.0)	14 (22.6)	0.778
Onset of symptoms (years), median (IQR) [min-max]	8 (11) [2-20]	10 (13) [3-37]	**0.021**
Diagnosis of insomnia (years), median (IQR) [min-max]	1 (3.25) [1-10]	3 (2) [1-15]	**0.003**
Insomnia Severity Index, median (IQR) [min-max]	19 (8) [8-26]	19 (6) [12-28]	**0.048**
Pittsburgh Sleep Quality Index, median (IQR) [min-max]	17 (7) [10-20]	19 (4) [6-21]	**0.007**

SD: standard deviation, IQR: interquartile range

Table 3. Comparison of Anger scores, TEMPS-A parameters, and alexithymia between groups

Median (IQR) [min-max]	Patients without sexual dysfunction (n=30)	Patients with sexual dysfunction (n=62)	p
Trait anger	22 (8) [16-29]	19.5 (6.25) [13-37]	**0.028**
Anger in	15 (8.5) [10-25]	21 (10) [10-29]	**0.032**
Anger out	16 (6) [13-22]	14.5 (8) [9-27]	**0.029**
Anger control	18 (10.5) [11-30]	21 (6) [13-31]	0.676
Depressive temperament	7 (4.25) [2-15]	8.5 (8) [1-16]	**0.026**
Cyclothymic temperament	9 (7.25) [1-18]	8 (11) [1-17]	0.547
Hyperthymic temperament	8 (9.25) [0-17]	8 (6) [0-16]	**0.013**
Irritable temperament	7 (7.25) [0-12]	3 (5.25) [0-14]	0.164
Anxious temperament	4 (9) [0-21]	12 (12) [0-21]	**0.008**
Alexithymia	62 (22.75) [41-74]	52 (19) [28-84]	0.223

TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, San Diego Auto-questionnaire, IQR: interquartile range

Table 4. Correlation analysis between ASEX score, ISI score and TEMPS-A parameters

	ASEX	ISI	Depressive T	Anxious T	Cyclothymic T	Hyperthymic T	Irritable T
ASEX	-	r=0.214 **p=0.041**	r=0.261 p=**0.012**	r=0.286 p=**0.007**	r=0.091 p=0.390	r=-0.189 p=0.071	r=-0.168 p=0.110
ISI	r=0.214 **p=0.041**	-	r=0.113 p=0.282	r=0.162 p=0.132	r=0.336 **p=0.001**	r=0.007 p=0.944	r=0.032 p=0.765
Depressive T	r=0.261 p=**0.012**	r=0.113 p=0.282	-	r=0.630 p<**0.001**	r=0.629 p<**0.001**	r=0.068 p=0.522	r=0.528 p<**0.001**
Anxious T	r=0.286 p=**0.007**	r=0.162 p=0.132	r=0.630 p<**0.001**	-	r=0.735 **p<0.001**	r=0.169 p=0.115	r=0.644 **p<0.001**
Cyclothymic T	r=0.091 p=0.390	r=0.336 **p=0.001**	r=0.629 p<**0.001**	r=0.735 **p<0.001**	-	r=0.247 p=**0.018**	r=0.767 p<**0.001**
Hyperthymic T	r=-0.189 p=0.071	r=0.007 p=0.944	r=0.068 p=0.522	r=0.169 p=0.115	r=0.247 p=**0.018**	-	r=0.443 p<**0.001**
Irritable T	r=-0.168 p=0.110	r=0.032 p=0.765	r=0.528 p<**0.001**	r=0.644 **p<0.001**	r=0.767 p<**0.001**	r=0.443 p<**0.001**	-

ASEX: Arizona Sexual Experiences Scale, ISI: Insomnia severity index, TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, San Diego Auto-questionnaire, T: Temperament

significantly higher in patients with SD (p=0.026, and p=0.008, respectively), whereas hyperthymic temperament was significantly higher in patients without SD (p=0.013). Alexithymia score was not significantly different between groups (p=0.223).

Correlation analysis was performed to demonstrate the association between ASEX score with ISI and TEMPS-A parameters (Table 4). ASEX score was significantly correlated with the ISI score (r=0.214, p=0.041). Among TEMPS-A parameters; depressive and anxious temperament was significantly correlated with ASEX score (r=0.261, p=0.012; r=0.286, p=0.007, respectively). Multivariate linear regression analysis was performed to demonstrate the predictors of ISI. Among TEMPS-A parameters, alexithymia, and ASEX score; depressive temperament, cyclothymic temperament, and irritable temperament were predictors of ISI (p=0.001, Table 5). Multivariate logistic regression analysis was performed to demonstrate the predictors of SD. Among age, gender, ISI score, PSQI score, anxious, depressive, and hyperthymic temperaments; hyperthymic temperament was independent predictor of SD (p=0.001, Table 6).

Table 5. Multivariate linear regression analysis to determinate predictors of ISI

	B	Beta	t	95% CI	p
Depressive T	-0.294	-0.327	-2.500	-0.529 - -0.060	**0.014**
Cyclothymic T	0.514	0.641	3.580	0.228 - 0.800	**0.001**
Hyperthymic T	0.046	0.055	0.499	-0.138 - 0.230	0.619
Irritable T	-0.358	-0.355	-2.000	-0.713 - -0.002	**0.049**
Anxious T	-0.003	0.103	-0.031	-0.209 - 0.203	0.975
Alexithymia	0.052	0.165	1.555	-0.015 - 0.119	0.124
ASEX	0.085	0.153	1.278	-0.047 - 0.216	0.205

ISI: insomnia severity index, CI: confidence interval, T: Temperament, ASEX: Arizona Sexual Experiences Scale

Table 6. Multivariate logistic regression analysis to determinate predictors of SD

	OR	95% CI	p
Age	1.023	0.955 - 1.097	0.511
Gender	2.423	0.680 - 8.631	0.172
ISI score	1.011	0.882 - 1.159	0.872
Total PSQI score	1.175	0.988 - 1.398	0.069
Anxious temperament	1.078	0.952 - 1.222	0.236
Depressive temperament	1.032	0.875 - 1.218	0.707
Hyperthymic temperament	0.857	0.770 - 0.954	**0.005**

SD: sexual dysfunction, OR: odds ratio, CI: confidence interval, ISI: insomnia severity index, PSQI: Pittsburgh Sleep Quality Index

DISCUSSION

Because of the different diagnostic and screening methods, the prevalence of insomnia varies from 6-76.3%. The environmental factors affect the prevalence of insomnia. Sociodemographic factors such as sex, age, marital status, income, education, occupation, and somatic or psychiatric conditions are associated with insomnia.

Sex differences are widely mentioned in the sleep literature. In a study, it was shown that insomnia in females is 1.5 times higher than in males (20). Similarly, we found that insomnia was more common in females (n=68, 73,9%). Because females are more likely to mention their somatic symptoms (21). In previous studies, it was shown that females with insomnia attend to hospital more than males (22,23).

Benbir et al. (24) searched the prevalence of insomnia in Turkey. They observed that the prevalence of insomnia is 12.2% while the prevalence of any symptom of insomnia is 51%. In their study, of the 5021 participants, 51.7% were female, 14.8% were aged 18-24 years, 47.6% were aged 25-44 years, 29.3% were aged 45-64 years, and 8.3% were older than 64 years. In another study, individuals in the 55 to 64-year age group had a higher rate of insomnia (25). Therefore, the prevalence of insomnia increases with age, and then peaks in the 45-64 years of age. Similarly, participants with 54.9±8.7 years mostly reported insomnia in our study. Stressors related to retirement, taking care of parents, supporting children, and increase risk of medical illness may be the reasons for insomnia in this age group (26).

Previous studies in Western countries mentioned that divorced, separated, and widowed subjects had a higher prevalence of insomnia (27). Similarly, Benbir et al. (24) also observed that insomnia was more in divorced people. On the contrary, in our study 62 (67.4%) patient was married. This can be explained by stressful life events caused by marriage, problems with the children, and other family members.

In a study, it was shown that insomnia was higher in unemployed people and housewives than people with a regular salary (24). Similar to this data only 21 (22.8%) patients were working in our study. Although smoking and drinking are risk factors for insomnia in some studies (28,29), there were contemporary results also (25). In our study, only 19 (20.7%) patients were smoking and 20 (21.7%) patients had alcohol consumption. The impact of smoking and drinking on insomnia needs to be further explored.

The relation between sleep, the endocrine system, and sexual functions are not yet clearly understood. Studies on the prevalence and characteristics of SD in patients reporting symptoms of sleep disorders are scarce and limited to specific research groups. Most data refer to the SD in the population of male with obstructive sleep apnea syndrome and the SD in the perimenopausal female with comorbid insomnias (30). They suggest that insufficient sleep duration and sleep quality affect sexual responses negatively. Kalmbach et al. (31) demonstrated that higher sleep duration was associated with higher sexual desire. In our study, 62 (67.4%) of the 92 insomnia patients included in the study had SD; ISI and PSQI scores were significantly higher in this group.

Previous studies reported a broad range of prevalence rates due to different age groups, dysfunction description, data selection and collection, duration of dysfunction, and the severity of the dysfunction. Therefore, epidemiological studies indicate that nearly 20-30% of adult males and 40-45% of adult females have at least one SD. As the age increases, the prevalence of SD also increases. Although some studies show that SD increases especially after the age of 50, some studies have not shown its relevance to age (32). In our study, when patients with and without SD were compared, there was no significant difference in age. But, the rate of women in the group with SD was significantly higher. Vulvovaginal atrophy and hypoactive sexual desire disorder can be seen due to hormonal changes during menopause. This situation may cause impairment in the sexual functions of women in this age group.

In the literature, it was shown that females with vaginismus were at a higher risk of depressive, cyclothymic, anxious, and irritable temperament than controls, although there were no significant differences in the hyperthymic temperament scores between groups (33). Similarly, in our study, the group with SD had significantly higher scores of depressive and anxious temperaments; however, hyperthymic scores were significantly higher in the group without SD. Depressive and anxious temperament may be considered to have negative effects on sexual functions, both by causing the development of depressive and anxious symptoms leading to anhedonia, lack of energy, and decrease in desire. Previous studies defined hyperthymic temperament as extraversion (34). They found hyperthymic temperament was protective against any anxiety disorder and mood disorder (35). Perhaps this is why hyperthymic temperament was higher in the group without SD.

Anger is an emotional conflict that can interrupt SD. The role of anger in SD was investigated in previous studies. Anger in reactions and anger-related behaviors scores were found significantly higher in patients with SD than

without SD (36). On the contrary, in 2000, the Massachusetts Male Aging Study (37) showed that anger in and anger out do not predict the presence of ED. Muscatello et al. (38) showed that trait anger was not related to sexual motivation; but trait anger was related to sexual interpersonal behavior, especially in males. In another study, Iannuzzo et al. (39) demonstrated that anger-prone individuals aim to meet their own needs and desires, but not their partner's. So, they are interested in sexual pleasure. In our study anger in score was significantly higher in patients with SD. This can be explained by anger can tense the body, so it prevents the relaxation that has a negative impact on sexual pleasure. Or, anger can be experienced as problematic and dangerous that results in anxiety and confusion and leads to SD.

In previous studies, it was shown that greater alexithymia was associated with ED, hypoactive sexual desire, and premature ejaculation (11). Studies with female samples show associations of alexithymia with SD. Berunger et al. (40) showed the correlation of greater alexithymia with more erectile difficulties, while not with difficulties delaying ejaculation during vaginal intercourse in nonclinical sample. In our study alexithymia score was not significantly different between groups. Therefore, further researches are needed to investigate if alexithymia is more related to some SD.

Our study had several limitations. Firstly, the study sample size was small. This may affect the power of the correlation analysis. Secondly, our study was cross-sectional and comes with some limitations. The cross-sectional nature of the study does not allow one to draw strong conclusions about the clinical utility of the proposed questionnaires. Our study population did not cover the younger age population. Further prospective studies are needed to demonstrate the effect of age on the study results.

CONCLUSION

In conclusion, psychological factors should also be considered in studies conducted on the relationship between insomnia and SD. The results of the current study showed that ISI score, depressive and anxious temperament was significantly correlated with ASEX score. Depressive, cyclothymic, and irritable temperament were predictors of ISI. Hyperthymic temperament was predictor of SD. Further research is needed on temperament characteristics, alexithymia and anger issues. Thus, patients can be approached more comprehensively.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of University of Health Sciences Erenköy Mental and Neurological Diseases Training and Research Hospital (17.06.2019, 41).

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Subacute Lung Injury Associated with Heated Tobacco Products

Isıtılmış Tütün Ürünü İlişkili Subakut Akciğer Hasarı

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ABSTRACT

Heated tobacco products release nicotine without burning tobacco with an electronically controlled heating system. 56-year-old male patient admitted with sudden onset of chest pain and shortness of breath. He had been using a heated tobacco product (I quit ordinary smoking, IQOS) for 2.5 years. Thoracic computed tomography scan revealed pleural-based atelectasis and fibroatelectatic changes in the lower lobe of the right lung, pleural fluid in the right upper lobe, fibroatelectatic changes and pleural thickening in the left lung. Biopsy taken with video-assisted thoracic surgery (VATS) showed lymphoid aggregation in nodular form and widespread anthracosis around the lung, fibrillar material that double-refracting the light in the alveoli, hyaline membrane-like material in the alveoli, type 2 pneumocyte hyperplasia, an interstitial organization, and a subacute lung injury picture with exogenous lipoid material. These findings were evaluated in accordance with toxic substance-induced chemical pneumonia. It was thought that it might be related to 2.5 years of using heated tobacco product.
Keywords: Tobacco products; tobacco smoking; lung injury.

ÖZ

Isıtılmış tütün ürünleri elektronik olarak kontrol edilen bir ısıtma sistemi ile tütün yakmadan nikotin açığa çıkaran ürünlerdir. 56 yaşında erkek hasta ani başlangıçlı nefes darlığı ve göğüs ağrısı şikayetleriyle başvurdu. 2,5 yıldır ısıtılmış tütün ürünü (I quit ordinary smoking, IQOS) kullanıyordu. Toraks bilgisayarlı tomografi tetkikinde sağ akciğer alt lobda plevra tabanlı atelektazi ve fibroatelektatik değişiklikler, sağ üst lobda plevral sıvı, sol akciğerde fibroatelektatik değişiklikler ve plevral kalınlaşma izlendi. Video yardımlı toraks cerrahisi (video-assisted thoracic surgery, VATS) ile alınan biyopsi de akciğerde nodüler halinde lenfoid agregasyon ve çevresinde yaygın antrakoz, alveol içinde ışığı çift kıran fibriler materyal, alveol içinde hyalin membran benzeri materyal, tip 2 pnömosit hiperplazisi, interstisyel bir organizasyon ve ekzojen lipoid materyalin izlenmekte olduğu bir subakut akciğer hasarı tablosu izlendi. Bu bulgular toksik madde kaynaklı kimyasal pnömoni ile uyumlu olarak değerlendirildi. Bunun hastanın 2,5 yıllık ısıtılmış tütün kullanımına bağlı olduğu düşünüldü.
Anahtar kelimeler: Tütün; tütün içimi; akciğer hasarı.

INTRODUCTION

In conventional cigarettes, when tobacco is heated above 600 °C, burning occurs and the result is smoke containing harmful chemical (1). Heated tobacco products, heat tobacco up to 350 °C, releasing volatile components (2). Heated tobacco products release nicotine-containing emissions without burning tobacco using an electronically controlled heating element. Tobacco companies marketing the heated

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tobacco products using the messages claiming to be safer than cigarettes (3). The I quit ordinary smoking (IQOS) device, a heated tobacco product, was developed by Philip Morris International (PMI) and launched in international markets in mid-2014. It was available in 51 countries by May 2020 (4).

The variety and prevalence of nicotine-containing devices such as heated tobacco products are increasing day by day. However, there is less information about their effects on the lungs.

CASE REPORT

A 56-year-old male admitted with sudden onset of chest pain and shortness of breath. The patient had a history of chronic obstructive lung disease, coronary artery disease, and previous pulmonary embolism. He had smoked approximately 25 pack years and quit. He had been using a heated tobacco product, IQOS, for 2.5 years. There was no history of any other substance use. The initial bloodwork during the admission of the patient: WBC: 7.99×10^3 /uL, N: 70.6%, L: 17.2%. The procalcitonin level was 0.06 ng/ml, ESR was 54 mm/h, CRP was 42.2 µg/L. The other all laboratory tests were negative. Pleural-based parenchymal infiltration and accompanying fibroatelectatic densities in the posterior basilar segment of the right lung were observed on the computed tomografy (CT) scan. Because of clinical deterioration on antibiotics, progression on chest X-ray, and no growth on cultures, repeat thoracic CT examination was performed. Pleural-based atelectasis and fibroatelectatic changes in the lower lobe of the right lung, pleural fluid in the right upper lobe, fibroatelectatic changes in the left lung and pleural thickening were observed (Figure 1). Partial decortication and wedge resection was performed with video-assisted thoracic surgery (VATS). Visceral aspect of lung parenchyma as a result of the pathological examination of the material taken. Subpleural fibrotic thickening is seen (Figure 2.A), subpleural fibrosis and anthracotic pigments in lung parenchyma (Figure 2.B), nodular lymphoid aggregates in perialveolar areas (Figure 2.C), prominent interstitial edema is seen (Figure 2.D), hyaline membrane like material is present in alveoli (Figure 2.E). A fibrillary protein like foreign material is present in many alveoli (shown in circles) in HE stained sections (Figure 3.A). These materials are also birefringent in polarized microscopy (as indicated with arrows, Figure 3.B). Presence of such birefringent material in alveoli is not physiological and most likely represents a deposition of inhaled dust (Figure 3). These findings were evaluated in accordance with chemical pneumonia caused by one or more toxic substances. The general condition was found to be compatible with subacute lung injury.

DISCUSSION

In the United States in 2019, acute respiratory diseases that mimic a viral disease that cannot be explained by any other disease were detected in patients with a history of e-cigarette use in the last ninety days. This disease was named with e-cigarette or vaping product use-associated lung injury (EVALI) when evaluated by imaging methods and histopathologically.

EVALI patients typically had a nonspecific clinical presentation characterized by a combination of respiratory, gastrointestinal, and constitutional symptoms. CT findings in EVALI were most commonly pneumonia and diffuse alveolar damage. Less common forms of lung injury have been reported, including acute eosinophilic pneumonia and widespread alveolar bleeding. Histological findings were not specific. There were macrophages in alveoli containing lipid and granular brown-black refractile foreign material. There was a small amount of neutrophils and eosinophils. There was prominent type 2 pneumocyte

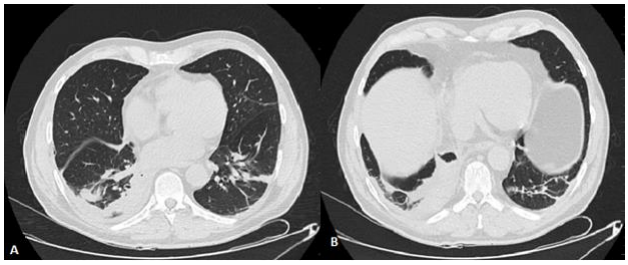

Figure 1. Pleural-based atelectasis and fibroatelectatic changes in the lower lobe of the right lung, fibroatelectatic changes in the left lung and pleural thickening

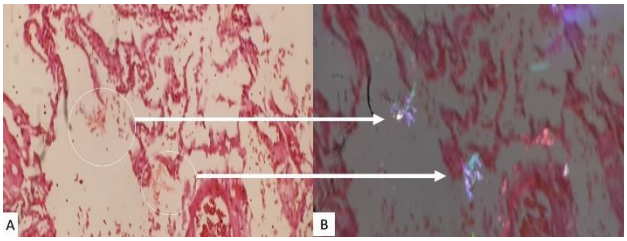

Figure 3. A) A fibrillary protein like foreign material in many alveoli (shown in circles) in HE stained sections, **B)** These materials are also birefringent in polarized microscopy (as indicated with arrows)

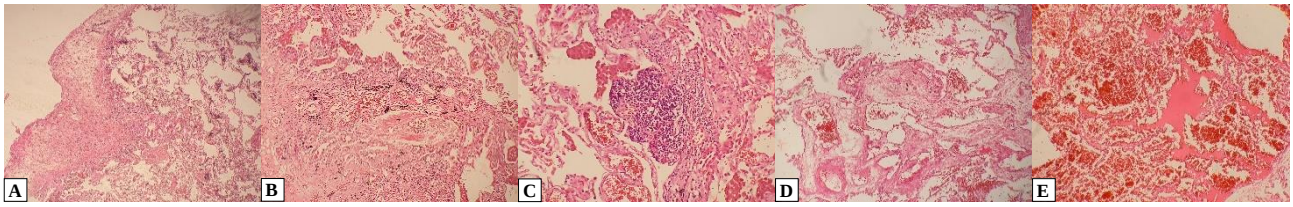

Figure 2. Visceral aspect of lung parenchyma, **A)** Subpleural fibrotic thickening (H&E, x20), **B)** Subpleural fibrosis and antrachotic pigments in lung parenchyma (H&E, x20), **C)** Nodular lymphoid aggregates in perialevolar areas (H&E, x20), **D)** Prominent interstitial edema (H&E, x10), **E)** Hyalin membrane like material in alveoli (H&E, x20)

hyperplasia with prominent nucleolus and multinucleation. There were also scattered mitotic figures. Findings showed patterns of acute lung injury, including acute fibrinous pneumonitis, diffuse alveolar damage, or pneumonia often seen with bronchiolitis and bronchiolitis. Foamy macrophages and pneumocyte vacuolization were seen in all cases. Pigmented macrophages were sometimes present but not a dominant feature. It has been reported that the findings suggest an airway-centered chemical pneumonitis from one or more inhaled toxic substances (5,6).

In our case, a subacute lung injury was observed in which lymphoid aggregation in nodular form with surrounding extensive anthracosis, fibrillar material that double-refracting the light in the alveoli, hyaline membrane-like material in the alveoli, fibrotic nodules, type 2 pneumocyte hyperplasia, an interstitial organization and exogenous lipoid material were seen.

Although e-cigarette use-associated lung injury have been defined, there is no definition associated with the use of heated tobacco products in the literature. Although tobacco producers introduce heated tobacco products to the market as a safer product compared to cigarettes, studies have shown that the emissions of heated tobacco products also have a cytotoxic effect on the human bronchial epithelium (7). Heated tobacco products also emit significant levels of tobacco-specific nitrosamines similar to conventional cigarettes. Although heated tobacco products emit lower amounts of tobacco-specific nitrosamines than flammable cigarettes, the amounts are significantly higher than e-cigarettes (8-10).

In conclusion, the presence of biopsy findings due to toxic or irritant substance inhalation in our patient might be related to the heated tobacco product he was using. We would like to emphasize that chemical pneumonia should not be forgotten in cases of pneumonia that do not respond to conventional treatments in individuals smoking such products. Although tobacco companies present a less harmful alternative to traditional cigarettes, it is clear that further studies are needed on products of the heated type.

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Convalescent (Immune) Plasma Followed by Intravenous Immunoglobulin Infusion in an Adolescent with Severe COVID-19

Şiddetli COVID-19'lu Ergende Konvalesan (İmmün) Plazmayı İzleyen İntravenöz İmmünoglobülin İnfüzyonu

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ABSTRACT

Coronavirus disease 2019 (COVID-19) pandemic has drawn attention over old immunotherapeutic agents such as convalescent (immune) plasma (CIP). Here, an adolescent with severe COVID-19 case requiring CIP and intravenous immunoglobulin (IVIG) treatments is described. A 17-year-old male patient was brought to the emergency room with complaints of fever, fatigue, and severe cough. Two doses of CIP were infused to the patient because of the increase in persisting fever, dyspnea, and acute phase reactant levels after the third day of routine protocol treatment. IVIG therapy was begun for 2 days at a dose of 1 g/kg/day due to resurgence in acute phase reactants and progressing radiological findings following CIP transfusion. Our patient avoided mechanical ventilation and showed immediate clinical and radiological improvement with CIP transfusion followed by IVIG therapy. Timely initiation of CIP treatment followed by IVIG prevented the disease from worsening and helped to reduce the requirement for mechanical ventilation.
Keywords: Convalescent (immune) plasma; COVID-19; intravenous immunoglobulin.

ÖZ

Koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) pandemisi konvalesan (immün) plazma (CIP) gibi eski immünoterapötik ajanların üzerine dikkati çekmiştir. Burada, CIP ve intravenöz immünoglobulin (IVIG) tedavisi gerektiren şiddetli COVID-19 hastalığı olan bir adolesan olgu sunulmaktadır. 17 yaşında erkek hasta ateş, yorgunluk ve şiddetli öksürük yakınmalarıyla acil servise getirildi. Hastaya rutin protokol tedavisinin üçüncü gününden sonra devam eden ateş, nefes darlığı ve akut faz reaktan düzeylerindeki artış nedeniyle iki doz CIP infüzyonu uygulandı. CIP transfüzyonu sonrası akut faz reaktanlarında yeniden artış ve ilerleyen radyolojik bulgular nedeniyle 2 gün süreyle 1 g/kg/gün dozunda IVIG tedavisine başlandı. Hastamız CIP transfüzyonunu takiben IVIG tedavisiyle hemen klinik ve radyolojik iyileşme gösterdi ve mekanik vantilatör uygulamasından kurtuldu. CIP tedavisinin zamanında başlatılması ve ardından verilen IVIG, hastalığın kötüleşmesini önlemiş ve mekanik ventilasyon ihtiyacının azaltılmasına yardımcı olmuştur.
Anahtar kelimeler: Konvalesan (immün) plazma; COVID-19; intravenöz immunoglobulin.

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INTRODUCTION

The immune response is vital for the control and improvement of viral infections. The literature reports different immunotherapeutic modalities including convalescent (immune) plasma (CIP) therapy and intravenous immunoglobulin (IVIG) and their beneficial outcomes in coronavirus disease 2019 (COVID-19) (1-7). Here, an adolescent case with severe COVID-19 disease requiring CIP and IVIG treatments to recover is discussed under the light of current literature.

CASE REPORT

A 17-year-old male patient without any known illness was brought to the emergency room with complaints of fever, fatigue, and severe cough. His complaints had started 22 days before hospitalization, with insistent weakness, sore throat, runny nose, and mild cough. No pneumonia signs were detected in computed tomography (CT) one week after symptoms onset, and symptomatic treatment was given. On the 11th day of his complaints, he represented with persistent nasal discharge, paroxysmal and severe cough, causing dyspnea; and antibiotherapy and 5-day methylprednisolone (1mg/kg/day) were given. Two days later, the patient had increased weakness, severe cough with phlegm, and a persistent fever of 38-39 °C despite acetaminophen intake. He was hospitalized to our general pediatric wards due to positive (severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR) test and nonspecific pneumonia findings (diffuse nodular consolidations in both hemithorax) in thorax CT scan (Figure 1a, 1b).

At the admission his general condition was moderate; physical examinations demonstrated decreased lung sounds and diffuse crackles on both lungs. Mild lymphopenia (1290/μL), transient elevation of AST/ALT, high CRP (115, n: <5) mg/L, and normal D-dimer were detected. The patient was started on ceftriaxone 4 g/bid (50 mg/kg/day) and hydroxychloroquine 400 mg/bid (5 mg/kg base/day) for 5 days.

On the third day of admission, subcostal-intercostal retractions and dyspnea began with exercise and talking. Lymphopenia deepened as 945/μL, CRP increased up to 147 mg/L. The postero-anterior chest x-ray showed an increase in bilateral lung infiltration areas. Hydroxychloroquine therapy was changed to favipiravir 3200 mg/day at two doses of loading and 1200 mg/day for maintenance in a total of 5 days. Vancomycin 2 g/day and azithromycin 250 mg/day were added. High flow nasal cannula (HFNC) oxygen therapy was initiated after being transferred to pediatric intensive care unit.

On the fourth day of his admission, increase in dyspnea was observed with exercise and talking. Fibrinogen was 462 (n: 202-430) mg/dl, ferritin: 1036 (n: 4.6-204) ng/mL and IL-6: 21.95 (n: 0-7) pg/mL were elevated (Table 1). First dose of CIP was administered because of the increase in persisting fever, dyspnea, and acute phase reactant levels. Enoxaparin 4000 IU/day was added into his treatment.

On the fifth day, postero-anterior x-ray showed increased infiltration in the right lung. On the sixth day, pathologic lung sounds in the right were increased, considered as consolidation. A second dose of CIP was administered, because the consolidation developed in the right lung and the acute phase reactants were still above the normal, despite a decreasing trend.

On the seventh day, pathologic right lung sounds were decreased and there were crackles in the left lung. Blood gas analysis showed high lactate level (3.4 mmol/L). The thorax CT demonstrated an increase of infiltration extending through to upper segments (Figure 2a, 2b). Since resurgence in acute phase reactants and progressing radiological findings, IVIG therapy was begun for 2 days at a dose of 1 g/kg/day.

On the eighth day, the patient stated significant regression in his exercise dyspnea and cough. Blood gas analysis showed mild respiratory alkalosis (pH: 7.48, pCO_2: 32.8 mmHg). On the ninth day, following the second dose of IVIG, auscultation findings of both lungs were significantly lessened, the patient started to be able to eat comfortably, and activity increase was observed. The HFNC oxygen flow was started to reduce. Chest X-rays progressively demonstrated diffuse consolidation regions containing air bronchograms in both lungs at the 8th thru 9th day of admission (Figure 3a, 3b). On the tenth day, the pulmonary auscultation findings turned back to normal. Chest X-ray showed partial resolution of consolidation regions in both lungs (Figure 3c). On the eleventh day, he was transferred to general pediatric wards. He was discharged on 14th day and ceftriaxone was discontinued.

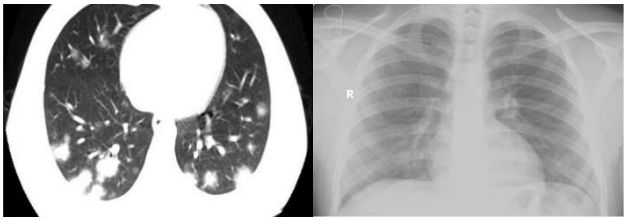

Figure 1a. Although CT shows diffuse nodular consolidations in both hemithorax (nonspesific findings for COVID-19 pneumonia); **1b.** chest X-ray seems to be roughly normal at the day of hospitalization

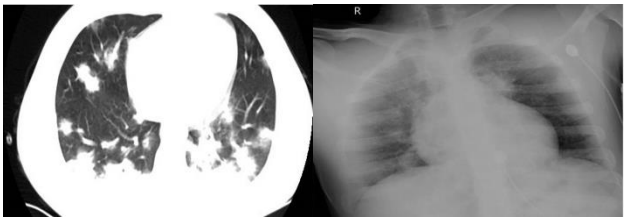

Figure 2a. CT shows diffuse peripheral and central focal patchy ground-glass opacities and air bronchograms in both hemithorax (specific for COVID-19 pneumonia) at the 7th day of hospitalization; **2b.** Chest X-ray also demonstrates diffuse consolidation regions containing air bronchograms at the same day of admission

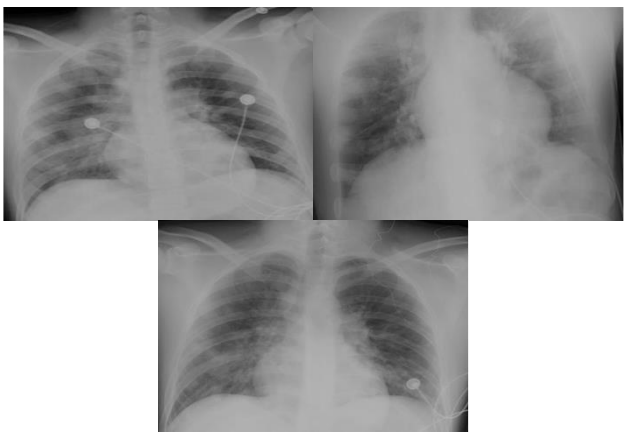

Figure 3a. Chest X-rays progressively demonstrate diffuse consolidation regions containing air bronchograms in both lungs at the 8th and **3b.** 9th day of admission; **3c.** it shows partial resolution of consolidation regions in both lungs at the 10th day of admission

Table 1. Change in acute phase reactants of our patient during hospitalization

	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
WBC (/μL)	13300	5130	4800	5520	5170	5880	7430	5540	4640
Neutrophil (/μL)	10800	3690	3280	3210	3510	3270	5550	3740	2900
Lymphocyte (/μL)	1290	945	1080	1580	1230	2030	1260	1290	1170
CRP (mg/L, n: <5)	115	147	95	65	58	48	87	72	48
Procalcitonin (ng/mL, n: <0.5)			0.33	0.19	0.15	0.11	0.10	0.06	0.04
D-dimer (μgFEU/L, n: <500)	154	266	494	327	316	931	434	716	2630
Fibrinogen (mg/dL, n: 202-430)			462	462	443	434	614	417	380
Ferritin (ng/mL, n: 4.6-204)			1036	890	694	979	1230	780	634
hsTn-I (ng/L, n: 0-15.6)		4.7	5.4	3.7	1.9	2.2	2.7	1.2	1.3

WBC: white blood cell, CRP: C-reactive protein, hsTn-I: high sensitivity troponin I, n: normal

DISCUSSION

Systematic reviews evaluated many studies providing clinical outcome data on the utilization of immunotherapies for the treatment of COVID-19, including CIP and IVIG administration. It seemed that immunotherapy as a supplementary therapy together with other routine cares could be an effective and safe method (6-8). However, Cochrane review by Chai et al. (9) stated that they are uncertain whether CIP is beneficial for people admitted to hospital with COVID-19.

Although there are no strict guidelines for IVIG use in COVID-19, the FDA approved the application of CIP to the severe or immediately life-threatening COVID-19 cases (10). According to the guidelines, severe disease is described as one or more of the following: Dyspnea, tachypnea ≥30/min, blood oxygen saturation ≤93%, PaO_2/FiO_2 <300 and pulmonary infiltrates >50% within 24-48 hours. Since our patient had started to have obvious dyspnea and tachypnea despite routine treatment, first dose of CIP transfusion was given on the fourth day of his admission.

IVIG might also be therapeutic option by the way of its nonspecific antiviral and immunomodulator actions. Even antibodies cross reacting with SARS-CoV-2 have been shown to be currently available IVIG preparations (11). Subgroup analysis of a research demonstrated that a high dose (>15 g/day) IVIG administration in the early phase (≤7 days after admission) revealed significant decline in 60-day mortality in the critical cases (12). However, some reviews still conclude that the present data is inadequate to advocate the efficacy or safety of IVIG administration in the COVID-19 therapy (5).

Both CIP and IVIG could be used as a part of combined immunomodulatory therapies in COVID-19 (13-15). Successful management with plasma exchange subsequently IVIG administration in a critically ill COVID-19 case was described (16). However, in the literature, to the best of our knowledge, successful use of IVIG following CIP treatment in the same patient has not been described.

CONCLUSION

Immunotherapeutic agents as a supplementary therapy together with other routine cares in COVID-19 cases seem to be an effective and safe method. Earlier CIP transfusion followed by IVIG administration even in an adolescent with severe COVID-19 cases could be helpful to prevent mechanical ventilation and recover rapidly from COVID-19.

Informed Consent: Written informed consent was obtained from the parents of the patient for publication.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

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Author Contributions: Idea/Concept: ÖÖ, BE, MCD, HS; Design: ÖÖ, BE, MCD, HS; Data Collection/Processing: ÖÖ, BE, MCD, HS; Analysis/Interpretation: ÖÖ, BE, MCD, HS; Literature Review: ÖÖ, BE, MCD, HS; Drafting/Writing: ÖÖ, BE, MCD, HS; Critical Review: ÖÖ, BE, MCD, HS.

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Abscess Caused by *Streptococcus Constellatus* Secondary to Tooth Decay on Facial Skin: Case Report

Diş Çürümesine İkincil Olarak Yüzde Gelişen *Streptococcus Constellatus*'un Neden Olduğu Apse: Olgu Sunumu

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ABSTRACT

A six-year-old male patient with no known history of disease was presented due to an unfamiliar *Streptococcus constellatus* growth that developed alongside a tooth abscess and fistulized. The patient was admitted with redness, swelling, and tenderness in the left-most section of his face. We learned that the patient visited a dentist three weeks prior due to complaints of swelling in the jaw and tooth decay. The patient had a leukocyte count of 22,600/µL (neutrophil: 17,190/µL), C-reactive protein level was 105.2 mg/L. The patient was put on an intravenous treatment of clindamycin and cefoperazone-sulbactam. There was spontaneous discharge in the abscess. S. *constellatus* grew in abscess culture dish. The current therapy was changed to ampicillin-sulbactam. Observations on the seventh day of hospitalization showed that the lesion had subsided almost entirely. *S. constellatus* is a rare microorganism that produces abscesses and requires immediate medical attention.
Keywords: Abscess; child; *Streptococcus milleri*; *Streptococcus constellatus*; tooth decay.

ÖZ

Bilinen bir hastalık öyküsü olmayan altı yaşında bir erkek hasta, diş absesine ikincil gelişen ve fistülize olan cilt apsesinden alınan mikrobiyolojik incelemede alışık olmadığımız bir etken olan *Streptococcus constellatus* üremesi sebebiyle sunuldu. Hasta yüzün sol yarısında izlenen kızarıklık, şişlik ve hassasiyet ile başvurdu. Hastanın üç hafta önce çenede şişlik ve diş çürüğü şikayetleri ile diş hekimine gittiği öğrenildi. Lökosit sayısı 22.600/µL (nötrofil: 17.190/µL), C-reaktif protein 105,2 mg/L idi. Hastaya intravenöz klindamisin ve sefoperazon-sulbaktam tedavisi başlandı. Apse kendiliğinden drene oldu. Kültürde *S. consellatus* üredi. Mevcut tedavi ampisilin-sulbaktam ile değiştirildi. Yatışının yedinci gününde lezyonun neredeyse tamamen gerilediği görüldü. *S. constellatus* nadir görülen ancak yol açtığı apseler nedeniyle dikkat edilmesi gereken bir mikroorganizmadır.
Anahtar kelimeler: Apse; çocuk; *Streptococcus milleri*; *Streptococcus constellatus*; diş çürüğü.

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INTRODUCTION

Streptococcus constellatus belongs to the *Streptococcus milleri* group (SMG) along with *Streptococcus intermedius* and *Streptococcus anginosus* (1). They are commonly found in oropharyngeal and gastrointestinal flora. However, they can produce abscesses in the abdominal cavity, lower respiratory tract, urogenital system, orofacial area, sinuses, and skin. They are capable of hematogenous spreading, potentially causing endocarditis or metastatic abscesses in the brain, liver, spleen,

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subdural space, and bones (2). They can be characterized by their tendency to produce abscesses (3). We present a six-year-old male patient with no known history of disease who developed a fistulized skin abscess alongside a dental abscess. *S. constellatus* was isolated in microbiological examinations as an uncommon pathogen. This case is presented as a literary contribution as a reminder of the possibility to isolate *S. constellatus* from skin abscesses.

CASE REPORT

A six-year-old male patient with no known disease history is presented with redness, swelling, and tenderness on the left-most section of his face (Figure 1). We learned that the patient visited a dentist three weeks prior due to complaints of swelling in the jaw and tooth decay. The patient received oral amoxicillin-clavulanic acid therapy for two weeks, with a one-week follow-up without any antibiotic treatment. However, he was admitted to hospital due to a worsening conditions. We were made aware by the patient's family that he could not receive the prescribed daily-dose of antibiotics due to his oral intake problems. Upon physical examination, we observed swelling, redness, and tenderness extending from the eye to the mandible of the left-most section of the patient's face. Further examinations revealed no pathological findings. The patient did not have a fever. A purulent discharge excreted from the lesion upon direct palpation. The abscess was drained and samples were submitted to the laboratory for culture tests and Gram-staining (Figure 2). The patient's blood tests revealed a leukocyte count of 22,600/μL, an absolute neutrophil count of 17,190/μL, hemoglobin levels were 12.5 g/L, a platelet count of 515,000/μL, and C-reactive protein levels of 105.2 mg/L. There were no significant findings on the computed tomography (CT) brain scans. Orbital CT scans revealed an edematous appearance in the skin and subcutaneous tissues anteriorly at the level of the left orbit. We identified an increase of soft tissue densities subcutaneously in that area. The patient was examined by an ophthalmologist. No pathological findings were found other than preseptal cellulitis. The patient was diagnosed with "preseptal cellulitis and fistulized skin abscess secondary to a dental abscess" and was admitted to the pediatric infectious diseases inpatient clinic. Blood samples were collected for culture tests, and the patient was put on an intravenous treatment of clindamycin and cefoperazone-sulbactam. Gram-staining of samples revealed polymorphonuclear leukocyte and a moderate density of gram-positive cocci distribution. By the third day of hospitalization, *S. constellatus* had grown in the abscess cultures. So, the current therapy was changed to ampicillin-sulbactam. There were no signs of growth in the blood culture tests. During patient follow-up, it was observed that the facial swelling, redness, and tenderness had subsided almost entirely by the seventh day of antibiotic treatment (Figures 3, 4). The patient was discharged from the hospital with advice given to the patient's family.

DISCUSSION

The SMG includes the *S. constellatus*, *S. anginosus*, and *S. intermedius* species. These Gram-positive, catalase-negative cocci are predominantly commensals isolated from the oropharynx, gastrointestinal and urogenital tracts (4). However, they can attain pathogenic characteristics and cause pyogenic infections in the central nervous system, abdominal cavity, odontogenic infections, deep neck infections, endocarditis, and abscess formation. The reasons for this remain unclear. *S. constellatus* is reportedly associated with diseases such as Lemierre's syndrome, odontogenic cerebral abscesses, cavernous sinus thrombosis orbital cellulitis, and necrotizing orbital cellulitis. Moreover, there has been a case of *S. constellatus* reportedly causing chronic osteomyelitis in a patient with Cogan's syndrome, an autoimmune disease. Further studies show that *S. constellatus* caused vertebral osteomyelitis in a patient with an atrial septal defect and an odontogenic infection (5). Similarly, our patient visited the dentist due to tooth decay and subsequent dental abscess, meaning odontogenic pathology.

In a study investigating infections associated with *S. constellatus* in children, the patients' age range was found to be between 3-15 years-of-age and predominantly male. Of those patients, 56.8% did not have a fever, while 62% developed leukocytosis (6). Consistent with the aforementioned study, our patient was a six-year-old male. Fever was not present before or during the hospital admission. Tests in blood samples revealed leukocytosis (22,600/μL). In the study conducted by Clarridge et al. (3) *S. constellatus* was reported as the causative organism of abscesses in 41 of the 54 samples (76%) collected from

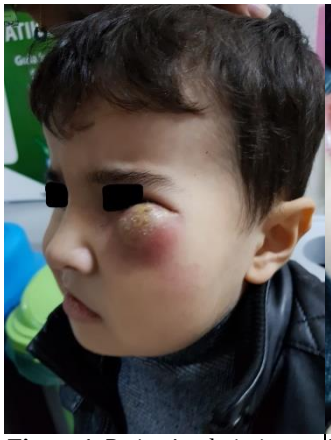

Figure 1. Patient's admission

Figure 2. During the drainage

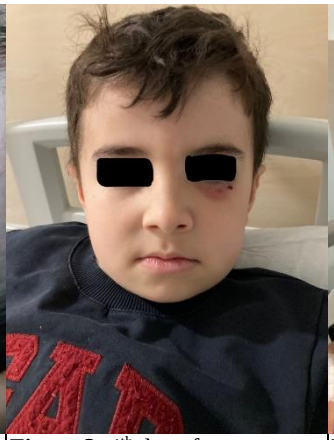

Figure 3. 4th day of treatment

Figure 4. 7th day of treatment

patients. It was found that most abscesses originated from soft tissue (n=13). Other origins were intra-abdominal-perirectal (n=8), pleuropulmonary (n=7), odontogenic-neck (n=2), genitourinary (n=2), and the central nervous system (n=2). Compared to other species of SMG, *S. constellatus* can cause a wider range of infections, including odontogenic and intra-abdominal diseases (3). In the study performed by Faden et al. (6) *S. constellatus* was predominately isolated from perforated acute appendicitis samples. Intra-abdominal abscesses were also found in half of the perforated acute appendicitis cases. Furthermore, *S. constellatus* was isolated from acute osteomyelitis samples and samples collected from dental abscesses, hand abscesses, and peritonsillar abscesses. However, growth did not occur in blood cultures. *S. constellatus* may sometimes lead to respiratory tract infections, which can cause pleural effusions or abscesses. However, they are easily treatable (7). In rare cases, it may cause bacteremia (2). It has been observed that bacteremia occurs most often after a tooth or soft tissue infection (4). It is presumed that immunocompromised patients, such as those with diabetes mellitus, chronic kidney disease, hepatobiliary disease, neoplasias, or undergoing chemotherapy, are at higher risk of bacteremia (8). Our patient was not immunocompromised. There was no detectable growth in the blood culture.

Adults with thrombosis have been more frequently associated with *S. constellatus* caused head and neck infections than those caused by other SMG members (9). Our patient had no symptoms of thrombosis. However, it is imperative to remain vigilant for complications during patient follow-ups due to the higher risk of thrombosis caused by *S. constellatus*.

Penicillin and cephalosporin are effective treatment options for *S. constellatus* infections. The susceptibility of S. constellatus to tetracycline, clindamycin, or erythromycin is reportedly varied. Antibiotic treatment for pyogenic abscesses should be chosen based on the antibiotic susceptibility test results, along with aspiration and drainage. In cases where the abscesses cannot be drained, penicillin and cephalosporin show promising results in treating *S. constellatus* infections (3,10). We learned that the patient had received amoxicillin-clavulanic acid therapy orally irregularly for two weeks before admission. Upon direct palpation, a purulent discharge from the abscess was observed. The abscess was immediately drained. The patient was then hospitalized, and treatment began with clindamycin and cefoperazone-sulbactam. *S. constellatus* had grown in the abscess cultures and so the current therapy was changed to ampicillin-sulbactam. On the seventh day of hospitalization, it was observed that the patient's swelling, redness, and tenderness had subsided almost entirely. He was then discharged and advised on treatment and follow-up procedures.

Although *S. constellatus* is not a commonly found species of bacteria, associated infections should be carefully monitored as the likelihood of complications and its pathogenic capacity may be overlooked.

Informed Consent: Written informed consent was obtained from the patient for publication and accompanying images.

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Inflammatory Rectal Polyp with Osseous Metaplasia: Is It a Distinctive Disease Entity?

Kemik Metaplazisinin Eşlik Ettiği İnflamatuar Rektal Polip: Ayrı Bir Antite mi?

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ABSTRACT

Osseous metaplasia is a heterotopic bone formation and it is encountered rarely in the gastrointestinal tract, especially in benign lesions. Although its pathophysiology is not known clearly, there are several theories suggested. In this case report, we presented a case of bone formation in a rectal inflammatory polyp presenting with rectal bleeding in a 7-year-old girl. Therewithal, we reviewed the literature and summarized the subject of osseous metaplasia in colon polyps. We detected some similarities in the cases presented that suggest it might be a distinctive disease entity. Osseous metaplasia in colorectal polyps is extremely rare. To our knowledge, this is the eleventh reported case of osseous metaplasia in a rectal inflammatory polyp. Although osseous metaplasia in colon polyps has not a significant effect on both clinic and the prognosis, it can be confusing when encountered in microscopic examination.
Keywords: Osseous metaplasia; heterotopic ossification; rectal polyp; colonic polyps; ectopic ossification.

ÖZ

Osseoz metaplazi, heterotopik kemik oluşumudur ve gastrointestinal sistemde, özellikle benign lezyonlarda nadiren karşılaşılır. Patofizyolojisi net olarak bilinmemekle birlikte bazı teoriler öne sürülmüştür. Bu olgu sunumunda, 7 yaşında bir kız çocuğunda rektal kanama ile bulgu veren kemik metaplazili inflamatuar rektal polip olgusunu sunduk. Aynı zamanda literatürü taradık ve kolon poliplerinde osseoz metaplazi olgularını özetledik. Sunulan vakalarda bunun spesifik bir antite olabileceğini düşündürten, bazı benzerlikler saptadık. Kolorektal poliplerde osseoz metaplazi oldukça nadirdir. Saptadığımız kadarıyla olgumuz osseoz metaplazi içeren on birinci rektal inflamatuar polip vakasıdır. Kolon poliplerinde osseoz metaplazi hem klinik hem de prognoz üzerinde önemli bir etkiye sahip olmasa da, mikroskopik incelemede karşılaşıldığında kafa karıştırıcı olabilir.
Anahtar kelimeler: Osseoz metaplazi; heterotopik ossifikasyon; rektal polip; kolon polipleri; ektopik kemikleşme.

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INTRODUCTION

Osseous metaplasia (heterotopic bone formation) is rarely encountered in the gastrointestinal tract (1). Most of the reported cases are associated with malignant lesions and are extremely rare in benign colonic polyps (2-4). Very few cases have been reported, especially in the pediatric population. Although its pathophysiology is not known clearly, there are several theories suggested (5).

In this case report, we presented a case of bone formation in a rectal inflammatory polyp presenting with rectal bleeding in a 7-year-old girl.

CASE REPORT

A seven-year-old girl applied to the clinic with the complaint of rectal bleeding following defecation for the last week. There was no previous history of constipation or rectal bleeding. On physical examination, a petiolate polypoid lesion with a diameter of approximately 1.5 cm was observed in the knee-elbow position, 3 cm above the dentate line and at 1 o'clock in the rectum. There was no history of rectal bleeding in the family. In addition, no pathology was detected in the laboratory findings, hemoglobin level was 12.9 g/dl. The polyp was excised under general anesthesia. No other polypoid lesions were detected from up to the first 10 cm of the rectum.

On macroscopic examination, a pink-skin colored polypoid tissue measured 1.7x1.5x0.5 cm was observed. In the microscopic examination; the polyp surface was ulcerated and covered with fibrinopurulent debris. Acute-chronic inflammation was observed with the development of granulation tissue in the stroma (Figure 1). Elongated, dilated, mucin-filled and some ruptured colonic glands were observed in this enflamed stroma. Some of the ruptured glands showed mucin scattered into the stroma (Figure 2). Multiple foci of bone formation surrounded by osteoblasts were observed in the stroma, whereas no bone-marrow tissue was observed (Figure 3).

Consent was obtained from the patient.

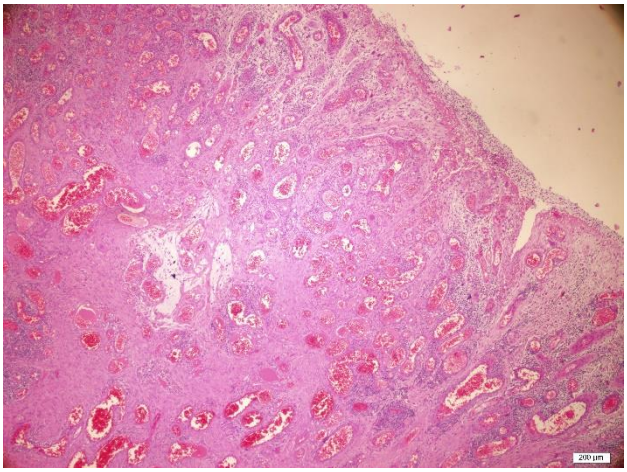

Figure 1. Histopathological specimen showing an ulcerated polyp with underlying inflammation and granulation tissue formation (H&E, x40)

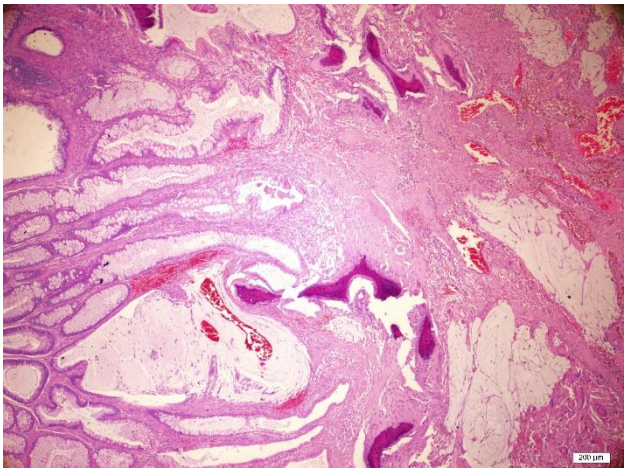

Figure 2. Histopathological specimen showing elongated, dilated, some ruptured colonic glands and areas of osseous metaplasia within an inflamed stroma (H&E, x40)

DISCUSSION

Heterotopic bone formation is not a common finding in colon polyps. To the best of our knowledge, 28 cases have been presented in the English literature so far (6-22). Eight of them were dysplastic, whereas others were inflammatory and juvenile polyps. The cases of colon polyps including osseous metaplasia that we detected as a result of the literature review are summarized in Table 1. When the cases are classified as dysplastic and non-dysplastic; the male:female ratio in the non-dysplastic (juvenile and inflammatory polyps) group is calculated as 4:1. The majority of cases are young patients. While one patient in this group is 74 years old, the age range of the other patients is 3-39 (mean age 17.2). The lesion was in the anal canal in 1 case, in the rectosigmoid are in 2 cases and in the rectum in other cases. Polyp sizes vary between 5-95 mm. Although the size of one of these 20 non-dysplastic polyps is unknown, 16 (80%) are 2 cm or less.

The pathogenesis of osseous metaplasia is not fully known, and different mechanisms have been suggested. In 1964, Marks and Atkinson (13) suggested that osseous metaplasia may develop as a result of transformation of fibroblasts into mesodermal tissue types such as osteoblasts and chondroblasts.

A case of rectal adenocarcinoma with heterotopic ossification was reported in the literature review of study by Ansari et al. (23) in which 52 cases of osseous metaplasia in the gastrointestinal system was included. Adenocarcinoma was diagnosed in 47 of 52 cases, and osseous metaplasia was mostly seen with primary tumors. Furthermore, mucin production has been observed widely in these tumors. They reported that necrosis, inflammation, calcification, increased vascularization and extracellular mucin accumulation were associated with heterotopic bone formation in tumors. Mucin was also present in the stroma of our case.

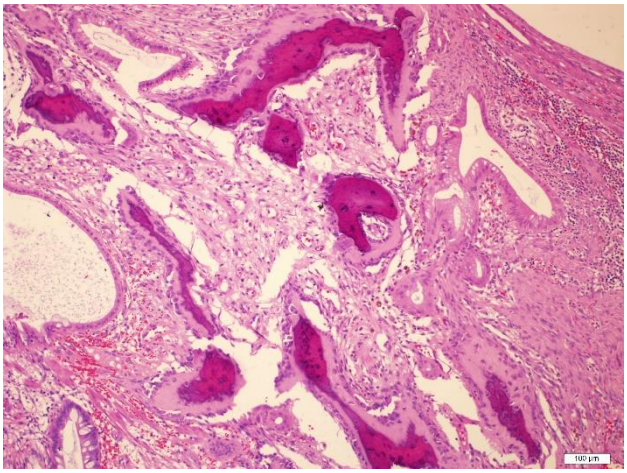

Figure 3. Histopathological specimen showing bone formation that some of them surrounded by osteoblasts (H&E, x100)

Table 1. Summary of our and previously reported cases of osseous metaplasia in colorectal polyps

No	Year	Author	Age	Gender	Site	Size (mm)
Dysplastic (3 of tubulovillous adenoma, 3 of tubular adenoma, 2 of traditional serrated adenoma)						
1	1994	Groisman (6)	67	Male	Rectum	18
2	1996	Cavazza (6)	Unknown	Unknown	Unknown	Unknown
3	1999	McPherson (7)	73	Male	Cecum	20
4	2000	Rothstein (8)	Unknown	Unknown	Sigmoid colon	25
5	2005	Al-Daraji (9)	85	Female	Sigmoid colon	15
6	2008	White (10)	63	Female	Transverse colon	Unknown
7	2010	Wilsher (11)	50	Male	Rectosigmoid	25
8	2012	Montalvo (12)	62	Male	Rectum	50
Juvenile Polyp						
9	1964	Marks (13)	10	Male	Rectum	Unknown
10	1992	Drut (14)	5	Male	Rectosigmoid	10
11	1992	Drut (14)	4	Male	Rectum	5
12	1994	Groisman (6)	3	Female	Rectum	20
13	2009	Ahmed (15)	17	Male	Rectum	18
14	2012	Bhat (16)	5	Female	Rectum	15
15	2013	Garg (17)	6	Male	Rectum	13
16	2016	Naimi (18)	10	Male	Rectum	30
17	2018	Haynes (19)	6	Male	Rectosigmoid	15
Inflammatory polyp						
18	1981	Sperling (20)	25	Male	Rectum	10
19	1992	Castelli (6)	22	Female	Rectum	10
20	2009	Oono (3)	39	Male	Rectum	12
21	2012	Odum (14)	74	Male	Rectum	10
22	2013	Bhattacharya (21)	14	Male	Rectum	10
23	2014	Zemheri (6)	9	Male	Rectum	8
24	2016	Stevanovic (2)	31	Male	Anal canal	57
25	2019	Lim (22)	30	Male	Rectum	18
26	2019	Amir (4)	10	Male	Rectum	95
27	2020	Wood (5)	17	Male	Rectum	12
28	2020	Our case	7	Female	Rectum	17

In some recent studies, it has been reported that the expression of bone morphogenetic proteins (BMPs) plays a role in the pathogenesis of bone metaplasia. In their study published in 2001; Imai et al. (24) showed that BMP-2, BMP-4, BMP-5 and BMP-6 are expressed in colonic adenocarcinomas with osseous metaplasia. In their study published in 2003; Kawai et al. (25) transferred the human BMP-2 gene to the skeletal muscle of rats by cutaneous electroporation and observed the formation of ectopic bone consisting of active osteoblasts and osteoclasts in all rats.

Wood et al. (5) presented a case of inflammatory rectal polyp with osseous metaplasia in a 17-year-old patient in their recent article and suggested that this may be a distinctive disease entity. We share the same perspective with them. Considering the presented cases of colon polyps with osseous metaplasia, the fact that most of the non-dysplastic patients are young male patients and almost all of them are located in the rectum suggest that this may be a specific entity and that recurrent traumas caused by defecation may also play role in the pathogenesis.

In conclusion, we present a rare case of osseous metaplasia in inflammatory rectal polyp. Although osseous metaplasia in colon polyps has not a significant effect on both clinic and the prognosis, it can be confusing when encountered in microscopic examination. It will be better to know that seeing osseous metaplasia will not change our original definition.

Informed Consent: Written informed consent was obtained from the patient for publication and accompanying images.

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Author Contributions: Idea/Concept: CBI; Design: CBI; Data Collection/Processing: CBI, MB, AS; Analysis/Interpretation: CBI, MB; Literature Review: CBI; Drafting/Writing: CBI; Critical Review: CBI, HÖU.

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AUTHOR GUIDELINES

SCIENTIFIC RESPONSIBILITY

In terms of scientific publishing standards, articles to be submitted should be prepared in accordance with the criteria of the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME) and the Committee of Publication Ethics (COPE).

- All articles must be complied with the research and publication ethics. The responsibility of the articles belongs to the authors.
- Articles are required to have not been published in anywhere previously, and/or are not in the evaluation process for publication.
- Articles must be submitted with the Copyright Transfer Form signed by all authors to begin the evaluation process. For authors' order, the signature order in the Copyright Transfer Form is based on.
- The corresponding author is responsible for the final version of the article on behalf of all authors.

ETHICAL RESPONSIBILITY

- Compliance with The Principles of Helsinki Declaration (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) is required in all studies including "human" factor. In this kind of studies, authors must state that they perform the study in compliance with these principles, they have taken the approval from ethics committee of their institution and the "informed consent" from people participating the study, in the MATERIAL AND METHODS section.
- If "animal" factor was used in the study, authors must state that they have protected the animal rights in line with the principles of Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) and they have taken the approval from ethics committee of their institution, in the MATERIAL AND METHODS section.
- In case reports, informed consent must be taken from patients.
- The information of the ethics committee approval should be indicated together with the name of the committee, approval date and number, in the MATERIAL AND METHODS section.
- If there is a direct-indirect commercial relation or an institution giving financial support in the study, authors must state that they have no commercial relationship with the commercial product, medicine, company etc. used, or if any, what kind of a relationship they have (consultant, other agreements), in the cover letter to the editor.
- The authors are responsible for reporting all personal and financial relationships that may be related with the study. It is necessary to state clearly whether there is any conflict of interest related to the submission and/or evaluation of the article.
- Compliance of the articles with the scientific and ethical rules is responsibility of authors.

SUBMISSION FILES

Articles must be uploaded to the system as separate files as described below.

Copyright Transfer Form: The Copyright Transfer Form to be obtained from the system during the submission must be signed by all authors in accordance with the authorship order in the article.

Cover Letter: Type of the article, the statement that has not been published previously in anywhere before, and/or not in the evaluation process for publication, if any, the people and institutions supporting the study financially and the relationship of these institutions with authors (if not, there is no relationship) must be stated. The names, academic titles, institutions, contact information and e-mail addresses of at least two reviewers suggested in relation to the subject of the article and not related to the authors and their institutions should be written. Editors' right to choose the reviewers are reserved.

Title Page: It must include the title of article (English and Turkish), short title not exceeding 40 characters, names, academic titles, ORCID® numbers, institutions, e-mail addresses of all authors, and also name, correspondence address, phone number, email address of the corresponding author. If the article has been presented previously in a scientific meeting; the name, date and place of the meeting (if not, not presented) should be stated.

Main Text: The title of the article (English and Turkish), short title not exceeding 40 characters, Abstract (English and Turkish), Keywords (English and Turkish), Main Text (sectioned according to the type of article submitted), References, Tables and Figures should be included.

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Note: If there are figures, pictures or photographs in the article, each of them must be uploaded as separate files.

SECTIONS THAT SHOULD BE USED ACCORDING TO THE TYPE OF ARTICLE

Research Article

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 200-250 words.
ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".
ÖZ, should be structured as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç".

Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 150-200 words.

Case Report

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES

- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each to be on a separate page.
- The table headings should be written at top of the table (Table 1. Table heading) and the figure captions should be written below the figure (Figure 1. Figure caption) as their first letters being upper case.
- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
- The figures and photographs should be upload as separate files in .png, .jpg, etc. format and at least 300 dpi resolution.
- Captions of figure and photograph should be given on a separate page respectively, after the page including last table.
- If figure, picture, table, graphic etc. which have been published before is used, written permission must be taken and it should be stated in the explanation of figures, pictures, tables, graphics. The legal responsibility in this regard belongs the authors.

ACKNOWLEDGEMENT

- If any conflict of interest, financial support, donation and other editorial (English/Turkish evaluation) and/or technical support, it must be stated in this section before the REFERENCES section.

REFERENCES

- References should be numbered according to the order of use and stated with numbers in parentheses as (1) or (1,2) or (3-5) at the end of the relevant sentence in the text.
- Reference list should be formed according to the reference order used in the text.
- If the number of authors are 6 or less, all authors should be specified, if there are 7 or more "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Book:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili kişisel ve finansal tüm ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve açıklama olarak belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

Makale:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

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Kitap:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:

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